GRAZER CONTROL OF BACTERIAL ABUNDANCE IN A FRESHWATER POND COMMUNITY

by

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Abstract

Metazoan grazing of bacteria represents a potential pathway for the transfer of bacterial production to higher trophic levels. Freshwater cladocerans of the genus *Daphnia* are able to reduce bacterial abundance in lakes, but many experiments have been restricted to the summer months. It is therefore necessary to test the generality of *Daphnia*'s role across seasons and across a broad range of food web configurations. The impact of mechanical grazing inhibition of Daphnid bacterivory, a potential outcome of algal blooms, has also not been addressed.

I tested the ability of each of three crustacean zooplankton species, *Daphnia pulex* Leydig, *Bosmina longirostris* (O.F.M.), and *Skistodiaptomus oregonensis* (Lillj.), and a mixed rotifer community, to control bacterial abundance in 80 litre enclosures suspended in a freshwater pond. Experiments were conducted in August 1995 and some treatments were repeated in a second experiment in October 1995 to test for seasonal differences in grazer impact. Bacterial cell abundances at the end of the summer experiment were found to be significantly lower in *Daphnia* enclosures (1.87×10^6 cells ml^{-1}) than in *B. longirostris* (3.91×10^6 cells ml^{-1}) and *S. oregonensis* (4.69×10^6 cells ml^{-1}) enclosures, using repeated measures ANOVA. Bacterial abundances were also low in the absence of macrozooplankton in both summer (2.47×10^6 cells ml^{-1}) and fall (1.19×10^6 cells ml^{-1}). In contrast to the results observed in summer, *Daphnia* enclosures sustained high bacterial abundances in the fall. Daphnid grazing of bacteria appears to have been influenced by seasonal shifts in algae composition. The presence of a bloom of *Elakatothrix* sp. coincided with significantly higher bacterial cells abundances in *Daphnia* enclosures (2.76×10^6 cells ml^{-1} and 3.65×10^6 cells

ml^{-1}), while in both seasons, grazing by *Daphnia* reduced rotifer and ciliate abundances. Daphnid grazing of bacteria appears to be more susceptible to changes in grazing behaviour than other components of the food web. Thus, the presence of *Daphnia* can be expected to have a detectable effect on bacterial abundance, but the direction of impact may differ seasonally as algal composition changes. Smaller zooplankton are not able to reduce bacterial abundance, but the absence of macrozooplankton can also result in low bacterial abundances, due to the loss of indirect influences of macrozooplankton on the microbial food web.

An experiment conducted to determine the impact of suspended particles on Daphnid grazing of bacteria resulted in an increase in bacterial abundance when grazing was inhibited by the presence of glass fibre filaments. The filaments also resulted in a modest increase of bacterial abundance in the absence of macrozooplankton grazers. Mechanical interference with *Daphnia* grazing may mitigate *Daphnia*'s potential for top-down control of the microbial food web. Suspended inorganic filaments were able to increase bacterial cell abundances in the absence of any substrate additions, indicating that the increased spatial heterogeneity and complexity afforded by suspended particles can cause a detectable enhancement of the microbial food web.

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1. General introduction

It has been nearly two decades since limnologists and oceanographers began the task of integrating the microbial food webs into the classical theories of aquatic food web structure and function (Azam et al. 1983). This surge of interest in aquatic microbial ecology swelled on the heels of several methodological innovations which permitted ecologists to measure the density (Porter and Feig 1980), productivity (Fuhrman and Azam 1982), and diversity (Fuhrman et al. 1992) of aquatic bacteria. The ability to quantify changes at the base of the heterotrophic microbial food web allowed researchers to study the ecology of microbes and their grazers in relation to autotrophic and heterotrophic production, and eventually to integrate the "microbial loop" into the algae-zooplankton-fish model of lake food webs. This has permitted more accurate estimation of carbon flows and nutrient cycling in aquatic ecosystems.

The nature of the relationship between the microbial and classical food webs was the subject of much early controversy (Ducklow et al. 1986, Sherr et al. 1986), as it became clear that in some systems (see Geertz-Hansen et al. 1987, Jeppesen et al. 1992), the secondary productivity of the microbial food web could be channelled to the macrozooplankton and thus become available to fish (Stockner and Porter 1988). In freshwater systems, researchers focussed their efforts on the distinguishing characteristics of food webs with microbial "links" as opposed to those exhibiting microbial "sinks" for organic carbon (Porter et al. 1988, Stockner and Porter 1988). It rapidly became clear that the freshwater cladoceran *Daphnia* was a key determinant of the fate of bacteria production (Stockner and Porter 1988, Gude

1988, Pace et al. 1990, Christoffersen et al. 1993), though this conclusion was reached in spite of some contradictory evidence (Pace and Funke 1991).

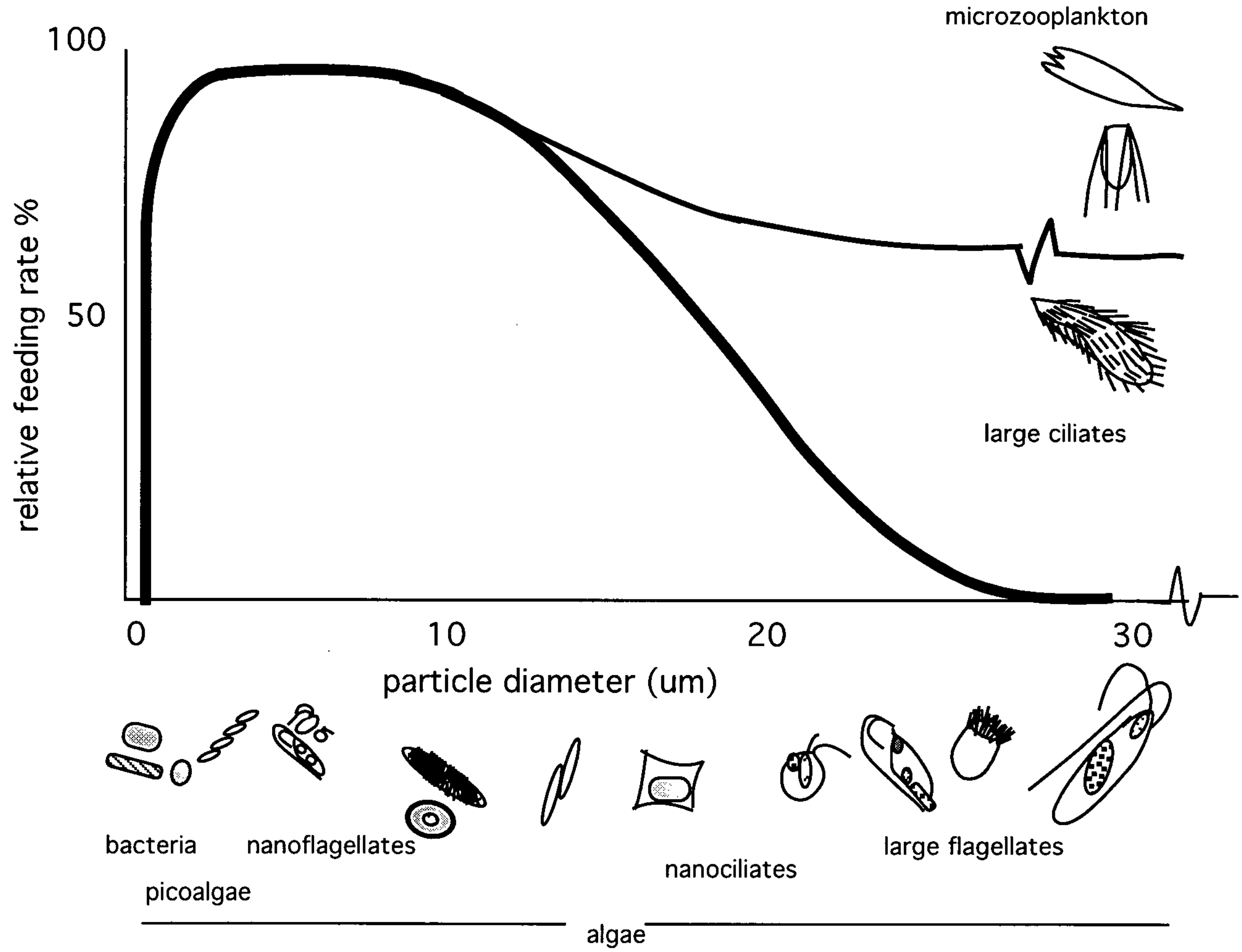
Daphnia has long been considered a "keystone" species in freshwater ecosystems (Stockner and Porter 1988). In the absence of fish or invertebrate predation, *Daphnia* are competitively superior to most small-bodied zooplankton due to their relatively non-selective feeding behaviour (Hall et al. 1976). The preferred particle size spectrum for Cladocera is shown in Figure 1 (Jurgens 1995 after Gliwicz 1980). *Daphnia* is capable of grazing a much wider array of available algal resources (including particles as large as 150 μm) and can suppress microzooplankton by both interference competition and exploitative competition simultaneously (Wickham and Gilbert 1991, 1993). It is thus not surprising to find that *Daphnia* spp. are often the most quantitatively significant links between the classical and microbial food web in lakes where they occur. The presence or absence of *Daphnia* can determine the magnitude of energy and nutrient transfer between the microbial and algae-zooplankton-fish pathways of the larger lake food web.

1.1 Trophic interactions in microbial food webs

The major components of the microbial and classical food webs are depicted in Figure 2. The term "classical food web" is used by microbial ecologists to refer to the pathways of the lake food web traditionally considered to be based on autotrophic production. Thus the autotrophic algae fix inorganic carbon through photosynthesis and take up inorganic nutrients. Algae also release DOC (dissolved organic carbon), which becomes part of the DOM (dissolved organic matter) pool shown in Figure 2. The algae are consumed by herbivorous

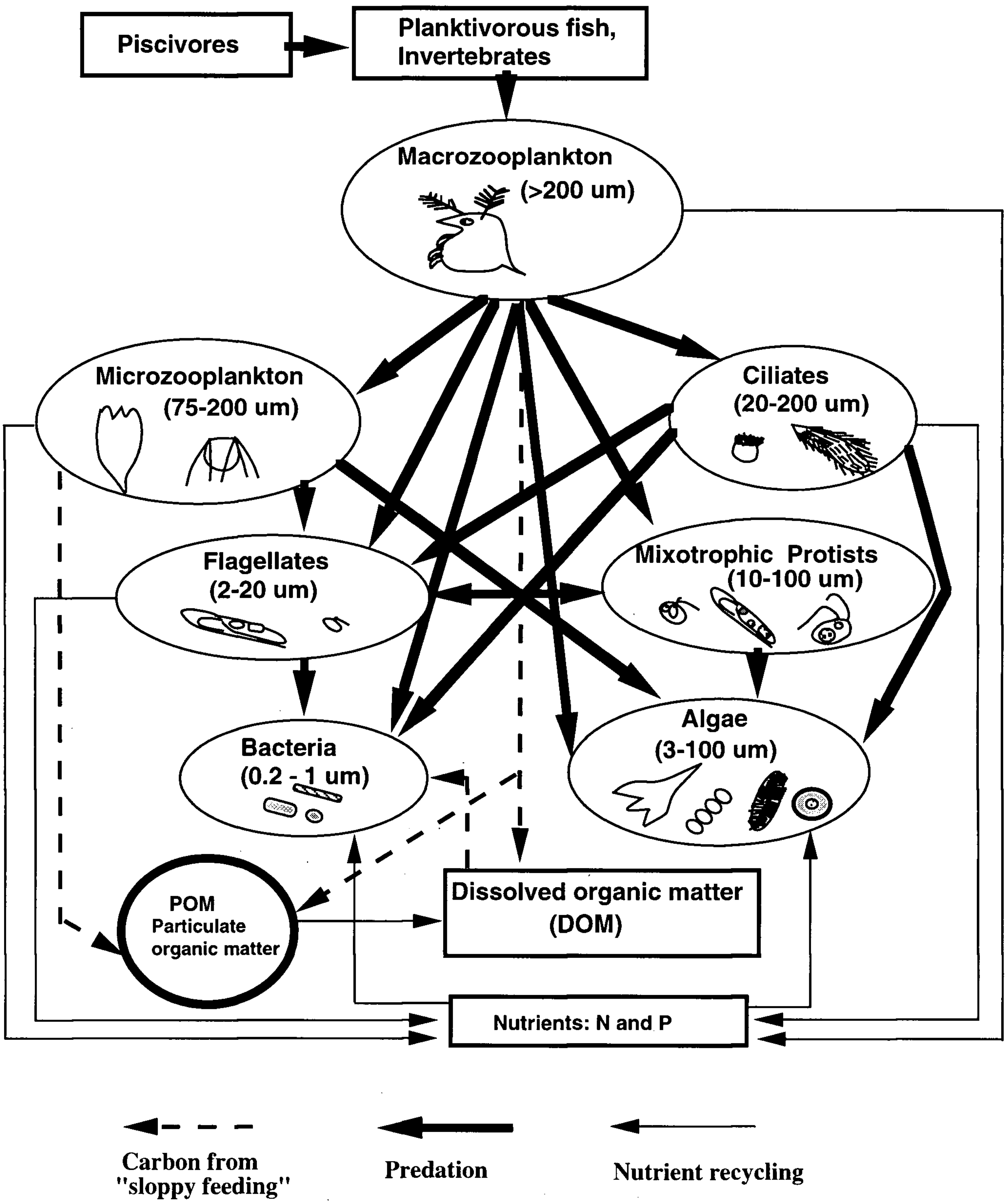
Figure 1.

Relative filtering rate of cladocerans for different particle sizes. The fine line indicates that filtering rates on large protozoa do not conform to the model.



(modified from Jurgens 1994, originally from Gliwicz 1980)

Figure 2. The microbial and classical lake food webs



zooplankton which convert algal carbon into animal biomass, excrete particulate organic matter and nutrients, and release algal cell contents into the surrounding water through "sloppy feeding". The herbivorous zooplankton are consumed either by carnivorous zooplankton or planktivorous fish, both of which return organic matter to the DOM and POM (particulate organic matter) pools through excretion. In some systems, planktivorous fish are consumed by piscivorous fish.

In literature prior to the mid-seventies, a microbial decomposer fauna was recognized, but its ecological role was restricted to the remineralization of refractory carbon compounds in the DOM pool (and therefore nutrient cycling). Modern convention now characterizes the bacterioplankton as a component of the heterotrophic food web (i.e. secondary productivity as distinguished from primary productivity), in which bacteria compete actively with algae for limiting nutrients (Currie and Kalff 1984, Currie et al. 1986, Currie 1990). At the base of the microbial food web, bacteria utilize the DOM pool to produce their biomass. Most bacterial cells are less than 1 μm in length and are vulnerable to direct grazing by protists. Small heterotrophic flagellates in the nanoplankton size range (2 - 20 μm) are the major grazers of the bacterioplankton, but larger flagellates and ciliates may graze bacteria as well (Sanders et al. 1989). Flagellates and ciliates may also graze algae, and prey on each other. Some microbial predators, unlike metazoan zooplankton, are capable of grazing prey which are equal to or larger than their own body size. Some species of algae are mixotrophic, grazing bacteria in addition to photosynthesizing, and they can be important grazers of bacteria in some systems (Boraas et al. 1988, Porter 1988, Sanders et al. 1989). Collectively these various microbes and protista are termed the microbial food web.

Where the algae are concerned, it is clear that the microbial and classical food webs are not functionally distinct components of the whole lake food web. Many of the organisms traditionally called "algae" are in fact autotrophic protists which are the same size as their heterotrophic counterparts, while some are mixotrophs which cannot be conveniently classified in the traditional autotroph/heterotroph food web paradigm. On average, about 40% of primary production fluxes through the bacteria in the photic zone of lakes and oceans (Cole et al. 1988)[1]. If the flow of carbon from the bacterioplankton and heterotrophic protists to macrozooplankton is of low magnitude (i.e. if neither bacteria nor flagellates and ciliates are grazed substantially by zooplankton), heterotrophic production by the bacteria is respired without reaching higher trophic levels. In such situations, the dynamics of the microbial components of the lake food web are of lesser importance to those who wish to understand the dynamics of algal, zooplankton and fish populations. In any event, to understand the dynamics of the microbial food web, it is necessary to quantify the biomass, productivity, and interactive pathways of its components. For questions involving the quantitative importance of the microbial food web to the processes of the classical food web, answers are often found in the zooplankton, and a cladoceran of the genus *Daphnia* often proves to be the determining factor.

[1]Bacterial production is about 20% of primary production in the photic zone (30% on an areal basis), and bacteria have a growth efficiency estimated at 50%. As a comparison, zooplankton production is about 12% of primary production (see Cole et al. 1988).

1.2 Other studies

A number of whole-lake and enclosure studies have addressed, directly or indirectly, the influence of grazer community structure on bacterial abundance (Riemann and Sondergaard 1986, Geertz-Hansen et al. 1987, Jeppesen et al. 1992, Markosova and Jezek 1993, Jurgens et al. 1994a, Pace and Cole 1996, Sarnelle 1997). Small enclosures studies tend to be well replicated and often involve direct manipulation of zooplankton abundances (Brett et al. 1994, Jurgens et al. 1994a, Sarnelle 1997). Zooplankton communities in large enclosure experiments are usually unreplicated (Riemann and Sondergaard 1986, Geertz-Hansen et al. 1987, Markosova and Jezek 1993) and indirectly manipulated using the presence/absence of fish (Riemann and Sondergaard 1986, Geertz-Hansen et al. 1987, Jeppesen et al. 1992, Markosova and Jezek 1993, Pace and Cole 1996). Manual zooplankton removal/addition is also common (Brett et al. 1994, Jurgens et al. 1994a, Sarnelle 1997). In all studies except Jurgens et al. 1994a, the presence of *Daphnia* caused a decrease in bacterial abundance. Bacterial abundance was elevated in the presence of small zooplankton grazers, and two studies were successful in maintaining a metazoan grazer-free treatment where bacterial abundance was lower than that observed in the presence of *Daphnia* (Brett et al. 1994, Jurgens et al. 1994a).

There have been two attempts to separate the impact of the various small zooplankton species in "no *Daphnia*" treatments (Brett et al. 1994, Jurgens et al. 1994a), though only the study of Brett et al. (1994) attempted single-species manipulations. In one study, *Bosmina longirostris* has been observed to stimulate bacterial production in contrast to *Daphnia*'s top-down control of biomass and production (Jeppesen et al. 1992). In another study, copepods exerted top-down control on ciliate abundance but bacterial abundance remained the same as that found in enclosures with no metazoan grazers (Jurgens et al. 1994a). The presence of *Daphnia* leads to a decrease in cell size in studies where bacterial biovolumes were measured (Jeppesen et al. 1992, Jurgens et al. 1994a).

1.3 *Daphnia* vs. small zooplankton in microbial food webs

A summary of the known and predicted effects of *Daphnia* vs. small zooplankton grazing in lake food webs is given in Table 1 (modified from Jurgens 1994). This model was developed from the various lines of evidence for the impact of *Daphnia*, and also small zooplankton, on both the microbial and classical food webs. Other versions of this model have been mentioned in the literature (Gude 1988, 1990, Stockner and Porter 1988). Its predictions have been validated to various degrees (Jurgens 1994). The food web features described under a "*Daphnia* dominant" grazer community are analogous to the conditions observed in the absence of planktivorous fish populations, where large zooplankton such as *Daphnia* are mostly free from predation pressure and can attain high population densities. A "small zooplankton dominant" community would typically be observed under heavy size-specific planktivory such as that imposed by planktivorous fish or large invertebrate predators. The trophic cascade hypothesis is implicit in this model, which essentially characterizes food webs under "top down" control (Carpenter et al. 1985).

Table 1. Important characteristics of systems dominated by *Daphnia* versus those dominated by smaller zooplankton, as observed in temperate, eutrophic lakes. Modified from Jurgens 1994.

Food Web Component	Dominance of *Daphnia* (planktivorous fish absent)	Dominance of small zooplankton (planktivorous fish present)
Phytoplankton	low biomass high grazing losses top down control	high biomass and diversity nutrient limitation bottom up control mixotrophy
Zooplankton	Daphnia	small cladocerans (*Bosmina*), rotifers, copepods
Protozoa	low numbers and diversity	high numbers and diversity numerous interactions bacterivorous, algivorous and mixotrophic species
Bacteria	moderate bacterial abundance and biomass low morphological diversity small cell sizes high ratio of bacterial to primary production	high numbers and biomass high morphological diversity grazing resistant forms: filaments, aggregates, attached bacteria low ratio of bacterial to primary production
Detritus	low standing stock, rapid turnover	high standing stock aggregates colonized by bacteria and protozoans
Nutrients	elevated levels of dissolved nutrients	nutrients bound in biomass, dissolved pools exhausted

1.4 Rationale and design

The experiments in this study were designed to further investigate the interaction pathways between zooplankton and the microbial loop. Its has been asserted that *Daphnia* grazing may directly decrease bacterial abundance in freshwater lakes, while also stimulating productivity of the remaining bacteria indirectly via nutrient recycling and the release of algal carbon due to grazing (Jurgens 1994). The use of abundance as a measure of bacterioplankton dynamics can be problematic, as changes in the relative abundance of metabolically active cells can be masked by the greater abundance of dormant cells (del Giorgio and Scarborough 1995). The grazing impact of *Daphnia*, however, is potentially large enough to have a measurable effect on bacterial cell abundances. The ability of a predator to control the biomass of prey is strong indicator of top-down control of the food web (Carpenter et al. 1985, Carpenter et al. 1987, McQueen et al. 1989, Psenner and Sommaruga 1992). I therefore wished to assess the ability of *Daphnia* to suppress bacterial abundance, and contrast this with the grazing impact of smaller zooplankton species not known to exert top-down influence on the microbial loop.

In seeking to establish and quantify metazoan links to the microbial food web, the impacts of particular grazer species are difficult to study in isolation. Only rarely are grazer "monocultures" (other than *Daphnia*) assessed for grazing impact in open lake water enclosures (Brett et al. 1994). The species-specific impacts of non-Daphnid zooplankton (especially non-cladocerans) on microbial food webs are usually inferred from laboratory studies of grazing rates on bacteria, protists and algae (Porter et al. 1983, Bleiwas and Stokes 1985, DeBiase et al. 1990, Sanders and Wickham 1993).

The experiments conducted in this study were designed to tease apart the impacts of 3 zooplankton grazers (*Daphnia pulex* Leydig 1860, *Bosmina longirostris* (O. F. Müller), and *Skistodiaptomus oregonensis* Lilljeborg 1889) and a mixed rotifer community (*Keratella cochlearis* Bory de St. Vincent and *Polyarthra* c.f. *vulgaris* Carlin 1943) on the microbial food web in the water column of a freshwater pond. While single-species impacts on the microbial food web have been studied previously (Brett et al. 1994), the small zooplankton species I examined have not been tested in isolation for their ability to control bacterial abundance. My enclosures were larger than those often used for measuring short-term microbial responses (Brett et al. 1994, Sarnelle 1997), and as I felt that previous failures to detect *Daphnia*'s impact on bacterial abundance were the result of experimental time scales that were too short. The durations of my experiments were 16 and 19 days.

Central to the model of zooplankton-microbial food web interactions tested in this study is the generality of a particular grazer's impact on the algae, protista, and bacteria in the food web. Most limnological experiments take place in the summer months, and data from early spring, late fall and winter are generally sparse. A number of food web parameters can alter *Daphnia*'s clearance rates and retention efficiency for bacteria (Lampert 1987a, Porter et al. 1983). Algal composition, algal density, nutrient availability and abiotic factors such as temperature and turbidity all vary seasonally, and all can affect the feeding behaviour of *Daphnia* (Lampert 1987a). While the influence of these factors on *Daphnia* grazing is acknowledged (Jurgens 1994), the consequences for the microbial loop have not been comprehensively investigated *in situ*. I chose to repeat experiments seasonally to test the generality of *Daphnia*'s impact. Therefore the *Daphnia* treatments included in the summer experiment were repeated in the autumn of the same year (1995).

Top down control by the grazer implies an ability to graze the available algae and microbes to the extent that standing stocks of bacteria and primary producers are reduced. But selection pressures of zooplankton on their prey, as well as bottom-up changes in nutrient regimes and abiotic factors, can induce responses in the algae that render the flora less vulnerable to grazing. Noxious, unpalatable, indigestible or colonial algae may be favoured, which are unavailable to zooplankton grazers. Inedible and sometimes inhibitory species often come to dominate the flora in the presence of *Daphnia* (Lampert et al. 1986, Sommer et al. 1986). While the inhibitory effects of algal toxins on *Daphnia* have been extensively studied, less is known about mechanical interference of filamentous algae with filter-feeding zooplankton (Webster and Peters 1978, Lampert 1987b). Inorganic particles (eg. suspended sediments) have been shown to interfere with the grazing of zooplankton populations (Kirk and Gilbert 1990, Kirk 1991), and so the possibility remains strong that there is a mechanical component to algal interference with zooplankton grazing. A number of food web parameters, such as the composition and abundance of algae, and also the presence of other particles (detritus) can alter *Daphnia*'s clearance rates and retention efficiency for bacteria (Lampert 1987a, Porter et al. 1983).

Therefore, in addition to seasonal replication of the main experiment in the Fall, the impact of "model filamentous algae" on *Daphnia* grazing on bacteria was assessed. In conjunction with this, the effect of (inorganic) filament addition on bacteria density was tested in the absence of grazing pressure. Bacterial growth is stimulated by the presence of surface area for attachment, as exemplified by the well-recognized problem of wall growth in experimental enclosures. Bacterial attachment to organic particles is common in freshwater, with attached bacteria comprising about 3% of the total bacterioplankton abundance (Kirchman 1983). Attached bacteria form a greater percentage of the total population (though never more than 10%) in the summer and fall than at other times of the year (Kirchman 1983). Attached bacteria are larger and metabolically more active than the free living bacteria (Kirchman 1983, Simon 1987, Gude 1990). The relative susceptibility of particle-bound bacteria to metazoan grazing varies according to grazer species (Schoenberg and Maccubbin 1985). It is possible that the presence of filamentous particles could stimulate bacteria growth by providing increased surface area for attachment. Aggregated growth forms also provide bacteria with refuge from protistan grazers (Gude 1990). Senescent algal blooms enhance the microbial food web by releasing organic carbon, but in providing a physical matrix for bacterial attachment they may also contribute a "mechanical" enhancement of microbial growth. Such an effect would increase the enhancement of the microbial food web in the latter stages of filamentous algal blooms. It is therefore likely that glass fibre filaments will inhibit *Daphnia*'s grazing on all components of the microbial food web, and increase bacterial abundance by providing increased surface area for attachment and growth.

1.5 Zooplankton

The zooplankton communities in this experiment were manipulated to include only one species of macrozooplankton. Microzooplankton (rotifers) were also manipulated and were present in all the experimental zooplankton communities. The individual zooplankters used in my experiments are common limnetic species that have been subject to investigations of their impact on classical lake food webs. In the case of *Daphnia*, much is already known about its relationship to the microbial component of lake food webs. For *Bosmina*, *S. oregonensis* and the rotifers *K. cochlearis* and *P. vulgaris*, studies of microbial food web interaction are less common, as often the smaller zooplankton are studied collectively where they co-occur. The feeding behaviours of the zooplankton employed in this study, and their potential impact on the microbial loop, are summarized below.

1.5-1 *Daphnia*

The dominance of *Daphnia* in freshwater food webs is a direct result of its competitive superiority over smaller zooplankton in grazing the < 20 µm algal size fraction (Hall et al. 1976, Gliwicz 1990). Competitive superiority and vulnerability to predation are positively related in the Cladocera (Bengtsson 1987), and it is the interaction of these major factors which structure zooplankton communities. In the absence of fish predation, larger bodied Cladocera are often able to competitively exclude smaller zooplankton, though the controversy surrounding this issue has hardly been settled (Dodson 1974, Romanovsky 1985, Bengtsson 1987, Gliwicz and Lampert 1993). *Daphnia pulex* has been shown to suppress the density of *Bosmina longirostris*, copepod nauplii and rotifers (Vanni 1986). *Daphnia*'s dominance as a pelagic grazer has spawned numerous investigations of its uniquely prodigious grazing ability (see Lampert 1987a for an extensive review).

Daphnia spp. are filter feeders, with specialized feeding limbs having fine meshes which are able to retain particles less than 1 μm in diameter. *Daphnia pulex*, with a mean filter-mesh size of about 0.4 μm (Brendelberger 1985) is able to retain the larger bacteria (~1 μm), but its filtering efficiency on the more numerous smaller cells (<0.5 μm) is poor (Brendelberger 1991). However, large bacteria have higher growth rates and are responsible for more bacterial productivity than the smaller cells (Sherr et al. 1992). *Daphnia* is morphologically able to selectively crop the metabolically more active fraction of the bacterioplankton. The upper size limit on *Daphnia*'s ingestion capability is correlated with the animal's body size (maximum length of adult animals ~3.5 mm for the largest *Daphnia* species). Juveniles have finer meshes than adults (Brendelberger 1991), and filter mesh size is a phenotypically plastic trait that is developmentally responsive to food levels experienced by neonates (Lampert 1994). The smaller filter meshes of juveniles allow them to be more efficient feeders on the smallest size fraction of the planktonic food spectrum (Brendelberger 1991). In general, *Daphnia* clearance rates are highest on algae below 20 μm (Gliwicz 1980 in Jurgens 1994), but clearance rates on large, soft-bodied protozoa can also be relatively high (Jurgens 1994). Feeding rates are influenced by food concentration, temperature, light, oxygen and pH, with nanoplanktonic algae comprising the most preferred component of the food spectrum.

Daphnia pulex is capable of adjusting its feeding behaviour to lower its intake of low quality food and increase its ingestion of preferred species. *Daphnia* cannot completely avoid grazing unwanted algae, and has been shown to be fairly non-selective when offered simple mixtures of food (DeMott 1982). *Daphnia* cannot reject individual particles, as food collected on its filter screens is transported *en masse* along the food groove to the mouth. The entire food groove may be cleaned by a rejection movement of the post-abdominal claw, but edible algae are removed along with the undesirable items (Lampert 1987a).

Adult *Daphnia pulex* has been shown to feed on bacteria with clearance rates between 0.23 and 1.05 ml ind^{-1} h^{-1} (Jurgens 1994). Some studies have reported *Daphnia*'s grazing of bacteria to be enhanced when algal density is low (Sanders et al. 1989, Jurgens et al. 1994b), while other investigators report that the presence of larger particles enhances the retention efficiency for bacteria (Porter et al. 1983, Urabe and Watanabe 1991). "Clogging" of the filter meshes with larger (edible) algae may reduce the effective mesh size of the filtering limbs, while very low algal abundance may promote an increase in filtering rate for *Daphnia* with a concomitant increase in feeding rate on bacteria.

Daphnia preys upon most of the major components of microbial food webs. *Daphnia* populations are able to suppress heterotrophic nanoflagellate abundance (Gude 1988, Weisse 1991, Jurgens and Stolpe 1995) and *Daphnia ambigua* is able to grow and reproduce on a diet of heterotrophic flagellates (Sanders and Porter 1990). Ciliates alone are not sufficient food for *Daphnia* (DeBiase et al. 1990), but *Daphnia* are able to graze small ciliates with the same efficiency as algae (Sanders and Wickham 1993) and can suppress ciliate abundance (Jack and Gilbert 1994). *Daphnia* is thus able to graze both the bacteria and bacterivorous protists. When its population density is high, it can clear the water of almost all edible algae and protists (with the exception of filamentous or colonial algae). This well known phenomenon has been termed the "clearwater phase" where it occurs seasonally in lakes (Lampert et al. 1986, Sommer et al. 1986).

1.5-2 *Bosmina*

Bosmina longirostris is a small bodied cladoceran which is capable of dual feeding modes. Its thoracic limbs are modified for both large-particle capture and small-particle filtering (DeMott and Kerfoot 1982, Bleiwas and Stokes 1985). *Bosmina* shows a strong preference for algal prey items over bacteria-sized particles, and will stop feeding in a pure bacterial suspension. Preconditioning on bacterial food sources only increased its preference for algae in grazing experiments (DeMott 1982). *Bosmina*'s feeding mode is fundamentally different from that of *Daphnia*, and for this reason it is a highly selective feeder capable of efficiently avoiding ingestion of undesirable items (Burns 1968 in DeMott 1982). *Bosmina* has a large advantage in ingestion rate per unit biomass over that of *Daphnia* at low food concentrations. However, *Bosmina*'s clearance rate is very sensitive to changes in food concentration, and at higher food concentrations its weight-specific ingestion rate is similar to that of *Daphnia* (DeMott 1982). Though it prefers algae in the <20 μm size range, it is able to collect the larger cells in this size class more quickly. Most probably small particles are collected by filtration while the larger algae are captured by grasping (Bleiwas and Stokes 1985). It has been speculated that *Bosmina*'s continuous swimming behaviour may increase its encounter rate with preferred prey items, which it could search out and actively grasp (DeMott 1982).

Daphnia relies on passive filtering and rejection mechanisms to avoid ingesting low quality food, while *Bosmina* is able to actively select high quality particles. Thus in situations where algal concentrations are high but the edible fraction is small, *Bosmina* can coexist with *Daphnia* even in the absence of fish predation. Where *Daphnia* feeds with low selectivity, *Bosmina* undergoes dietary switching and can discriminate between individual species of algae (DeMott and Kerfoot 1982). While the differences in feeding mode between *Daphnia* and *Bosmina* predict more complicated competitive outcomes than those suggested by the size-efficiency hypothesis (Dodson 1974, Hall et al. 1976), *Bosmina*'s feeding modes dictate that its impact on the microbial food web must also be fundamentally different from that of *Daphnia*.

Bosmina's dual feeding mode allows it to selectively feed on highly edible flagellated algae, particularly when these prey items are present at low densities (Demott and Kerfoot 1982). The population growth rate of *Bosmina* has been correlated with flagellate density (Demott and Kerfoot 1982), and it has been shown to prefer grazing on flagellated algal cells over non-flagellated algae (Bogdan and Gilbert 1982). Flagellated algae can be autotrophic or mixotrophic, and are usually categorized separately from the heterotrophic flagellates in the literature. This designation is an artificial one where crustacean zooplankton are concerned, as heterotrophic protists are equal in quality to autotrophs as food for zooplankton (DeBiase et al. 1990, Sanders and Porter 1990, Sanders and Wickham 1993, Sanders et al. 1994). The potential of *Bosmina* to graze heterotrophic flagellates has not been tested experimentally. However, as with algae, the suitability of individual flagellate species as food for Cladocera likely varies, and where edible heterotrophic flagellates are present, *Bosmina* has the potential to feed on them. In addition to its avoidance of bacteria as a food, *Bosmina* could increase bacterial standing stock still further by grazing heterotrophic nanoflagellates, which are the main bacterial predators (Fenchel 1982). *Bosmina* has been reported to capture ciliates at rates higher than its clearance rates for phytoplankton (Jack and Gilbert 1993), and ciliates are also well-documented bacterial grazers (Weisse and Muller 1990, Muller et al. 1991). Higher bacterial abundances are predicted in the presence of *Bosmina* than in *Daphnia*-dominated communities. If *Bosmina* were to graze bacterial predators selectively, bacteria standing stocks would be further enhanced.

