STUDIES ON ZOOPLANKTON FEEDING ECOLOGY AND RESOURCE UTILIZATION IN A SUB-TROPICAL HYPERTROPHIC IMPOUNDMENT (HARTBEESPOORT DAM, SOUTH AFRICA)

Thesis Submitted in Fulfilment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY
of Rhodes University

by

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December 1987

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ABSTRACT

Various aspects of the feeding ecology of zooplankton are described for hypertrophic Hartbeespoort Dam, where the phytoplankton is dominated by the cyanophyte *Microcystis*. The study considers zooplankton succession, community grazing rates, and species-specific filtration rates on *Microcystis* colonies and natural bacterioplankton. Seasonal abundance of the main herbivorous zooplankton between 1981 and 1986 is described both in respect of biomass and specific densities. *In situ* community grazing rates were measured from January 1983 to March 1985 using ^{14}C-labelled *Chlorella*. Zooplankton succession and community grazing rates are examined in relation to food quantity and quality.

Experiments measuring species-specific filtration rates on labelled *Chlorella* and *Microcystis* colony fractions revealed low filtration rates for small-bodied cladoceran species on cyanophyte colonies. *Daphnia* fed significantly on *Microcystis* colonies up to 60–100 µm but *Daphnia* filtration rates on *Chlorella* were suppressed by ∿70% during the mid-summer increase in *Microcystis* abundance. Filtration rates of small cladoceran species were not suppressed by *Microcystis*, which was not an important food resource. Cladoceran filtration rate:body length models were developed for *Chlorella* and *Microcystis* colony fractions as food. Multiple regression models explained variance in filtration rates on these foods as a function of body length, food type and size, grazer species and temperature (in order of significance). Inclusion of food quality factors such as cyanophyte colony size seems justified in models of plankton feeding in eutrophic or hypertrophic lakes.

Methods for *in situ* measurement of zooplankton filtration rates on ^{3}H-thymidine-labelled natural bacteria were improved for use under hypertrophic conditions, and associated isotope-adsorption errors were measured. Community, species-specific and length-specific filtration rates on bacterioplankton were measured (late-spring to late-summer 1986-87). *Ceriodaphnia* exhibited no preference for bacteria or *Chlorella*. Other cladocerans preferred the algal food. Algal/bacterial selectivity coefficients of the zooplankton community revealed an increased algal preference following the mid-

summer shift to phytoplankton dominance by largely inedible *Microcystis*. This implies that bacterioplankton is not an important food resource for the summer cladoceran community. Estimates of the contribution of bacterial carbon to the daily zooplankton carbon requirements are low.

The implications of all results are discussed in relation to seasonal succession, the 'clear-water phase', and biomanipulation in this hypertrophic reservoir.

PREFACE

The work described in this thesis was carried out in the Limnology Division of the National Institute for Water Research, Council for Scientific and Industrial Research, Pretoria, under the external supervision of Dr R C Hart. These studies represent original work by the author and have not been submitted in any form to another University. Where use was made of the work of others, it has been duly acknowledged in the text.

Sections of this thesis have been structured on the format of independent manuscripts. The need for integration of information between chapters therefore required some unavoidable duplication of material.

ACKNOWLEDGEMENTS

The work described in this thesis would not have been completed without the help of many others. I take this opportunity to thank the following people.

My sincere thanks are due to Dr R.C. Hart, my supervisor, for encouragement and thought-provoking advice and discussion whenever possible despite our geographical separation. His guidance and criticism deserve special thanks.

I am greatly indebted to Ms S. Combrink for her excellent and proficient technical assistance over five years of this study. In particular her friendly nature and competent assistance with all field, laboratory and microscope work made completion of this study possible.

The National Institute for Water Research, CSIR, under the direction of Dr D.F. Toerien, and the Limnology Division headed by Dr F.M. Chutter provided all the facilities and financial support for this study. I am also indebted to other members of the Hartbeespoort Dam Ecosystem Programme research team. Their team spirit, effort and co-operation is deeply appreciated. I particularly wish to thank Ms A.M. Pais-Madeira, Dr P.J. Ashton, Dr J. Thornton, Mr W.E. Scott and Mr P.H. McMillan.

My special thanks are also due to:
Dr R.D. Robarts who provided unpublished data on natural bacteria and who made valuable suggestions regarding the use of ^{3}H-thymidine in the radiolabelling of bacteria.

Ms T. Zohary for unpublished data on phytoplankton succession, valuable discussions concerning the biology of *Microcystis* in Hartbeespoort Dam, and with whom the trial of doctoral thesis writing was shared.

Ms L.M. Sephton for assistance with bacterial staining and enumeration techniques and her cheerful temperament during field work.

Dr K.L. Cochrane for guidance relating to statistical and regression analysis.

Dr J. Galpin of the National Research Institute for Mathematical Sciences, CSIR, for guidance relating to multiple regression analysis.

Finally, I would like to thank my wife Desirée for her patience and encouragement, and my parents for provision of my earlier education which made completion of this thesis possible.

1. INTRODUCTION

1.1 The study of zooplankton feeding ecology under hypertrophic conditions

Arising from the intention of the National Institute for Water Research (NIWR) to develop an ecosystem model for hypertrophic Hartbeespoort Dam (Cochrane *et al.* 1987), the need for estimation of model parameters relating to nutrient fluxes, the compilation of a database for model calibration, and comparison of model outputs, all required the monitoring of a number of variables of the zooplankton community. Routine measurements of community biomass, species composition and zooplankton numbers were carried out, but particular emphasis was placed on zooplankton grazing. Information from zooplankton feeding studies under extremely nutrient enriched or hypertrophic conditions, such as those in Hartbeespoort Dam (Scott *et al.* 1980), has been infrequently or only recently reported in the literature (e.g. Okamoto 1984, Schoenberg and Carlson 1984, Hanazato and Yasuno 1985). Furthermore inconsistencies exist in the filter-feeding responses of various zooplankton species to cyanophyte foods of unicellular or colonial morphology. Consequently the direction of this research on zooplankton filter-feeding in hypertrophic Hartbeespoort Dam progressed towards the examination of

(i) zooplankton succession in relation to feeding rates and food quality,
(ii) the types of foods utilized and feeding limitations, and
(iii) zooplankton/resource interrelations in this excessively enriched system.

Questions concerning resource utilization by zooplankton in eutrophic lakes, where cyanophyte blooms occur frequently and seasonally, have been the subject of much examination since the studies by Hrbáček (1964), Gliwicz (1969a, 1969b), Hillbricht-Ilkowska (1972) and Hillbricht-Ilkowska *et al.* (1972) on zooplankton grazing, selectivity and ecological energy transfer efficiencies within the plankton. These early

studies focussed attention on differences between phytoplankton-zooplankton interactions in lakes of varying nutrient and trophic status. Supported subsequently by Gliwicz (1977) and Edmondson and Litt (1982), these studies also highlighted an association between seasonal declines in zooplankton biomass, or shifts in community composition to smaller bodied crustaceans and rotifers, with the seasonal occurrence of cyanophyte blooms; such successional events occurring also in association with seasonal increases in water temperature, increasing lake enrichment, and with size-selective predation by planktivorous fish (Brooks and Dodson 1965).

Early studies on the nutritional value of phytoplankton to the zooplankton grazers indicated that cyanophytes (including *Microcystis aeruginosa* in some cases) were only poorly ingested and assimilated by zooplankton (Sorokin 1968, Arnold 1971, Schindler 1971). However, De Bernardi *et al.* (1981) reported the growth and reproduction of *Daphnia* spp. on *Microcystis* of unicellular and small colony form.

Cyanophyte colony or filament size has been identified as a further factor limiting filter-feeding on this food by zooplankton. Phytoplankton resistance to zooplankton grazing by the formation of large colonies or production of toxins, and resistance to zooplankton digestion by development of gelatinous sheaths (Porter 1973, 1975, 1987, Porter and Orcutt 1980, Lampert 1981, 1982), are factors which contribute to subsequent shifts in zooplankton community structure and the frequent mid-summer decline in populations of large herbivores such as *Daphnia* (Threlkeld 1985, Sommer *et al.* 1986).

The mechanism by which large herbivores are generally adversely affected by mid-summer increases in abundance of cyanophyte colonies or filaments, while the smaller grazers remain largely unaffected, has also been examined (Gliwicz 1977, Webster and Peters 1978, Gliwicz and Siedlar 1980, Porter and Orcutt 1980, Porter and McDonough 1984). Reduction in the feeding efficiency of large herbivores in the presence of abundant large cyanophyte particles has been attributed to narrowing of

the carapace gape in cladocerans, clogging of filter-feeding mechanisms and increased rejection of food particles and food boluses. Thus there follows a reduction in feeding rates on the more highly nutritive co-occurring and edible phytoplankton types, an increase in the energetic cost of filter-feeding, or an increase in the ingestion of less nutritious and even toxic cyanophytes. The feeding efficiency of small cladocerans may be largely unaltered in the presence of these large food particles following from their often greater selectivity for nanoplankton and bacterioplankton foods and their better ability to survive at low food resource levels than the larger grazers such as *Daphnia* (DeMott and Kerfoot 1982, Pace *et al.* 1983).

On the basis of early reports on the low nutritive value of cyanophytes and observed successional events within the zooplankton community of Hartbeespoort Dam, Seaman (1977) suggested that *Microcystis* was essentially not utilized as a food resource by most zooplankton species in this impoundment. In Hartbeespoort Dam *Microcystis* forms extensive blooms annually at early summer, which continue to grow throughout the summer and persist as extensive surface accumulations and in consolidated scums throughout autumn and much of the winter period (Robarts and Zohary 1984, NIWR 1985, Zohary 1985). Therefore, under these hypertrophic conditions, the zooplankton community experiences extreme fluctuations in food quality and the availability of food resources. The shift that takes place, between a phytoplankton community composed largely of chlorophytes and cryptophytes only in spring, to a community composed almost entirely of *Microcystis* of colonial morphology from early summer to late winter, is both pronounced and occurs at a fairly predictable time annually (Robarts and Zohary 1984, NIWR 1985).

Under the hypertrophic conditions outlined above a number of questions arise concerning zooplankton filtration rates that have not been specifically or adequately reported in the literature, due to the paucity of detailed information on grazing rates over a number of seasonal cycles under hypertrophic conditions elsewhere. These questions are:

(i) What are the community grazing rates of the zooplankton community in this hypertrophic impoundment and how do seasonal changes in the phytoplankton influence zooplankton seasonal succession and community grazing rates?

(ii) Does grazing markedly influence phytoplankton abundance and composition? (i.e. by contributing to the low algal biomass of the clear-water phase, and by promoting cyanophyte dominance in summer);

(iii) Can any relationships predicting community grazing rates be derived for this hypertrophic lake? (e.g. using zooplankton biomass, food resource levels or water temperature);

(iv) To what extent can the major herbivores filter-feed upon the dominant phytoplankter *Microcystis*?

(v) What are the implications of these results to zooplankton succession and to biomanipulation programmes where intensive grazing on algae and cyanophytes is intended?

(vi) Can zooplankton filtration rates on *Microcystis* be predicted based on variation in the size of both grazers and *Microcystis* colonies?

(vii) What are the filtration rates of the main grazer species on natural, free-living bacterioplankton and do these rates indicate any species or body length-specific bacterial feeding preference?

(viii) To what extent does the zooplankton community depend on natural bacteria in summer when edible algal food resource levels are low?

In this study, the filter-feeding rates of the zooplankton community of hypertrophic Hartbeespoort Dam are examined under conditions of both widely varying food quality (phytoplankton type) and resource availability (colony size). Zooplankton

grazing rates are measured using a chlorophyte food throughout two annual cycles to quantify seasonal grazing rates on a generally palatable food under otherwise natural seston levels and *in situ* conditions. Thereafter the filtration rates of the major grazer species on colony size-fractions of natural *Microcystis* are measured separately from spring to autumn. Differences in the filtration rates of zooplankton grazers of various body length-classes on *Microcystis* of various colony fractions are examined. Controversy exists around whether zooplankton, particularly large cladocerans, are able to effectively graze cyanophytes to the extent that the development of blooms can be retarded or prevented. Sections of this study aim to contribute significantly to knowledge on the potential impact of zooplankton grazers on nuisance *Microcystis*. They represent the most extensive study reported so far on the filtration rates of a number of zooplankton taxa measured *in situ* on *Microcystis* colony fractions under the natural seston conditions of a hypertrophic impoundment.

Energy transfer in lakes between producers and consumers has been generally shown to become increasingly indirect, involving decomposition processes and pathways as lake trophy increases (Gliwicz 1969a, Hillbricht-Ilkowska 1972). In the presence of only low concentrations of palatable phytoplankton species and the predominance of cyanophyte colonies of a size largely unsuitable for ingestion by the summer zooplankton community of Hartbeespoort Dam, the filtration rates of zooplankton species on natural lake bacteria were also measured. Free-living bacteria were examined as an alternative and potentially important food resource composed of cell sizes approaching the lower end of the particle size-spectrum utilized by many zooplankters. During the course of this study, shortcomings in methods described in the literature to measure zooplankton grazing on natural lake bacteria were encountered when applied *in situ* in hypertrophic Hartbeespoort Dam. Consequently some emphasis was placed on the identification, measurement and reduction of sources of error to improve both the method and the reliability of *in situ* measurements of bacterioplankton grazing rates by macrozooplankton under hypertrophic conditions.

1.2 The Study Area: Hartbeespoort Dam

Hartbeespoort Dam (area 20 km^2, max. depth 32.5 m, mean depth 9.6 m) near the large urban areas of Pretoria and Johannesburg, South Africa (25 ° 43'S, 27 ° 51'E) (Figure 1.0) has been described as a hypertrophic, warm, monomictic impoundment by Scott *et al.* (1980). Extreme enrichment of the lake has occurred in parallel with rapid urban growth in the northern Johannesburg area, from which industrial effluent and secondary treated domestic sewage comprise more than 90% of the nutrient load (Robarts 1985).

High total nitrogen and phosphorus loads of 76 and 20 g m^{-2} a^{-1} respectively (hydrological year 1982-83) and the high resultant mean annual chlorophyll concentration of 94 mg Chl. *a* m^{-3} over the euphotic zone maintain the lake's hypertrophic condition (NIWR 1985, Robarts 1985). Underwater light attenuation due to algal self-shading typically limits primary production (Robarts 1984, Robarts and Zohary 1985). Maximum chlorophyll *a* concentrations of almost 3 g m^{-3} (Robarts 1985) and primary production of over 3 g C m^{-2} h^{-1} have been measured (Robarts 1984). During the spring clear-water phase (late August to early November) chlorophyte and cryptophyte species may briefly dominate the phytoplankton (NIWR 1985). Thereafter the phytoplankton assemblage is dominated from late November to early August by the cyanophyte *Microcystis aeruginosa*, which from December to May (austral summer to early winter) may make up 90-99% of the phytoplankton biovolume (Robarts and Zohary 1984). A low wind speed regime allows the buoyant *Microcystis* to increase in colony size in the absence of wind mixing, thus decreasing light attenuation whilst remaining primarily within the upper water column (Robarts and Zohary 1984). Thus the net-phytoplankton is usually dominated by *Microcystis* which in calm weather is mainly composed of large colonies of >1 mm and reaching up to 50 mm in length (Robarts and Zohary 1984).

From August to early April thermal stratification leads to development of an anoxic hypolimnion usually below 10 m depth

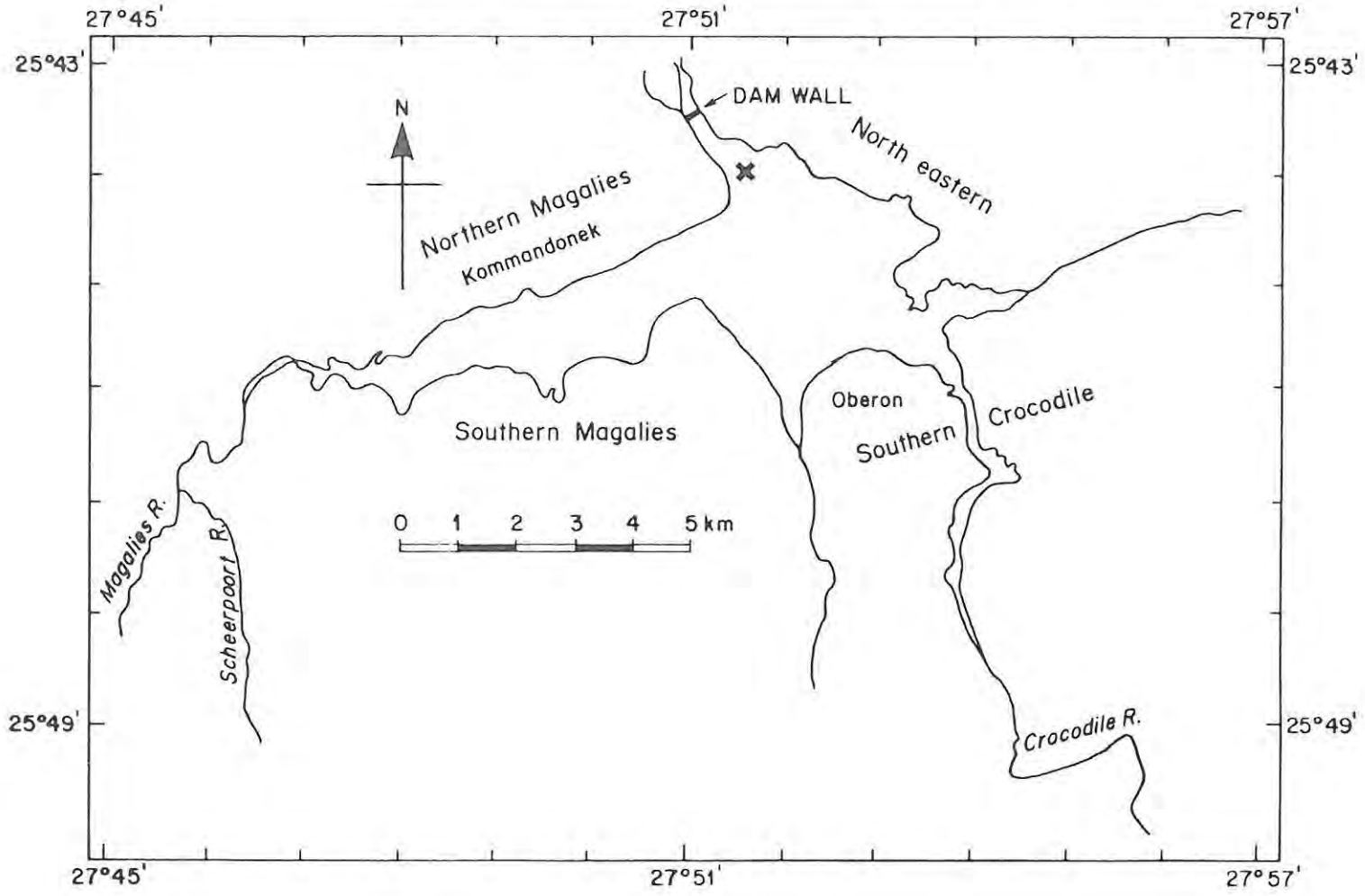


Figure 1.0: Hartbeespoort Dam and the location of the main basin sampling station (marked as X).

(NIWR 1985), although an oxycline at 4 m depth immediately prior to overturn has been recorded (Robarts *et al.* 1982). Generally the hypolimnion contains up to 40% of the lake volume (Scott *et al.* 1980). Seasonality in bacterial number and their metabolic activity in Hartbeespoort Dam has been reported by Robarts and Sephton (1984, 1987) and Robarts (1987). In addition to numerous publications on the bacterial, algal and fish populations of Hartbeespoort Dam, a comprehensive report on the limnology of this reservoir has also been published (NIWR 1985).

Zooplankton species composition and abundance at six sampling sites in Hartbeespoort Dam was previously studied between October 1972 and December 1974 by Seaman (1977). He suggested that the majority of the seston in Hartbeespoort Dam, primarily large *Microcystis* colonies, is likely to be too large to be utilized by filter-feeding zooplankton. This aspect of cyanophyte colony size limitation to grazing has received much attention in my study on zooplankton feeding ecology in this hypertrophic system.

Seaman reported a strong similarity in the seasonal population dynamics recorded between his six sampling points (spatial and temporal homogeneity); an exception noted was one station situated near the main lake inflow from the Crocodile River which had a generally lower biomass, and during periods of turbid inflow had higher population densities of *Moina micrura* and lower densities of *Daphnia* spp. (Seaman 1977).

Feeding studies were not carried out by Seaman, but positive or negative associations were evident between the occurrence and abundance of the main zooplankton species and the phytoplankton composition noted. Comparison of seasonal data on the percentage composition of cyanophytes, chlorophytes and diatoms during Seaman's study period (October 1972 to May 1973, Figure 42 in Seaman 1977) with data recorded during this study period (August 1981 to December 1986, NIWR 1985, T. Zohary unpublished data, and Figure 2.2) show very similar patterns indicating that events in the seasonal succession of phytoplankton species do not differ between these two study periods.

1.3 Zooplankton feeding studies: an overview of techniques

There have been many methods used to examine zooplankton feeding ecology in the laboratory or *in situ*, employing a variety of techniques or combinations of techniques. These methods fall largely into five categories:

(i) visual examination of gut contents (eg. Burns 1968, 1969a, Gliwicz 1969a, Wilson 1973, Horn 1981);

(ii) records of zooplankton growth and development under varying dietary conditions (eg. Cushing 1959, De Bernardi *et al.* 1979);

(iii) quantitative relationships between changes in the numbers of food and consumer organisms by measurement of decreases in food concentration by zooplankton activity (eg. Gauld 1951, Gliwicz 1968, Porter 1972, 1973, Hayward and Gallup 1976, Kersting and van der Leeuw 1976, Harbiston and McAlister 1980);

(iv) ingestion rates of radioisotopically labelled foods (eg. Nauwerck 1959, Marshall and Orr 1952, 1955, Monakov and Sorokin 1961, Rigler 1961, Schindler 1968, Haney 1971, 1973, and many others); and

(v) by direct observation of limb beat and mandible movement (eg. McMahon and Rigler 1963, Starkweather 1978, Watts and Young 1980) or more recently by using high speed cinematographic techniques (eg. Rosenberg 1980, Koel and Strickler 1981, Price *et al.* 1983, Scavia *et al.* 1984).

Early attempts to estimate grazing activity were based on comparisons made following repeated phytoplankton sampling over a known period. Losses of algal cells assumed to be due to zooplankton grazing could be estimated over time in the natural phytoplankton population if cell division rates, death and sedimentation rates of the algal species were known (Uhlman 1971). Enright (1969) used differences in diatom

concentration as an index of grazing and explained diel variations in diatom number as being due to zooplankton grazing. Cushing (1976) using the data of Nauwerck (1963) attributed algal population fluctuations and seasonal changes in species composition over time as being largely due to zooplankton grazing, but Reynolds *et al.* (1982) showed that generalizations about algal mortality and grazing loss without taking into account differences between species does not support such assumptions.

Observations on changes in phytoplankton densities have also been carried out over shorter time periods using containers and enclosures with or without zooplankton (Gauld 1951 and more recently in various biomanipulation studies). An example of this technique was provided by Porter (1972) using natural phytoplankton populations isolated and incubated for four days *in situ* in 0.5 m^3 polythene bags. These bags either contained the natural plankton community, increased numbers of grazers, or water depleted of grazers by filtration. Porter's results, although not providing grazing rates, demonstrated that marked changes in the composition of phytoplankton can occur following variable zooplankton grazing activity. Densities of many small or nanoplankton algal species were reduced by grazing whereas the larger desmids, dinoflagellates, chrysophytes and cyanophyte colonies were unaffected (Porter 1972, 1973). Large gelatinous chlorophytes increased in number with high grazing activity. This was attributed to their indigestibility due to the sheath protecting the cells, the possible uptake of nutrients during gut passage, and their resultant competitive advantage over other algae (Porter 1973, 1975, 1976). These studies highlighted the complexity of zooplankton/phytoplankton interrelationships involving both direct and indirect effects.

Direct examination of zooplankton gut contents has frequently been carried out to determine zooplankton feeding habits. This method also does not permit a quantitative measure of grazing rate due to the rapid breakdown of some algae, but does allow analysis of qualitative feeding habits and food

selection. Gliwicz (1969a) carried out an extensive study of zooplankton feeding by analysis of gut contents for zooplankton in lakes ranging from oligotrophic to eutrophic. Horn (1981) used detailed gut content analysis in conjunction with laboratory experiments to quantify phytoplankton losses due to grazing in a reservoir. Gut content analysis techniques using fluorescence microscopy (Gerber and Marshall 1974) and fluorescence methods using a fluorimeter for detecting chlorophyll and phaeophytins (Mackas and Bohrer 1976, Dagg 1983) have provided data on *in situ* grazing, but again conversion of these measurements to absolute ingestion rates is difficult.

Zooplankton feeding rates have also been measured using the electronic particle counter (Coulter counter) which can be used not only to record the number of cells in a suspension but also their size range (volume, μm^3). Kersting and Holterman (1973) using the Coulter counter concluded that *Daphnia magna* feeds non-selectively over a particle size range of 2-165 μm^3. Kersting and van der Leeuw (1976) examined the effects of cell concentration and temperature on *Daphnia magna* feeding rates and confirmed the earlier results of McMahon and Rigler (1965) and McMahon (1965) who used radiolabelling techniques. Riger (1971), Bogdan and McNaught (1975) and Kersting (1978) warned, however, that a major error in feeding experiments using the Coulter counter is the problem of contamination by faecal particles which may be easily resuspended and reconsumed. This can lead to an underestimation of feeding rates. Another potential problem with this method is that algal growth rate in the control chamber without zooplankton may be adversely affected since excretory products from zooplankton are not available to the algae (Rigler 1971). However, use of the Coulter counter does provide valuable data on food particle size selection by zooplankton (Kersting 1978, Harbiston and McAlister 1980). Richman *et al.* (1977) established the size range of particles and the optimum particle size ingested by three *Diaptomus* species and *Eurytemora affinis*. Nival and Nival (1976) used the Coulter counter to determine the size spectrum filtration efficiency of *Acartia clausi* and obtained a good fit of observed results to expected

data obtained from measurements of the maxillae setae and setule mesh dimensions.

A variety of methods have been used to measure *in situ* feeding. The fluorescence technique of Mackas and Bohrer (1976) who examined the gut contents of freshly collected copepods, and the experiments of Porter (1972, 1973) on plankton contained in polythene bags are examples of *in situ* feeding studies. Other workers have used *in situ* feeding chambers specially designed to trap, isolate and treat the natural zooplankton community over a controlled time at a desired depth. Gliwicz (1968) carried out *in situ* grazing experiments using two 3 ℓ capacity chambers. One chamber acted as a control containing actively grazing zooplankton whilst into the other chamber an anaesthetizing drug was introduced which rapidly inactivated the zooplankton. After a period of 4 h the difference between the number of food particles in the control and experimental chambers provided a quantitative measure of zooplankton grazing rate. This method also provided information on the proportions of the food available (algal type and particle size) that is consumed by the natural zooplankton community (Gliwicz 1969a, 1969b). However, due to the long experimental duration of 4 h, this method is also not free from error due to resuspension of faecal material.

Radioisotopes have been widely used in the study of zooplankton feeding in both laboratory studies and *in situ* experiments, and allow the use of very reliable techniques for direct measurement of grazing and assimilation rates (Sorokin 1968, Rigler 1971, Krylov 1980). A major part of the literature on zooplankton nutrition is based on radiolabelling techniques, especially in recent years.

Radiolabelling methods were first developed in the early 1950's. Marshall and Orr (1952, 1955) investigated feeding and assimilation in the marine copepod *Calanus finmarchicus* using ^{32}P labelled algae, measuring isotope uptake and incorporation into eggs and faeces. Malovitskaya and Sorokin (1961) and Monakov and Sorokin (1961) used ^{14}C to label algae

in simple experiments that determined isotope removal and hence cell removal after zooplankton were fed on labelled food of a known specific activity and cell concentration. This method was essentially very similar to the cell count method using the Coulter counter. Error due to resuspension of labelled faeces remains a problem, while in addition, estimates of respiratory ^{14}C loss by algae and zooplankton are required.

Nauwerck (1959) used algae labelled with ^{14}C in feeding experiments of a duration shorter than the gut passage time of food in the zooplankton species studied. This method eliminated errors not only due to defaecation, but also of excretion of isotope not yet fully assimilated by the end of the experiment.

By the end of the 1960's experimental procedures using radioisotopes provided data on ingestion, assimilation, excretion and respiration (Rigler 1961, Sorokin 1966, 1968), food size selection and effects of food concentration (McMahon and Rigler 1965, Richman 1966, Burns and Rigler 1967, Schindler 1968), and the influence of temperature on filtering rates (Burns and Rigler 1967, Schindler 1968).

All of these studies were carried out in the laboratory under controlled conditions on species in isolation fed on cultured foods. Caution must be exercised in extrapolating the results of these experiments to natural communities made up of a variety of species and subjected to many varying external influences normally eliminated under laboratory conditions. Furthermore, containment, handling effects due to the transfer of animals between food media and acclimation are all factors that can influence measurements in the laboratory. Such disturbances to feeding behaviour may be very important in experiments with a short duration, usually <10 min (Krylov 1980).

Haney (1971) developed and tested a technique for measurement of zooplankton grazing rates *in situ*, thereby avoiding problems that arise when attempting to extrapolate results of

laboratory studies to feeding in the field under natural conditions. Haney used a feeding chamber similar to that of Gliwicz (1968), but released radiolabelled food instead of an anaesthetic. Zooplankton fed in this medium for ~5 minutes, a period less than their gut passage time. Using this *in situ* technique Haney was able to measure the community gut passage time (10-12 min), community filtration rates and, after sorting and separation of organisms in the samples, individual species gut passage times and filtration rates could also be determined. Further studies (Haney 1973, Haney and Hall 1975) demonstrated the versatility and sensitivity of this *in situ* radiolabelling technique.

Subsequently very many zooplankton feeding studies have used variations and modifications to the Gliwicz-Haney technique. Extensive *in situ* studies have used labelled natural lake seston (eg. Gulati *et al.* 1982, Persson 1985a, 1985b) or natural bacteria (Forsyth and James 1984). Other studies have highlighted and examined problems and contributed to the reduction of errors due to isotope loss from samples following preservation (Holtby and Knoechel 1981, Persson 1982), problems associated with lack of homogeneity of label uptake (Lampert 1977a, Gulati *et al.* 1982, Lampert and Taylor 1985), or have examined containment or 'bottle effects', handling and acclimation effects and made comparisons between true *in situ* measurements and other less direct techniques (Roman and Rublee 1980, Forsyth and James 1984, Chow-Fraser 1986).

Further mention and discussion of the recent extensive literature relating to zooplankton feeding is restricted to other sections of the study reported here.

2. ZOOPLANKTON POPULATIONS AND SEASONAL SUCCESSION IN HARTBEESPOORT DAM

2.1 Introduction

Phytoplankton succession or periodicity (Reynolds 1984) generally involves shifts from small celled chlorophyte and cryptophyte forms in spring to larger or colonial, sheathed cyanophytes in summer. Shifts in zooplankton from large - to small-bodied types which occur both seasonally and with increasing trophic status of waterbodies have been noted and studied by many aquatic ecologists (Porter 1976, Gliwicz 1977). Recently, some attention has focused upon commonly recurring events within lacustrine planktonic succession, the timing of which is annually predictable (Geller 1980, Sommer *et al.* 1986).

Of the many recognisable events occurring throughout the annual cycle of plankton succession (Sommer *et al.* 1986), the spring clear-water phase (Lampert 1978, Lampert and Schober 1978) with its associated high zooplankton grazing rates (Gulati *et al.* 1982, Lampert *et al.* 1986) is frequently pronounced and conspicuous. Following this event there often occurs a mid-summer decline in large-cladoceran populations (Gliwicz 1985, Larsson *et al.* 1985, Threlkeld 1985), and a shift to a largely 'inedible' algal or cyanophyte phytoplankton community in summer in response to nutrient recycling, changing N:P concentrations, temperature and zooplankton grazing pressure amongst other factors (Porter 1973, 1976, 1977, Reynolds *et al.* 1982, Thompson *et al.* 1982, Schoenberg and Carlson 1984). Consequently the summer zooplankton community in many eutrophic and mesotrophic lakes is frequently composed of smaller-bodied species. Community biomass at this time is often low and may be limited by a combination of fish predation (Brooks and Dodson 1965, Gliwicz 1985), interference to feeding mechanisms by inedible algae and cyanophyte colonies (Webster and Peters 1978, Gliwicz and Siedlar 1980, Porter and Orcutt 1980, Porter and McDonough 1984), and food limitation, particularly in hypertrophic lakes

where the zooplankton may depend on decomposing cyanophytes as a supplementary food resource (Gliwicz 1969a, Hillbricht-Ilkowska *et al.* 1972, Hanazato and Yasuno 1987).

These and many other commonly occurring events and processes in the seasonal succession of plankton communities in lakes studied by the Plankton Ecology Group (PEG) have been summarised into a descriptive model by Sommer *et al.* (1986). This PEG-model consists of 24 sequential statements based on events occurring in many well-studied lakes of varying trophy. Hypertrophic Hartbeespoort Dam lies as an extreme point on the spectrum of lake trophic type and is subtropical, a geographical region poorly represented in the PEG-model.

An aspect clearly highlighted in the successional PEG-model (Sommer *et al.* 1986) was the importance of zooplankton/phytoplankton interactions. Many of the commonly occurring processes described were governed by zooplankton grazing pressure on the changing phytoplankton resource and, via feedbacks, the resources available to the zooplankton were identified as important in driving zooplankton succession, particularly in the more eutrophic lakes. Sommer *et al.* (1986) challenged the philosophy that the phytoplankton is a physically controlled community. Their analysis showed that biological processes such as resource limitation, grazing, competition and predation determine the successional progress with physical factors superimposing 'random noise upon the process of autogenic succession'.

This chapter describes the population fluctuations of the major zooplankton species in Hartbeespoort Dam. Key successional events are outlined and the interrelations between the zooplankton community and the food resources available in this hypertrophic impoundment provide a background against which the subsequent in-depth feeding studies can be viewed.

2.2 Methods

Samples were collected weekly from January 1981 to December

1986 using a vertically hauled nylon plankton net (60 µm mesh; mouth aperture 0.1 m^2) at a fixed station (Figure 1.0) in the main basin of the lake (maximum depth 32.5 m at full supply level). One 4% sugar-formalin preserved sample (Haney and Hall 1972) was subsampled following the method of Allanson and Kerrich (1961) and the species present enumerated in a grid-marked counting dish under a scanning, low power binocular microscope (Zeiss). A minimum of 200 organisms were usually counted in each subsample.

Three unpreserved samples were collected for dry biomass determination. Most of the phytoplankton component, consisting predominantly of buoyant *Microcystis* colonies was removed soon after death and sedimentation of zooplankton and natural flotation of cyanophyte colonies in closed jars kept at ~4 °C. Low speed centrifugation of samples in tapering glass tubes allowed further separation and removal of the less dense phytoplankton component from the zooplankton samples. Samples were dried in an oven for 48 h at 50 °C and allowed to cool in a desiccator before weighing. Total dry weight of zooplankton was measured to 0.1 mg using a Mettler balance. The mean biomass of the three samples was then calculated.

In addition to vertical hauls using a plankton net, discrete samples were taken at fixed depths at the same sampling point during the course of *in situ* feeding rate measurement (see Section 3.2 for further details and description of sampling device). Zooplankton samples were collected every two weeks from January 1983 to March 1985 using a Gliwicz-Haney style grazing chamber of 3 ℓ capacity. Samples were taken at 0.5, 2, 4, 6, 8, 10, 15 and 20 m below the lake surface during winter (the full supply depth of 32 m at the sampling point did not occur throughout this study period). Following lake stratification, discrete sampling was not carried out below the oxycline. Physical, chemical and other biological parameters were measured weekly or fortnightly at the same station by other members of the NIWR during the course of the Hartbeespoort Dam Ecosystem Study. After zooplankton species identification and enumeration the community biomass was determined for each sample as described above.

All data files collated were analyzed using either the CSIR Cyber 750 mainframe or personal computer systems. The SPSS analytical package (Nie *et al.* 1975) was employed on the Cyber 750 computer and the Statgraphics package (Version 2.1, STSC Inc.) was used with IBM compatable personal computers (Olivetti M21 or M24).

2.3 Results

Figure 2.0a shows 3-point moving average fluctuations in the total zooplankton community biomass over 6 years which reveal a repeated seasonal pattern. Annually the highest community biomass was recorded during the spring-early summer period from August to late November. As water temperatures continued to rise into mid-summer (Figure 2.1), the community biomass characteristically dropped sharply in December of each year. Thereafter during the mid to late summer (January - March) the community biomass was typically lowest.

With the autumn decline in water temperature during April and May, community biomass again rose but was usually low during the coldest period (June - July). This bimodality in total zooplankton biomass is particularly evident in a rigorously smoothed plot (9-point moving average, Figure 2.0b). The community biomass maxima in spring (marked as S) are generally high and may persist for 3 to 4 months. Both summer and mid-winter minima are evident. The second rise in community biomass in autumn (marked as A) is usually a minor event. Exceptions to this pattern occurred in the winters of 1982 and 1985 when no mid-winter biomass minimum was evident.

Seasonal population fluctuations of the major crustacean zooplankton species that contributed to the total community biomass are shown in Figure 2.1. These crustaceans were sufficiently numerous in Hartbeespoort Dam also to allow examination of aspects of species-specific feeding behaviour (see Section 4).

The cladocerans *Daphnia pulex* and *D. longispina*, due to their

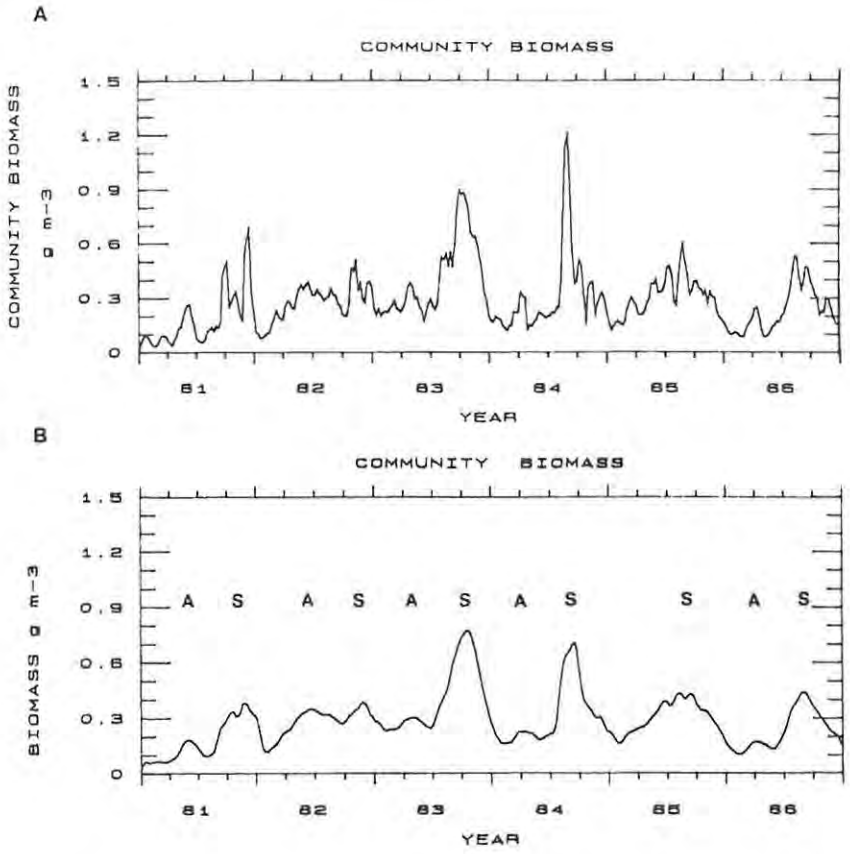


Figure 2.0: Zooplankton community biomass in Hartbeespoort Dam over 6 years.
A. Data smoothed by moving average of 3 points;
B. Rigorous smoothing (n = 9) to highlight basic seasonal patterns.
S = spring biomass peak;
A = autumn biomass peak.

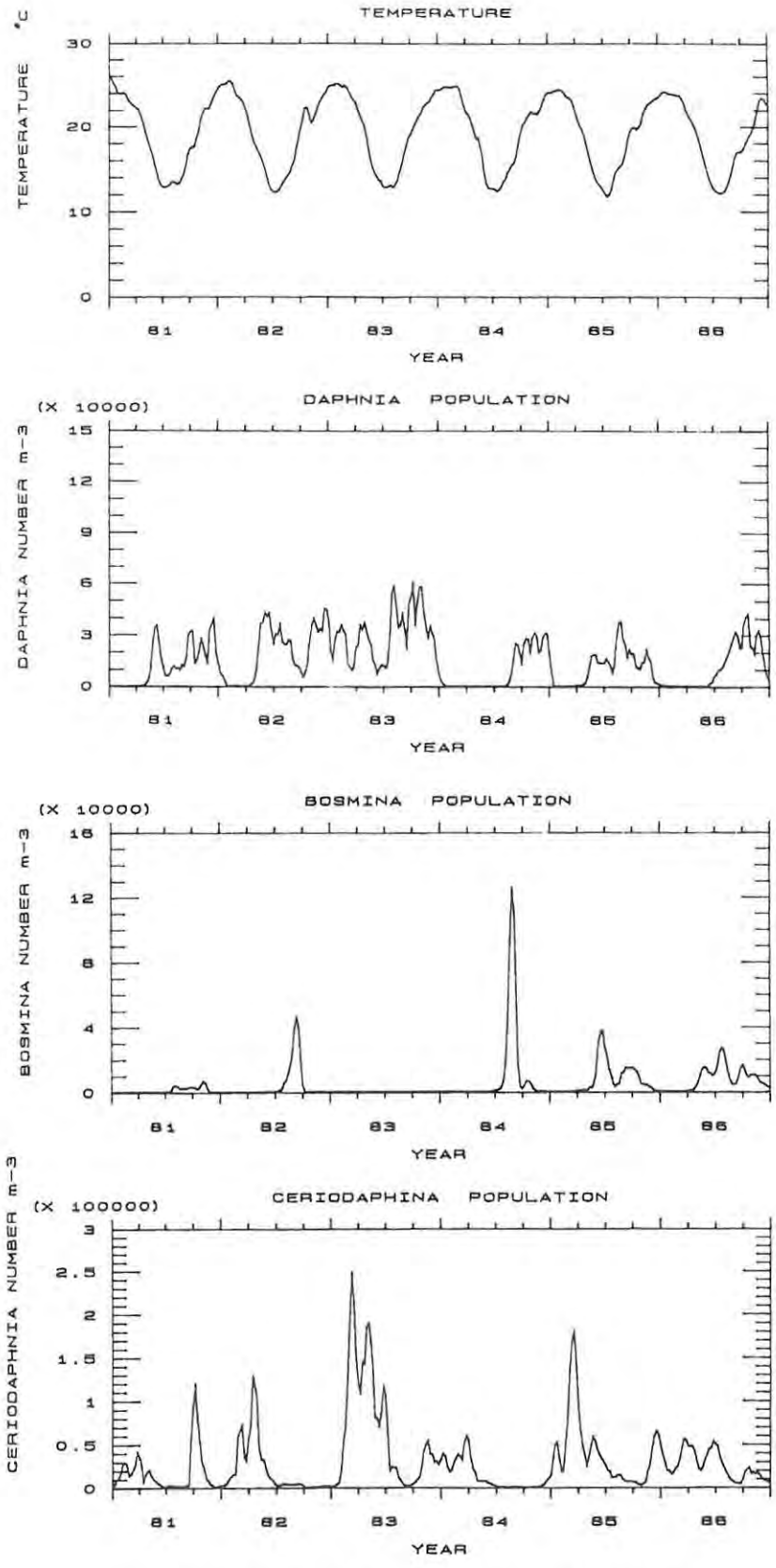


Figure 2.1: Seasonal fluctuations in water temperature and numbers of the seven major zooplankton species common over 6 years in Hartbeespoort Dam. Data smoothed (n = 3).

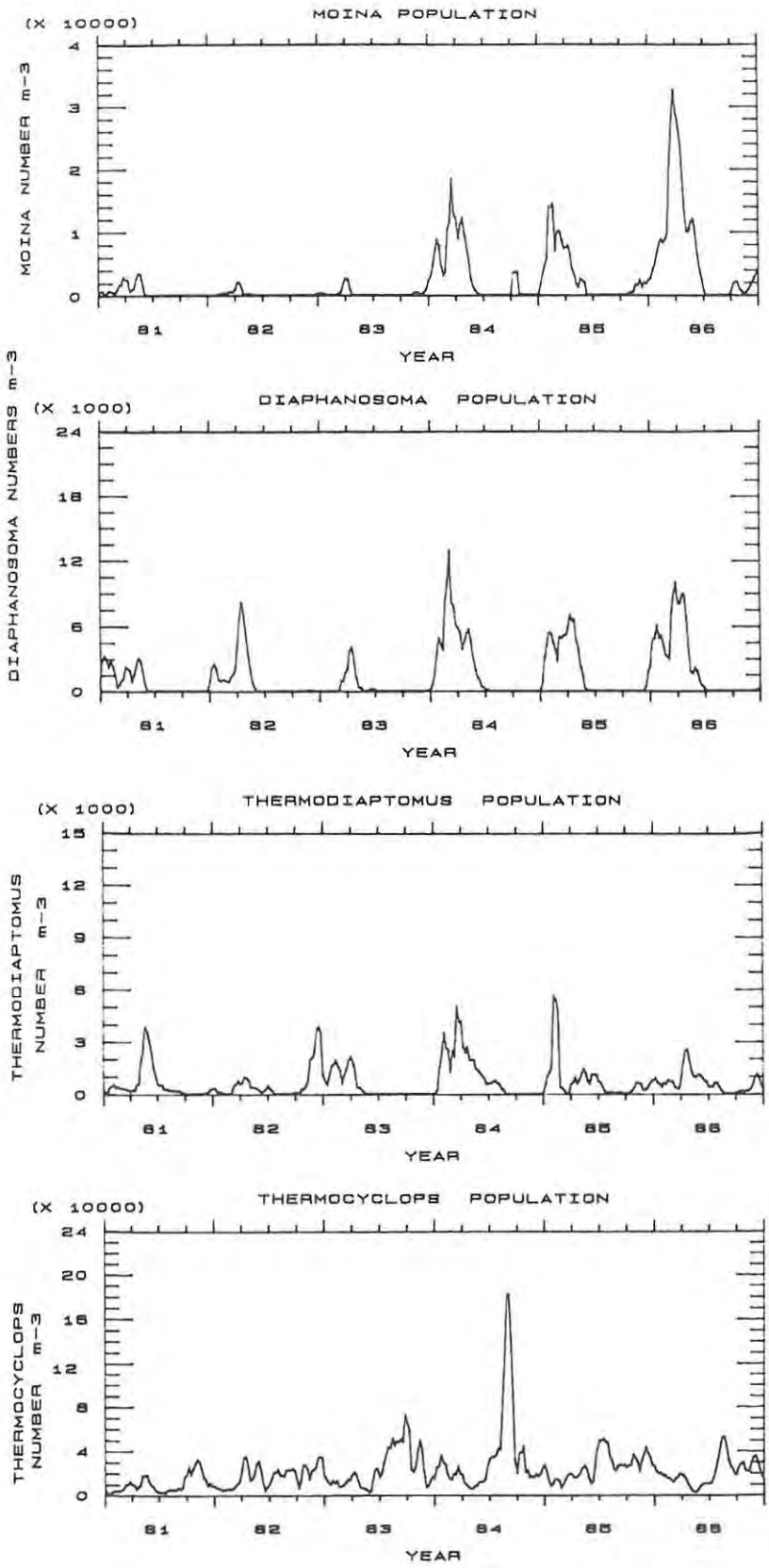


Figure 2.1: (continued).

large adult size (up to 2.25 mm) and abundance in winter and spring, were the herbivores that contributed most to the main peaks in community biomass (Figure 2.0) and to periods of maximum community grazing rates (Section 3). Correlation of *Daphnia* numbers with community biomass was significant (Pearson correlation coefficient: $r = 0.606$, $n = 286$, $p<0.001$) with *Daphnia* number accounting for 37% of variance in community biomass. Fluctuations in community biomass were also significantly influenced by the generally common and frequently abundant cyclopoid copepod *Thermocyclops oblongatus*, population densities of which also explained 37% of the variance in community biomass ($r = 0.606$, $n = 286$, $p<0.001$).

The *Daphnia* population characteristically reached its maximum densities in spring. Thereafter a rapid mid-summer decline in the *Daphnia* population occurred annually during December - January, and *Daphnia* was typically absent during the late summer and early autumn when the smaller cladoceran *Ceriodaphnia reticulata* was usually the most abundant zooplankter. An exception to this pattern occurred in the summer of 1982-83 when the *Daphnia* population did not decline, but persisted with erratic peaks throughout the summer period.

Species succession was evident within the genus *Daphnia*. Separate enumeration of *D. pulex* and *D. longispina* was not carried out routinely. Due to their very close morphological similarities, dissection of adults for examination of spines or combs on the abdominal claw is necessary to guarantee accurate separation of *Daphnia* into these species. Periodic examination of adult *Daphnia* showed that *D. longispina* led the annual cool season increase and was succeeded approximately 1 - 2 months later by *D. pulex*. *D. longispina* did not contribute to the annual late-spring *Daphnia* peak. Following the persistence of *D. pulex* through the summer of 1982-83 *D. longispina* was not recorded during the autumn and winter of 1983.

Bosmina longirostris was usually associated with *Daphnia* during early spring. From 1984-86 *Bosmina* reached its brief

population maxima before that of the *Daphnia* population. During the first three years of the study, however, *Bosmina* densities were either lower or it was present over a shorter period when the first rise in the *Daphnia* population reached very high densities. Of particular note was the absence of *Bosmina* during the spring of 1983 following the persistence of *Daphnia pulex* through the previous summer and autumn in unusually high numbers.

The mid-summer to autumn herbivore community in Hartbeespoort Dam was composed mainly of the small bodied cladocerans *Ceriodaphnia reticulata, Moina micrura* and *Diaphanosoma excisum*. Of these genera the *Ceriodaphnia* population dominated numerically and was present over a longer period (usually December to July-August). *Ceriodaphnia* briefly co-existed with *Daphnia* during both the decline in *Daphnia* numbers and early during the re-establishment of the *Daphnia* population, but was usually absent or only sparsely represented during the spring *Daphnia* population maxima.

Moina and *Diaphanosoma* were generally both present only between late December and June when the *Daphnia* population was waning, absent or re-developing. Both *Moina* and *Diaphanosoma* population densities were negatively correlated with *Daphnia* abundance ($r = -0.295$, $n = 286$, $p<0.001$; $r = -0.391$, $n = 286$, $p<0.001$ respectively). Population densities of *Moina* and *Diaphanosoma* were significantly associated during their annual period of co-existence ($r = 0.657$, $n = 286$, $p<0.001$). The numbers of *Moina* recorded during the first three years of the study period were low and both *Moina* and *Diaphanosoma* population densities were low during 1983 when unusually high *Daphnia* population densities were recorded throughout the late summer.

Copepod species diversity was low in this hypertrophic impoundment. Only the calanoid *Thermodiaptomus syngenes* and cyclopoid *Thermocyclops oblongatus* were commonly recorded. Seasonality within the copepod populations were not as clearly marked as that noted for the cladoceran species. *Thermodiaptomus* was

generally most abundant from mid-summer to late autumn. *Thermocyclops*, however, exhibited erratic population fluctuations throughout the study. Population maxima of *Thermocyclops* from 1984-86 almost coincided with the highest peaks in the *Bosmina* population (Figure 2.1). Correlation of *Thermocyclops* and *Bosmina* numbers during periods of their coexistence revealed a significant positive association ($r = 0.736$, $n = 133$, $p<0.001$). This suggested that a predator-prey interrelationship may exist between this raptorial omnivorous or carnivorous copepod and the small cladoceran. Further correlation between the numbers of these species when present together showed that, by introduction of a one week lag in the *Bosmina* database, the positive association with *Thermocyclops* improved slightly ($r = 0.789$, $p<0.001$).

Planktonic rotifer species were also frequently present in Hartbeespoort Dam throughout this study, but with the exception of *Brachionus calyciflorus* the small rotifer species were not sufficiently numerous during feeding experiments carried out on individual zooplankton species (Section 4) to enable aspects of their feeding biology to be examined separately. Whilst occasional high population maxima of rotifer species were recorded, their contribution to total community biomass was slight. The principle rotifer species recorded were *Brachionus calyciflorus*, *Keratella cochlearis*, *Polyarthra vulgaris*, *Hexarthra mira*, *Filinia pejleri* (deep in the water column) and on rare occasions *Lecane luna* and the predaceous *Asplanchna brightwelli*.