1.5-3 Copepods

Calanoid copepods such as *Skistodiaptomus oregonensis* are known to be highly discriminant grazers of freshwater algae (Butler et al. 1989). Copepods are capable of passive filter feeding on small particles, but their predominant feeding mode involves the capture and ingestion of larger cells. Some species have been shown to prefer larger algae and flagellates over smaller cells. Diaptomid copepods detect their prey primarily by mechanoreception and select their food actively (DeMott and Watson 1991). *S. oregonensis* itself is capable of a high degree of taste discrimination in accepting or rejecting prey items and the cells are usually tasted at the mouth before rejection (Demott and Watson 1991). When offered flavoured beads coated in algal extract, it showed a preference for flavoured beads and could discriminate among the "flavours" of algal species. In contrast, *Daphnia* shows very little taste discrimination, while *Bosmina* showed a modest taste response (Kerfoot and Kirk 1991). This is consistent with both cladocerans' abilities to feed selectively on algae and protists.

Skistodiaptomus oregonensis could not grow and reproduce on a bacterial diet, and though it reproduced well on abundant algal food, it achieved a higher reproductive rate when fed a mixed diet of algae and ciliates (Sanders et al. 1996). High reproductive rates were also achieved on a diet of ciliates alone. *S. oregonensis* did not thrive when its algal diet was supplemented with a heterotrophic nanoflagellate known to be suitable as a food source for *Daphnia* (Sanders et al. 1996).

It is likely that copepods will not exert direct control on bacterial biomass, though they may be able to influence it through their grazing impact on bacterivorous ciliates and larger flagellates. *S. oregonensis* has low filtering rates compared to Cladocera of similar body size (Knochel and Holtby 1986). However, if copepods graze very selectively and at high rates they may be able to influence ciliate community structure (Burns and Gilbert 1993), and hence exert control of the microbial food web indirectly through predation on microbial grazers. Copepods can be significant predators on ciliates in marine microbial food webs, and also in freshwater systems (Sanders et al. 1996, Burns and Gilbert 1993, Sanders and Wickham 1993).

1.5-4 Rotifers

The position of rotifers relative to the microbial food web has been subject to some dispute. Though in some systems they constitute a large fraction of the zooplankton biomass, their impact on the trophic dynamics of lake food webs is seldom regarded as consequential (Bogdan and Gilbert 1982). However, at high abundances they have been found to have higher grazing rates on nanoplankton than Crustacea (Sanders et al. 1994). Recent synthesis indicates that rotifers are unlikely to exert top-down control over the microbial food web, though they may be able to alter the species composition and size spectrum of its components (Arndt 1993).

It is difficult to separate rotifers as one "compartment" of lake food webs, as their range of body sizes, usually 100 to 500 μm in length, overlaps those of large microbial grazers and small Crustacea (Pennak 1989). In addition, they have a range of feeding modes which allows for bacterivory, herbivory, and raptorial or "grasping" capture of single cells (Pennak 1989, Bogdan et al. 1980, Bogdan and Gilbert 1987). Many are omnivorous filter feeders which will consume any potential food item that falls within their preferred size range (Arndt 1993).

The most abundant rotifer species in the South Campus experimental pond were *Keratella cochlearis* and *Polyarthra* c.f *vulgaris*, which constituted most of the rotifer fauna added to (or inadvertently present in) experimental enclosures. Both are common limnetic rotifer species (Pennak 1989). *K. cochlearis* has demonstrated an ability to feed on bacteria (Bogdan and Gilbert 1987, Sanders et al. 1989), though it may also show some selectivity for algal over bacterial cells (Bogdan et al. 1980, Gilbert and Bogdan 1981, Bogdan and Gilbert 1982.) It is a filter feeder capable of concentrating small particles in its feeding current and ingesting them en masse (Bogdan et al. 1980; Starkweather 1980; Bogdan and Gilbert 1987). *Polyarthra* prefers food in the 1-40μm range, and feeds on single, flagellated cells (Gilbert and Bogdan 1981; Bogdan and Gilbert 1982, 1987; Arndt 1993). It is a much more selective grazer than *K. cochlearis* (Gilbert and Bogdan 1981), and does not graze bacteria (Sanders et al. 1989). For both species, the presence of a flagellum on an algal cell facilitates capture of the cell by the rotifer, though only *Polyarthra* seems to require this feature (Gilbert and Bogdan 1981). *K. cochlearis* has been observed to grasp algal cells by the flagellum in order to facilitate ingestion (Pourriot 1977). *Polyarthra* has also been observed to feed on species of *Bodo*, a nanoflagellate genus which includes bacterivorous species (Buikema et al. 1978).

1.5-5 Predictions

The predictions regarding bacterial abundance, as well as ciliate and rotifer densities, are summarized for each treatment in Table 2.

Table 2. A summary of the predictions regarding food web parameters for treatments in the Summer and Fall experiments. Note that some treatments were not performed in both seasons.

Treatment	Predictions for Summer	Predictions for Fall
DAPHNIA *Daphnia pulex*	low bacterial abundance suppression of ciliates and rotifers	low bacterial abundance suppression of ciliates and rotifers
BOSMINA *Bosmina longirostris*	high bacterial abundance suppression of ciliates	NA
COPEPOD *S. oregonensis*	high bacterial abundance suppression of ciliates	high bacterial abundance suppression of ciliates
ROTIFER *K. cochlearis/P. vulgaris*	high bacterial abundance high ciliate abundance highest rotifer abundance	high bacterial abundance high ciliate abundance highest rotifer abundance
DAPHNIA+F *Daphnia pulex* under grazing inhibition	NA	high bacterial abundance moderate ciliate abundance moderate rotifer abundance
FILAMENT Suspended glass fibre filaments	NA	increase bacterial abundance

2. Methods

2.1 Design

Two experiments were performed to elucidate the potential influence of zooplankton grazers on the microbial food web of a small pond. The experiments were designed to detect whether a particular grazer community (ideally consisting of a single grazer species) could control bacterial abundance. These experiments exposed microbial food webs to simplified, strongly manipulated grazer communities over a time scale which encompassed many generations of the bacterial prey populations.

Experiment 1 took place from August 9 to August 24, 1995. Experiment 2 was run from October 19 until November 4, 1995. The second experiment was designed to repeat treatments from experiment 1 later in the season. I will refer to experiment 1 as "Summer" and experiment 2 as "Fall" when making seasonal comparisons of the results. Some treatments from the Summer experiment could not be run in the Fall, and therefore two new treatments were added to the Fall experiment.

The basic structure of both experiments included five grazer treatments with three replicate enclosures for each treatment. Treatments were randomly assigned to enclosures (15 out of the 20 enclosures were "experimental"). The treatments applied are described in Table 3. In Summer, treatments were selected to represent "*Daphnia*-dominated" (DAPHNIA treatment) and "small zooplankton-dominated" communities (BOSMINA, COPEPOD and ROTIFER treatments). Prior to treatment addition, each enclosure contained a natural pond phytoplankton/microbial community from which metazoan grazers had been removed. In the

Table 3. Initial treatments added to enclosures 48 hours after filling of the bags with 54 μm filtered water. Stocking densities are given in Table 4 a-c.

#	Season	Treatment	Description	Number stocked per bag	Biomass stocked per bag (mg dry weight)	Mean weight of individuals stocked (μg ± 1 standard error)	Mean length of individuals stocked (mm)
1	Summer	**Daphnia**	*Daphnia pulex* adults (> 1 mm)	350	19	54.7 ± 1.4	2.47
2	Summer	**Bosmina**	*Bosmina longirostris*	9600	9.3	0.97 ± 0.074	0.33
3	Summer	**Rotifer**	*Keratella cochlearis* *Polyarthra vulgaris* copepod nauplii (not counted)	1200	0.051†	0.043*	not measured
4	Summer	**Copepod**	*Diaptomus oregonensis* adults and copepodites	1920	19.2**	10	not measured
5	Summer	**No Grazer**	no zooplankton added				
6	Fall	**Daphnia**	*Daphnia pulex* adults (> 1 mm)	950	17	17.8 ± 0.4	1.5
9	Fall	**Daphnia + F**	Daphnia pulex adults (<1 mm) glass fibre filaments	950 8 x 10^7	17	17.8 ± 0.4	1.5
7	Fall	**Filament**	glass fibre filaments no zooplankton added	8 x 10^7			
8	Fall	**Rotifer**	*Keratella cochlearis* *Polyarthra vulgaris*	1200	0.051†	0.043*	not measured
10	Fall	**No Grazer**	no zooplankton added				

†not measured directly; calculated using estimated biomass per individual from literature values
*not measured directly; estimated from average of literature values of biomass for both species
** based on biomass given in Dumont et al. 1975 for calanoid copepods; very likely an overestimate

Fall, two new treatments were added to investigate the effects of mechanical interference on *Daphnia* grazing.

There is no established protocol for determining the appropriate duration of an experiment for a container of a given size. On the basis of a pilot study conducted in early spring, I estimated that seven days would be required to detect any effects, and doubled this estimate as a safety margin. The container size chosen was based on the need to hand-sort sufficient zooplankton for three replicates of each treatment while minimizing the handling time for the organisms.

2.2 The G.G.E. Scudder Experimental Ponds

All experiments were conducted in Pond 13 of the G.G.E. Scudder Experimental Ponds on the University of British Columbia campus. The ponds are located in a clearing adjacent to old second-growth BC temperate rainforest. There are 13 morphologically identical ponds in close proximity. These artificial ponds were constructed in 1990, each with dimensions of 23 m X 23 m, with the sides of the pond sloping at a 3:1 ratio to a maximum depth of 3 m (Schluter 1994). They have a natural bottom substrate covering a thick plastic liner (Schluter 1994). The initial substrate depth was 30 cm, consisting primarily of sand and Texada limestone, but the deposition of sediment has increased at the centre of the pond due to slumping from the littoral zone. The pH of Pond 13 is slightly alkaline, and remained at or near 8.5 during the time of my experiments.

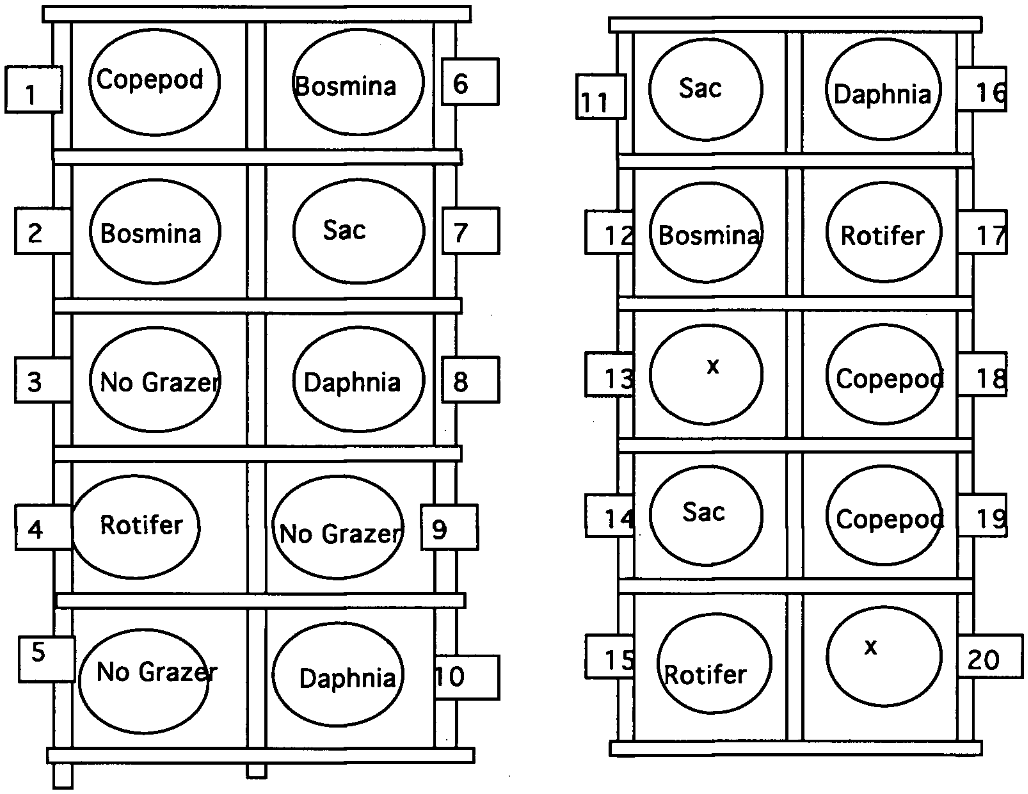
In 1991, the ponds had initially been stocked with zooplankton and macrophyte vegetation from Paxton Lake, a mesotrophic lake on Texada Island. Pond 13 has been unmanipulated since that time and serves as one of the "control" ponds in a long term study of the impact of stickleback on zooplankton and benthic communities. In Summer and Fall 1995, Pond 13 exhibited a typical small-bodied zooplankton community. Its late summer zooplankton assemblage consisted primarily of the copepod *Skistodiaptomus oregonensis* along with the cladocerans *Bosmina longirostris* and *Diaphanosoma brachyurum*. The most abundant rotifer species were *Keratella cochlearis* and *Polyarthra* c.f. *vulgaris*. Pond 13 is fishless, however it had high densities of *Chaoborus* sp. larvae in summer 1995 and experienced heavy invertebrate planktivory. There were no *D. pulex* in Pond 13 in the Summer or Fall of 1995, and *Daphnia* has made only sporadic appearances in the pond in recent years.

The bottom of Pond 13 is obscured by a thick carpeting of macrophytes. The water remains clear throughout the year and the bottom vegetation is always visible. This is in contrast the "fish" ponds adjacent to it, which have frequent algal blooms that greatly reduce water clarity. Pond 13 has come to adequately represent a "natural" pond in terms of its limnetic plankton and benthic invertebrate communities. Its recent origin has resulted in a system with low species diversity that facilitates manipulation and monitoring of its components.

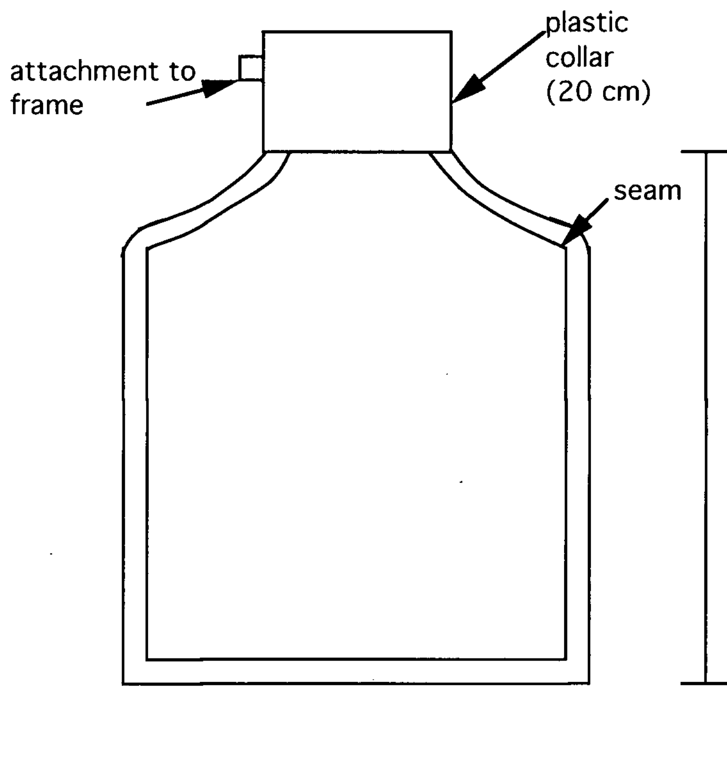
2.3 Enclosures

The enclosures were built of 6 mil (0.15 mm) clear polyethylene sheeting, with a maximum capacity of 100 litres (see Figure 3). The polyethylene sheeting was washed thoroughly with phosphate-free soap to removed any binders or lubricants remaining from the

Figure 3. Placement of treatments in enclosures in the Summer experiment. The "sac" enclosures were sampled for zooplankton at the midway through the experiment. X denotes an unused enclosure.



Construction of enclosures:



manufacturing process. The enclosures were tied into wooden floating frames which separated the open enclosures from the pond surface by a height of 10 cm. The two floating wooden frames were anchored in the centre of Pond 13. The dimensions of the enclosure bags and the position of assigned treatments are shown in Figure 3.

The pond water which was used to fill the enclosures, was collected with a battery-powered plankton pump at approximately 1 m depth in the centre of Pond 13. The water was filtered through a 54 μm plankton net into 70 litre plastic containers and mixed thoroughly. Each batch of filtrate was divided and distributed equally across all enclosures until the enclosures were filled to 80 litres in volume. This process ensured that initial conditions were nearly identical for all enclosures. In both experiments, a natural algal/microbial Pond 13 community was retained in the enclosures, while all macrozooplankton and most of the rotifers were removed by the filtration. Further reducing the filter mesh size to screen out all rotifers would also have screened out large protozoa and algae. This would have prevented a natural microbial community from developing. Large dinoflagellates (*Ceratium* sp.) and other large algal cells were also removed by the filtering; any further reduction in large algae was not desirable. The presence of rotifers was unavoidable in all experimental enclosures.

A solution of potassium phosphate and potassium nitrate, in an atomic N:P ratio of 20:1, was added to filled enclosures, for a total phosphorus addition of 10 μg/L and a nitrogen addition of 90.4 μg/L. This was done to ensure that the enclosures would be able to develop sufficient algal biomass before zooplankton were added, as well as sustain algal growth throughout the experiment. The bags were allowed to stand for 48 h prior to macroand microzooplankton additions to permit the algae and protista to recover numerically from the effects of pumping and filtering.

Each enclosure opening was covered with nylon mesh window screening to reduce illumination in the enclosures. All organisms were necessarily restricted to the upper 1 m of the water column and therefore were unable to migrate in response light levels. Shading reduced the possibility that light levels in the enclosures would be harmful to the plankton. Enclosures were thoroughly mixed twice daily using a long plastic stirring rod with a small paddle at the tip. Care was taken to stir gently while bringing up water from the bottom of the bag and loosening settled detritus. The enclosures had very little natural turbulence, and the stirring protocol prevented algae and nutrients from "settling out" of the enclosure system.

Shortly after the zooplankton addition in the Fall experiment, filaments were added to enclosures receiving the glass fibre filament treatment. The filament solution was prepared by sonicating Whatman GF/F and GF/C filters in distilled water, until the filters were completely dispersed. The filters had previously been ashed at 450 °C for 24 h (Brinch-Iversen and King 1990). The fibre solution was settled and the filament density estimated; the filament solution was then added in aliquots to the bags to achieve an initial density of 1000 filaments per ml. Throughout the experiment there was considerable loss of filaments due to settling despite the stirring protocol. To counteract this, additions of filament solution were given to the enclosures several times throughout the 18 day experimental run. Preliminary laboratory estimates indicated that all of the filaments would have settled out of solution after 24 h; stirring occurred every 12 h. Filament densities were therefore quite variable. Filament density in recently stirred enclosures was 4200 ± 1300 ml^{-1} (mean $\pm$ 1 standard error) on the final day of the Fall experiment.

2.4 Preparation of treatments

2.4-1 *Daphnia*

Daphnia pulex used in experiments were collected from an ornamental pond north of Main Library, on the University of British Columbia campus. There are *Daphnia* present in "Library Pond" from early spring until late fall. They were easily collected in large numbers using a 10 X 20 cm aquarium net. *Daphnia* were sorted and counted in the lab; adults estimated to be larger than 1 mm were selected using a large-bore pipette. Any individuals exhibiting ephippia (resting egg cases) were excluded. The sorted stock was then sampled to obtain a size distribution for the experimental animals. *Daphnia* were added to the enclosures less than 24 h after collection, and were kept incubated in the dark at 16°C until the bags were stocked. Stocking densities were 5 individuals L^{-1} in experiment 1 and 12 individuals L^{-1} in experiment 2; the total biomass stocked per enclosure was 19 mg in Summer and 17 mg in Fall (Table 3). This biomass of *Daphnia* was chosen to give a final population filtering capacity (allowing for reproduction) of about 1/3 of the enclosure per day. *D. pulex* size in the source populations (August vs. October) differed substantially (Table 4a); stock densities were adjusted in Experiment 2 to maintain comparable *Daphnia* biomass between experiments (Table 3) .

2.4-2 *Bosmina*

Bosmina longirostris added to experimental enclosures were collected from Pond 13 using a plankton net with a mesh size of 54 μm, and sorted in the lab overnight prior to addition to the enclosures. *Bosmina* were separated by hand from the other plankton; this

Table 4a. Initial *Daphnia* sizes and filtering rates for both experiments.

Month	Mean Length (µm)	Mean Weight (µg)	Peterson[1] (ml ind^{-1} d^{-1})	Haney[2] (ml ind^{-1} d^{-1})	Number L^{-1}
August	2467	54.7	31.6	27.2	5
October	1523	17.8	14.4	10.6	12

[1] calculated from the equation for hourly grazing rate on natural bacteria; Peterson et al. 1978
[2] Haney 1985

Table 4b. Initial *Bosmina* size and estimated clearance rates in the Summer experiment

Month	Mean Length (µm)	Mean Weight (µg)	Filtering rate on bacteria (ml ind^{-1} d^{-1})	Filtering rate on flagellates (ml ind^{-1} d^{-1})	Number L^{-1}
August	332	0.97	0.43	1.87	120

Table 4c. Initial copepod density and estimated clearance rates in the Summer experiment.

Month	Mean Weight (µg)	Filtering rate on ciliates (ml ind^{-1} d^{-1})	Filtering rate on flagellates (ml ind^{-1} d^{-1})	Number L^{-1}
August	10*	7.68	4.8	20

*weight is an estimate from literature (Dumont et al. 1975) was facilitated by *Bosmina*'s phototactic response and relatively rapid swimming speed. Initial stocking densities were targeted at 100 individuals per litre with a 20% allowance for handling mortality, therefore 120 individuals were added for each litre of enclosure volume. Assuming some eventual biomass increase, this density was roughly estimated to allow a population filtering capacity (L pop^{-1} d^{-1}) of 1/3 of the enclosure volume. Animals were stocked in enclosures within 24 h of collection, after being kept in Nalgene carboys overnight in a dark incubator at 16°C. Initial *Bosmina* size and filtering capacity is given in Table 4b.

2.4-3 Copepods

After most of the *Bosmina* had been removed from the collected plankton, the remaining *Skistodiaptomus oregonensis* could only be separated from the co-occurring *Diaphanosoma brachyurum* by allowing the concentrated animals to remain in the 20 L collection carboys overnight (in a dark incubator at 16°C). *S. oregonensis* survived this treatment, but *D. brachyurum* eventually collided with the walls of the container and adhered or were trapped at the air/water interface. Few *Diaphanosoma* were left alive after 24 h, and the remaining zooplankton in the carboy were almost exclusively *S. oregonensis*. Copepods were stocked at an initial density of 24 animals per litre of enclosure volume, which included a 20% allowance for handling mortality. As copepod filtering rates are much lower per unit biomass than those of Cladocera, an estimated copepod biomass equal to that of *Daphnia* was added (Table 4c). Biomass estimates were made using values given for calanoid copepods in Dumont et al. 1975.

2.4-4 Rotifers

Rotifers were harvested from experimental ponds which had high rotifer densities at the time the experiments were conducted; source ponds were chosen based on their low densities of *S. oregonensis* nauplii, which could not be separated from the collected rotifers. The stock of rotifers added was combined from Ponds 3, 5, and 7, all of which contain limnetic or benthic stickleback. Rotifers were collected with a 54 μm mesh-size plankton net, and the collected plankton was passed through a 118 μm sieve to gently filter out all large zooplankton and most of the larger copepod nauplii. Small nauplii could not be separated from the rotifers by filtration. After the 118 μm filtration, rotifers showed some mortality due to sieving. Further reductions in mesh size increased handling mortality. To reduce the stress on the rotifers, they were harvested, concentrated, their abundance estimated, and the stock added to enclosures as rapidly as possible (within 1 h of collection). Initial stocking densities were 1,200 individuals per litre. Despite this precaution, some mortality of rotifers in the stocking carboys was observed. I did not attempt to equalize rotifer biomasses to that of *Daphnia* (200 $\mu g\ L^{-1}$), though biomass estimates for *K. cochlearis* in Dumont et al. 1975 indicate that the biomass of rotifers added may have been as much as half the *Daphnia* biomass.

2.5 Collection of Samples

Enclosures were sampled for bacteria and algae daily between noon and 14:00. Each bag was thoroughly stirred prior to sampling, and let stand for a few minutes to allow large detritus to settle. Three depth-integrated water samples were then taken from each bag using a weighted length of polyethylene tubing (1 cm diameter). The subsamples from different areas of the bag were pooled (approx. 250 ml total volume). Any macrozooplankton captured were removed with a pipette and returned to the enclosure. The water sample was gently mixed and a 15 ml sub-sample for bacterial counts was taken and preserved with 2% glutaraldehyde. The remainder of the sample was preserved with acid Lugol's solution. Temperature was recorded daily[2] in enclosure 1, initially with a probe accompanying the pH meter and later with a hand-held thermometer after the probe proved unreliable. pH was sampled with a portable pH meter (manually corrected for temperature) in all enclosures every 2 or 3 days[3].

Rotifers in the ROTIFER enclosures were sampled at the midpoint of each experiment (August 17, 1995 and October 25, 1995), to give an estimate of rotifer densities. Two additional enclosure bags were included in the Fall experiment which had been treated in the same fashion as the ROTIFER enclosures, but the inoculum of harvested rotifers was heat-killed before addition to the enclosures. These "killed rotifer" treatments were sampled for zooplankton at the same time as the other "rotifer" enclosures. The volume of the midpoint sample was 2 L from each enclosure. Midpoint macrozooplankton samples could not be taken from the experimental enclosures, though samples were taken from non-experimental enclosures included for this purpose. These samples were counted to determine whether added zooplankton survived until the midpoint of the experiment, and are not included in the presentation of results. Final rotifer and macrozooplankton samples were taken at the end of

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[2]temperature samples were omitted on a few dates in the Summer experiment

[3]a malfunctioning probe necessitated the exclusion of some Summer pH sampling

each experiment by emptying the entire contents of the enclosure through a 54 μm plankton net and preserving all zooplankton in sugared formalin.

2.6 Processing of samples

2.6-1 Zooplankton

Macrozooplankton samples were counted using a Wild M5 dissecting microscope equipped with a drawing tube, digitizer pad and associated microcomputer. Subsamples were taken using a plankton splitter and counted to give a minimum of 300 individuals of the main taxa present. With the exception of rotifers, all individual zooplankton counted were also measured for length.

Macrozooplankton masses were calculated using the equations given in Table 5. Any individuals which could not be measured due to poor orientation or physical damage to the organism were assigned the mean weight for that taxon for that particular replicate. Mass for each individual was calculated from length-weight regressions and summed to give the total biomass for the sample. This obviates the need for any correction factors associated with the use of mean zooplankton length to estimate biomass for the sample (see McCauley 1984 for a review). Mean length is a more accurate measure when measuring only 30-50 animals in a sample, but as I measured many more individuals (about 300 per sample), summing individual calculated weights provides a better estimate.

Rotifers were enumerated using the digitizer pad; accurate lengths could not be determined using the digitizer and microscope available; biomasses were calculated using the average species-specific biomass values available from the literature (Table 6).

Table 5. Length-weight regressions used in the calculation of zooplankton biomasses. Equations used are of the form ln W = ln a + b ln L, where L= length and W = mass of the zooplankter. Masses were calculated for each individual in a sample, and the total used to estimate zooplankton biomass for that sample.

Species Name	**a**	**b**	**units** (length/weight)	**Source**
Daphnia pulex	0.00624	2.4	mm/mg	Paloheimo et al. 1982
Diaphanosoma brachyurum	0	3.0468	mm/ug	McCauley 1984 (Bottrell et al. 1976)
Bosmina longirostris	0	2.5294	mm/ug	McCauley 1984 (Bottrel et al. 1976)
Chydorus sphaericus	0	3.636	mm/ug	McCauley 1984 (Rosen 1981)
Simocephalus vetulus	7.43	3.28	mm/ug	Dumont 1975
Skistodiaptomus oregonensis nauplia	0	2.1547	mm/ug	Malley et al. 1989
Skistodiaptomus oregonensis copepodite	0	2.4235	mm/ug	Malley et al. 1989
Skistodiaptomus oregonensis adult	0	2.5384	mm/ug	Malley et al. 1989

Table 6. Literature values of rotifer mass used in zooplankton biomass calculations.

Species Name	Weight (ug per ind)	Reference
Keratella cochlearis	0.0105	Ruttner-Kolisko 1977 in Malley et al. 1989
Keratella cochlearis	0.005	Hall et al. 1970 in Malley et al. 1989
Keratella cochlearis	0.0035	Nauwerk 1963 in Malley et al. 1989
Keratella cochlearis	0.005	Lewis 1979 in Malley et al. 1989
Keratella cochlearis	0.001	Berman et al. 1982 in Malley et al. 1989
Keratella cochlearis	0.07	Bottrell 1976 in Malley et al. 1989
Keratella cochlearis	0.11	Dumont et al. 1975
Keratella cochlearis	0.049	Schindler and Noven 1971 in Malley et al. 1989
Keratella cochlearis	0.07	Makarewicz and Likens 1979 in Malley et al. 1989
Keratella cochlearis	0.013	Comita 1972 in Malley et al. 19889
Keratella cochlearis	**0.0337**	**mean value used in biomass calculations**
Keratella quadrata	0.35	Dumont et al. 1975
Keratella quadrata	0.32	Dumont et al. 1975
Keratella quadrata	**0.335**	**mean value used in biomass calculations**
Lecane sp.	0.028	Malley et al. 1989
Lecane sp.	0.2	Bottrell et al. 1976
Lecane sp.	0.038	Malley et al. 1989
Lecane sp.	**0.08867**	**mean value used in biomass calculations**
Polyarthra vulgaris	0.02	Lewis 1979 in Malley et al. 1989
Polyarthra vulgaris	0.098	Schindler and Noven 1971 in Malley et al. 1989
Polyarthra vulgaris	0.043	Doohan 1973 in Malley et al. 1989
Polyarthra vulgaris	0.06	Makarewicz and Likens 1979 in Malley et al. 1989
Polyarthra vulgaris	0.0385	Nauwerck 1963 in Malley et al. 1989
Polyarthra vulgaris	**0.0519**	**mean value used in biomass calculations**
Synchaeta sp.	0.013	Malley et al. 1989
Synchaeta sp.	0.156	Malley et al. 1989
Synchaeta sp.	0.07	Malley et al. 1989
Synchaeta sp.	0.366	Malley et al. 1989
Synchaeta sp.	0.27	Dumont 1975
Synchaeta sp.	0.26	Dumont 1975
Synchaeta sp.	**0.1892**	**mean value used in biomass calculations**

Table 7a. Filtering rate equations used to calculate filtering capacity of *Daphnia pulex* populations in Summer and Fall experimental enclosures. Filtering rates are expressed as ml individual^{-1} h^{-1}. L= length (mm).

Source	Equation	r	Temperature	Food Item	Time
Peterson et al. 1977	$F = 0.294\ L^{1.66}$	0.93	8 °C	natural bacteria	midnight
Haney 1985	$F = 4.467\ L^{2.00}$	0.98	3.1 - 4.0 °C	labelled yeast (tracer cells)	night

Table 7b. Filtering rate equations used to calculate filtering capacity of *Bosmina longirostris* populations in Summer enclosures. Filtering rates are expressed as ml individual^{-1} h^{-1}. L= length (mm).

Source	Equation	r^2	Temperature	Food Item	Time
DeMott 1982	$F = 0.106\ L^{1.663}$	0.69	15 °C	aerobacter	night
DeMott 1982	$F = .598\ L^{1.87}$	0.87	15°C	chlamydomonas	night

Table 7c. Per capita clearance rates of S. oregonesis, K. cochlearis and P. vulgaris used to estimate community filtering rates in experimental enclosures.

Source	Species	Prey	Clearance rate
Sanders and Wickham 1993	*S. oregonensis*	mixed ciliates <30μm	0.32 ml ind^{-1} h^{-1}
Sanders and Wickham 1993	*S. oregonensis*	paraphysomonas	0.2 ml ind^{-1} h^{-1}
Sanders et al. 1994	rotifers	^{14}C-labelled flagellate	0.051 ml ind^{-1} h^{-1}
Bogdan et al. 1980	*Polyarthra dolichoptera*	bacteria	0.01 μl ind^{-1} h^{-1}
Bogdan et al. 1980	*Polyarthra dolichoptera*	chlamydomonas	1.69 μl ind^{-1} h^{-1}
Bogdan et al. 1980	*Keratella cochlearis*	bacteria	0.29-0.46 μl ind^{-1} h^{-1}
Bogdan et al. 1980	*Keratella cochlearis*	chlamydomonas	0.76-6.41 μl ind^{-1} h^{-1}

2.6-2 Filtering capacity

Total filtering capacity of the *Daphnia* added to enclosures in both experiments was calculated from the length-weight regressions in Table 7a. Daily (or hourly) filtering rates for each individual animal in a sample were calculated and summed. This filtering capacity was expressed as total volume filtered by the *Daphnia pulex* population per day. The total volume of the enclosure was divided by this number to estimate the amount of time required by the *Daphnia* population in an enclosure hypothetically to filter all the water in the bag. Similar calculations were made for the BOSMINA, COPEPOD, and ROTIFER treatments. The equations used are summarized in Table 7b-c. Where possible, clearance rates for each species on bacteria and flagellates were calculated, but as the clearance rates for *S. oregonensis* were highest on ciliate and negligible on bacteria, ciliate and flagellate clearance rates were employed for this species. In the case of *Bosmina*, it was possible to use published regressions of clearance rate to body length to calculate population clearance rates (L population^{-1} day^{-1}). For *S. oregonensis* and rotifers, only measured per capita rates were available. The mean filtering capacities of the *Daphnia*, *Bosmina*, *S. oregonensis* and *rotifer* (not reported) populations are expressed as the estimated time required for the population to filter the entire volume of the enclosure.

2.6-3 Bacteria

Gluteraldehyde-preserved bacterial samples were refrigerated at 5°C immediately after collection for storage until processing. A two millilitre sub-sample was stained with 4, 6 diamidino-2-diphenylindole (DAPI) at a concentration of 5.8 μg/ml, and filtered under low vacuum onto a 0.22 μm black membrane filter (Millipore). The filter was mounted onto a glass slide and frozen until counting (Porter and Feig 1980). Slides were made from each sample within one month of collection. All samples were subject to the same storage time.

Slides were viewed using a Nikon inverted microscope equipped for epifluorescence. High bacterial density in the samples precluded efficient direct counting using the epifluorescence microscope, as fading of the stain often occurred before all cells in the field view could be counted. This made it necessary to develop a counting method which would accurately and quickly record the entire field view for later counting. After trial tests to determine accuracy of image recording, samples to be counted were photographed using TMAX 400 film set at 5 s exposure time. This method allowed me to record the presence and shape of even weakly fluorescing cells. Bacterial counts were made directly from the film negative using a dissecting scope with 16X magnification. A 2 cm grid in the centre of each negative was examined and counted, as focus irregularities near the edges of the film made it undesirable to count the entire photograph. A minimum of 10 randomly chosen frames (approx 1000 cells) were counted for each sample. This is in accordance with other methods for bacteria counting (Kirchman 1993). The area counted was larger than the area usually counted with the microscope eyepiece graticule (grid) used in direct counting, and this increased the accuracy of the count.

2.6-4 Ciliates

Lugol-preserved samples were settled in 25 ml counting chambers and enumerated for ciliates at 150X magnification. The entire area of the chamber was counted. Results were reported as total ciliate numbers per litre. Ciliates were identified to the order level (Pennak 1989), but as abundances of individual taxa were often too low to extrapolate densities for each species, only the total for all taxa could be reliably estimated. In addition, it is not advisable to attempt precise taxonomic identification for ciliates preserved in Lugol's. Protargol staining is generally required for accurate determination to the species level.

2.6-5 Glass Fibre Filaments and Green Algae

Final densities of glass fibre filaments added to "FILAMENT" treatments were estimated from algae samples taken on the final day of the experiment. Subsamples of the Lugol-preserved algal samples were settled in 25 ml settling chambers and counted at 600X magnification. A bloom of gelatinous green algae, *Elakatothrix* sp. (Wille 1898), occurred in the second experiment. Its densities were estimated by counting a minimum of 300 cells (usually about 5 fields) at 600X magnification.

2.7 Statistics

All means are reported $\pm$ one standard error.

Though bacteria were sampled daily, a subset of the samples was chosen and counted to give estimates of bacterial abundance throughout each experiment. In the Summer experiment, samples from the DAPHNIA, BOSMINA and NO GRAZER treatments were counted on

Table 8a. A list of samples (indicating number of replicates) counted for each treatment in the Summer experiment. Blank cells indicate that no samples were counted on that date. Samples in bold text were included in the repeated measures ANOVA.

Date	Experiment	DAPHNIA	BOSMINA	COPEPOD	(failed copepod)	ROTIFER
August 9	**Summer**	**3**	**3**	**3**	**3**	**3**
August 11	Summer	3	3			3
August 12	**Summer**	**3**	**3**	**3**	**3**	**2***
August 14	Summer	2*	3			3
August 17	**Summer**	**3**	**3**	**3**	**3**	**3**
August 21	Summer	3	3			2*
August 23	**Summer**	**3**	**3**	**3**	**3**	**3**
August 24	**Summer**	**3**	**3**	**3**	**3**	**3**

* replicate sample missing due to errors in processing.

Table 8b. A list of samples counted for each treatment in the Fall experiment. One sample was counted from each enclosure, for a total of three replicates per treatment. No samples were omitted or lost due to accident.

Date	Experiment	DAPHNIA	DAPHNIA+F	COPEPOD	FILAMENT	ROTIFER
October 18	Fall	3	3	3	3	3
October 25	Fall	3	3	3	3	3
October 30	Fall	3	3	3	3	3
November 2	Fall	3	3	3	3	3
November 4	Fall	3	3	3	3	3

more dates than the ROTIFER and COPEPOD treatments. The samples counted for both experiments are listed in Table 8a-b. For both Summer and Fall experiments, the complete data set for statistical analysis consisted of 5 sample dates. Bacterial abundance measurements were natural log-transformed and examined statistically using a repeated measures ANOVA procedure with SPSS 7.5 statistics software. When several measurements are made on the same experimental unit (each enclosure on 5 sample dates), this procedure assumes a correlation of the measurements within the same enclosure and separates this from the total variation. It is therefore possible to partition the effects of time (the Date variable) from the measurements of treatment effects (the Treatment variable). Date and Date*Treatment interaction are within-subject effects. Each enclosure is therefore analogous to a "block" in a randomized complete block design. The between-subjects aspect of the analysis examines the variation due to "between enclosures" effects (i.e. the treatments applied). One limitation of this procedure is that a missing sample will result in the exclusion of the enclosure from the analysis. This was the case for enclosure 9 on August 12th, as the bacteria sample from this enclosure was damaged during processing. In order to avoid exclusion of the enclosure from the statistical analysis, the missing value was replaced with an estimate, which was generated using a linear regression of abundance measurements from enclosure 9. This aspect of the analysis is discussed in Appendix 2.

For each date in the bacteria analysis, *post-hoc* comparisons were performed using the Tukey HSD procedure. For some dates in the repeated measures analysis, the homogeneity of variance assumption was violated. ANOVA is generally robust to violations of this assumption, but this not universally true (Kirk 1982, Winer et al 1991). No transformation of the data was able to stabilize variance in these instances. Results are reported as obtained and the outcome of the Levene test for homogeneity of variance is given where significant results indicate that caution is warranted. I consider it unlikely that a small departure from a nominal significance level of 0.05 warrants concern that the observed treatment effects are a statistical artifact.

3. Results Part I: Persistence of treatments

In order to determine if the desired treatments were successfully implemented, I examined and compared the abundance and biomass of the zooplankton in the enclosures at the end of each experiment. The following results are split into two main sections: (1) an evaluation of the "success" of the treatment implementation (did the experimental manipulation produce the desired zooplankton communities under comparison?) and (2) an evaluation of the effect of each treatment on the microbial food web (what was the impact of each zooplankton community?). Figure 4a and 4b summarize the organizational framework for evaluating and presenting results.

The mean zooplankton densities for each treatment on the final day of each experiment are given in Figure 5a (Summer) and 5b (Fall). The biomass estimates for zooplankton in each treatment are given in Figure 6a (Summer) and 6b (Fall). The densities, biomass, and filtering capacities of the zooplankton treatments will be discussed below. In some treatments the final zooplankton species composition differed from the initial single-species treatment added to bags at the start of each experiment, and these outcomes are also noted below.

3.1 *Daphnia*

Figures 6a and 6b illustrate the high zooplankton biomass in enclosures with added *Daphnia* relative to other treatments. As expected, final *Daphnia* abundances exceeded the stocking densities of 5 and 12 individuals L^{-1} in both the Summer and Fall treatments. Final

Figure 4a. Summary of results for the Summer experiment

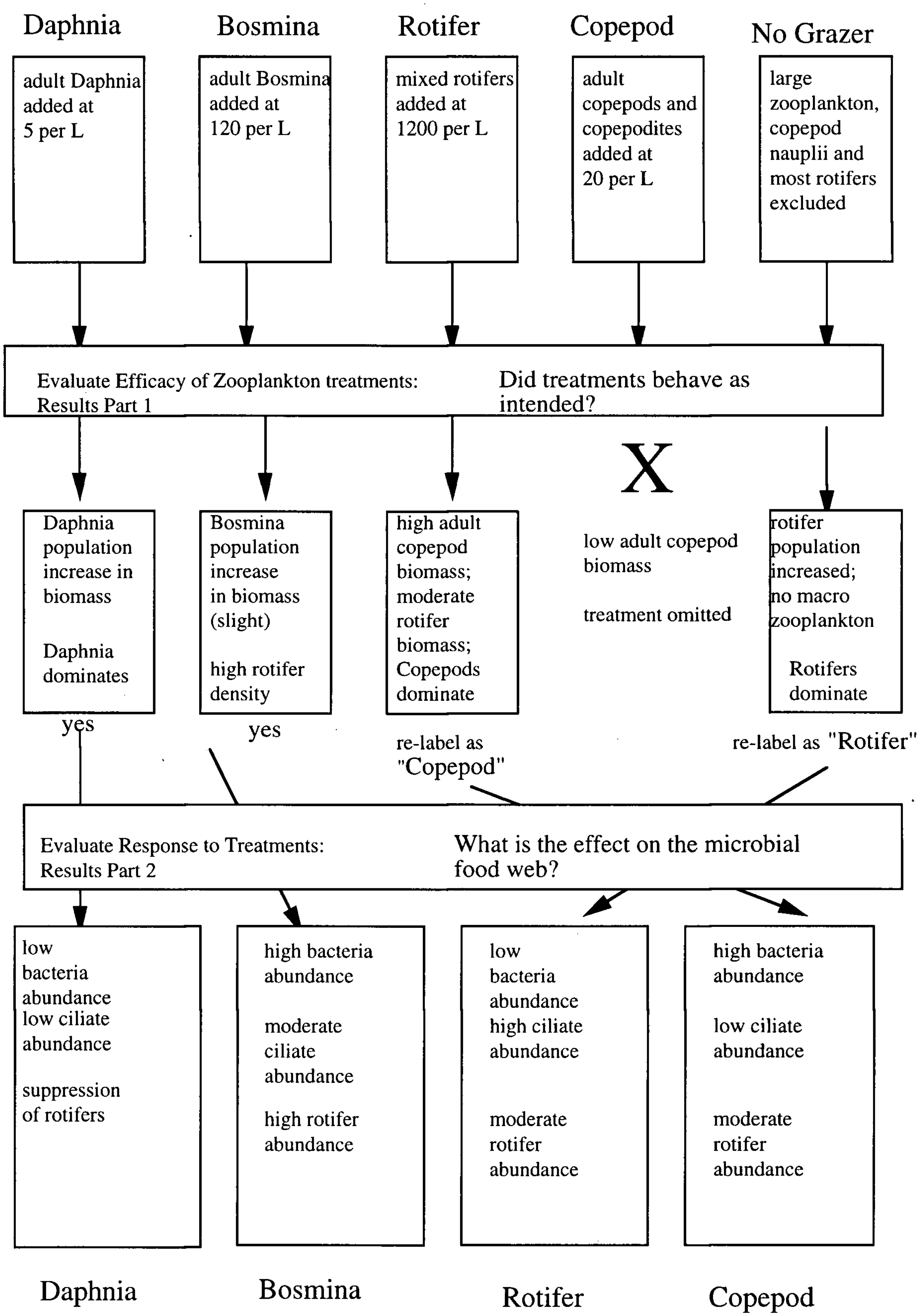


Figure 4B. Summary of results for the Fall experiment

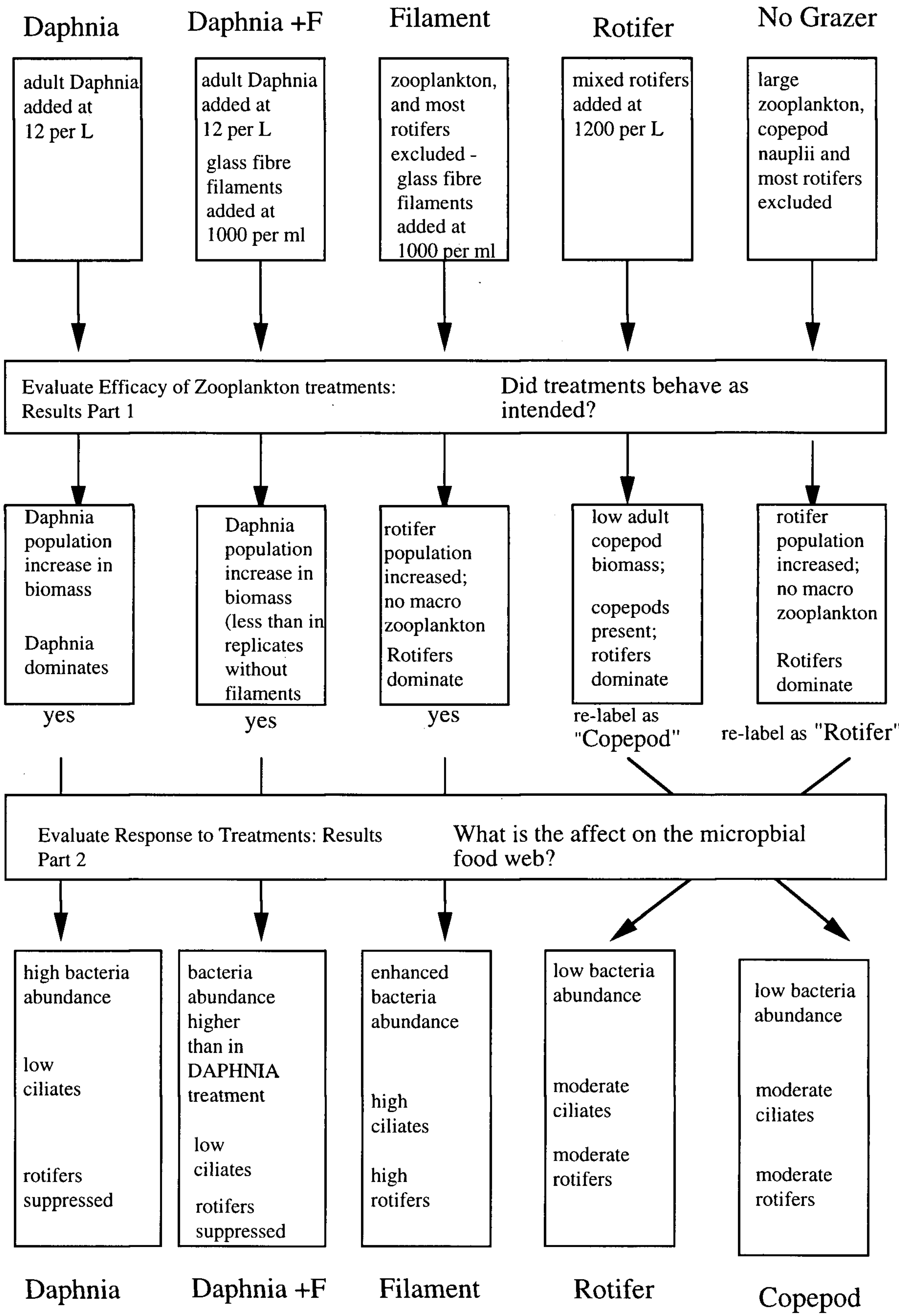


Figure 5a. Final zooplankton abundance in Summer enclosures. Values given are the mean of 3 replicates. Error bars indicate 1 standard error.

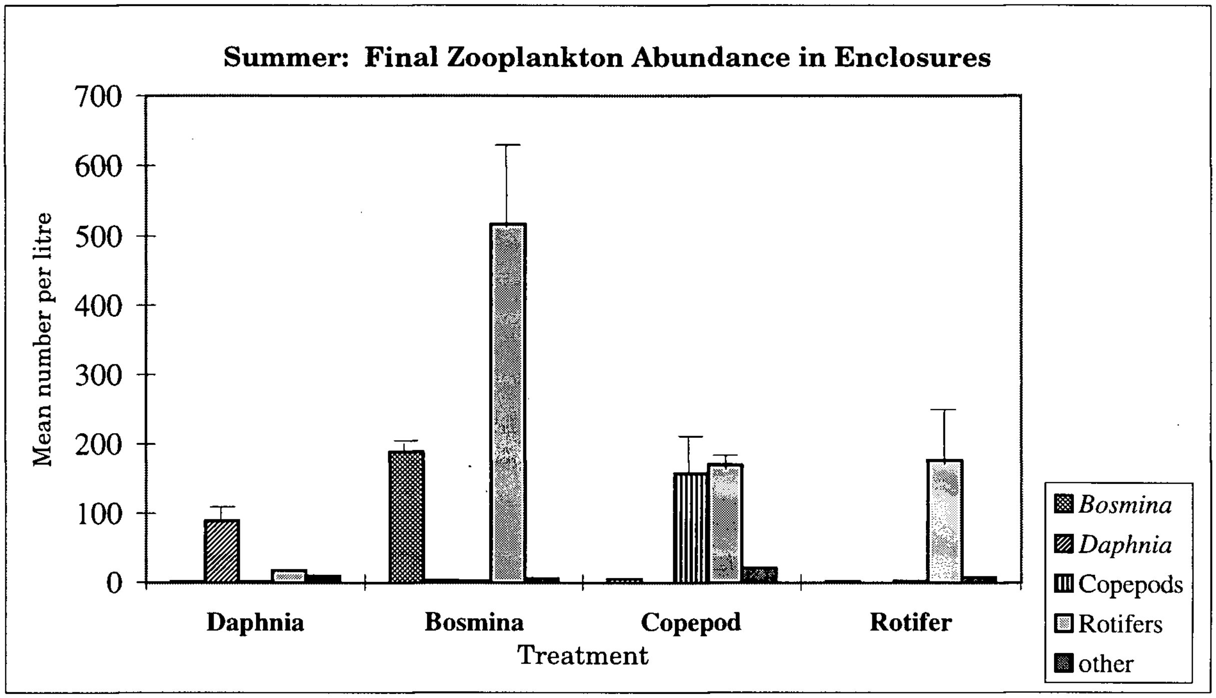


Figure 5b. Final zooplankton abundance in the Fall enclosures. Values given are the mean of 3 replicates.

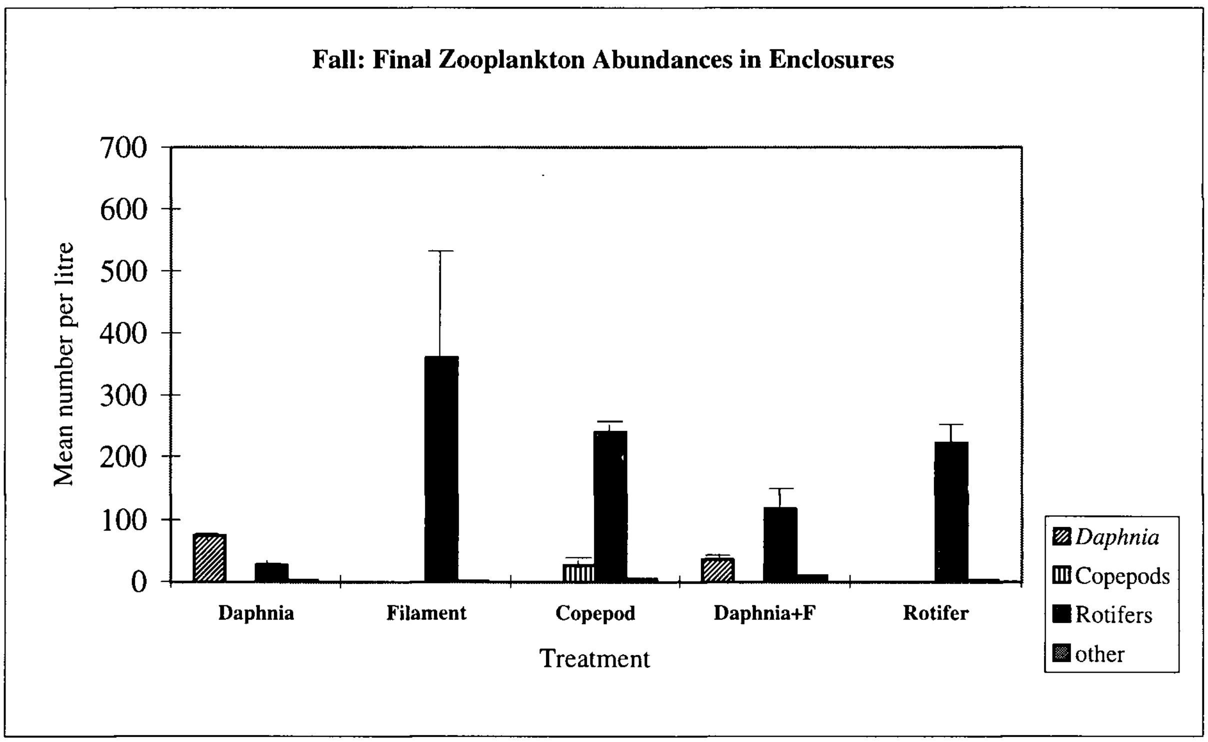


Figure 6a. Zooplankton biomass on the final day of the Summer experiment.
Daphnia, Bosmina and other zooplankton biomasses were calculated using length-weight regressions (Table 5); rotifer biomasses were estimated using literature values (Table 6). Error bars indicate 1 standard error.

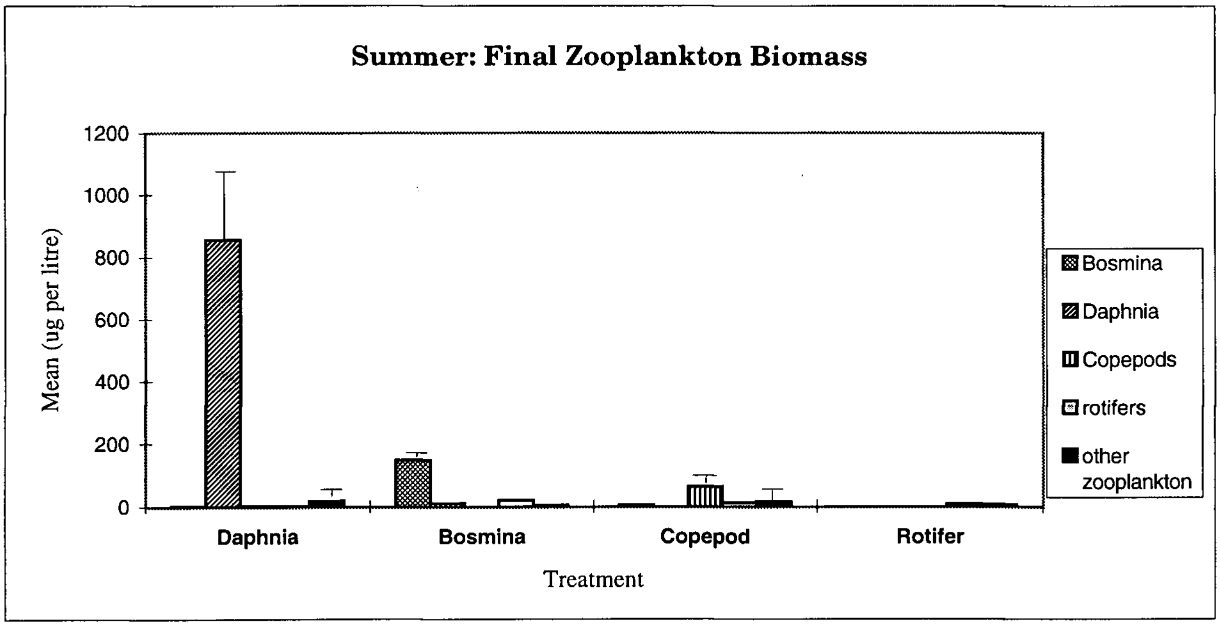
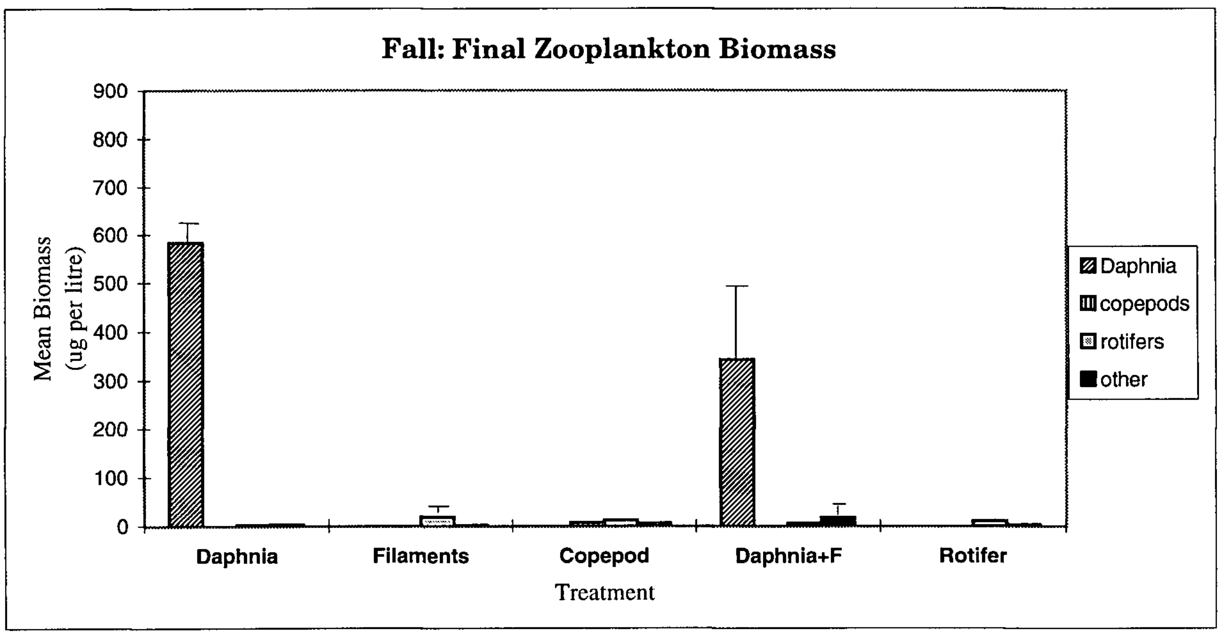


Figure 6b. Zooplankton biomass on the final day of the Fall experiment.
Daphnia, Bosmina and other zooplankton biomasses were calculated using length-weight regressions (Table 5); rotifer biomasses were estimated using literature values (Table 6).



Summer *Daphnia* density was 89 ± 28 individuals L^{-1} while the final Fall density was 74 ± 5 individuals L^{-1} in the Fall DAPHNIA and 35 ± 11 individuals L^{-1} in the DAPHNIA+F treatment. High levels of reproduction of *Daphnia* resulted in a threefold increase in biomass over the course of the Summer experiment.

Daphnia abundance in the Fall DAPHNIA treatment (74 ± 5 individuals L^{-1}) was similar to that observed in the Summer DAPHNIA treatment. The source populations in Summer and Fall differed in their size distributions (refer to Table 4 in Methods), and the number of *Daphnia* stocked per enclosure was increased in Fall to maintain similar *Daphnia* biomasses between seasons. This attempt to equalize the biomass could not influence subsequent reproductive behaviour of the *Daphnia* in response to seasonal differences in experimental conditions. Fortunately for the purposes of comparison, *Daphnia pulex*'s population dynamics resulted in final population densities and biomasses that were nearly equal in the Summer and Fall DAPHNIA treatments (Figure 7). Despite the significantly lower mean weight of individuals in the final *Daphnia* population of the Fall DAPHNIA treatment (8.1μg vs. 9.5 μg, ANOVA of DAPHNIA and DAPHNIA+F treatments: $F_{(2, 2078)}=3.244$, $p=0.04$, Tukey HSD comparison mean difference 1.3638, $p= 0.04$), the biomass of *Daphnia pulex* was not significantly different between any of the 3 treatments in which *Daphnia* were added (ANOVA $F_{(2,6)}=2.465$, $p=0.16$). The size and weight distributions for the Summer DAPHNIA and Fall DAPHNIA are given in Figures 8 and 9. None of the *Daphnia* in the Fall treatments attained the large sizes common in the Summer populations (>2.5mm) but there was a distinct cohort of adult animals larger than 1.5 mm in length, survivors from the initial zooplankton addition. Given that the mean length of *Daphnia* added to Fall enclosures was 1.5 mm at the

Figure 7. Abundance and biomass of *Daphnia pulex* in the 3 treatments in both experiments to which *Daphnia* were added. Error bars indicate 1 standard error.

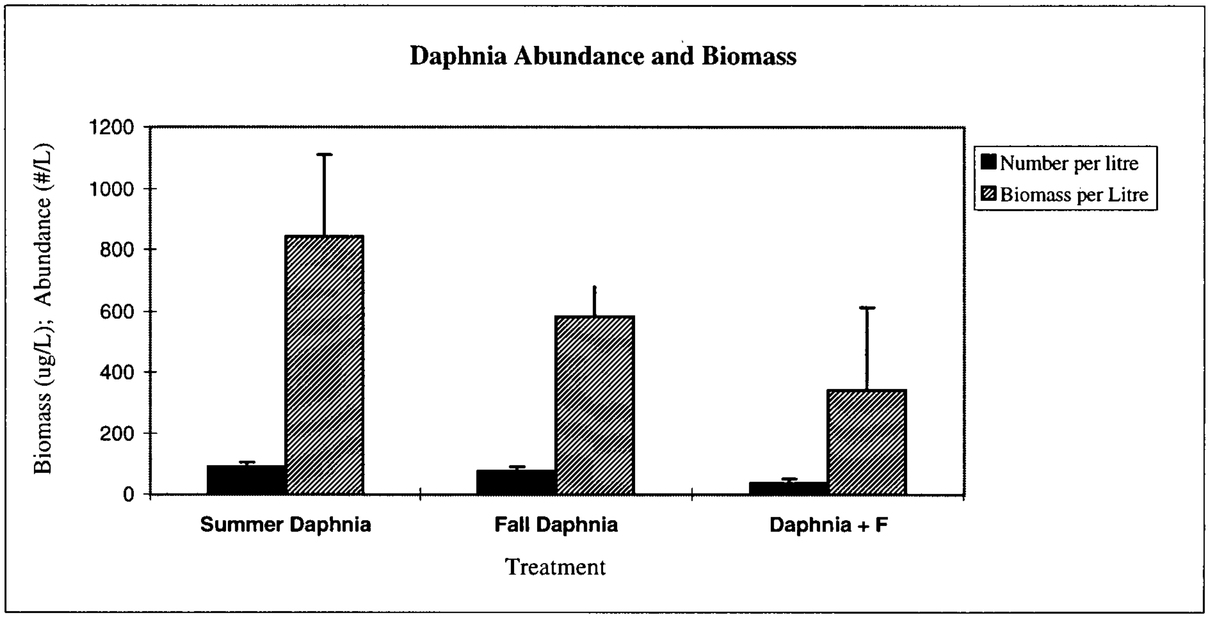
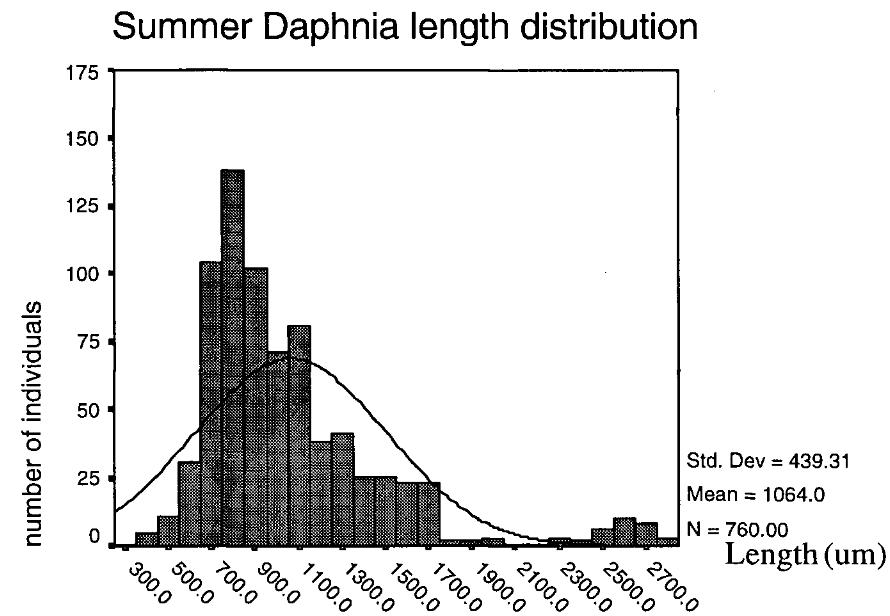
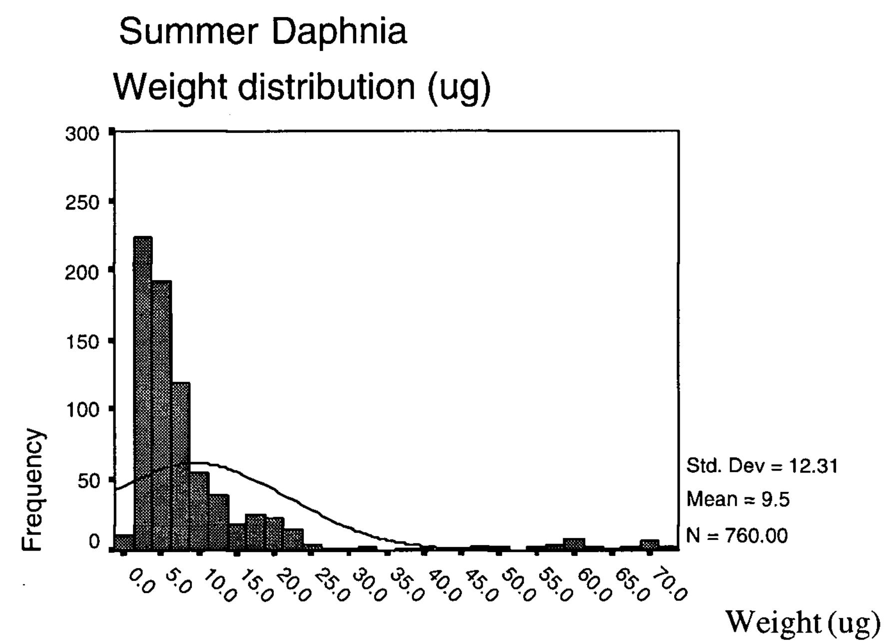


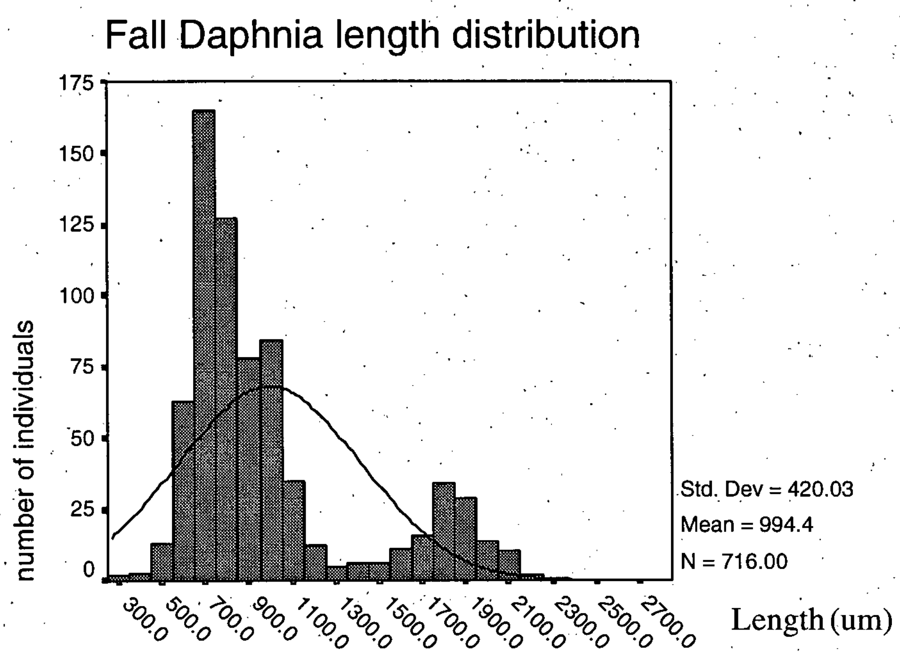
Figure 8. Summer Daphnia length and weight distributions for individuals counted (samples pooled for allthree enclosures sampled.