Neither species-specific nor community biomass levels were significantly correlated with epilimnetic water temperature. On the other hand, population changes within the zooplankton community accompanied successional changes in the phytoplankton resources available. Data were examined on the phytoplankton species composition and chlorophyll *a* concentrations in Hartbeespoort Dam measured simultaneously throughout the study period (Robarts and Zohary 1984, NIWR 1985, T. Zohary unpublished data).

The regular seasonal succession within the phytoplankton community described by Robarts and Zohary (1984) and NIWR (1985) occurred annually from 1981 to 1986. Typically phytoplankton biovolume was dominated for a minimum of eight months by *Microcystis aeruginosa* a characteristic of which, peculiar to Hartbeespoort Dam, being its ability to persist in high densities throughout the winter period of low growth and primary productivity whereas, in other eutrophic lakes, *Microcystis* usually fails to maintain its dominance through the winter (T. Zohary pers. comm.). A shift in phytoplankton species composition to brief dominance by a number of chlorophyte and cryptophyte species occurs annually in August. This chlorophyte-cryptophyte phase persists until mid-November during which time water transparency or Secchi depth is frequently greatest (NIWR 1985). This late-spring clear-water phase is analogous to the marked spring event frequently recorded in northern temperate lakes (Lampert 1985, Lampert *et al.* 1986, Sommer *et al.* 1986). Thereafter, by mid-summer, *Microcystis* again dominates the phytoplankton and reaches a maximum of over 90% of the phytoplankton biovolume from February to May. In addition to this marked annual shift between dominance by *Microcystis* or by chlorophytes and cryptophytes, the filamentous diatom *Melosira granulata* may be briefly abundant in mid-winter when *Microcystis* densities begin to decline and the lake is vertically mixed throughout the water column. *Melosira* may again briefly increase during the decline of the chlorophyte-cryptophyte phase in November (Robarts and Zohary 1984, NIWR 1985, T. Zohary unpublished data).

Fluctuations in the concentrations of the phytoplankton resource available to the zooplankton, as shown by integrated chlorophyll *a* concentrations over the upper 4 m of the water column, do not reveal a clear seasonal pattern (Figure 2.2). Extreme variability in chlorophyll *a* concentrations measured in Hartbeespoort Dam occur principally due to both the diel and seasonal effects of wind which strongly influences the distribution of buoyant surface accumulations of *Microcystis* over the upper water column of the lake (T. Zohary pers. comm.). Thus seasonal patterns in chlorophyll *a* concentra-

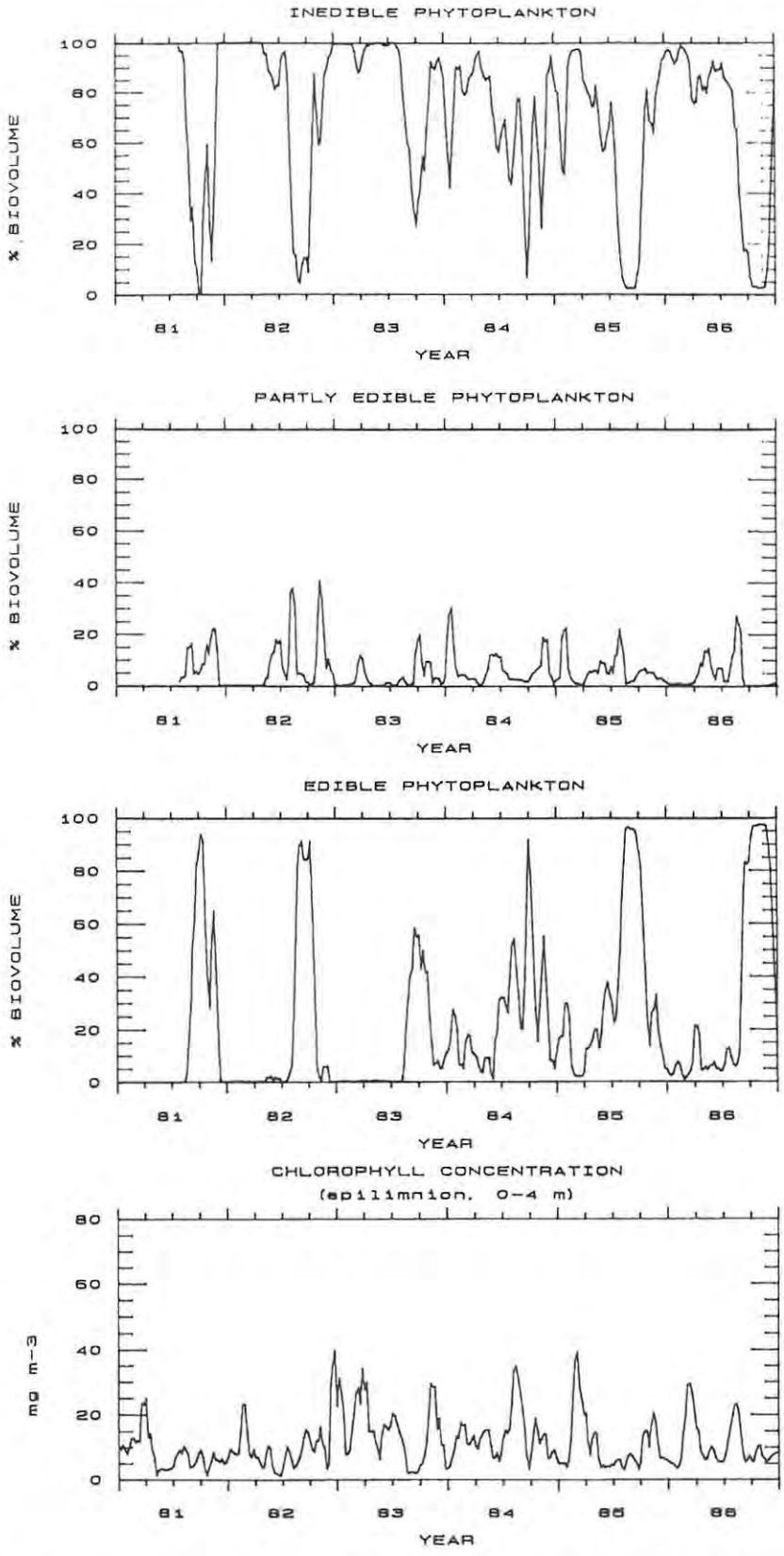


Figure 2.2: Seasonal composition of phytoplankton (% biovolume) in Hartbeespoort Dam after subdivision into categories based on their availability to zooplankton grazers (see details in text), and seasonal chlorophyll *a* concentrations (epilimnetic chlorophyll *a*, 0-4 m, sampled by hosepipe). Data smoothed (n = 3)

tions are easily masked by extreme weekly variations. Coupled with the presence of the majority of chlorophyll *a* contained in large buoyant *Microcystis* colonies over most of the year, chlorophyll *a* was therefore not regarded as as a good indicator of food resource level available to the zooplankton. Consequently, in hypertrophic Hartbeespoort Dam, phytoplankton food quality (species, cell and colony type and size) rather than food quantity (chlorophyll *a*) was examined as a potentially better indicator of resource availability to the zooplankton community.

On the basis of particle size limitations and feeding preferences reported in the literature (summarized by Morgan 1980 and Thompson *et al.* 1982), and based on the results reported in Sections 3 and 4, the availability of the phytoplankton resource to filter-feeding zooplankton in Hartbeespoort Dam changes markedly with the pronounced shifts in phytoplankton species during seasonal succession. Therefore the phytoplankton present, classified according to species, cell or colony type and size, were categorized as being either largely inedible, partially edible or edible. The 'largely inedible' category was composed entirely of *Microcystis*, which in Hartbeespoort Dam is predominantly in a large colony form (Robarts and Zohary 1984) larger than the upper colony size limit to ingestion by zooplankton (Thompson *et al.* 1982, and data presented in Section 4). 'Partially edible' phytoplankton mainly included the long filamentous diatom *Melosira* (Thompson *et al.* 1982), the occasionally sheathed or 'resistant' form of *Oocystis* sp. (Porter 1973, 1977) and the small cyanophyte *Pseudanabaena* sp. Although the cell size (≅4.0 µm x 2.5 µm) and short filament length of *Pseudanabaena* is within a size range readily acceptable to the filter-feeding zooplankton species present, in Hartbeespoort Dam it is almost exclusively present only as an epiphyte on or within the mucilaginous sheath of larger *Microcystis* colonies (W.E. Scott and T. Zohary unpublished data). Consequently *Pseudanabaena* was regarded as being only partly available as a food resource to the zooplankton. The 'edible' phytoplankton category included a number of taxonomic groups, principally the flagellates

Cryptomonas spp and *Chroomonas* spp, the chlorophytes *Coelastrum* sp, *Ankistrodesmus* sp and non-sheathed *Oocystis* sp, and the diatom *Cyclotella* sp amongst other minor contributions from various unicellular taxa.

Figure 2.2 also shows the percentage of the phytoplankton biovolume classified as largely inedible, partially edible and edible as categorized above. The mid- to late-summer phytoplankton dominance by largely inedible *Microcystis* colonies (frequently >95% of biovolume) is clearly evident. Edible chlorophytes and cryptophytes dominate annually during spring and early summer (August to late November). In addition erratic fluctuations occur in the proportion of partly edible phytoplankton due mainly to the often bimodal maxima in *Melosira* abundance, principally occurring in winter and secondarily in early summer (NIWR 1985), and also due to the association between *Pseudanabaena* and *Microcystis* colonies (T. Zohary, pers. comm.).

Comparison of seasonal patterns in zooplankton community biomass and population densities of the major herbivores (Figures 2.0 and 2.1) with phytoplankton resource categories (Figure 2.2) shows positive association between some zooplankton population densities (*Daphnia* and *Bosmina*) and the proportion of edible phytoplankton present. The presence of high population densities of other herbivores (*Cerdiodaphnia*, *Moina*, *Diaphanosoma* and *Thermodiaptomus*) were positively associated with high proportions of the largely inedible (*Microcystis*) phytoplankton resource.

2.4 Discussion

The importance of food quality rather than food quantity or temperature in governing the large scale seasonal fluctuations of zooplankton standing stocks and population densities of the various species in this subtropical impoundment has also been noted in many temperate lakes, particularly with increasing trophy (see Lampert 1985, Sommer *et al.* 1986). Irrespective of the causes of seasonal change in the phytoplankton commun-

ity, (for example whether due to physical and chemical factors or whether food quality decreases in response to increased zooplankton biomass and grazing, or vice versa, or both occur in a cyclical sequence; see various scenarios in Bergquist *et al.* 1985, Gulati *et al.* 1985, Infante and Edmondson 1985, Kerfoot *et al.* 1985) shifts in phytoplankton composition and thus food quality are of paramount importance to the zooplankton community of eutrophic lakes. The strong influence of the phytoplankton resource categories identified in Hartbeespoort Dam on the zooplankton community structure and standing stock suggests that under hypertrophic conditions changing food quality is a primary factor driving zooplankton succession, diminishing the relative impact of direct physical factors or of chlorophyll *a* concentrations *per se* (Figures 2.0 - 2.2).

The predictability of regularly occurring seasonal events in both the phytoplankton and zooplankton successions in Hartbeespoort Dam (Figures 2.1 and 2.2) is further emphasized by the cyclical patterns evident when the weekly data gathered over the six year study period is grouped into twelve monthly data sets (Figures 2.3 and 2.4). This analysis (showing monthly median, interquartile range and full range) highlights the strong seasonality in phytoplankton resources and corresponding positive or negative associations between phytoplankton resource categories and the major herbivorous species.

As indicated in Figure 2.2 the spring chlorophyte - cryptophyte or edible phytoplankton phase usually extends annually from August to late November. Monthly grouping of data show that composition by edible forms is typically highest in September and October. As expected, composition by largely inedible phytoplankton (*Microcystis*) has a converse seasonal pattern. Differences between these main two categories are due to the relatively minor monthly variations in the partly edible category. Clearly the quality and availability (large cyanophyte colonies) of phytoplankton resources is low in late summer, the lowest level occurring in March when not only is *Microcystis* most abundant (NIWR 1985) but also both the edible

and partly edible food categories are simultaneously lowest (Figure 2.3).

A very different seasonal pattern of phytoplankton resource levels in the lake is presented by similar analysis of data on chlorophyll *a* concentrations (Figure 2.3). In late summer chlorophyll *a* concentrations are usually maximal when *Microcystis* is most abundant and accumulates in dense surface blooms or scums (Robarts and Zohary 1984, NIWR 1985). The generally low chlorophyll *a* concentrations of mid-winter are followed by irregular monthly variations. Interestingly during September, when the edible phytoplankton component is high, the chlorophyll *a* concentration is often at its lowest level (Figure 2.3). Consequently each year mean monthly zooplankton community biomass is either negatively correlated with chlorophyll *a* concentration or these parameters are unlinked in this hypertrophic system (Table 2.0). Whilst a lag in the response of zooplankton community biomass to changing chlorophyll *a* concentration could explain such a lack of association between chlorophyll *a* and herbivore populations or community biomass, in Hartbeespoort Dam pronounced fluctuations of these variables are of longer duration, occurring seasonally. In fact, introduction of a lag generally increased the significance of the negative correlation between mean monthly chlorophyll *a* concentration and mean monthly community biomass (Table 2.0) except for 1986 when this association was positive, although not significant. This negative association reflects the inhibitory effect of the abundant *Microcystis* colonies (Section 4), which contribute most to chlorophyll concentrations, on the zooplankton community biomass.

Comparison between monthly data sets of phytoplankton resource levels and zooplankton community biomass or herbivore population densities over six years in Hartbeespoort Dam (Figures 2.3 and 2.4) further support the view that seasonal succession within the predominantly cladoceran herbivore community occurs primarily in response to food quality rather than quantity (chlorophyll *a*). Community biomass is maximal during the

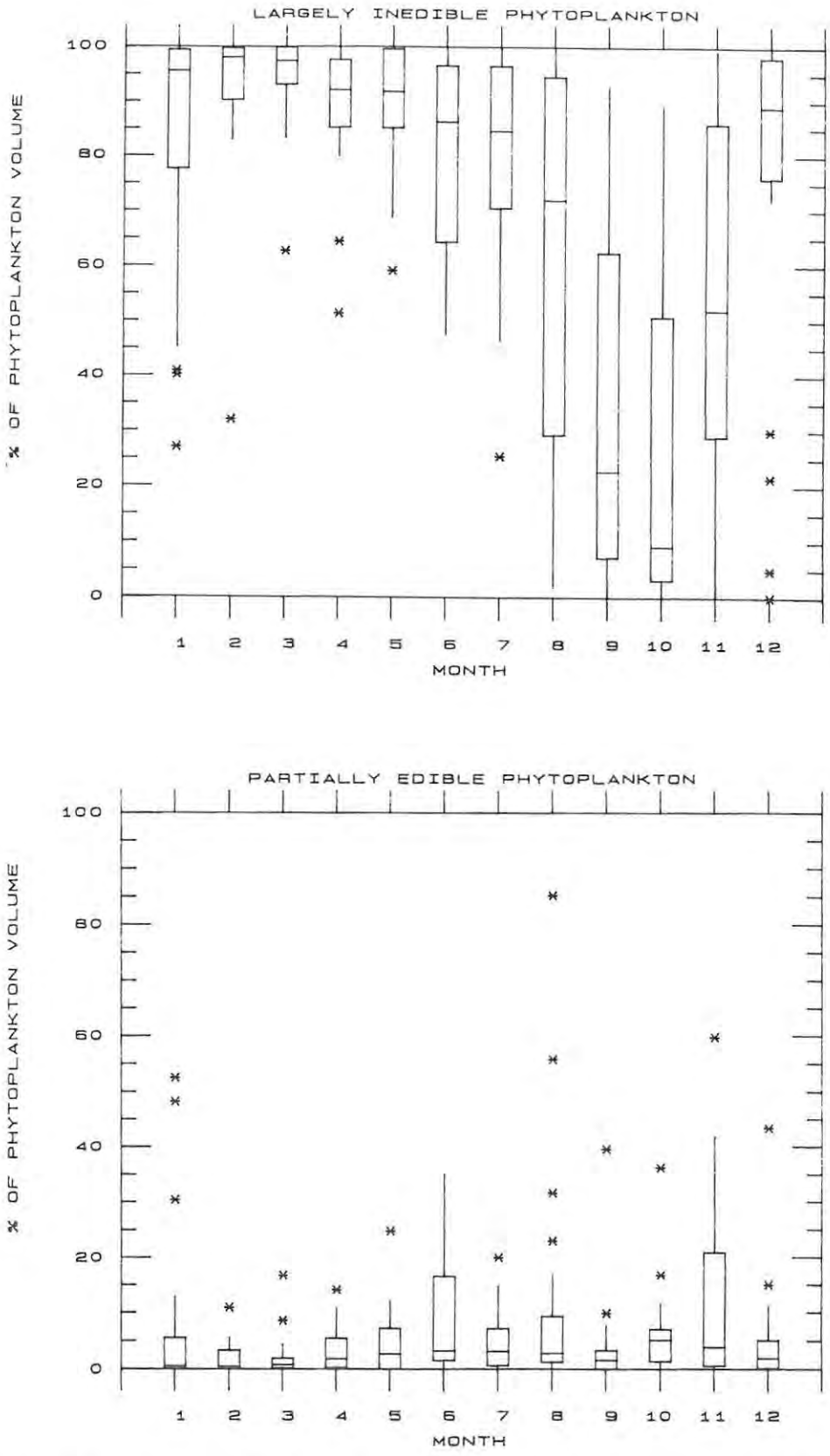
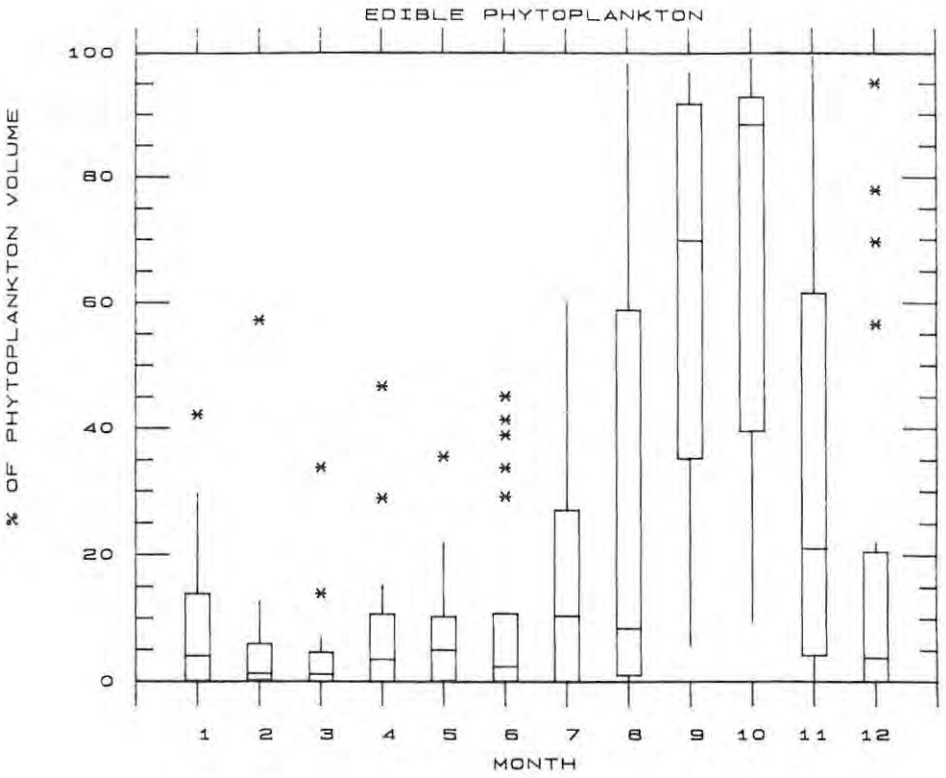
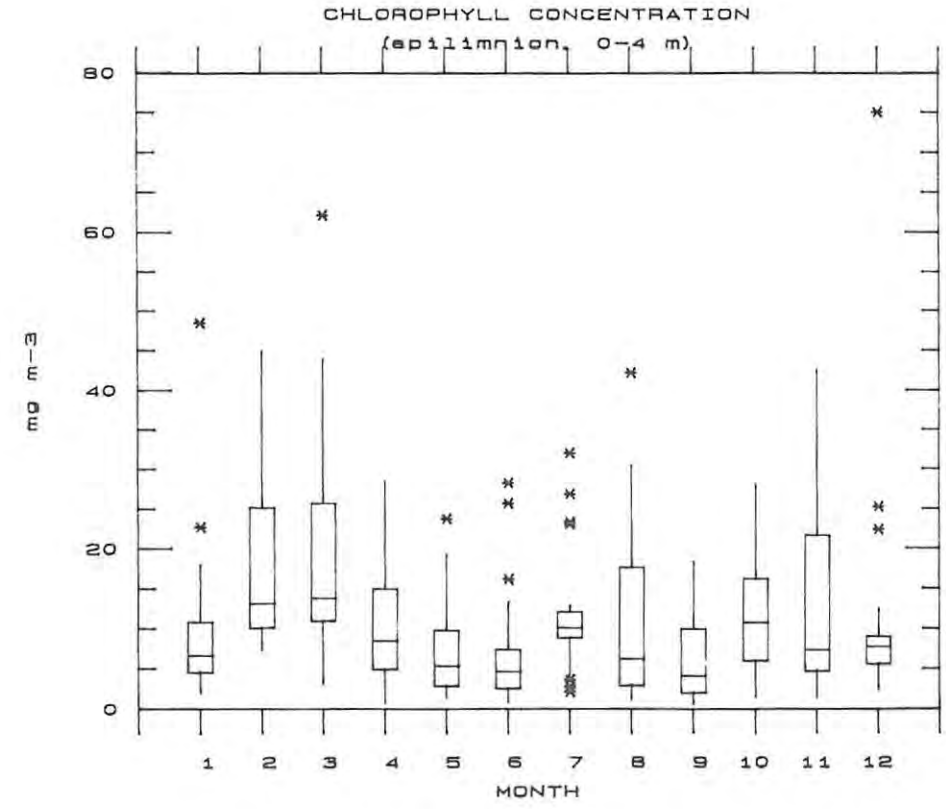


Figure 2.3: Frequency of phytoplankton composition (%) biovolume), after subdivision into resource categories, and chlorophyll *a* concentrations, after grouping of weekly data over 6 years into monthly datasets. Horizontal line = median; box = upper and lower quartile range; vertical line = range; * = points lying beyond 1.5 times the interquartile range.





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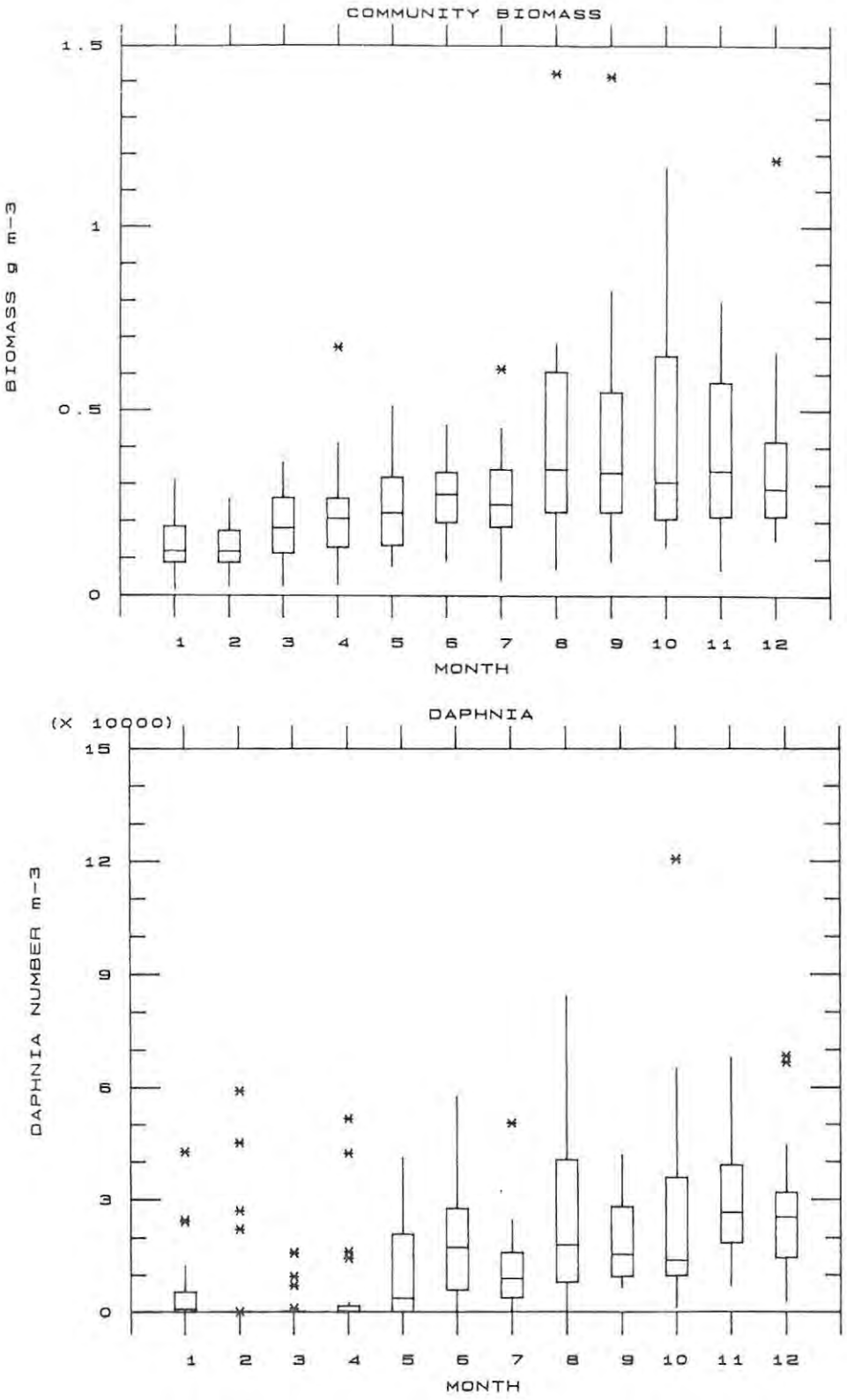
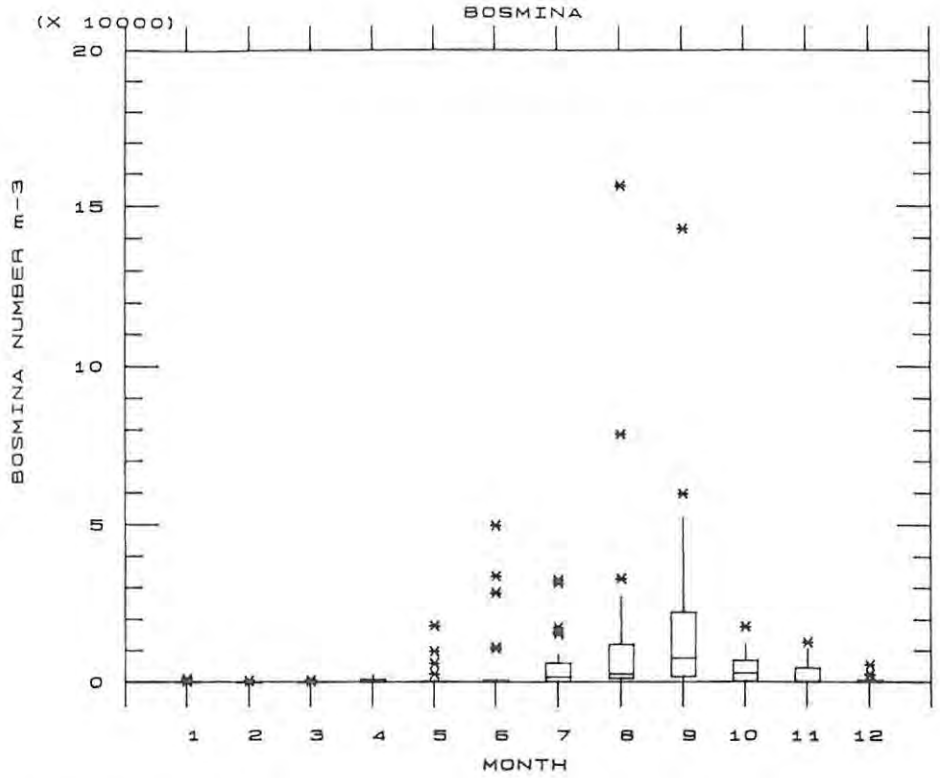
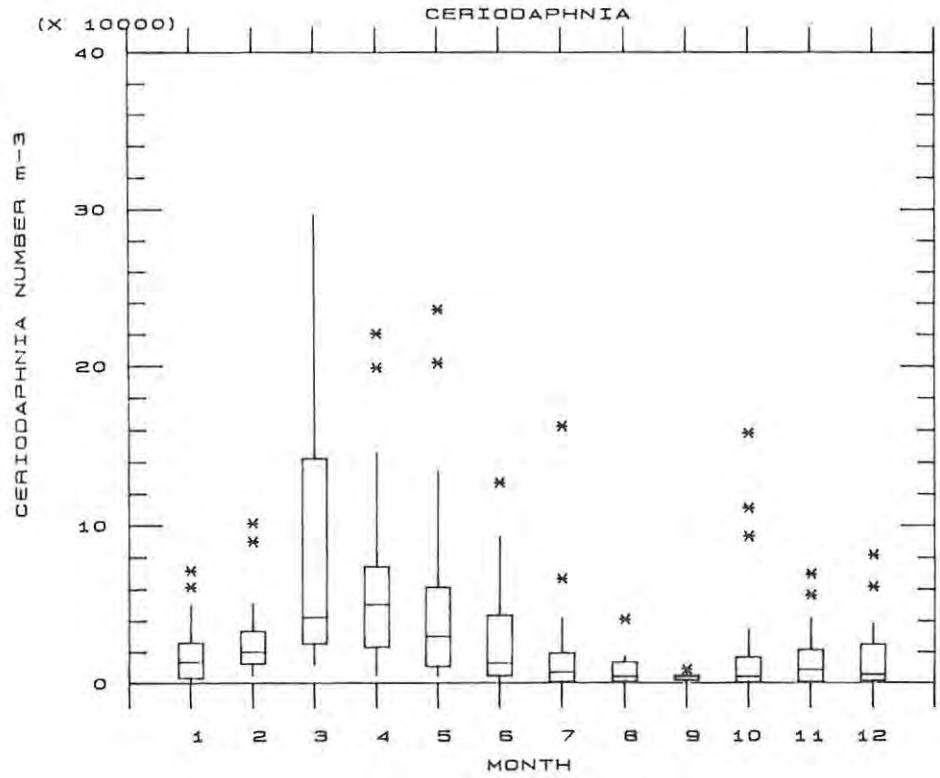


Figure 2.4: Frequency of population densities of the 5 major grazers and of community biomass in Hartbeespoort Dam after grouping of weekly data over six years into monthly datasets, showing median, interquartile range, range and outlying points (see legend to Figure 2.3).





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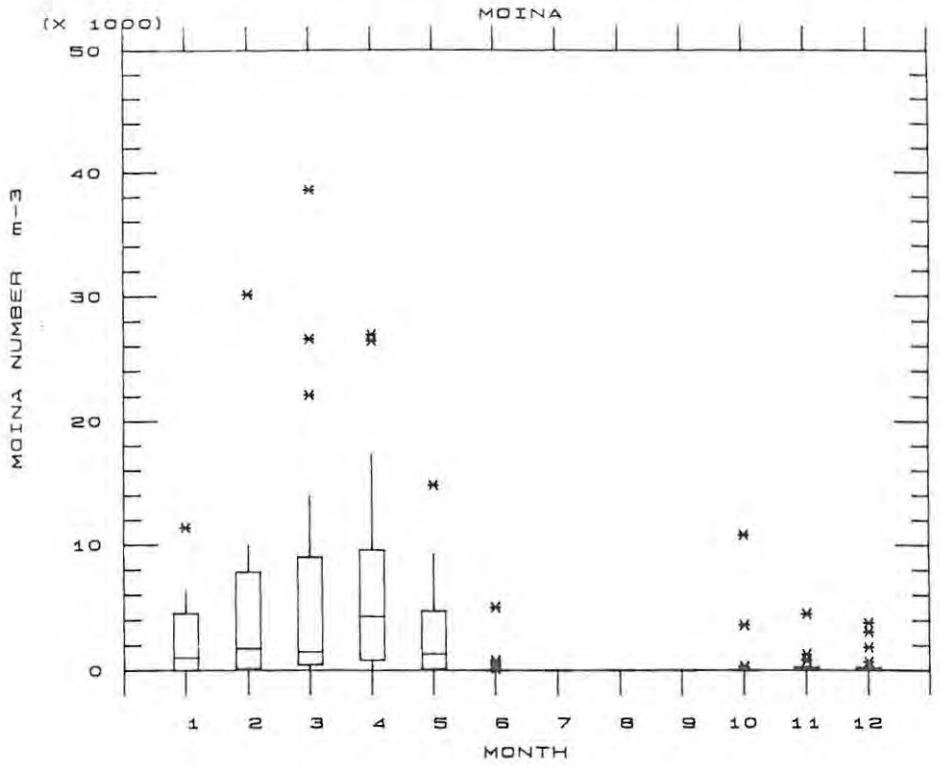


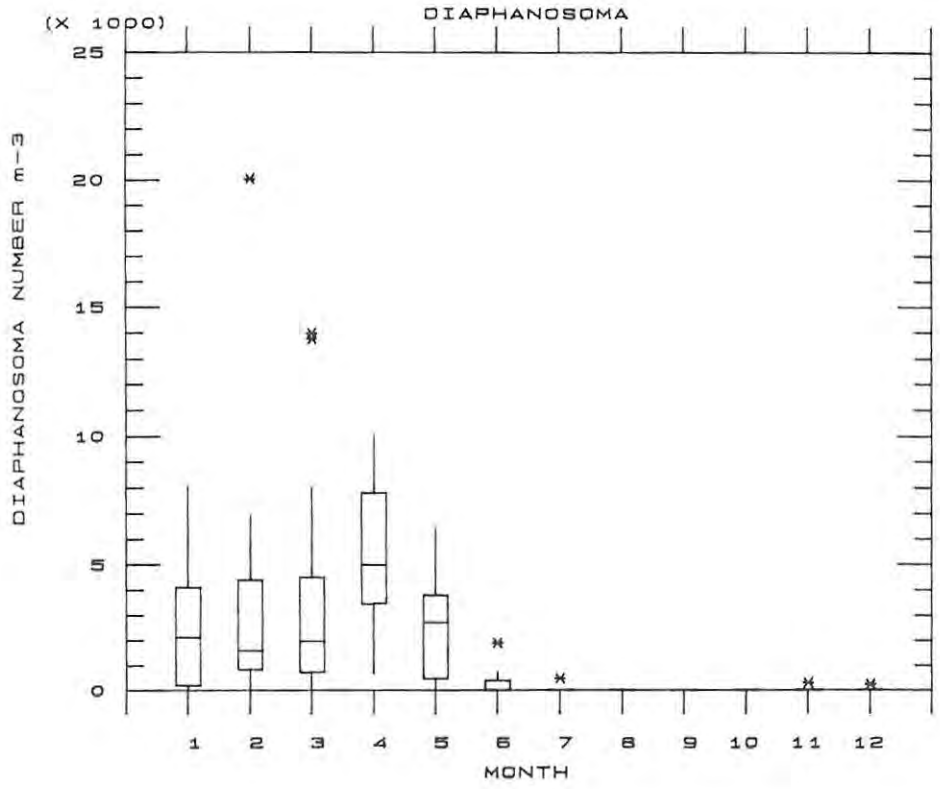


Figure 2.4: (continued).

Table 2.0. Correlation between monthly mean zooplankton community biomass (g dry wt m^{-3}) and monthly mean integrated epilimnetic chlorophyll *a* concentration (mg m^{-3}) (ln transformed data).

Year	r (direct correlation)	p	r (correlation incorporating one month lag)	p
1981	-0.676	0.016*	-0.447	0.168
1982	-0.233	0.467	-0.227	0.502
1983	-0.495	0.102	-0.688	0.019*
1984	+0.034	0.917	-0.622	0.041*
1985	-0.408	0.188	-0.290	0.387
1986	+0.211	0.511	+0.323	0.333

* significant ($p<0.05$)

spring chlorophyte-cryptophyte phase and is minimal following the return to *Microcystis* dominance in January and February.

Ceriodaphnia, *Moina* and *Diaphanosoma* populations were either absent or at their lowest densities during the spring chlorophyte-cryptophyte phase, but undergo rapid population growth in January as *Microcystis* domination became fully established. Maximum *Ceriodaphnia* population densities occurred mainly in March followed in April by maxima of *Moina* and *Diaphanosoma* after *Microcystis* dominance of almost 90% had generally prevailed over the preceding 4-5 months (Figures 2.3 and 2.4). Conversely, for five of the years studied, the *Daphnia* population was notably absent during the late summer when *Microcystis* exceeded 90-95% of phytoplankton biovolume. The exceptions shown in Figure 2.4 as outlying individually plotted points between January and April were due to the unusual presence of *Daphnia* in 1983. Similarly, *Bosmina* was generally absent during months (December - June) when *Microcystis* usually exceeded ≅85% of the phytoplankton biovolume. The period of growth of the *Bosmina* population was restricted to the period when the chlorophyte - cryptophyte composition was increasing.

The frequent coexistence and competitive interaction between *Daphnia* and *Bosmina* populations in many lakes has been the subject of attention in the literature. Aspects of their feeding efficiency and subsequent fecundity under conditions of varying phytoplankton resources have been examined by DeMott (1982), DeMott and Kerfoot (1982), Goulden *et al.* (1982), Kerfoot *et al.* (1985) and Vaga *et al.* (1985). The better foraging efficiency of *Bosmina* than *Daphnia* on low densities of algal flagellates (DeMott and Kerfoot 1982, Kerfoot *et al.* 1985) and *Bosmina*'s resistance to starvation mortality when resources are limited during intense competition (Goulden *et al.* 1982) have been postulated as primary reasons for the ability of *Bosmina* to coexist during periods of high grazing pressure (see Section 3) and high population densities of the larger herbivore *Daphnia*. Neither hypothesis can be clearly regarded as being more applicable than the

other to events occurring in Hartbeespoort Dam. Rather, events influencing competition between *Daphnia* and *Bosmina* in Hartbeespoort Dam appear to be linked to the different abilities of these cladocerans to utilize the type of algae available during the spring, as indicated by Sarnelle (1986).

Close comparison of *Daphnia* population fluctuations in spring with the proportion of partly edible phytoplankton present (Figure 2.1 with 2.2 and Figure 2.3 with 2.4) reveals the presence of corresponding peaks in *Daphnia* numbers occurring with maxima of partly edible phytoplankton in June and November, whereas *Bosmina* maxima are strictly limited to corresponding maxima or increases in 'edible' chlorophytes and cryptophytes. From this it may be concluded that the partly edible category (principally composed of the filamentous diatom *Melosira*) is utilized more by the large herbivore *Daphnia* than by small *Bosmina*. Sarnelle (1986), in a study on the effects of food quality on cladoceran fecundity and population growth, noted that *Daphnia* better utilized larger colonial algae and diatoms than *Bosmina*, which was limited to a small food size range.

Reliance of *Daphnia* on partly edible phytoplankton during its winter growth is particularly apparent in both 1982 and 1983 (Figures 2.1 and 2.2). Correlation between weekly estimates of *Daphnia* numbers and percentages of partly edible phytoplankton biovolume during each of the six years studied showed that significant positive associations existed in both 1982 and 1983 ($r = 0.280$, $p < 0.05$; $r = 0.488$, $p < 0.001$ respectively). The reason for *Daphnia*'s greater reliance on the partly edible phytoplankton category only during 1982 and 1983 and its subsequent survival throughout the late summer period are not clearly evident. Atypical events in species succession within the phytoplankton during that period were not pronounced (NIWR 1985).

Thus the spring period of *Daphnia* and *Bosmina* coexistance in Hartbeespoort Dam is evidently due to some degree of resource partitioning between these cladocerans due to different

feeding strategies (i.e. foraging in *Bosmina*, DeMott and Kerfoot 1982; less selective feeding by *Daphnia*, Sarnelle 1986) in combination with intense competition on edible phytoplankton where niche overlap is likely. Therefore it appears that a combination of hypotheses (*Bosmina*'s foraging and higher feeding efficiency on flagellates, *Daphnia*'s wider resource utilization, *Bosmina*'s better survivorship under conditions of food limitation during competition) may explain the interactions between these cladocerans and the resultant population fluctuations. In addition, predation pressure on *Bosmina* exerted by *Thermocyclops*, or by fish on *Daphnia*, may also influence the outcome of this spring competition on the available resources. Further insight into the mechanisms driving interspecific competition between the cladoceran populations would depend upon a sampling frequency of less than one week or specific event related sampling.

Microcystis returns to dominance annually during December, following the spring chlorophyte-cryptophyte peak and associated *Daphnia* phase. Subsequently *Daphnia* numbers drop sharply and *Ceriodaphnia* dominates the small-bodied cladoceran community over the summer. This seasonal shift occurs frequently in eutrophic lakes (Sommer *et al.* 1986). Competitive exclusion or coexistence of *Daphnia* and *Ceriodaphnia* in relation to their utilization of resources have been examined in detail by Neill (1975), Lynch (1978), Pace *et al.* (1983) and Romanovsky and Feniova (1985). These studies predict that the annual switch between *Daphnia* and *Ceriodaphnia* dominance is based on changes in available resources. *Daphnia* dominates when food is abundant generally in spring; however, as food becomes limited, *Ceriodaphnia* survivorship is better than that of juvenile *Daphnia*, and *Ceriodaphnia* out-competes its larger rival. In Hartbeespoort Dam, whilst the phytoplankton remains abundant in mid-summer, food quality changes to predominantly largely inedible *Microcystis* colonies. Therefore severe food limitation occurs during December concomitant with the predicted switch in cladoceran dominance.

Daphnia and *Ceriodaphnia* usually coexist for one or two months

at both the beginning and end of their respective annual phases. *Ceriodaphnia* numbers diminish as improvement in the partly edible resource levels support the growth of the *Daphnia* population in mid-winter. The converse occurs in mid-summer. In addition to this general temporal separation of *Daphnia* and *Ceriodaphnia*, spatial separation between their population maxima also occurs during their periods of coexistance. Daytime maximum numbers of *Ceriodaphnia*, recorded during discrete depth sampling in 1983 and 1984, occurred near the lake bottom or in deep water nearer the oxycline when the lake was stratified (Figures 2.5 and 2.6). The *Daphnia* population maximum usually occurred above that of *Ceriodaphnia* (at 2-4 m depth) both at the start and end of the *Daphnia* phase. This vertical separation of these cladocerans, also noted in Hartbeespoort Dam by Connell (1978), indicates that these herbivores may not be competing for the same food resources but their periods of coexistance are based on spatial resource partitioning. This partitioning is further demonstrated by their temporal separation which is supported by the study of Pace *et al.* (1983).

Edible and partly edible phytoplankton resources are virtually absent during the summer period of *Ceriodaphnia* population growth and maximum abundance. Coupled with *Ceriodaphnia*'s inability to feed upon *Microcystis* colonies >20 µm and its low filtration rates on small colonies or unicells of *Microcystis* (see results presented in Section 4), the rapidly growing *Ceriodaphnia* population is clearly not resource limited but is very likely dependent upon decomposing *Microcystis* and/or attached bacteria, and free-living bacteria.

Pace *et al.* (1983) demonstrated that *Ceriodaphnia* was able to grow on bacteria due to its higher efficiency of utilization rather than any higher biomass-specific filtration rates on bacteria than *Daphnia*. In Hartbeespoort Dam the presence of abundant *Ceriodaphnia* situated well below the euphotic and diel mixed depths during summer coexistence with *Daphnia*, or close to the lake bottom during winter coexistence, also indicates that phytoplankton is not necessarily a primary food resource of *Ceriodaphnia* in this hypertrophic impoundment.

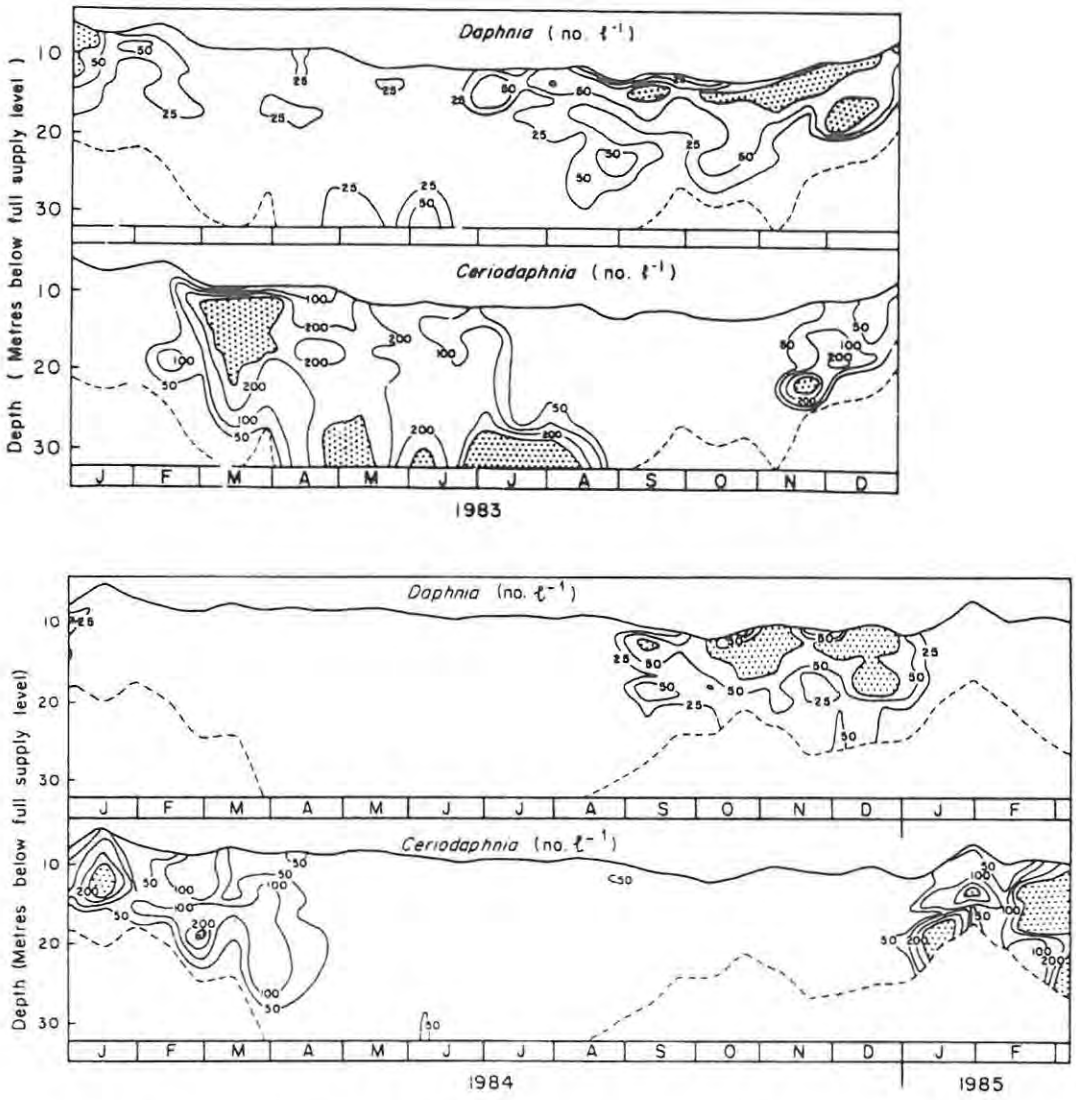


Figure 2.5: Isopleths of *Daphnia* and *Ceriodaphnia* numbers throughout the water column. Stippled area = *Daphnia* >100 ℓ^{-1} or *Ceriodaphnia* >300 ℓ^{-1}. Oxycline shown by broken line.

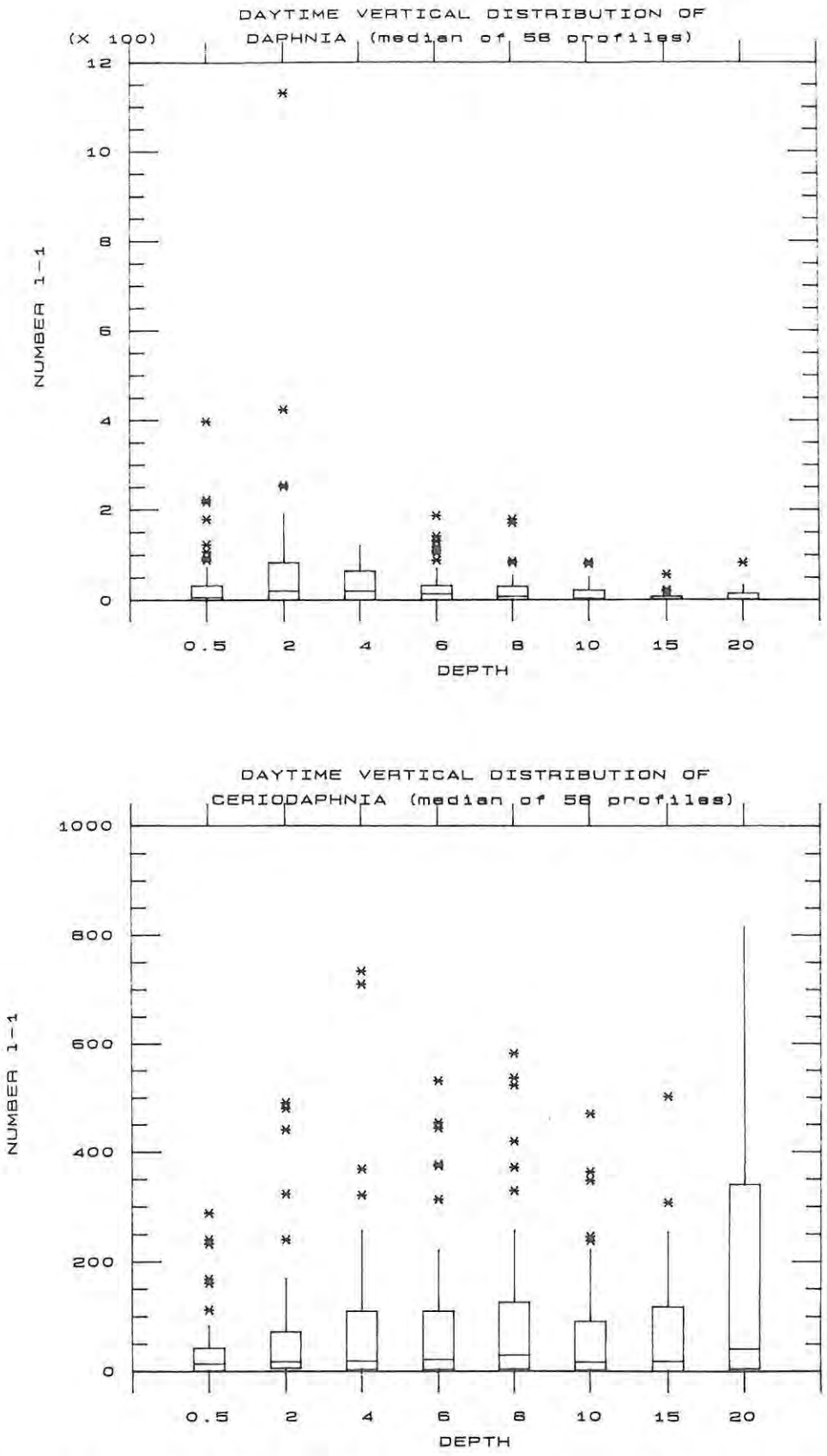


Figure 2.6: Frequency of *Daphnia* and *Ceriodaphnia* numbers occurring vertically throughout the water column after grouping of fortnightly data from January 1983 to March 1985 into depth-specific datasets, showing median, interquartile range, range and outlying points (see legend to Figure 2.3).

Similarly both the *Moina* and *Diaphanosoma* populations, which develop when *Daphnia* is absent during the summer period of maximal *Microcystis* abundance and minimal edible phytoplankton resource levels, may also rely heavily upon decomposing *Microcystis* and bacteria (Hanazato and Yasuno 1985). Recently Hanazato and Yasuno (1987) have shown that *Moina micrura* did not utilize *Microcystis* even when colonies were of an edible size (see also Section 4), but *Moina macrocopa* did assimilate *Microcystis*. However, both the presence of *Moina micrura* during the summer blooms of *Microcystis* and its survival in laboratory experiments when reared on *Microcystis* (presumed to be decomposing), also led Hanazato and Yasuno (1987) to the conclusion that the summer *Moina* population in eutrophic lakes can grow and survive on bacteria and decomposing cyanophytes.