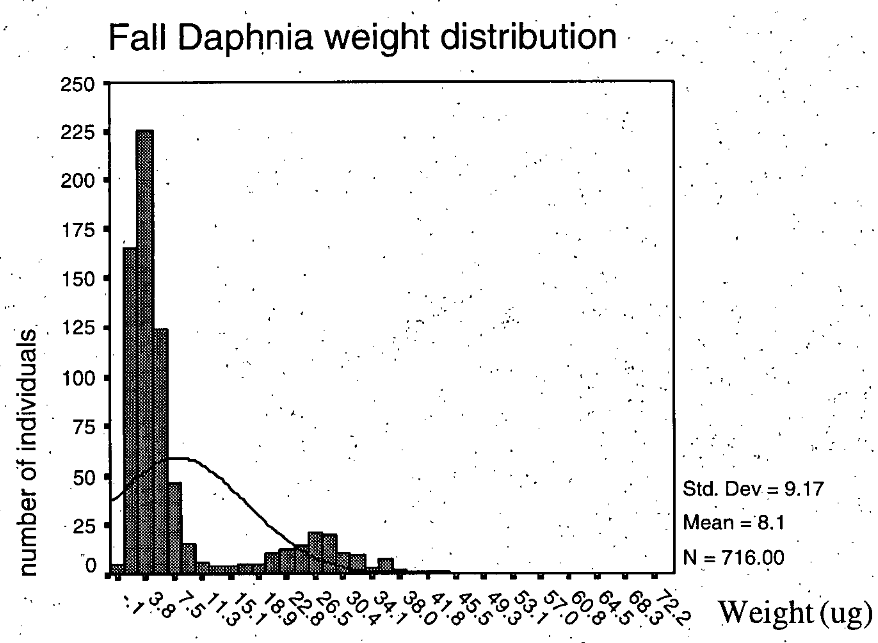




	N		Mean		Std. Deviation
	Valid Statistic	Missing Statistic	Statistic	Std. Error	Statistic
LENGTH	760	0	1064.0112	15.9355	439.3113
Weight (ug)	760	0	9.4897	.4464	12.3056

Figure 9. Length and weight distributions of Daphnia in the Fall Daphnia samples (all three enclosures pooled).





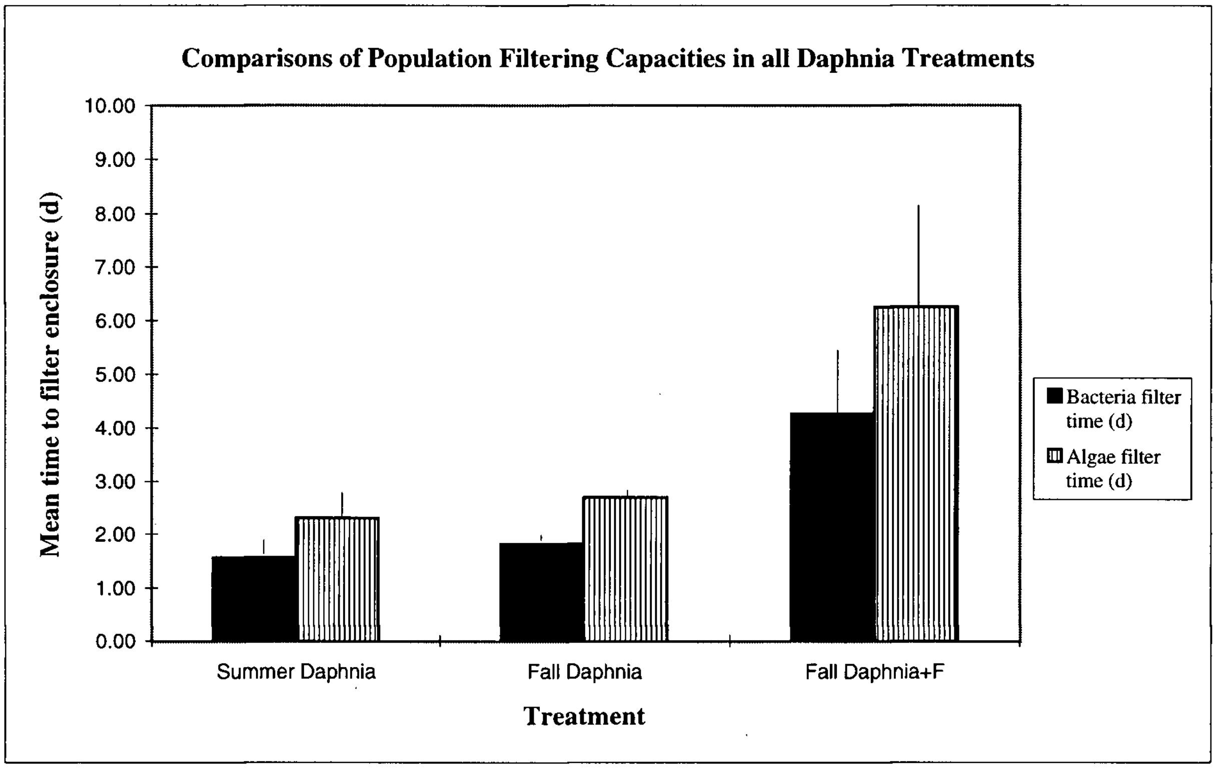
	N	Minimum	Maximum	Mean	Std. Deviation
Weight (ug)	716	.41	43.91	8.1175	9.1742
LENGTH	716	321.33	2254.57	994.4382	420.0318
Valid N (listwise)	716				

start of the experiment, it is obvious that some growth of individuals had taken place (Figure 9). In both Summer and Fall experiments numerous juveniles were present. Despite the differences in the size and weight distributions of the Summer and Fall DAPHNIA treatments, they were equivalent in terms of biomass and from this perspective can be considered seasonal replicates.

The abundance of *Daphnia* in the DAPHNIA+F treatment was lower and somewhat more variable between enclosures (35 $\pm$ 11 individuals L^{-1}). Two enclosures in the DAPHNIA+F treatment had fewer than 25 *Daphnia* L^{-1} while a third had a *Daphnia* density similar to the Fall DAPHNIA enclosures. The difference in *Daphnia* biomass between the Fall DAPHNIA and DAPHNIA+F treatments was not significant (ANOVA of all DAPHNIA treatments: $F_{(2,6)}=2.465$, $p= 0.16$). *Daphnia* abundance was also lower in the DAPHNIA+F experiment than in the Fall DAPHNIA treatment, but not significantly so (ANOVA of all DAPHNIA treatments: $F_{(2,6)}=6.601$, $p=0.03$; Tukey HSD comparison for Fall DAPHNIA and DAPHNIA+F: mean difference = -38.5 individuals L^{-1}, $p=0.1$). This indicates that the presence of glass fibre filaments curtailed the growth of the *Daphnia* populations in the DAPHNIA+F enclosures, and the surviving individuals were larger in size than those in the Fall DAPHNIA (filament-free) enclosures (Figures 9 and 12). This difference in the size distribution is resulted in a difference in the mean weight of individual *Daphnia* between the two DAPHNIA treatments in Fall; 8.1 $\pm$ .4 μg in the Fall DAPHNIA treatment and 9.1 $\pm$ .4 μg in DAPHNIA+F.

Biomass alone does not necessarily indicate equivalency for the Summer and Fall DAPHNIA treatments. Community filtering rate must also be considered. I used three regressions from the literature to generate estimates of the filtering capacity of *Daphnia pulex*

Figure 10. Estimated filtering capacities of *Daphnia* populations in experimental enclosures, expressed as the time required for the zooplankton to clear the entire enclosure volume of bacteria and algae. Filtering capacity is estimated from the equations of Haney 1985 and Petersen et al. 1978. Error bars represent 1 standard error.



in the experimental enclosures in all *Daphnia* treatments. The equations used are given in Table 7. The results are compared for all *Daphnia pulex* treatments in Figure 10, stated as the estimated time required by the *Daphnia* populations to clear all the water of algae or bacteria (bag turnover time). The final estimates of bag turnover time were log-transformed and compared using ANOVA, but no significant differences were detected, although in the case of filtering capacity on bacteria, the result approached significance ($F_{(2,6)}$= 4.418, p=0.07). However the observed power of the ANOVA was low (.526), mostly likely due to the large variance in estimated filtering capacities of the DAPHNIA+F enclosures. As Figure 10 illustrates, estimated enclosure clearance times are not appreciably different between the Summer and Fall DAPHNIA treatments. This indicates that the differences in the size distributions of the *Daphnia pulex* populations in the Summer and Fall DAPHNIA treatments do not translate into predictable differences in the potential grazing impact of *Daphnia* in the enclosures. The Summer and Fall treatments appear to be equivalent in their potential to influence the microbial and algal food webs if biomass and size distribution are used to predict their impact.

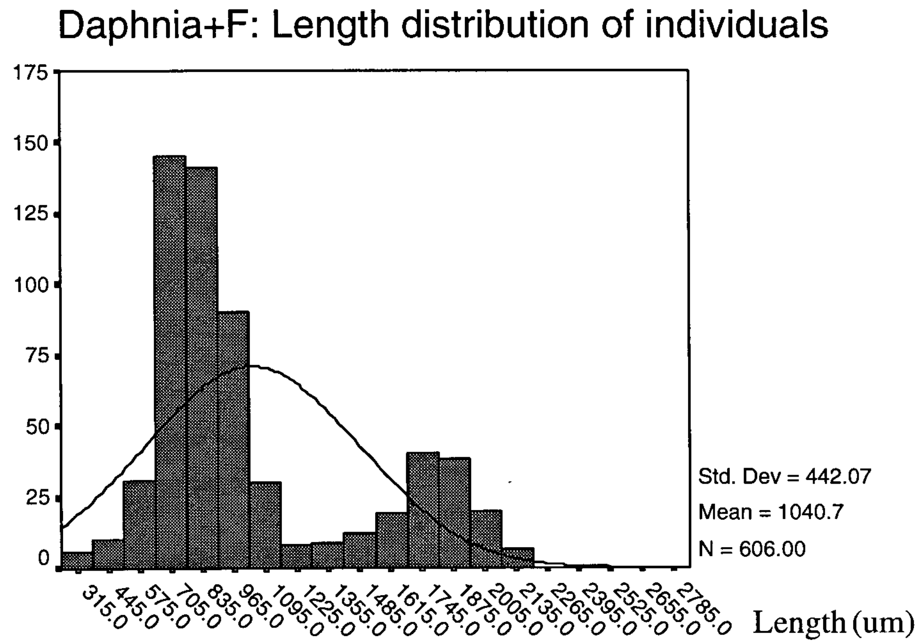
3.2 *Daphnia* and Filaments

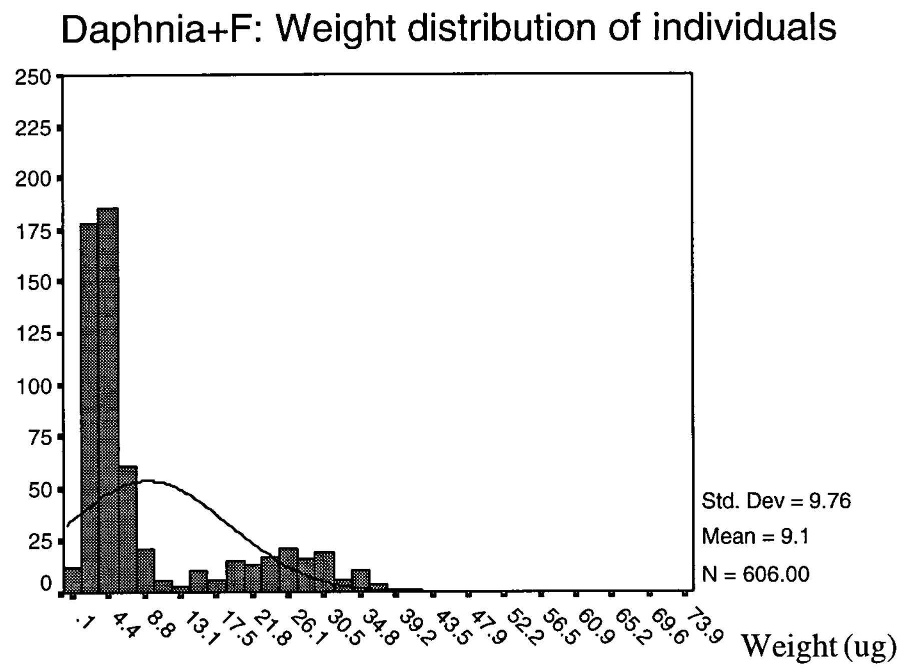
Initially the DAPHNIA+F treatment received the same stock population of *Daphnia pulex* as the enclosures in the Fall DAPHNIA treatment. Therefore, differences in the *Daphnia* population between the DAPHNIA+F and the Summer/Fall DAPHNIA treatments are the result of glass fibre filament addition and as such constitute a measurable treatment effect. The mean *Daphnia* biomass in this treatment, though lower than the other *Daphnia* treatments, was not significantly different (see above) but the mean abundance of *Daphnia* in the DAPHNIA+F enclosures was significantly different from both the Summer and Fall DAPHNIA treatments (ANOVA: $F_{(2,6)}$= 6.601, p=0.03; Tukey HSD test significantly different for the Summer DAPHNIA-DAPHNIA+F comparison, mean difference 53.67, p= 0.03). The population filtering capacity for algae and bacteria in this treatment was much lower (enclosure turnover time greater- Figure 10) and approached significance (see above). In two of the three DAPHNIA+F enclosures, *Daphnia* density was less than 25 individuals L^{-1}. There was very little increase in *Daphnia* abundance in the DAPHNIA+F treatment relative to the Fall DAPHNIA treatment. Though the mean length and weight per individual was significantly different between the Summer and Fall DAPHNIA treatments, this was not the case in the DAPHNIA+F treatment (Summer DAPHNIA-DAPHNIA+F Tukey HSD comparison, mean difference in weight 0.35μg, mean difference in length, 23μm, p>0.58 for both). Figure 11 displays the weight and length distributions for the final DAPHNIA+F *D. pulex* populations. When compared to the Fall DAPHNIA treatment (Figure 9), the size distribution is skewed towards the larger size classes. This indicates that the presence of glass fibre filaments reduced the survival of *Daphnia* juveniles and/or reduced the reproductive rate of the adults.

3.3 *Bosmina*

Final *Bosmina* abundances exceeded the target population density of 100 individuals L^1 and increased above the stocking density of 120 individuals L^{-1}. The final mean *Bosmina* density in enclosures was 188 $\pm$ 17 individuals L^{-1} (Figure 5a). In comparison with the Summer DAPHNIA treatment, the population increase in BOSMINA over the course of the

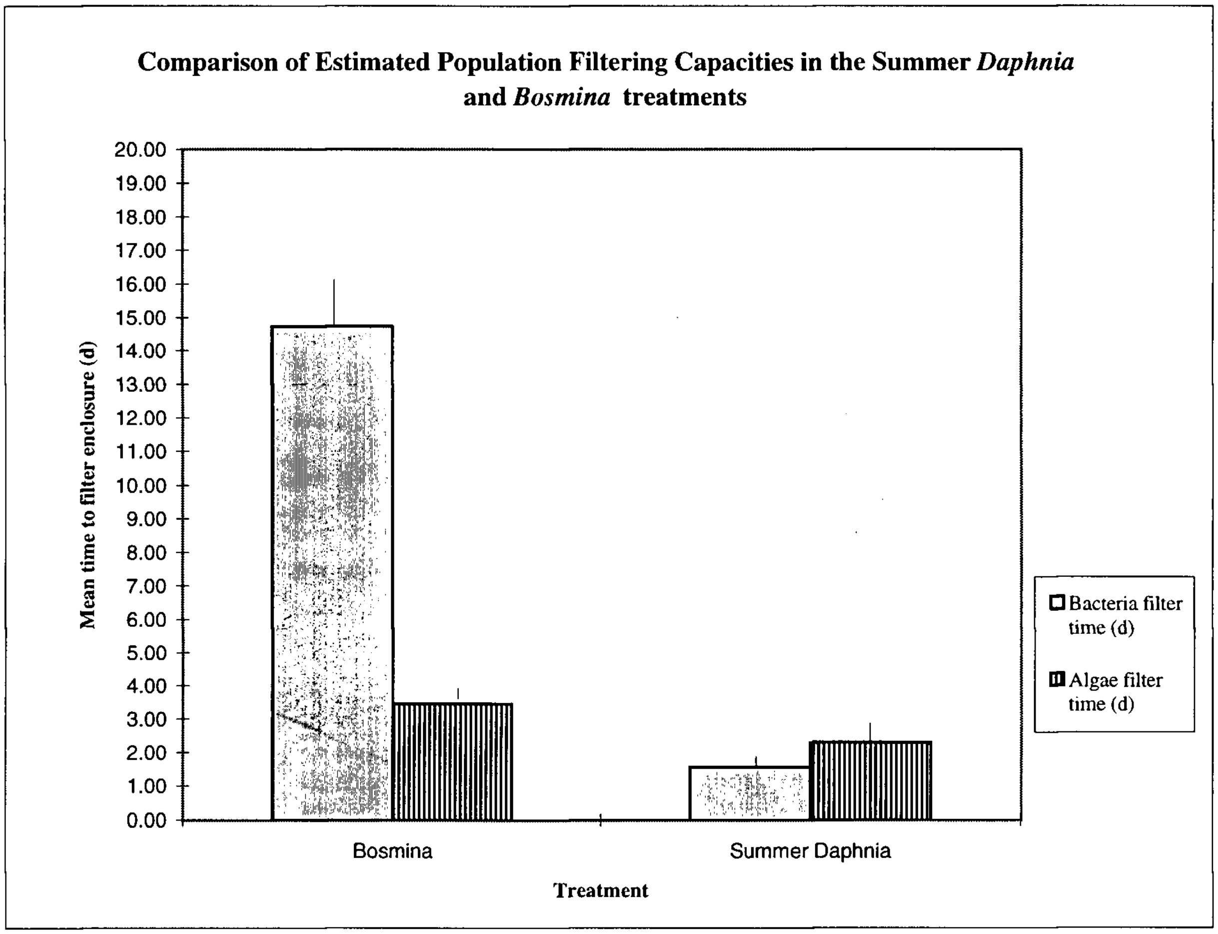
Figure 11. Length and weight of Daphnia counted in final zooplankton sampled from the three Daphnia+F enclosures (pooled).





	N	Minimum	Maximum	Mean	Std. Deviation
LENGTH	606	307.92	2196.30	1040.7277	442.0679
Weight (ug)	606	.37	41.23	9.1392	9.7606
Valid N (listwise)	606				

Figure 12. Estimated filtering capacities of *Bosmina* and *Daphnia* populations in experimental, enclosures expressed as the time required for the zooplankton to clear the entire enclosure volume of bacteria and algae. Filtering capacity is estimated from the equations of Haney 1985, Petersen et al. 1978, and DeMott 1982.
Error bars represent 1 standard error.



experiment was modest. In terms of numbers this increase amounted to only 1.5 times the original stocking density, and therefore the final biomass observed is quite low relative to the zooplankton biomass in the DAPHNIA treatment. The estimated mean filtering capacity of the BOSMINA enclosures, in comparison with the DAPHNIA treatment, is shown in Figure 12. While the estimated turnover time for bacteria is quite long in the BOSMINA treatment, the estimated turnover time for algae is similar for the two treatments. This indicates that the BOSMINA treatment was able to "sweep" approximately the same daily volume of enclosure water as the Summer DAPHNIA treatment, but the two treatments differed in clearances rates for the algal and bacteria size fractions.

3.4 Copepods

The final densities of adult copepods in the enclosures which originally received *S. oregonensis* additions was quite low (< 4 individuals L^{-1}). The absence of adult copepods had been noted early in the experiment after visual assessment of enclosures during sampling. It is therefore assumed that *S. oregonensis* did not survive the handling procedure during the experimental set-up. For this reason, all 3 "copepod" enclosures in the Summer experiment were excluded from further analysis. This did not result in the total exclusion of a "copepod" grazer type from the Summer experimental design, however. While the adult copepods deliberately added to enclosures died as a consequence of handling, the nauplii inadvertently added to ROTIFER enclosures survived and reached maturity during the experiment.

Figure 13a. Life stage distribution of copepods in enclosures at the end of the Summer experiment. Values given are the mean of 3 replicate enclosures (2 in the *Bosmina* treatment). Error bars indicate 1 standard error.

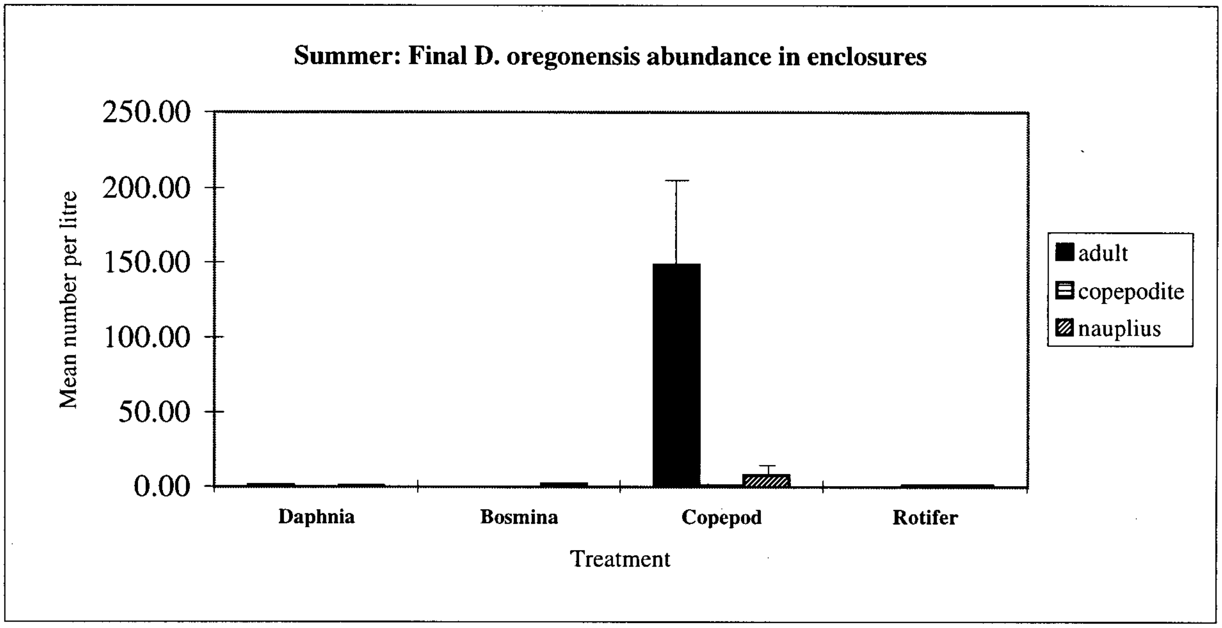
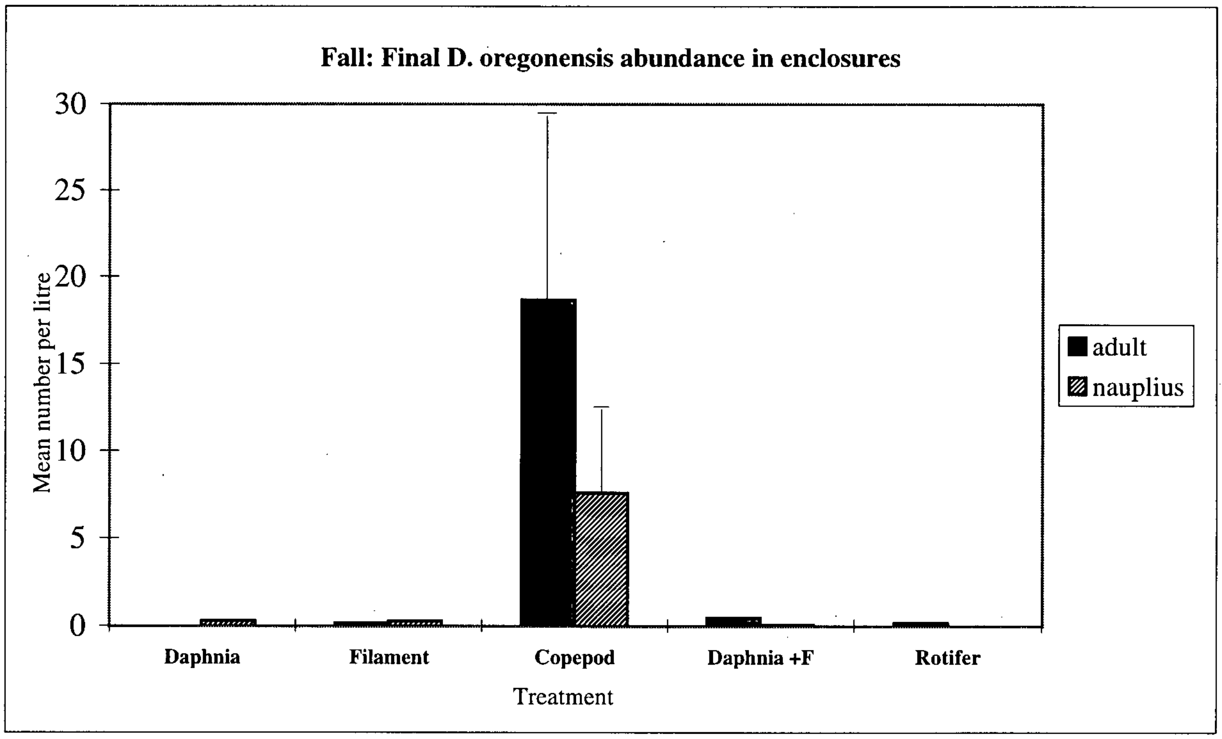


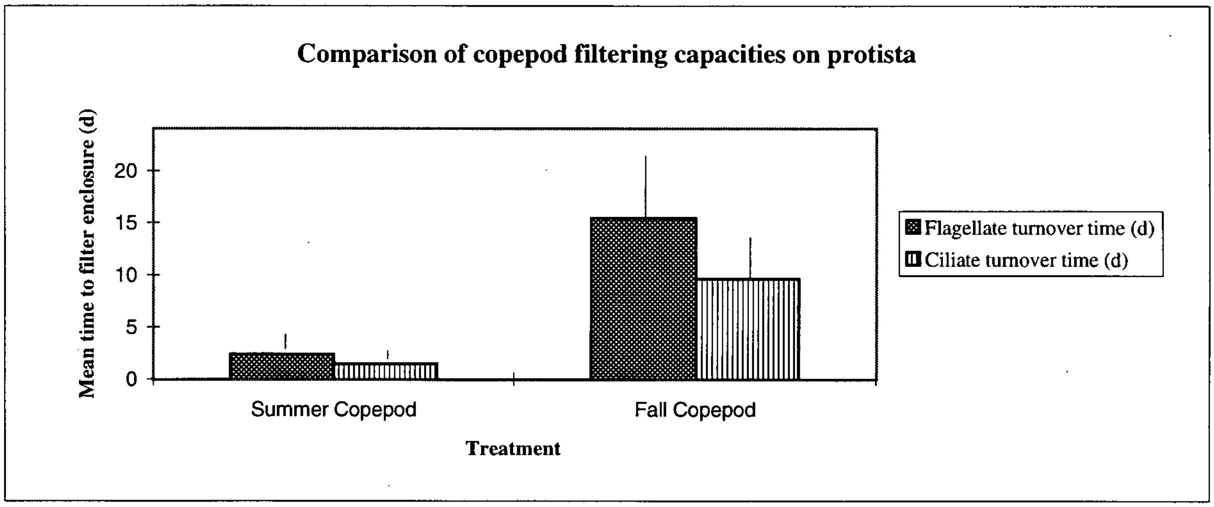
Figure 13b. Life stage distribution of copepods in enclosures at the end of the Fall experiment. Values given are the mean of 3 replicate enclosures. Error bars indicate 1 standard error.



The copepod densities in the "ROTIFER" enclosures are given in Figure 13a-b. Two of the Summer ROTIFER enclosures had copepod numbers in excess of 150 individuals L^{-1}. The mean copepod density for the entire treatment was 156.9 $\pm$ 110.2 individuals L^{-1}. There were also copepods present in the Fall ROTIFER enclosures (Figure 13b), though at much lower abundance than in Summer. The majority of copepods present in the ROTIFER enclosures were adult or late stage copepodites; animals of this size would have been excluded from the enclosures by the initial filtration of enclosure water. Therefore, adult copepods present in these enclosures at the end of the experiment must have been added as nauplii contaminating the initial "rotifer" stock. The high copepod density in the Summer "ROTIFER" treatment had changed the nature of the grazer community from rotifer-dominated to copepod-dominated . In order to reflect this change, the former "ROTIFER" treatments have been renamed as "COPEPOD" treatments in what follows. The Summer COPEPOD treatment had high copepod abundances, but the Fall COPEPOD treatment had very low copepod abundances, so that in effect the "Fall COPEPOD" treatment is very similar (in terms of the grazer community) to the "Fall ROTIFER" treatment discussed below.

The estimated filtering capacities (on flagellates and ciliates) for the Summer and Fall COPEPOD treatments are given in Figure 14. These estimates are based on per capita clearance rates measured for *S. oregonensis* (Sanders and Wickham 1993). *S. oregonensis* is not bactivorous, however its clearance rates on ciliates can be high. The copepod population would have been able to "sweep" the same enclosure volume per day as the DAPHNIA and BOSMINA treatments, if grazing on ciliates. This is not the case for the Fall COPEPOD treatment, where copepod density was low. Therefore the estimated enclosure "turnover"

Figure 14. Estimated time required by the copepod population to filter all of the enclosure volume, using flagellates and ciliates as reference prey items.
Error bars indicate 1 standard error.



time for this treatment was quite long and not comparable to the DAPHNIA and BOSMINA treatments.

3.5 Rotifers

At the end of the Summer experiment, I became concerned about my ability to evaluate the successful implementation of the "rotifer" treatment. I had therefore included an informal comparison "treatment" in the Fall experiment which was designed to provide information on the success of rotifer population additions. Two additional enclosures received heat-killed rotifer inoculum concurrent with the addition of live inoculum to experimental enclosures. At the midpoint of the Fall experiment, the mean rotifer abundance in the killed controls was not significantly different from enclosures where live rotifers had been added ($t = 1.456$, $df=3$, $p=0.24$, see Figure 15a). The killed controls were also sampled for rotifers on the final day of the experiment. Rotifer densities in enclosures with no zooplankton added were not different from either the enclosures with rotifers added or the "killed rotifer" controls (Figure 15b). The rotifer abundances at the midpoint of the fall experiment apparently reached the target density of 1000 ind L^{-1} before declining to the levels observed in the final samples, but this was not related to the addition of rotifers to the enclosure bags.

The results above suggest that the addition of live rotifers to enclosures was not likely responsible for observed rotifer densities at the end of the Fall experiment. The similarity of the treated enclosures to the "no grazer added" and "killed control" enclosures at the midpoint of the Fall experiment suggests that the added rotifers died soon after inoculation. As the

Figure 15a. Rotifers at the midpoint in the Fall experiment. The animals were added to the Rotifer enclosures (3 replicates) at densities of 1200 individuals per litre; while the Killed Rotifer enclosures (2 replicates) recieved heat-killed inoculum.
Error bars indicate one standard error.

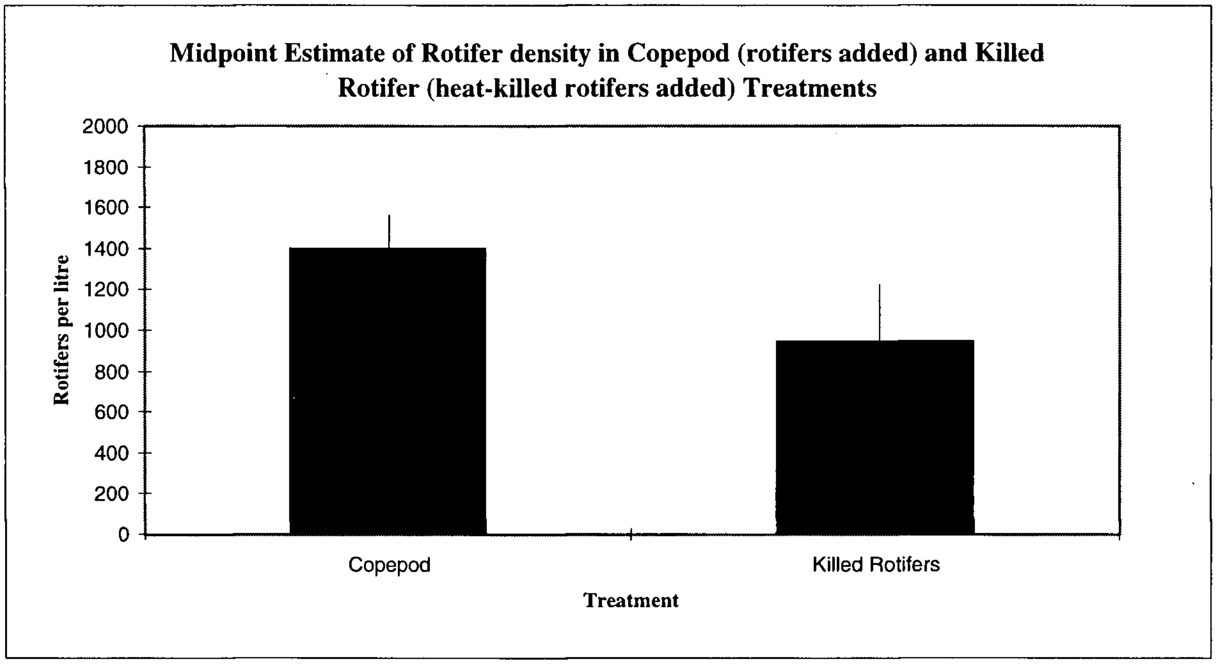
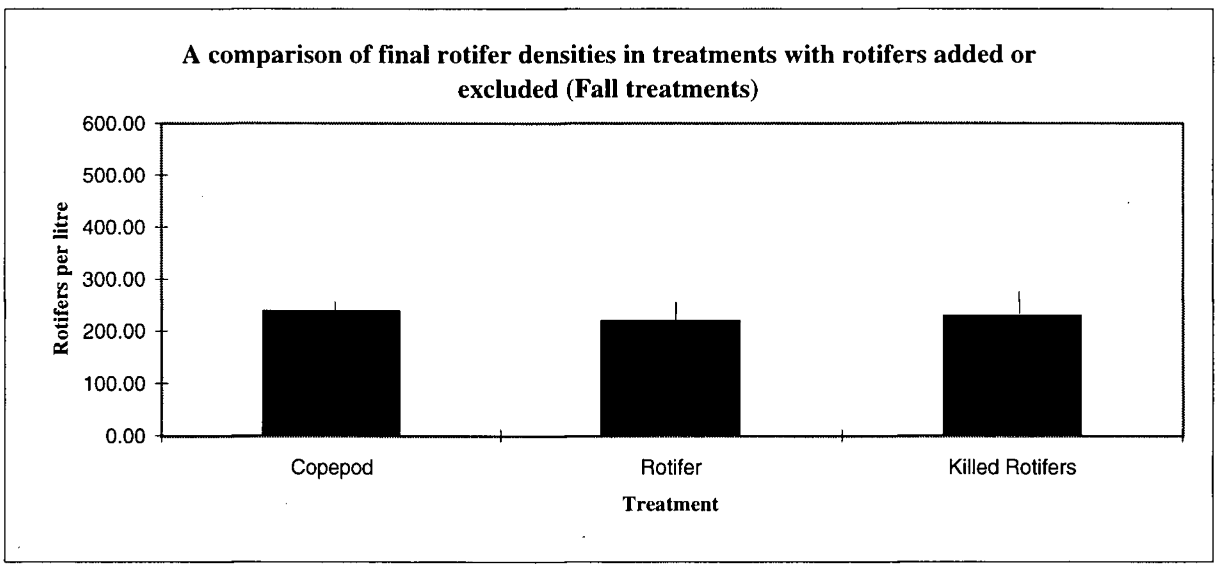


Figure 15b. Rotifer densities in the Fall experiment. The animals were added to the Rotifer enclosures at densities of 1200 individuals per litre; rotifers were initially reduced by filtering, while the Killed Rotifer enclosures (2 replicates) received heat-killed rotifer inoculum. Error bars indicate 1 standard error.



Summer "no grazer" treatment had the same mean rotifer abundance as treatments where live rotifers were added, it is likely that the rotifer additions in the first experiment also had no effect on final rotifer abundance. The total rotifer abundance in the Summer ROTIFER enclosures was 170 $\pm$ 33 individuals L^{-1}, substantially below the 1000 L^{-1} stocking density.

Furthermore, rotifers were present in all enclosures except those which contained *Daphnia.* Initial rotifer populations were drastically reduced, but not eliminated, by filtration. By the end of both Summer and Fall experiments, the rotifer populations had increased. These rotifer populations somewhat confound all the grazer treatments, but their biomass and filtration capacity (not shown)[4] was likely much lower than the DAPHNIA, BOSMINA and COPEPOD grazer populations. Only those enclosures which received no zooplankton additions could be considered "rotifer-dominated" at the end of the experiment (but the copepod presence in the Fall COPEPOD treatment is very limited and this community is effectively rotifer-dominated as well).

In the Summer and Fall "NO GRAZER" treatments, rotifers constituted the dominant fraction of the metazoan grazer community (Figure 6a and 6b). These treatments are therefore referred to as "ROTIFER" treatments in the text below. This signifies that there is no treatment in the design which completely excludes all but the microbial grazers.

The estimated filtering capacities of *K. cochlearis* and *P. vulgaris* on nanoplankton and bacteria vary by an order of magnitude in the literature (Sanders et al. 1994). Some estimates of *P. vulgaris*'s clearance rate on bacteria would indicate potential enclosure

[4]Filtration capacity was estimated using per capita rates available in the literature, but the range of possible values (for both *K. cochlearis* and *P. vulgaris*) was quite large, and I chose not to present the results in detail. Clearance rates for rotifers can range from 0.001 to 0.072 ml $rotifer^{-1}$ hr^{-1} (Sanders et al. 1994).

turnover times of more than 130 years for some rotifer populations in the Fall experiment. However, a measured clearance rate found in another study for a mixed *Keratella/Polyarthra* community in situ (Sanders, et al. 1994) would give respectable turnover times on the order of 3-5 days for the rotifer populations in the Summer Enclosures, when feeding on nanoflagellates. Comparable values were obtained for the Fall experiment. Based on the wide range of literature values, the equivalency of "enclosure turnover time" between the ROTIFER enclosures and the other treatments cannot be reliably assessed without measured grazing rates. It is within the realm of possibility that the Summer and Fall ROTIFER treatments were characterized by grazing pressures on nanoplankton roughly equivalent to those in the macrozooplankton-dominated enclosures. However, the enclosures containing *Bosmina* and *S. oregonensis* populations have community clearance rates which combine those of macrozooplankton and rotifers, and are therefore higher than those in the Summer and Fall ROTIFER treatments.

3.6 Other zooplankton

Figure 5a indicates that in all treatments there are a few zooplankters characterized as "other" (their biomass is also indicated in Figure 6a). A few individuals of *Sida crystallina* (O.F.Müller) 1875, *Diaphanosoma brachyurum* (Liéven) 1848, *Chydorus sphaericus* (O.F.Müller), *Simocephalous vetulus* Schødler 1858 , *Ceriodaphnia* sp. Dana 1853 and the occasional ostracod were present in some final zooplankton samples. Their appearance in enclosures was sporadic and unrelated to the treatments; it is likely that such individuals escaped into the enclosures during the filtering process (perhaps as eggs), or perhaps were

Figure 16a. Noon temperatures measured in the Summer experiment (prior to daily sampling).

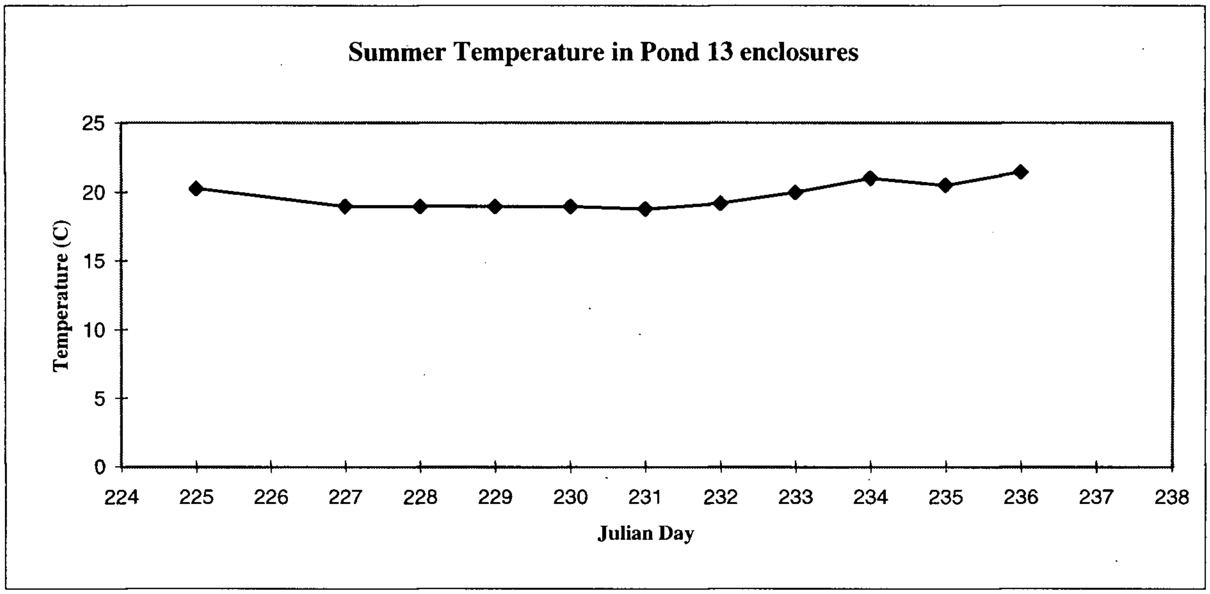
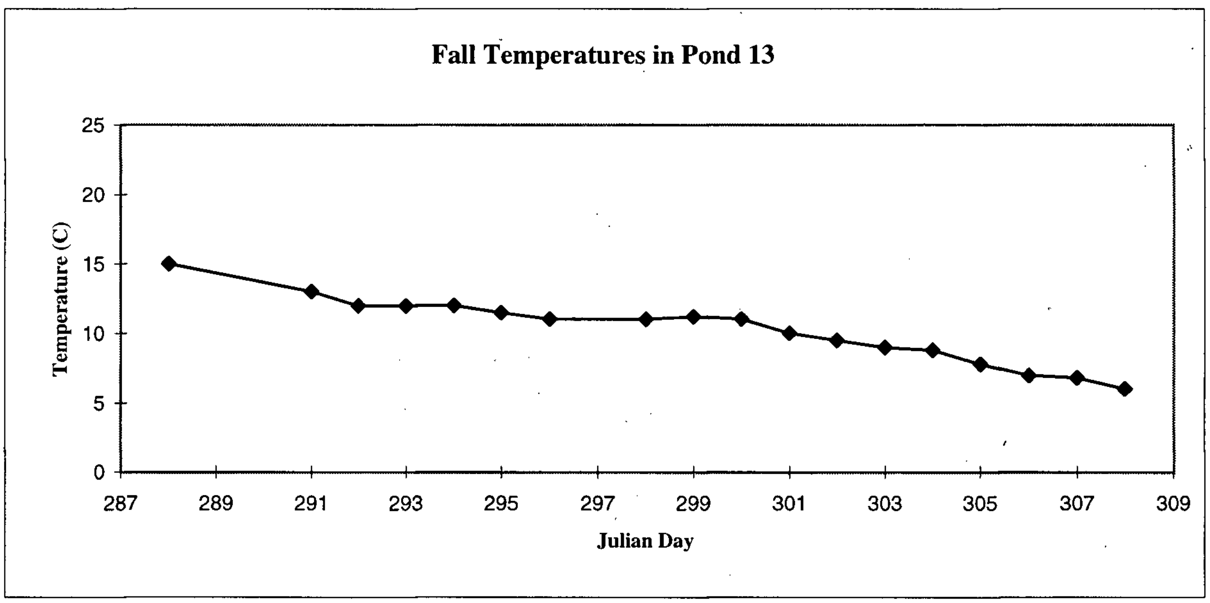


Figure 16b. Noon temperatures measured in the Fall experiment (prior to daily sampling).



added accidently along with the treatment zooplankton. As these uninvited guests were never abundant, they were not important components of the grazer community in any of the experimental enclosures. Still fewer of these stray zooplankton were present in the Fall enclosures.

3.7 Temperature

Daily temperature measurements were part of the sampling protocol in both Summer and Fall experiments, however due to a malfunction of the probe used to determine pH and temperature, some measurements are missing from the Summer experiment. The results obtained are presented in Figure 16a-b. Weather conditions were generally cloudy with light showers for the first half of the Summer experiment, and water temperature was stable near 19°C. The final week of the experiment was characterized by hot, sunny weather and resulted in a warming trend with enclosure temperatures rising to 21.5°C.

The temperature pattern in the Fall experiment was much different. The Fall experiment was set up during a period of warm weather and water temperature was 15°C. Coincident with the onset of sampling, the weather became much colder with frequent episodes of rain. Water temperatures declined gradually throughout the experiment, to a low of 6°C.

The pH measurements in enclosures in both experiments ranged between 8 and 8.9, and on most dates were approximately 8.6, with very small variability between enclosures on any given day.

4. Part II : Numerical responses to grazer manipulations

4.1 Bacterial abundance

To place the observed variation in bacterial abundance in perspective, Figure 17 shows the range of bacterial abundance (all individual estimates pooled) for both Summer and Fall experiments. The minimum and maximum values are given in Table 9. The range observed experimentally is compared to literature values of bacterial abundance recorded from a variety of freshwater ecosystems. The range of variation in bacterial abundance observed in this study encompasses a large fraction of the variation in bacteria density across diverse freshwater ecosystems. The literature values chosen consist mainly of seasonal minima and maxima for particular lakes, though some observations may also have been taken over shorter periods of time. Year to year variation within lakes is also included in the literature data set.

The lowest single estimate of bacterial abundance observed in my experiments was 9.1 x 10^5 cells ml^{-1} in the Fall experiment, while the highest observed was 6.8 x 10^6 cells ml^{-1} in the Summer. A sample taken from open water at the centre of Pond 13 on August 12 (at the same depth as the enclosures) was 2.29 x 10^6 cells ml^{-1}. The enclosures appear to be appropriate models of the natural bacterial abundance in Pond 13.

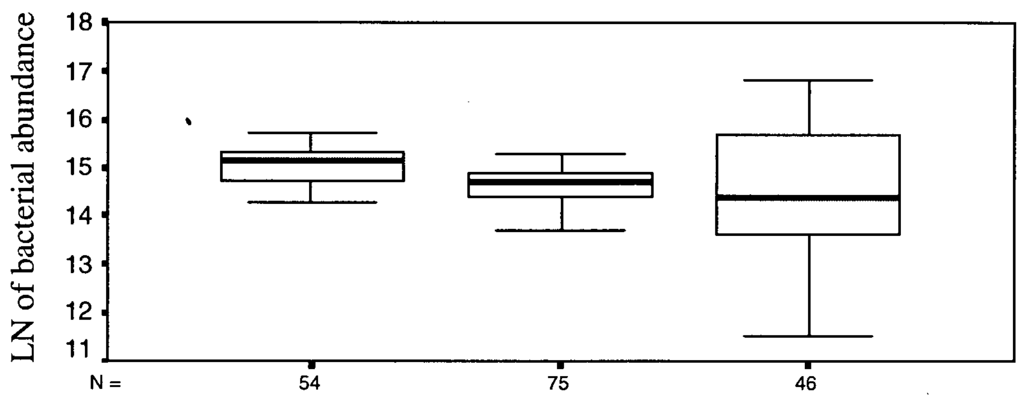
4.1-1 Bacterial abundance- Summer

Bacterial abundances observed in replicate enclosures of each treatment in the Summer experiment are shown in Figure 18a, b, c and d. The sample dates included in the statistical analysis are indicated in Table 8a. The results of the ANOVA procedure are summarized in

Figure 17. Bacterial abundance measurements

Boxplots of experiments and literature values

Estimates from each experiment are pooled



Source of abundance measurements

Literature: pooled high and low values from freshwater habitats

Blue line represents mean; error bars indicate range of values

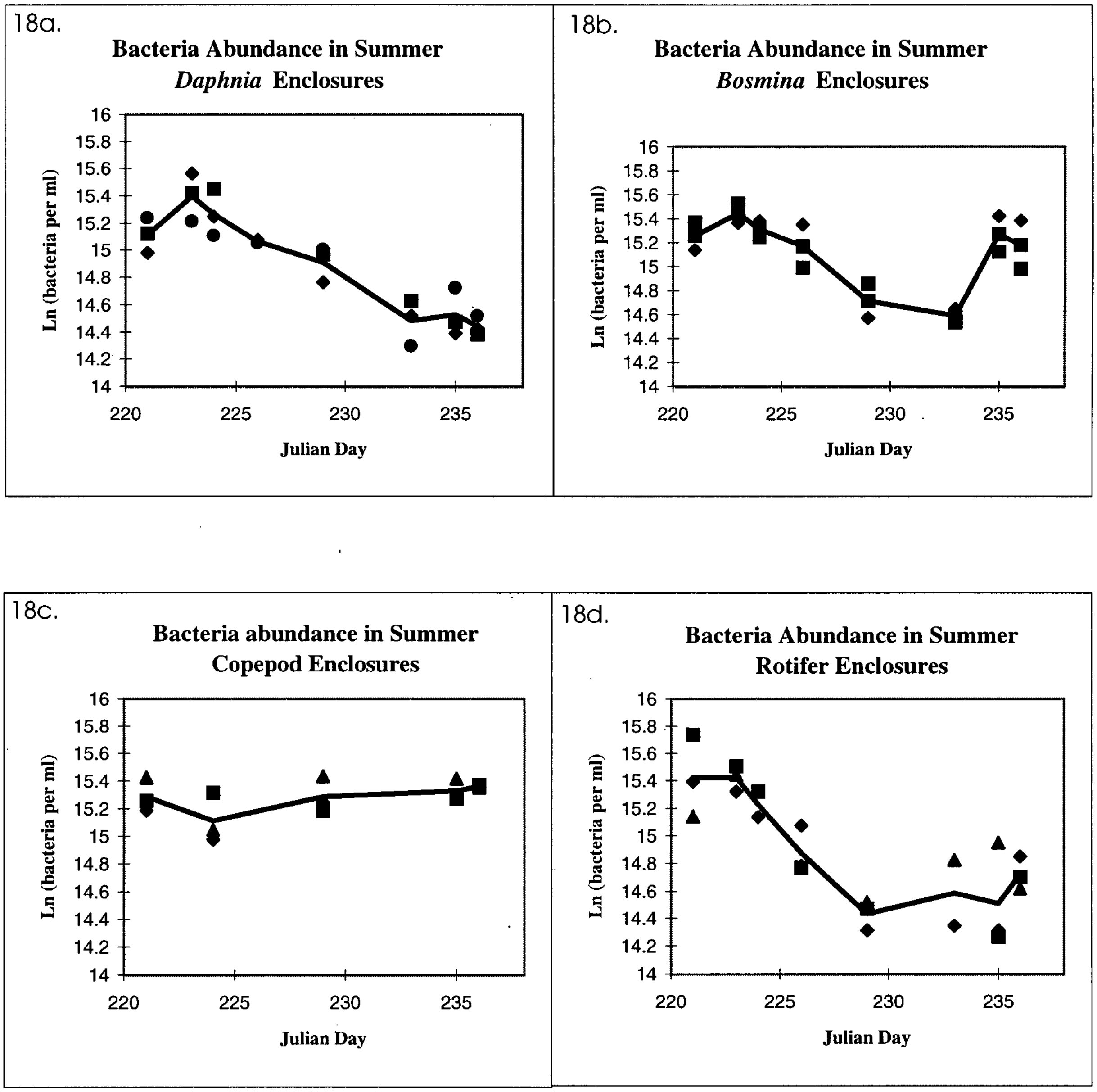
References:

Bennet et al. 1990, Berninger et al. 1991Bird and Kalff 1984, Gude 1988, Gude 1991, Hardy et al. 1986, Markosova and Jezek 1993, Psenner and Sommaruga 1992, Weisse 1990.

Table 9. Range of bacterial abundance estimates observed during the two experiments in this study. A comparison with a range literature values is shown in Figure 18. Values are given as cells ml^{-1}.

Experiment	# of observations	minimum	maximum	mean	Std. Error
Summer	54	1.6×10^6	6.8×10^6	3.6×10^6	1.6×10^5
Fall	75	9.1×10^5	4.3×10^6	2.4×10^6	9×10^4
Both seasons	129	9.1×10^5	6.8×10^6	2.9×10^6	1×10^5

Figure 18. Bacterial cell numbers in Summer enclosures are shown. Points represent individual enclosures. The Dark line represents the mean of 3 replicates for the Daphnia, Copepod and Rotifer treatments. The Bosmina treatment has 2 replicates.



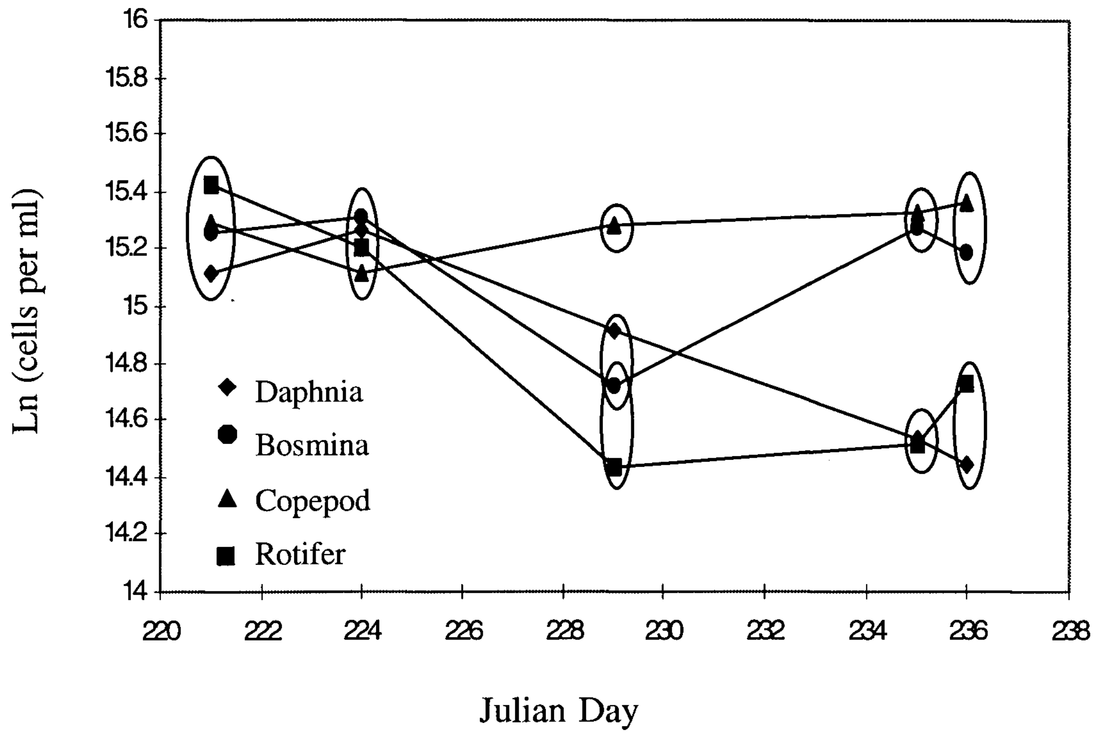
Appendix 1. The within-subjects effects indicate a significant effect of sample date ($F_{(4,28)}$= 12.54, p<.001), and a significant interaction of date and treatment ($F_{(12,28)}$= 6.472, p<.001). The between-subjects effects indicate a significant effect of treatment ($F_{(3,7)}$= 32.586, p<.001). Error variances were unequal for two dates at the end of the experiment (Appendix 1, Table C), and α in this case may differ from the stated level of 0.05. However, differences between treatments are obvious from visual inspection of Figure 18. As an example, mean bacterial abundance between the DAPHNIA and COPEPOD treatments differs by 2.5 times on the final day of the experiment and the abundance values for individual enclosures do not overlap. I consider this difference to be real and biologically significant.

Differences between particular treatment means were examined *post-hoc* using Tukey HSD comparisons. All treatments were compared on each sample date; the results obtained are summarized in Table 10. No treatment differences were discernable prior to August 17 (Julian day 229, the midpoint of the experiment), and so the table displays only the results for sample dates where significant differences between treatments were detected. The four treatment means are shown together in Figure 19 for all dates included in the repeated measures ANOVA. It is apparent that bacterial abundance in the COPEPOD treatment remained at or near the same level for the duration of the experiment. Bacterial abundance in the DAPHNIA and ROTIFER treatments declined throughout the experiment and was significantly less than the COPEPOD and BOSMINA treatments when the experiment was concluded. The BOSMINA treatment showed an initial decline in abundance similar to that of the DAPHNIA treatment, but after August 17 bacterial abundance in this treatment increased to match that observed in the COPEPOD treatment.

Table 10. Tukey multiple comparison test results following repeated measures ANOVA of natural log-transformed bacteria abundances (5 dates) in the Summer experiment. No comparisons before August 17th (August 9, August 12) were significant (not shown). Significant differences are indicated in bold type, $\alpha = 0.05$

Date	Treatment (i)	Treatment (j)	Mean difference (i - j)	Standard Error	Sig.
August 17	Daphnia	Bosmina	.1957	.125	.452
		Copepod	**-.3769**	**.112**	**.046**
		Rotifer	**.4765**	**.112**	**.015**
	Bosmina	Copepod	**-.5726**	**.125**	**.011**
		Rotifer	.2808	.125	.200
	Copepod	Rotifer	**.8534**	**.112**	**.001**
August 23	Daphnia	Bosmina	**-.7467**	**.220**	**.045**
		Copepod	**-.8021**	**.197**	**.028**
		Rotifer	.01524	.197	1
	Bosmina	Copepod	-.0554	.220	.994
		Rotifer	**.7619**	**.220**	**.041**
	Copepod	Rotifer	**.8173**	**.197**	**.018**
August 24	Daphnia	Bosmina	**-.7420**	**.119**	**.002**
		Copepod	**-.9230**	**.106**	**<.001**
		Rotifer	-.2843	.106	.115
	Bosmina	Copepod	-.1811	.119	.473
		Rotifer	**.4577**	**.119**	**.025**
	Copepod	Rotifer	**.6387**	**.106**	**.002**

Figure 19. Mean of natural log-transformed bacteria abundance for the Daphnia, Bosmina, Rotifer, and Copepod treatments in the Summer experiment. Homogeneous subsets are indicated by ellipses.



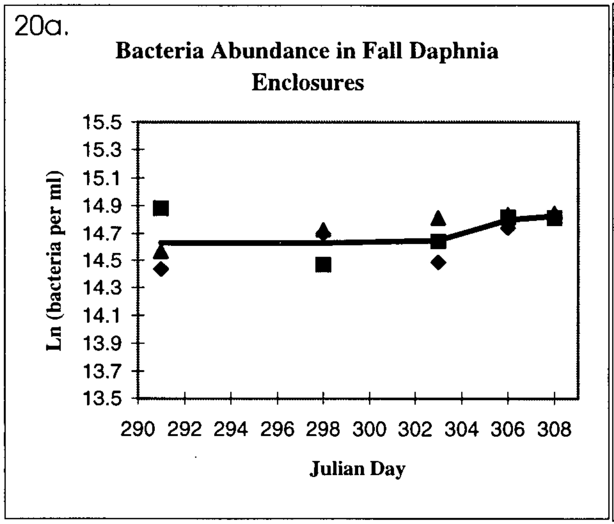
4.1-2 Bacterial abundance- Fall

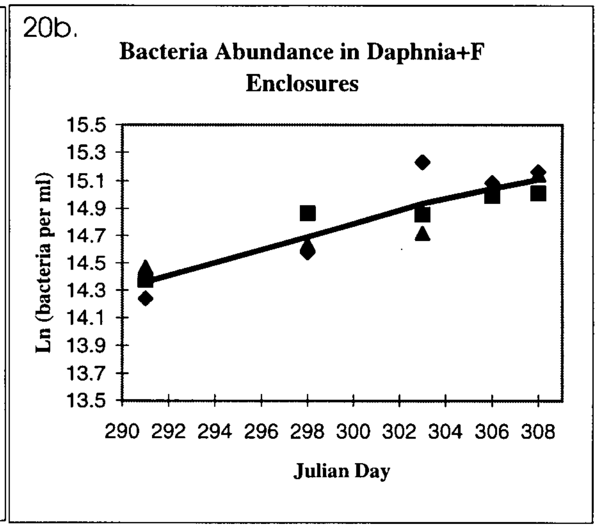
The repeated measures ANOVA of natural log-transformed bacterial abundance in the Fall experiment includes 5 sample dates (Table 8b). The ANOVA results are given in Appendix 1 Table D, E, and F. The within subject effect of sample date ($F_{(4,40)}$= 13.474, p<0.001) and the date x treatment interaction ($F_{(16,40)}$= 5.348, p<0.001) are significant. There is also a significant effect of treatment ($F_{(4,10)}$=18.426, p<0.001). Again, despite log-transformation of the data, variances were not equal for all sample dates (Appendix , Table F). However, this assumption of the ANOVA is only violated for a single sample date and ANOVA is generally thought to be robust to violations of this assumption in many circumstances (for a discussion see Winer et al. 1991 or Kirk 1982).

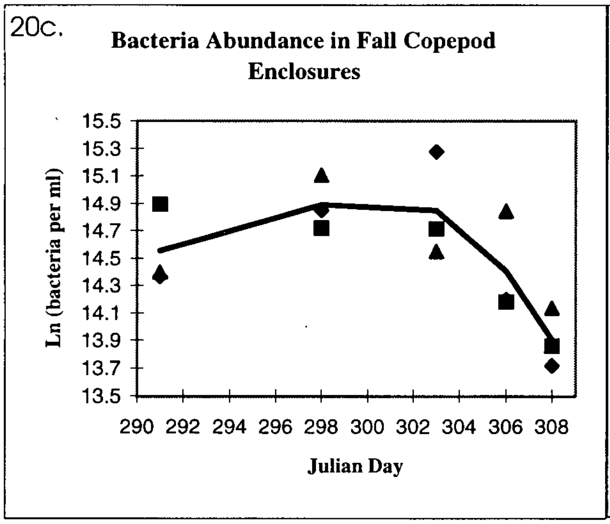
The bacterial abundances for each Fall treatment are shown in Figure 20a-e. The effect of *Daphnia* on bacterial abundance in this experiment is immediately apparent. Bacterial abundance in the DAPHNIA treatment remained constant and a modest increase occurred in the DAPHNIA+F treatment. This is in stark contrast to the COPEPOD, ROTIFER and FILAMENT treatments, which show modest increases in bacterial abundance up to the mid-point of the experiment, followed in each case by a steep decline. This pattern also contrasts markedly with that observed in the Summer experiment, in which the COPEPOD treatment had a constant (and high) bacterial abundance while the DAPHNIA treatment showed a decline.

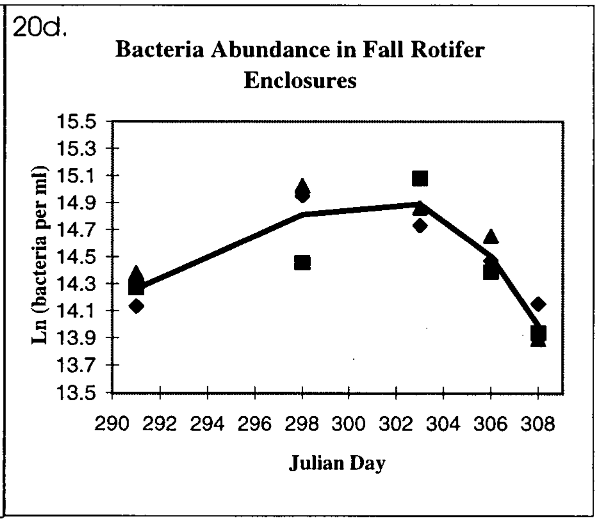
Tukey HSD *post-hoc* comparisons of individual treatments (on each sample date) did not detect any differences among treatments prior to November 2 (Julian day 306); this is also apparent in visual inspection of Figure 20. The results obtained from the Tukey HSD multiple comparisons are given in Table 11 for the dates where significant effects were

Figure 20. Natural log-transformed bacterial cell numbers in Fall enclosures are shown. Points represent individual enclosures. The dark line represents the mean of 3 replicates for the Daphnia, Daphnia+F, Copepod and Rotifer treatments.









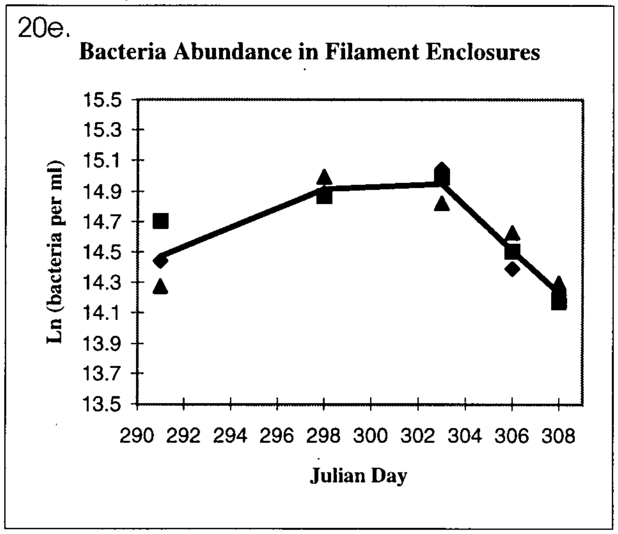


Table 11. Tukey multiple comparison test results based on repeated measures ANOVA of natural log-transformed bacterial abundances (5 dates) in the Fall experiment. No comparisons before November 2nd (October 18, October 25, October 30) were significant (not shown). Significant differences at $\alpha = .05$ are indicated in bold type.

Date	Treatment (i)	Treatment (j)	Mean difference (i - j)	Standard Error	Sig.
November 2	Daphnia	Filament	.2901	.154	.385
		Copepod	.3900		.160
		Daphnia+F	-.2456		.533
		Rotifer	.2926		.378
	Filament	Copepod	.0999		.963
		Daphnia+F	**-.5357**		**.038**
		Rotifer	.00246		1
	Copepod	Daphnia+F	**-.6356**		**.014**
		Rotifer	-.0974		.966
	Daphnia+F	Rotifer	**-.5382**		**.037**
November 4	Daphnia	Filament	**.5937**	**.100**	**.001**
		Copepod	**.9240**		**<.001**
		Daphnia+F	-.2778		.112
		Rotifer	**.8358**		**<.001**
	Filament	Copepod	**.3303**		**.050**
		Daphnia+F	**-.8715**		**<.001**
		Rotifer	.2421		.189
	Copepod	Daphnia+F	**-1.2018**		**<.001**
		Rotifer	-.0882		.899
	Daphnia+F	Rotifer	**1.1136**		**<.001**

detected. The FILAMENT, ROTIFER and COPEPOD treatments are significantly different from DAPHNIA+F on November 2nd; the DAPHNIA treatment is significantly different from DAPHNIA+F at an α of 0.053. By the final day of the experiment, the DAPHNIA and the DAPHNIA+F treatments are significantly different from the other treatments but not from each other (Figure 21). Additionally, the FILAMENT treatment has a final bacterial abundance significantly higher than that observed in the COPEPOD and ROTIFER treatments.

4.1-3 Bacterial abundance- Seasonal comparison of DAPHNIA, COPEPOD and ROTIFER

The three treatments performed in both experiments can be compared to illustrate the seasonal differences in their grazing impact on the microbial web, as implied by changes in bacterial abundance. Figures 22, 23 and 24 depict bacterial abundance under the repeated treatments in both experiments. The data given are natural log-transformed and each point represents the mean of three replicates. The Summer experiment took place over 16 sampling days, the Fall experiment over 18; for ease of comparison abundances are given according to the time elapsed in each experiment rather than by Julian date. Repeated measures ANOVA could not be performed on these data, because too few samples were taken at the same time relative to the onset of sampling in each experiment. Bacterial abundance as a whole was lower in the Fall than in the Summer (t-test on log-transformed abundance measurements pooled for each season, $t=6.662$, $df=157$, $\alpha=.05$, $p<.001$). There were large differences in water temperature between the Summer and Fall experiments. Despite this, when the time course of abundance changes are compared, significant differences among treatments in either experiment are only apparent after the midpoint.

Figure 21. Mean of natural log-transformed bacteria abundance for the Daphnia, Daphnia+F, Filament, Copepod and Rotifer treatments in the Fall experiment. Homogeneous subsets as determined by Tukey multiple comparisons are given by ellipses. See Appendix 1 Table G for significance levels.

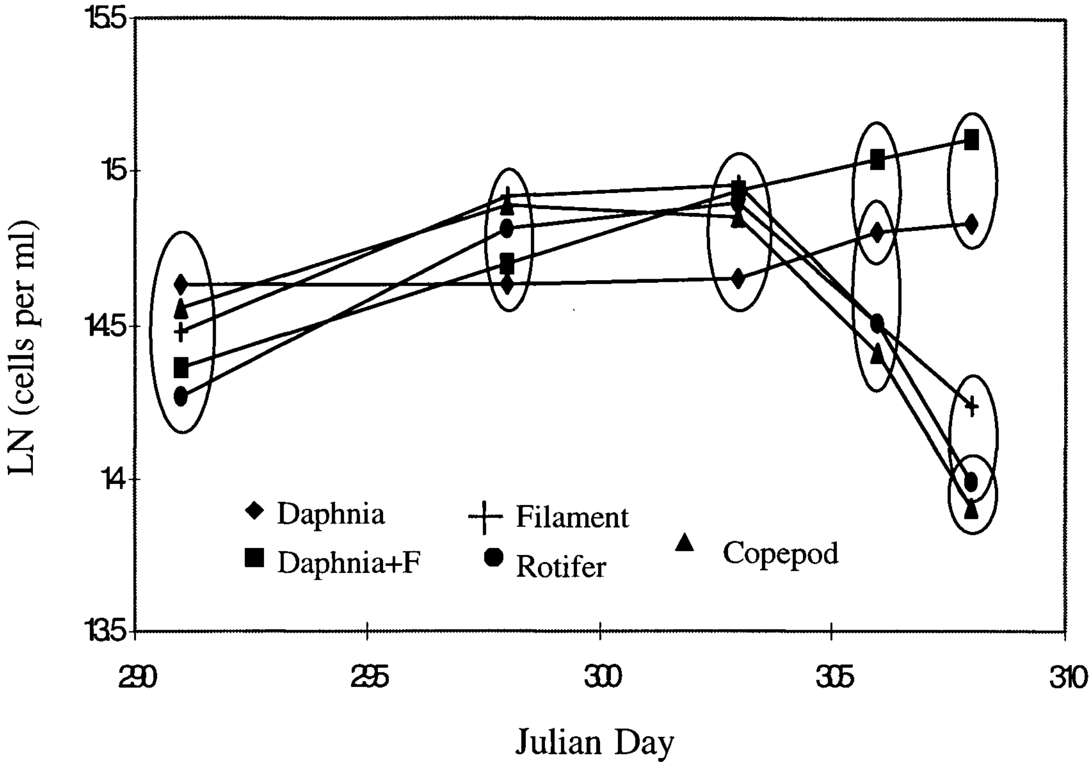


Figure 22. A comparison of natural log-transformed bacteria abundances in the Summer and Fall Daphnia treatments. The Summer experiment lasted 1 6 days, the Fall experiment 18. Data points represent the mean of three replicates.