The effects of large algal colonies and filaments on zooplankton of different sizes has been studied by Webster and Peters (1978), Gliwicz (1980), Porter and McDonough (1984), Infante and Abella (1985) and Chow-Frazer and Sprules (1986). These studies showed that increasing concentrations of large cyanobacterial colonies reduced the filtration rates of large cladocerans and increased their particle rejection rates. This increases the energetic cost of their feeding when seston is dominated by large particles, and reduces brood size, favouring a shift from large to small bodied cladocerans during algal seasonal succession in eutrophic lakes. The shift from *Daphnia* to *Ceriodaphnia* in Hartbeespoort Dam approximately one month after the change from the chlorophyte - cryptophyte phase to the *Microcystis* phase indicates that, following the reduction in edible algae available, feeding by *Daphnia* is hindered by the presence of dense *Microcystis* blooms (see results in Section 4).

Gliwicz (1985), in his study and review of food limitation versus predation effects, stated that shifts in zooplankton community structure towards dominance by small zooplankters is driven principally by food quality and food limitation in cyanophyte dominated eutrophic lakes, with fish predation usually of secondary importance. Examination of data gathered

in Hartbeespoort Dam on the number of gravid adults of *Daphnia* and *Ceriodaphnia* and their brood size over most of the six year study period shows strong seasonality in the number of eggs produced which is influenced by seasonal food quality (Figures 2.7 and 2.9).

During development of *Daphnia* populations in May of 1982, 1985 and 1986, both the number of gravid females and the number of eggs they carried (brood size) was high. Subsequently the population fecundity measured as the eggs per adult female (Ghilarov 1985) was also relatively high in those years. Collection of egg data commenced too late in 1981 to record this event and in 1983, following unusually high *Daphnia* densities over the previous summer, no pronounced autumn increase in *Daphnia* fecundity occurred. In 1984 *Daphnia* fecundity was low and the initial mid-winter peak of egg production and population growth was absent. Consequently the *Daphnia* phase was short-lived in 1984 and strictly limited to the edible phytoplankton period.

Regardless of the rate of *Daphnia* population growth and egg production, *Daphnia* fecundity from August to December each year was lower than during the mid-winter period, whilst the population density usually continued to rise during the spring chlorophyte-cryptophyte period. Many field studies have confirmed laboratory experiments demonstrating the dependence of cladoceran fecundity or brood size upon food quantity or quality (e.g. Lampert 1977b, Webster and Peters 1978, Lampert and Schoder 1980, Ghilarov 1985, Larsson *et al.* 1985, Orcutt 1985, Threlkeld 1985, Vanni 1986). In particular, food limitation occurring during periods of high population densities, grazing pressure and competition for resources, or even quite brief shifts to lower food quality, have been identified as causes of reduced *Daphnia* fecundity leading to a mid-summer decline or disappearance of the population. In Hartbeespoort Dam a marked lowering of *Daphnia* egg production during the period of maximal *Daphnia* densities and highest annual grazing rates (see Section 3) strongly indicates that food limitation during the intense grazing pressure of this spring clear-water

and edible phytoplankton phase can occur. Such an event has been identified previously (Lampert 1985) as an important factor controlling zooplankton standing crop.

The high rate of increase (as reflected in instantaneous rates of population change; r) in *Daphnia* populations when food levels are high, and *Daphnia*'s susceptibility to low resource levels (lower r) have been well documented (Neill 1975, Lampert 1977b, DeMott and Kerfoot 1982, Kerfoot *et al.* 1985, Orcutt 1985, Threlkeld 1985). Towards the end of the spring edible phytoplankton period in Hartbeespoort Dam a slight rise in the total population fecundity of *Daphnia* during October and November arises primarily from an increase in brood size rather than a substantial increase in the number of gravid adults (Figures. 2.7 and 2.8). However, egg production again drops with the onset of *Microcystis* dominance and lower food quality. Larsson *et al.* (1985) demonstrated that even a brief cyanophyte bloom (≅3 weeks) could temporarily depress *Daphnia* fecundity and Threlkeld (1985) showed that such short events can reduce *Daphnia*'s birth rate through lowering egg production or more significantly by increasing the rate of food quality mediated abortion of eggs and developing embryos. Of those daphnids surviving into January in Hartbeespoort Dam when *Microcystis* usually comprises over 90% of the phytoplankton biovolume, the number of gravid adults, the eggs per adult and their brood sizes were low. This is indicative of both quantitative and qualitative limitations to population growth arising from interference to efficient filter-feeding by these large cladocerans due to clogging of their filtering apparatus or increased rejection of food particles or boluses (Webster and Peters 1978).

Consequently, in Hartbeespoort Dam, the primary factors leading to the shift in zooplankton community structure from large - to small-bodied herbivores are the spring change to edible phytoplankton species, the subsequent food limitation evident following rapid population growth, the shift back to largely inedible phytoplankton forms (large *Microcystis* colonies) and their interference with *Daphnia*'s feeding efficiency and reproductive rate.

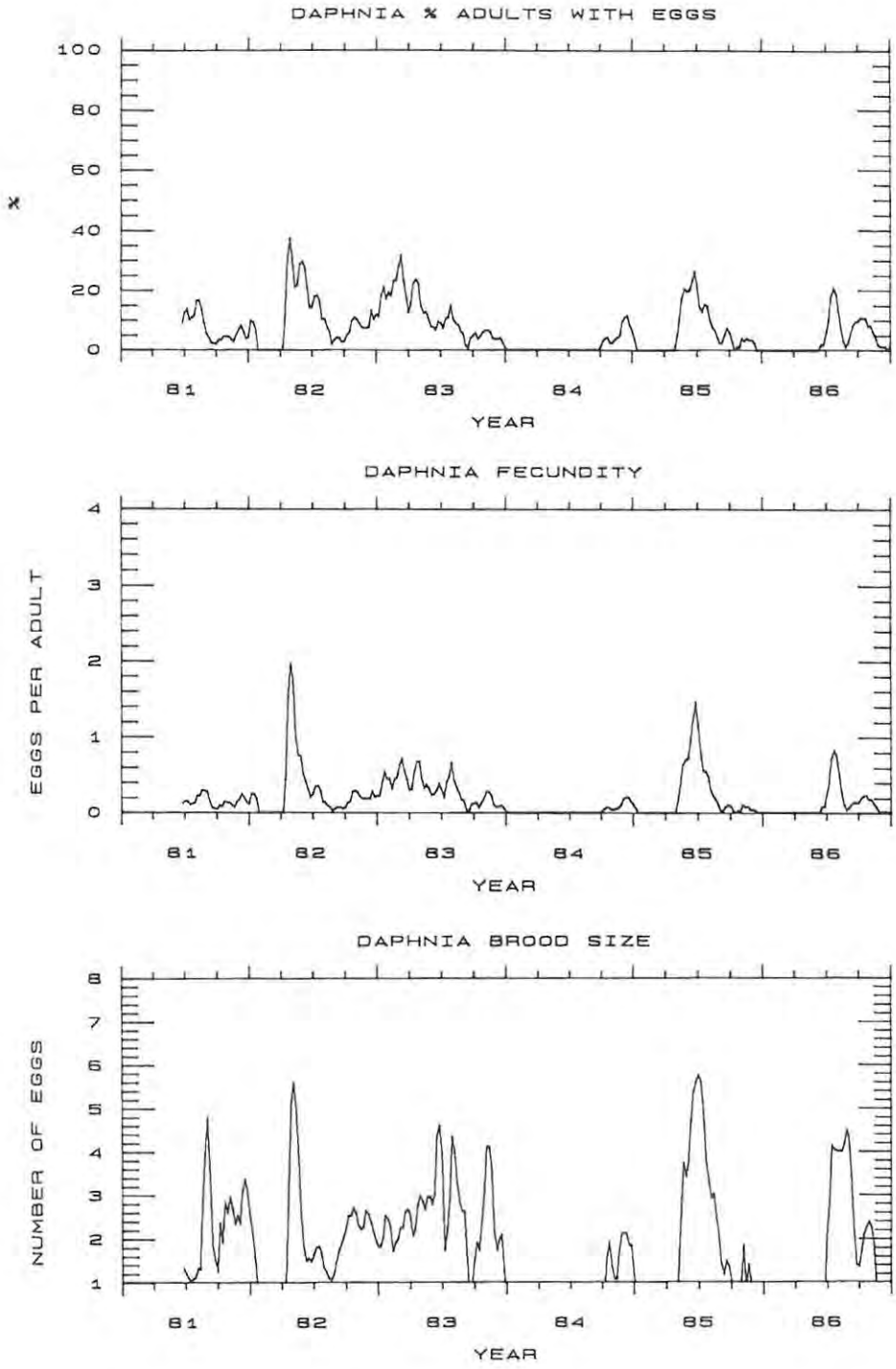


Figure 2.7: *Daphnia*: % of adults carrying asexual eggs, the number of eggs per adult female and the average number of eggs per brood. Data smoothed (n = 3).

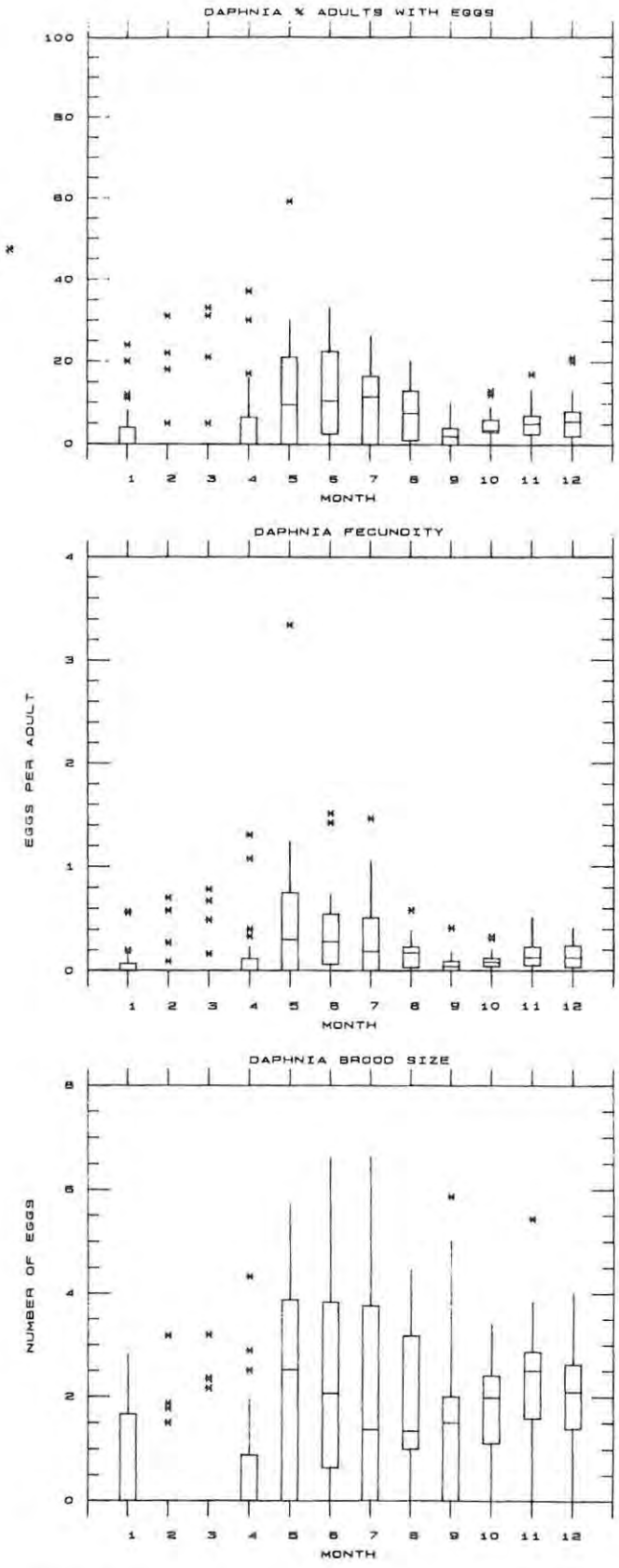


Figure 2.8: Frequency of gravid *Daphnia*, eggs per adult female and brood size after grouping of weekly data over 5½ years into monthly datasets, showing median, interquartile range, range and outlying points (see legend to Figure 2.3).

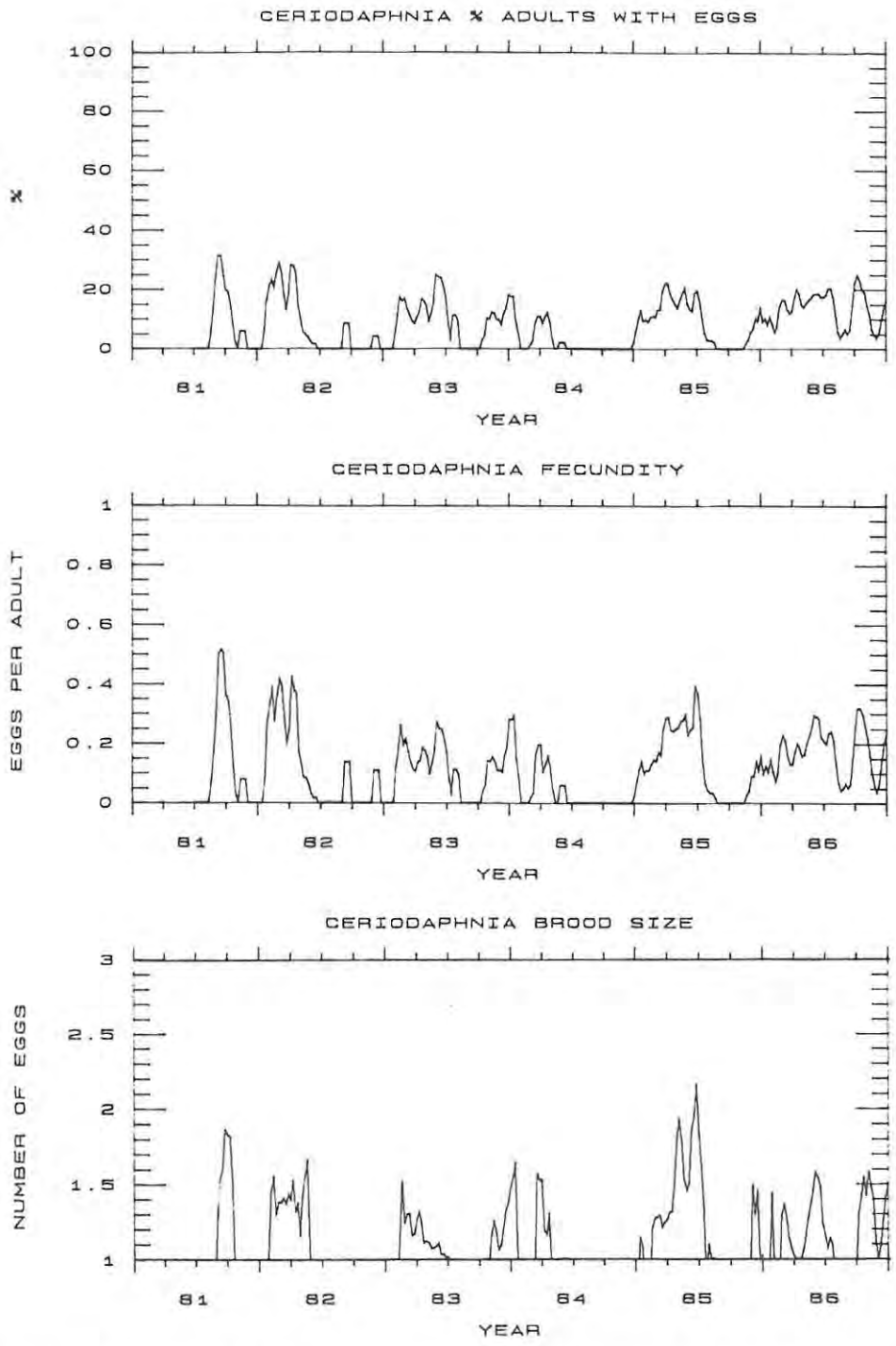


Figure 2.9: *Ceriodaphnia*: % of adults carrying asexual eggs, the number of eggs per adult female and the average number of eggs per brood. Data smoothed (n = 3).

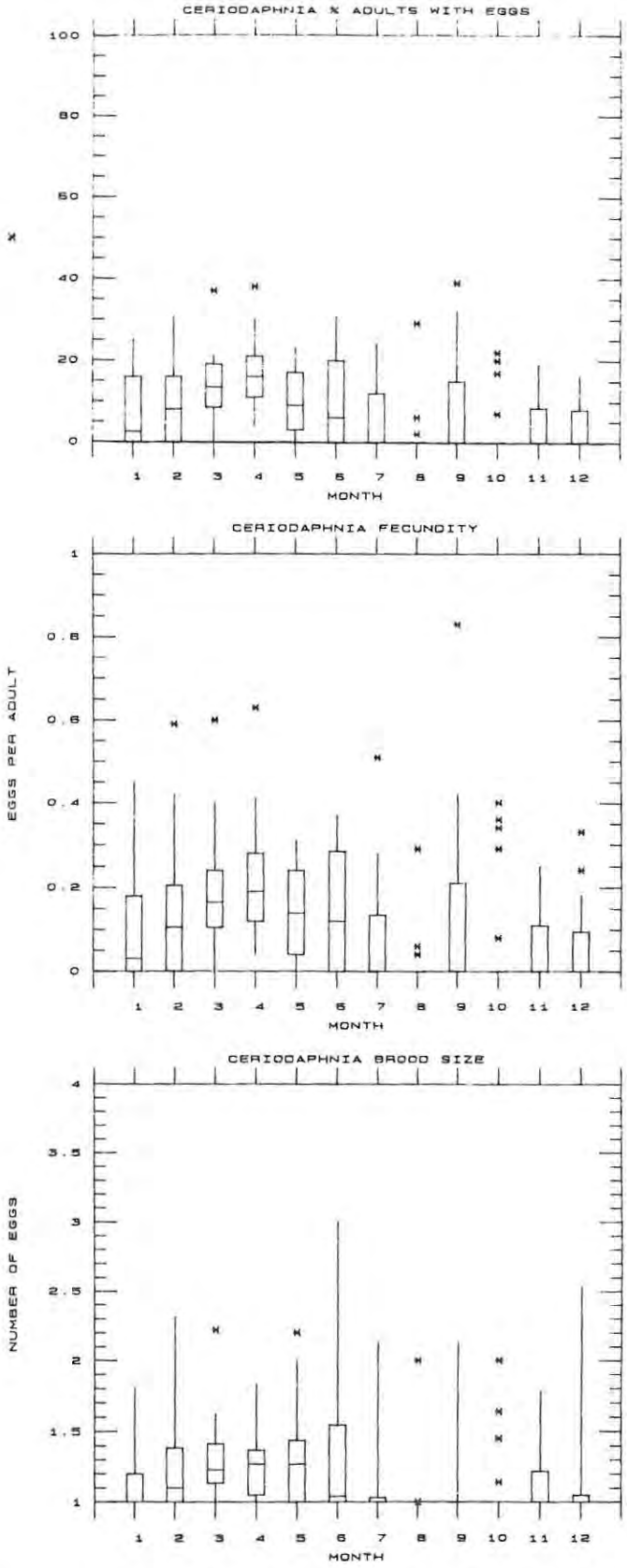


Figure 2.10: Frequency of gravid *Ceriodaphnia*, eggs per adult female and brood size after grouping of weekly data over 5½ years into monthly datasets, showing median, inter-quartile range, range and outlying points (see legend to Figure 2.3).

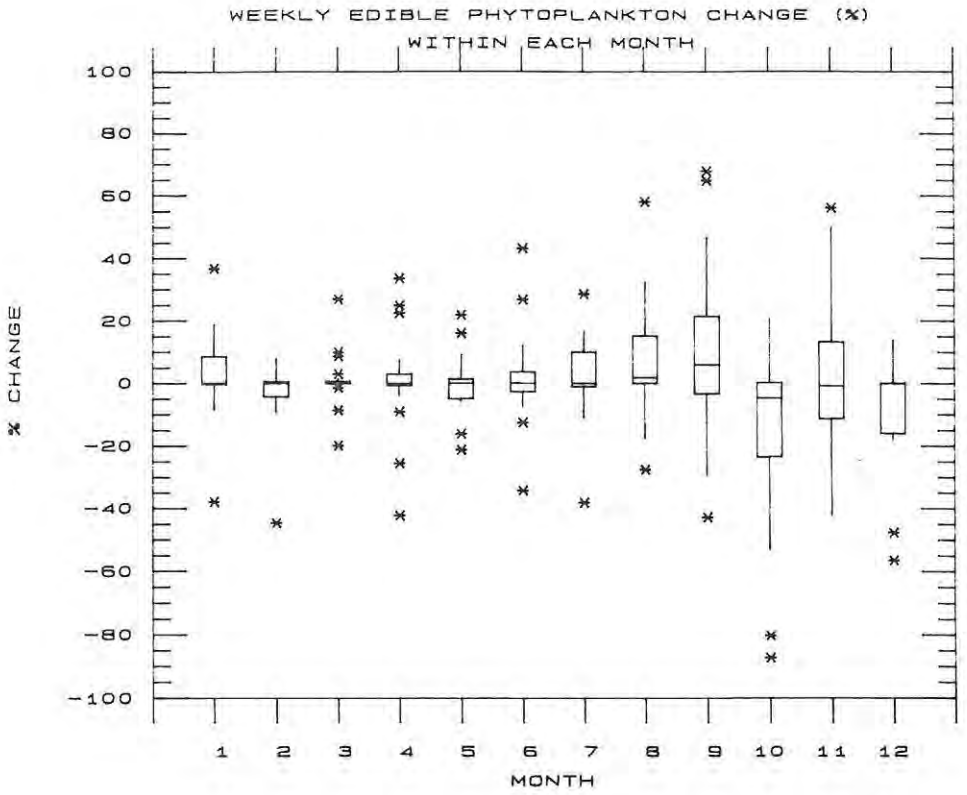
Evidence to support high fish predation pressure as being largely responsible for this shift in herbivore community size structure (Brooks and Dodson 1965) is not clearly forthcoming. A principal indication that fish predation is a primary cause of declining abundance of the larger bodied cladoceran populations is the decline in cladoceran numbers whilst reproductive rate and brood size either do not diminish or even increase when coexisting with zooplanktivorous fish (Lynch 1979, Vanni 1986). In Hartbeespoort Dam, whilst the general monthly trend in Figure 2.8 shows a decline in *Daphnia* brood size from November to January accompanying their mid-summer decline associated with decreasing food quality, examination of this event for each year studied (Figure 2.7) shows that in 1981 *Daphnia*'s brood size remained fairly high immediately before their disappearance. That this is a symptom of more intense predation by fish during the summer of 1981-82 is not easily supported by other data. One of the primary zooplanktivores in Hartbeespoort Dam, young *Oreochromis mossambicus*, was less abundant during 1981-82 than in the following two years (NIWR 1985). During the winter of 1981 severe cold-shock induced mortality of *Oreochromis* juveniles spawned in 1980-81 resulted in a 75-99% reduction of numbers within that year class. Winter mortality in the following two years was estimated at 25-74% and only 0-24% of the juvenile year-classes respectively (NIWR 1985). Thus it appears likely that fish predation pressure on large *Daphnia* was lower in the spring-summer of 1981-82. Regardless of the relatively high *Daphnia* brood size prior to its mid-summer decline in 1981-82, it cannot be concluded in the light of events in following years that fish predation is the primary cause of the annual shift in zooplankton community size structure in Hartbeespoort Dam.

Seasonality of egg production by *Ceriodaphnia* was also pronounced. Fecundity and brood size increased during the late summer *Microcystis* phase. *Ceriodaphnia* fecundity was usually highest in April (Figures. 2.9 and 2.10) following periods of almost complete dominance by largely inedible phytoplankton (Figure 2.2). Conversely *Ceriodaphnia* fecundity was usually

low during the spring chlorophyte - cryptophyte phase. Exceptions occurred in 1981 and 1986 when large but short-lived increases in egg number were recorded.

The significant increase in *Ceriodaphnia* brood size when food quality was at its lowest (correlation coefficient; $r = 0.129$, $n = 239$, $p<0.05$) indicates that *Ceriodaphnia* is not food limited during summer when large inedible *Microcystis* colonies which are not utilized by *Ceriodaphnia* dominate (see also Section 4). Alternative resources available during the late summer are bacteria, decomposing *Microcystis* colonies or very low densities of edible algal species existing between the abundant large *Microcystis* colonies. The increase in *Ceriodaphnia* fecundity and rapid growth of the *Ceriodaphnia* population when the edible phytoplankton resource was lowest implies that both attached bacteria and the abundant free-living bacteria, composed of rods and particularly of very small cocci (Robarts and Sephton 1984), are the primary summer resources utilized by this small-bodied cladoceran community in Hartbeespoort Dam. This is examined further in Section 5.

Although Hartbeespoort Dam is subtropical and hypertrophic, with primary production light limited (self shading) rather than nutrient limited (Robarts 1984), sequences of events occurring seasonally within the zooplankton community are similar to those described in other eutrophic lakes and as predicted by the PEG-model (Sommer *et al.* 1986). Many of the seasonal successional events are, however, very pronounced in Hartbeespoort Dam, particularly the annual changes in zooplankton size structure and community composition that occur in response to the marked changes in food quality. In addition, as growth of the large particle size cyanophyte resource occurs in summer, *Daphnia* fecundity and brood size are depressed, indicating that interference to feeding efficiency may play a major role in species succession. Both the apparent absence of strong influence by physical factors and the spring period of food limitation, as indicated by the low *Daphnia* brood size during the chlorophyte-cryptophyte phase evident in Hartbeespoort Dam, were also outlined by Sommer *et al.* (1986).



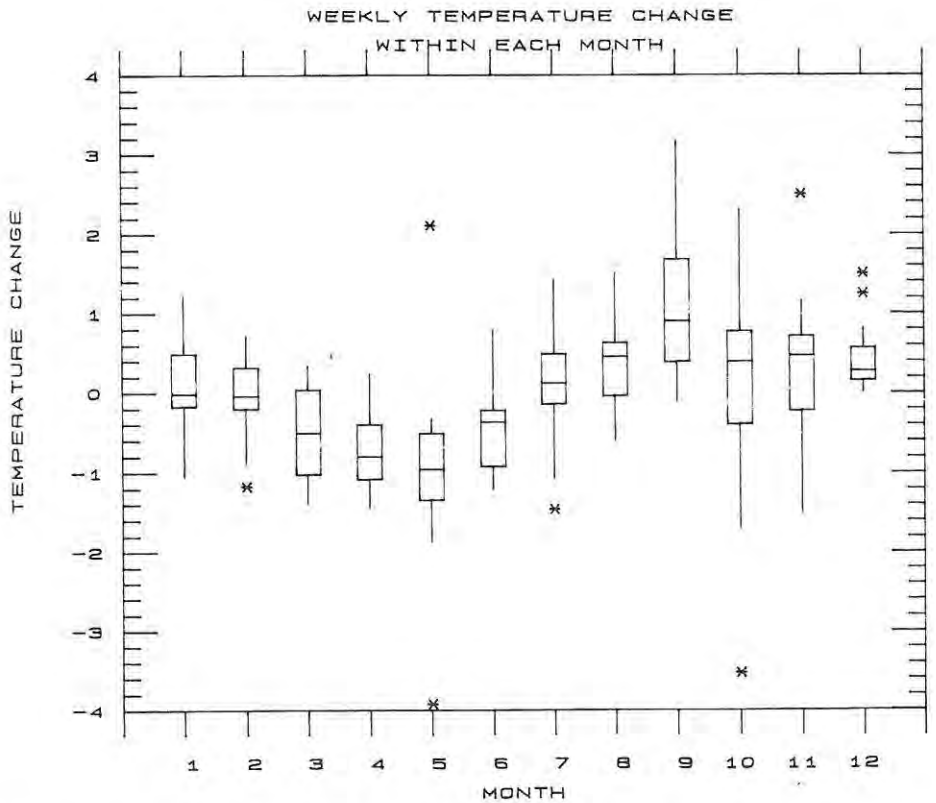


Figure 2.11: Differences between consecutive weekly measurements of edible phytoplankton composition (change in % biovolume) and water temperature (change in °C) after grouping of data gathered over 6 years into monthly datasets, showing median, interquartile range, range and outlying points (see legend to Figure 2.3).

Examination of weekly changes occurring in the proportion of edible phytoplankton present within each month over the six year study period further demonstrates the occurrence of resource depression and probable food limitation in spring. During October each year when *Daphnia* population densities are generally high the proportion of edible algae present decreases (Figure 2.11) presumably due to selective feeding and intense grazing pressure.

Whilst the influence of temperature on zooplankton may be partially indirect via the phytoplankton or fish, direct temperature effects may still be important in controlling events that initiate the process of autogenic succession. For example, recovery and regrowth of the *Daphnia* population in Hartbeespoort Dam usually commences annually in May with a sudden and pronounced increase in fecundity and brood size (Figures 2.7 and 2.8). Whilst an increase in the proportion of partly edible phytoplankton (e.g. *Melosira*) occurs in winter, any noticeable improvement in food level does not usually occur until June. So initially the reappearance of *Daphnia* commences while *Microcystis* is still abundant, although approaching the end of its dominance.

A possible stimulus initiating the hatching of resting eggs (Schwartz and Herbert 1987) and an increase in *Daphnia* egg production in May may be low temperature or temperature change (decrease) rather than change in food resource levels. Change in weekly epilimnetic water temperature occurring within each month over the six year period is shown in Figure 2.11. Greatest weekly increase in temperature occurs in September and greatest decrease in May, usually of almost 1 °C in both cases. Therefore, the direct influence of temperature in 'switching on' successional events, upon which other factors then modify or control subsequent events, must not be overlooked.

3. ZOOPLANKTON COMMUNITY GRAZING

3.1 Introduction

Zooplankton grazing has been shown by Porter (1973, 1976) to be advantageous to the development of abundant populations of large colonial or sheathed algae. Both increased grazing pressure on more palatable chlorophyte and cryptophyte forms, and viable gut passage of ingested sheathed forms (Porter 1975, 1976) contribute to this effect. A decline in grazer-mediated control of algae in eutrophic lakes dominated by cyanophytes has been questioned by Schoenberg and Carlson (1984). They highlighted conflicting results reported in the literature concerning the ingestion and assimilation rates of zooplankton fed unicellular and colonial cyanophyte suspensions (Sorokin 1968, Arnold 1971, Schindler 1971, Moriarty *et al.* 1973, De Bernardi *et al.* 1981) and the effects of toxic strains of algae on filter-feeding rates (Porter and Orcutt 1980, Lampert 1981, 1982). Carlson and Schoenberg (1983) suggested that cyanophyte populations may be suppressed by the grazing influences of large-bodied zooplankton following artificial manipulation of eutrophic lakes, such as by the 'biomanipulative' reduction in number of fish predators. However, it is likely that with the development of extremely large cyanophyte colonies (eg. *Microcystis aeruginosa*) in hypertrophic lakes (Robarts and Zohary 1984) nuisance blooms will still develop, as large colonies exceed the maximum size limit (≅60 μm) for ingestion by filter-feeding herbivorous zooplankton (Thompson *et al.* 1982).

Grazing pressure on phytoplankton may be affected by interference to filter-feeding processes and reduced filtration efficiency by the presence of cyanophyte filaments (Webster and Peters 1978), reduction in the zooplankton community size frequency following fish predation (Brooks and Dodson 1965), and interspecific competition, often characterized by mid-summer declines in cladoceran numbers or by shifts to smaller species (DeMott and Kerfoot 1982, Goulden *et al.*, 1982, Threlkeld 1985, and others). These events may also limit

zooplankton utilization of cyanophytes which, in the absence of severe nutrient limitation in eutrophic lakes, may develop into surface blooms in summer. This chapter examines seasonal zooplankton community grazing rates in hypertrophic Hartbeespoort Dam, in which for 8 - 10 months of each year the phytoplankton resource is dominated by net-phytoplankton, largely *Microcystis*, composed mainly of large colonies >1 mm in length or diameter (Robarts and Zohary 1984, see also Sections 1 and 2). The suggestion of Carlson and Schoenberg (1983) that large zooplankton herbivores, by high grazing pressure, can effectively control algal blooms in eutrophic lakes is examined by measurement of *in situ* community grazing rates on a palatable algal species. Intensive examination of species-specific filtration rates on *Microcystis* is reported in Section 4.

When compared to temperate lake studies, there is a paucity of information on the interrelationships between zooplankton community grazing and phytoplankton food resources under sub-tropical lake conditions (Sommer *et al.* 1986). Comparisons are made with the zooplankton feeding studies carried out in another sub-tropical lake (Hart 1984, 1986) and in eutrophic lakes (e.g. Gulati *et al.* 1982). Using the extensive database gathered on community grazing in Hartbeespoort Dam, multiple regression analysis similar to that performed by Hart (1984 and 1986) was carried out to allow comparison with the disparate functions of temperature and seston with grazing rates obtained by Hart (1986).

3.2 Methods

3.2.1 Rationale for the use of an *in situ* experimental approach

Factors requiring consideration in this study included: the hypertrophic condition of Hartbeespoort Dam; its associated extremely abundant phytoplankton dominated for most of the year by a large colony form (*Microcystis*); and the need to obtain representative measurements of zooplankton feeding rates under natural conditions.

Intuitively, a preferred experimental approach involves the following criteria: measurement of filter-feeding *in situ*, where the zooplankton remain within their ambient physical and biological conditions; and the introduction of minimal handling stresses or crowding of animals. Stress due to disturbance or 'handling' of zooplankton in experiments has been been shown to alter feeding activities (Peters and Downing 1984). Chow-Fraser (1986) has also shown that feeding rates of concentrated zooplankton communities either measured immediately, or after a 24 h acclimation period, differed significantly from *in situ* rates measured using the technique of Haney (1971). Similarly, Forsyth and James (1984) also compared results obtained using the *in situ* Haney technique with laboratory procedures. They found zooplankton clearance rates of both phytoplankton and bacteria recorded using the Haney chamber to be highest 'when handling stress was minimal and environmental conditions more closely approximated those in nature'. Therefore the *in situ* Haney technique avoids problems associated with effects of collection, artificial concentration, handling and acclimation on measurements of zooplankton grazing activity.

The Haney technique also avoids major alteration of the size and type of the natural phytoplankton assemblage present. In hypertrophic conditions where net-phytoplankton frequently predominates, any concentration of the zooplankton during 'on-board' or 'lake-shore' experiments will also alter the natural particle size frequency and net-phytoplankton composition within the feeding medium. Due to the variable response of zooplankton to feeding on cyanophyte colonies in many studies, as summarised by Schoenberg and Carlson (1984; see Section 3.1), it was essential to avoid the exclusion of *Microcystis* colonies and their influences on the feeding response of zooplankters in the feeding experiments. Furthermore, due to seasonally variable colony sizes of *Microcystis* and variation in the toxicity of the *Microcystis* types present (i.e. occurrence of the potentially toxic strain *M. aeruginosa* forma *aeruginosa* or non-toxic *M. aeruginosa* forma *flos-aquae*, Scott *et al.* 1981, NIWR 1985) the use of a cultured unicellu-

lar alga (*Chlorella* sp.) as a standardized radiolabelled food source in experiments was favoured over use of the extremely variable natural phytoplankton community.

A readily palatable unicellular chlorophyte was chosen to avoid use of a potentially toxic food, whilst the *in situ* experiments do not remove any influences to filtration rates of both toxicity within the varying natural phytoplankton, or of the natural food-particle size distribution. The use of low concentrations of *Chlorella* of a uniform cell size and high specific activity, whilst not significantly altering the food resource available and thus the filter-feeding behaviour of the zooplankton, enables variation in the zooplankton response to changes in the natural phytoplankton assemblage to be reflected by corresponding long-term changes in community grazing rates. Furthermore use of a monoalgal culture as the radiolabelled food in *in situ* feeding experiments also obviates problems associated with heterogeneity of radiolabel uptake by different algal species. This has been known to cause errors in the measurement of filtration rates when using the natural seston assemblage (Gulati *et al.* 1982, Lampert and Taylor 1985).

A further advantage of the use of a standard unicellular food is that of greater ease in comparison of the data obtained with that of other studies. Whilst natural seston has been used in some of these studies (eg. Gulati *et al.* 1982), the extremely different nature of the seston occurring in hypertrophic Hartbeespoort Dam (i.e. almost complete composition by *Microcystis* for many weeks or months) would to a large degree invalidate the conclusions drawn from such inter-study comparisons. The filtration rates measured cannot be used to calculate ingestion rates of the natural seston in Hartbeespoort Dam due to unpalatability and frequent rejection of particles, filaments or colonies (Webster and Peters 1978) but the rates measured here reflect the *in situ* filter-feeding activity of the zooplankton community on a palatable alga under the varying natural seston conditions of this hypertrophic impoundment.

3.2.2 Application of the Haney technique

Zooplankton community grazing rates were measured every two weeks from January to 1983 to March 1985 within 2 h of midday over a vertical profile through the aerobic water column. Experiments were conducted at the main basin sampling station of the lake (Figure 1.0) using a method based on that of Haney (1971), as modified by Hart (1986), and further altered to suit the hypertrophic conditions of abundant *Microcystis* colonies. In outline, the Haney procedure involves the capture of zooplankton in a grazing chamber at the desired depth, release of radiolabelled algae into the chamber for a short feeding duration, retrieval and filtration to retain zooplankton, and solubilization of the zooplankton to allow counts of radioactive algae within their guts to be determined.

The design of the grazing chamber (Figure 3.0) was based on a double Van Dorn water sampling bottle (2 x 3 litres capacity) which was rebuilt using clear perspex. Additions to the Van Dorn bottles included externally fixed syringe holders and injecting mechanisms that triggered simultaneously with closing of the doors of the grazing chamber. Thus the contents of the syringes could be injected rapidly into either one or both sides of the chamber. Tests using the same volume of a dye within each syringe as the volume of labelled algae used showed that on closure of the chamber's doors mixing throughout each compartment, although not instantaneous, occurred with ≅5 s due to the T-shaped injection ports.

During the regular community grazing rate experiments, radiolabelled alga was injected into one of the two compartments only. Experiments comparing feeding rates on different foods or measuring community grazing as a control in experiments measuring individual species grazing rates (see Section 4) required release of labelled foods into both compartments of the grazing chamber.

The unicellular chlorophyte *Chlorella* sp. (NIWR culture

collection No. WR 1007) was used as the radiolabelled food and was considered to be an acceptable food for most herbivorous zooplankters due to its spherical shape (cell diameter ≅ 8.5 μm) lack of spines or sheath, and its common use in other feeding studies. *Chlorella* was easily maintained in batch culture in the laboratory (culture medium BG11; W.E. Scott pers. comm.) and from these sources small volumes of log-phase culture were withdrawn for radiolabelling.

Radioisotope was added to a growing *Chlorella* culture at a concentration of 2 μCi $m\ell^{-1}$ of $Na_2{}^{14}CO_3$ (Amersham International). After incubation for 24 h at room temperature the labelled algae were centrifuged and resuspended twice in fresh culture medium to remove isotope not bound within the algal cells. Comparison between the chlorophyll concentration in the *Chlorella* culture and in the euphotic zone of the lake allowed the amount of labelled algae introduced into the grazing chamber to be limited to under 10% of the natural chlorophyll present. This ensured that the zooplankton grazing rates would not be altered by a sudden increase in available food. Due to extreme variation in the amount of chlorophyll and unicellular phytoplankton forms present (Robarts and Zohary 1984, NIWR 1985) precise standardization of the amount of tracer cells used was not undertaken.

Feeding experiments were carried out *in situ* at depths of 0.5, 2, 4, 6, 8, 10, 15 and 20 m below the lake surface. When an anoxic hypolimnion was present, grazing experiments were not carried out below the oxycline. The duration of each experiment varied between 5 and 6 min, measured from closure of the chamber and simultaneous automatic injection of labelled algae into one of the two compartments, to completion of drainage of half of the sample volume. Zooplankton from both compartments of the grazing chamber were retained on 60 μm mesh nylon nets. Organisms from the compartment not treated with labelled algae were stored with 4% formalin for identification, enumeration and dry biomass determination in the laboratory as described in Section 2.2.

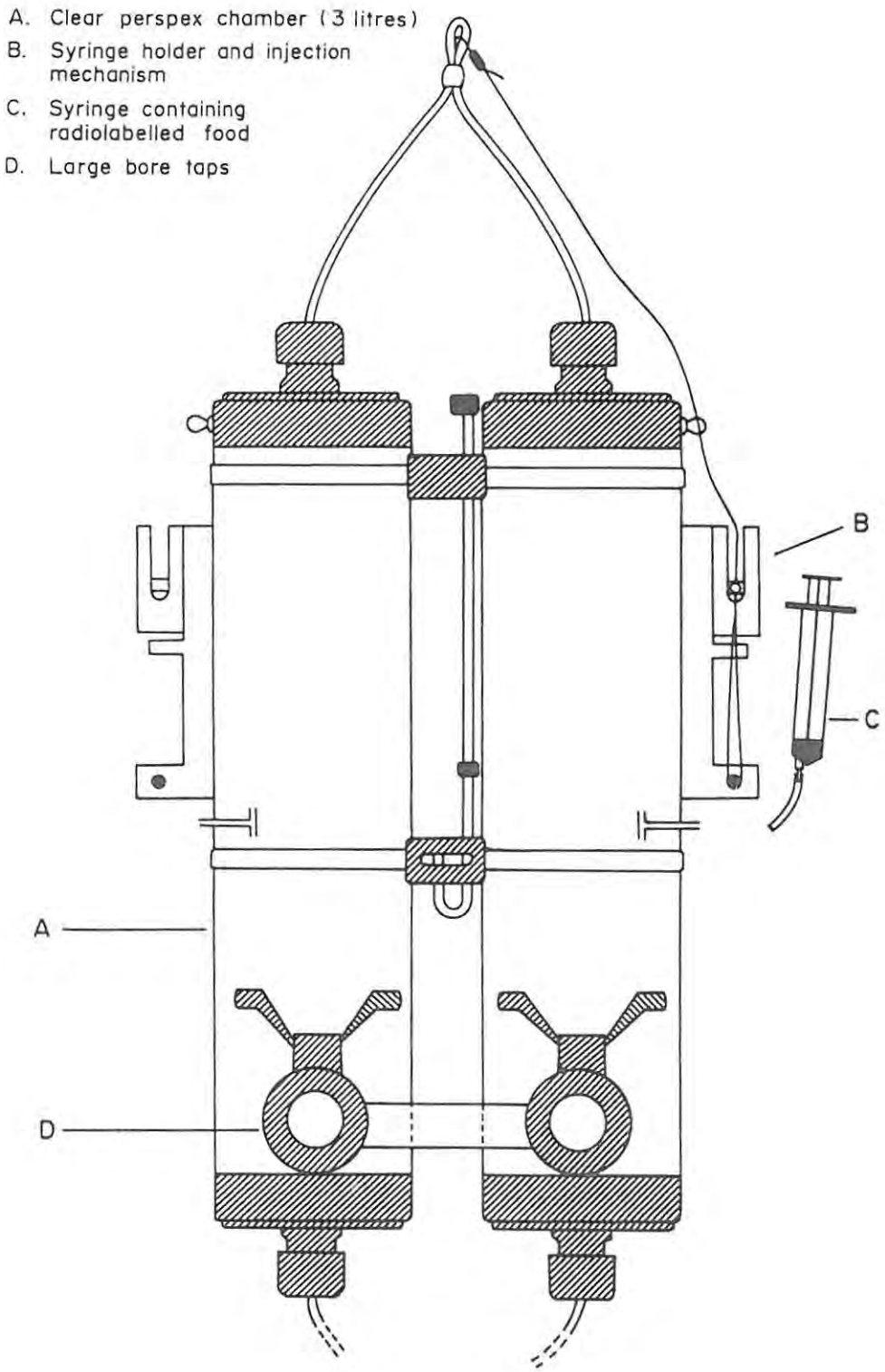


Figure 3.0: Gliwicz-Haney style *in situ* grazing chamber, modified from a double Van Dorn water sampler.

Zooplankton exposed to the labelled algae were immediately immobilised in carbonated water (soda water), killed in a 4% formalin bath and rinsed. Depending upon the amount of *Microcystis* present, the samples were either filtered onto a 60 µm mesh disc and immediately placed into a scintillation vial with 1 mℓ of 0.5 N quarternary ammonium hydroxide (Soluene 350, Packard) or were washed temporarily into a 200 mℓ jar if *Microcystis* was so abundant that immediate filtration onto the 60 µm mesh disc was not possible due to clogging. The filtered lakewater used for the washing process had a few drops of detergent added to reduce surface tension and prevent any dead zooplankters adhering to the surface. The samples treated in this way were allowed to stand for 10 - 15 minutes during which time the dead zooplankton settled whilst the buoyant *Microcystis* colonies collected at or near the surface and were removed by suction. Microscopic examination of this waste water and *Microcystis* showed that zooplankters were not inadvertently removed during this process. After the removal of *Microcystis* colonies the zooplankton sample was filtered onto a 60 µm mesh disc and solubilized as described above.

A 200 mℓ sample of water from the unlabelled compartment was also collected after passage through the 60 µm mesh for determination of the amount of suspended particulate matter (seston) <60 µm present. Sub-samples of 50 mℓ were filtered onto pre-weighed 0.45 µm membrane filters and dried for 24 hours at 50 °C before reweighing to measure the dry weight of suspended particulates present (mg ℓ^{-1}). This provided only an indication of the natural resource level present since not all of this particulate matter may be ingested. Some particles >60 µm may also be utilized. High levels of inorganic suspended matter occurred infrequently in Hartbeespoort Dam, especially during the severe drought period from 1982 - 1985 (NIWR 1985).

Two 10 mℓ samples of medium from the grazing chamber were each filtered through 0.45 µm membrane filters to retain samples of labelled algae for determination of the specific activity of

the medium using 10 mℓ Filter Count (Packard). Zooplankton samples, after solubilization for at least 6 h at 50 °C were allowed to cool before addition of 10 mℓ Dimilume (Packard). Radioassay was carried out with a liquid scintillation counter (Packard Tricarb 300C) using the external standard method of quench correction based on ^{14}C standards. Correction for counting efficiency and quenching permitted convertion of c.p.m. to d.p.m.. Calculations of the community filtration rate (CFR, mℓ h^{-1}) and community grazing rate (CGR, % d^{-1}) were based on the equations of Haney (1973).

$$\text{Community Filtration Rate (m}\ell\ h^{-1}) = \frac{\text{d.p.m. of zooplankton}}{\text{d.p.m. of feeding suspension (m}\ell^{-1})} \times \frac{60}{\text{duration of experiment (min)}}$$

$$\text{Community Grazing Rate (\% d}^{-1}) = \text{CFR (m}\ell\ h^{-1}) \times \frac{24}{\text{volume of chamber (m}\ell)} \times 100$$

Biomass specific filtration or grazing rates (SFR and SGR) were calculated by dividing the rates calculated above by the biomass of the herbivores present (SFR, mℓ mg dry weight^{-1} h^{-1}; SGR, % mg dry wt^{-1} d^{-1}). Filtration or grazing rates are not equivalent to ingestion rates but are comparable to the term 'clearance rates'. From measurement of community and specific filtration and grazing rates over a vertical profile at the limnetic station, integrated rates were calculated for the water column by planimetric integration of volumes represented by the various sampling depths over the aerobic water column (>1.0 mg O_2 ℓ^{-1}).

In addition to these regular bi-monthly measurements of zooplankton community feeding activity the minimum gut passage time of animals present and the existence of diel variations in feeding activity were also examined early during the study period.

3.3 Results

3.3.1 Duration of feeding experiments: gut clearance time

During the measurement of grazing rates it is essential that

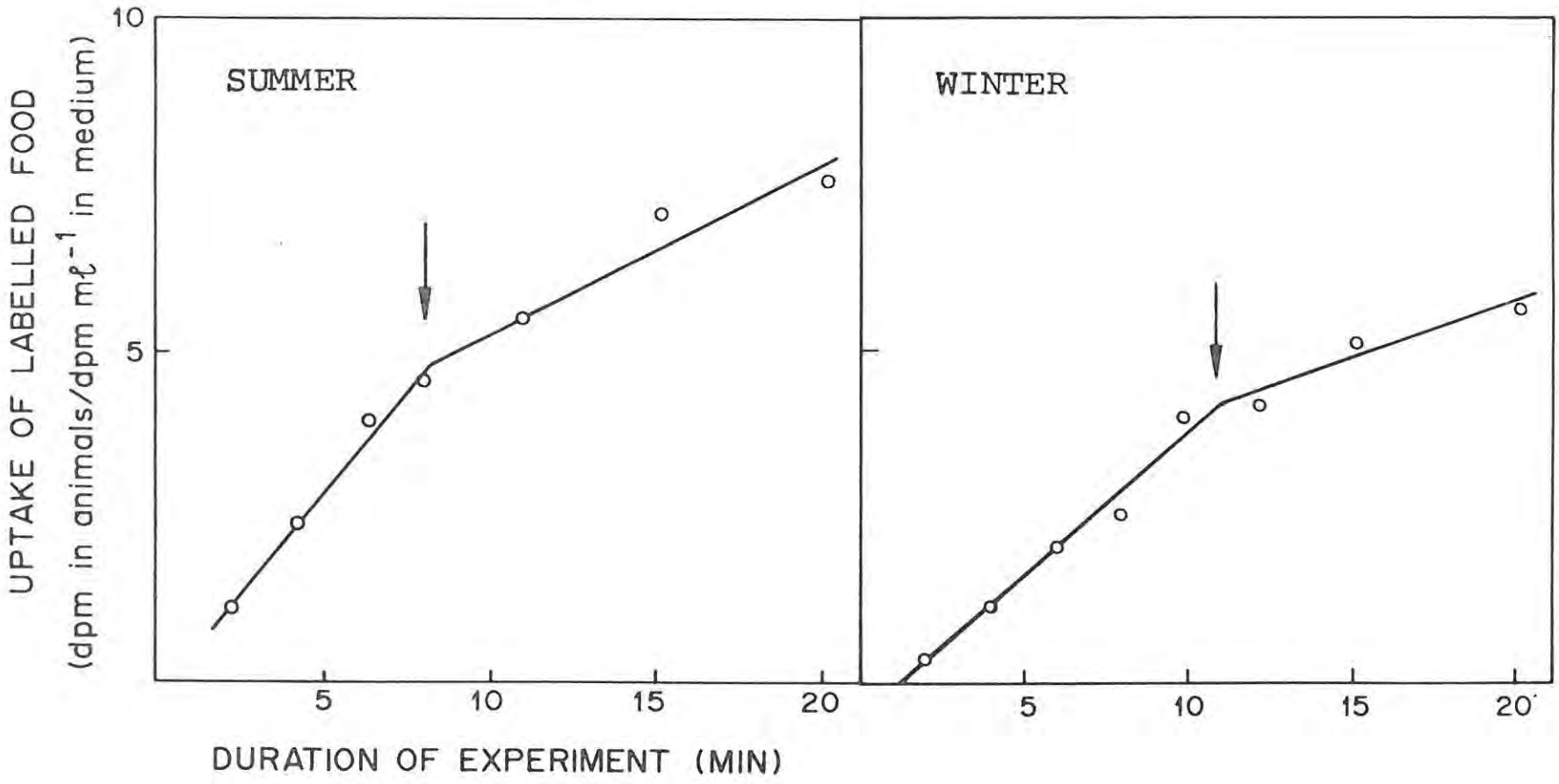


Figure 3.1: Zooplankton uptake of labelled *Chlorella* over time. Inflection point (arrow), located by linear regressions, indicates approximate minimum gut passage times in summer and winter.

the duration of the experiment does not exceed the time taken for the labelled food ingested to pass through the gut of the zooplankton present. At the start of this study the minimum gut passage time of the zooplankton community present in summer, at a water temperature of 24.6 °C, was measured by conducting a series of experiments of variable duration (2-20 min) at a fixed depth (Haney 1971). The uptake of labelled food, or increase in isotope body-burden, versus time was linear for the first 8 min, implying a constant community filtration rate (Figure 3.1a). Thereafter the apparent reduction in filtration rate was a result of loss of labelled food by defaecation. As variations in the gut passage time of each of the zooplankton species present was expected, particularly of the smaller forms, so the duration of experimental exposure to labelled algae was limited to ≅ 6 min. This duration is below the gut passage times of the zooplankton species present as also recorded in other studies (Geller 1975, Starkweather and Gilbert 1977).

A minimum gut passage time of ≅11 min (inflection point in Figure 3.1b) was measured when the series of experiments was repeated in winter at a water temperature of 13.4 °C . These gut clearance rates are similar to those reported by Hart (1986; 10 - 12 min) measured in spring in Lake Le Roux, South Africa. The influence of temperature on gut passage times has been reported in other studies. Haney (1971) measured a gut passage time >15 min at 17 °C and from ∿3-10 min at 22-25 °C . Bogdan and McNaught (1975) also reported 10 min. for gut clearance at 24 °C and Riemann and Bosselmann (1984) recorded 45 min at 1-4 °C .

3.3.2 Diel variation in grazing rates

Diel surveys were carried out during periods of lake stratification in summer and vertical mixing in winter on the 26-27.1. 1984 and 9-10.8.1984 respectively. Discrete measurements of community grazing rates, population densities, total community biomass and herbivore biomass were carried out at the usual profile sampling depths down to the oxycline in summer and to

just above the lake bottom (20 m) in winter. Samples were collected and feeding experiments conducted every 4 h during the summer survey and every 3 h during the winter survey.

Contrary to the results of Connell (1978), who reported that reversed vertical migration (day-time ascent and nocturnal descent) occurred in Hartbeespoort Dam, some zooplankton species ascended at night during the summer diel survey. For the majority of zooplankton species present during these surveys, no change in vertical distribution was evident. The most pronounced diel fluctuation in vertical distribution was exhibited by *Ceriodaphnia*, the population maxima of which remained below the depth of 1% of surface illumination during the day, as shown in Figure 3.2. Populations of *Moina*, *Thermocyclops* and *Keratella* showed only weak and insignificant upward night-time migration (mean number during day or night at each depth; $p>0.10$).

Examination of community grazing rates, total biomass, total herbivore biomass and species density revealed no evidence of vertical migration or changes in grazing activity. Percentage distributions of biomass and grazing rate throughout the water column, calculated to avoid variation due to the possibly patchy distribution of zooplankton, also showed no significant difference between day-time and night-time results ($p>0.10$; paired Students t test) as shown in Figure 3.3.

Absence of diel variations in community filtration rate and vertical distribution in another South African impoundment (Lake Le Roux) was demonstrated by Hart (1984). Results from northern temperate diel grazing studies vary considerably. Haney and Hall (1975) first reported extreme diel variation of feeding activity in four lakes in Michigan, Canada. They found that up to 85.7% of the daily grazing activity by *Daphnia* occurred at night and emphasised the importance of taking cognisance of diel variations in feeding studies (Haney and Hall 1975, Haney 1985). However, recently other studies of Canadian lakes have revealed little or no day-night influences on species or community filtration rates (Chow-Fraser

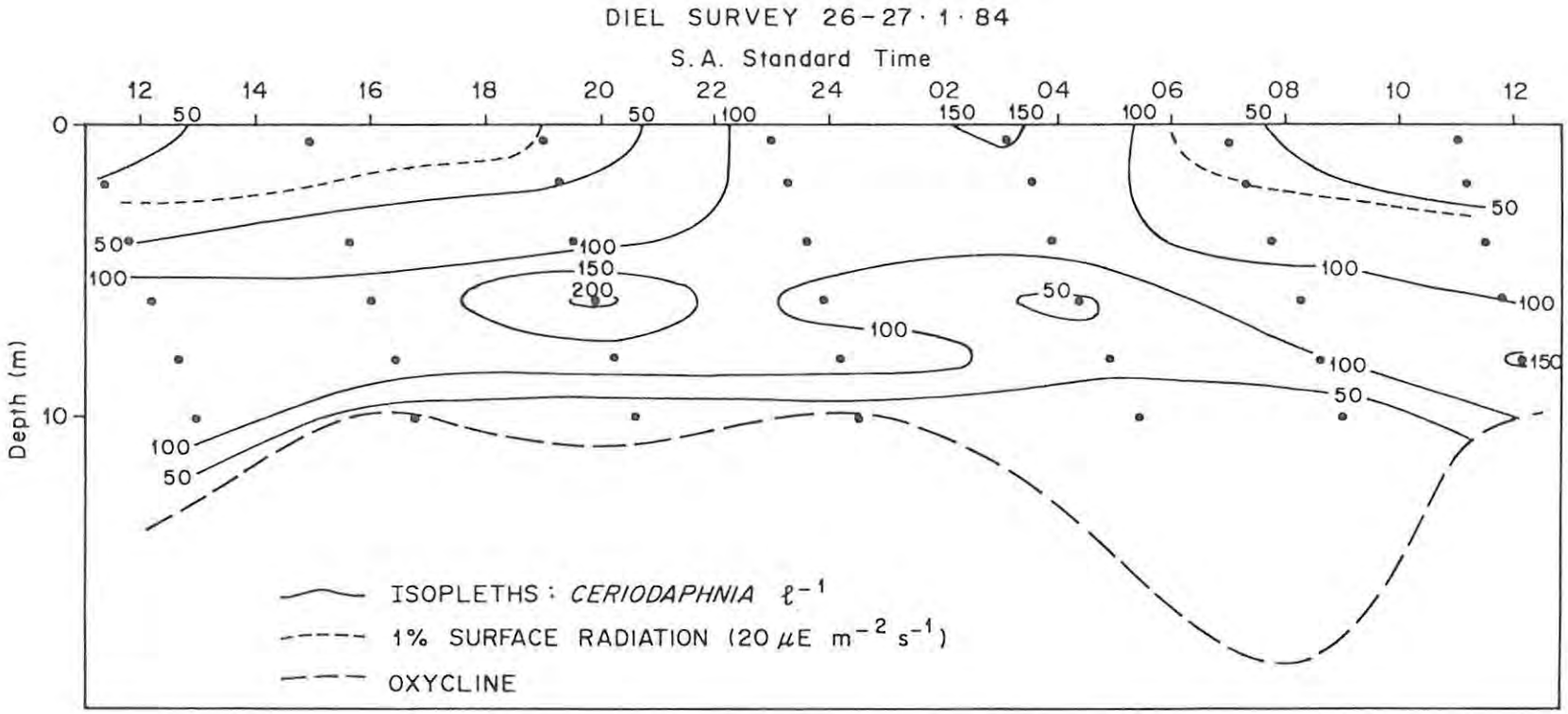


Figure 3.2: Vertical and temporal distribution of *Ceriodaphnia* during the diel survey on 26-27.1.1984.

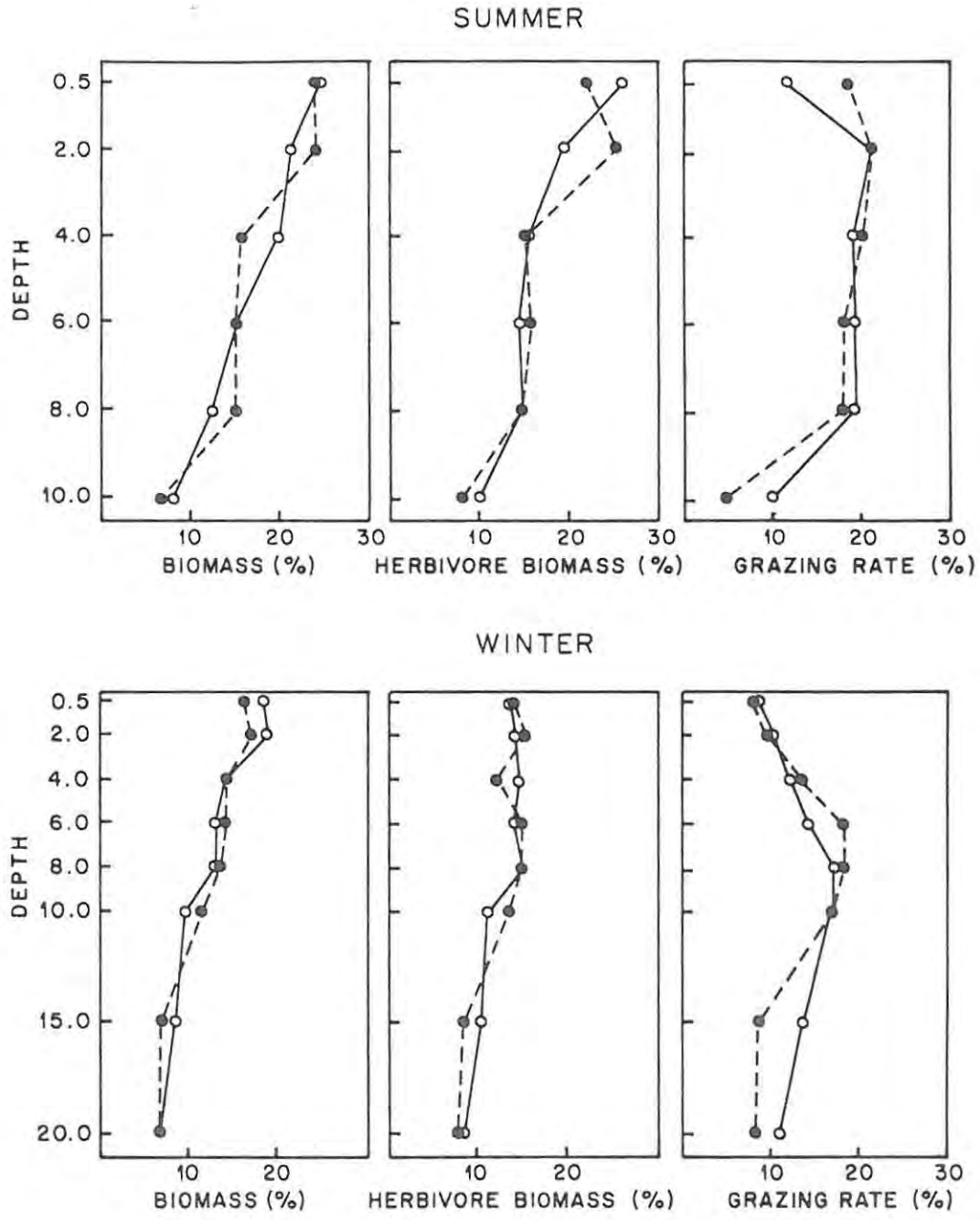


Figure 3.3: Vertical distribution of zooplankton biomass and grazing activity during diel surveys. Solid line = day; broken line = night.

and Knoechel 1985, Knoechel and Holtby 1986a). It seems likely therefore that diel variations in zooplankton distribution or feeding activity are to a large degree site and community specific, requiring individual assessment in each study programme.

3.3.3 Seasonal community grazing rates

A total of 58 vertical profiles of bimonthly *in situ* community filtration and grazing rate measurements were made between January 1983 and March 1985 yielding 386 depth-specific experimental results. Temporal and vertical variations in CGR in the main basin of Hartbeespoort Dam are shown in Figure 3.4 in relation to concurrent variations in the total biomass of herbivorous zooplankton. Depth-specific CGR and herbivore biomass varied seasonally; highest values of both variables occurred throughout the spring period of edible phytoplankton dominance and to a lesser degree in deep water during the autumn after overturn. The highest CGR measured (581.5% d^{-1} at 2 m in November 1983) was associated with a dense aggregation or swarm of *Daphnia* (1130.7 ℓ^{-1}). Most CGRs of >200% d^{-1} were associated with high numbers of this herbivore and, when *Daphnia* was present, CGR was strongly correlated with *Daphnia* abundance ($r = 0.71$, $n = 236$, $p<0.001$). Periods of lowest CGR and herbivore biomass generally occurred in mid-summer (late January and early February) and in mid-winter (June and July).

The distribution of high numbers of the two major herbivores in Hartbeespoort Dam, *Daphnia* and *Ceriodaphnia* (Figure 2.5, Section 2) corresponded with high CGR and biomass values (Figure 3.4). CGR and CFR varied with depth in association with the daytime vertical distribution of the major herbivores. CGR maxima in spring occurred high in the water column where *Daphnia* was abundant, and autumn-winter CGR maxima occurred in deep water when *Ceriodaphnia* was dominant. However, annually, the vertical variation in depth-specific CGR or CFR was not marked (Figure 3.5), being generally highest at 2.0 m and lowest at 0.5 and 15 m.

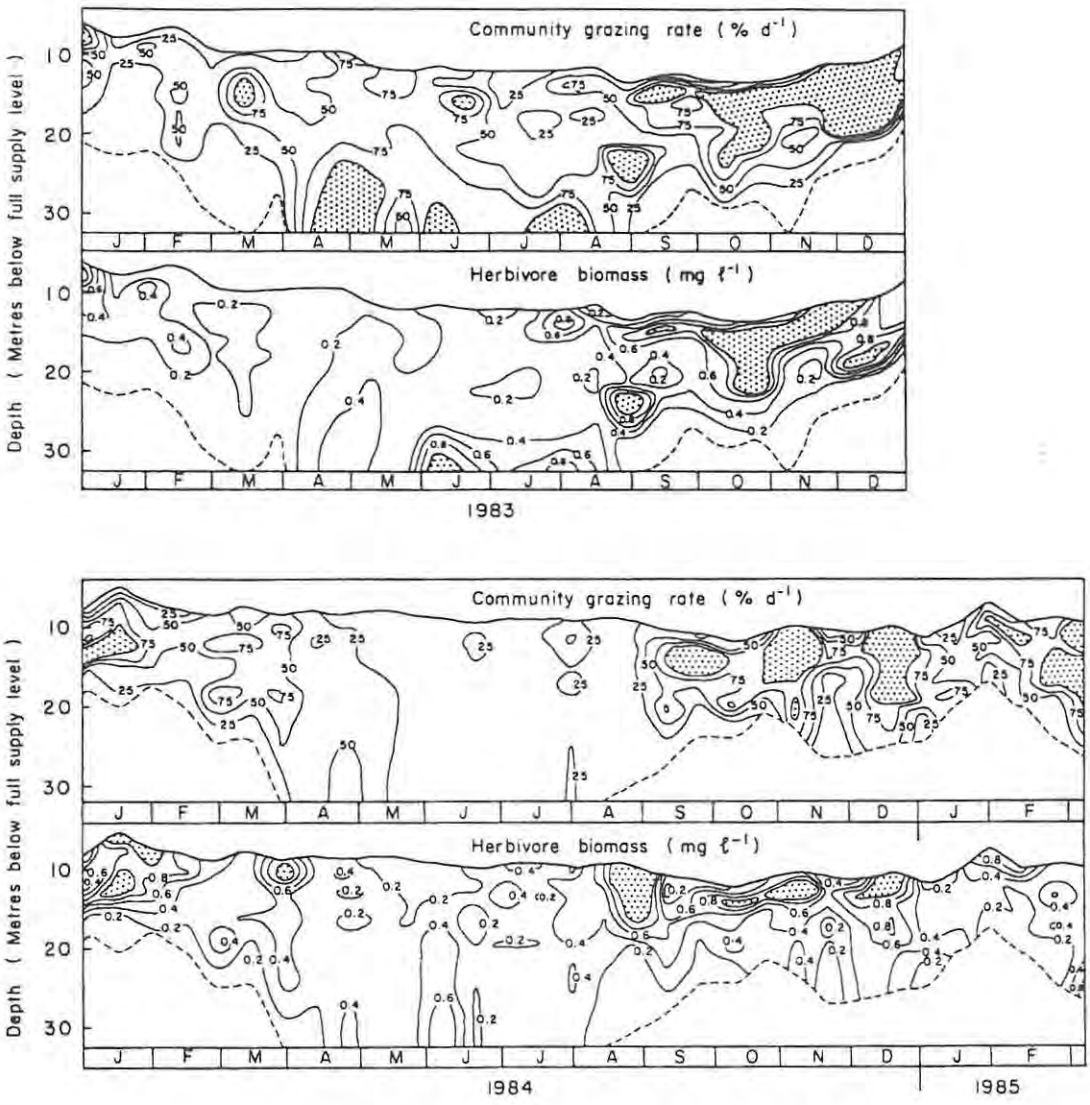


Figure 3.4: Isopleths of CGR and herbivore biomass throughout the water column. Stippled area = CGR >100% d^{-1}; herbivore biomass >1.0 mg ℓ^{-1}. Oxycline shown by broken line.

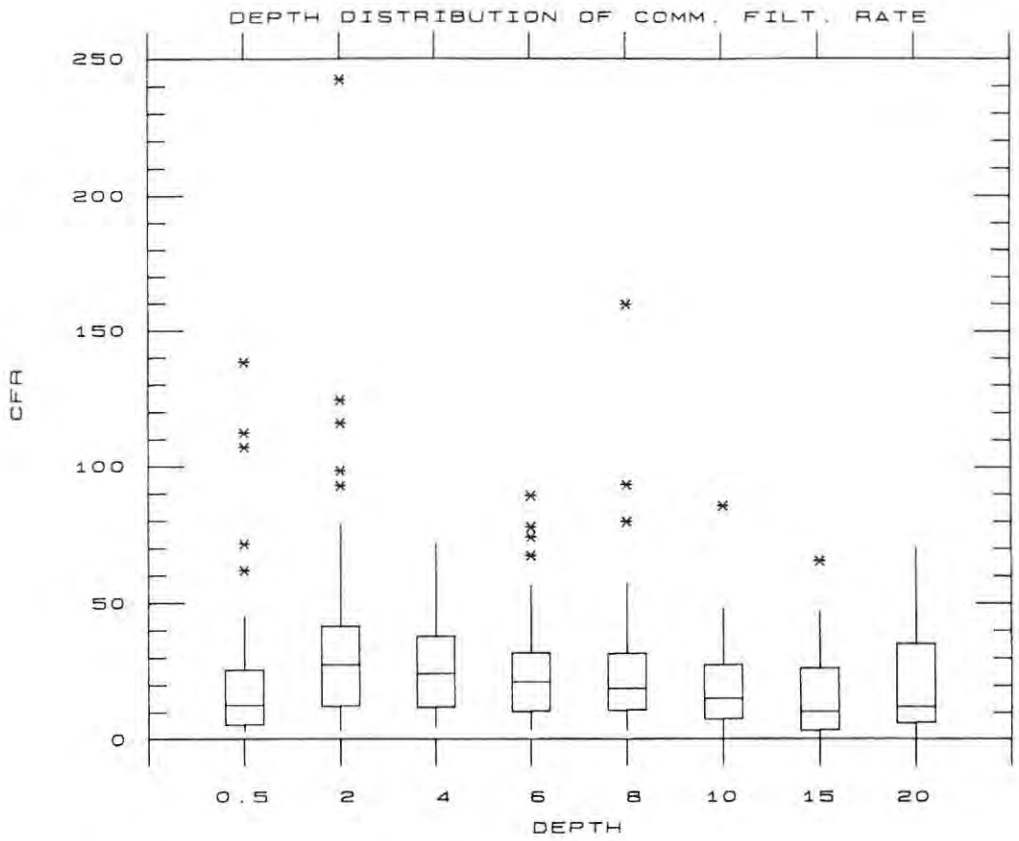


Figure 3.5: Frequency of CFR ($m\ell\ h^{-1}$) at each sampling depth, showing median, interquartile range, range and outlying points (see legend of Figure 2.3).

CFR was most strongly correlated with herbivore biomass, which accounted for 43.4% of the observed variation in CFR ($r = 0.659$, $n = 386$, $p<0.001$). CFR was best described by a linear function of herbivore biomass. Examination of Figure 3.6 shows the potential for this linear relationship to be very greatly influenced by the outlying data point representing high CFR and biomass in November 1983 (CFR = 242.3 $m\ell\ h^{-1}$ at a herbivore biomass of 4.12 mg dry weight ℓ^{-1}). The linear regression was therefore repeated following exclusion of this outlying data point. Comparison of slopes, intercepts and correlation coefficients showed that this relationship of CFR expressed as a linear function of herbivore biomass was not influenced by the extreme point (inclusion of outlyer, $a = 7.24$, $b = 41.25$, $r = 0.659$: exclusion of outlyer, $a = 7.23$, $b = 41.25$, $r = 0.659$).

CFR expressed as both linear and exponential functions of temperature were significant ($r = 0.23$, $p<0.001$ in both cases) but these relationships were not supported as temperatures rose above ~25 °C (Figure 3.7). With highest temperatures in mid-summer, CFR was low following the shift from a *Daphnia* dominated high biomass community to a *Ceriodaphnia* dominated low biomass community. This occurred in association with the annual mid-summer shift in phytoplankton dominance from edible chlorophytes and cryptophytes to largely inedible *Microcystis* (see Section 2).

No significant correlations existed between depth-specific CFR and food resources expressed either as the dry weight of total seston <60 µm or as chlorophyll *a* concentration (Figures 3.8 and 3.9). In both cases the CFR was high when these resources were low and *vice versa*, the relationship between these variables loosely conforming to that of a negative power function.

Identification of variables that significantly accounted for most of the variance in depth-specific CFR was carried out by stepwise multiple regression analysis. CFR was expressed in terms of herbivore biomass, temperature, chlorophyll *a* concen-

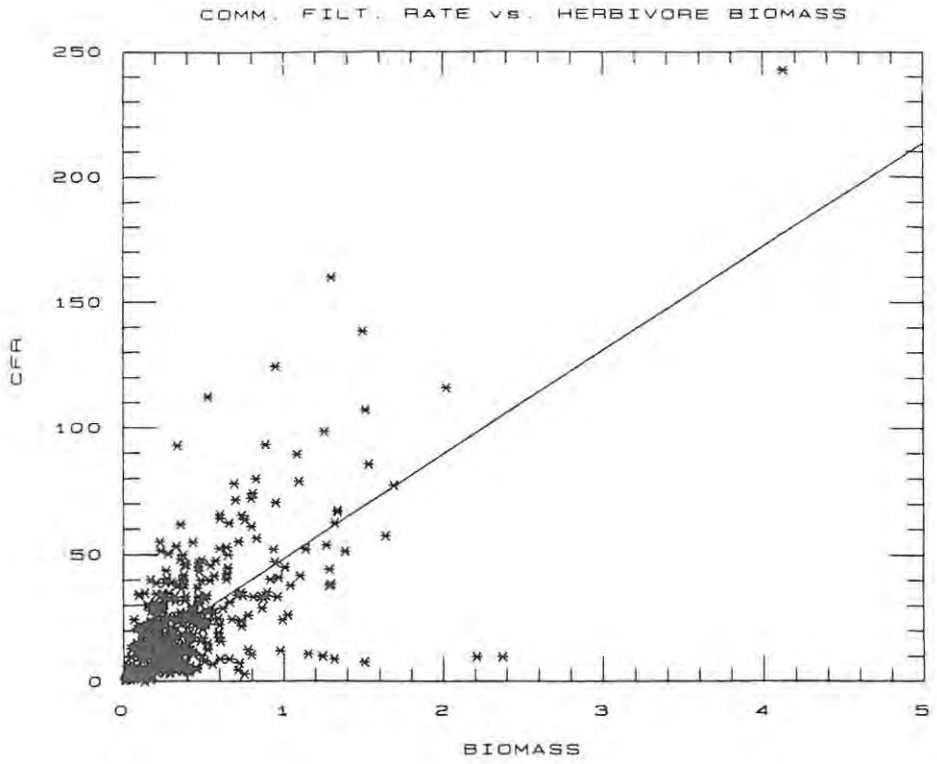


Figure 3.6: Scatter-plot and linear regression of CFR (mℓ h^{-1}) as a function of herbivore biomass (mg ℓ^{-1}).

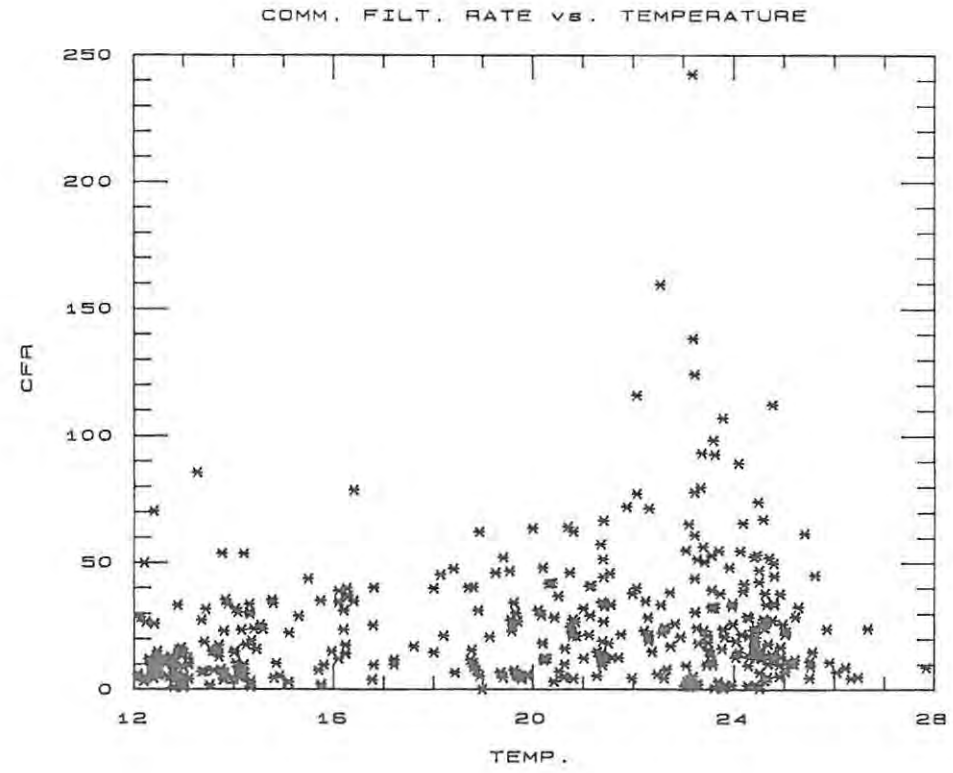


Figure 3.7: Scatter-plot of CFR (mℓ h^{-1}) against temperature (°C)

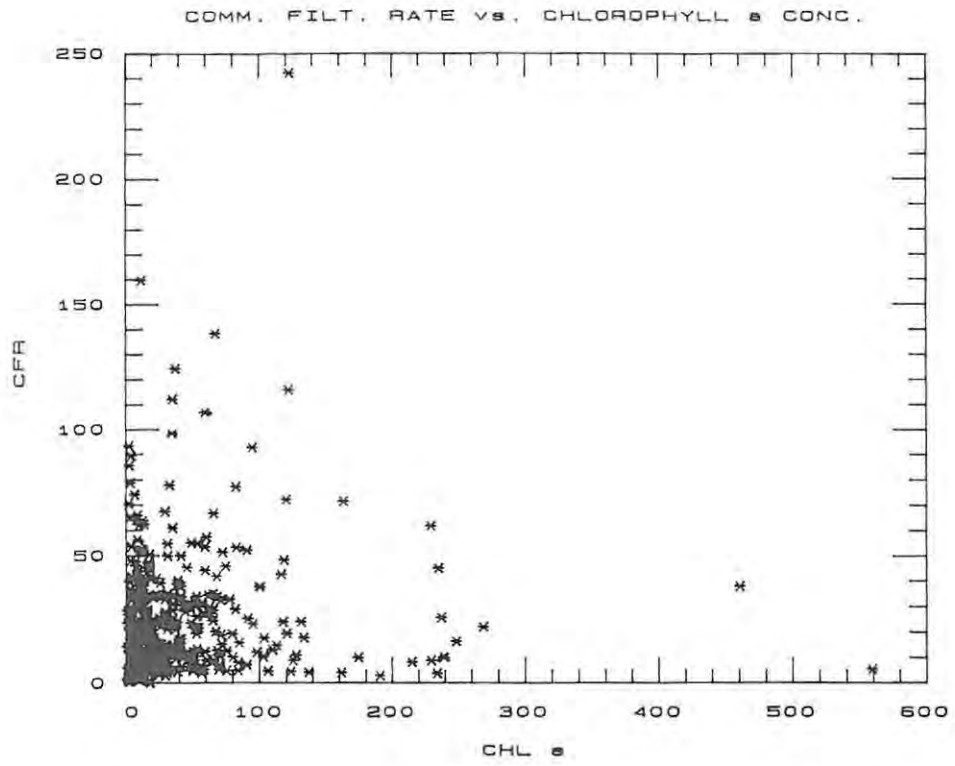


Figure 3.8: Scatter-plot of CFR (mℓ h^{-1}) against chlorophyll *a* concentration (µg ℓ^{-1})

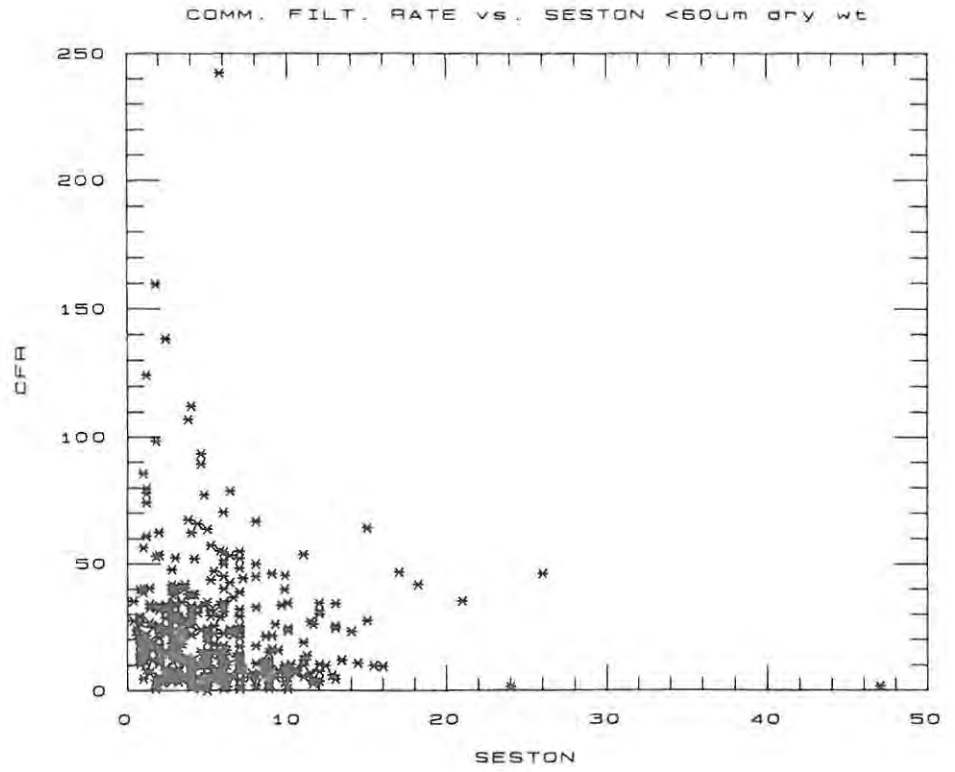


Figure 3.9: Scatter-plot of CFR (mℓ h^{-1}) against seston <60 µm (mg ℓ^{-1})

Table 3.0. Stepwise multiple regression analysis of depth-specific CFR. Variance in CFR explained as a function of herbivore biomass (B, mg ℓ^{-1}), temperature (T, °C), chlorophyll *a* concentration (C, µg ℓ^{-1}) and seston <60 µm dry weight (s, mg ℓ^{-1}) in order of entry into the model.

Variable	Coefficient	R^2	ΔR^2	F ratio
Constant	-9.45			
B	40.97	0.436	0.436	310.24
T	1.11	0.472	0.036	28.44
C	-0.07	0.498	0.026	17.16
S	-0.50	0.505	0.007	5.32

Degrees of freedom: Model = 5, Residual = 354 $p<0.001$

Table 3.1. Stepwise multiple regression analysis of depth-specific CFR. Variance in CFR explained as a function of herbivore biomass (B, mg ℓ^{-1}), temperature (T, °C), chlorophyll *a* concentration (C, µg ℓ^{-1}), their squares (B^2, T^2, C^2) and their interaction terms (BT, BC, TC). Significant terms shown only, in order of entry in the model.

Variable	Coefficient	R^2	ΔR^2	F ratio
Constant	9.07			
BT	3.03	0.497	0.497	65.75
BC	-0.18	0.543	0.046	38.92
B^2	5.80	0.549	0.003	10.88
B	-19.39	0.556	0.007	5.03
C^2	0.0001	0.561	0.005	3.96

Degrees of freedom: Model = 6, Residual = 368 $p<0.001$

tration, dry weight of seston <60 µm and depth. In a CFR regression model based on these variables, variance explained by depth was not significant ($F = 1.17$, $p>0.5$). Therefore this variable was excluded from further analyses.

As expected from Figures 3.6 - 3.9, herbivore biomass accounted for most (over 43%) of the variance in depth-specific CFR. Temperature and chlorophyll *a* concentration explained a further 3.6% and 2.6% respectively (Table 3.0). Whilst reduction in unexplained variance by the inclusion of seston in the model was significant, model improvement by seston was slight ($\Delta R^2 = 0.7\%$) and its influence potentially a duplication of that of chlorophyll *a* concentration. Therefore seston was also excluded from further analyses.

The influence of herbivore biomass (B), temperature (T), chlorophyll *a* concentration (C), their squares and their interaction terms (B^2, T^2, C^2 and BT, BC, TC) on depth-specific CFR was also examined by stepwise multiple regression. Of the terms entering this regression model and contributing significantly to a reduction in unexplained variance in CFR, the interaction term BT accounted for almost 50% of the observed variance (Table 3.1). All terms based on or including herbivore biomass entered the regression model as significant variables with the interaction between herbivore biomass and chlorophyll *a* concentration further reducing unexplained variance by 4.6%.

From the multiple regression equation of the interactive influences of herbivore biomass and temperature as expressed by the second-order polynomial function

$$CFR = 3.30BT - 31.35B + 1.13B^2 + 8.33T - 0.23T^2 - 61.74 \quad (3.1)$$
$$(R^2 = 0.525;\ F = 83.9;\ df\ 5, 385;\ p<0.001)$$

the CFR predictions derived can be represented graphically as response surface plots shown both in 3-dimensional surface form and as contour plots for clarity of interpretation (Figure 3.10a and b). The slight negative influence of high

temperatures on CFR when herbivore biomass was low (during the mid-summer *Ceriodaphnia* phase) is evident in these response surface plots (see also Figure 3.7), whilst the interaction of biomass and temperature had a strong positive influence on CFR.

The interaction of herbivore biomass and chlorophyll *a* concentration also had a marked influence on CFR. Depth-specific CFR expressed as a function of biomass and chlorophyll was:

$$CFR = -0.18BC + 41.33B + 7.27B^2 + 0.012C + 0.0001C^2 + 7.98 \quad (3.2)$$

$$(R^2 = 0.483;\ F = 68.6;\ df\ 5, 373;\ p<0.001)$$

Response surface plots (Figure 3.11a and b) show the marked negative influence of increasing chlorophyll *a* concentration on CFR (highest chlorophyll values typically due to dense *Microcystis* blooms; see Section 2) which becomes steadily more pronounced as herbivore biomass increases (highest biomass typically occurs when *Daphnia* is most abundant in early summer; see Section 2).

Stepwise multiple regression analysis of the influence of the principle zooplankton taxa on CFR was carried out using *Daphnia* and *Ceriodaphnia* numbers, total number of all rotifer species, and *Thermocyclops* number. *Daphnia* and *Ceriodaphnia* were selected as they were the dominant herbivore species characteristic of either the late winter - early summer *Daphnia* phase (typically co-occurring with *Bosmina*) or the *Ceriodaphnia* phase (typically co-occurring with *Moina* and *Diaphanosoma*) as described in detail in Section 2. The influence of rotifers was included in this CFR model in a combined all-species form as their individual effect on CFR was expected to be only slight. *Thermocyclops* was included as it is generally very abundant in Hartbeespoort Dam, but was not regarded as being a likely herbivore.

The regression model of CFR by species including the forced entry of non-significant variables is shown in Table 3.2. As

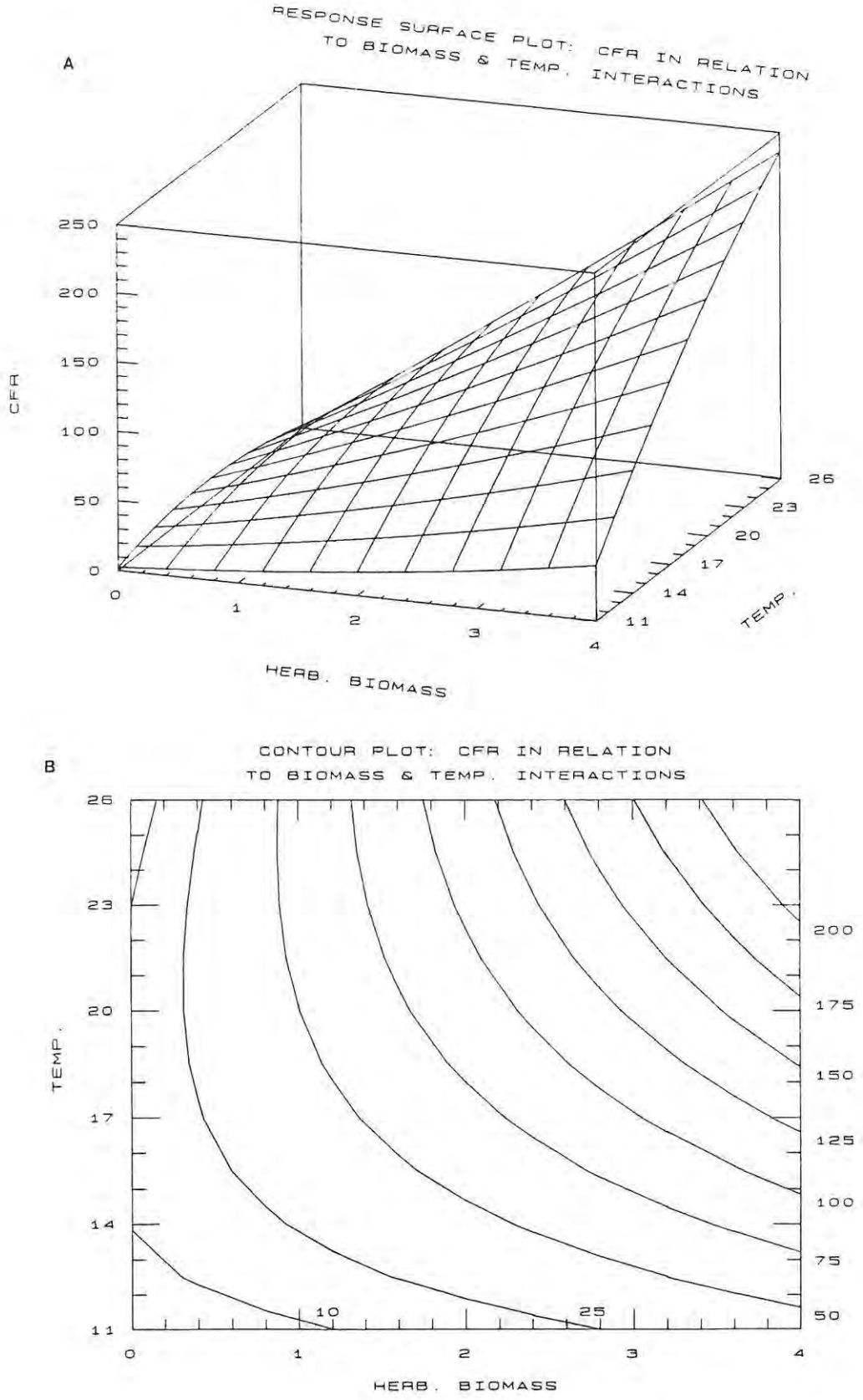


Figure 3.10: Response surface plots of CFR ($m\ell\ h^{-1}$) in relation to interactions of herbivore biomass (mg ℓ^{-1}) and temperature (°C) expressed in their polynomial form; (A) three-dimensional and (B) contour plot.

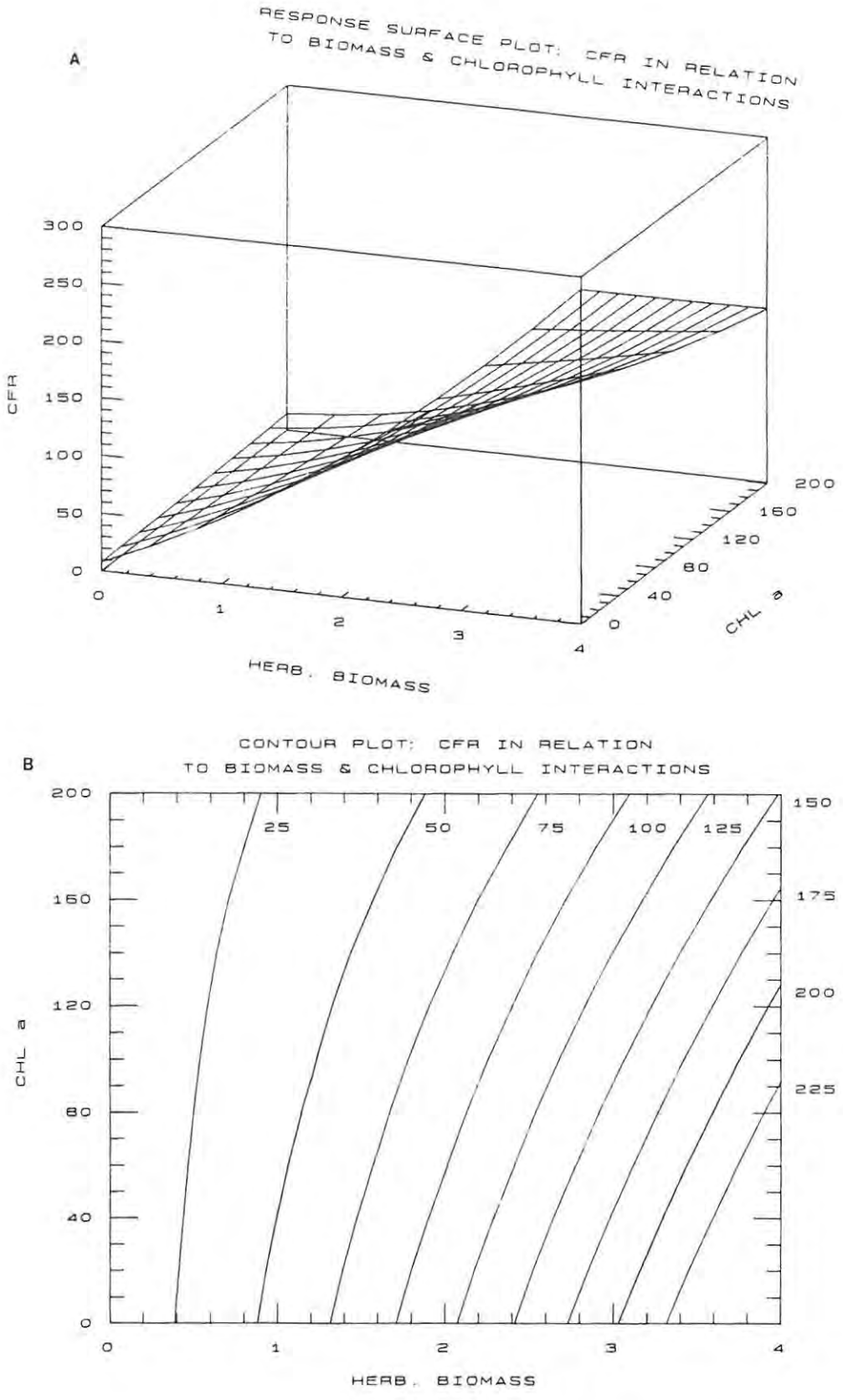


Figure 3.11: Response surface plots of CFR (mℓ h^{-1}) in relation to interactions of herbivore biomass (mg ℓ^{-1}) and chlorophyll *a* concentration (µg ℓ^{-1}) expressed in their polynomial form; (A) three-dimensional and (B) contour plot.

anticipated, inclusion of *Daphnia* and *Ceriodaphnia* numbers accounted for much of the variance in depth-specific CFR (R^2 = 0.590). However, the abundance of rotifer species had no significant influence on this CFR model, whilst the negligible influence of *Thermocyclops* supports the assumption that this cyclopoid copepod is a likely carnivore.

Data for each experimental profile gathered over the aerobic water column (>1.0 mg O_2 ℓ^{-1}) were integrated by volume weighting (vertical compartmentalization). These resulting integrated rates and values which incorporate vertical variations in temperature, zooplankton biomass and CGR are shown in Figure 3.12. Integrated community grazing rates (ΣCGR) varied from 10.7 to 260.2% d^{-1} and was low both during periods of highest (January - February) and lowest water temperatures (June - August). High ΣCGR (>100% d^{-1}) occurred in association with high integrated herbivore biomass (ΣB) usually during the late winter - early summer edible phytoplankton/ *Daphnia* phase. An exception arose in March 1985 when a ΣCGR of 141.3% d^{-1} was recorded when *Ceriodaphnia* was particularly abundant throughout the aerobic water column (245.3 - 501.3 animals ℓ^{-1}) whilst integrated total herbivore biomass was low. Generally ΣCGR was low while *Ceriodaphnia* dominated the characteristically low biomass zooplankton community of summer - autumn. Thereafter ΣCGR was very low during the mid-winter periods of 1983 and 1984 (28.3 and 10.7% d^{-1} respectively) before rising to spring - early summer maxima in both years. Annually, with the return to dominance of *Microcystis* at mid-summer, the *Daphnia* population declined rapidly and *Ceriodaphnia* became the dominant herbivore of the small bodied/low biomass community (see Section 2). A characteristic of this event was the distinct reduction in ΣCGR in January and February (Figure 3.12). In 1983 and 1985 this decline in ΣCGR was brief following development of extremely high *Ceriodaphnia* population densities by March in 1983 and by as early as mid-February in 1985 (Figure 2.5, Section 2). In 1984, when *Ceriodaphnia* failed to develop full community dominance, and was supplanted by *Moina* and *Diaphanosoma*, autumn ΣCGRs were lower than in 1983 (Figure 3.12).

Table 3.2. Stepwise multiple regression analysis of depth-specific CFR. Variance in CFR explained as a function of zooplankton taxon (see text for details). Non-significant terms included in order of entry into the model.

Variable	Coefficient	R^2	ΔR^2	F ratio
Constant	12.88			
Daphnia	0.24	0.474	0.474	473.95
Ceriodaphnia	0.07	0.590	0.116	105.66
Total rotifers	-0.0007	0.590	0.000	0.49*
Thermocyclops	-0.0013	0.590	0.000	0.08*

Degrees of freedom: Model = 5, Residual = 354 $p<0.001$ *$p>0.5$

Table 3.3. Stepwise multiple regression analysis of integrated CGR. Variance in ΣCFR explained as a function of integrated herbivore biomass (ΣB, mg ℓ^{-1}), integrated temperature (ΣT, °C) and integrated chlorophyll *a* concentration (ΣC, μg ℓ^{-1}).

Variable	Coefficient	R^2	ΔR^2	F ratio
Constant	-34.56			
ΣB	112.77	0.485	0.485	46.59
ΣT	2.70	0.535	0.050	5.69
ΣC	-0.01	0.540	0.005	0.64*

Degrees of freedom: Model = 4, Residual = 52, $p<0.001$ *$p>0.5$

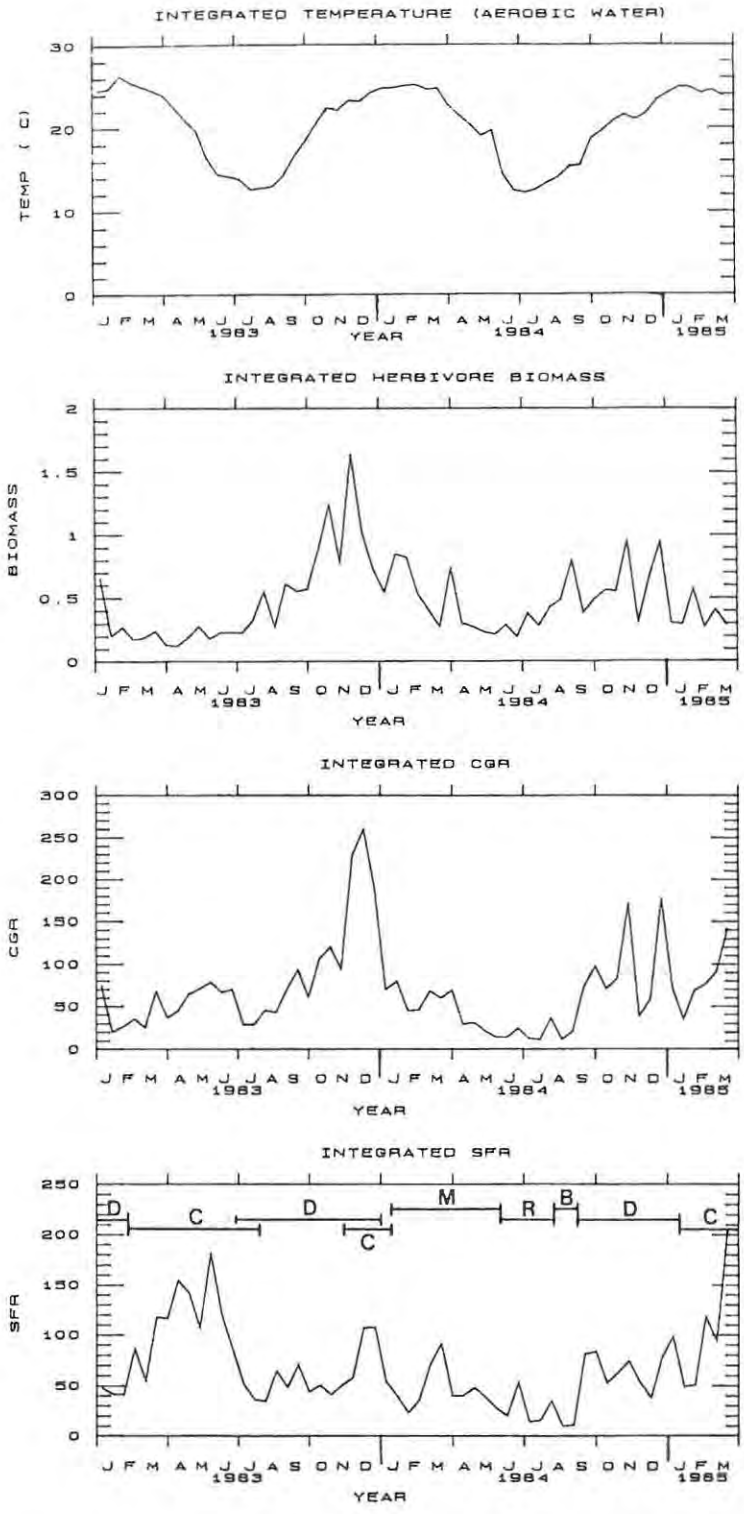


Figure 3.12: Seasonal variation of integrated temperature (°C), herbivore biomass (mg ℓ^{-1}), CGR (% d^{-1}) and SFR (mg h^{-1}) over the total aerobic water column. Periods of dominance and co-dominance of *Daphnia* (D), *Ceriodaphnia* (C), *Moina* and *Diaphanosoma* (M), rotifers (R) and *Bosmina* (B) are shown by horizontal bars.

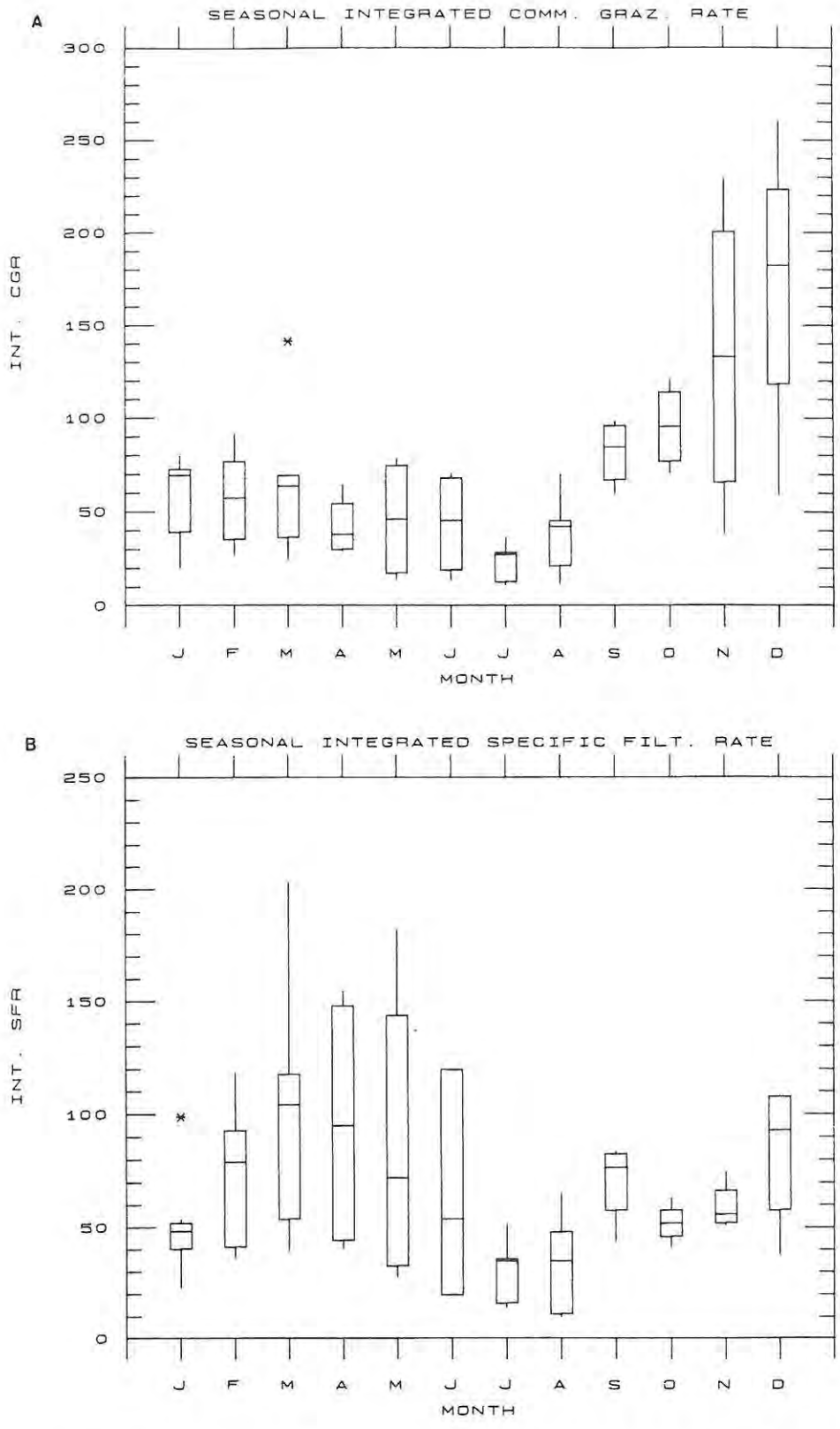


Figure 3.13: Frequency of ΣCGR (% d^{-1}) and ΣSFR (mℓ mg^{-1} h^{-1}) after grouping of bimonthly data over 27 months into monthly datasets, showing median, interquartile range, range and outlying points (see legend of Figure 2.3).