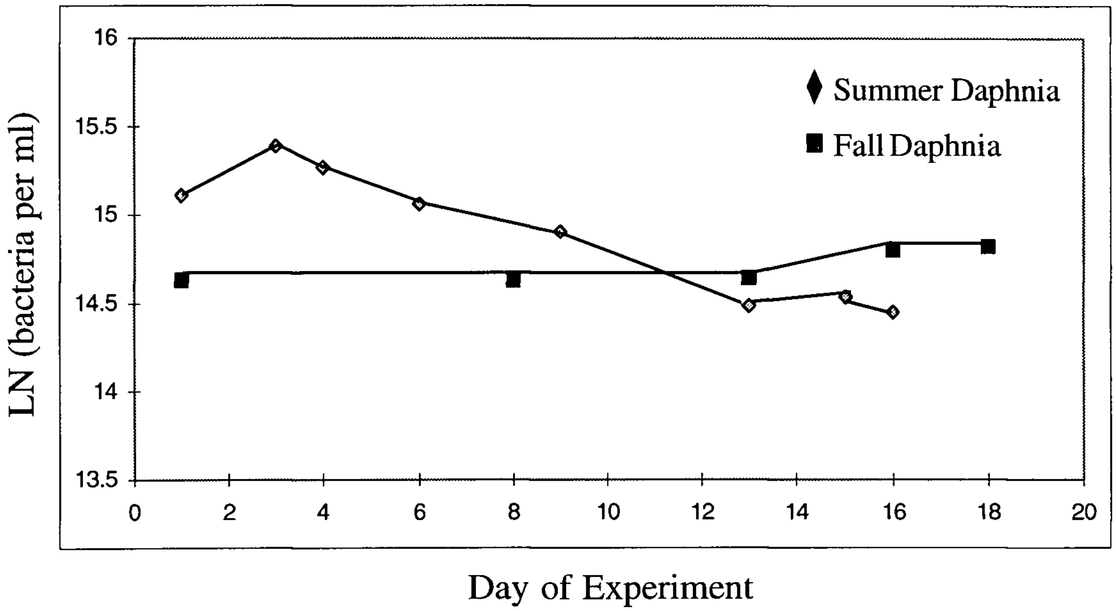


Figure 23. A comparison of natural log-transformed bacteria abundances in the Summer and Fall Rotifer treatments. The Summer experiment lasted 16 days, the Fall experiment 18. Data points represent the mean of three replicates.

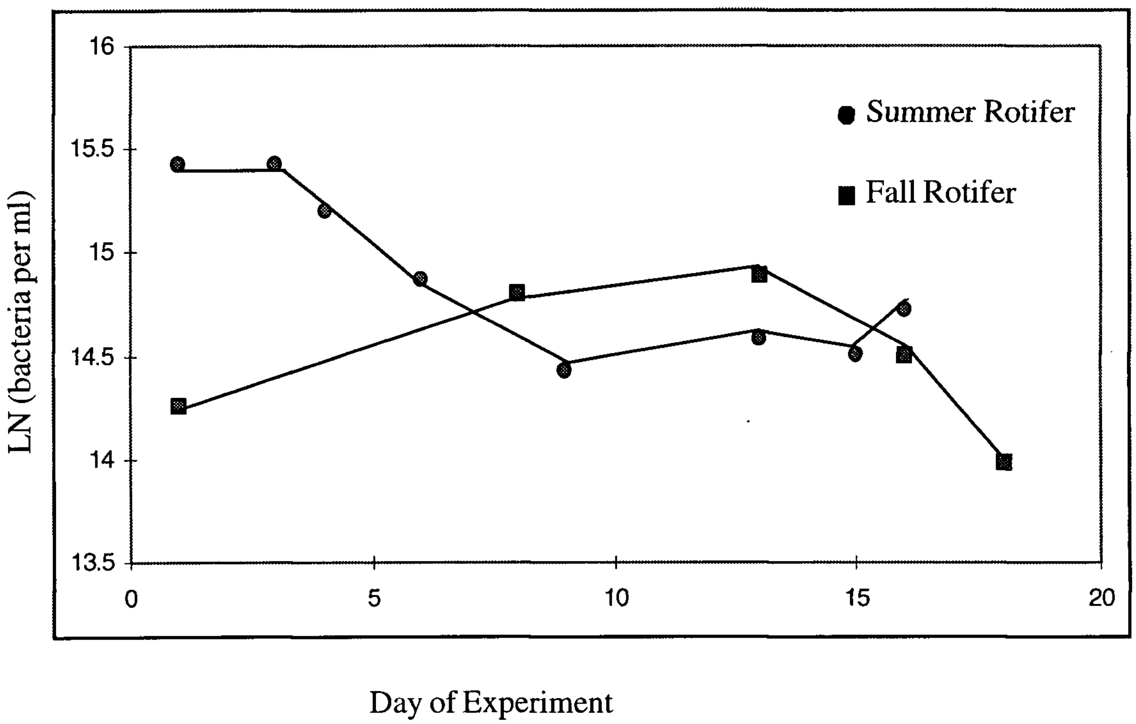


Figure 24. A comparison of natural log-transformed bacteria abundances in the Summer and Fall Copepod treatments. The Summer experiment lasted 16 days, the Fall experiment 18. Data points represent the mean of three replicates.

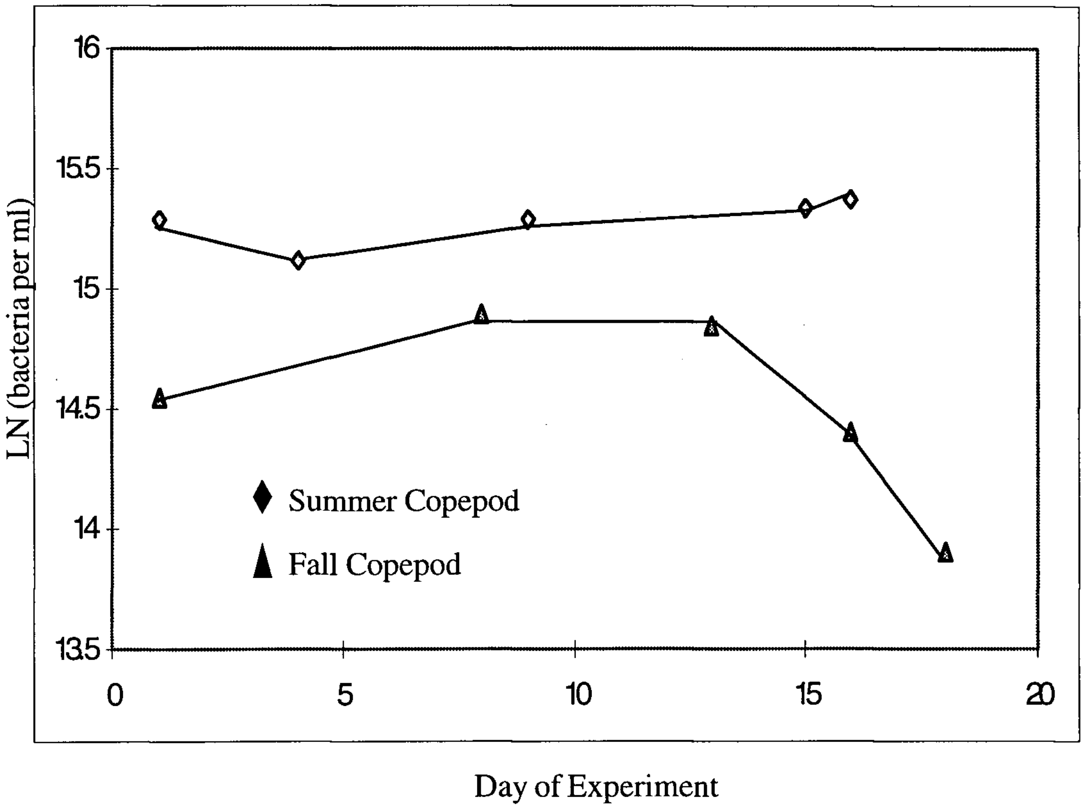


Figure 22 illustrates the decline in bacterial abundance observed in the Summer DAPHNIA experiment relative to the gradual increase seen in the Fall. While overall bacterial abundance does not differ markedly between the two seasons, the maintenance of a high bacterial abundance in the DAPHNIA treatment in the Fall experiment occurs in opposition to the general seasonal trend to lower bacterial numbers.

The bacterial abundance pattern in the ROTIFER treatment is shown in Figure 23. The Summer treatment shows a gradual decline in abundance, followed by a slight recovery towards the end of the experiment. The Fall pattern shows a gradual increase in abundance, followed by a sharp decline. This pattern in bacterial abundance is similar to that observed in the FILAMENT (Figure 20e) and COPEPOD (Figure 20c) treatments in the Fall experiment. There is a decline in bacteria standing stock observed for the ROTIFER treatment in both seasons.

In contrast, the COPEPOD treatment exhibits high bacterial abundance in Summer, and a decline in bacterial abundance in Fall (Figure 24). The Summer COPEPOD enclosures were *S. oregonensis*-dominated, but copepod populations did not increase in Fall enclosures to the same extent as in Summer. The Fall COPEPOD and Fall ROTIFER enclosures thus had similar zooplankton communities and exhibited the same trend in bacterial abundance, while the Summer COPEPOD enclosures had bacterial abundances similar to the BOSMINA enclosures.

4.2 Response of rotifers to treatments

In addition to the initial attempts to manipulate rotifer biomass in what eventually became the "COPEPOD" treatment, rotifer abundance and species composition in the DAPHNIA, BOSMINA, ROTIFER and FILAMENT treatments exhibited a response to the various zooplankton additions (or lack thereof). Due to the mesh size used to filter enclosure water, low numbers of rotifers were initially present in all bags.

Rotifer abundances in the enclosures on the final day of the Summer experiment are given in Figure 25a. Growth of the rotifer population was suppressed in the DAPHNIA treatment. The COPEPOD enclosures had rotifer populations similar to the ROTIFER enclosures, despite the addition of 1200 rotifers L^{-1} to the former. There appears to be a slight enhancement of rotifer numbers in the BOSMINA enclosures. The results for the Fall enclosures are similar, with the two *Daphnia* enclosures showing greatly reduced rotifer numbers, while the Fall COPEPOD treatment shows no increase in rotifer densities over that observed in the ROTIFER treatment.

Table 12a gives the results of a one-way ANOVA comparison of log-transformed rotifer abundances across all treatments in the Summer and Fall experiments. There is a significant effect of treatment ($F_{(8,17)}$= 7.926, p< .001), however despite log-transformation of the data, the variances were not homogenous (Table 12b) and therefore the reported p-values may be inaccurate. Differences between specific treatments were determined in *post-hoc* testing using the Tukey HSD procedure; the significance levels of the testing outcomes are given in Appendix 1 Table G. Log-transformed abundances are displayed graphically in their homogeneous subsets in Figure 26.

The comparison of rotifer abundances across all treatments in both seasons indicates a strong inhibition of rotifer population growth in the presence of *Daphnia.* This effect of *Daphnia* appears to be ameliorated by the presence of glass fibre filaments in the DAPHNIA+F

Table 12a. ANOVA comparison of natural log-transformed rotifer abundance on the final day of sampling. Includes all treatments from both the Summer and Fall experiments, $\alpha = .05$

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	29.634	8	3.704	7.926	<.001
Error	7.945	17	.467		
Total	37.579	25			

[†]R squared = .789 (adjusted R squared = .689)

Table 12b. Levene's test of equality of error variances: tests the null hypothesis that the error variance of the natural log-transformed rotifer abundance is equal across treatments. The dependent variable is the natural log-tranformed rotifer abundance on the final day of sampling. Treatments from both the Summer and Fall experiments were included.

Variable	F	df 1	df 2	Sig.
Rotifer Abundance	2.978	8	17	.028

Figure 25a. Total rotifer abundance on the final day of the Summer experiment (all species). Abundance values are given as a mean of 3 replicates (2 replicates in the Bosmina treatment).

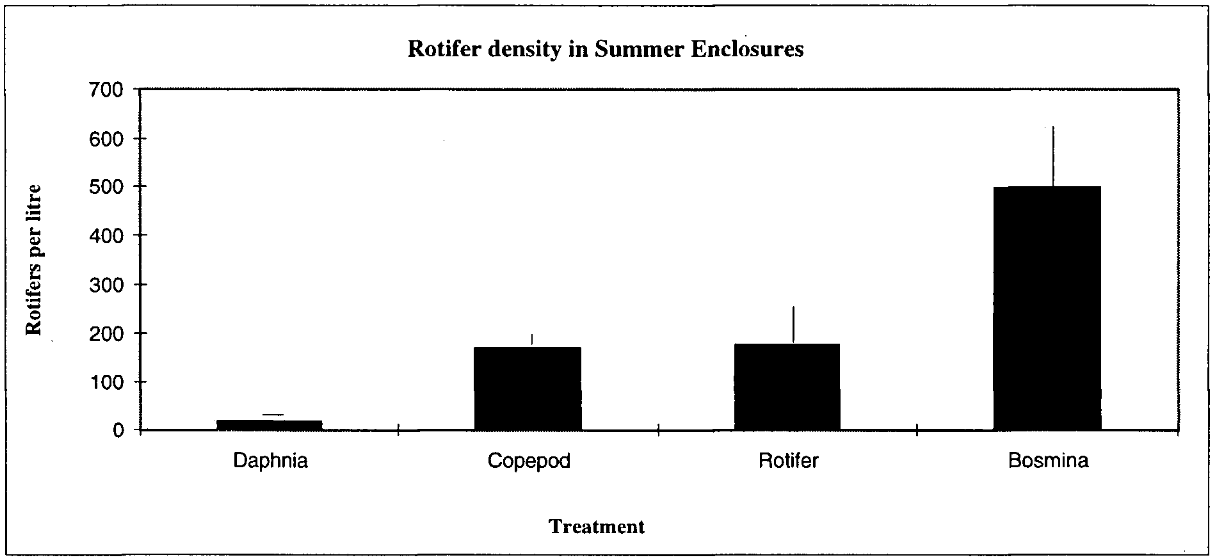


Figure 25b. Total rotifer abundance on the final day of the Fall experiment (all species). Abundance values are given as a mean of 3 replicates per treatment.

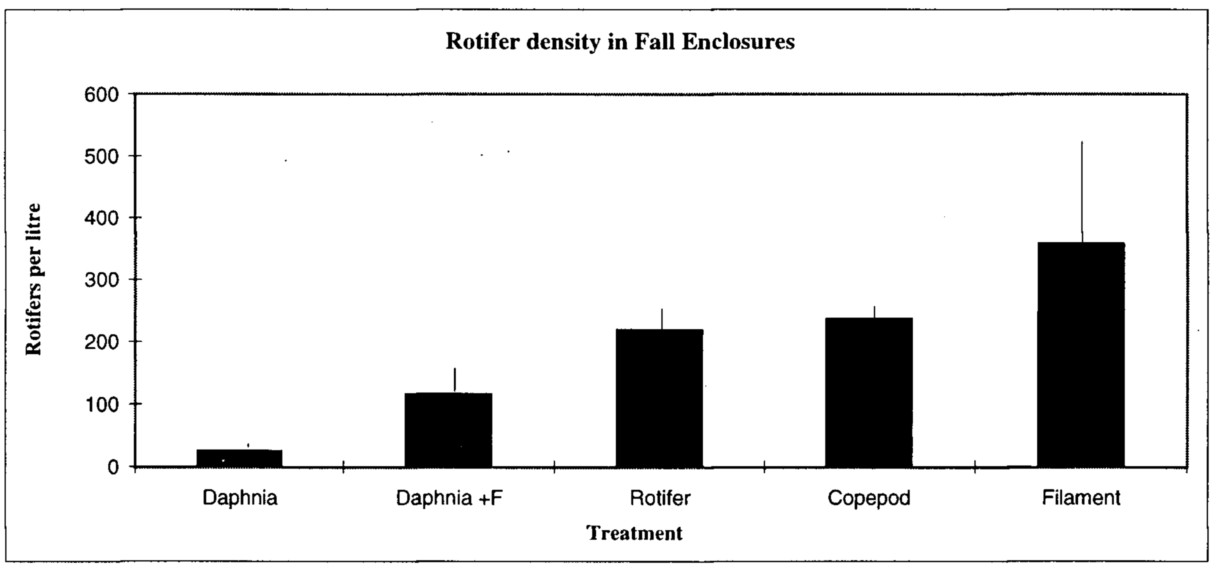
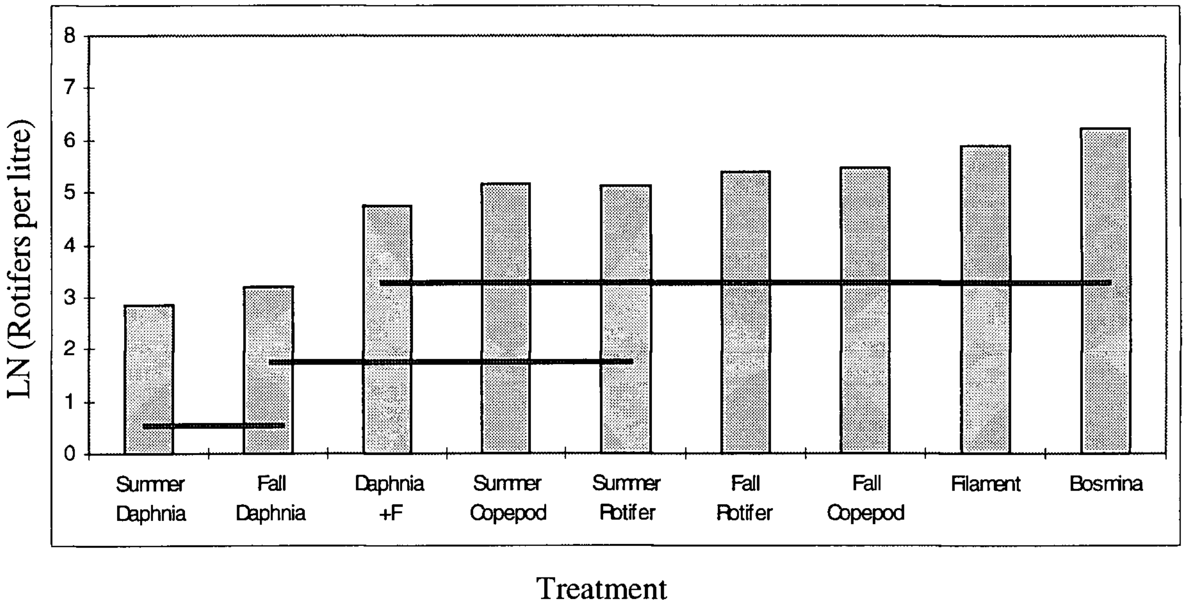


Figure 26. Natural log-transformed rotifer abundance on the final day of sampling both experiments. Homogenous subsets (Tukey multiple comparison, Appendix 1 Table G) are indicated by the solid lines. Note that the Daphnia+F treatment is significantly different from the Summer Daphnia at the 0.053 level)



treatment (Fig. 27), which has rotifer abundances similar to the ROTIFER and COPEPOD treatments in Summer and Fall. Rotifer densities tended to be higher in the Fall treatments, but the highest abundances were recorded in the Summer in the Bosmina enclosures.

While the patterns in rotifer abundance remained similar across treatments in both the Summer and Fall experiments, the species composition of the rotifer community differed between seasons. Figure 27 illustrates the major change in species composition. In Summer *Keratella cochlearis* was most abundant while in the fall *Polyarthra* c.f. *vulgaris* were more numerous. *Lecane* spp. (2 species) were also relatively abundant in the Summer COPEPOD enclosures at a mean density of 81 individuals L^{-1}. *Lecane* spp. were present in the other Summer enclosures at low densities (< 15 individuals L^{-1}), but were not recorded in the Fall zooplankton samples. *Keratella quadrata* was present in some enclosures at very low densities (~ 1 individual L^{-1}), absent from others, and was never abundant.

4.3 Response of ciliates

Ciliates were present in all enclosures, and there were significant differences in ciliate abundance across treatments in both experiments (ANOVA: $F_{(8,17)}$= 6.838, $p<.001$) . In a pattern similar to that observed in rotifer densities, ciliates abundance is lowest in the Summer and Fall DAPHNIA treatments (Figure 28a and 28b). The highest ciliate densities were recorded in the ROTIFER and FILAMENT treatments. Contrasts between all treatments were compared using the Tukey multiple comparison procedure, and the homogenous subsets are shown graphically in Figure 29. The Summer and Fall DAPHNIA, Summer COPEPOD, and BOSMINA treatments all had low final ciliate abundances. The lowest ciliate density was

Figure 27. Abundance estimates of the most common rotifer species in both the Summer and Fall experiments. Values given are the mean of 3 replicates for each treatment (2 in the Bosmina treatment). These two species comprised most of the rotifer populations in enclosures.

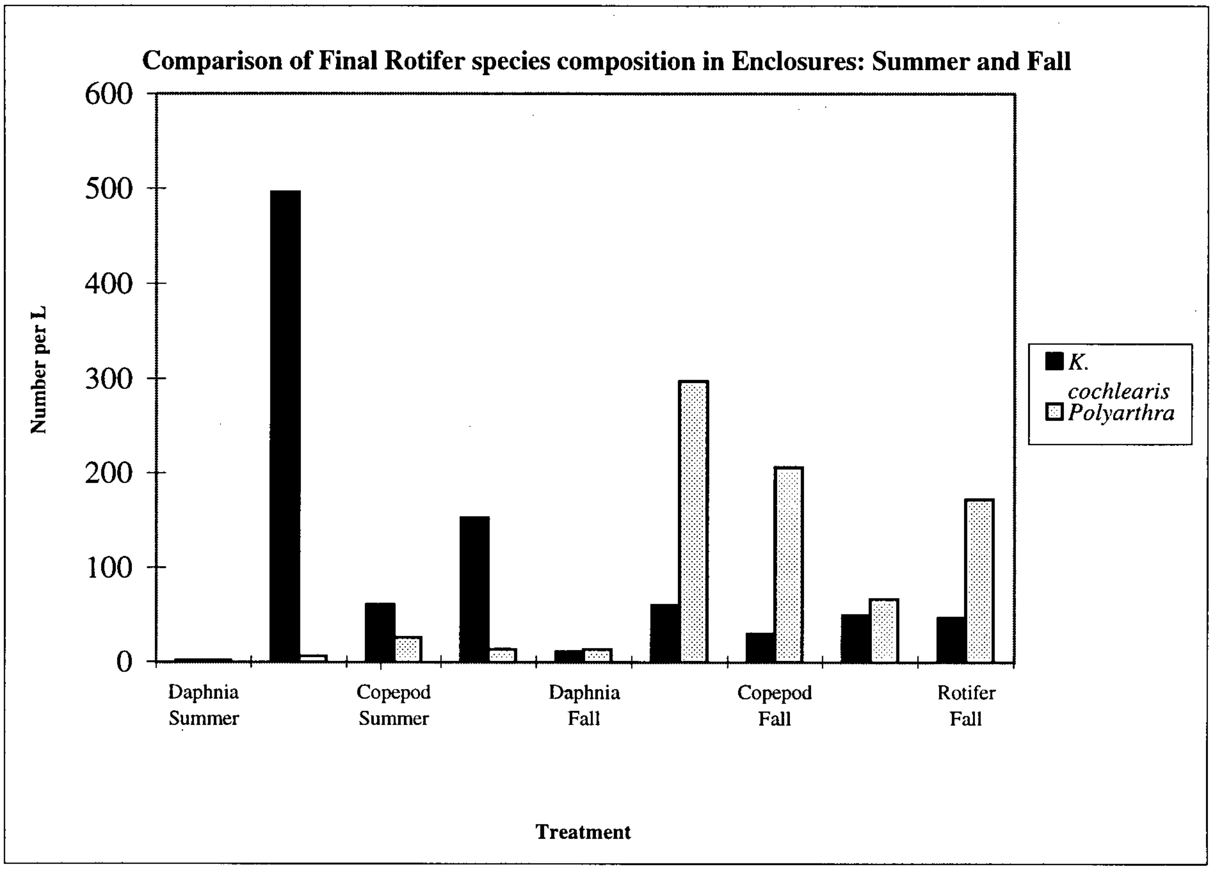


Figure 28a. Ciliate abundance in the Daphnia, Bosmina, Rotifer and Copepod treatments on the final day of the Summer experiment. Densities given are means of 3 replicates (2 in the Bosmina treatment). Error bars indicate 1 standard error.

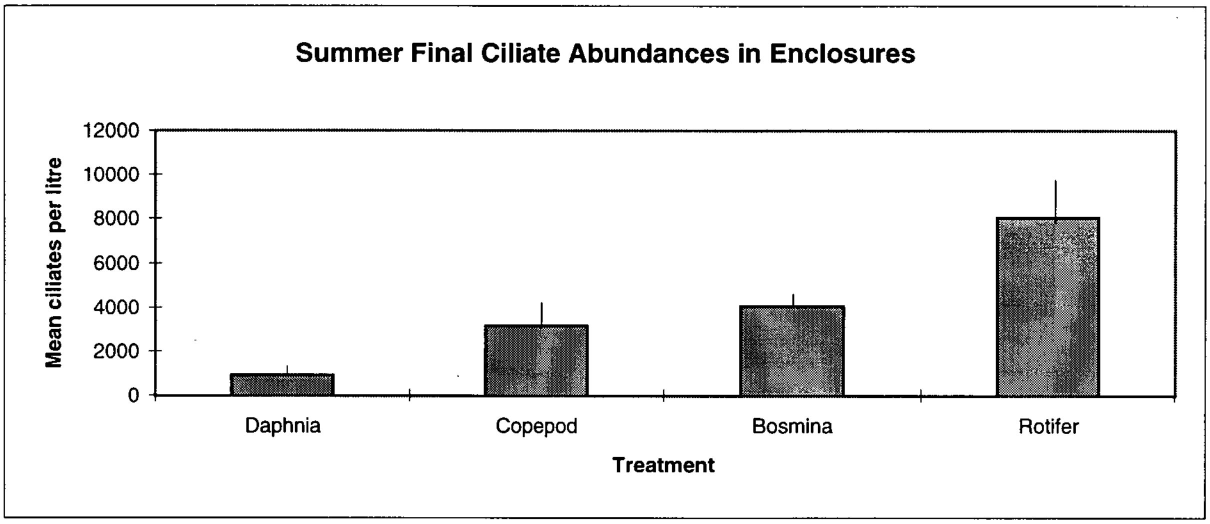


Figure 28b. Ciliate abundance in the Daphnia, Daphnia+F, Filament, Rotifer and Copepod treatments on thefinal day of the Summer experiment. Densities given are means of 3 replicates. Error bars indicate 1 standard error.

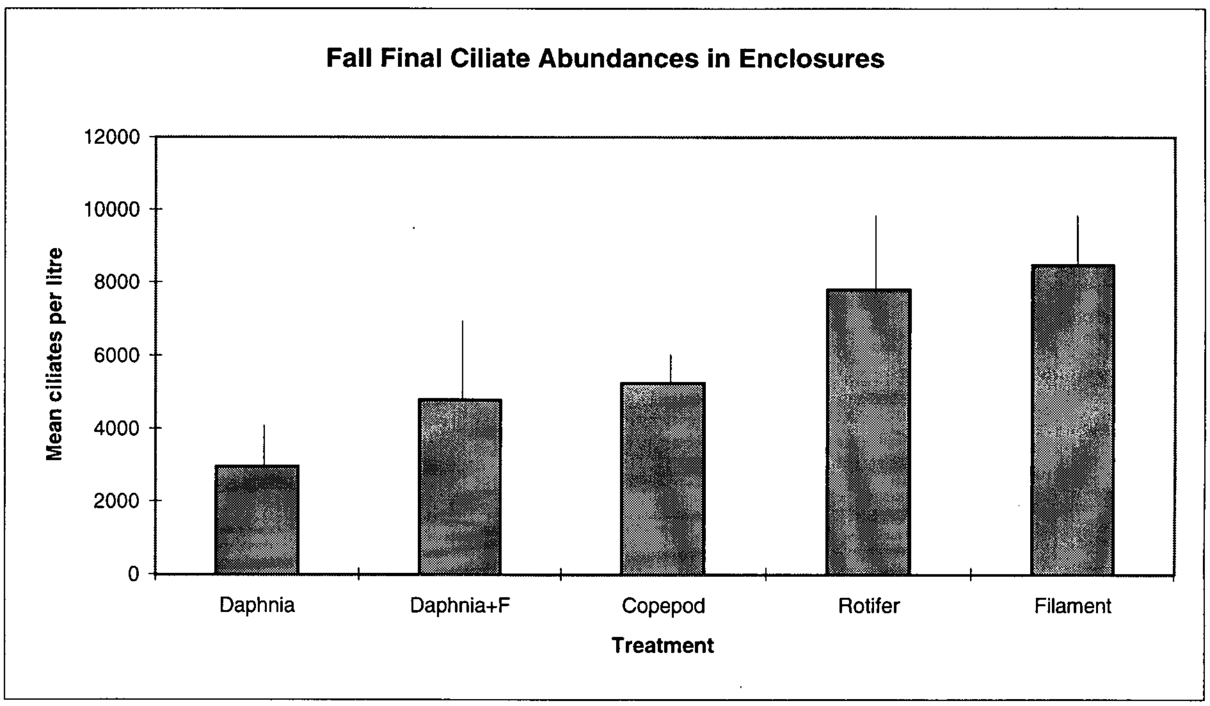


Figure 29. Natural log-transformed ciliate abundances on the final day of the S Summer and Fall experiments. Homogenous subsets (Tukey mutliple comparison) are shown by the black bars.

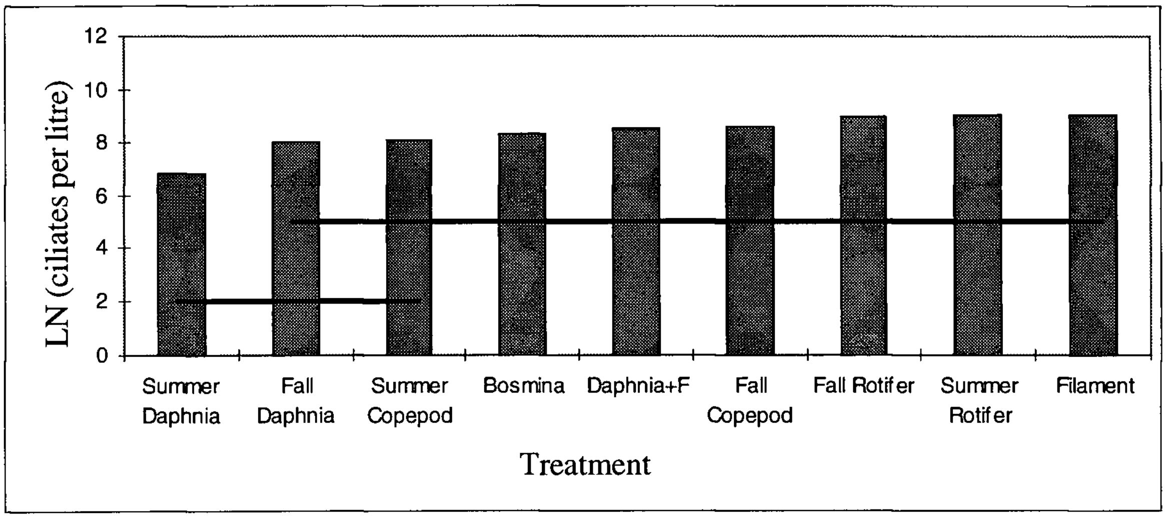


Figure 30a. Species composition of the ciliate community in the Summer enclosures. Densities are given as the mean of 3 replicate enclosures (2 in Bosmina) Identification is made to the Order level except where indicated.

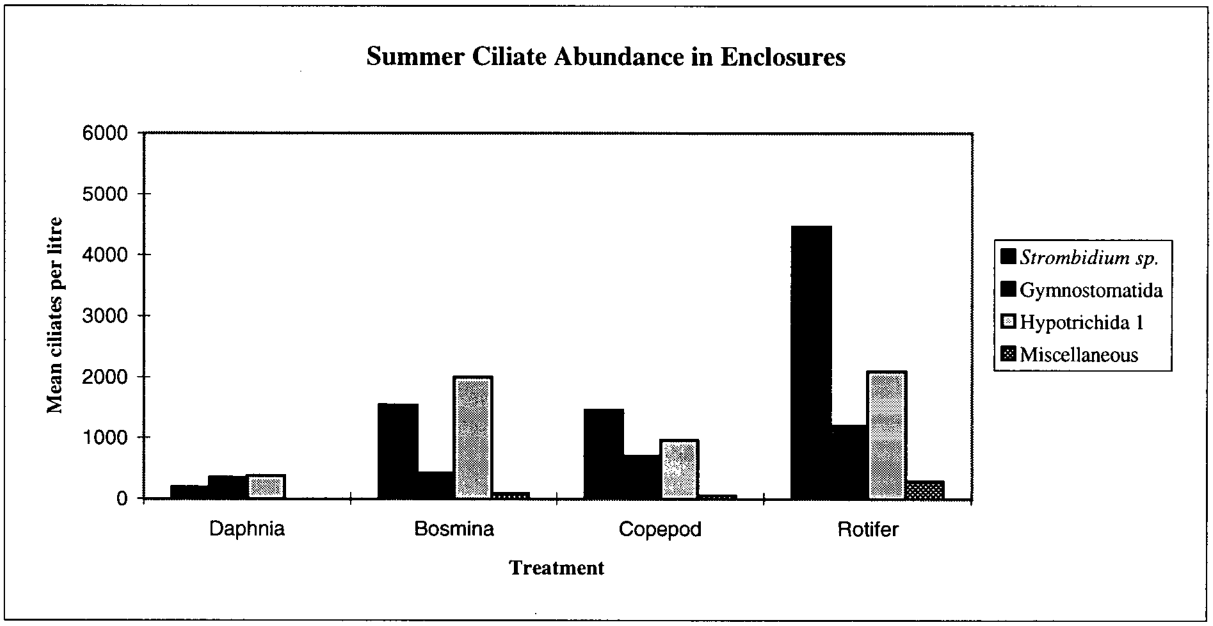
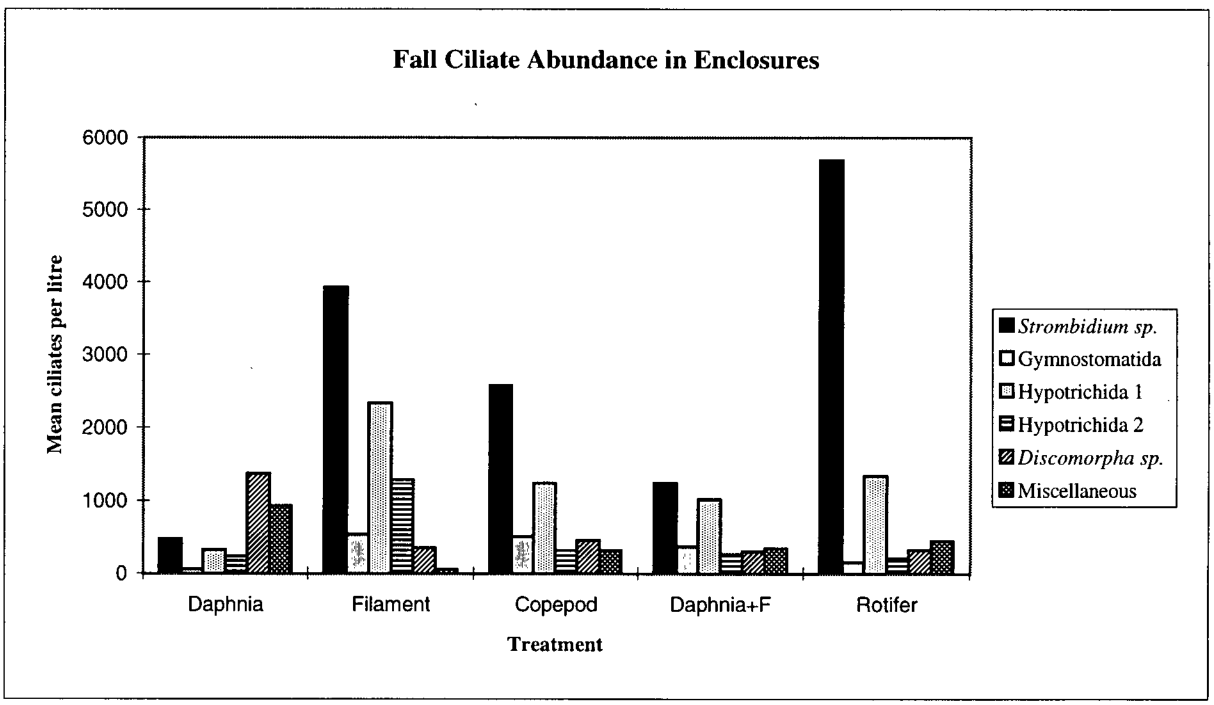


Figure 30b. Species composition of the ciliate community in the Fall enclosures. Densities are given as the mean of 3 replicate enclosures. Identification is made to the Order level except where indicated.



found in the Summer DAPHNIA treatment, which was significantly different from the DAPHNIA+F, Fall COPEPOD, Fall ROTIFER, Summer ROTIFER and FILAMENT treatments.

Ciliates were identified to the Order level in most instances. In the majority of the ciliate sub-samples, total ciliates counted numbered less than 200 (in a 25 ml settling chamber). Counts of this magnitude are acceptable for an assessment of total ciliate abundance. The counts of particular species are small subsets of the total count, and are too low in absolute numbers to allow accurate estimates of their density. With this caveat, the relative abundances of the most abundant ciliate groups identified are shown in Figure 30a (Summer) and Figure 30b (Fall).

The most commonly observed species observed in both Summer and Fall experiments was *Strombidium* sp. Other ciliates observed were counted and described as "morphotypes" and later identified. The Gymnostomatida, Hypotrichida type 1 and Hypotrichida type 2 ciliates observed were all single species, and in the case of the Gymnostomatida and Hypotrichida 1, the same species was observed in both experiments. Hypotrichida 2 was also relatively common in the Fall experiment but was not observed in Summer. A species of *Discomorpha* sp. was also frequently observed in the Fall enclosures, but only in low relative abundance. In general ciliate abundance was higher at the end of the Fall treatments (excluding *Daphnia* treatments), and higher treatments where large metazoan grazers had been excluded. The Fall increase in abundance is largely due to an increase in the most common species, *Strombidium* sp. (Figures 30a and 30b).

4.4 Response of algae

Lugol-preserved samples were settled for the counting of ciliates; algae were not enumerated. In the process of counting ciliates, however, some general observation of the algal abundance and diversity in enclosures were made. Lugol-preserved samples from the final sampling date of each experiment were examined. The flora present remained typical of that observed in Pond 13 in a pilot study conducted in May 1995. Algae samples from Pond 13 were visually inspected prior to the Summer experiment using an inverted microscope at 100X magnification and the composition remained typical of that observed in the late spring. The most common algae in Pond 13 are small chrysophytes and small cryptomonads. In the spring the green alga *Selenastrum* sp. was present in high abundances but was rarely observed in the samples from the Summer and Fall experiments. Another gelatinous green alga, *Elakatothrix* sp. was present in the spring and in the Summer experiment, though at relatively low abundance. Typically there are dinoflagellates such as *Ceratium* sp. and other large algal species present in Pond 13. These cells were excluded from the enclosures by the initial filtration and were present only in low numbers. In the case of *Ceratium*, cells were observed at high abundance in the Pond during initial surveys of microzooplankton prior to the experiment. Large *Volvox* colonies were also present in the Pond prior to the Summer experiment and were noted in the zooplankton tows taken from Pond 13 during the harvesting of *Bosmina* and *S. oregonensis*. *Arthrodesmus* sp. and *Neurocytium* sp. were present in the spring and in the Summer enclosures, but at very low abundance.

In the Summer enclosures, there was a particularly visible effect of the DAPHNIA treatment on the algae present. A large bloom of *Volvox* occurred in Summer in all the *Daphnia* enclosures (2940 $\pm$ 355 colonies L^{-1}) which was not observed in the other treatments. This bloom turned the water in the enclosures a murky green while the other bags remained clear. Inspection of the samples indicated that there were few edible algal cells in the DAPHNIA enclosures on the last day of the experiment; only broken *Volvox* colonies and a few cells (and large ciliates) were present in the samples. In contrast, the samples from the other treatments contained abundant edible algae in *Daphnia*'s preferred feeding range.

Volvox blooms also occurred in the Fall DAPHNIA enclosures, but the intensity of the water colour never reached the deep green observed in Summer. In counts of colony densities on the final sampling day of the Fall experiment, *Volvox* densities were 465 $\pm$ 35 colonies L^{-1} in the DAPHNIA enclosures and 577 $\pm$ 125 colonies L^{-1} in the DAPHNIA+F enclosures. *Volvox* colonies were present in the other enclosures, but were not abundant. In contrast to the Summer DAPHNIA enclosures, Fall enclosures containing *Daphnia* also had small edible algal cells present. Abundance of edible cells appeared to be somewhat less than that observed in the other Fall enclosures, but did not approach the "clear water" state seen in the Summer DAPHNIA treatment. However, algal diversity was somewhat lower in the Fall enclosures due to the appearance of an algal bloom described below.

Inspection of the algal samples from the final day of the Fall experiment indicated that a bloom of *Elakatothrix sp.* was present in all the enclosures. This gelatinous green alga can form sheets, but was present in the samples as single cells, although occasionally two or more

Figure 31. Cell numbers of Elakatothrix sp. in the Fall enclosures. The densities given for the Daphnia, Daphnia+F, Rotifer, Copepod and Filament treatments are mean values for 3 replicate enclosures. Error bars represent 1 standard error.

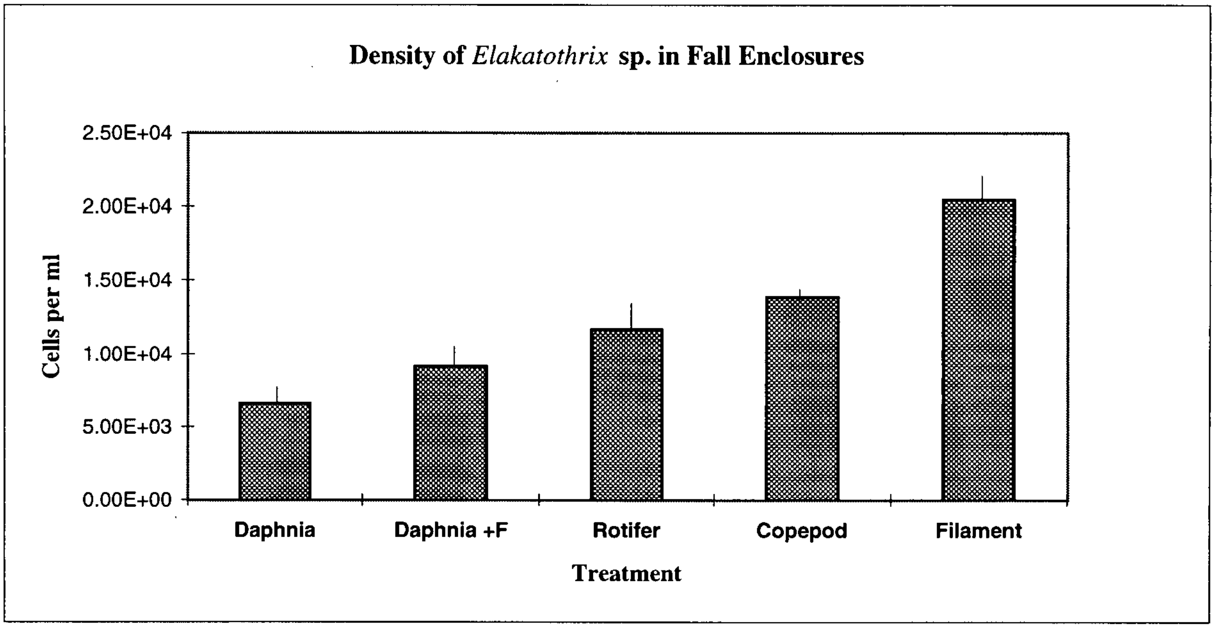
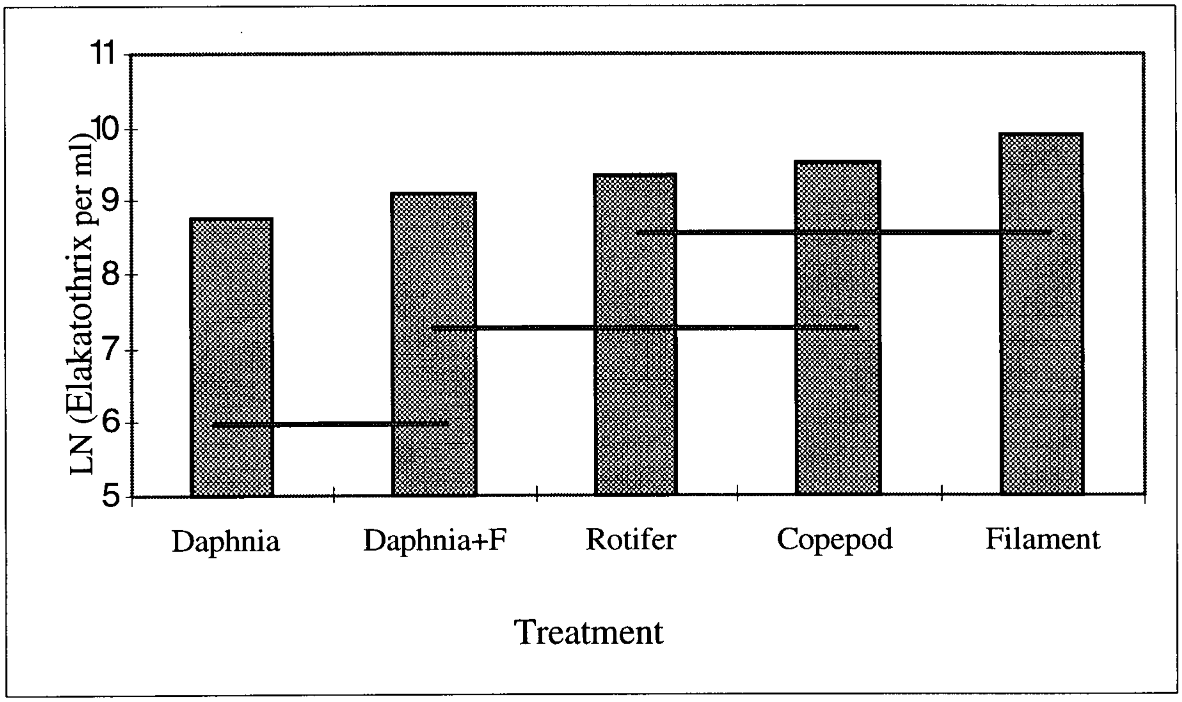


Table 13. ANOVA of natural log-transformed *Elakatothirx* sp. density on the final day of the Fall experiment.

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	2.305	4	.576	11.715	.001
Error	.492	10	.04918		
Corrected Total	2.796	14			

[†]R squared = .824 (Adjusted R squared = .754)

Figure 32. Natural log-transformed Elakatothrix densities in the Fall experiment. Homgenous subsets according to the Tukey multiple comparison procedure are indicated. The Daphnia -Rotifer comparison is significantly different at 0.053, all other comparisons are significant at <.05.



cells appeared to share a gelatinous matrix. The matrix itself did not stain and was not observed under the light microscope, however the cells adhered to the settling chambers with a particularly vexing tenacity, and a gelatinous sheath covering the single cells was inferred. Because a bloom of such magnitude is likely to influence the filtering behaviour of the metazoan grazers, the densities of *Elakatothrix sp.* were determined for all treatments on the final day of the Fall experiment. The estimated abundances are shown in Figure 31. Densities were lowest in enclosures with *Daphnia* and highest in the FILAMENT treatment. The difference in *Elakatothrix* densities was significant between treatments (Table 13). Homogenous subsets as determined by Tukey HSD multiple comparison tests are shown graphically in Figure 32. Comparison of the treatment means indicated that the FILAMENT treatment had significantly larger *Elakatothrix* densities than the six enclosures containing *Daphnia*. *Elakatothrix* in the DAPHNIA treatment was also significantly lower than the COPEPOD treatment.

4.5 Filament effects - A re-analysis

In the Fall experiment, the Fall DAPHNIA - DAPHNIA+F and Fall ROTIFER - FILAMENT treatment pairs were designed, independent of the seasonal comparison, to detect evidence of mechanical interference on *Daphnia* grazing and to determine the potential impact on microbial food webs. The comparison of the DAPHNIA and DAPHNIA+F treatments explores this question, while the comparison of the FILAMENT and ROTIFER treatment effects can be examined to detect any enhancement of the microbial food web due to inhibition of microbial grazers and/or the availability of increased surface area for microbial attachment.

Accordingly, these four treatments were re-analyzed together (excluding the COPEPOD treatment as there was no COPEPOD+F treatment to complete the design). Again, repeated measures ANOVA was employed to examine differences in bacterial abundance, while the ciliate, rotifer, and green algae abundances were compared using ANOVA for a single (final) sampling date. These analyses are similar to those described above, the only difference being the exclusion of samples from Fall COPEPOD enclosures.

The exclusion of the COPEPOD treatment from the statistical analysis resulted in homogenous variances for the entire (5 date) bacteria data set. Thus the problems encountered in the original analysis (violation of the ANOVA assumptions) are not an issue in the filament/no filament comparisons. The results are given in Tables 14a, b and c; the main results are of course similar to those obtained in the initial analysis above. *Post-hoc* multiple comparisons were made using the Tukey HSD procedure. No comparisons before the November 2nd sampling date were significant. The results from the November 2nd and November 4th sampling dates are given in Table 14d. On November 2nd, the DAPHNIA treatment is distinct from the ROTIFER and FILAMENT treatments, and though not nominally different from the DAPHNIA+F treatment, p=0.054, a contrast which had become fully significant by the November 4th sampling date. By the final day of the experiment, all four treatments are significantly different. In the initial analysis (which included the Fall COPEPOD treatment), the need to compare five treatments and the increased inequalities in variance, rendered the test too low in power to detect the more subtle effect of glass fibre filament addition. An examination of Figure 21 illustrated clearly that the effect of *Daphnia* in enclosures produced a much more pronounced enhancement of bacterial abundance; the

Table 14a. Repeated measures ANOVA of natural log-transformed bacterial abundance, comparing the Daphnia, Daphnia+F, Filament and No Grazer treatments from the Fall Experiment, 5 dates (α= .05)

Within-subject effects	Sum of Squares	df	Mean Square	F	Sig.
Date	1.431	4	.358	13.354	<.001
Date * Treatment	2.639	12	.220	8.209	<.001
Error (Date)	.857	32	.02679		

Table 14b. Repeated measures ANOVA of natural log-transformed bacterial abundance , comparing the Daphnia, Daphnia+F, Filament and No Grazer treatments from the Fall Experiment, 5 dates (α= .05)

Between-subject Effects	Sum of Squares	df	Mean Square	F	Sig.
Treatment	.906	3	.302	24.170	<.001
Error	.09991	8	.01249		

Table 14c. Levene's test of equality of error variances: tests the null hypothesis that the error variance of the natural log-transformed bacteria abundance in the enclosures is equal for the Daphnia, Daphnia+F, Filament and No Grazer treatments.

Sample date	F	df 1	df 2	Sig.
October 18	.885	3	8	.489
October 25	3.905	3	8	.055
October 30	.890	3	8	.487
November 2	1.346	3	8	.327
November 4	3.818	3	8	.058

Table 14d. Tukey HSD multiple comparison tests between the bacterial abundances in the Daphnia, Daphnia+F, Filament and Rotifer enclosures in the Fall experiment. Repeated measures ANOVA results are found in Table 14a-c. Bacterial abundances have been log-transformed. Treatment comparisons which were also significant in an analysis which included the Fall Rotifer treatment are shown in *italic* type.

Date	Treatment (i)	Treatment (j)	Mean difference (i - j)	Standard Error	Sig.
November 2	Daphnia	Filament	**.2901**	**.078**	**.024**
		Daphnia+F	-.2456	.078	.054
		Rotifer	**.2926**	**.078**	**.023**
	Filament	*Daphnia+F*	**-.5357**	**.078**	**.001**
		Rotifer	.002460	.078	1
	Daphnia+F	*Rotifer*	**.5382**	**.078**	**.001**
November 4	Daphnia	*Filament*	**.5937**	**.071**	**<.001**
		Daphnia+F	**-.2778**	**.071**	**.019**
		Rotifer	**.8358**	**.071**	**<.001**
	Filament	*Daphnia+F*	**-.8715**	**.071**	**<.001**
		Rotifer	**.2421**	**.071**	**.038**
	Daphnia+F	*Rotifer*	**1.1136**	**.071**	**<.001**

increased abundance due to "filaments" can be seen to be smaller. But whether *Daphnia* were present or not, the addition of glass fibre filaments always resulted in a higher bacterial abundance than would otherwise be observed.

The pattern seen in other response variables is less clear. Though there is a trend for the treatment mean density of ciliates, rotifers and *Elakatothrix* sp. to be higher in treatments which received filaments than in the corresponding treatment without filaments, in no case are the DAPHNIA-DAPHNIA+F and FILAMENT-ROTIFER pairs significantly different with regard to these variables (results not shown). At the level of the individual enclosures, most commonly two out of three enclosures followed this trend.

5. <u>Discussion</u>

5.1 Zooplankton biomass

As expected, there were increases in zooplankton biomass within treatments over the course of the experiment, as the zooplankton populations reproduced in enclosures. The *Daphnia* treatments attained high population densities, despite the fact that initial densities stocked were low to moderate when compared to similar enclosure studies (Brett et al. 1994; Jurgens et al. 1994a). As there were no *Daphnia* in Pond 13, the animals had to be harvested from a nearby pond where the food web was dissimilar. This source environment was probably low in food; some of the *Daphnia* in Library Pond exhibited ephippia, the pond was heavily shaded, and the food web was likely detritus-driven. Once these *Daphnia* were released into the comparatively lush environment of the enclosures, their growth and reproductive rates (inferred from population growth and changes in size distribution) were high. Based on an estimate of population size at the midpoint of the experiment (data not shown), the *Daphnia* populations had reached their final abundance levels by the midpoint of the Summer experiment. Though *Daphnia* densities were high, much higher population sizes, with pronounced effects on bacterial abundance, have been recorded after lake colonization by *Daphnia* (Jurgens et al. 1994b).

Unlike *Daphnia, Bosmina* was initially present in Pond 13, but at low abundances relative to *S. oregonensis* and *D. brachyurum*. The 120 *Bosmina* L^{-1} added to enclosures was higher than ambient density in the Pond, and population increase was modest. Individual *Bosmina* showed increases in biomass and some reproduction did occur, but population growth was not so large as that observed for *Daphnia.* The *Bosmina* populations were apparently close to equilibrium density in the enclosures.

The final copepod biomass in Summer enclosures was calculated (using the species- and instar-specific regressions given in Culver et al. 1985) to be smaller than that of *Daphnia* or *Bosmina.* The biomass of copepods in the Fall COPEPOD treatment was almost negligible, and I consider the Fall COPEPOD and Fall ROTIFER treatments to be effectively the same. Given the large differences in body size and growth rates of the various grazers, equalizing biomasses is not possible on an experimental time scale that allows reproduction and growth to occur. In evaluating the species-specific effects of the grazers, comparisons of population filtering capacities are much more instructive, and are discussed below.

5.2 Effectiveness of treatments

Examination of the zooplankton filtering capacities at the end of both experiments indicated that the DAPHNIA and BOSMINA treatments were successfully imposed as intended, and with comparable grazing pressures between treatments. Estimation of the Summer and Fall DAPHNIA population filtering capacities indicated enclosure turnover times of less than three days. The Summer BOSMINA treatment had an estimated enclosure turnover time only slightly larger, when flagellates were considered the "reference" prey. *Bosmina*'s estimated filtering capacity on bacteria was much lower, but the Summer DAPHNIA and BOSMINA treatments differ in this respect due to contrasts in feeding behaviour and not due to biomass differences between treatments. This is also true for *S. oregonensis* in the Summer COPEPOD treatment; while the estimated population clearance rates on flagellates were low for this treatment, when feeding on ciliates the copepods could be expected to clear the entire enclosure in less than two days. All of the macrozooplankton treatments had the potential to exert substantial grazing pressure on the microbial food web by the end of the experiments. Their different impacts, both direct and indirect, are due to species-specific differences in feeding behaviour. It is also likely that some of the indirect impacts of each species are a result of species-specific differences in nutrient recycling (excretion), but that possibility was not addressed in this study.

The time span of the experiment allowed rotifer populations to grow in all treatments except those containing *Daphnia.* The treatments imposed resulted in very different grazer communities between enclosures, but only in the *Daphnia* enclosures were the "single species" treatments maintained. The ubiquitous presence of rotifer populations in all the non-*Daphnia* treatments does not diminish their comparative value, however. *Daphnia* can reduce rotifer populations in lakes (Neill 1984); this has not been observed for *Bosmina* or *S. oregonensis.* In each of these treatments, the crustacean grazers have the potential for higher grazing rates than the rotifers. Rotifer populations alone are not expected to exert a strong direct influence through grazing microbial food webs, though they may have important indirect impacts on nutrient cycling (Arndt 1993). The presence/absence of rotifers in experimental enclosures is rightly considered an indirect effect of the larger metazoan grazers present, and it is the sum of both direct and indirect impacts that is of interest in this study.

The Summer "rotifer" treatment, though not successful in enhancing rotifer abundance above naturally occurring levels, functioned effectively as the COPEPOD treatment by the end of the experiment. Given the failure of stocked *S. oregonensis* populations to thrive after experimental manipulation, it is fortuitous, in terms of the experimental design, that *S. oregonensis* nauplii fared much better than the adults.

The NO GRAZER-turned-ROTIFER treatment had been intended to function as a microbial grazer community, but by the time treatment effects began to appear in the other enclosures, the grazer community in the "NO GRAZER" enclosures was rotifer-dominated. In an experiment performed by Brett et al. (1994), each of their treatments also contained rotifer populations, but at a somewhat lower density than I observed in my studies. Their "removal" treatment is equivalent to my Summer ROTIFER treatment. Brett et al. (1994) considered their removal treatment to be "grazer-free" despite rotifer abundances of approximately 75 $\pm$ 44.7 individuals L^{-1}. Comparably, mean rotifer abundance in my Summer ROTIFER treatment was 176 $\pm$ 80 individuals L^{-1}. Given the nature of sampling error for rotifer counts, these abundances are not appreciably different (Ruttner-Kolisko 1977). The microbe-only community structure intended for my NO GRAZER treatment exists naturally only in Antarctic lakes. Though such a treatment would have provided an interesting contrast to the grazer treatments, its loss from the design does not reduce the generality of the results.

5.3 *Daphnia*-rotifer interactions

The interactions of macrozooplankton and rotifers can have an indirect influence on the microbial food web. *Daphnia* virtually excluded rotifers from the enclosures in both the Summer and Fall experiments. *Daphnia* suppression of rotifer populations is well known from both laboratory (Burns and Gilbert 1986a, b; MacIssac and Gilbert 1991) and field experiments (Neill 1984; Wickham and Gilbert 1991). Rotifer abundances can be suppressed by exploitative or interference competition, or some combination of both (Gilbert 1988a).

Daphnia pulex has been shown to interfere with *Keratella cochlearis* by catching a rotifer in its feeding current and drawing it into the carapace. The rotifer may be rejected immediately with little effect, or drawn up the food grove towards the mouth, with retention time increasing the probability of lethal effects to the rotifer and occasionally resulting in its ingestion (Burns and Gilbert 1986a). *Daphnia* can also suppress *Keratella* by exploitative competition (MacIssac and Gilbert 1991).

Unlike *K. cochlearis*, at least one species of *Polyarthra* is able to escape capture by *Daphnia* (Gilbert 1988b). It has been suggested that this response of *Polyarthra* is affected by container size, with *Polyarthra* being suppressed by *Daphnia* in small enclosures but not in larger ones (Sarnelle 1997). One possible explanation for this is that long incubation times may increase the probability of encounter for *Daphnia* and *Polyarthra* in small enclosures (Wickham and Gilbert 1991), resulting in a stronger measured effect. However, my enclosures were much larger than the glass jars used previously (Wickham and Gilbert 1991), and it is possible that the high grazing pressure of *Daphnia* resulted in both exploitative and interference competition with *Polyarthra*, as *Daphnia* heavily grazed all edible algae in the enclosures. *Polyarthra* was greatly suppressed by *Daphnia* in both the Summer and Fall experiments.

5.4 *Bosmina*-rotifer interactions

The BOSMINA treatment, though successful in maintaining a *Bosmina*-dominated community, resulted in an interesting but difficult-to-interpret enhancement of rotifer abundances. It would be expected that *Bosmina longirostris*'s body size (maximum ~ 450 μm in length) is too small to allow interaction with rotifers by direct interference in the same manner as *Daphnia* (Wickham and Gilbert 1991). Given that *K. cochlearis*, *P. vulgaris* and *Bosmina* may all compete for the same preferred food (small flagellates), it is difficult to explain why rotifer abundances would be highest in the presence of *Bosmina.* It suggests that nutrient cycling or other indirect effects of the presence of *Bosmina longirostris* may enhance both the microbial food web and the microzooplankton.

5.5 Productivity and nutrient cycling

Though bacterial production was not measured in this study, the model outlined in Table 1 predicts a high ratio of bacterial production to primary production under *Daphnia* grazing (Jurgens 1994; see also Jeppesen et al. 1992) . *Daphnia* may decrease bacterial abundance by cropping bacteria cells directly, but grazing releases algal carbon and recycles potentially limiting nutrients, both of which can stimulate bacterial growth (Olsen et al. 1986, Jurgens 1994). Low levels of grazing may allow the indirect benefits to be of greater magnitude than the negative impact of direct grazing. However, algae were grazed to very low levels in my Summer DAPHNIA enclosures. I suspect that in the Summer DAPHNIA enclosures bacterial productivity was negatively affected by the reduction in algal biomass (and the concomitant reduction of available carbon substrates). The contention that moderate grazing may enhance productivity (Sterner 1986) has yet to be definitively tested for bacterioplankton. Ultimately, however, bacterial production in a *Daphnia*-dominated system may be channelled to higher trophic levels, while in food webs dominated by small zooplankton, most of the bacterial production is respired within the microbial food web. Enhancement of bacterial production and turnover by the indirect effects of grazing is of little importance to the classical lake food web if bacterial carbon does not pass into the zooplankton via direct pathways. Even if *Daphnia* were to decrease bacterial productivity in absolute terms, it is able to convert bacterial production into metazoan biomass, while smaller cladocerans and copepods cannot.

5.6 *Daphnia* and bacteria in Summer

The effect of the Summer DAPHNIA treatment relative to the BOSMINA and Summer COPEPOD treatments upholds the model of *Daphnia* interactions in microbial food webs (Table 1). *Daphnia* was able to graze down all the algae in the enclosure and hold bacterial abundance low through both direct and indirect effects. This stands in contrast to the Summer COPEPOD and BOSMINA treatments, where bacterial abundances were relatively high.

The Summer DAPHNIA treatment had a bacteria standing stock similar to the Summer ROTIFER treatment, but in no other way were the food webs similar. There were grazable algae remaining in Summer ROTIFER enclosures, but little other than *Volvox* colonies remained in the Summer DAPHNIA enclosures. Ciliate densities in the Summer ROTIFER enclosures were significantly higher than those in the DAPHNIA treatment, and rotifers were all but excluded from the Summer DAPHNIA enclosures. The ROTIFER treatment has the potential for high protistan grazing pressure on bacteria. Heterotrophic flagellates (main bacterivores) were likely grazed by the rotifer community in the ROTIFER enclosure, but neither *Keratella* nor *Polyarthra* feeds on ciliates (Buikema et al. 1978, Gilbert and Bogdan 1981, Arndt 1993). However, the absence of small zooplankton from ROTIFER enclosures such as *Bosmina* and copepods may also have had an indirect impact on the bacteria by decreasing nutrient recycling. The absence of macrozooplankton grazers probably denies bacteria the algal carbon made available to them by sloppy feeding. My results indicate that the loss of the positive indirect effects of small zooplankton on the microbial food web has the same consequence for bacterial abundance as high *Daphnia* grazing. In the Summer DAPHNIA enclosures, algae were grazed down to such low levels that the *Daphnia* were able to consume a substantial portion of the bacterial standing stock, and also deny bacteria the substrates (algal exudates) needed for growth.

5.7 *Daphnia* and bacteria in Fall

In the Fall experiment, the difference between the high and low bacteria densities resulting from treatment effects was even more pronounced than that seen in the summer. However, in the case of DAPHNIA treatments, the effect was opposite to that seen in the Summer experiment. As the *Daphnia* biomass (and filtering capacity) were the same in the Summer and Fall DAPHNIA treatments, this difference in outcome does not result from a difference in the grazer community. The high bacterial abundance in Fall suggests that the pathway for *Daphnia*'s direct effects on bacteria had been inhibited. *Daphnia*'s impact on ciliates and rotifers remained similar to the Summer treatment. The observed bloom of

Elakatothrix, present in Fall enclosures but not Summer, likely altered *Daphnia*'s filtering of the bacterial size fraction. *Elakatothrix* densities were lowest in the *Daphnia* enclosures, which is indicative of some cropping of the algae. Saturation of *Daphnia*'s feeding by high food concentration results in a plateau of ingestion rate and a decline in overall filtering rate (Lampert 1987a). *Daphnia* feeds inefficiently on bacteria and is only able to ingest the largest fraction of the available cells (Brendelberger 1991). The bloom of algae likely saturated *Daphnia*'s ingestion rates. This may have occurred if the algae were a good food source, and if not, the interference of so many low quality food particles would also reduce filtering rates. *Daphnia*'s impact on the microbial food web can thus be heavily influenced by the bottom up mechanisms which drive nutrient regimes and the development of algal blooms. My results indicate that *Daphnia* will always exert some kind of top-down control of microbial food webs, but the outcome may be either indirect enhancement of bacterial density or direct suppression of bacterial abundance.

The existence of a large algal bloom in the Fall enclosures may also indicate an increase in nutrient availability. Both algae and bacteria require dissolved nutrients for growth, and actively compete for them (Currie and Kalff 1984). Bacteria are also dependant on dissolved carbon substrates, which may be increased in the presence of an algal bloom (through algal exudation/lysis and "sloppy feeding" of grazers).

5.8 Other zooplankton and bacteria

Unfortunately, *Bosmina*'s impact on bacteria cannot be assessed in the presence of the *Elakatothrix* bloom, as the animals were not available for experimental collection in Fall. The evidence regarding *Bosmina* is specific to summer only, and as predicted, bacterial abundance was high in the *Bosmina*-dominated community. This is consistent with evidence that *Bosmina* does not graze bacteria directly (Bogdan and Gilbert 1982, Hart 1996). Though *Bosmina* could potentially graze bacterial predators in the < 20 μm size fraction (heterotrophic nanoflagellates), this cannot be determined from the data available. As stated above, *Bosmina*'s effect on ciliate abundance was moderate, despite its potentially high clearance rates for ciliates (Sanders and Wickham 1993). Rotifer abundances were higher in the *Bosmina*-dominated community than in the ROTIFER communities, and yet *Bosmina* has been shown to selectively graze the small flagellates which are also the preferred food of *K. cochlearis* and *P. vulgaris* (Bogdan and Gilbert 1982). Enhancement of bacterial abundance is expected in a *Bosmina*-dominated community (Table 1), and it would appear that protistan and rotifer populations benefit from *Bosmina*'s presence as well. *Bosmina*'s dual feeding mode allows it to co-exist with *Daphnia* rather than becoming excluded by exploitative competition (DeMott 1982). Its relationships with microzooplankton competitors may also be complex, but the design of this study does not allow this possibility to be fully assessed.

The high bacterial abundances seen in the Summer COPEPOD treatment are also in keeping with the predictions of the *Daphnia* vs. small zooplankton model (Table 1), though the pathways for the food web interactions are different. When compared to the ROTIFER treatment, enclosures with copepods demonstrated the predicted high bacteria densities, while the rotifer dominated enclosures did not. Rotifer-dominated zooplankton communities are maintained by planktivorous fish predation on macrozooplankton in some lakes, but more often an abiotic factor such as pH excludes other competing zooplankton (Arndt 1993). The effects of rotifers on microbial food webs have been reviewed, but their impact is seldom studied in isolation (Arndt 1993). Though *K. cochlearis* is capable of grazing bacteria, *P. vulgaris* is not (Sanders et al. 1989), and in both Summer and Fall enclosures, the rotifer dominated communities expressed low bacteria densities. This leads me to suspect that it is the combination of both 1) unfettered protistan grazing pressure and 2) the loss of the positive indirect effects of macrozooplankton grazing (nutrient recycling and sloppy feeding) which dictated bacterial abundance in the ROTIFER treatments. In the absence of direct grazing measurements and nutrient measurements, this conclusion is purely speculative. My results, in general, demonstrate the high bacterial abundances predicted for small zooplankton-dominated communities, but microzooplankton and microbial grazers do not fit this pattern in isolation. Usually the term "small zooplankton dominated" is used to encompass mixed copepod, small cladoceran and rotifer communities, but the data in this study indicate that rotifers are not equivalent to the macrozooplankton groups in their impact on the microbial food web.