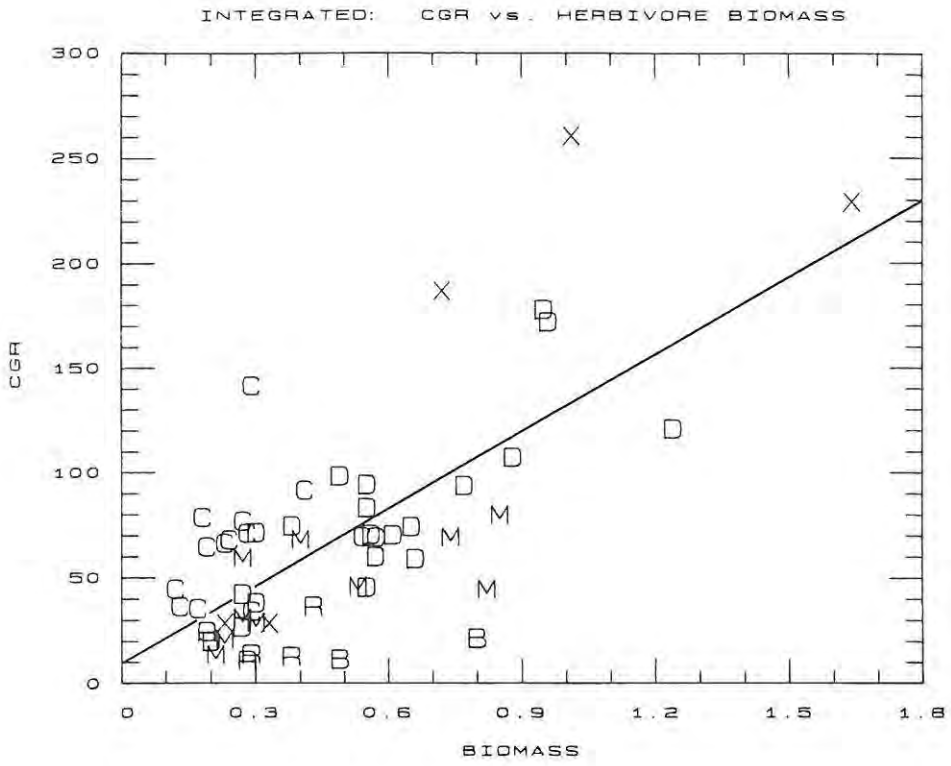


Figure 3.14: ΣCGR (% d^{-1}) in relation to Σherbivore biomass (mg ℓ^{-1}). Points coded by dominant grazer as in Figure 3.12. X = *Daphnia : Ceriodaphnia* co-dominance.

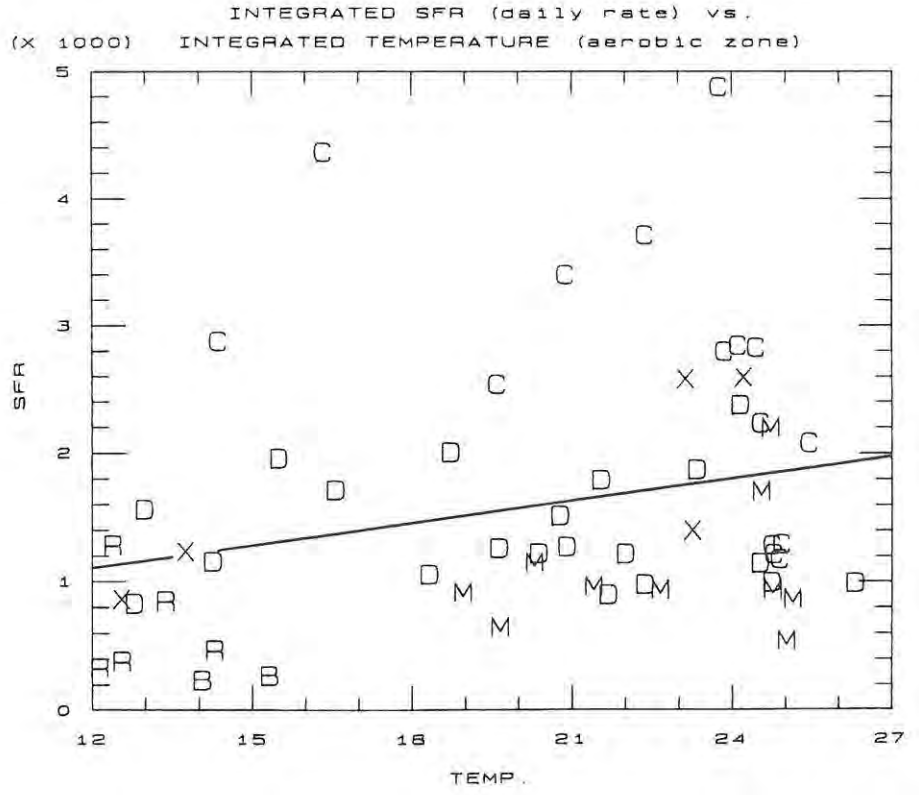


Figure 3.15: ΣSFR (mℓ mg^{-1} d^{-1}) in relation to Σtemperature (°C). Points coded by dominant grazer as in Figure 3.12. X = *Daphnia : Ceriodaphnia* co-dominance.

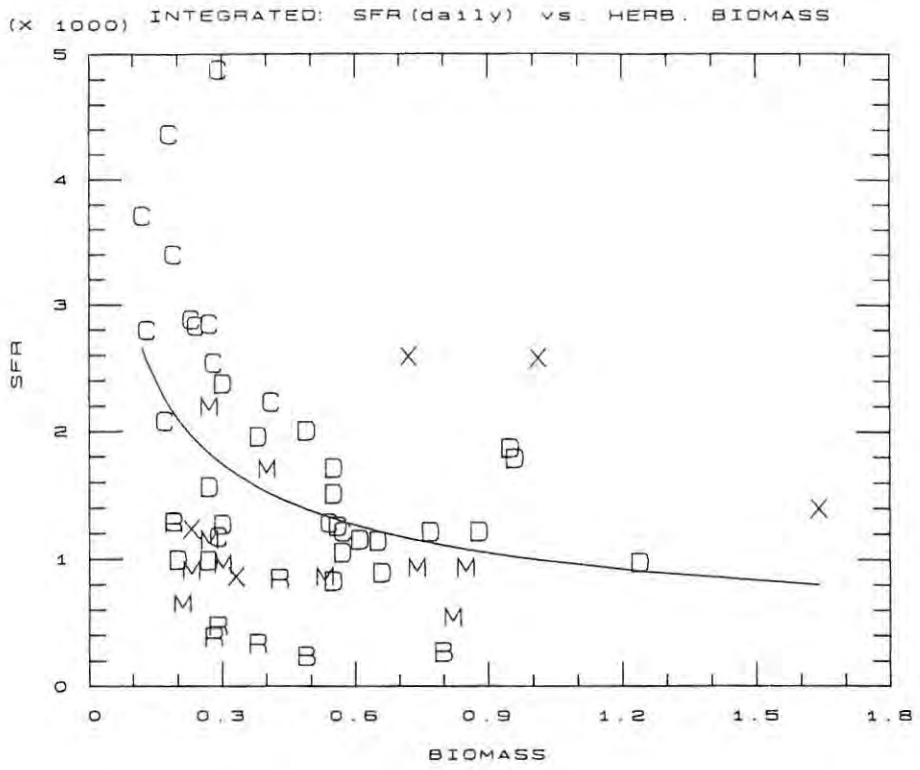


Figure 3.16: ΣSFR (mℓ mg^{-1} d^{-1}) in relation to Σherbivore biomass (mg ℓ^{-1}). Points coded by dominant grazer as in Figure 3.12. X = *Daphnia : Ceriodaphnia* co-dominance.

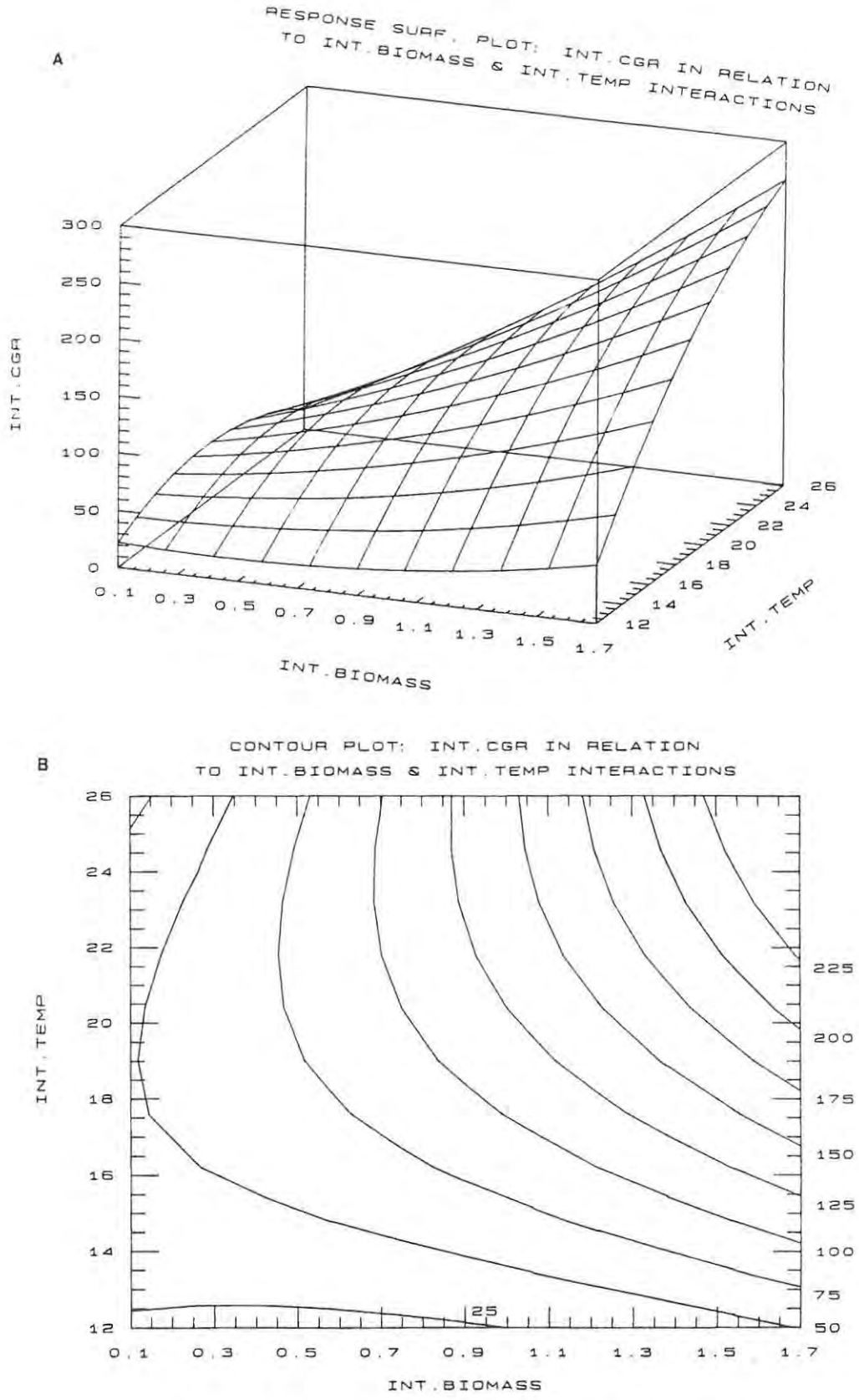


Figure 3.17: Response surface plots of ΣCGR (% d^{-1}) in relation to interactions of Σherbivore biomass (mg ℓ^{-1}) and Σ temperature (°C) expressed in their polynomial form; (A) three-dimensional and (B) contour plot.

Integrated biomass specific filtration rates of the community (ΣSFR, mℓ mg^{-1} dry weight h^{-1}) also varied seasonally in relation to the composition of the zooplankton grazer community. ΣSFR was highest when *Ceriodaphnia* was abundant and ΣB was generally low. Annually, the lowest ΣSFRs occurred in mid-summer and mid-winter (Figures 3.12 and 3.13). The former low was associated with the transition period between the *Daphnia* and *Ceriodaphnia* phases when *Microcystis* abundance was increasing, and the latter with low water temperatures.

Relationships were examined between both ΣCGR and ΣSFR and the depth-integrals of variables previously identified as important predictors of depth-specific CGR. ΣCGR was a significant linear function of ΣB ($r = 0.699$, $n = 57$, $p<0.001$, Figure 3.14) whilst the linear relationship of ΣSFR with integrated temperature (ΣT) was less pronounced ($r = 0.103$, $p<0.02$, Figure 3.15) as was also the case in the depth-specific correlations. No significant relationship existed between ΣCGR and integrated chlorophyll *a* concentration (ΣC).

ΣSFR (mℓ mg^{-1} h^{-1}) was best expressed as a negative power function of ΣB (Figure 3.16; line drawn from Equation 3.3 after conversion to daily rate)

$$\Sigma SFR = 41.6\ (\Sigma B^{-0.46}) \qquad (3.3)$$
$$(r^2 = 0.393,\ F = 90.3,\ p<0.001)$$

which follows from the inclusion of ΣB in the calculation of ΣSFR (i.e. ΣSFR = ΣCFR/ΣB). Linear correlation between ΣSFR and ΣT was weaker than between their depth-specific values ($r = 0.259$, $p = 0.51$, Figure 3.15).

Regressions plotted for both ΣCGR and ΣSFR (Figures 3.14 and 3.15) include coded points to further highlight any influence associated with the structure of the grazer community on these integrated feeding relationships. Generally, low ΣCGR but high ΣSFR were associated with abundant *Ceriodaphnia* whilst both ΣCGR and ΣSFR were low when *Moina* and *Diaphanosoma*, rotifers and *Bosmina* were abundant during 1984.

Stepwise multiple regression of ΣCGR in terms of ΣB, ΣT and ΣT (Table 3.3) again shows the significance of herbivore biomass and temperature as predictors of community grazing rather than food resource concentration *per se* (if food quality factors are ignored, see Section 2). A multiple regression model of ΣCGR in terms of the interactive influence of ΣB and ΣT was derived:

$$\Sigma CGR = 9.82\Sigma BT - 137.39\Sigma B + 20.98\Sigma B^2 + 21.193\Sigma T - 0.59\Sigma T^2 - 145.77 \quad (3.4)$$

$$(R^2 = 0.583;\ F = 14.3;\ df\ 5, 56;\ p<0.001)$$

Response surface plots of ΣCFR predictions based on this polynomial function (Figures 3.17a and b) are similar to those derived on depth-specific CFR data (Figure 3.10), but show a more pronounced negative influence of high ΣT on ΣCFR. When herbivore biomass is low, ΣCFR is maximal between 18-20 °C, above which grazing rates decline as the temperature rises to its mid-summer maximum.

3.4 Discussion

Zooplankton CGR calculated as % per day represents the potential maximum percentage of suspended particulates in the surrounding medium, of similar size to the radiolabelled cells used, that may be filtered by the zooplankton present. However, not all natural food particles encountered may be ingested by zooplankton. In addition to rejection of some food particles, the patchy distribution of phytoplankton, preferential filtration in natural mixtures of food, and the low grazing efficiency or filtration rates of zooplankton on large algae (particularly *Microcystis*; see Section 4) prevents removal of all food resources during periods of localized high grazing activity. Therefore CGR and CFR measured using *Chlorella* represent the maximum grazing or filtration rates independent of variations in food type and palatability.

Seasonality in CGR over the 27 month study period was very pronounced (Figures 3.4 and 3.12). Grouping of ΣCGR into

monthly datasets summarises and highlights this seasonality (Figure 3.13a). High integrated CGRs occurred in November - December (usually >100% d^{-1}) at the end of the period of edible phytoplankton abundance when *Daphnia* was most abundant and chlorophyte and cryptophyte components of the phytoplankton were diminishing rapidly as the density of *Microcystis* was increasing (Section 2). Throughout the aerobic water column the edible phytoplankton species therefore undergo extremely high grazing pressure at this time of maximum herbivore biomass. The virtual absence of chlorophytes in sediment traps in Hartbeespoort Dam during November and December (NIWR 1985) supports the view that the further development of edible phytoplankton species is severely limited by high zooplankton grazing pressure. Thus, these results do not support the suggestion made by Schoenberg and Carlson (1984) that large cladocerans such as *Daphnia*, through direct heavy grazing pressure and associated abiotic factors, can retard the development of cyanophytes in hypertrophic systems. In hypertrophic Hartbeespoort Dam *Daphnia*, which are abundant in spring, can by intensive grazing lead to the rapid decline of edible phytoplankton species and thereby may promote the complete dominance of the phytoplankton by *Microcystis* throughout most of the year.

ΣSFR exhibited a very different seasonal pattern to that of ΣCGR (Figure 3.13b). ΣSFR was low in January each year following the return to phytoplankton dominance of *Microcystis*, but as the summer zooplankton community, characterized by the small bodied cladocerans *Ceriodaphnia, Moina* and *Diaphanosoma* (*Ceriodaphnia* phase), became established so the community ΣSFR rose. Maximum biomass specific filtration typically occurred in March - April when dominance by *Microcystis* often approached 100%. Thereafter ΣSFR declined to a mid-winter minimum in July.

3.4.1 Taxonomic influences on grazing

High SFRs during the late summer *Ceriodaphnia* phase may be attributed to the particularly high filtration rate of *Cerio-*

daphnia in relation to its size (noted also by Chow-Fraser and Knoechel 1985, Knoechel and Holtby 1986a and b, and in Section 4). They occur primarily when only low concentrations of edible phytoplankton resources are available. This high SFR indicates that feeding by the *Ceriodaphnia* dominated community during summer-autumn is not inhibited by *Microcystis* toxicity (Lampert 1981) nor by interference to efficient filter-feeding in the presence of abundant cyanophyte colonies (Webster and Peters 1978, Gliwicz and Siedlar 1980, Porter and McDonough 1984).

During the *Daphnia* phase (early spring to mid-summer) SFR rose from the mid-winter minimum (Figure 3.13b), but was not as high as during the *Ceriodaphnia* phase (mid-summer to late autumn). The highest SFRs of the *Daphnia* phase usually occurred in December when the edible phytoplankton resource was most severely depleted following high grazing pressure (compare Figure 3.13b with Figure 2.3 of Section 2). This indicates that as grazing intensity on palatable algae (eg. *Chlorella*) increases, resource limitation plays a major role in the succession of zooplankton species from a large to a small herbivore community in Hartbeespoort Dam. Furthermore, as *Microcystis* dominance becomes established in January, so the SFR of the remaining, and still declining, *Daphnia* population is very low indicating the low filtration efficiencies of large herbivores in the presence of abundant large *Microcystis* colonies (see further evidence of this influence in Section 4.4.1 'Species-specific filtration rates ...').

The influence of the composition of the grazer community on rates measured is further demonstrated in scatter-plots (Figures 3.14 - 3.16) coded by the dominant herbivore present as indicated in Figure 3.12. High ΣCGRs occurred when either *Daphnia* was numerous or when both *Daphnia* and *Ceriodaphnia* were abundant at mid-summer (Figure 3.14). Highest grazing rates were recorded at the end of the *Daphnia* phase when SFR and *Daphnia* numbers were also high. Both ΣSGR and ΣSFR were low when *Daphnia* and *Ceriodaphnia* were co-dominant at mid-winter).

During the *Ceriodaphnia* phase both ΣCGR and herbivore biomass were low, but occasionally during this phase *Moina* and *Diaphanosoma* were also abundant and during 1984 were co-dominant. Co-dominance by these cladocerans was characterized by higher herbivore biomass but similar or slightly lower ΣCGR than during *Ceriodaphnia* dominance (Figure 3.14). Consequently ΣSFR was typically high during *Ceriodaphnia* dominance and lower during *Moina* and *Diaphanosoma* co-dominance (Figure 3.16).

Brief periods of dominance by other grazers, rotifers and *Bosmina* (indicated in Figures 3.12 and 3.14 - 3.16) were characterized by lowest ΣCGR and ΣSFR values. Whilst low ΣCGR was expected of the small body size - low biomass rotifer community, low ΣSFR indicates that *Chlorella* is not necessarily a preferred food of these small filter-feeders. Low size-specific grazing on *Chlorella* by *Bosmina* and the rotifer *Brachionus* was recorded during species-specific experiments and is reported in Section 4.

Hart (1984, 1986) showed that in another subtropical African impoundment (turbid Lake Le Roux) copepods exerted a stronger influence on CFR than cladocerans. Unfortunately due to the very different trophic status and hence zooplankton community composition between Lake Le Roux and Hartbeespoort Dam, only limited comparison between these studies can be made. Of the two copepods present in Hartbeespoort Dam, *Thermocyclops* was common but is not a herbivore, and *Thermodiaptomus* was not sufficiently numerous to greatly influence CFR or SFR. General comparison between SFRs (daily rates) of Lake Le Roux (Hart 1984, Figure 4; 1986, Figure 7) and of Hartbeespoort Dam (Figures 3.14 - 3.16), which removes effects of dissimilarities in herbivore biomass, shows a general similarity in the magnitude of SFR in these subtropical impoundments. Further comparison with those rates reported for a number of northern temperate lakes of varying trophy by Gulati *et al.* (1982) requires conversion of SFR units (mℓ mg^{-1} zoop. dry weight d^{-1}) to mℓ mg^{-1} zoop. C d^{-1}. Assuming a carbon content of 40% of zooplankton dry weight (Lampert 1985) daily SFRs recorded

in Dutch lakes (Gulati *et al.* 1982) were generally 2-4 times lower than converted rates for Hartbeespoort Dam and also lower than for Lake Le Roux (Hart 1984, 1986). This reflects differences due probably to both the species composition of the zooplankton communities and the temperature regimes between these studies.

3.4.2 Temperature influences on grazing

In common with other feeding studies, zooplankton community grazing in Hartbeespoort Dam showed a biphasic response to temperature (eg. Gulati 1977, Horn 1981, Hart 1986). In many cases grazing rates have been found to be maximal at around 20 °C (see Gulati *et al.* 1982 and Hart 1986) and decrease with further increase in temperature. Hart (1984, 1986) showed the optimal temperature for high CFR and SFR in Lake Le Roux to be 20 °C whereas, in Hartbeespoort Dam, grazing rates continue to rise above this temperature value. Figure 3.7 shows maximal grazing between ∿22-24 °C . Similarly SFR was also maximal at ∿24 °C (Figure 3.15) but was also strongly influenced by grazer community composition. Above 24 °C both CGR and SFR declined sharply to very low values at the highest temperatures recorded (i.e. from ∿26 °C to almost 28 °C, Figure 3.7).

In controlled laboratory studies (Burns and Rigler 1967, Burns 1968, Hayward and Gallup 1976), this decline in CGR and SFR at high temperatures may be attributed to physiological responses. Comparable conclusions cannot easily be drawn from *in situ* experiments in Hartbeespoort Dam due to associated multivariate environmental factors. Rather the negative influence of high temperatures in Hartbeespoort Dam occurs in association with both the shift in the quality of the food resource available (i.e. from edible algae to largely inedible cyanophyte colonies) and the shift in dominant herbivore (i.e. from *Daphnia* to *Ceriodaphnia* phases).

This negative influence of temperatures >24 °C on CGR and SFR occurs primarily when herbivore biomass is low at the onset of

the *Ceriodaphnia* phase when grazing rates are low. Expression of CGR in terms of temperature in combination with biomass in Equations 3.1 and 3.4, or as their respective response surface plots (Figures 3.10 and 3.17) therefore yields estimates of low mid-summer grazing rates in Hartbeespoort Dam similar to measured values when temperature is high while total biomass is low (see below).

In general ΣSFR may be described as a linear function of temperature (Figure 3.15) although in Hartbeespoort Dam the significance of this relationship lay just outside the 5% level ($r = 0.259$, $p = 0.051$). Gulati *et al.* (1982) also found this relationship to be linear and similarly described by a low positive slope. However, Hart (1986) found that both depth-specific and integrated SFR declined with increasing temperature. This anomaly was regarded as possibly being due to methodological problems or the influence of food concentration. Whilst the oligotrophic and turbid condition of Lake Le Roux differs markedly from Hartbeespoort Dam, results presented here indicate that a positive correlation between SFR and temperature also occurs under subtropical conditions and maximal grazing activity can occur at an optimum temperature of between 22-24 °C .

Clearly, changes in temperature have an effect on grazing activity in Hartbeespoort Dam, but the influence of temperature is not necessarily of primary importance. Changes in herbivore biomass and species (particularly *Daphnia*) governed by preceding food quality factors have more marked influences on filter-feeding activity. This is examined in greater detail in Section 4 where the combined influence of body-size, grazer species, temperature and food type factors on filtration rates are examined.

3.4.3 Seston concentration influence on grazing

Due to the regular abundance of large *Microcystis* colonies in Hartbeespoort Dam, chlorophyll *a* concentration was shown in Section 2 to be a very poor indicator of food resource level

and hence of zooplankton biomass. This is further supported by Equation 3.2 and Figure 3.11 which indicate the inhibitory effect of high chlorophyll *a* concentrations on CGR. Consequently, as Hartbeespoort Dam is essentially a clear-water impoundment with low levels of silt or suspended inorganic particles (NIWR 1985), the dry weight of seston (<60 µm) was used as an indicator of available food resource level, excluding large *Microcystis* colonies.

High seston concentrations were recorded when *Microcystis* was abundant. This may be primarily attributed to cyanophyte colonies <60 µm in cross-section. Therefore seston concentration may also be a poor indicator of edible food resource level in Hartbeespoort Dam. Depth-specific seston concentrations frequently reached ∿16 mg ℓ^{-1}. The maximum recorded level of 47 mg ℓ^{-1}, occurred when CGR was very low (Figure 3.9). Assuming a 40% carbon content of this largely organic seston (dry weight), 16 mg ℓ^{-1} is equal to 6 400 µg C ℓ^{-1}. This value is approximately 5 times that recorded by Hart (1986) in Lake Le Roux, but is not dissimilar to values recorded by Gulati *et al.* (1982) for shallow eutrophic Dutch lakes. However, quantifiable comparisons with these studies are not reliable since Gulati *et al.* measured seston <33 µm, Hart <35 µm and this study <60 µm. Nevertheless, in all three studies SFR declined as the quantity of seston increased. In Lake Le Roux, Hart (1986) found SFR to decline as a linear function of seston level, although results were also generally in agreement with those of Gulati (1983).

With regard to detection of an incipient limiting level (food concentration) below which specific-filtration rate is maximal, results presented in Figure 3.9 and Equation 3.3 represent a grazing response to food concentration intermediate between the results of Hart (1986), under low algal resource conditions, and Gulati *et al.* (1982), under varying trophic and food conditions. Whilst an incipient limiting level of ∿1 mg seston C ℓ^{-1} can be estimated from Gulati *et al.* (1982; in their Figure 12), in Hartbeespoort Dam an incipient limiting level cannot be clearly defined. Slow reduction in SFR

with increasing seston levels rather indicates poor utilization of the largely inedible cyanophyte resource present in Hartbeespoort Dam. This difference between less enriched northern temperate conditions and hypertrophic Hartbeespoort Dam is shown further by the absence of any correlation between herbivore biomass and seston (r^2 = 0.0001, $p>0.85$) whereas Gulati (1985) found herbivore biomass to be a positive linear function of seston level.

3.4.4 Grazing rate prediction

Herbivore biomass, water temperature and chlorophyll *a* concentration were used in regression models, derived from stepwise multiple regression analysis, to estimate depth-specific or integrated CGR (Equations 3.1, 3.2 and 3.4). Chlorophyll *a* concentrations measured in Hartbeespoort Dam are extremely variable due to the distribution by wind of surface blooms of buoyant *Microcystis* (NIWR 1985, T. Zohary pers. comm.). Chlorophyll *a* concentration is generally an unreliable variable to use for CGR prediction under these hypertrophic conditions (Tables 3.0 and 3.3). Furthermore, chlorophyll *a* concentrations in Hartbeespoort Dam are not representative of the amount of edible food resource available to zooplankton (see Figures 2.2 and 2.3, Section 2). Consequently, herbivore biomass and temperature were chosen as predictors of CGR in Hartbeespoort Dam.

Figure 3.18 shows multiple regression model predictions of grazing rates (from Equation 3.4) throughout the water column of Hartbeespoort Dam (ΣCGR) over the 27 month study period. The mean value of ΣCGR predicted by Equation 3.4 was 68.28% d^{-1} and the mean of measured values was 68.22% d^{-1} (range: model, 20.6-233.5% d^{-1}; measured, 10.7-260.2% d^{-1}). Bimonthly predictions using herbivore biomass and temperature closely simulated the observed seasonal variations in ΣCGR (Figure 3.18).

Greatest discrepancies between observed and predicted grazing rates occurred in late December 1983, the autumn of 1984 and

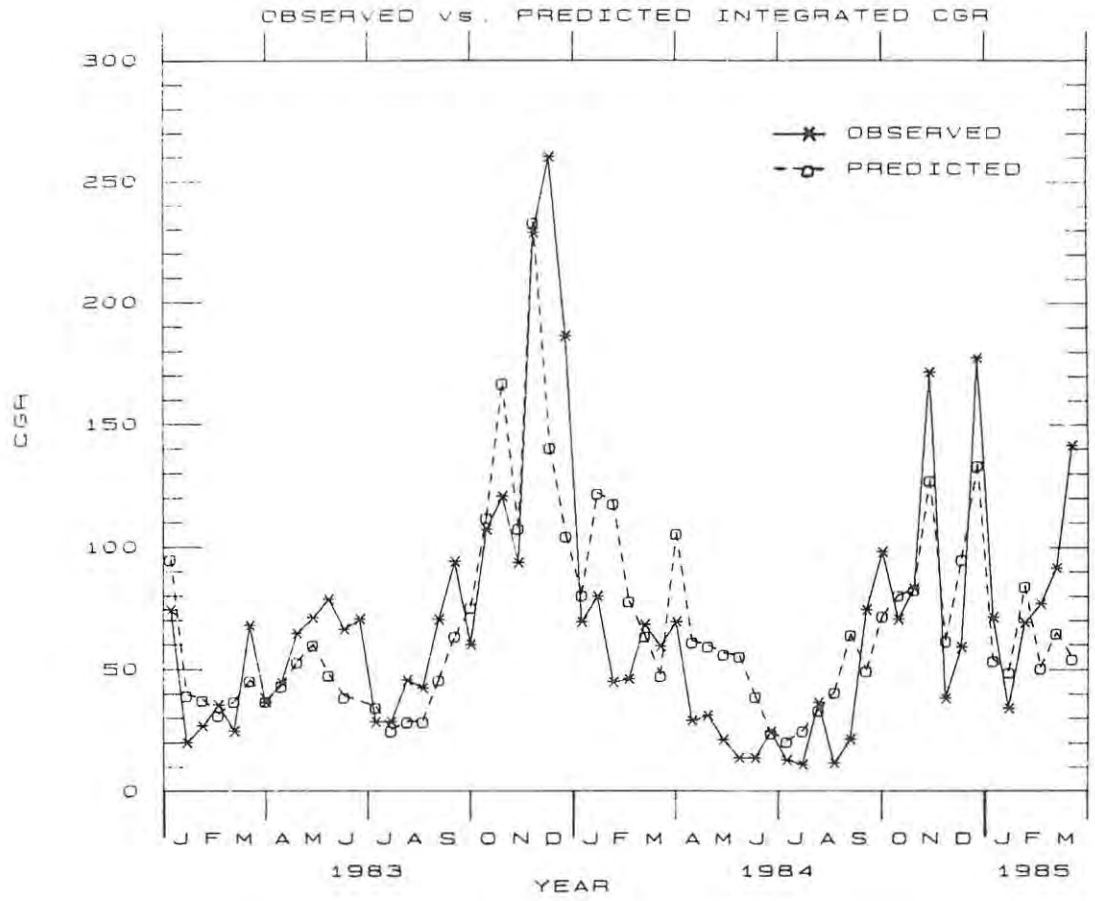


Figure 3.18: Measured and estimated ΣCGR (% d^{-1}) over 27 months (aerobic water column). Predictions estimated from text Equation 3.4

in March 1985. Failure of the model to predict CGR closely in summer arose when both CGR and *Ceriodaphnia* numbers were high but biomass was very low. Overestimation of CGR in the autumn of 1984 occurred when *Moina* and *Diaphanosoma* dominated the grazer community, and biomass-specific grazing was characteristically low (Figure 3.16). Hence the relatively large contribution of *Moina* and *Diaphanosoma* to total biomass, but their low contribution to CGR led to overestimation of CGR at these times. Similarly, dominance by rotifers and *Bosmina* also had the same effect on CGR predictions due to their low specific filtration rates on *Chlorella* (see also Section 4). Generally, however, this CGR model simulated grazing rates well in Hartbeespoort Dam, particularly when herbivore biomass was composed mainly of *Daphnia* (following from *Daphnia*'s greatest influence on CGR).

3.4.5 General conclusions

Seasonality of CGR and SGR measured using *Chlorella* in Hartbeespoort Dam is not primarily under the direct influence of temperature but is regulated by changes in dominance between the main herbivores *Daphnia* and *Ceriodaphnia*, which in turn are associated with changes in natural food availability. Variations in the amount of edible phytoplankton (Figure 2.3) largely correspond with variations in CGR (Figure 3.13a) except at the end of the edible phytoplankton phase. The sharp decline in edible phytoplankton occurs when CGR continues to rise rapidly to the annual maximum in December. This provides strong evidence that the extremely high grazing pressure in Hartbeespoort Dam limits the further development of chlorophytes and cryptophytes, and enhances or promotes complete dominance of *Microcystis* by mid-summer. This implies that biomanipulation strategies, aimed only at increasing zooplankton grazing pressure by large herbivores on the phytoplankton (Carlson and Schoenberg 1983), without also taking cognisance of selective feeding and the outcome of interspecific competition in the phytoplankton community is not likely to aid in the reduction of cyanophyte bloom formation and chlorophyll concentrations in Hartbeespoort Dam.

Integrated CGRs are high throughout the year in Hartbeespoort Dam when compared with seasonal grazing studies carried out on other lakes, which show more marked reductions to lower grazing rates during winter (Haney 1973, Gulati *et al.* 1982, Hart 1986). There is no indication of toxic inhibition of *in situ* CGRs and SGRs by *Microcystis* (Lampert 1981, 1982). High and low CGRs in Hartbeespoort Dam occurred during periods when the potentially toxic strain, *M. aeruginosa* forma *aeruginosa* (Scott *et al.* 1981) was present and when the non-toxic strain *M. aeruginosa* forma *flos-aquae* was present (April-September in most years). ΣCGR and ΣSFR values during June-July when the toxic strain was usually absent (Scott *et al.* 1981, NIWR 1985) were generally far lower than at other times of the year when potentially toxic *Microcystis* occurred, suggesting that any toxic inhibition was slight. High SFRs measured in Hartbeespoort Dam when compared to studies in other eutrophic waters (Gulati *et al.* 1982, Gulati 1983) also indicate the absence of toxic inhibition to grazing.

High SFRs during summer may not only be attributed to the low biomass of this small body-size summer community, but may also indicate heightened selectivity for edible foods such as labelled-*Chlorella* at this period of predominantly inedible phytoplankton resource. Such high food selectivity would be advantageous to the summer zooplankton grazers feeding in the presence of largely inedible or 'nutritionally poor' large *Microcystis* colonies. High filtration rates of the small cladoceran species on *Chlorella* when expressed in relation to filtration rates on unicells or small colonies of *Microcystis* is demonstrated in Section 4.

The annual mid-summer decline of the *Daphnia* population following its abundance in spring, as well as high grazing pressure on chlorophytes and cryptophytes and the shift in algal species to *Microcystis* dominance does not support the views of Carlson and Schoenberg (1983) that large cladocerans can utilize and thus limit cyanophyte blooms in hypertrophic lakes. Growth of the summer grazer community (*Ceriodaphnia* phase) may depend to a greater extent on other particles such

as bacteria (Pace *et al.* 1983) or suspended detritus particles. Zooplankton avoid the regions of low dissolved oxygen at the interface between epilimnion and anoxic hypolimnion (Figure 3.4). In contrast, the study by Haney (1973) showed that grazing was occasionally highest at this boundary where high numbers of bacteria provide the zooplankton with an important food source. In Hartbeespoort Dam, however, bacterial numbers are very high throughout the water column and the density of epilimnetic bacteria usually exceeds that of hypolimnetic bacteria (Robarts and Sephton 1984). Zooplankton filtration rates *in situ* on natural bacteria are examined in Section 5.

Gliwicz (1977) showed that small-bodied cladocerans such as *Ceriodaphnia*, with a lower particle size limit to ingestion, replace large cladocerans such as *Daphnia* in the presence of abundant large cyanophyte particles when low concentrations of available food particles exist. In Hartbeespoort Dam the low biomass *Ceriodaphnia* phase, typically occurring from late summer to winter when the edible phytoplankton volume is low, and the high SGRs of the *Ceriodaphnia* dominated community support the view of Gliwicz (1977) that feeding and growth of small-bodied cladoceran populations is not hindered by abundant large cyanophyte filaments or colonies. Studies on zooplankton grazing and food selection by Gliwicz (1969a, 1969b), and subsequently the study by Hillbricht-Ilkowska *et al.* (1972) on phytoplankton and zooplankton of lakes of different trophic status, showed that zooplankton grazing pressure on phytoplankton decreases as trophic status increases and algal composition changes to colonial cyanobacteria. Utilization of primary production thus becomes increasingly indirect via suspended detritus and bacteria. In Hartbeespoort Dam the high phytoplankton biomass, primarily composed of large colonies of *Microcystis*, is under-utilized by the low zooplankton biomass except during the spring period of chlorophyte and cryptophyte abundance.

4. FILTRATION RATES OF THE MAJOR ZOOPLANKTON SPECIES ON *MICROCYSTIS* COLONIES AND *CHLORELLA*

4.1 Introduction

Size selection of algal particles by zooplankton has been identified as an important factor influencing the phytoplankton community structure of lakes (Porter 1973, 1977). Phytoplankton size distribution and compositional shifts in community structure have been shown to occur in response to grazing by zooplankton assemblages of varying size distribution (Gliwicz 1980, Carpenter and Kitchell 1984, Bergquist *et al.* 1985). Porter (1977) stated that high zooplankton grazing pressure may depress nanoplankton populations and promote the development of algal and cyanophyte blooms composed of large colonies that are resistant to grazing. Conversely Schoenberg and Carlson (1984) argue that both direct and indirect effects of grazing by large herbivorous zooplankters can control cyanophyte blooms.

Variations in the shape of algal cells, presence or absence of a gelatinous sheath, chemical composition, colony formation and size are factors to be considered when examining zooplankton grazing impact on any phytoplankton assemblage. The ability of filter-feeding zooplankton to ingest colonial or filamentous cyanophytes has been examined in a variety of experimental procedures using numerous phytoplankton and zooplankton species (Sorokin 1968, Arnold 1971, Schindler 1971, Webster and Peters 1978, De Bernardi *et al.* 1981). Controversy surrounding the edibility of cyanophytes has been highlighted by Schoenberg and Carlson (1984). Much of the difficulty in comparing results of the various studies arises from absence of information regarding variations in the size of phytoplankton colonies or filaments and the body-length of the zooplankton species examined.

Okamoto (1984) measured size selective feeding using size fractionated and labelled natural phytoplankton in laboratory studies on the zooplankton of Lake Biwa. He stated that

ideally zooplankton filtering rates should be determined individually on the major species comprising the phytoplankton assemblage. McCauley and Downing (1985) highlighted the paucity of measurements of *in situ* particle size selection and stressed the need for inclusion of such information in plankton simulation models. Pace *et al.* (1983) stated that models based on animal body size rather than on species may benefit plankton community theory.

Numerous studies of the factors governing zooplankton feeding activity have highlighted variability in filtration rates between species and between animals of differing length and also between type, size and concentration of food, and ambient temperatures. Furthermore most of this information, gathered in controlled laboratory studies, has been applied to describe changes in filtration rates *in situ* where combinations of these factors present a complex picture of synergistic and antagonistic effects influencing zooplankton filtration rates. Thus development and application of a simple model to avoid the need for specialized labour-intensive direct measurements and provide a reasonably accurate estimate of zooplankton community feeding activity in a lake seemed unlikely. Using published data from many laboratory studies, regression models were produced by Peters and Downing (1984) which identified animal size as the most important of many predictive variables influencing filtration rates of freshwater cladocerans, marine calanoids and of all zooplankton combined. Knoechel and Holtby (1986a) found that, in Lake St George, up to 93% of variance in cladoceran community filtration rate measured *in situ* using labelled yeast (6 µm cell diameter) could be explained by body length alone. Subsequently, using cultured bacteria (1 µm) and the colonial chlorophyte alga *Pandorina* sp. (20 µm mean colony diameter) Knoechel and Holtby (1986b) recorded similar results. In a combined model based on the results obtained for the three foods tested *in situ*, 87% of the variance in cladoceran community filtration rate on foods of 1-20 µm particle diameter was explained by body length irrespective of species, temperature or time of day (Knoechel and Holtby 1986b).

This section firstly presents information on the *in situ* filtration rates of the gravimetrically important zooplankton species on fractionated and labelled colonies of *Microcystis aeruginosa* naturally present in hypertrophic Hartbeespoort Dam. Secondly, it presents filtration rate : body length relationships (FR:L) and models. From the disproportionately low biomass of zooplankton in Hartbeespoort Dam (NIWR 1985) relative to the extremely high phytoplankton standing stock and production (Robarts 1984 and 1985, NIWR 1985), principally of large colonies of *Microcystis*, it is reasonable to infer that the development of zooplankton biomass is limited by the size and nature of the food available in the absence of clear indications that fish predation is the principle factor limiting zooplankton biomass (Section 2). Therefore feeding by zooplankton species on the abundant *Microcystis* present in Hartbeespoort Dam was examined both during the spring period of high community grazing rates ('*Daphnia* phase') and during the mid-summer period of low community grazing rates ('*Ceriodaphnia* phase') when large bodied and small bodied herbivores dominated respectively (Section 2).

The models of Knoechel and Holtby (1986a and 1986b) are extended to include larger food particles such as cyanophyte colonies which usually dominate the summer phytoplankton communities of eutrophic and hypertrophic lakes. Taking cognisance of the particle size distribution of the food resources or of food quality criteria and edibility of foods available to zooplankton in many lakes may not improve filtration rate predictions but may merely introduce unnecessary complexity in models. However, in highly eutrophic or hypertrophic conditions, such as those prevailing in Hartbeespoort Dam, prolonged cyanophyte blooms occur annually and may strongly influence zooplankton standing stock and species succession (Sommer *et al.* 1986, Sections 2 and 3). Modelling of phytoplankton grazing losses and zooplankton biomass may therefore depend heavily upon inclusion of factors recognising pronounced changes in food quality with season or lake trophy, as indicated by Chow-Fraser and Knoechel (1985) and as in the ecosystem model of Hartbeespoort Dam described by Cochrane *et*

al. (1987). Food particle size limitation to grazing and low filtration efficiencies of cladocerans in the presence of abundant cyanophytes (Thompson *et al.* 1982) plus interference to filter-feeding and increased particle rejection (Webster and Peters 1978, Porter and McDonough 1984) are factors which can significantly alter cladoceran FR:L relationships in hypertrophic waters.

4.2 Additional methods

Zooplankton filtration rates were measured at a fixed limnetic station in the main basin of Hartbeespoort Dam during periods of *Daphnia* dominance in the austral spring and *Ceriodaphnia* dominance from the austral mid-summer to autumn 1984-85.

Natural *Microcystis* colonies collected from the impoundment and cultured unicellular *Chlorella* sp. (WR 1007, NIWR culture collection: cell diameter ≅8.5 µm) were used as labelled foods in separate experiments. A log-phase *Chlorella* culture was incubated as described in Section 3.2.2 for 24 h on a low speed magnetic stirrer at room temperature. *Microcystis* colonies were similarly labelled, but incubation with isotope was allowed to continue for 40-60 h to increase homogeneity of label uptake. Continuous low speed stirring was not carried out as this caused the gradual disintegration of colonies. Instead, colonies were periodically agitated during incubation to ensure even contact of the buoyant colonies with the labelling medium. Size fractionation of labelled *Microcystis* colonies was undertaken by differential filtration through nylon screens. Fragmentation of large colonies by brief vigorous shaking broke colonies without disrupting the integrity of individual cells. Rinsing of *Microcystis* colonies by gentle agitation on nylon screens of 100, 60, 40 and 20 µm mesh apertures was carried out to obtain discrete colony size-classes of 5-20, 20-40, 40-60 and 60-100 µm . The rinsing and fractionation procedure was undertaken repeatedly to ensure discrete size-classing, which was checked by microscopic examination. Low vacuum filtration was not possible as *Microcystis* colonies broke or distorted and passed through the

narrow apertures. Preparation of colony size-classes prior to incubation with radioisotope failed due to clumping of colonies to form larger colonies during incubation, or fragmentation of colonies if stirred sufficiently to avoid clumping.

Filtration rates were determined *in situ* using a modification of the flowchart procedure given by Hart (1986). Parallel feeding experiments of 7-8 min duration were carried out simultaneously in both chambers of the double grazing chamber using the same labelled food type and size fraction. Zooplankton from each compartment of the chamber were retained on a 60 µm mesh net, immobilized in carbonated water and killed in a 4% formalin bath. The zooplankton from one of the experiments was preserved with acid-Lugol's solution and transported to the laboratory. Zooplankton from the second experiment was immediately filtered onto a 60 µm mesh disc and solubilized with 1 mℓ Soluene 350 (Packard) in a scintillation vial. This provided a simultaneous measurement of the community feeding rate without loss of isotope due to preservation (Holtby and Knoechel 1981). Two 20 mℓ aliquots of medium from both compartments of the grazing chamber were each filtered onto 0.45 µm membrane filters and the specific activity of the algae released into the feeding medium in each experiment was determined using 10 mℓ Filter Count (Packard). Experiments using labelled *Chlorella* or labelled *Microcystis* colonies were carried out on the same date at the same depth within 2 h of midday. To ensure that changes in food availability did not influence feeding behaviour, the radiolabelled food released was limited to an amount <10% of ambient food levels as determined by chlorophyll *a* measurements (Section 3). Food concentrations were not kept constant.

In the laboratory, the preserved zooplankton was sorted into species and body length-classes under a binocular microscope within 24 h. Animals were measured from the top of the head the edge of the posterior margin of the carapace, excluding spines. No helmeted forms occurred in the lake. *Daphnia* were sorted into seven 0.25 mm interval body length-classes from the smallest animals (0.5 mm body length) to the largest

animals (2.5 mm body length). Individuals from the smaller cladoceran species *Ceriodaphnia reticulata*, *Moina micrura*, *Diaphanosoma excisum* and *Bosmina longirostris* generally had lower specific activities due to their lower filtration rates. Therefore, coupled with their frequent paucity in experiments, the small-bodied caldoceran species were fractionated into either 2 or 3 length-classes. With little variation in the body length of mature individuals of the calanoid copepod *Thermodiaptomus syngenes* and the rotifer *Brachionus calyciflorus* only the mean body length was recorded. When animals were abundant during experiments, up to 200 or more individuals in a length-class were solubilized in a single scintillation vial containing 0.5 mℓ Soluene 350 (Packard). When infrequent , a minimum of 5 animals >1.0 mm or 10 animals <1.0 mm were regarded as necessary to obtain an accurate measure of filtration rate for each body-length class. All animals sorted into each body length-class were solubilized in a vial containing 0.5 mℓ Soluene 350 and activity counted using Dimilume 30 (Packard).

Calculations of filter-feeding rates (mℓ animal^{-1} h^{-1}) were based on the equations of Haney (1973) after correction of counts for background radiation and isotope loss during preservation (Holtby and Knoechel 1981). Comparison of results of experiments measuring community filtration rates (processed in the field) with the total filtration of all individual animals and any unsorted material (preserved samples) measured simultaneously enabled isotope loss to be estimated for each experiment. This single correction factor was then applied equally to all species present within an experiment. The magnitude of isotope loss measured from all 124 *in situ* experiments was 42.2% ± 3.5% when using *Chlorella* and 45.3% ± 3.0% when using *Microcystis*. These average isotope loss measurements are very similar to the loss reported by Gulati *et al.* (1982) of 42 ± 10% using formalin preservation, and to losses reported by Holtby and Knoechel (1981) using formalin (57%) and acid Lugol's solution (40%).

Average dry weights of *Daphnia* of each body length-class were determined by weighing batches of up to ≅100 animals in each length-class after drying at 50 °C for 24 h .

Stepwise multiple regression analysis was performed using the Statgraphics software package (STSC Inc.). Nominal-scale variables were used to indicate both zooplankton species and food type (*Chlorella* or four *Microcystis* fractions). Since these nominal-scale variables represent a coded classification and contain no numeric measurement, indicator or 'dummy' variables were created to allow examination of species and food effects in multiple regression models.

The Statgraphics package uses dummy variables coded in binary form as described by Kim and Kohout (1975) for use in SPSS (Nie *et al.* 1975). Dummy variables account for the main effects of a nominal-scale variable with k-1 degrees of freedom (where k is the number of unique factor or code levels, e.g. species or food types). Therefore one of the coded levels or dummy variables created must be excluded from the regression equation. This excluded dummy variable becomes the 'reference category' against which the effects of other dummy variables are interpreted, so there is no loss of information (Kim and Kohout 1975). *Daphnia* was the species used as the reference category when species effects were examined, and *Chlorella* was the reference category for food type. Consequently the influences of these variables, chosen because of their strong influences on the models examined, whilst still included in these regression analyses, do not appear as separately listed variables in the tabulated results of the stepwise regressions performed.

4.3 Results

From a total of 124 *in situ* operations, between spring 1984 and autumn 1985, 603 species-specific determinations of filtration rate were established using *Chlorella* and *Microcystis* colonies. Due to the paucity of large *Daphnia*, only a few results were obtained for the three largest length-classes

(from 1.75 mm to 2.5 mm) which were therefore excluded from species-specific analysis by length-class (n = 584; Table 4.0). Pilot experiments revealed that *Microcystis* colonies of 100-150 µm diameter were not ingested. Therefore subsequent experiments were restricted to size-classes below 100 µm diameter. Colonies of 60-100 µm diameter can be regarded as being at the upper size limit for filter-feeding zooplankton in Hartbeespoort Dam. Water temperature varied from 16.4 to 25.1 °C over this spring to autumn study period.

4.3.1 Analysis of species-specific filtration rates

Figure 4.0 shows that species-specific filtration rates on the various foods were power functions ($Y = aX^b$) of cladoceran length. For the majority of zooplankton species present, filtration rates on *Chlorella* were higher than on all size-classes of *Microcystis*. Exceptions to this were small *Bosmina longirostris* of 0.2-0.4 mm length (Figure 4.0) and the rotifer *Brachionus calyciflorus* (Table 4.1) for which filtration rates on *Chlorella* were very low. *Daphnia pulex* had the highest filtration rate on *Chlorella* with a maximum of 1.790 mℓ animal^{-1} h^{-1} being recorded for the 1.50-1.75 mm length-class (mean 1.510 mℓ animal^{-1} h^{-1}). For the largest length-classes of other cladocerans, maximum filtration rates on *Chlorella* (mℓ animal^{-1} h^{-1}) were: *Ceriodaphnia reticulata* 0.254 (mean 0.124); *Moina micrura* 0.469 (mean 0.256); *Diaphanosoma excisum* 0.320 (mean 0.193) and *Bosmina longirostris* 0.184 (mean 0.093).

All *Daphnia* length-classes filtered unicellular *Microcystis* and small *Microcystis* colonies (5-20 µm) at about half (mean = 51.6 ± 3.8%) the rate on *Chlorella* (∿8.5 µm cell diameter) (Figure 4.1). The filtration rate of small *Daphnia* declined as *Microcystis* colony size increased (Figure 4.0). Calculation of the relative filtration rate is similar to that for the filtration efficiency of Okamoto (1984) but is based on the mean filtration rate of *Chlorella*, the optimally filtered food for all zooplankters except *Bosmina* of 0.2-0.4 mm body length. Relative filtration rates of small *Daphnia* dropped

Table 4.0: Number of *in situ* measurements of zooplankton filtration rates using *Chlorella* or *Microcystis* colonies of up to 60–100 µm .

	Chlorella	*Microcystis* colonies
Daphnia 1.75 - 2.5 mm	13	6
Daphnia 0.5 - 1.75 mm	61	85
Bosmina	24	44
Ceriodaphnia	62	53
Moina	45	54
Diaphanosoma	49	28
Thermodiaptomus	29	22
Brachionus	6	22
Total n	276	314
Grand Total		603

Table 4.1: Filtration rates (µℓ animal^{-1} h^{-1}) of adult stages of *Thermodiaptomus syngenes* and *Brachionus calyciflorus* on *Microcystis* colonies and *Chlorella*. Means and standard error shown.

	mean body length (mm)	*Microcystis* colony classes 5–20 µm	20–40 µm	40–60 µm	60–100 µm	*Chlorella*
Thermodiaptomus	1.250	67.4 ± 18.0	22.6 ± 1.4	16.8 ± 5.2	11.8 ± 4.9	387.8 ± 45.9
Brachionus	0.313	38.0 ± 6.6	87.1 ± 11.6	65.0 ± 10.0	10.0 ± 3.7	4.7 ± 0.8

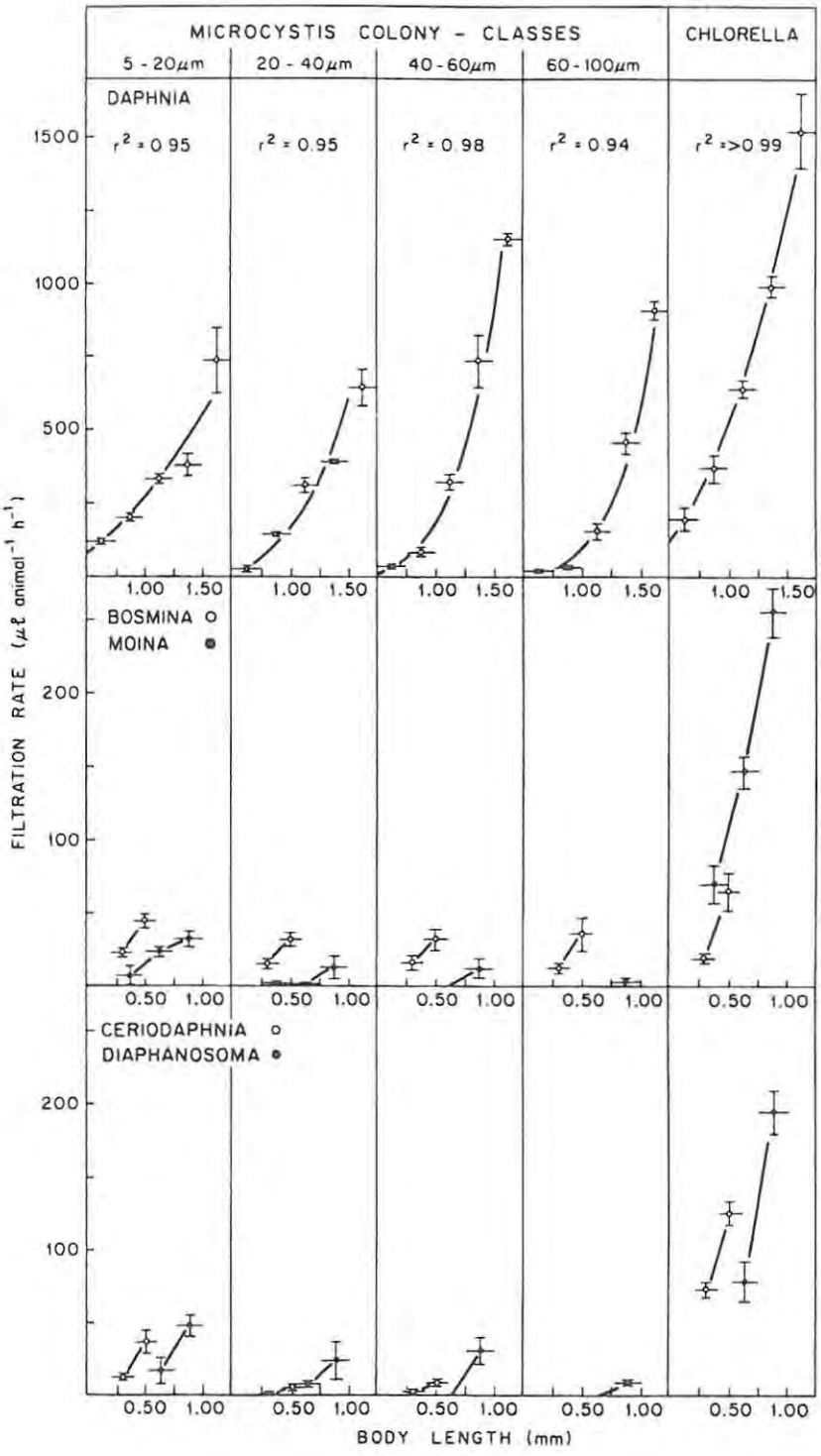


Figure 4.0: Mean filtration rates of body length-classes of the major cladocerans in Hartbeespoort Dam on four *Microcystis* colony size fractions and on *Chlorella*. Vertical bars indicate standard errors.

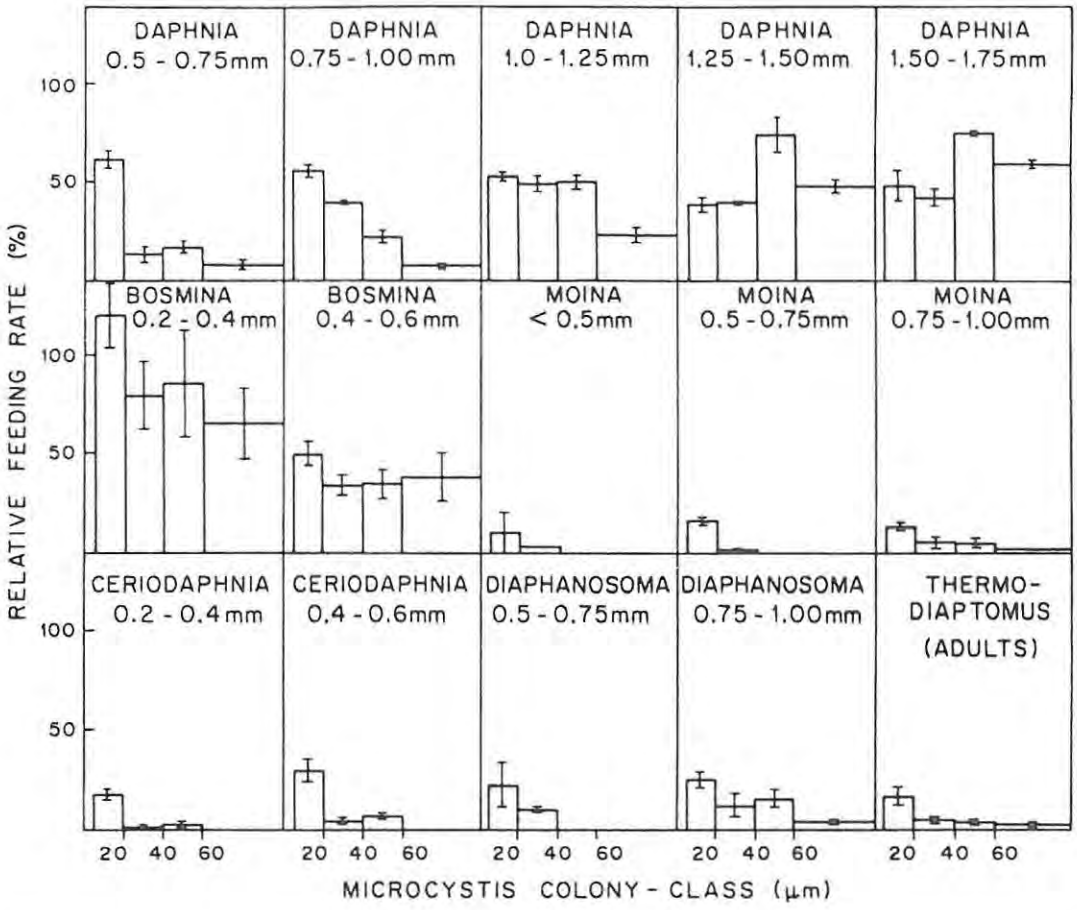


Figure 4.1: Relative filtration rates (filtration efficiencies) of cladoceran length-classes and adult *Thermodiaptomus* on four *Microcystis* colony size fractions. Results expressed in relation to filtration rates on *Chlorella* (100%). Vertical bars indicate standard error.

most sharply (Figure 4.1) showing that small *Daphnia* are limited to feeding principally on small colonies or unicellular *Microcystis*. Both the absolute and relative filtration rates of *Daphnia* >1.25 mm were highest when fed on the 40-60 µm colony size-class, indicating an ability to filter large phytoplankton particles from the water. Large *Daphnia* also had a higher relative filtration rate on large *Microcystis* (60-100 µm) than on small colonies (5-20 µm and 20-40 µm).

The relationship between *Daphnia* dry weight (Y, µg) and length (X, mm) for each body length-class was $Y = 9.49X^{2.07}$ ($r^2 = 0.89$, $n = 420$). Length-weight transformations based on this equation show that biomass-specific filtration rates of *Daphnia* on *Microcystis* and *Chlorella* were linear (Figure 4.2), but varied in slope and intercept depending upon food type. Comparison of the slopes in Figure 4.2, using the method of Zar (1974), shows that when feeding on the 5-20 µm and 20-40 µm *Microcystis* colony size-classes the biomass-specific filtration rates of *Daphnia* were significantly different from the rates measured on *Chlorella* ($p<0.0001$ in both cases). As size limitation to grazing on *Microcystis* colonies <20 µm diameter is probably absent, the significantly lower biomass-specific filtration rate of *Daphnia* on small *Microcystis* colonies compared to *Chlorella* may be due to the presence of physical or chemical cues that reduce the 'taste' preference of *Daphnia* for *Microcystis*. For *Daphnia* feeding on *Microcystis* of 40-60 µm the slope shown in Figure 4.2 did not differ significantly from that for *Chlorella* ($p>0,20$) due to the high filtration rates of large *Daphnia* (>1,25 mm body length) on *Microcystis*. However, particle size limitation to grazing was evident for small *Daphnia* (Figure 4.2) shown by the significantly depressed intercept compared to that for *Chlorella* ($p<0.005$). When feeding on the 60-100 µm *Microcystis* colony size-class, both the slope and intercept of the biomass-specific filtration rate of *Daphnia* differed from that measured on *Chlorella* (slope $p<0.05$; intercept $p<0.02$) showing the combined effects of low taste preference for *Microcystis* and large particle size limitation to feeding on *Microcystis* colonies larger than 60 µm in diameter. Above 100 µm particle

size no grazing was recorded. Observed biomass-specific filtration rates for each *Daphnia* length-class and rates predicted from the linear equations fitted to relationships shown in Figure 4.2 are given in Figure 4.3; the predicted rates highlight *Daphnia*'s high filtration rate on *Chlorella* and the depressive effects of large *Microcystis* colony size on feeding rates of small daphnids. As expected, particle size had little or no influence on food selection in all *Daphnia* length-classes when feeding on *Chlorella* or the 5-20 µm *Microcystis* colony size-class. Reductions in specific filtration rate (Figure 4.3) probably mediated by food size became increasingly evident in the 0.50-0.75 mm and 0.75- 1.00 mm *Daphnia* length-classes as *Microcystis* colony size increased.

With the exception of *Bosmina*, especially smaller individuals, relative filtration rates of other cladocerans on *Microcystis* were very low in comparison to *Daphnia* (Figure 4.1). The relative filtration rate of *Ceriodaphnia* 0.4-0.6 mm in length was highest at only 29.6%. Both *Ceriodaphnia* and *Moina* fed mainly on the 5-20 µm colony size-class, probably reflecting a particle size limitation to their feeding activity. *Diaphanosoma* were less restricted by *Microcystis* colony size, but filtration rates of *Diaphanosoma* on *Microcystis* remained very low relative to *Chlorella* (Figures 4.0 and 4.1). The filtration rate of *Bosmina* on *Microcystis* was largely independent of colony size. Its high relative filtration rates on *Microcystis* reflect its low filtration rate on *Chlorella*, particularly by smaller *Bosmina* (0.2-0.4 mm body length), rather than high absolute rates on *Microcystis*. (Figures 4.0 and 4.1). Similarly, filtration rates of *Brachionus* were lower on *Chlorella* than on *Microcystis*, where maximal filtration by *Brachionus* focused on the 20-40 µm colony size-class (Table 4.1). Conversely filtration rates of adult stages of the calanoid copepod *Thermodiaptomus syngenes* on all *Microcystis* colony size-classes were very low compared to filtration rates on *Chlorella* (Figure 4.1 and Table 4.1).

Figure 4.4 summarises the mean filtration rates of each crustacean zooplankter on *Chlorella* during their seasonal

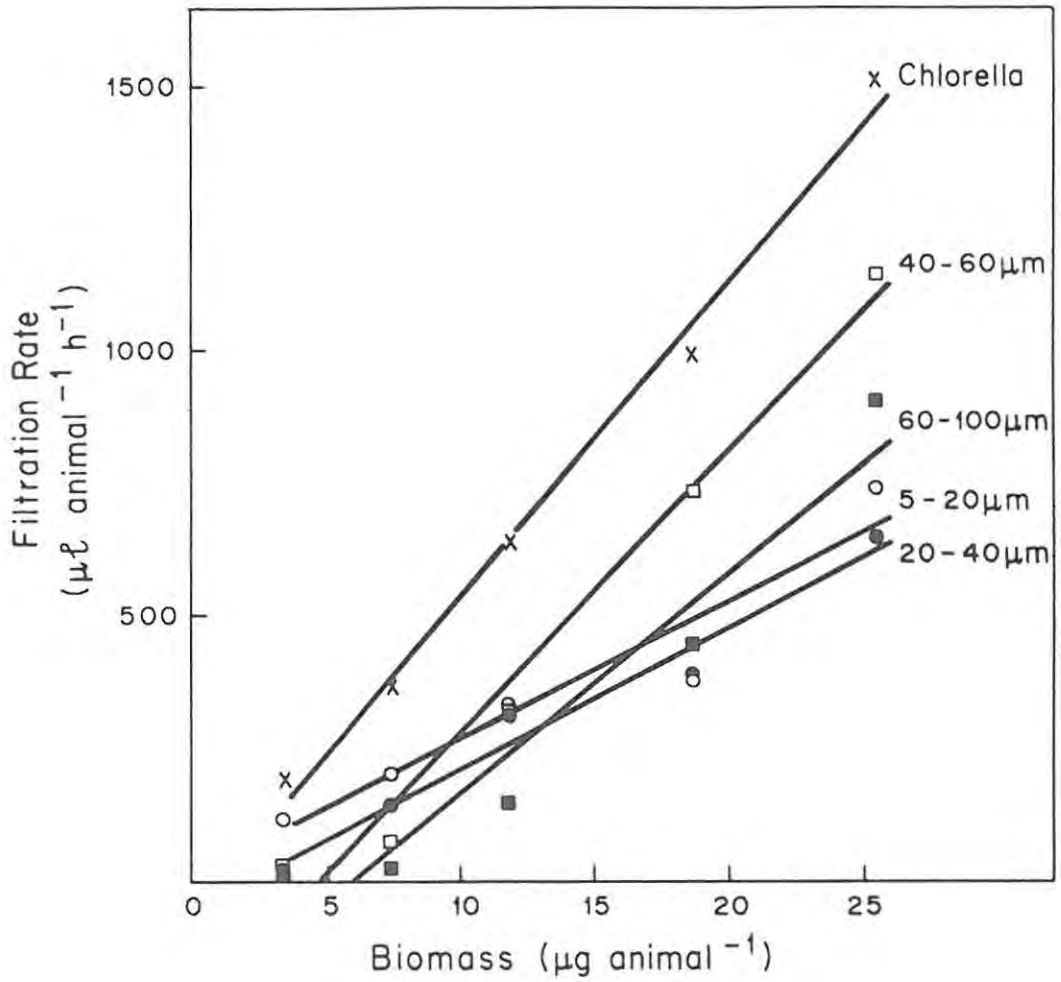


Figure 4.2: Relationships between *Daphnia* biomass and filtration rate on the four *Microcystis* colony size fractions and on *Chlorella*.

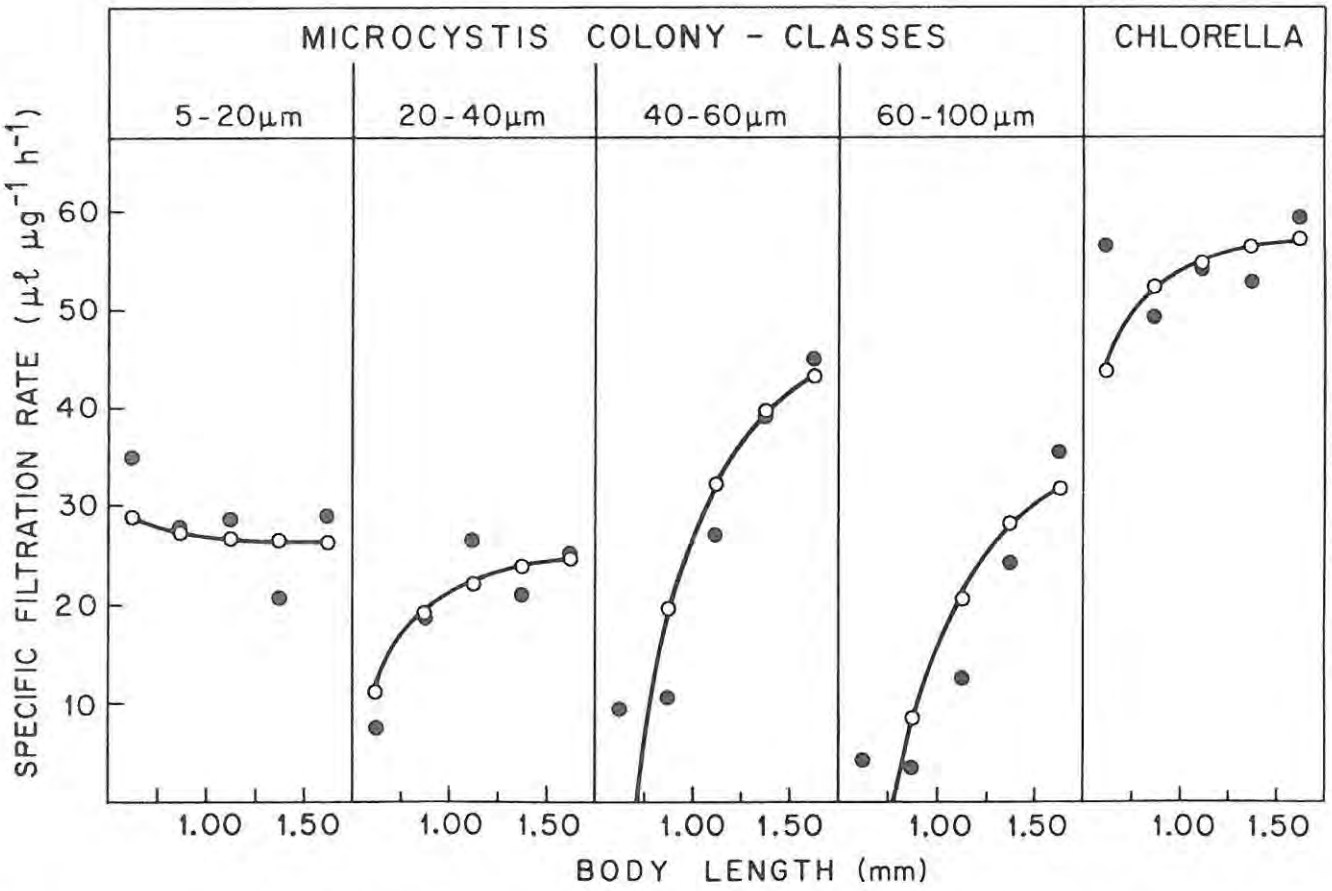


Figure 4.3: Closed circles: observed specific filtration rates of five length-classes of *Daphnia* on four colony size-classes of *Microcystis* and on *Chlorella*. Open circles: specific filtration rates predicted from the relationships in Figure 4.2. Lines drawn by eye for predicted values.

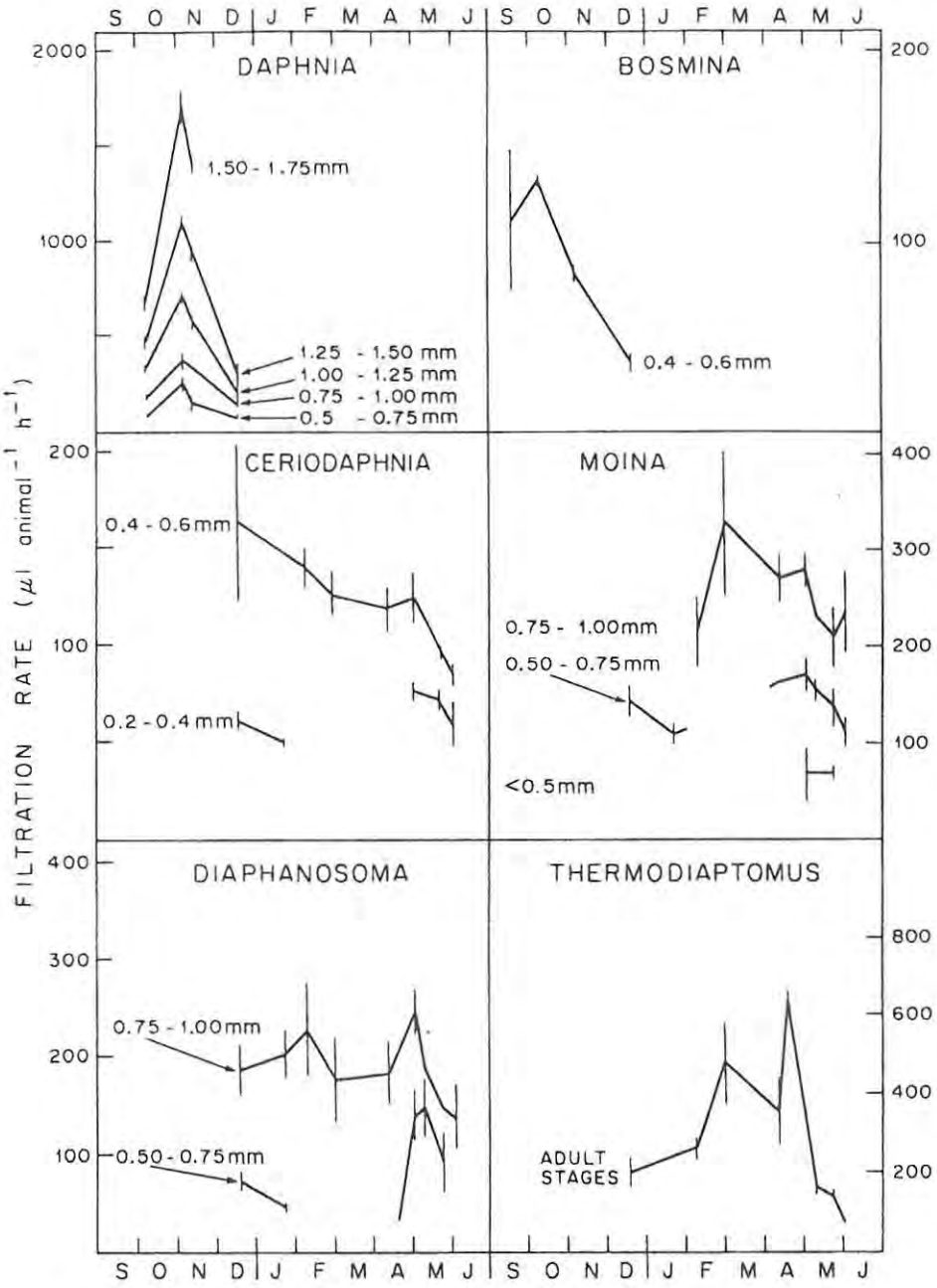


Figure 4.4: Seasonal filtration rates of crustaceans on *Chlorella* measured in Hartbeespoort Dam during 1984-85. Mean value for each date plotted with standard error (vertical bars).

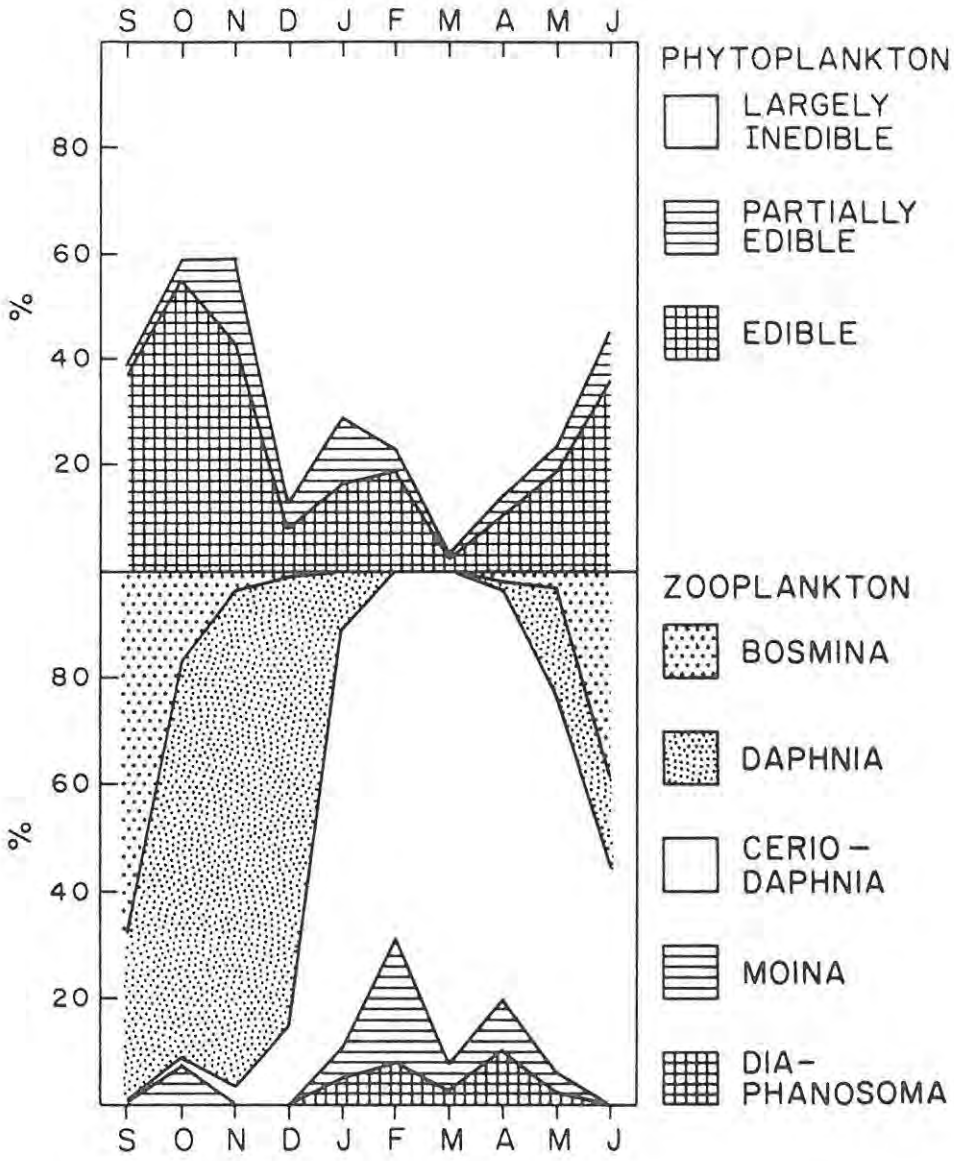


Figure 4.5: Seasonal succession of Cladocera (as % of total cladoceran number) in relation to phytoplankton composition (as % of algal biovolume), expressed as monthly changes in the contribution by different edibility fractions (Section 2) during 1984-85. Phytoplankton data from T. Zohary (unpublished).

occurrences in the water column. Grazing on *Chlorella* was measured from spring to late autumn, providing seasonal information on species filtration rates of a variety of zooplankters on a readily acceptable cell size food in the presence of the natural phytoplankton assemblage of varying food type and size. During this period temperature variations were 20.1-23.4 °C when *Daphnia* was present, 16.4-23.4 °C when *Bosmina* was present and 16.4-25.1 °C when other crustacean species were present during the experiments. The algal food resource as classified in terms of edibility criteria (see Section 2) and the composition of the cladoceran community over the duration of these feeding experiments using labelled fractionated *Microcystis* is summarized in Figure 4.5. With the presence of abundant edible phytoplankton food forms in spring (Figure 4.5) the high *Daphnia* filtration rates in October rose to a maximum in November (Figure 4.4) before declining sharply with the reappearance of abundant *Microcystis* colonies in December. *Bosmina* was similarly abundant with high filtration rates when the phytoplankton was mainly composed of edible forms, but the filtration rate and population density of *Bosmina* diminished immediately preceding the spring peak in *Daphnia* number and feeding activity. Filtration rates of *Ceriodaphnia* on *Chlorella* were high immediately following the mid-summer disappearance of *Daphnia* and the shift to dominance by the small-bodied cladoceran assemblage, as characterized by the marked December-January increase in the *Ceriodaphnia* population (Figure 4.5). *Ceriodaphnia*, *Moina* and *Diaphanosoma* typically co-exist during the period of highest *Microcystis* biomass (see also Section 2). Decreases in filtration rate were evident in the *Ceriodaphnia*, *Moina* and *Diaphanosoma* populations from early May when the water temperature fell below 20 °C .

4.3.2 Filtration rate-body length relationships within the zooplankton community

Disregarding taxa, individual zooplankter filtration rate (FR, mℓ animal^{-1} d^{-1}) on all food types tested was a significant function (r^2 = 0.429, $p<0.001$) of body length (L, mm):

$$FR = 3.841\ L^{1.824} \quad (4.1)$$

Separate regression analyses carried out for each food type tested revealed similarly significant relationships between filtration rate (FR) and body length (L) (FR as $\mu\ell$ animal^{-1} h^{-1} in Table 4.2). The strongest FR:L correlation, calculated for all taxa combined, was obtained with the unicellular chlorophyte *Chlorella* as food (Table 4.2) which when exhibited as a power relationship (FR = mℓ animal^{-1} d^{-1}, L = mm) was:

$$FR = 6.387\ L^{1.744} \quad (4.2)$$

The FR:L relationship for all zooplankters on all foods (Equation 4.1) was further analyzed using stepwise multiple regression (Table 4.3) to examine additional influences of temperature and of food size and type or zooplankton species, which were entered into the models as coded indicator or 'dummy' variables (Kim and Kohout 1975). Stepwise inclusion in the model of body length, coded food type (*Chlorella* as reference category) and temperature increased the explained variance from 42.9 to 57.7% (Table 4.3, Model A). Food size and type accounted for a total of 13.8% of the variance and temperature entered the model last, accounting for only 1.0% of the variance.

Analysis of the FR:L model by coded zooplankton species (with *Daphnia* as reference category; Table 4.3, Model B) decreased the residual variance by 7.6% (i.e. 3.5 + 3.1 + 0.7 + 0.3%) whilst temperature improved the model by 3.6%. Of the separate zooplankton species included, the calanoid copepod *Thermodiaptomus* had the most influence (3.5%) on model variance. Filtration rates of *Thermodiaptomus* on *Chlorella* resembled those of similar body length cladocerans, but were markedly lower when feeding on *Microcystis* (Figure 4.6a and b). The structure and operation of feeding mechanisms of calanoid copepods are different from those of cladocerans (Paffenhöfer 1984). Filtration rates of *Thermodiaptomus* deviated from the general relationships derived predominantly from cladoceran FR:L data. Similarly, rotifer feeding mechanisms (Gilbert and Starkweather 1978) and the resultant filtration rates of *Brachionus* differed when compared to small cladoceran filtration rates, being generally lower when feeding on *Chlorella*

Table 4.2: Regression model parameters of ln transformed zooplankton FR:L relationships on *Chlorella* or *Microcystis* in four colony size fractions, measured *in situ* in Hartbeespoort Dam. Regression equation: ln FR = ln a + b ln L where FR = filtration rate in $\mu\ell$ animal^{-1} h^{-1}, L = length in mm . All slopes significant at $p<0.001$. Cladoceran models exclude data from the rotifer *Brachionus* and copepod *Thermodiaptomus*.

		Chlorella	*Microcystis* 5-20 µm	20-40 µm	40-60 µm	60-100 µm
ALL ZOOPLANKTON	a	5.584	4.593	4.061	4.631	4.436
	b	1.744	1.589	1.288	2.353	2.631
	r^2	0.660	0.441	0.206	0.472	0.532
	n	289	97	60	64	50
Sample mean	x	-0.343	-0.412	-0.506	-0.308	-0.054
	y	4.985	3.938	3.409	3.906	4.293
Sample variance	x	0.286	0.255	0.285	0.339	0.291
	y	1.321	1.460	2.297	3.977	3.786
CLADOCERANS	a	5.644	4.833	4.484	4.857	4.623
	b	1.682	2.054	2.523	3.053	3.164
	r^2	0.700	0.577	0.567	0.674	0.662
	n	254	83	47	56	42
Sample mean	x	-0.390	-0.422	-0.479	-0.288	0.021
	y	4.989	3.967	3.277	3.978	4.690
Sample variance	x	0.273	0.222	0.241	0.319	0.223
	y	1.102	1.623	2.708	4.408	3.370

Table 4.3: Stepwise multiple regression analysis of filtration rates of all zooplankton species on all food types. Variance in ln filtration rate explained as a function of: (Model A) ln body length, food type and temperature; (Model B) ln body length, species and temperature, in order of entry of variables into the models. Food type and zooplankton species entered as binary coded dummy variables with *Chlorella* and *Daphnia* as reference categories (Kim and Kohout 1975) and therefore not represented.

Variable	Coefficient	R^2	ΔR^2	F ratio
MODEL A				
Constant	4.474			
Ln length	1.724	0.429	0.429	421.53
20-40 µm	-1.159	0.458	0.029	64.03
60-100 µm	-1.046	0.486	0.028	56.13
40-60 µm	-1.142	0.519	0.033	54.24
5-20 µm	-0.826	0.567	0.048	47.92
Temperature	0.055	0.577	0.010	13.35
Degrees of freedom : Model = 7, Residual = 553				
MODEL B				
Constant	3.329			
Ln length	1.868	0.429	0.429	123.85
Thermodiaptomus	-1.160	0.464	0.035	47.33
Temperature	0.105	0.500	0.036	44.62
Diaphanosoma	-0.852	0.531	0.031	28.34
Moina	-0.393	0.538	0.007	5.12
Brachionus	0.342	0.541	0.003	1.23*
Bosmina	-0.102	0.541	0.000	0.19*
Ceriodaphnia	0.020	0.541	0.000	0.01*
Degrees of freedom : Model = 9, Residual = 551				

$p<0.001$; * $p>0.5$

Table 4.4: Stepwise multiple regression of cladoceran filtration rates on all food types. Variance in ln filtration rate explained as a function of ln body length, food type, zooplankton species and temperature in order of entry into the model. Coded dummy variables as in Table 4.3.

Variable	Coefficient	R^2	ΔR^2	F ratio
Constant	5.665			
Ln length	2.079	0.508	0.508	276.24
20-40 µm	-1.607	0.554	0.046	163.74
40-60 µm	-1.348	0.589	0.035	134.25
60-100 µm	-1.407	0.617	0.028	109.65
5-20 µm	-0.966	0.669	0.052	95.12
Diaphanosoma	-1.094	0.716	0.047	83.22
Moina	-0.735	0.743	0.027	31.74
Temperature	0.025	0.745	0.002	3.98
Ceriodaphnia	-0.145	0.746	0.001	0.76*
Bosmina	-0.031	0.746	0.000	0.03*

Degrees of freedom: Model = 11, Residual = 471 $p<0.001$; * $p>0.5$

Table 4.5: Stepwise multiple regression of cladoceran filtration rate *in situ* on *Chlorella*. Variance in ln filtration rate explained as a function of ln body length, cladoceran species and temperature in order of entry into the model. Species entered as binary coded dummy variables; *Daphnia* as reference category and therefore not represented.

Variable	Coefficient	R^2	ΔR^2	F ratio
Constant	6.200			
Ln length	2.017	0.700	0.700	395.96
Ceriodaphnia	0.653	0.772	0.072	22.31
Diaphanosoma	-0.400	0.795	0.023	16.98
Temperature	-0.025	0.800	0.005	7.28
Bosmina	-0.278	0.808	0.008	3.36
Moina	-0.141	0.809	0.001	1.82*

Degrees of freedom: Model = 7, Residual = 247
$p<0.05$; *$p>0.05$

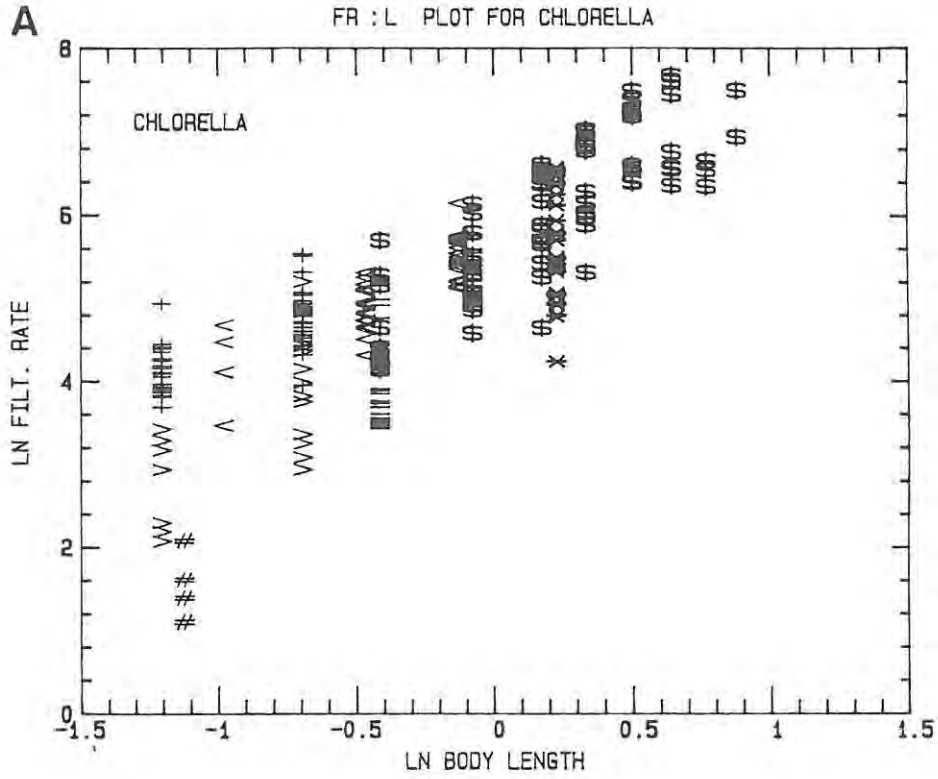


Figure 4.6: Ln:Ln scatter-plots of individual zooplankton filtration rate ($\mu\ell$ animal^{-1} h^{-1}) against animal body lenth (mm) when feeding *in situ* on labelled *Chlorella* (A) or on four colony size fractions of *Microcystis* (B). Species coded as: *Daphnia* \$; *Ceriodaphnia* +; *Moina* <; *Diaphanosoma* -; *Bosmina* >; *Thermodiaptomus* * and *Brachionus*.

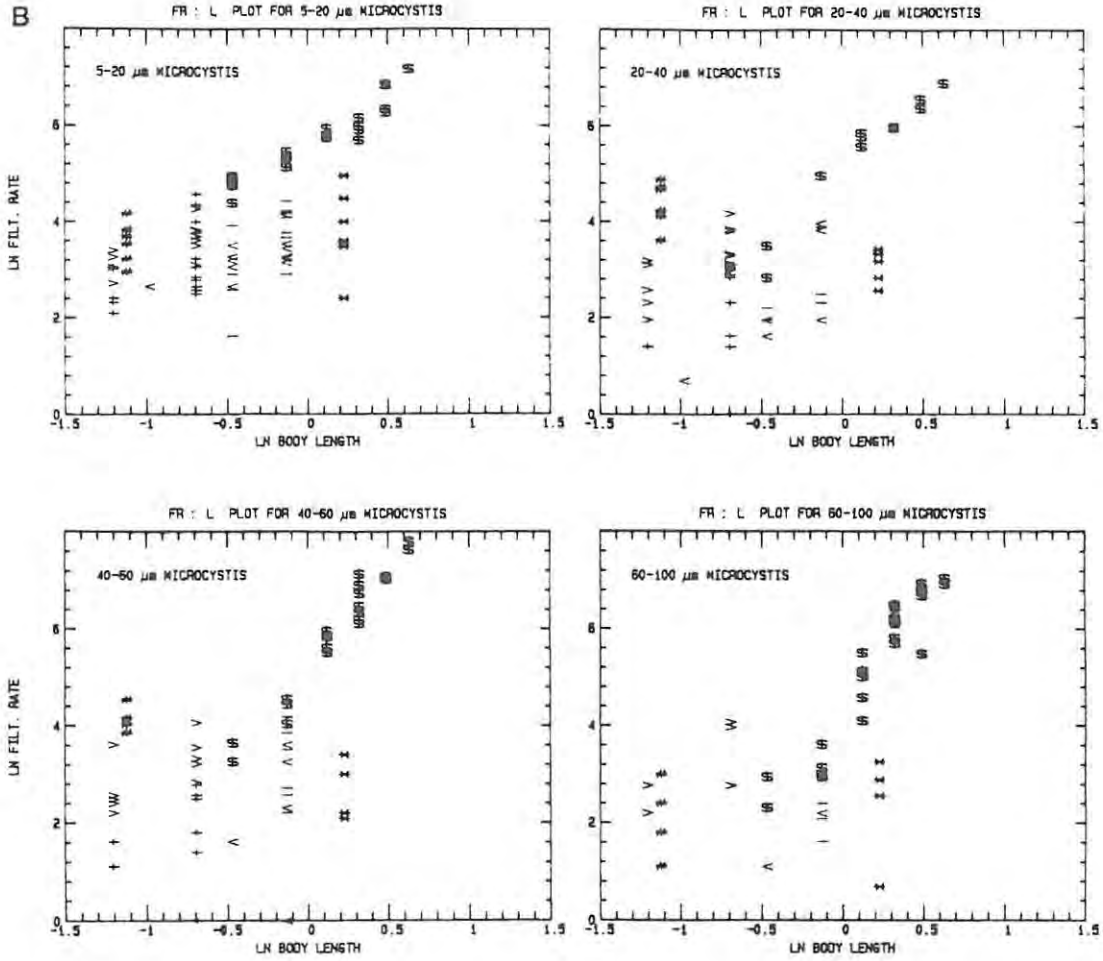


Figure 4.6: continued.

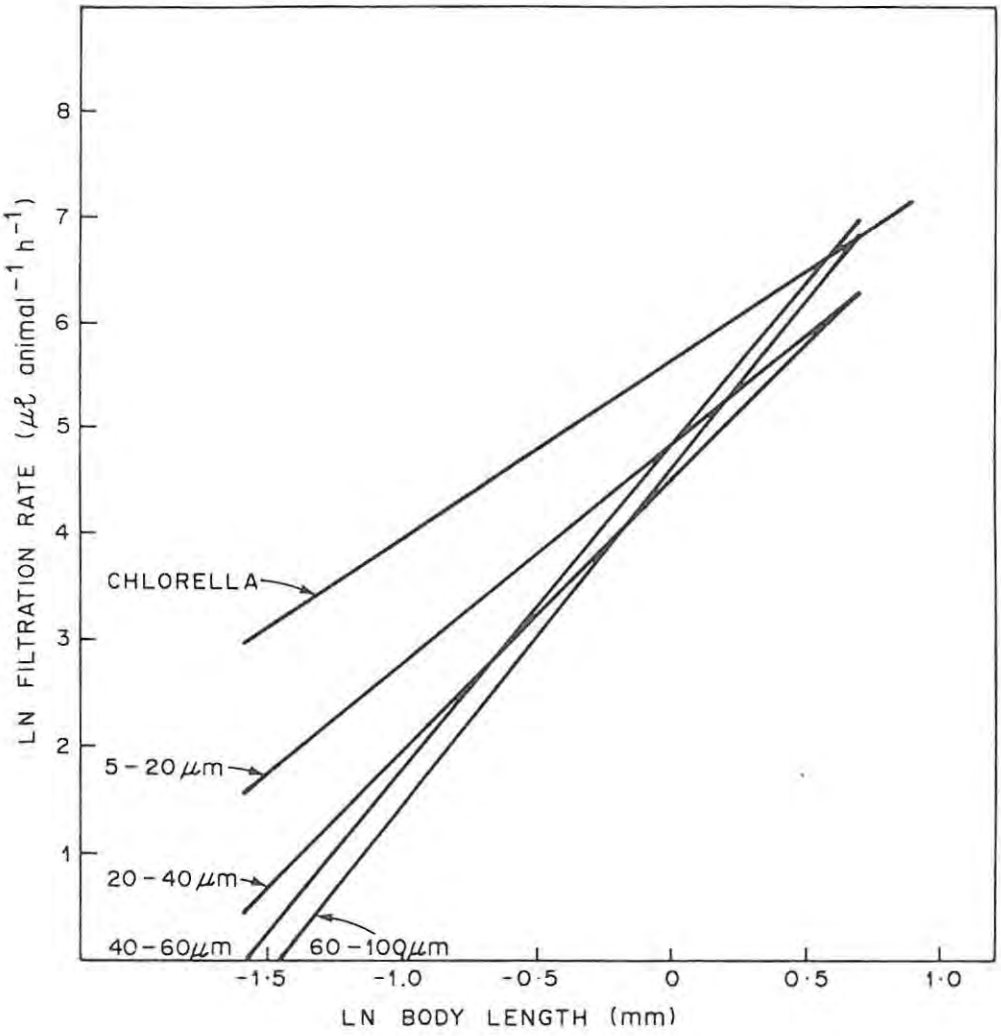


Figure 4.7: Transformed cladoceran FR:L linear regression models on *Chlorella* and four colony size fractions of *Microcystis* in Hartbeespoort Dam.

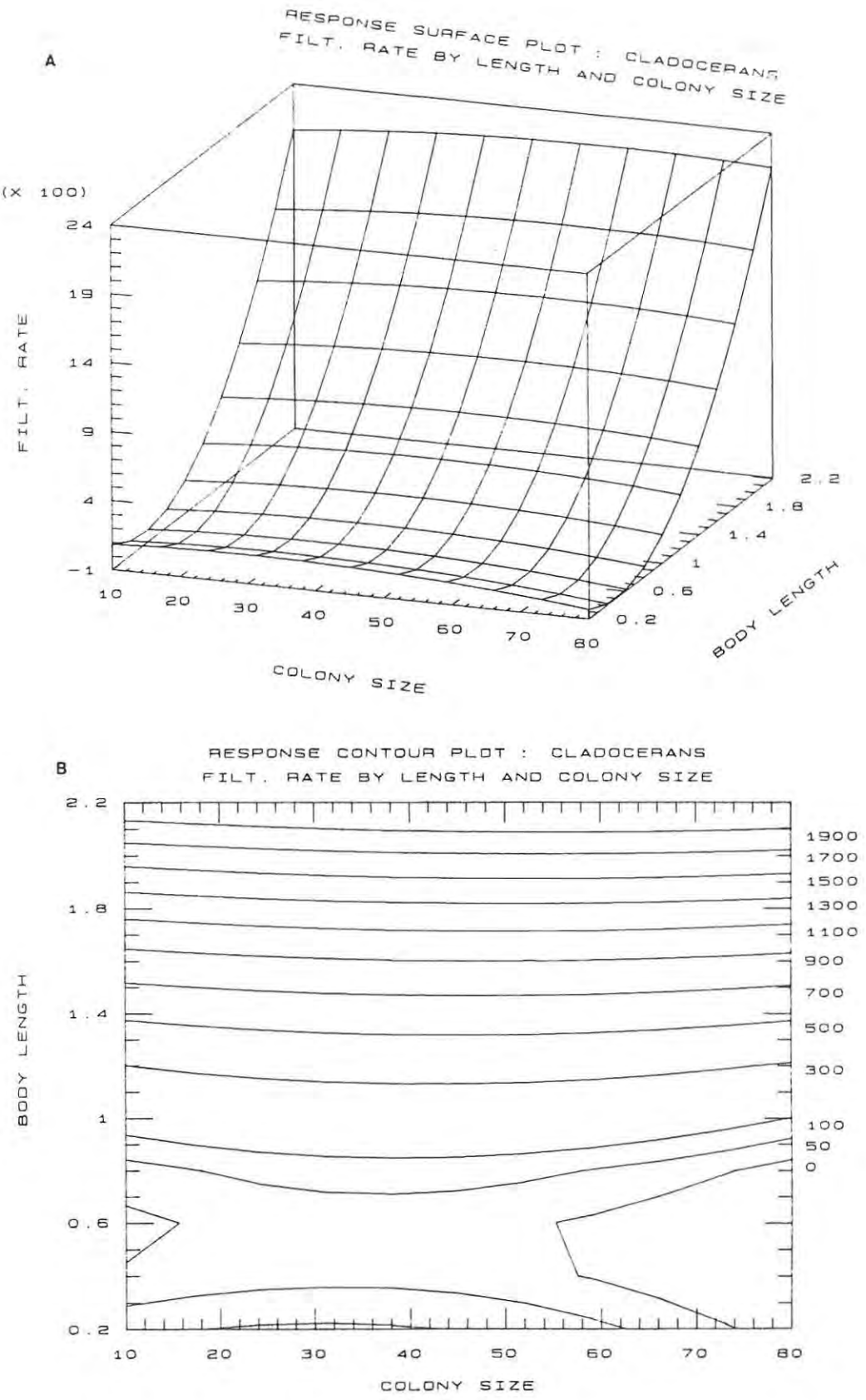


Figure 4.8: Response surface plot of cladoceran filtration rate (µℓ animal^{-1} h^{-1}) on *Microcystis* colonies in relation to the interactions of body length (mm) and colony size (diameter, µm) expressed in their polynomial form; (A) three-dimensional and (B) contour plot.

Table 4.6: Significant differences between the slopes of regression models describing cladoceran filtration rate as a function of body length. Separate regressions were fitted to feeding rates on *Chlorella* or *Microcystis* in four colony size fractions, measured *in situ* in Hartbeespoort Dam. Where slopes are not different, then significant differences between intercepts are shown. Formulae of Zar (1974) were used to derive the t statistic. For data obtained using the 60-100 µm colony fraction, comparison of intercepts with models for other food fractions was not performed due to a marked difference in mean body length which prejudiced the validity of the test.

Cladoceran Models	Degrees of freedom	t
DIFFERENCES BETWEEN SLOPES		
Chlorella/20-40 µm	297	3.472
40-60 µm	306	6.413
60-100 µm	292	5.674
5-20 µm /40-60 µm	135	3.001*
60-100 µm	121	2.959*
DIFFERENCES BETWEEN INTERCEPTS		
Chlorella/ 5-20 µm	334	8.507

$p<0.001$; *$p<0.005$

and higher when feeding on *Microcystis* colonies (Figures 4.6a and b). Therefore the FR:L models were restricted to include only data for cladoceran species. This restriction reduced the unexplained variance in FR:L models for each food type, particularly for the *Microcystis* colony fractions, as summarized in Table 4.2.