5.9 Zooplankton-ciliate interactions

The interaction of *Daphnia* and ciliates has been less well studied than that of *Daphnia* and rotifers, but the evidence points to the same general conclusions. *Daphnia* are able to suppress ciliates by both interference and exploitative competition (Neill 1984, Gilbert 1988a, Wickham and Gilbert 1991). Unlike rotifers, ciliates can also form a nutritive component of *Daphnia*'s diet, though as for algae, the nutritive value of a particular ciliate species may differ for the various zooplankton taxa (DeBiase et al. 1990, Sanders et al. 1989, Sanders and Wickham 1993). Many ciliates have escape responses which would also dictate species-specific vulnerability to zooplankton predators (Wickham and Gilbert 1991, Jack and Gilbert 1993, Sarnelle 1997).

Daphnia pulex has been shown to depress ciliate abundance while in the same study *Bosmina longirostris* did not (Wickham and Gilbert 1991). Though ciliate densities in the BOSMINA treatment in this study were somewhat less than that found in the rotifer dominated enclosures, the ciliate abundance under *Bosmina* was higher than in the DAPHNIA treatments. This occurred despite the known potential of *Bosmina* to feed on ciliates (Sanders and Wickham 1993). The Summer and Fall DAPHNIA treatments had the lowest ciliate abundances observed in the study, while the DAPHNIA+F treatment (grazing interference) did not exhibit this suppression to the same degree. The Summer COPEPOD treatment also had relatively low ciliate abundances; *Skistodiaptomus oregonensis* has been shown to thrive on a diet of ciliates in the laboratory (Sanders et al. 1996), and likely preyed heavily on ciliates in the enclosures.

The *Elakatothrix* bloom which probably depressed *Daphnia*'s grazing on bacteria did not substantively alter *Daphnia*'s effect on ciliate abundance. *Daphnia*'s grazing of ciliates is likely to be a function of encounter rate and the defensive mechanisms of the ciliate (Jack and Gilbert 1993). *Daphnia*'s clearance rate on ciliates is more a function of encounter rate than particle retention, and may not be affected by mechanical interference to the same extent as that for bacteria. A similar enhancement of bacterial abundance by the indirect pathway of predation on ciliates was also seen in the *S. oregonensis*-dominated community of the Summer COPEPOD treatment.

5.10 Algal blooms and grazing interference

One major objective of this study was to assess seasonal differences in the response of bacterial abundance to zooplankton grazing. By repeating treatments in time as well as replicating within an experiment, it is possible to assess the general applicability of the results. While the rotifer-dominated communities had similar effects on bacterial abundance across seasons, the impact of *Daphnia* varied with seasonal differences in algal abundance and diversity.

Algal blooms are a common feature of lake phytoplankton dynamics. Late summer algal communities often exhibit increased abundance of inedible algae in response to zooplankton grazing, while successional and grazer induced shifts in dissolved nutrient ratios may favour blooms of filamentous cyanobacteria or indigestible gelatinous green algae (Sommer et al. 1986). Though it is not possible to conclusively determine the cause of the Fall algal bloom observed in the experimental enclosures, rainfall may have provided substantial nutrient inputs to the ponds and the enclosures. The precipitation-weighted average nitrogen content of rainfall near the University of British Columbia , measured in 1991 in the Georgia Basin (Strait of Georgia), has been estimated at 17 $\pm$ 2.5 μM [NO_3+NH_4] (Mackas and Harrison 1997). Nutrient inputs to the enclosures via rainfall may have precipitated the observed bloom of *Elakatothrix* sp., as rainfall occurred almost daily during the fall experiment. Irrespective of its origin, the occurrence of a Fall *Elakatothrix* bloom in the experimental enclosures allows the effects of zooplankton grazing on bacterial abundance to be tested for a food web configuration not explicitly addressed by the model in Table 1.

Though *Daphnia*'s feeding responses to food concentration and food quality have been well characterized in the lab (Lampert 1987a), this wide range of potential responses is often overlooked in both predictive models and field experiments. As my results suggest, algal blooms which alter *Daphnia*'s grazing behaviour may allow the bacterioplankton to escape top-down control by macrozooplankton. Algal filaments also enhance bacterial growth during algal senescence and lysis.

Many species of cyanobacteria can inhibit *Daphnia* grazing. Often they are toxic to zooplankton, and filamentous forms can mechanically inhibit grazing (Lampert 1987b). Model filaments have been used to investigate this mechanical effect, but their success has been somewhat limited (Webster and Peters 1978). More commonly, natural filaments have been used to illustrate the mechanics of grazing inhibition (Lampert 1987b). The use of natural filaments to determine mechanical interference of *Daphnia* grazing on bacteria is problematic; the filaments may release organic substrates as they decay, and enhance bacterial growth still further while the zooplankton grazing is inhibited. By using a model filament with no nutritive value to the *Daphnia* or the bacteria, I was able to detect the effect of mechanical interference on *Daphnia* grazing bacteria, without providing additional substrates for bacteria growth. However, the significantly enhanced bacterial abundance in the FILAMENT treatment, indicates that the physical presence of suspended filaments can enhance bacterial abundance independent of nutritional effects.

The addition of filaments in the absence of large zooplankton (FILAMENT treatment) provided suspended particles which enhanced microbial growth. Bacteria are known to attach to suspended organic particles in both marine and freshwater environments (Simon 1987). The productivity and cell size of attached bacteria are much greater than free living forms (Kirchman 1983, Simon 1987). Much of this productivity increase is thought to be provided by increased substrate availability in the vicinity of flocculent organic matter. However, this study demonstrates that bacterial growth or biomass can be stimulated by increased (inorganic) surface area available for attachment. Adding glass fibre filaments increased the spatial complexity and effective "surface area" of the enclosure environment. It is possible that algal blooms function in a similar way. Not only do filamentous algae inhibit zooplankton grazing, they also provide a physical matrix for enhanced microbial activity. This appears to be the case in the FILAMENT treatment, where bacterial abundance was significantly higher than in the Fall ROTIFER treatment (filament free). There was a slight trend for other components of the FILAMENT food web (ciliates, rotifers and *Elakatothrix)* to be higher as well, but not significantly so.

The results of the Fall experiment suggest, that in environments where suspended inorganic particles interfere with *Daphnia* grazing (Kirk and Gilbert 1990, Kirk 1991), bacteria densities may be enhanced. The DAPHNIA+F treatment demonstrates this enhancement, while the increased bacterial abundance in the FILAMENT treatments suggests that the increase is due to both grazing inhibition and increased particle surface area for microbial attachment. The increased spatial complexity generated by suspended particles has a measurable impact on microbial processes.

5.11 Comparison to other studies

The Summer experiment agrees well with results from other enclosure studies of bacterial abundance. Bacterial abundance was low in the DAPHNIA treatment as predicted by the model outlined in Jurgens (1994). Bacterial abundance was high under the BOSMINA treatment, in accordance with the results obtained by Jeppesen et al. (1992) for a community dominated by *B. longirostris*, and Geertz-Hansen et al. (1987) for *B. coregoni*-dominated enclosures. The high bacterial abundance I observed in the Summer COPEPOD treatment is supported by inspection of the results of Brett et al. (1994), who found a significant increase in bacterial abundance in small enclosures containing *Diaptomus novamexicanus* relative to those dominated by *Daphnia rosea.* In previous studies comparable to mine, the impact of rotifers has not been examined in the absence of other small zooplankton. The closest example is the "removal" treatment of Brett et al. (1994) which had somewhat lower rotifer abundances than my "rotifer-dominated" enclosures. None of the zooplankton treatments in Brett et al. (1994) had bacterial densities significantly different from the "removal" treatment, where bacterial abundance was intermediate between the *Daphnia* and small zooplankton treatments.

In contrast, the enhancement of bacterial abundance in the Fall DAPHNIA treatments goes against the trend in other studies towards reduced abundance. As most other studies took place in the Summer, it is possible that seasonality may play a greater role in bacteria-zooplankton interactions than has been investigated to date. Experiments conducted in one season cannot necessarily be extrapolated to other time periods (Brett et al. 1994). A wider range of food web states needs to be examined before an all-encompassing model of *Daphnia*'s impact can be validated. My study confirms the results of previous studies for the Summer food web condition, but the opposite effect observed in the Fall indicates that the impact of *Daphnia* on bacterioplankton has not yet been fully characterized. *Daphnia* can have a large impact on bacteria where it is abundant, but the balance of its direct and indirect impacts on the microbial food web may differ seasonally. The presence of *Daphnia* can be a strong predictor of bacterial abundance. However, knowledge of other factors affecting *Daphnia* grazing, such as food quality, quantity and/or the presence of inhibitory algal blooms must also be factored into any predictive model. While the magnitude of *Daphnia*'s impact on microbial food webs is often large, the effect on bacterial abundance is not always negative.

References

Arndt, H. (1993) Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates) - a review. Hydrobiol. 255/256: 231-246.

Azam, F, T. Fenchel, J.G. Field, J.S. Gray, LA. Meyer-Reil, F. Thingstad. (1983) The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10:257-263.

Bengtsson, J. (1987) Competitive dominance among Cladocera: Are single-factor explanations enough? Hydrobiol. 145: 245-257.

Bennet, S.J., R.W. Sanders and K.G. Porter. (1990) Heterotrophic, autotrophic, and mixotrophic nanoflagellates: Seasonal abundances and bacterivory in a eutrophic lake. Limnol. Oceanogr. 35: 1821-1832.

Berninger, Ulrike-G., B.J. Finlay and P. Kuuppo-Leinikki. (1991) Protozoan control of bacterial abundances in freshwater. Limnol. Oceanogr. 36: 139-147.

Bird, D.F. and J. Kalff (1984): Empirical relationships between bacterial abundance and chlorophyll concentration in fresh and marine waters. Can. J. Fish. Aquat. Sci. 41: 1015-1023.

Bleiwas, A.H. and P.M. Stokes. (1985) Collection of large and small food particles by Bosmina. Limnol. Oceanogr. 30: 1090-1092.

Bogdan, K.G. and J.J. Gilbert. (1982) Seasonal patterns of feeding by natural populations of Keratella, Polyarthra, and Bosmina: Clearance rates, selectivities, and contributions to community grazing. Limnol. Oceanogr. 27: 918-934.

Bogdan, K.G. and J.J. Gilbert. (1987) Quantitative comparison of food niches in some freshwater zooplankton. A multi-tracer-cell approach. Oecologia 72: 331-340.

Bogdan, K.G., J.J. Gilbert and P.L. Starkweather. (1980) *In situ* clearance rates of planktonic rotifers. Hydrobiol. 73: 73-77.

Boraas, M.E., K.W. Estep, P.W. Johnson and J.McN. Sieburth. (1988) Phagotrophic phototrophs: The ecological significance of mixotrophy. J. Protozool. 35: 249-252.

Bottrell, H., A. Duncan, Z.M. Gliwicz, E. Grygierek, A. Herzig, A. Hillbricht-Ilkowska, H. Kurasawa, P. Larsson and T. Weglenska. (1976) A review of some problems in zooplankton production studies. Norw. J. Zool. 24: 419-456.

Brendelberger, H. (1985) Filter mesh size and retention efficiencies for small particles: Comparative studies with Cladocera. Archiv. Hydrobiol. Beih. Ergebn. Limnol. 21: 135-146.

Brendelberger, H. (1991) Filter mesh size of cladocerans predicts retention efficiency for bacteria. Limnol. Oceanogr. 36: 884-894.

Brett, M.T., K. Wiackowski, F.S. Lubnow, A. Mueller-Solger, J.J. Elser and C.R. Goldman. (1994) Species-dependent effects of zooplankton on planktonic ecosystem processes in Castle Lake, California. Ecology 75: 2243-2254.

Brinch-Iversen, J. and G.M. King. (1990) Effects of substrate concentration, growth state, and oxygen availability on relationships among bacterial carbon, nitrogen and phospholipid phosphorus content. FEMS Microbiol. Ecol. 74: 345-356.

Buikema, A.L., J.D. Miller and W.H. Yongue. (1978) Effects of algae and protozoans on the dynamics of *Polyarthra vulgaris*. Verh. Internat. Verein. Limnol. 20: 2395-2399.

Burns, C.W., and J.J. Gilbert. (1986a) Direct observations of the mechanisms of interference between *Daphnia* and *Keratella cochlearis*. Limnol. Oceanogr. 31: 859-866.

Burns, C.W., and J.J. Gilbert. (1986b) Effects of daphnid size and density on interference between *Daphnia* and *Keratella cochlearis*. Limnol. Oceanogr. 31: 848-858.

Burns, C.W. and J.J. Gilbert (1993). Predation on ciliates by freshwater calanoid copepods: rates of predation and relative vulnerabilities of prey. Freshwater Biology 30: 377-393.

Butler, N.M., Suttle,CA. and W.E. Neill. (1989) Discrimination by freshwater zooplankton between single algal cells differing in nutritional status. Oecologia 78: 368-372.

Carpenter, S.R. J.F. Kitchell and J.R. Hodgson. (1985) Cascading trophic interactions and lake productivity. BioScience 35: 634-639.

Carpenter, S.R., J.F. Kitchell, J.R. Hodgson, P.A. Cochran, J.J. Elser, M.M. Elser, D.M. Lodge, D. Kretchmer, X. He and C.N. von Ende. (1987) Regulation of lake primary productivity by food web structure. Ecology 68: 1863-1876.

Christoffersen, K., B. Reimann, L.R. Hansen, A. Klysner, H. Soernsen. (1990) Qualitative importance of the microbial loop and plankton community structure in a eutrophic lake during a bloom of cyanobacteria. Microb. Ecol. 20: 253-272

Christofferson, K., B. Riemann, A. Klysner and M. Sondergaard. (1993) Potential role of fish predation and natural populations of zooplankton in structuring a plankton community in eutrophic lake water. Limnol. Oceanogr. 38: 561-573.

Currie, D.J. (1990) Large-scale variability and interactions among phytoplankton, bacterioplankton and phosphorus. Limnol. Oceanogr. 35: 1437-1455.

Currie, D.J. and J. Kalff. (1984) A comparison of the abilities of freshwater algae and bacteria to aquire and retain phosphorus. Limnol. Oceanogr. 29: 298-310.

Currie, D.J., E. Bentzen and J. Kalff. (1986) Does algal-bacterial phosphorus partioning vary among lakes? A comparative study of orthophosphate uptake and alkaline phosphatase activity in freshwater. Can. J. Fish. Aquat. Sci. 43: 311-318.

DeBiase, A.E., R.W. Sanders and K.G. Porter. (1990) Relative nutritional value of ciliate protozoa and algae as food for Daphnia. Microb. Ecol. 19: 199-210.

del Giorgio, P.A. and G. Scarborough. (1995) Increase in the proportion of metabolically active bacteria along gradients of enrichment in freshwater and marine plankton: implications for estimates of bacterial growth and production rates. J. Plankton Res. 17: 1905-1924.

DeMott, W.R. (1982) Feeding selectivities and relative ingestion rates of *Daphnia* and *Bosmina.* Limnol. Oceanogr. 27: 518-527.

Demott, W.R. and W.C. Kerfoot. (1982) Competition among cladocerans: nature of the interaction between *Daphnia* and *Bosmina.* Ecology 63: 1949-1966.

Demott, W.R. and M.D. Watson. (1991) Remote detection of algae by copepods: responses to algal size, odors and motility. J. Plankton Res. 13: 1203-1222.

Dodson, S.I. (1974) Zooplankton competition and predation: an experimental test of the size-efficiency hypothesis. Ecology 55: 605-613.

Ducklow, H.W., D.A. Purdie, P.J.LeB.Williams and J.M. Davies. (1986) Bacterioplankton: A sink for carbon in a coastal marine plankton community. Science 232: 865-867.

Dumont, H.J., I. Van de Velde and S. Dumont. (1975) The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. Oecologia 19: 75-97.

Fenchel, T. (1982) Ecology of heterotrophic microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. Mar. Ecol. Prog. Ser. 9: 35-42.

Fuhrman, J.A. and F. Azam. (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: Evaluation and field results. Mar. Biol. 66: 109-120.

Fuhrman, J.A., K. McCallum and A.A. Davis. (1992) Novel major archaebacterial group from marine plankton. Nature 356: 148-149.

Geertz-Hansen, O., M. Olesen, P. Koefoed Bjornsen, J. Brenner Larsen and B. Riemann. (1987) Zooplankton consumption of bacteria in a eutrophic lake and in experimental enclosures. Arch. Hydrobiol. 110: 553-563.

Gilbert, J.J. (1988a) Suppression of rotifer populations by *Daphnia*: A review of the evidence, the mechanisms, and the effects on zooplankton community structure. Limnol. Oceanogr. 33: 1286-1303.

Gilbert, J.J. (1988b) Susceptibilities of ten rotifer species to interference from *Daphnia pulex*. Ecology 69: 1826-1838.

Gilbert, J.J. and K.G. Bogdan. (1981) Selectivity of *Polyarthra* and *Keratella* for flagellate and aflagellate cells. Verh. Internat. Verein. Limnol. 21: 1515-1521.

Gliwicz, Z.M. (1990) Food thresholds and body size in cladocerans. Nature 343: 638-640.

Gliwicz, Z.M. (1980) Filtering rates, food size selection, and feeding rates in cladocerans - another aspect of interspecific competition in filter-feeding zooplankton. Am. Soc. Limnol. Ocean. Spec. Symp 3: 282-292.

Gliwicz, Z.M. and W. Lampert (1993) Body-size related survival of cladocerans in a trophic gradient: an enclosure study. Arch. Hydrobiol. 129: 1-23.

Gude, H. (1988) Direct and indirect influences of crustacean zooplankton on bacterioplankton of Lake Constance. Hydrobiol. 159: 63-73.

Gude, H. (1990) The role of grazing on bacteria in plankton succession. Chap. 9. In: Phytoplankton Ecology: Succession in plankton communities. 1st ed. (Ed: Sommer,U.) Springer-Verlag, Berlin Heidelberg, 337-364.

Gude, H. (1991) Bacterial production and the flow of organic matter in Lake Constance. Chap. 25. In: Large Lakes, Eds. Tilzer and Serruya, Springer-Verlag, New York, 489-502.

Hall, D.J., S.T. Threlkeld, C. Burns and P.H. Crowley. (1976) The size-efficiency hypothesis and the size structure of zooplankton communities. Ann. Rev. Ecol. Syst. 7: 177-208.

Hardy, F.J., K.S. Shortreed and J.G. Stockner (1986) Bacterioplankton, phytoplankton, and zooplankton communities in a British Columbia coastal lake before and after nutrient reduction. Can. J. Fish. Sci. 43: 1504-1514.

Hart, R.C. (1996) Naupliar and copepodite growth and survival of two freshwater calanoids at various food levels: Demographic contrasts, similarities, and food needs. Limnol. Oceanogr. 41: 648-658.

Jack, J.D. and J.J. Gilbert. (1993) Susceptibilities of different-sized ciliates to direct suppression by small and large cladocerans. Freshwater Biology 29: 19-29.

Jack, J.D. and J.J. Gilbert. (1994) Effects of *Daphnia* on microzooplankton communities. J. Plankton Res. 16: 1499-1512.

Jeppesen, E., O. Sortkjaer, M. Sondergaard and M. Erlandsen. (1992) Impact of a trophic cascade on heterotrophic bacterioplankton production in two shallow fish-manipulated lakes. Arch. Hydrobiol. Beih. Ergebn. Limnol. 37: 219-231.

Jurgens, K. and G. Stolpe. (1995) Seasonal dynamics of crustacean zooplankton, heterotrophic nanoflagellates and bacteria in a shallow, eutrophic lake. Freshwater Biology 33: 27-38.

Jurgens, K., H. Arndt and K.O. Rothhaupt. (1994a) Zooplankton-mediated changes of bacterial community structure. Microb. Ecol. 27: 27-42.

Jurgens, K., J.M. Gasol, R. Massana and C. Pedros-Alio. (1994b) Control of heterotrophic bacteria and protozoans by *Daphnia pulex* in the epilimnion of Lake Ciso. Arch. Hydrobiol. 131: 55-78.

Jurgens, K. (1994) Impact of *Daphnia* on planktonic microbial food webs- a review. Mar. Mic. Food Webs 8: 295-324.

Kerfoot, W.C. and K.L. Kirk. (1991) Degree of taste discrimination among suspension-feeding cladocerans and copepods: Implications for detritivory and herbivory. Limnol. Oceanogr. 36: 1107-1123.

Kirchman, D.L. (1993) Statistical Analysis of Direct Counts of Microbial Abundance. Chap. 14. In: Handbook of Methods in Aquatic Microbial Ecology. ed. P.F. Kemp et al., Lewis Publishers, Boca Raton, p.117-120.

Kirchman, D.L. (1983) The production of bacteria attached to particles suspended in a freshwater pond. Limnol. Oceanogr. 28: 858-872.

Kirk, K.L. (1991) Inorganic particles alter competition in grazing plankton: the role of selective feeding. Ecology 72: 915-923.

Kirk, K.L. and J.J. Gilbert. (1990) Suspended clay and the population dynamics of planktonic rotifers and cladocerans. Ecology 71: 1741-1755.

Kirk, R.E. (1982) Experimental design: procedures for the behavioral sciences. 2nd ed. Brooks/Cole Publishing Company, Belmont, California. 911 p.

Knochel, R. and L.B. Holtby. (1986) Construction and validation of a body-length-based model for the prediction of cladoceran community filtering rates. Limnol. Oceanogr. 31: 1-16.

Lampert, W. (1987a) Feeding and nutrition in *Daphnia.* Mem. Ist. Ital. Idrobiol. 45: 143-192.

Lampert, W. (1987b) Laboratory studies on zooplankton-cyanobacteria interactions. New Zealand Journal of Marine and Freshwater Research 21: 483-490.

Lampert, W. (1994) Phenotypic plasticity of the filter screens in *Daphnia*: Adapataion to a low-food environment. Limnol. Oceanogr. 39: 997-1006.

Lampert, W., W. Fleckner, H. Rai and B.E. Taylor. (1986) Phytoplankton control by grazing zooplankton: A study on the spring clear-water phase. Limnol. Oceanogr. 31: 478-490.

Malley, D.F., S.G. Lawrence, M.A. MacIver and W.J. Findlay. (1989) Range of variation in estimates of dry weight for planktonic crustacea and rotifera from temperate North American lakes. Can. Tech. Rep. Fish. Aquat. Sci. 1666, 49p.

MacAuley, E. (1984) The estimation of the abundance and biomass of zooplankton in samples. In: A manual on methods for the assessment of secondary productivity in fresh waters. (Ed. Downing, J.A., Rigler, F.H.). Blackwell Scientific, Boston, 228-265.

MacIssac, H.J. and J.J. Gilbert. (1991) Discrimination between exploitative and interference competition between cladocera and *Keratella cochlearis*. Ecology 72: 924-937.

Mackas, D. L. and P. J. Harrison. (1997) Nitrogenous nutrient sources and sinks in the Juan de Fuca Strait/Strait of Georgia/Puget Sound estuarine system: Assessing the potential for eutrophication. Estuarine and Coastal Shelf Science 44: 1-21.

Markosova, R. and J. Jezek. (1993) Bacterioplankton interactions with *Daphnia* and algae in experiemtnal enclosures. Hydrobiol. 264: 85-99.

McQueen, D.J., M.R.S. Johannes, J.L. Post,JR and D.R.S. Lean. (1989) Bottom-up and top-down impacts on freshwater pelagic community structure. Ecol. Monog. 59: 289-309

Muller, H., A. Schone, R.M. Pinto-Coelho, A. Schweizer and T. Weisse. (1991) Seasonal succession of ciliates in Lake Constance. Microb. Ecol. 21: 119-138.

Neill, W.E. (1984) Regulation of rotifer densities by crustacean zooplankton in an oligotrophic montane lake in British Columbia. Oecologia 61: 175-181.

Olsen, Y., A. Jensen, H. Reinertsen, K.Y. Borsheim, M. Heldal and A. Langeland. (1986) Dependence of the rate of release of phosphorus by zooplankton on the P:C ratio in the food supply, as calculated by a recycling model. Limnol. Oceanogr. 31: 34-44.

Pace, M.L. and J.J. Cole. (1996) Regulation of bacteria by resources and predation tested in whole-lake experiments. Limnol. Oceanogr. 41: 1448-1460.

Pace, M.L., G.B. McManus and S.E.G. Findlay. (1990) Planktonic community structure determines the fate of bacterial production in a temperate lake. Limnol. Oceanogr. 35: 795-808.

Pace, M.L. and E. Funke. (1991) Regulation of planktonic microbial communities by nutrients and herbivores. Ecology 72: 904-914.

Pennak, R.W. (1989) Fresh-water invertebrates of the United States. 3rd ed. John Wiley and Sons, Toronto. 628 p.

Porter, K.G. and Y.S. Feig. (1980): The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25: 943-948.

Porter, K.G., Y.S. Feig and E.F. Vetter. (1983) Morphology, flow regimes, and filtering rates of Daphnia, Ceriodaphnia, and Bosmina fed natural bacteria. Oecologia 58: 156-163.

Porter, K.G., H. Paerl, R. Hodson, M. Pace, J. Priscu, B. Reimann, D. Scavia and J. Stockner. (1988) Microbial interactions in lake food webs. In: Complex Interactions in Lake Communities. (Ed: Carpenter,S.R.) Springer-Verlag, New York, NY, 209-227.

Porter, K.G. (1988) Phagotrophic phytoflagellates in microbial food webs. Hydrobiol. 159: 89-97.

Pourriot, R. (1977) Food and feeding habits of Rotifera. Arch. Hydrobiol. Beih. Ergebn. Limnol. 8: 243-260.

Psenner, R. and R. Sommaruga. (1992) Are rapid changes in bacterial biomass caused by shifts from top-down to bottom-up control? Limnol. Oceanogr. 37: 1092-1100.

Riemann, B. and M. Sondergaard. (1986) Regulation of bacterial secondary production in two eutrophic lakes and in experimental enclosures. J. Plankton Res. 8: 519-536.

Romanovsky, Y.E. (1985) Food limitation and life-history strategies in cladoceran crustaceans. Arch. Hydrobiol. 21: 363-372.

Ruttner-Kolisko, A. (1977) Comparison of various sampling techniques, and results of repeated sampling of planktonic rotifers. Arch. Hydrobiol. Beih. Ergebn. Limnol. 8: 13-18.

Sanders, R.W. and S.A. Wickham. (1993) Planktonic protozoa and metazoa: predation, food quality and population control. Mar. Mic. Food Webs 7: 197-223.

Sanders, R.W., C.E. Williamson, P.L. Stutzman, R.E. Moeller, C.E. Goulden and R. Aoki-Goldsmith. (1966) Reproductive success of herbivorous zooplankton fed algal and non-algal food resources. Limnol. Oceanogr. 41: 1295-1305.

Sanders, R.W., D.A. Leeper, C.H. King and K.G. Porter. (1994) Grazing by rotifers and crustacean zooplankton on nanoplanktonic protists. Hydrobiol. 288: 167-181.

Sanders, R.W. and K. Porter. (1990) Bacterivorous flagellates as food sources for the freshwater crustacean zooplankter *Daphnia ambigua.* Limnol. Oceanogr. 35: 188-191.

Sanders, R.W., K.G. Porter, S.J. Bennett and A.E. DeBiase (1989) Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. Limnol. Oceanogr. 34: 673-687.

Sarnelle, O. (1997) *Daphnia* effects on microzooplankton: comparisons of enclosure and whole-lake responses. Ecology 78: 913-928.

Schluter, D. (1994) Experimental evidence that competition promotes divergence in adaptive radiation. Science 266: 798-801.

Schoenberg, S.A. (1990) Short-term productivity responses of algae and bacteria to zooplankton grazing in two freshwater lakes. Freshwater Biology 23: 395-410.

Schoenberg, S.A. and A.E. Maccubbin. (1985) Relative feeding rates on free and particle-bound bacteria by freshwater macrozooplankton. Limnol. Oceanogr. 30: 1084-1090.

Sherr, E.B., B.F. Sherr and L.J. Albright. (1986) Bacteria: Link or sink? Science 235: 88-89

Sherr, B.F.; E.B. Sherr and J. McDaniel. (1992) Effect of protistan grazing on the frequency of dividing cells in bacterioplankton assemblages. Appl. Environ. Microbiol. 58: 2381-2385.

Simon, M. (1987) Biomass and production of small and large free-living and attached bacteria in Lake Constance. Limnol. Oceanogr. 32: 591-607.

Sommer, U., M.Z. Gliwicz, W. Lampert and A. Duncan. (1986) The PEG-model of seasonal succession of planktonic events in fresh waters. Arch. Hydrobiol. 106: 433-471.

Starkweather, P.L. (1980) Aspects of the feeding behaviour and trophic ecology of supension feeding rotifers. Hydrobiol. 73: 63-72.

Sterner, R.W. (1986) Herbivores' direct and indirect effects on algal populations. Science 231: 605-606.

Stockner, J.G. and K.G. Porter. (1988) Microbial food webs in freshwater planktonic cosystems. Chap. 5. In: Complex interactions in lake communities. (Ed: Carpenter,S.R.) Springer-Verlag, New York, NY, 69-83.

Urabe, J. and Y. Watanabe (1991) Effect of food concentration on the assimilation and production efficiencies of *Daphnia galeata* G.O. Sars (Crustacea: Cladocera). Funct. Ecol. 3: 635-641.

Vanni, M.J. (1986) Competition in zooplankton communities: suppression of small species by *Daphnia pulex*. Limnol. Oceanogr. 31: 1039-1056.

Webster, K.E. and R.H. Peters. (1978) Some size dependent inhibitions of larger cladoceran filterers in filamentous suspensions. Limnol. Oceanogr. 23: 1238-1245.

Weisse, T. (1990) Trophic interactions among heterotrophic microplankton, nanoplankton, and bacteria in Lake Constance. Hydrobiol. 191: 111-122.

Weisse, T. (1991) The annual cycle of heterotrophic freshwater nanoflagellates: role of bottom-up versus top-down control. J. Plankton Res. 13: 167-185.

Weisse, T. and H. Muller. (1990) Significance of heterotrophic nanoflagellates and ciliates in large lakes: evidence from Lake Constance. Chap. 29. In: Large Lakes: Ecological structure and function. (Eds: Tilzer,M.M. and C. Serruya) Springer-Verlag, New York, 540-555.

Wickham, S.A. and J.J. Gilbert. (1991) Relative vulnerabilities of natural rotifer and ciliate communities to cladocerans: laboratory and field experiments. Freshwater Biology 26: 77-86.

Wickham, S.A. and J.J. Gilbert. (1993) The comparative importance of comptetion and predation by *Daphnia* on ciliated protists. Arch. Hydrobiol. 126: 289-313.

Winer, B.J., D.R. Brown, and K.M. Michels.(1991) Statistical principles in experimental design. 3rd ed. McGraw-Hill Inc., Toronto. 1057 p.

Appendix 1

Table A. Repeated measures ANOVA of natural log-transformed bacterial abundance in Summer enclosures (5 dates), α=.05

Within-subject Effects	Sum of Squares	df	Mean Square	F	Sig.
Date	1.685	4	.421	12.54	<.001
Date * Treatment	2.610	12	.217	6.472	<.001
Error (Date)	.941	28	0.03360		

Table B. Repeated measures ANOVA of log-transformed bacterial abundance in Summer enclosures (5 dates), α= .05

Between-subject Effects	Sum of Squares	df	Mean Square	F	Sig.
Treatment	1.959	3	.653	32.586	<.001
Error	.140	7	0.02004		

Table C. Levene's test of equality of error variances: tests the null hypothesis that the error variance of the natural log-transformed bacterial abundance in the Summer enclosures is equal across sampling dates

Sample date	F	df 1	df 2	Sig.
August 9	1.093	3	7	.413
August 12	.759	3	7	.552
August 17	.864	3	7	.503
August 23	**4.562**	**3**	**7**	**.045**
August 24	**14.416**	**3**	**7**	**.002**

Appendix 1

Table D. Repeated measures ANOVA of natural log-transformed bacterial abundance in Fall enclosures (5 dates).

Within-subject effects	Sum of Squares	df	Mean Square	F	Sig.
Date	2.311	4	.578	13.474	<.001
Date * Treatment	3.670	16	.229	5.348	<.001
Error	1.715	40	.04288		

Table E. Repeated measures ANOVA of natural log-transformed bacterial abundance in Fall enclosures (5 dates).

Between-subject Effects	Sum of Squares	df	Mean Square	F	Sig.
Treatment	1.144	4	.286	18.426	<.001
Error	.155	10	.01553		

Table F. Levene's test of equality of error variances: tests the null hypothesis that the error variance of the natural log-transformed bacterial abundance in the Fall enclosures is equal across sampling dates.

Sample date	F	df 1	df 2	Sig.
October 18	1.467	4	10	.283
October 25	2.594	4	10	.101
October 30	1.870	4	10	.192
November 2	**6.759**	**4**	**10**	**.007**
November 4	3.239	4	10	.060

Appendix 1

Table G. Tukey multiple comparison test of natural log-transformed rotifer abundance in enclosures. All treatments from both experiments were compared using ANOVA; significance levels are indicated in the table, with significance at $\alpha = .05$ given in bold type.

Bonferroni Multiple Comparison	Summer Daphnia	Bosmina	Summer Rotifer	Summer No Grazer	Fall Daphnia	Filament	Fall Rotifer	Daphnia +F	Fall No Grazer
Summer Daphnia	■								
Bosmina	**.001**	■							
Summer Rotifer	**.009**	.708	■						
Summer No Grazer	**.033**	.384	.999	■					
Fall Daphnia	.989	**.004**	.057	.176	■				
Filament	**.002**	.976	.995	.869	**.012**	■			
Fall Rotifer	**.003**	.946	.999	.933	**.017**	1	■		
Daphnia+F	**.053**	.278	.991	1	.264	.746	.840	■	
Fall No Grazer	**.004**	.898	1	.971	**.024**	1	1	.910	■

Appendix 2 : Replacement of a missing value

Data involving repeated measurements of each experimental unit are appropriately evaluated using repeated measures ANOVA (Winer et al 1991). This analysis was performed using the SPSS 7.5 statistics software, and the procedure does not allow for any missing values in the data set. Thus, of the 8 dates for which samples were counted in the Summer experiment, only 5 sample dates had a complete set of samples from all enclosures. Some extra samples were counted for the DAPHNIA, ROTIFER and BOSMINA treatments, but those could not be included as the COPEPOD enclosure samples were not counted on those dates. Additionally, one sample for the ROTIFER treatment (enclosure 9) taken on August 12 was inadvertently damaged during processing. Any missing value in the data set results in the entire case (enclosure) being dropped from the analysis. Unfortunately, though the deleted data point occurs early in the experiment when no treatment effects are observed, dropping the entire enclosure from the analysis would influence the results seen at the end of the experiment, where significant results were obtained. With this in mind, I decided to replace the missing value with an estimate and thus allow all the remaining measurements for enclosure 9 to be included in the analysis of bacterial abundance..

To generate an estimate to replace the missing data point, all bacterial abundance values for enclosure 9 were used to generate a linear regression of bacteria density in the bag over the course of the experiment; the predicted value for the bacterial abundance in enclosure 9 on August 12 was then used to replace the missing value in the data set.