Stepwise multiple regression analysis of cladoceran filtration rate as a function of body length, temperature and of both food fraction and cladoceran species is presented in Table 4.4. After body length, *Microcystis* colony sizes (as 'dummy' variables) entered the regression model and accounted for an additional 16.1% (i.e. 4.6 + 3.5 + 2.8 + 5.2%) of the variance in filtration rate. Inclusion in the model of the influences of cladoceran species explained a further 7.5% of filtration rate variance. *Ceriodaphnia* and *Bosmina* had no significant influence on the model (*Daphnia* as the reference category), whilst temperature accounted for only 0.2% of the variance (Table 4.4). This analysis highlights the importance of incorporating the effects of the colony size of cyanophyte foods in regressions aimed at estimating cladoceran filtration rates from body length when cyanophytes are abundant. The lesser influences of cladoceran species and water temperature support their further exclusion from these models.

Similar analysis of data gathered using *Chlorella* showed that, when feeding on a generally palatable unicellular chlorophyte, 10.2% of the variance in cladoceran filtration rate was attributed to differences in species, with temperature entering as the last significant variable, improving the model by only 0.5% (Table 4.5). *Ceriodaphnia* had the most influence on model variance when feeding on *Chlorella*, due to its filtration rates being generally higher than other similarly sized cladocerans (Figure 4.6a).

Linear regressions of cladoceran FR:L relationships when feeding *in situ* on labelled *Microcystis* colonies (Table 4.2) are illustrated in Figure 4.7. No pattern of residual variance in relation to body-length was evident from visual

examination of appropriate residual scatter-plots, indicating that the transformation used was suitable, and the error variance stable. Significant differences in both slope and intercept (summarised in Table 4.6) show the effect of increasing cyanophyte colony size on cladoceran filtration rate as significant deviations from both the linear regression model for *Chlorella* and the linear model for the 5-20 µm *Microcystis* colony size fraction (Table 4.2 and Figure 4.7).

The difference in intercept between the two smallest foods, *Chlorella* (≅8.5 µm) and 5-20 µm *Microcystis* colonies, represents a reduction of over 50% in cladoceran filtration rate on the cyanophyte food compared to the chlorophyte food. The model for the 5-20 µm colony fraction did not differ significantly from that for the 20-40 µm fraction, but its slope did differ from those of the largest *Microcystis* fractions of 40-60 µm and 60-100 µm (Table 4.6). This shows that particle size limitation to feeding gradually becomes an important limiting factor to cladoceran filtration rates as cyanophyte colony dimensions increase. When compared, the three models for *Microcystis* colony size fractions of 20-40 µm, 40-60 µm and 60-100 µm did not differ significantly from each other in either slope or intercept. Therefore a combined regression model was produced for the cladoceran FR:L relationship when feeding on *Microcystis* colonies of 20-100 µm minimum and maximum dimensions. When untransformed this relationship was

$$FR = 2.655\ L^{2.894} \qquad (3.3)$$

($r^2 = 0.668$, $n = 145$, $p<0.001$).

The linear FR:L regressions on *Microcystis* colonies can also be summarised in a combined untransformed, multiple regression form. This polynomial expression highlights the interactive influences of body-length and *Microcystis* colony size on cladoceran filtration rate. Using the mid-point value of each body-length and colony size class, the equation derived was:

$$FR = 1.432LM - 880.660L + 772.691L^2 + 3.174M - 0.057M^2 + 201.464 \qquad (3.4)$$

($R^2 = 0.85$; df 5, 264; $p<0.001$)

where L = body-length (mm), M = *Microcystis* colony size (µm diameter). This equation is presented as response surface plots in Figure 4.8. Expression of cladoceran FR:L relationships in such a multivariate form is, however, likely to be of less value if applied in studies aimed at predicting filtering rates in ecosystem studies (i.e. in estimating potential phytoplankton grazing losses); detailed data on continuous size distributions of both zooplankton and phytoplankton are not usually available, thus discrete linear regression models may be more useful in most cases.

4.4 Discussion

4.4.1 Species-specific filtration rates and their implications to resource utilization and zooplankton succession

High grazing pressure on edible phytoplankton occurs in Hartbeespoort Dam in early summer as shown in Section 3 and by the high filtration rates of *Daphnia* on *Chlorella*. However, filtration rates were reduced through a combination of size-related influences. Firstly, a limitation to grazing imposed by large colony size, and secondly, low filtration rates on small *Microcystis* colonies. These factors were more pronounced in reducing the feeding impact of the small cladocerans *Ceriodaphnia*, *Moina* and *Diaphanosoma* on *Microcystis* (Figure 4.1) to the extent that this cyanophyte was essentially not utilized as a major food resource by this summer-autumn cladoceran community in Hartbeespoort Dam.

Increase in phytoplankton colony size has been regarded as an effective means of avoiding high zooplankton grazing pressure (Gliwicz 1977, Porter 1977, Webster and Peters 1978, De Bernardi *et al.* 1981). Thompson *et al.* (1982) classed *Microcystis* colonies >50-60 µm as being inedible to the filter-feeding zooplankton of Blelham Tarn, and smaller colonies were termed partially edible. In Hartbeespoort Dam the low feeding rates of zooplankton on *Microcystis* compared to feeding rates on *Chlorella* support this view, but the upper colony size limit to filtration is placed higher at 60-100 µm . This size

range corresponds to the maximum particle size limit of 70-80 µm measured by Burns (1968) based on ingestion of plastic beads by *D. schodleri*. Okamoto (1984), examining zooplankton in Lake Biwa, measured low filtration rates by *Daphnia longispina hyalina* on a natural phytoplankton size fraction of 70-150 µm (in the absence of *Microcystis*). In Hartbeespoort Dam low filtration rates by *Daphnia pulex* on the 60-100 µm *Microcystis* colony size-class and the absence of any filtration on the 100-150 µm colony size-class, which jointly cover the largest size fraction studied by Okamoto (1984), indicate that the majority of feeding may occur only on the smaller half of Okamoto's 70-150 µm phytoplankton fraction; but algal species and other palatability factors must also be considered. Accurate estimation of upper particle size limits to zooplankton grazing on phytoplankton clearly depends upon the species composition and size structure of each plankton community studied, although similar results have been obtained from different aquatic systems. In hypertrophic Hartbeespoort Dam *Microcystis* escapes the extremely high zooplankton grazing pressure in spring following formation of large colonies exceeding 60-100 µm. Concomitant improvements in the underwater light field due to packaging of the chlorophyll present in the form of larger particles (Kirk 1975) presents an additional advantage for large-sized *Microcystis* colonies which also tend to be more buoyant (T. Zohary pers. comm.).

If cladocerans such as *Daphnia* were entirely passive, non-selective filter-feeders then filtration rates on *Chlorella* and unicells or small colonies of *Microcystis* in mixtures would be similar. Food concentration, food mixtures and factors influencing feeding preferences were not examined in this study, but the low specific filtration rates of *Daphnia* on 5-20 µm *Microcystis* compared to rates on *Chlorella* indicate poor palatability of *Microcystis* (Figure 4.3). The origin of this response may be either mechanical and/or chemosensory, arising from the mucilaginous sheath of *Microcystis* cells and/or chemical cues providing specific information on palatability or nutritional quality (Poulet and Marsot 1978, Rassoulzadegan *et al.* 1984).

The sharp mid-summer (December-January) decline of the *Daphnia* population was immediately preceded by a marked reduction in individual filtration rates measured *in situ* on *Chlorella* (Figure 4.4). This occurred in association with a reduction in the edible and partly edible component of the phytoplankton in December (Figure 4.5) due to the antecedent high zooplankton filtration rates which promoted the return to phytoplankton dominance of *Microcystis*. This reduction in the *in situ* filtration rate of *Daphnia*, prior to the seasonal disappearance of *Daphnia* from Hartbeespoort Dam, strongly supports the views of Webster and Peters (1978) and Porter and McDonough (1984) that large filter-feeders are disadvantaged in the presence of abundant cyanophyte filaments and colonies due to the high energetic cost of particle rejection, or by utilization of the abundant but 'nutritionally poor' food. This favours a shift to smaller filter-feeding species. Fish predation is not excluded as a contributing factor. However, the marked reduction in feeding rate of *Daphnia* in December shown in Figure 4.4 indicates that this species shift is at least partly mediated by a reduction in the feeding efficiency of *Daphnia* and the food quality available to this large herbivore.

Comparison between the filtration rates of *Daphnia* measured on Hartbeespoort Dam and rates measured in other lakes shows that similar suppression of *Daphnia* filtration rate occurs when the phytoplankton composition changes to large algal forms or becomes dominated by 'inedible' *Microcystis* of colonies >60 µm. *Daphnia longispina hyalina* filtration rates measured by Okamoto (1984) on a <10 µm phytoplankton fraction over a range of body length-classes were very similar to results obtained on Hartbeespoort Dam using *Chlorella*. Okamoto (1984) noted that filtration efficiency on a large-sized algal fraction was reduced in the presence of the complex shaped desmid *Staurastrum dorsidentiferum*. Similarly, data presented by Thompson *et al.* (1982) on *Daphnia hyalina* in an experimental enclosure on Blelham Tarn showed filtration rates on *Chlorella* marginally higher than those measured for *D. pulex* in Hartbeespoort Dam during periods when edible algae dominated the

Table 4.7: Mean *Daphnia* filtration rates (FR) on *Chlorella* during periods of maximum filter-feeding activity when the phytoplankton was dominated by partly edible and edible forms and when dominated by large inedible *Microcystis* colonies in Blelham Tarn (Thompson *et al.* 1982, Tube B) and Hartbeespoort Dam (this study). Filtration efficiency is expressed as the ratio of FR during edible and inedible phases x 100. Values from Thompson *et al.* (1982) are approximations derived from their Figure 2.

Thompson *et al.* 1982				This study			
Daphnia body length (mm)	Filtration Rate (mℓ animal^{-1} h^{-1})			*Daphnia* body length (mm)	Filtration Rate (mℓ animal^{-1} h^{-1})		
	Partly edible + edible phase	*Microcystis* phase	Filtr. effic.(%)		Partly edible + edible phase	*Microcystis* phase	Filtr. effic.(%)
1.3 - 1.6	1.530	0.190 - 0.380	12-25	1.25 - 1.50	1.107	0.281	25
1.0 - 1.3	0.830	0.140 - 0.230	17-28	1.00 - 1.25	0.704	0.176	25
<1.0	0.510	0.090 - 0.140	18-28	0.75 - 1.00	0.364	0.131	36
				0.50 - 0.75	0.247	0.067	27

phytoplankton communities in both of these lakes. Thereafter, following a shift in phytoplankton composition in the experimental enclosure to 'overwhelming dominance' by large inedible *Microcystis* colonies, filtration rates of *D. hyalina* were reduced by an amount similar to that recorded for *D. pulex* in Hartbeespoort Dam (Table 4.7). Thompson *et al.* (1982) suggested that depression in filtration rate may have been due to interference to efficient feeding and more frequent particle rejection due to the presence of *Microcystis*. Toxic inhibition of filtration rates by *Microcystis* (Lampert 1981) was not implicated. Chow-Fraser and Sprules (1986) also reported a similar depression in *Daphnia* filtration rate due to interference by cyanophyte filaments. Comparison of filtration rates measured *in situ* in two lakes with and without *Anabaena* blooms showed that in the presence of *Anabaena* filaments, *Daphnia* spp. filtered *Chlorella* and *Scenedesmus* at only 36% of the rates in the filament free lake. Laboratory studies using combinations of labelled algal foods further supported the view that interference by *Anabaena* filaments lowered *Daphnia*'s filtration rate on other edible algae (Chow-Fraser and Sprules 1986). Similarly Zánkai and Ponyi (1986) reported that, during a bloom of *Anabaenopsis raciborskii* in part of Lake Balaton, individual zooplankton filtration rates diminished to only 30% of rates measured in the absence of the cyanophyte bloom.

There is no evidence to indicate that *Microcystis* toxicity depresses zooplankton filtration rates in Hartbeespoort Dam (see also in Section 3). The potentially toxic variety *M. aeruginosa* forma *aeruginosa* is typically present for 11-12 months of each year, being briefly absent in winter, usually in June (Scott *et al.* 1981, NIWR 1985). *M. aeruginosa* forma *aeruginosa* is present during the spring period of edible phytoplankton abundance (Figure 4.5) when zooplankton grazing activity is extremely high (Section 3). The non-toxic *M. aeruginosa* forma *flos-aquae*, which coexists with forma *aeruginosa* from April to September, becomes abundant in May and June when filtration rates of the small cladoceran community are lowest, presumably due to low water temperatures. These

changes in the variety of *Microcystis* present and their potential toxicity based on 'mouse killing' factors have been shown to be a poor indicator of their lethal or inhibitory effects to feeding in daphnids (Nizan *et al.* 1986).

The edible phytoplankton phase in Hartbeespoort Dam (approximately July - late November) is characterized by high numbers of both *Bosmina* and *Daphnia*. The *Bosmina* population typically declines when the *Daphnia* population reaches its highest numbers prior to its mid-summer disappearance (Section 2). Studies on the competition between these cladocerans (DeMott 1982, DeMott and Kerfoot 1982) have shown that *Bosmina* can feed more efficiently than *Daphnia* on large flagellates at low food concentrations. High filtration rates of *Bosmina* on *Chlorella* occur when the edible phytoplankton component (chlorophytes and cryptophytes) is increasing (Figures 4.4 and 4.5). The ability of *Bosmina* to collect large particles (Figure 4.1), and the decrease in *Bosmina* numbers and filtration rate as the *Daphnia* population and filtration activity reach a maximum, indicates the existence of competitive interactions between these cladocerans based on food resources during the edible phytoplankton phase in Hartbeespoort Dam.

The edible phytoplankton phase, which in 1984 extended from June to November, was principally composed of species of the diatom *Cyclotella* and the flagellates *Cryptomonas* and *Chroomonas* from July - September. In October there followed a shift to the chlorophytes *Coelastrum* and the appearance of a more 'resistant' sheathed form of *Oocystis* (T. Zohary unpublished data). Both the highest number of *Bosmina* recorded in Hartbeespoort Dam (≅150 animals ℓ^{-1}) and highest *in situ* filtration rates for *Bosmina* measured using *Chlorella* (Figure 4.4) occurred when flagellates were common. The subsequent decline in *Bosmina* number and filtration rate as chlorophyte and *Daphnia* abundance increased is consistent with DeMott's (1982) and DeMott and Kerfoot's (1982) observations that competition occurring between coexisting *Daphnia* and *Bosmina* populations is related to qualitative changes in food and *Bosmina's* ability to exploit edible flagellates better

than *Daphnia*. In Hartbeespoort Dam, growth of the *Daphnia* population annually excludes *Bosmina* during the latter part of the edible phytoplankton phase (Section 2).

The mean filtration rate of large *Bosmina* (0.4-0.6 mm length-class) of 0.093 mℓ animal^{-1} h^{-1} in Hartbeespoort Dam is high compared to the mean filtration rate of 0.039 mℓ animal^{-1} h^{-1} (>0.4 mm length) measured by Bleiwas and Stokes (1985) using a smaller form of *Chlorella* (average cell diameter of 5 µm). The ability of *Bosmina* to collect *Microcystis* colonies up to 60-100 µm (Figure 4.1) was surprising although positive selectivity for particles such as the large flagellate *Euglena* (45 x 6.5 µm) has been demonstrated by Bogdan and Gilbert (1982). The desmid *Cosmarium* (25-28 x 10-12 µm) was also shown to be collected very efficiently by *Bosmina* (Bleiwas and Stokes 1985). The large particle feeding mode of *Bosmina* (DeMott and Kerfoot 1982) may enable *Microcystis* colonies to be ingested, or colonies may be partially broken during this feeding activity.

Numerous studies (reviewed by Sommer *et al.* 1986) indicate that the shift in zooplankton community composition to smaller bodied species in eutrophic lakes is primarily driven by composition of the food resource and by resource limitation associated with summer cyanophyte blooms, whilst predatory selection of larger herbivores by fish plays an accompanying role of varying magnitude (Gliwicz 1985). Threlkeld (1985) suggested that only brief resource-mediated decreases in birth rate are sufficient to initiate declines in *Daphnia* populations. Larsson *et al.* (1985) demonstrated that a short period of reduced *Daphnia* growth and fecundity concomitant with a brief *Anabaena* bloom facilitated a shift to *Diaphanosoma* and *Bosmina*.

Studies on competition for resources amongst cladocerans also show that variations in growth and fecundity are strongly influenced by their ability to utilize the available foods. Kerfoot *et al.* (1985) and Orcutt (1985) showed that *Bosmina* and *Diaphanosoma* survived better than *Daphnia* at low food-

resource levels, for example when the phytoplankton was composed of largely inedible or resistant algal species or cyanophyte colonies; these smaller cladocerans showed rapid positive responses to reductions in the *Daphnia* population. *Daphnia* fed upon a wider variety of the food resources available, however, and dominated when food was abundant. The marked annual shifts in phytoplankton composition and the overwhelming abundance of large *Microcystis* colonies in hypertrophic Hartbeespoort Dam (Robarts and Zohary 1984) present an example of an extreme change in food resource availability to the zooplankton community. Food mediated shifts in cladoceran species composition comparable to those described above occur in Hartbeespoort Dam. Growth of the *Ceriodaphnia* population and its continued existence during the summer-autumn period, when edible phytoplankton resources are scarce and when its filtration rates on even small *Microcystis* colonies is low, indicates that a change occurs in the principle food resource utilized. *Ceriodaphnia* and the summer cladoceran community may rely partially on particles such as broken and decomposing *Microcystis* colonies or may depend to a large degree on the abundant free-living bacteria in Hartbeespoort Dam (Section 5).

The hypothesis of Gliwicz (1969a, 1969b) that indirect decomposition pathways via bacteria and suspended detritus become increasingly important to zooplankton as increasing trophic status leads to cyanophyte dominance has received much support. Recently Hanazato and Yasuno (1985) suggested that in eutrophic Lake Kasumigaura, which is dominated in summer by *Microcystis*, increased cladoceran production is prompted by high rates of decomposition of *Microcystis* and the associated bacterial flora. Subsequently, Hanazato and Yasuno (1987) demonstrated that *Moina micrura* did not grow or reproduce well even on edible-size *Microcystis* particles. Decomposed *Microcystis* was, however, a utilizable food (Hanazato and Yasuno 1987). This food resource may also be of importance to the *Ceriodaphnia* dominated summer zooplankton community in Hartbeespoort Dam, in addition to some ingestion of small colonies or unicellular *Microcystis* (as suggested in Section 2). Pace

et al. (1983) demonstrated that *Ceriodaphnia* efficiently utilizes and survives on bacteria which is not a primary resource for *Daphnia*. The increasing importance of this small food fraction as water temperatures increase in summer is another factor facilitating the shift to smaller cladoceran species.

Comparison of seasonal bacterial activity levels in Hartbeespoort Dam (Robarts and Sephton 1984) with zooplankton filter-feeding activity supports the likely importance of bacteria to the summer cladoceran population. Robarts and Sephton (1984) observed an increase in bacterial numbers in the epilimnion (0-8 m) which correlated significantly with increasing temperature until January when there occurred a marked drop in bacterial numbers to the low population densities of late summer. This pronounced decrease in bacterial numbers was associated with equally pronounced increases in heterotrophic activity and the bacterial specific activity index (activity per cell, Robarts and Sephton 1984). The sudden increase in bacterial activity and concomitant sharp drop in bacterial numbers occurred at the same time as the shift to *Ceriodaphnia* dominance, about 1 month after *Microcystis* dominated phytoplankton food resources in Hartbeespoort Dam. Low filtration rates of *Ceriodaphnia*, *Moina* and *Diaphanosoma* on *Microcystis* colonies (Figure 4.0) despite their high filtration rates on *Chlorella* in summer (Figure 4.4) point to the presence of potentially high zooplankton grazing pressure on the nanoplankton and bacterioplankton fractions (the 'high efficiency bacteria feeders' of Geller and Müller 1981). Thus the small bodied cladoceran community seemingly utilizes the smallest fraction of the summer food resource and feeds on bacteria and the low concentration of edible phytoplankton whilst avoiding the problem of filtration interference by abundant large *Microcystis* colonies (for example, by narrowing the carapace gape; Gliwicz and Siedlar 1980).

In conclusion the species-specific data presented identifies the upper colony size limit to zooplankton feeding on *Microcystis* in a hypertrophic lake and shows that low filtration

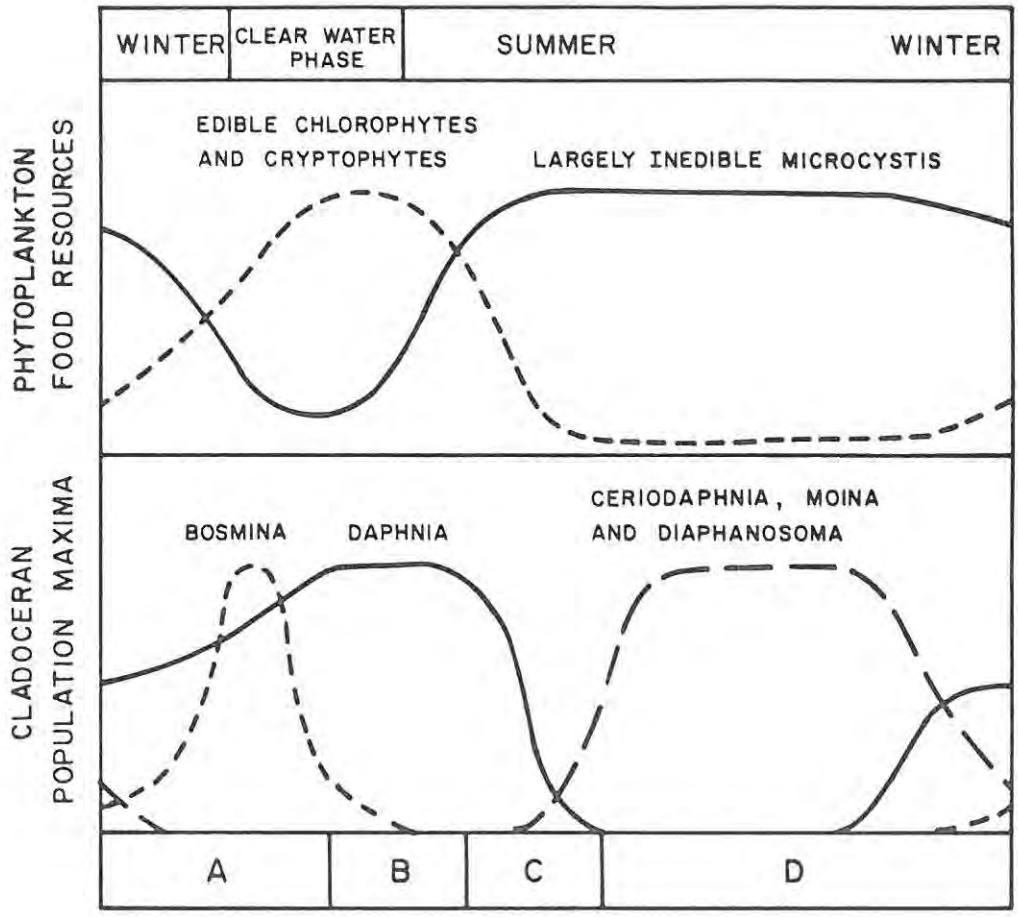


Figure 4.9: Schematic summary of phytoplankton and cladoceran succession in Hartbeespoort Dam. (A) *Daphnia* - *Bosmina* resource competition. (B) Maximum community grazing pressure. (C) Colony size limitation to feeding and reduced filtration efficiency. (D) Small particle utilization, bactivory and detritivory.

rates of the large herbivore *Daphnia* on *Microcystis* also reduces grazing pressure on edible sized *Microcystis* colonies up to 100 µm diameter. High *Bosmina* and *Daphnia* filtration rates and population densities occur when edible and partly edible phytoplankton species are abundant during the spring clear water phase (Figure 4.9). Reappearance of overwhelmingly abundant *Microcystis* of large colony form (Robarts and Zohary 1984) at mid-summer may be promoted by the antecedent high grazing pressure. Subsequently *Daphnia* filtration rates on *Chlorella* decline to only 25-36% of the rates measured during the edible phytoplankton phase implying interference by *Microcystis* colonies to efficient *Daphnia* filter-feeding. A shift in zooplankton community structure occurs annually to a smaller-bodied cladoceran species assemblage for which low filtration rates on *Microcystis* were measured during summer when edible phytoplankton food resources are typically very low. Evidence from changes in bacterial numbers and activity (Robarts and Sephton 1984) indicate that bacterioplankton potentially becomes an important food resource during the *Ceriodaphnia* phase of summer cyanophyte dominance in this hypertrophic impoundment (Figure 4.9). Consequently direct control of cyanophyte blooms through grazing by large herbivorous zooplankters such as *Daphnia* (Carlson and Schoenberg 1983, Schoenberg and Carlson 1984) is unlikely to succeed if colonies or filaments rapidly exceed dimensions of 60-100 µm, reduce filtration efficiency (Table 4.7), or are of low nutritional value. The high zooplankton community grazing activity measured in this hypertrophic lake (Section 3) is an environmental parameter of potential value in reducing phytoplankton biomass if the usual annual species shift to cyanophyte dominance can be moderated by food-web manipulation during implementation of lake management strategies. Data on size-selective limitations to grazing on problem cyanophytes such as *Microcystis* by zooplankton communities of known body size structure has practical application in lake management studies and in the improvement of models assessing phytoplankton grazing losses or predicting changes in the size structure of phytoplankton communities or standing stocks.

4.4.2 Cladoceran filtration rate - body-length model improvements for a *Microcystis* dominated hypertrophic reservoir

Inclusion of model variables which achieve major improvements in explained variance whilst keeping model parameters to a minimum is an important consideration in the development of simple models. In this study, cladoceran body length accounted for up to 70% of variance in filtration rate when measured *in situ* under hypertrophic conditions. Other major variables which significantly contributed to the explained variance were the particle size of the food resource and the cladoceran species.

Cladoceran species

Species-specific FR:L models may be of value if estimates of filtration rates are required for the purpose of studying interspecific competition and niche overlap. Given that major taxonomic differences exist in feeding behaviour (eg. *Bosmina* vs. *Daphnia*, DeMott 1982) ideally it is necessary to include such effects. But such subdivision may not be justified during broader plankton or ecosystem studies due to the introduction of unnecessary complexity. These models for whole cladoceran communities thus provide 'ballpark' estimates of community filtration rate based on simple body length data. Stepwise regression analysis showed that in the *Chlorella* model the dummy variables for species explained over 10% of the variance in filtration rate. This was a significant contribution which, in the absence of any major contribution to the model from temperature, showed that $\sim$ 19% of the observed variance remained unexplained by the parameters included here. As this study was carried out *in situ* in a hypertrophic lake over a period of 9 months it is likely that unexplained variance in cladoceran filtration rates was in part due to seasonal fluctuations in the ambient particle size distribution of the natural food resource present during all *in situ* experiments. Additional variance may also be due to grouping of individuals into body-length classes and to the assumption of equal isotope loss rates by all species.

In the cladoceran community of Hartbeespoort Dam, *Ceriodaphnia* was the first species to enter the *Chlorella-Daphnia* model in the stepwise analysis and alone accounted for 7.2% of the variance in cladoceran filtration rate (Table 4.5). In the scatter-plots (Figure 4.6a and b) *Ceriodaphnia's* filtration rates were usually higher than the trend for cladocerans in general when feeding on *Chlorella* and were lower when feeding on *Microcystis* colonies >20 µm . This was highlighted previously in terms of the high SFR exhibited during the *Ceriodaphnia* 'phase' in Hartbeespoort Dam at a time of low total community grazing rates and herbivore biomass (Figure 3.12). This occurred following the mid-summer shift to *Microcystis* dominance which almost excluded other phytoplankton species. Chow-Fraser and Knoechel (1985) and Knoechel and Holtby (1986a, 1986b) also found that filtration rates of *Ceriodaphnia* were higher than those of *Daphnia*, convincingly demonstrating that *Ceriodaphnia* feeds more efficiently than small *Daphnia* on *Chlorella* as shown earlier by Neill (1975) and Lynch (1978). Stepwise model entry of dummy variables representing the remaining species further reduced residual variance by only 3.2%.

In place of body length, herbivore biomass may also be an easily measured parameter which can be used to estimate zooplankton filtration rate by their linear function (Peters and Downing 1984, Lampert 1985). Most of the variation in zooplankton filtration rates in a turbid reservoir was explained by herbivore biomass (Hart 1984, 1986). Similarly, depth-specific and depth integrated zooplankton herbivore biomass explained 43.4 % and 48.9% respectively of the variance in seasonal community grazing rate in the aerobic water column of Hartbeespoort Dam (Section 3). Taxonomic subdivision of data and inclusion of data on natural variations in the particle size spectra and concentrations of natural food resources could also further significantly improve grazing rate predictions based on herbivore biomass.

Food particle or colony size

The influence of food particle size spectra and seston concen-

trations on zooplankton filtration rates *in situ* have been highlighted by Gulati *et al.* (1985), McCauley and Downing (1985) and Hart (1986). The earlier studies by Webster and Peters (1978) and Porter and McDonough (1984) showed how the presence of large cyanophyte colonies can adversely affect filtration efficiencies, particularly of large-bodied cladocerans such as *Daphnia*. In Hartbeespoort Dam the high filtration rates of the largest herbivore *Daphnia* on *Chlorella* during the spring clear water phase were much lower immediately following the reappearance of abundant *Microcystis* in summer. This reduction in *Daphnia's* feeding efficiency associated with summer dominance of the phytoplankton by *Microcystis*, when filtration rates only amounted to 25-35% of the maximal spring rates, implies that changes in natural food particle size spectra and food type are potentially important model parameters which may further significantly reduce residual variance.

The significant differences in both slope and intercept between models for *Chlorella* and *Microcystis* colony fractions of 5-20 µm, 20-40 µm, 40-60 µm and 60-100 µm (Table 4.6), and the considerable variance in filtration rate explained by labelled colony size fractions (13.8 and 16.1%) in the combined models of all food types tested (Tables 4.3 and 4.4) shows the significant contribution of food particle size to model improvement. Therefore increasing complexity by model subdivision based on particle size seems justified, particularly in eutrophic or hypertrophic lakes where cyanophytes are a major component of the food resource which, by colony or filament formation during blooms may radically increase the frequency of large food particles present, thereby influencing filtration rates.

Comparison of results from hypertrophic Hartbeespoort Dam with similar studies by Knoechel and Holtby (1986a, 1986b) showed that in less enriched Lake St George residual variance was lower. Lake St George, Canada, which Knoechel and Holtby (1986a) described as 'productive', is mesotrophic (Knowles *et al.* 1981), with a mean summer chlorophyll *a* concentration of

8 µg ℓ^{-1}. Body length alone accounted for 87%, 93% and 95% of variance in cladoceran filtration rate on cultured bacteria, yeast, and the chlorophyte *Pandorina* respectively, and almost 87% on a combined model of these foods (1-20 µm particle size range, Knoechel and Holtby 1986b). Hartbeespoort Dam by comparison has exceptionally high mean annual chlorophyll *a* concentrations ranging from 40-94 µg ℓ^{-1} over the euphotic depth (NIWR 1985). *Microcystis* blooms and dense surface accumulations occur for 9-10 months each year whereas only in spring do chlorophytes and cryptophytes dominate the phytoplankton. There is a need to take cognisance of food particle size over a prolonged period in Hartbeespoort Dam compared to the relatively brief summer periods when cyanophyte blooms occur in less enriched temperate lakes. Less extreme concentrations and variations in phytoplankton resources in Lake St George and the seasonal limitations of Knoechel and Holtby's (1986a) two month study probably contribute to lower unexplained variance in their individual cladoceran FR:L models on specific foods.

Feeding interference by cyanophytes

The models of cladoceran FR:L relationships in Hartbeespoort Dam are shown in Figure 4.10. This shows that the filtration rate of small cladocerans declines rapidly as particle size increases (reduced intercept and increased slope as particle size increases). When compared with the similar models of Chow-Fraser and Knoechel (1985) and Knoechel and Holtby (1986b), untransformed filtration rates calculated from equations in Table 4.8 for small cladocerans of 0.5-1.0 mm in length feeding on *Chlorella* in Hartbeespoort Dam were similar to those estimated for 10 Canadian lakes using *Chlorella* or *Scenedesmus* and rates in Lake St George measured using yeast. Filtration rates calculated for small cladocerans in Hartbeespoort Dam were also higher than the filtration rates reported on the other foods tested by Knoechel and Holtby (Table 4.8). Filtration rates of large cladocerans in Hartbeespoort Dam were, however, markedly lower than those calculated from the Lake St George models. For cladocerans of 2.0 mm in length

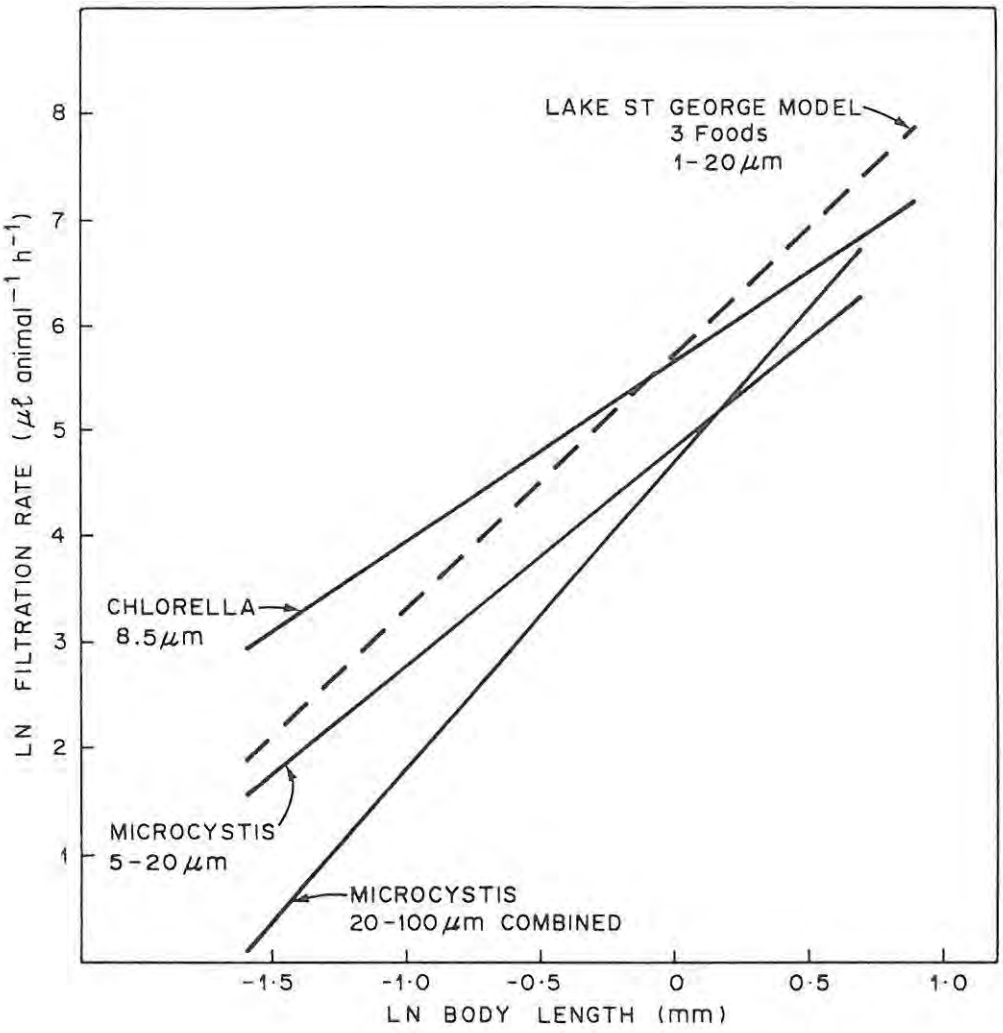


Figure 4.10: Comparison of the three significantly different cladoceran FR:L models from Hartbeespoort Dam with the combined model on foods of 1-20 μm particle size from Lake St George (Knoechel and Holtby 1986b).

Table 4.8: *Daphnia* and cladoceran community FR:L relationships measured *in situ* under various lake conditions reported in the literature and for Hartbeespoort Dam (this study). FR = filtration rate (mℓ animal^{-1} d^{-1}), L = body length (mm).

Source	Lake	Condition	Zooplankton	Food	Equation FR=	r^2
Chow-Fraser & Knoechel 1985	10 lakes	Applicable from oligo- to mesotrophy only.	Cladocerans	*Chlorella* or *Scenedesmus*	$6.31\ L^{2.45}$	0.59
Chow-Fraser & Sprules 1986	Three Mile	Mean summer Chl. *a* 7.5 µg ℓ^{-1}, *Anabaena* filaments common.	*Daphnia* spp.	*Chlorella*	$2.951\ L^{1.22}$	0.20
			Daphnia spp.	*Anabaena*	$3.467\ L^{1.80}$	0.44
	Gull	Mean summer Chl. *a* 1.8 µg ℓ^{-1}, *Anabaena* filaments infrequent.	*Daphnia* spp.	*Scenedesmus*	$11.482\ L^{2.01}$	0.65
			Daphnia spp.	*Anabaena*	$4.571\ L^{2.13}$	0.77
Haney 1985	Experimental pond	150 µm filtered water from Gull Lake.	*D. pulex* (day)	Yeast	$10.09\ L^{1.61}$	0.42
			D. pulex (night)	Yeast	$19.62\ L^{2.13}$	0.94
Chow-Fraser 1986	Picard		*Daphnia* spp.	*Chlorella*	$9.772\ L^{2.25}$	0.04
Knoechel & Holtby 1986a	St George	Mean summer Chl. *a* 8 µg ℓ^{-1},* productive.	Cladocerans	Yeast	$11.695\ L^{2.48}$	0.93
Knoechel & Holtby 1986b	St George	Mean summer Chl. *a* 8 µg ℓ^{-1},* productive.	Cladocerans	Bacteria	$5.105\ L^{2.176}$	0.87
			Cladocerans	*Pandorina*	$7.534\ L^{3.002}$	0.95
			Cladocerans	3 foods	$7.396\ L^{2.403}$	0.65
This study	Hartbees-poort Dam	Mean annual Chl. *a* 40-94 µg ℓ^{-1} (euphotic depth), hypertrophic, *Microcystis* colonies abundant for 9-10 months.	Cladocerans	*Chlorella*	$6.782\ L^{1.682}$	0.70
			Cladocerans	*Microcystis* 5-20 µm	$3.014\ L^{2.054}$	0.58
			Cladocerans	*Microcystis* 20-100 µm	$2.655\ L^{2.894}$	0.67
			D. pulex	*Chlorella*	$6.901\ L^{2.104}$	0.70

* from Knowles *et al.* (1981).

filtration rates calculated from the *Chlorella* model in Hartbeespoort Dam were only 33% of filtration rates on similarly sized yeast cells in Lake St George, 36% of rates on *Pandorina*, 94% of rates on cultured bacteria and 55% of rates calculated from the combined food model of Knoechel and Holtby (1986b). The markedly lower filtration rates of large cladocerans on *Chlorella* (8.5 µm) in Hartbeespoort Dam compared to yeast cells (6 µm) in Lake St George and *Chlorella* in 10 Canadian Lakes also support the hypothesis that the naturally abundant colonies of *Microcystis* in the hypertrophic lake interfere with the *in situ* feeding rate of large herbivores, particularly *Daphnia* (Webster and Peters 1978). Chow-Fraser and Knoechel (1985) acknowledged that their model was limited only to oligotrophic or mesotrophic lakes and that it does not apply where cyanophyte filaments are prevalent due to interference or 'edibility' criteria. When compared to the *Pandorina* model (<20 µm) from Lake St George (Knoechel and Holtby 1986b), cladoceran filtration rates on the 5-20 µm *Microcystis* colony fraction in Hartbeespoort Dam were much lower (40% at 1.0 mm length; 21% at 2.0 mm length). This suggests that feeding by cladocerans on this cyanophyte may possibly be partially regulated by chemosensory or 'taste' factors when particle size is not a limiting factor (Poulet and Marsot 1978, DeMott 1986).

Other studies examining *in situ* FR:L relationships on various foods were usually species-specific, with *Daphnia* the most frequent taxon examined in a number of lakes of varying trophic status (Table 4.8). In the study of Chow-Fraser and Sprules (1986) filtration rates of *Daphnia* spp. were significantly lower in Three Mile Lake, in which *Anabaena* filaments were common, than in the mainly filament-free Gull Lake. This was also partly attributed to interference with the feeding efficiency of *Daphnia* by cyanophyte filaments, resulting in a 64% reduction in filtration rate. Parameters for the cladoceran models on *Microcystis* in hypertrophic Hartbeespoort Dam were similar to those of the *Daphnia* model on *Anabaena* in eutrophic Three Mile Lake. Untransformed filtration rates solved for 1 mm cladocerans in Hartbeespoort Dam feeding on

<20 µm and >20 µm *Microcystis* colonies, and 1 mm *Daphnia* feeding on *Anabaena* in Three Mile Lake were respectively 66%, 58% and 76% of rates calculated from the *Anabaena* model from the less productive Gull Lake. In the absence of abundant cyanophyte colonies or filaments in Gull and Picard lakes (Table 4.8), *Daphnia's* filtration rates on unicellular chlorophytes were extremely high (Chow-Fraser 1986) and were still higher on yeast cells in Gull Lake water filtered to remove all filaments >150 µm (Haney 1985). Many of the high filtration rates reported for *Daphnia* species from *in situ* studies using unicellular food (Table 4.8) exceeded rates measured in controlled laboratory studies (McMahon 1965, Geller 1975, DeMott 1982).

Diel and temperature effects

Haney (1985) reported strong diel variations in *Daphnia* filtration rates in the Canadian lakes that he studied which could greatly influence predictions of daily filtration rates. However, in Hartbeespoort Dam no significant diel variation in community grazing rate was evident (Section 3). Day:night influences examined by Chow-Fraser and Knoechel (1985) were insignificant and in the study of Knoechel and Holtby (1986a) were also regarded as of minor importance only explaining 0.2% of variance in filtration rate in their stepwise multiple regression analysis. Temperature also had little influence on cladoceran filtration rates in Lake St George (0.6% of variance, Knoechel and Holtby 1986a). Similarly in multiple regression analyses of data from Hartbeespoort Dam, temperature was shown to have little influence on the FR:L relationship, accounting for 0.2 to 3.6% of the variance. Consequently both diel and temperature effects on the FR:L models in Hartbeespoort Dam can be largely disregarded.

Conclusions

Whilst numerous predictive species-specific FR:L models already exist and cladoceran community or total zooplankton models have been developed in which biomass or body length

account for the majority of explained variance (eg. Peters and Downing 1984, Knoechel and Holtby 1986a, 1986b), the presence of abundant cyanophytes in hypertrophic systems increases the importance of seston particle size as a variable significantly influencing filtration rates. In nutrient enriched waters, cyanophyte colonies influence the slope and intercepts of linear regression models estimating zooplankton filtration rates *in situ* on the more palatable or preferred foods such as chlorophytes. In addition the slopes and intercepts of linear models estimating filtration rates on these less palatable cyanophytes also vary significantly with colony or filament size. Whilst these models may not necessarily be generally applicable to all enriched, cyanophyte dominated systems, it is intended that by highlighting the changes in zooplankton FR:L models that occurred on *Microcystis* colonies in hypertrophic Hartbeespoort Dam, the suite of models described here will provide insight for the improvement of predictions of zooplankton clearance rates and phytoplankton grazing losses. Advancing the accuracy of such estimates in eutrophic and hypertrophic systems can be of value to environmental programmes aimed at lake management and restoration.

5. FILTRATION RATES OF THE MAJOR ZOOPLANKTON SPECIES ON NATURAL, FREE-LIVING, LAKE BACTERIA

5.1 Introduction

Bacteria have been identified as being an important resource utilized by many zooplankton grazers in addition to algal and cyanophyte foods (Gliwicz 1969b, Peterson *et al.* 1978, Hollibaugh *et al.* 1980, Riemann and Bosselmann 1984). With the frequently observed seasonal shift to a smaller-bodied zooplankton community (small crustaceans, rotifers and protozoans), particularly in eutrophic waters (Gliwicz 1969b), it has been assumed and recently determined that these generally small grazers rely more heavily on small-sized particles and feed more efficiently on bacteria than large filter-feeders (Geller and Müller 1981, Pedros-Alio and Brock 1983, Porter *et al.* 1983). This is certainly the case with regard to the microzooplankton grazers (protozoans such as flagellates and ciliates). These microzooplankters have been shown to play a major role in limiting bacterial biomass whilst heterotrophic activity is high in summer, or in limiting the number and, by size-selective grazing, limiting the cell size of bacteria (Pace 1982, Porter 1984, Wright and Coffin 1984, Servias *et al.* 1985, Güde 1986, Borsheim and Andersen 1987).

Bacterial grazing by macrozooplankton has also been the subject of intensive investigations (eg. Pace *et al.* 1983, Pedros-Alio and Brock 1983, Porter *et al.*, Borsheim and Olsen 1984, Forsyth and James 1984, Porter 1984, Riemann and Bosselmann 1984, Nagata 1985, Schoenberg and Maccubbin 1985, Bjornsen *et al.* 1986, Bern 1987, Borsheim and Andersen 1987). Laboratory studies have shown that the macrozooplankton, particularly cladocerans, can efficiently filter very small food particles and bacterial cells (Gophen *et al.* 1974, Peterson *et al.* 1978, Pace *et al.* 1983, Porter *et al.* 1983, Gophen and Geller 1984, Brendelberger 1985). Working closer to more natural or field conditions, other studies have used concentrated zooplankton populations or communities and natural, lake bacteria (Peterson *et al.* 1978, Griffiths and

Caperon 1979, Pedros-Alio and Brock 1983, Riemann and Bosselmann 1984, Bjornsen *et al.* 1986, Bern 1987, Borsheim and Andersen 1987). Several studies have shown that experimental artifacts can influence the conclusions reached from such studies. Bjornsen *et al.* (1986) found that zooplankton grazing rates on bacteria were 3 to 3.8 times lower when the grazers were concentrated than when natural concentrations were used in experimental tests.

Peterson *et al.* (1978), Porter *et al.* (1983) and Porter (1984) have also expressed caution when comparing or extrapolating laboratory results, often obtained using cultured bacteria, to filtration rates measured *in situ* using natural, lake bacteria. Not only do cultured and natural, lake bacteria differ greatly in cell size (cultured cells often 1-3 µm in length, natural cells often 0.15-1.0 µm in length) but they may also differ in surface properties such as charge or wettability that can alter the particle collection efficiencies of zooplankton (Gerristen and Porter 1982).

Forsyth and James (1984) compared clearance rates of bacterioplankton by zooplankton using four methods (changes in direct cell counts; changes in radioactivity of the bacterioplankton; radiolabel uptake by zooplankton under laboratory conditions; *in situ* measurement using a grazing chamber). They regarded grazing rates measured using a radiolabel (methyl-^{3}H-thymidine) as being reasonable estimations, and the *in situ* technique as most closely approximating true zooplankton grazing rates under natural environmental conditions with minimal effects due to stress.

True *in situ* studies on radiolabelled natural bacteria are also not free from sources of error. Many variations in methods and radiolabelled compounds used (acetate, glucose and methyl thymidine) have been reported, with the use of methyl-^{3}H-thymidine (Hollibaugh *et al.* 1980) in grazing studies gaining favour. This method has been further complemented by studies on methyl-^{3}H-thymidine uptake by natural bacteria (Fuhrman and Azam 1982, Servias *et al.* 1985) and on the

preparation and *in situ* use of labelled bacterioplankton (Roman and Rublee 1981, Forsyth and James 1984, Bjornsen *et al.* 1986, Bern 1987).

This section examines the filtration rates of the major zooplankton filter-feeders on natural, free-living bacteria in hypertrophic Hartbeespoort Dam. However, considerable problems were encountered regarding the reliability of results obtained using labelled bacterioplankton *in situ* under these hypertrophic conditions. Consequently, methodological problems in the experimental protocols initially followed (Hollibaugh *et al.* 1980, Roman and Rublee 1981) were examined with the aim of highlighting shortcomings, reducing errors and contributing to an improvement of *in situ* methods employed in bacterioplankton grazing studies.

5.2 *In situ* Measurement of Grazing on Natural Bacteria: Developments and Improvements to Methods

5.2.1 Pilot experiments using methyl-^{3}H-thymidine

Methyl-^{3}H-thymidine was selected as the compound most suitable for use as a radiolabel for natural bacteria from Hartbeespoort Dam, due to its successful use in other bacterial grazing studies as outlined above, and because of the nature of its uptake by bacteria. Methyl-^{3}H-thymidine is largely incorporated into bacterial macromolecules (up to 80% into DNA in bacteria from Hartbeespoort Dam) and is therefore metabolically fairly conservative (Robarts *et al.* 1986). Furthermore, low micromolar concentrations of methyl-^{3}H-thymidine have been shown to be selectively incorporated by bacteria and not by algae or cyanophytes (Roman and Rublee 1981, Fuhrman and Azam 1982, Bern 1985) allowing its use as a bacteria-specific label.

Roman and Rublee (1981) used methyl-^{3}H-thymidine released directly into a grazing chamber in their 1 h duration experiments on bacterioplankton grazing by marine zooplankton. In

other studies incubation of bacteria with radiolabel was allowed before the release of cells into the feeding medium, bacteria being either unrinsed or partially rinsed free of unincorporated radiolabel (Forsyth and James 1984, Riemann and Bosselmann 1984, Schoenberg and Maccubbin 1985, Bjornsen *et al.* 1986, Bern 1987, Borsheim and Andersen 1987). In these studies incubation times varied from as short as 1 h to 'overnight' (up to 24 h) using either the natural bacteria at lake concentrations or bacteria further concentrated by filtration, often after removal of algae and micrograzers (3 µm filtration).

Initially the labelling method described by Hollibaugh *et al.* (1980) was used in Hartbeespoort Dam in conjunction with the *in situ* grazing chamber technique. Lake water was filtered through 3 µm membrane filters and incubated for 1 h, which has been regarded as sufficient time for methyl-^{3}H-thymidine uptake by bacteria (Roman and Rublee 1981, R.D. Robarts pers. comm.). However, problems were experienced with this technique: firstly, it was not possible to replicate results of experiments carried out in series, or even in parallel using both compartments of the grazing chamber simultaneously; secondly, attempts to increase substantially the concentration of labelled natural bacteria (Table 5.0) or remove unincorporated radiolabel were unsuccessful.

As in *in situ* feeding experiments using algae, use of a concentrated suspension of labelled natural bacteria was regarded as being of importance, firstly because the zooplankton grazers were not concentrated, and secondly because the maximum volume of food 'spike' introduced into each compartment of the 3 ℓ grazing chamber was limited to 5 mℓ, and so was considerably diluted. In addition a concentrated suspension of labelled bacteria of high specific activity was needed to allow adequate ingestion of labelled cells to produce reliable results within the short duration of *in situ* feeding experiments (6-7 min) whilst confining increases in the total bacterial-food concentration present during each experiment to <3% (Table 5.3).

Concentration of natural bacteria by filtration using a 0.2 µm membrane filter (Hollibaugh *et al.* 1980, Pedros-Alio and Brock 1983) resulted in the occasional loss of many cells by their adhesion to the membrane or loss of some cells through the membrane filter. The latter was due to the extremely small size of natural free-living bacteria in Hartbeespoort Dam which included cocci with diameters of 0.2-0.5 µm (minimum cocci diameter ≅0.1 µm) and rods 1.0 x 0.3 to 3.5 x 0.7 µm (Robarts and Septhon 1984). 10-15% of small cocci in Hartbeespoort Dam have been found to pass through a 0.2 µm membrane filter under vacuum pressure (R.D. Robarts, pers. comm.).

Recently, photographic negatives of scanning electron micrographs of DAPI stained bacteria (DNA-specific stain) from Hartbeespoort Dam prepared by R.D. Robarts were sent to the Max-Planck-Institute for Limnology, Plön, West Germany for morphological analysis by C. Krambeck. The size distribution and cell volume of free-living bacteria from Hartbeespoort Dam was obtained by projection of these micrograph negatives onto a digitizer field, followed by image analysis using the digitizer-microcomputer system described by Krambeck *et al.* (1981) is summarized in Table 5.1.

Table 5.1 shows that the majority of bacteria in Hartbeespoort Dam are very small rods and cocci (<0.71 µm wide, with cell length averaging 2.15 x cell width). Computer analysis of bacterial size-classes showed that some cells in a 0-0.09 µm width-class were present whilst for the majority, cell widths lay between 0.09 - 0.25 µm, or more specifically 0.12 - 0.18 µm . These numerous, very small cells only contribute little to the total biovolume of bacteria present (<20%) but their recognition and enumeration highlights a potential problem and source of error in bacterioplankton grazing studies which has not generally been considered or examined in any detail.

Table 5.0: Number of bacteria (x 10^6 $m\ell^{-1}$) in labelled concentrated suspension following the concentration procedure of Hollibaugh *et al.* (1980) using 0.2 µm membrane filters. Bacteria counted using the DAPI technique (Robarts and Sephton 1981). Theoretical maximum concentration factor = 10 (250 mℓ reduced to 25 mℓ).

Date	Lakewater bacteria	0.2 µm 'concentrated' bacterial suspension	Concentration factor
4.9.85	7.5	2.8	0.4
5.9.85	9.8	20.8	2.1
11.9.85	11.6	18.3	1.6
12.9.85	12.1	9.8	0.8

Table 5.1: Summary of the dimensions of free-living bacteria in Hartbeespoort Dam. Analysis carried out by C. Krambeck on SEM negatives provided by R.D. Robarts

Max. cell width (rods and cocci)	Mean cell volume	Mean biovolume x 10^6 $m\ell^{-1}$	Mean length per width
<0.71 µm	0.013 μm^3	0.06 μm^3	215%

Mean of % biomass of cells up to 0.18 µm width = 19.31%
Mean of % biomass of cells above 0.18 µm width = 80.69%

Table 5.2: Activity present in edge region of 0.2 µm filters (31% of total filter area), after 3 x 5 mℓ rinses with autoclaved lakewater, expressed as % of total activity on the filter (± standard error).

Date	n	%
26. 9.85	5	21.6 ± 3.4
24.10.85	16	17.5 ± 1.0
6.11.85	10	20.3 ± 1.1
14.11.85	26	14.8 ± 0.5

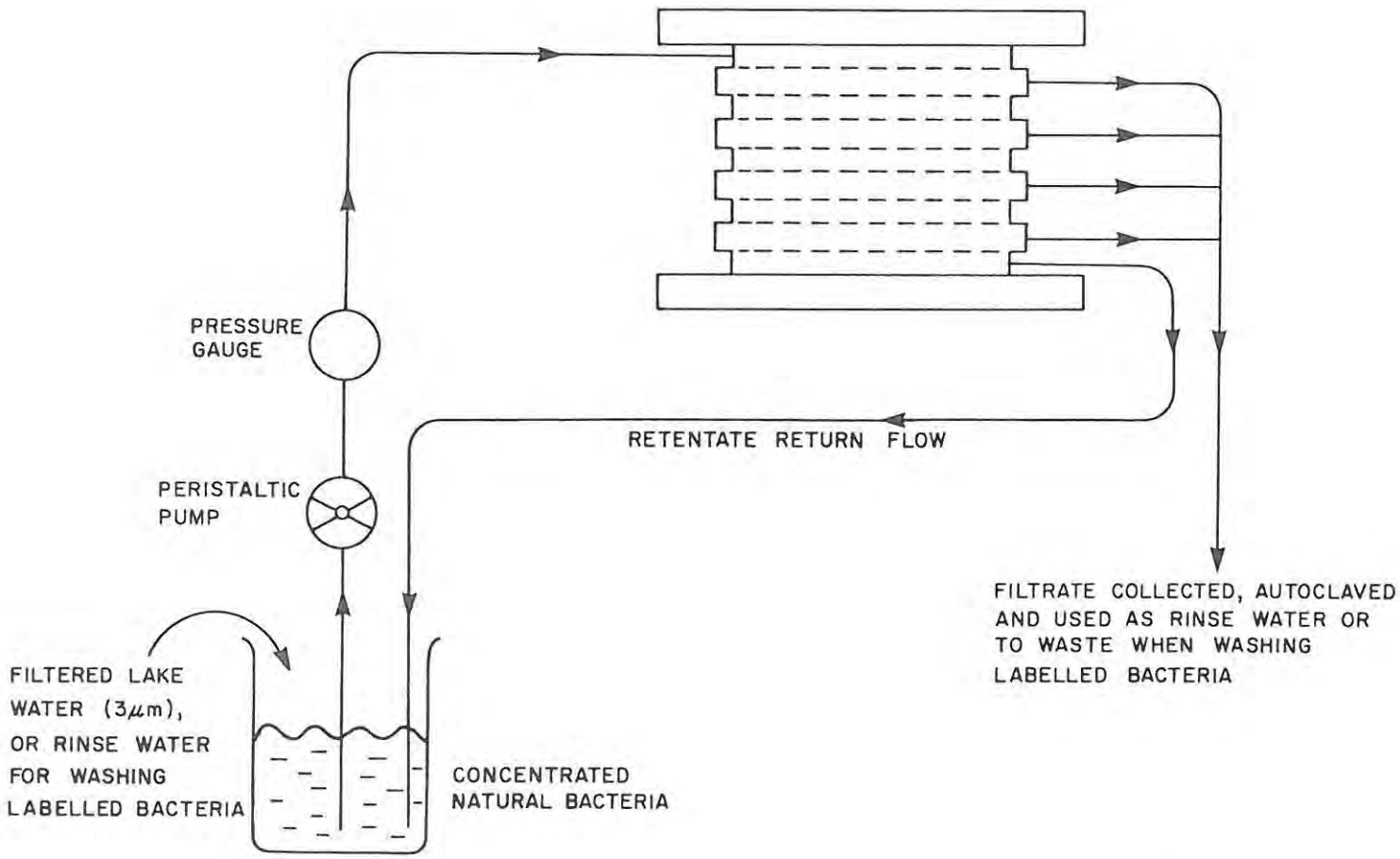


Figure 5.0: Cross-flow filtration system used to concentrate lake bacteria or wash labelled bacteria.

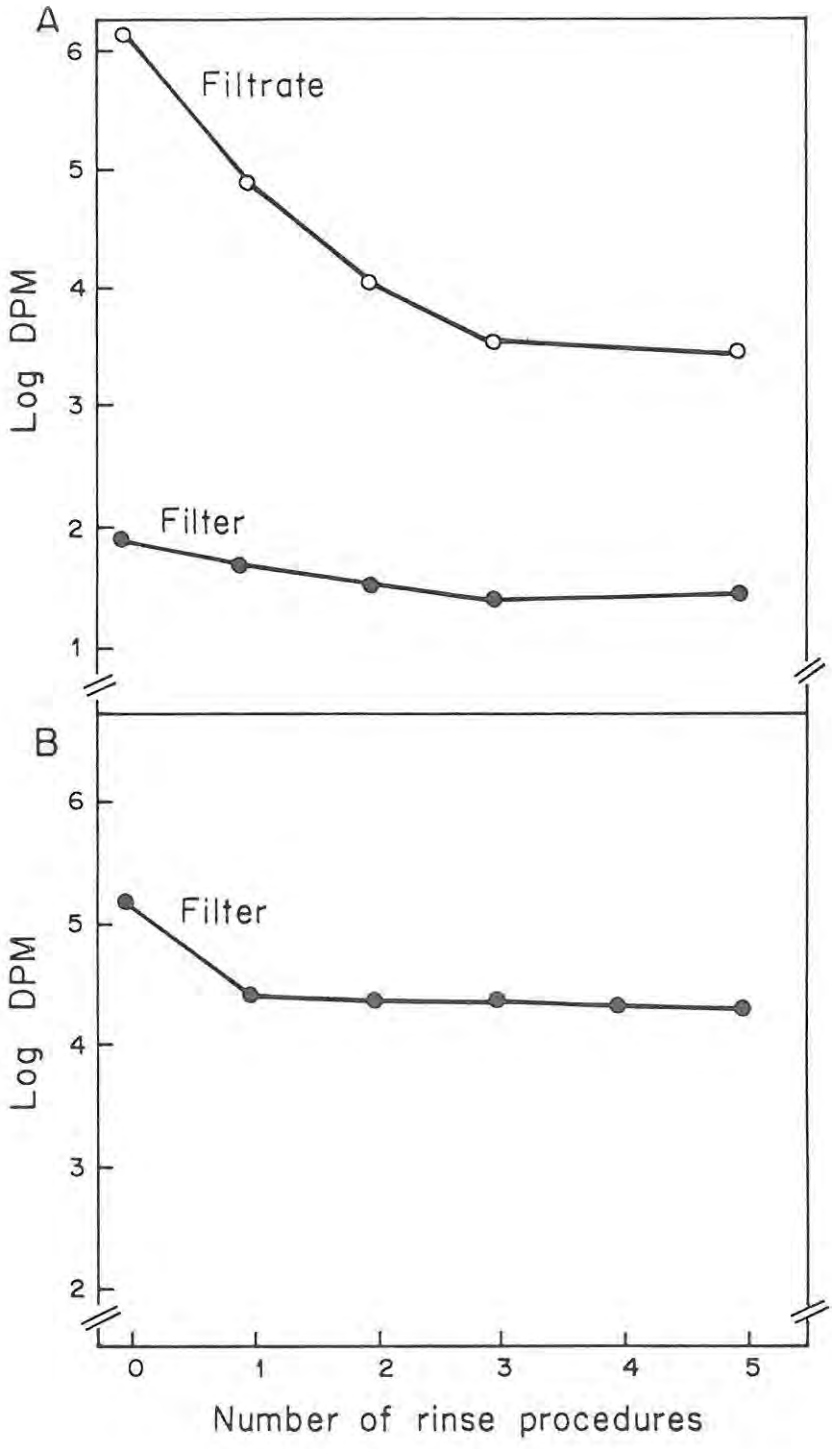


Figure 5.1: (a) Radioactivity of membrane filter and filtrate following successive rinsing procedures of a labelled bacteria suspension.
(b) Filter as in (a) but with additional rinsing (3 x 5 mℓ)

5.2.2 Concentration of natural bacteria using a tangential (cross-flow) ultra-filtration system

Tangential flow filtration was used to achieve very high concentrations of natural bacteria from large initial volumes of lakewater. 2-6 ℓ of lakewater was used depending upon the number of experiments planned and thus the volume of concentrated labelled bacteria required. Lakewater was prefiltered through a 10 µm mesh to remove macrozooplankton and *Microcystis* colonies, then filtered through glass fibre and 3 µm Nucleopore filters to remove all phytoplankton and microzooplankton grazers. Bacteria were then concentrated using a cross-flow ultra-filtration system (Minitan System, Millipore) fitted with a 100 000 NMW (nominal molecular weight) filter pack (Figure 5.0).

Using this system all bacterial cells, including the smallest cocci, were successfully retained and concentrated using only a low filtration pressure (0.8 kg cm^{-2}) and without clogging of the filter surfaces. Bacteria were counted using the DAPI technique described by Robarts and Sephton (1981). The retentate (200 mℓ of concentrated natural bacteria) was incubated in the dark with methyl-^{3}H-thymidine (initially 1 µCi $m\ell^{-1}$ for 1 h, later 2.5 µCi $m\ell^{-1}$ for 20 h after methodological improvements).

After incubation, the same cross-flow filtration system was used to wash unincorporated radiolabelled compounds from the labelled cells and to further concentrate the bacterial suspension to a final working volume (dependent upon the number of experiments and food 'spikes' required). Finally, the rinsed and concentrated labelled bacteria produced were again passed through a 3 µm membrane filter to remove any clumps or aggregations of bacteria that may have arisen during incubation and filtration.

5.2.3 Rinse procedures, contamination by unincorporated isotope and release of isotope from bacteria

Improvement of the above method for the preparation and use of

labelled natural free-living bacteria required examination of a number of potential problem areas.

Free radiolabel not bound within the bacteria cells is a potential source of error through contamination of membrane filters, adsorption to zooplankton exoskeletons and other particles, and to a much lesser degree through possible uptake of some radiolabel by unlabelled bacteria during the feeding experiments. Therefore methods were modified to allow for the washing of free isotope from the labelled bacteria, the adequate rinsing of membrane filters during experiments, and minimizing isotope release from labelled bacterial cells.

Washing of the concentrated labelled bacteria suspension ($\sim$ 200 mℓ) after incubation was carried out by further concentration of the bacterial suspension to approximately 50 mℓ followed by addition of 200 mℓ of autoclaved lakewater. This procedure was repeated to determine the number of times washing of cells was necessary. 2.5 mℓ of the washed labelled bacterial suspension was filtered onto a 47 mm diameter 0.2 µm filter after each rinse procedure. Activity on the filter and in 1 mℓ of the filtrate was counted with Filter Count (Packard). Results in Figure 5.1a show that initially free isotope contamination of the filter was very high, as confirmed by the high activity present in the filtrate. With repeated rinses of the bacterial suspension with autoclaved lakewater, contamination declined to a low and fairly constant level after 3 to 5 rinses. Results typical of this washing procedure are shown in Figure 5.1.

Figure 5.1b shows a result from a repeated series of rinse procedures (performed on a different date), but on this occasion the 0.2 µm filter was also rinsed three times with 5 mℓ aliquots of autoclaved lakewater to wash free isotope from the filter membrane. This additional rinsing of the 0.2 µm filter, used to determine the specific activity of food released during feeding experiments, further reduced contamination by free isotope. However, contamination was also detected in the filter edge (i.e. that part of the membrane

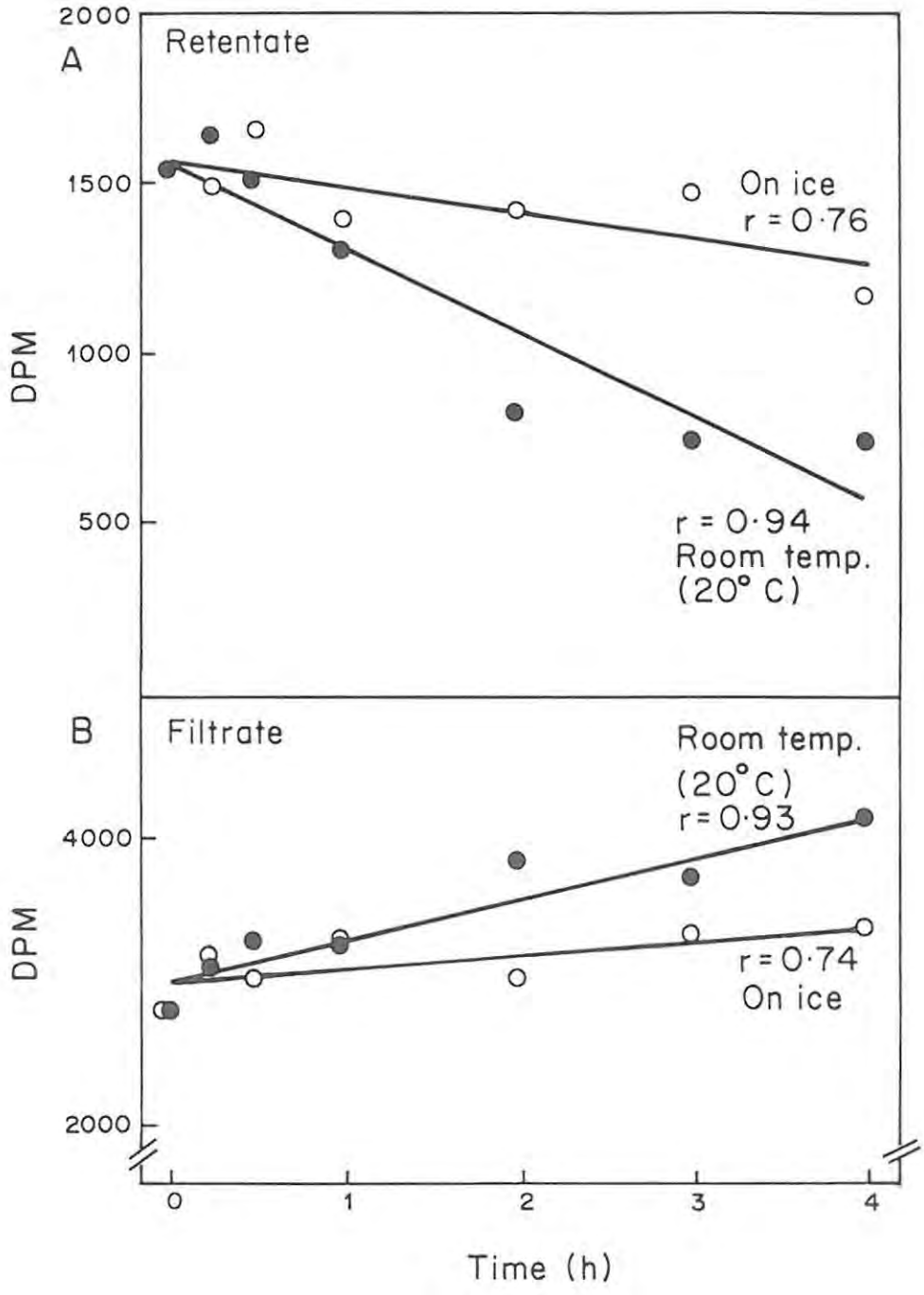


Figure 5.2: Radioactivity of labelled bacteria kept at room temperature or on ice.
(a) 1 mℓ of bacteria suspension retained on filter.
(b) 1 mℓ of the filtrate.

Table 5.3: Summary of pilot experiments examining methods for *in situ* measurement of bacterioplankton grazing by zooplankton. Community filtration rates (CFR) expressed as mℓ h^{-1} ± standard error (SE). Killed-control experiments (see text) were used to measure adsorption error. Dominant grazers in community: D = *Daphnia*, C = *Ceriodaphnia*.

Date	Isotope	Incubation (h)	Natural Bacteria x 10^6 mℓ$^{-1}$		% increase in food conc.	n	Mean CFR ± SE *Chlorella*	SE %	Mean CFR ± SE Bacteria	SE %	Adsorption error as % of CFR on Bacteria ± SE	Dominant Feeder
			Lake	Labelled								
26. 9.85	^{3}H-thymidine	1	16.84	39.71	0.4	4			66.82 ± 19.82	30	48.8 ± 15.1	D
3.10.85	^{3}H-thymidine	1	(LAB)	162.12	1.1	5			254.98 ± 31.55	12	32.4 ± 5.6	D
24.10.85	^{3}H-glucose	1				4			191.65 ± 75.19	39	122.9 ± 47.7	D
6.11.85	^{3}H-glucose	1				5			145.90 ± 26.17	18	96.0 ± 6.5	D
14.11.85	^{3}H-glucose	1				5	69.95 ± 7.75	11	188.12 ± 70.24	37	188.5 ± 83.9	D
21.11.85	^{3}H-glucose	14	(culture)	126.39		4	42.98 ± 6.17	14	26.93 ± 2.04	8	12.0 ± 3.1	D
6.12.85	^{3}H-thymidine	14	13.44	84.08	1.0	5	118.37 ± 9.11	8	191.45 ± 18.61	10	36.0 ± 8.3	D/C
11.12.85	^{3}H-glucose	14-20	18.40	175.71	1.6	5	69.78 ± 6.33	9	148.19 ± 15.87	11	104.8 ± 12.8	D/C
18.12.85	^{3}H-thymidine	14-20				5	31.06 ± 3.04	10	36.13 ± 3.15	9	74.4 ± 11.1	C
9. 1.86	^{14}C-glucose	14-20	18.03	148.98	1.4	5	45.13 ± 3.32	7	28.99 ± 2.80	10	32.4 ± 6.6	C
15. 1.86	^{14}C-glucose	14-20	10.82	180.00	2.8	5	38.03 ± 1.76	5	60.49 ± 3.33	6	46.2 ± 10.8	C
22. 1.86	^{14}C-glucose	14-20	14.36	106.33	1.2	5	48.98 ± 2.00	4	44.28 ± 5.21	12		C

filter clamped under the filter support, not retaining labelled bacteria whilst still being wetted by feeding medium). A filter cutter designed to remove the edge of the filter was used (Robarts *et al.* 1986), and activity in this edge portion was counted and found to be a source of error (Table 5.2), raising activity of the filter by an average of 18.6%. If this error was left uncorrected, the activity of labelled bacteria in the feeding medium during *in situ* experiments would be overestimated and consequently feeding rates on bacteria underestimated.

In addition to the various contamination errors examined above, isotope loss from the labelled bacteria was also measured. Rapid release of isotope from the bacteria would severely reduce their specific activity and hence reduce the uptake of activity during zooplankton feeding, thereby decreasing the sensitivity of *in situ* grazing measurements. Release of isotope from cells may also increase possible adsorption of isotope by other particles present and increase filter contamination error. The activity of labelled bacteria was therefore examined over a period of 4 h. Disintegrations per minute (d.p.m) of 3 mℓ aliquots of labelled bacteria kept at room temperature (20 °C) and kept on ice (<3 °C) were counted following filtration onto 0.2 µm filters. Activity in samples of filtrate was also counted. Figure 5.2a shows that the d.p.m of labelled bacteria kept on ice declined only slightly over 4 h when compared to the loss of isotope from bacteria kept at room temperature. Consequently isotope release into the filtrate occurred at a lower rate at low temperature than at room temperature (Fig. 5.2b). Therefore, after preparation, the labelled bacterial suspensions were kept on ice during transport and use in feeding experiments on Hartbeespoort Dam.

5.2.4 Adsorption error estimation, error reduction and outline of final procedure

The procedures described above were used to conduct numerous series of *in situ* experiments measuring zooplankton community

feeding rates on bacteria. Results of these trials are summarised in Table 5.3. Control experiments designed to measure adsorption of radiolabel to zooplankton (exoskeletons) and other particulates >60 µm present (i.e. abundant *Microcystis* colonies) were carried out in Hartbeespoort Dam. These killed-control or 'blank' experiments are equivalent to the 'blank' experiments of Roman and Rublee (1981), Pedros-Alio and Brock (1983) and Schoenberg and Maccubbin (1985). Adsorption of radiolabel to particles >60 µm in killed-control experiments (% error in Table 5.3) was measured using zooplankton collected using the grazing chamber and immobilized in soda water. Zooplankton immersed in soda water for >10 min either died or did not resume activity during killed-control experiments of the same duration as *in situ* experiments.

Results of initial *in situ* experiments at a fixed depth using concentrated bacteria labelled with ^{3}H-thymidine (carried out on 26.9.1985, Table 5.3) were extremely variable. Community filtration rates ranged from 26.23 - 117.42 mℓ h^{-1} and the standard error expressed as a percent of the mean (SE %) was high. Estimations of adsorption error in killed-control experiments were also very variable (range 25 - 87%) and SE % was also high. To eliminate zooplankton patchiness as a partial cause of the variability of the results, experiments were carried out next in the laboratory using 50 adult (∿2 mm body length) *Daphnia* per feeding trial. *Daphnia* filtration rates measured on natural bacteria were very high (3.10.1985, Table 5.3). Variability of results was lower in both the test and killed-control experiments than in the previous *in situ* trial, although variability was higher than expected for a laboratory study under controlled conditions. Another cause of this variability was the very low specific activity of the ^{3}H-thymidine labelled bacteria which resulted in low d.p.m counts after correction for background radiation.

In an attempt to increase label uptake by bacteria the labelled compound used was changed to ^{3}H-glucose which is also rapidly incorporated by bacteria. The specific activity of ^{3}H-glucose labelled bacteria was higher. However, variation

in the results of *in situ* experiments using ^{3}H-glucose was extremely high and the activity in killed-control experiments approached or exceeded that measured in feeding experiments (24.10.85 to 14.11.85, Table 5.3).

Due to the failure of these *in situ* experiments using natural bacteria, the validity of the method used was tested using a cultured non-pathogenic strain of *Salmonella*. To ensure a high specific activity, and thus a minimum of variability of results due to low specific activity of the label, the *Salmonella* culture was incubated with ^{3}H-glucose overnight (14 h). To further minimize adsorption error, experiments were performed in deep water essentially free of *Microcystis* colonies >60 µm. The rod shaped cells of *Salmonella* were all 1.5 x 1.0 µm, within the size range of free-living rod cells present in the dam but much larger than the more abundant cocci. Using identical procedures on 21.11.1985 to previous *in situ* experiments, the results obtained in four replicate experiments using both *Salmonella* and *Chlorella* were similar, with low variability and very low adsorption error in killed-control experiments (Table 5.3).

The success of these experiments using *Salmonella* showed that the problems experienced were not due to poor experimental design. In addition to greatly improving bacterial specific activity by the 14 h incubation period, these experiments indicated that high adsorption error may be attributable either to the abundant particulates usually present (*Microcystis* colonies) during *in situ* experiments in Hartbeespoort Dam or to the nature of radiolabelled compounds released from natural lake-bacteria. Experiments using methyl-^{3}H-thymidine labelled natural bacteria on 6.12.85, also in water mainly free from *Microcystis* colonies, yielded low estimates of adsorption error and lend support to the idea that much of the high adsorption error in experiments under hypertrophic conditions is due to adsorption of labelled compounds or excretory products to *Microcystis* colonies. However detailed examination of the nature of the adsorption error was beyond the scope of this study; efforts were confined to stabilizing

and estimating the magnitude of adsorption error to allow the *in situ* measurement of bacterioplankton grazing, particularly as experiments performed in the complete absence of *Microcystis* colonies cannot be regarded as typical of natural seston conditions and grazing responses in this hypertrophic impoundment.

A long incubation time had been avoided, firstly due to indications from studies reported in the literature that this was unnecessary as bacterial uptake of isotope is rapid, and secondly, in case confinement of natural free-living bacteria for many hours in small containers may increase the size of cells, which will thus no longer represent the natural size frequency distribution. Incubation of natural bacteria for 14 h with ^{3}H-thymidine on 6.12.1985 did result in an increase in label uptake by the bacterial suspension. No change in either the size of bacteria or of the proportions of cocci to rods was detected following incubation for up to 20 h, washing of cells, and further concentration immediately before use (Figure 5.3).

Subsequently, in experiments using methyl-^{3}H-thymidine, ^{3}H-glucose and ^{14}C-glucose with a 14-20 h incubation period (11.12.85 to 22.1.86) repeated community filtration rate measurements on bacteria were similar with a standard error similar to results obtained using *Chlorella* (Table 5.3). However adsorption error remained unacceptably high.

The adsorption error measured in killed-control experiments was examined using ^{14}C-glucose labelled bacteria. Experiments were conducted to determine if adsorption of radiolabelled compounds occurred over the duration of *in situ* experiments or immediately upon exposure of zooplankton and other particles to the labelled bacterial suspension (as assumed by the 'time zero' control experiments of Riemann and Bosselmann (1984) and Bjornsen *et al.* (1986)).

Figure 5.4 shows results of two series of experiments of varying duration comparing isotope uptake by inactive zoo-

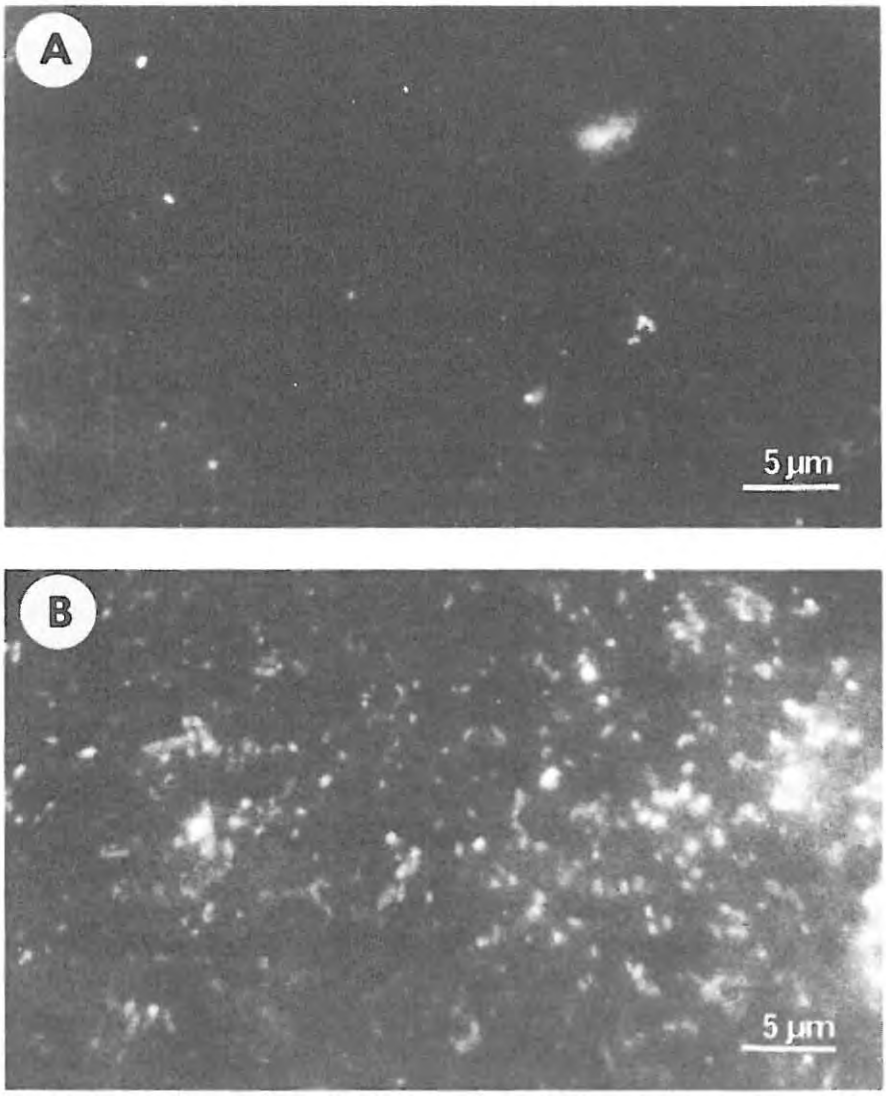


Figure 5.3: Photographs of natural lake bacteria from Hartbeespoort Dam. Bacteria stained using DAPI and viewed using an epifluorescent technique.
(a) Bacteria in lakewater.
(b) Bacteria after 20 h incubation with methyl-^{3}H-thymidine, concentration and rinsing.
Concentrated bacteria were diluted (1:5) to reduce the amount of fluorescence for photography.

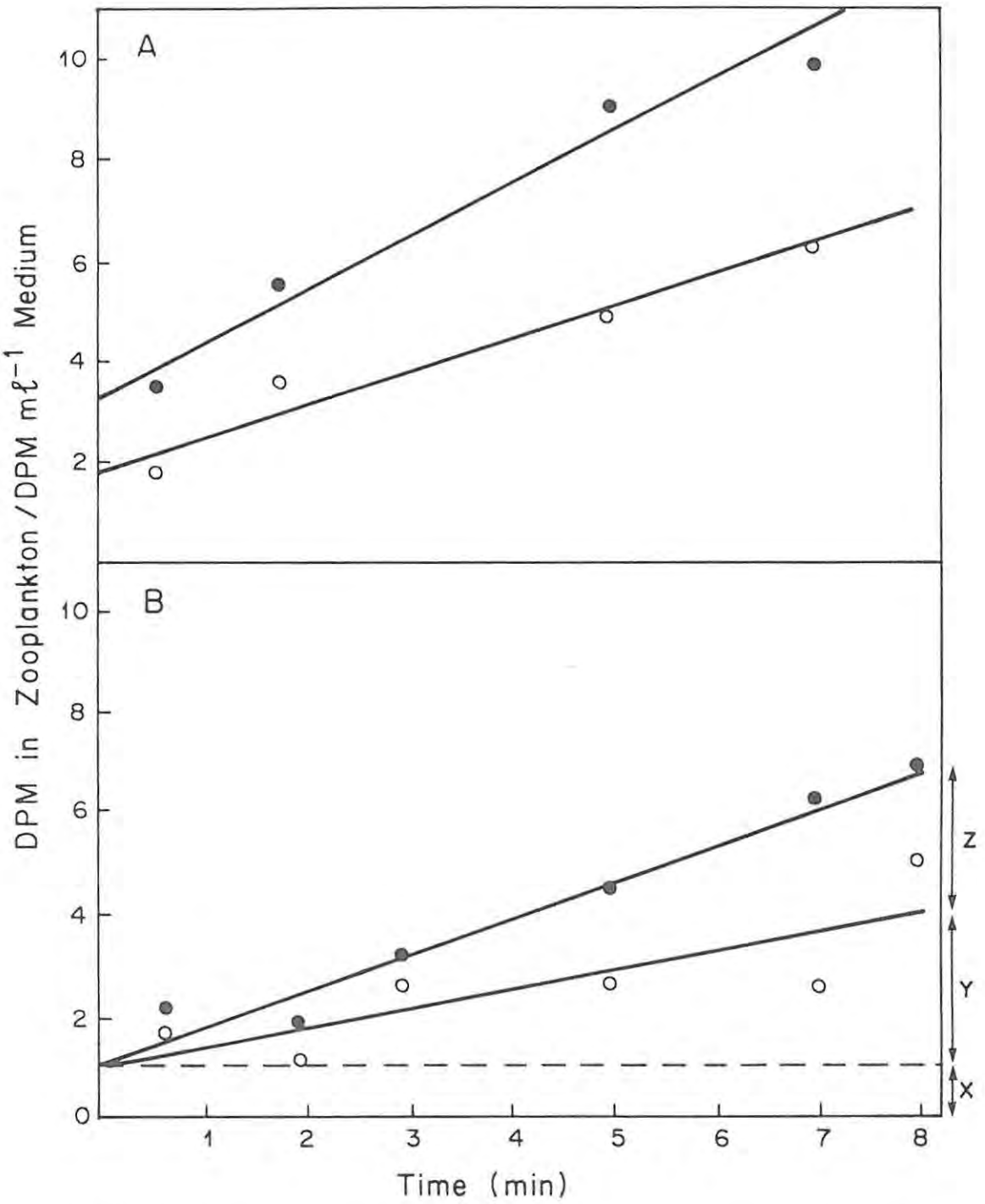


Figure 5.4: Adsorption error estimated using killed-control experiments. Isotope uptake by actively feeding zooplankton and associated large particles (closed circles) versus killed zooplankton and particles (open circles). X indicates initial adsorption error (time zero); Y indicates time-related adsorption; Z indicates isotope uptake due to filter-feeding on labelled bacteria.

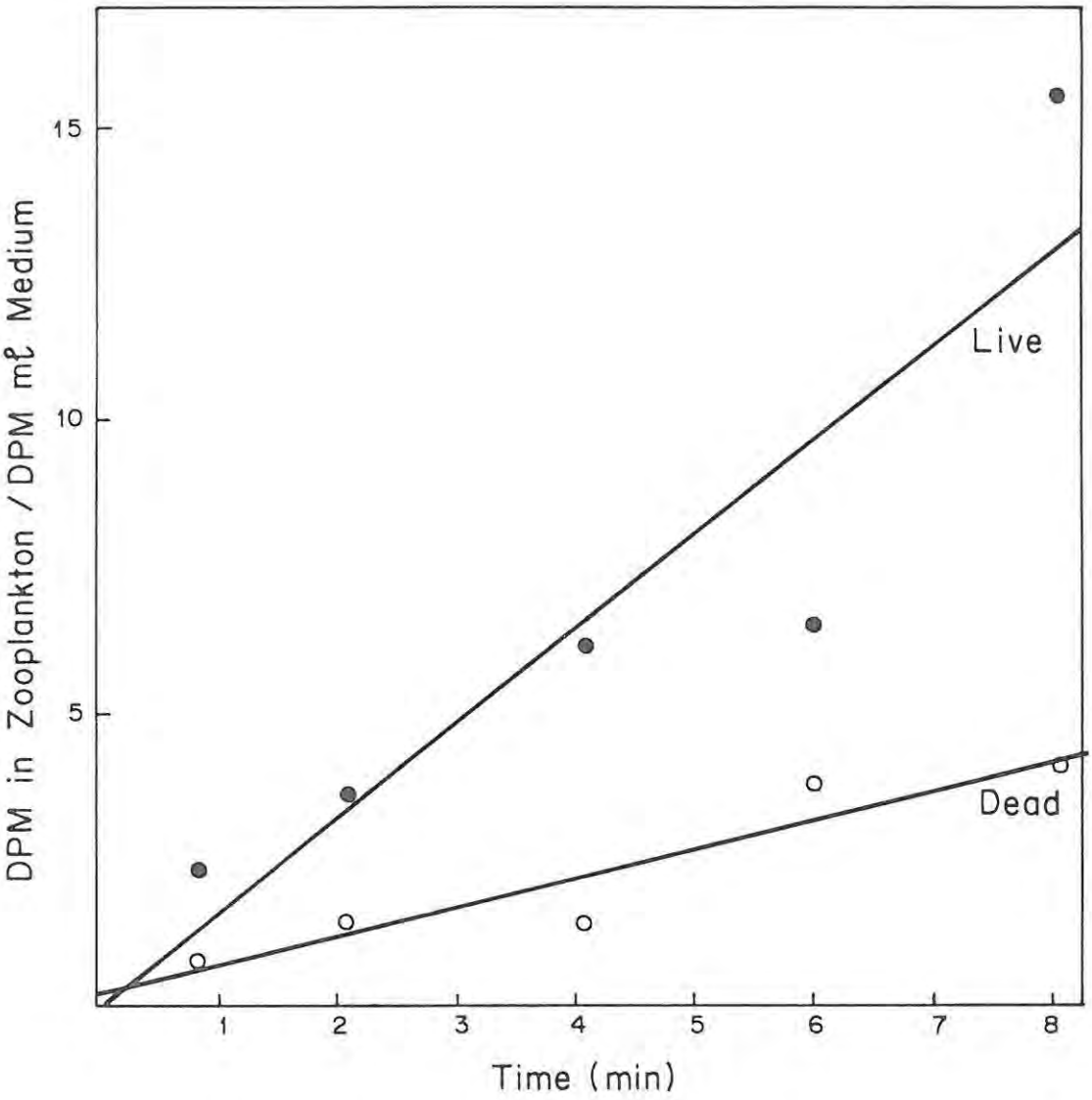


Figure 5.5: As in Figure 5.4 but after addition of unlabelled thymidine to the bacteria suspension after final rinsing and concentration. Initial adsorption error (time zero) and total time-related adsorption error both reduced.

Table 5.4: Summary of zooplankton community filtration rates (CFR; $m\ell\ h^{-1}$) measured *in situ* on labelled natural bacteria over the study period, and summary of adsorption error estimation using killed-control experiments. All bacteria labelled with methyl-^{3}H-thymidine for 20 h. Unlabelled thymidine added to bacterial suspension after washing and reconcentration of cells (see text for details). Dominant grazers in community: D = *Daphnia*, B = *Bosmina*, C = *Ceriodaphnia*, M = *Moina* and *Diaphanosoma*.

Date	Natural Bacteria x 10^6 $m\ell^{-1}$		% increase in food conc.	n	Mean CFR ± SE *Chlorella*	SE %	n	Mean CFR ± SE Bacteria	SE %	Adsorption error as % of CFR on Bacteria ± SE	Dominant Feeder
	Lake	Labelled									
7.10.86	9.69	48.47	0.8				3	7.03 ± 0.46	6	36.0 ± 1.0	D
16.10.86	13.65	224.01	2.7				4	30.27 ± 2.15	7	12.3 ± 1.4	D
6.11.86	9.77	32.00	0.6				3	12.42 ± 0.97	8	25.0 ± 5.0	B
20.11.86	7.99	132.40	2.8	1	16.14		4	26.76 ± 9.73	36	4.3 ± 0.9	D/C
27.11.86	8.98	160.20	3.0	5	7.49 ± 0.89	12	4	7.25 ± 0.76	11	13.7 ± 0.7	D/C
3.12.86	11.29	162.50	2.4	3	13.90 ± 2.16	16	4	20.97 ± 2.39	11	18.3 ± 2.4	D/C
18.12.86	6.39	162.24	4.2	3	40.32 ± 1.87	5	4	39.28 ± 2.64	7	1.0 ± 0	D/C
7. 1.87				3	13.51 ± 0.10	1	3	5.46 ± 0.22	4	4.0 ± 0.6	D/C
22. 1.87	7.98			3	33.50 ± 4.22	13	4	16.18 ± 1.22	8	5.7 ± 1.5	C
5. 2.87	6.87	69.87	1.7	3	30.85 ± 1.97	6	3	19.88 ± 2.55	13	6.0 ± 0.6	C
19. 2.87	6.49	73.72	1.9	3	3.12 ± 0.91	29	3	1.33 ± 0.41	30	6.0 ± 1.0	C
5. 3.87	6.32	81.10	2.1	3	15.81 ± 1.47	9	3	7.93 ± 0.95	12	8.3 ± 5.4	C
19. 3.87	6.49	89.52	2.3	3	28.04 ± 1.59	6	3	26.75 ± 0.70	3	1.7 ± 0.7	C/M

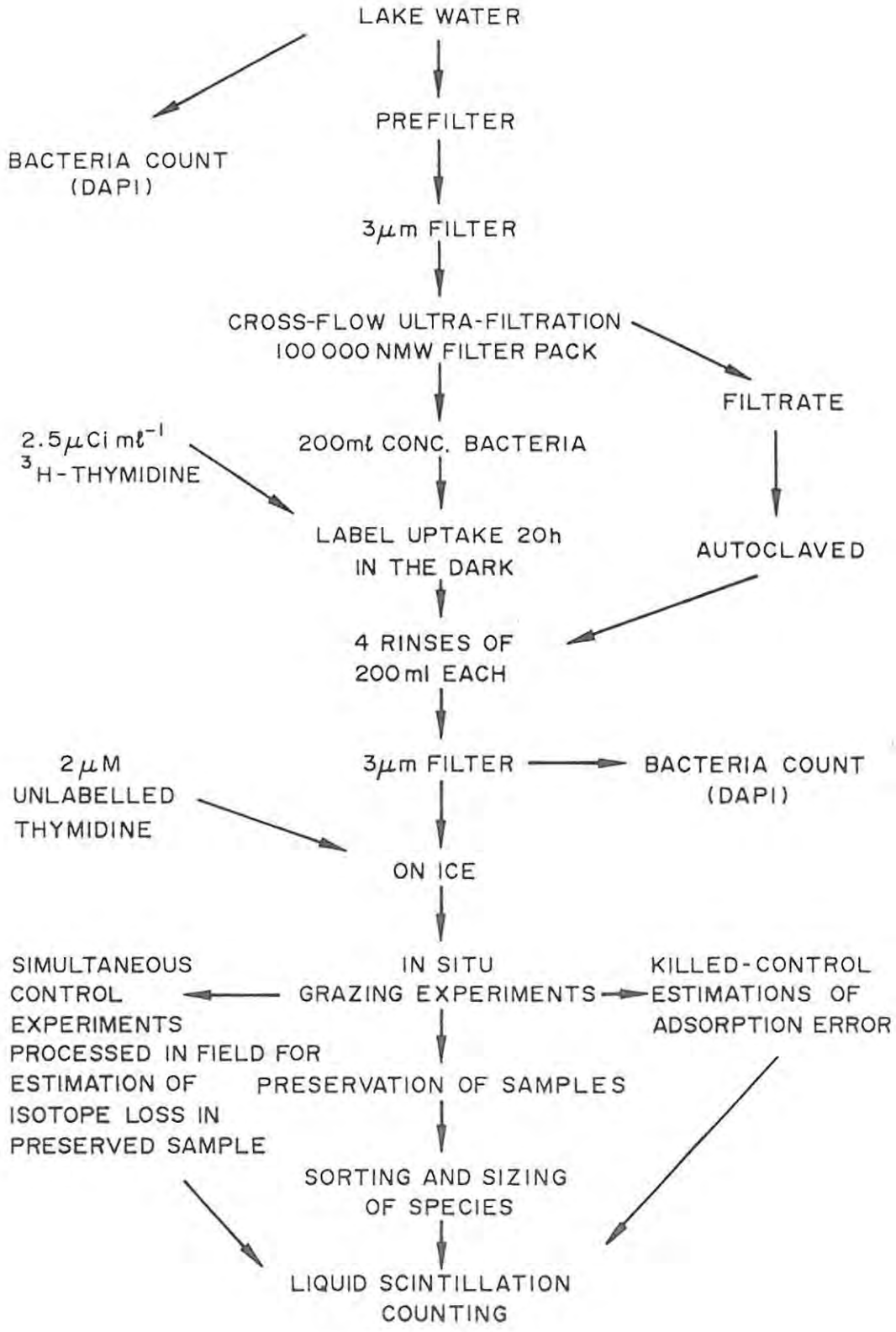


Figure 5.6: Outline of the procedure developed to measure zooplankton grazing *in situ* on natural lake bacterioplankton in Hartbeespoort Dam.

plankton exposed to a feeding medium containing labelled bacteria with uptake by actively feeding zooplankton *in situ*. In both series of experiments there is an immediate uptake of radiolabel by zooplankton and other particles retained by a 60 µm mesh, indicating initial adsorption as a source of some error. With both active and inactive zooplankton this error should be the same, (although in Figure 5.4a, the intercepts do not meet). The slope of the regression lines represent the rate of uptake of radiolabel. The higher rate of uptake in experiments with active zooplankton reveals the community filtration rate on natural bacteria above the rate of further adsorption of isotope by particles in experiments with inactive zooplankton. Adsorption error in both time series of experiments (Figure 5.4a and b) was approximately 60% at 6-7 minutes. Consequently procedures were examined to further reduce the magnitude of this error.

Reduction in the release of radiolabelled compounds by the labelled bacteria after washing of cells was identified as a likely additional method of reducing adsorption error. Keeping the labelled bacterial suspension on ice has been shown to reduce isotope loss into the surrounding medium (Figure 5.2). However, as much as 2-3 h delay in the use of the washed cells during transit from laboratory to lake sampling station, even though kept on ice, could allow considerable release of unbound radiolabel into solution, thereby increasing the potential for adsorption to particles during experiments. Therefore both reduction in rate of isotope release from cells and duration of release before use in experiments was achieved by use of a final lake-shore washing procedure combined with the addition of unlabelled thymidine (Hollibaugh *et al.* 1980).

The small and portable design of the cross-flow ultra-filtration system used (Minitan system) enabled washing of the labelled natural bacteria (4 x 200 mℓ rinses) and final concentration of the suspension to be carried out at a lake-shore site given a 240 volt electricity mains supply (or portable generator). Thereafter, immediately before final

3 µm filtration and placing of the bacterial suspension on ice, unlabelled thymidine was added to a final concentration of 1-2 µM. Hollibaugh *et al.* (1980) showed that addition of unlabelled thymidine reduced re-uptake of excreted radio-labelled compounds (uptake inhibited by 0-64%) and suggested that such addition could reduce 'blank' values. This was also found to be the case in Hartbeespoort Dam (Figure 5.5). In combination with washing and final concentration of cells at the lake-shore before use in experiments, the addition of unlabelled thymidine from 7.10.87 (Table 5.4) also resulted in a marked reduction of adsorption errors measured subsequently in regular *in situ* bacterioplankton grazing experiments, which routinely included killed-control experimental estimations of adsorption error.

An outline of the full set of procedures necessary for measurement of zooplankton community filtration rates on natural, free-living, lake bacteria under the hypertrophic conditions of Hartbeespoort Dam is presented in Figure 5.6. Measurement of species-specific filtration rates were carried out using acid-Lugol's solution preservation and corrected for isotope loss as outlined in Section 4. The average isotope loss measured for all 45 *in situ* experiments using methyl-^{3}H-thymidine was 57.6% ± 1.8%. This is higher than the isotope losses recorded using ^{14}C-labelled *Chlorella* or *Microcystis* (Section 4). ^{3}H loss during formalin or acid-Lugol's preservation of zooplankton samples has not been reported in the literature. This average loss of 57.6% lies within the range of isotope losses reported for ^{32}P and ^{14}C-labelled foods by Holtby and Knoechel (1981; up to 73%) and Persson (1982; up to 74%).

5.2.5 General comments on methods used to measure bacterioplankton grazing by zooplankton

The use of cross-flow ultra-filtration to concentrate and wash labelled natural bacteria without the loss of very small cocci is an improvement over methods described in the literature. Earlier studies have failed to take account of the very small

size of free-living cocci some of which in Hartbeespoort Dam have a diameter of <0.18 µm (Table 5.1) and can pass a 0.2 µm membrane filter (R.D. Robarts pers. comm.). This problem was not avoided in this study due to both the late recognition of the filtration problem and to difficulty in the filtration of lake water in the field using 0.1 µm membrane filters (rapid clogging).

Cross-flow concentration of these labelled cells is of value, particularly for *in situ* experiments where procedures such as the release of unincorporated radiolabel in long duration experiments and the concentration of zooplankton are not suitable. Labelled natural bacteria representing an increase over ambient lake bacteria concentrations of up to 4.2% (Table 5.4) have been used. This small change in food concentration is unlikely to alter the filter-feeding behaviour of zooplankton, but the increase produces d.p.m. values high enough for accurate calculation of their filtration rates.

Examination of possible errors has revealed considerable sources of contamination that have been largely ignored in many previous studies. Killed-control experiments to measure adsorption error using zooplankton, collected simultaneously in the second compartment of the grazing chamber, and immobilized using soda water were carried out in preference to using zooplankton killed by 5% formalin (Roman and Rublee 1981). Use of formalin kills unlabelled bacteria attached to particles, so reducing label uptake, and may alter surface charge and chemical properties thereby also influencing adsorption error estimation. The 'time zero' control experiments of Riemann and Bosselmann (1984), in which zooplankton were killed immediately on exposure to labelled bacteria, were also regarded as a less reliable estimate of error since adsorption error has been shown to be a function of experimental duration (Figures 5.4 and 5.5).

Of particular interest, Riemann and Bosselmann (1984) using their time zero control experiments measured an error of 8-58% of d.p.m in grazing experiments carried out on four dates.

Considering also that this does not include any further time-related adsorption error, this measured error is extremely high. There is no indication if these experiments were replicated, thus the extreme variability and magnitude of this error casts doubt upon the validity of their results. Riemann and Bosselmann (1984) acknowledged that their time zero controls 'contributed a large proportion of sample activity'. Harrison *et al.* (1987), in their study on marine microbial heterotrophic activity using tritiated thymidine and uridine, also questioned the validity of time zero blanks due to radiolabel uptake and adsorption during experiments, and stated that the problem of adsorption, common to many isotopic tracers, is not generally appreciated and not yet understood.

Work on heterotrophic activity in Hartbeespoort Dam has also revealed many problem areas. In addition to concentration-dependent adsorption of methyl-^{3}H-thymidine to membrane filters, adsorption of radiolabel to an as yet unidentified site during experiments has also been noted (R.D. Robarts pers. comm.). Adsorption errors during zooplankton grazing experiments on bacteria in Hartbeespoort Dam (Table 5.4) may be in part attributable to the abundant large particles of *Microcystis* present in these *in situ* experiments and thus may be exacerbated by hypertrophic conditions. However, information is not available to support this idea.

5.3 Results

5.3.1 Community filtration rates on natural bacteria

CFRs measured *in situ* on natural bacteria and on *Chlorella* (Figure 5.7a) were similar over the six months study period (spring 1986 to late summer 1987). CFRs on both of these foods fluctuated in relation to the abundance of zooplankton present in experiments. Before the end of December CFRs on natural bacteria were generally higher than rates measured using *Chlorella*; thereafter the converse prevailed. This change in community feeding 'preference' is shown in Figure

5.7b. Selectivity coefficients were calculated for each date as the ratio of mean CFR on *Chlorella* relative to the mean CFR on bacteria on each sampling date, after DeMott and Kerfoot (1982; in their Table 4 and Figures 10 and 11). The change in selectivity coefficient from values <1 ('preference' for natural bacteria) to values >1 ('preference' for *Chlorella*) occurred in association with both the annual mid-summer shift in phytoplankton composition, from dominance by edible chlorophytes and cryptophytes to dominance by largely inedible *Microcystis*, and with the decline in the numbers of natural bacteria present in Hartbeespoort Dam (Figure 5.8a and b).

5.3.2 Analysis of species-specific filtration rates

A total of 698 species-specific measurements of zooplankton filtration rate on natural lake bacteria and on *Chlorella* were obtained from 75 *in situ* feeding experiments (Table 5.5). As noted in Section 4 large *Daphnia* were generally scarce, so few feeding rate measurements were obtained on the largest *Daphnia* length-classes (from 1.75 mm to 2.25 mm) which were accordingly excluded from most of the species-specific analysis. Furthermore, the copepod *Thermodiaptomus syngenes* and the rotifer *Brachionus calyciflorus* were unfortunately largely absent during this study period using natural bacteria, so their filtration rates on this food could not be examined. Water temperature varied from 16.6 °C in early October 1986 to 25.6 °C in early March 1987.

Daphnia filtration rate on natural bacteria was, like that on *Chlorella*, a power function of animal body length (Figure 5.9 and see also Figure 4.0). Insufficient body length classes for other cladocerans prevented this relationship being explored for all species. Generally, mean species-specific filtration rates on *Chlorella* for each zooplankton length class for all dates combined were lower during the 1986-1987 study period than rates measured during 1985-1986 (Figure 4.0, Section 4). This was particularly marked within the *Daphnia* population, the mean filtration rate of the 1.50 - 1.75 mm length class on *Chlorella* being 0.960 mℓ animal^{-1} h^{-1} in 1986-1987 compared to 1.510 mℓ animal^{-1} h^{-1} in 1985-1986.

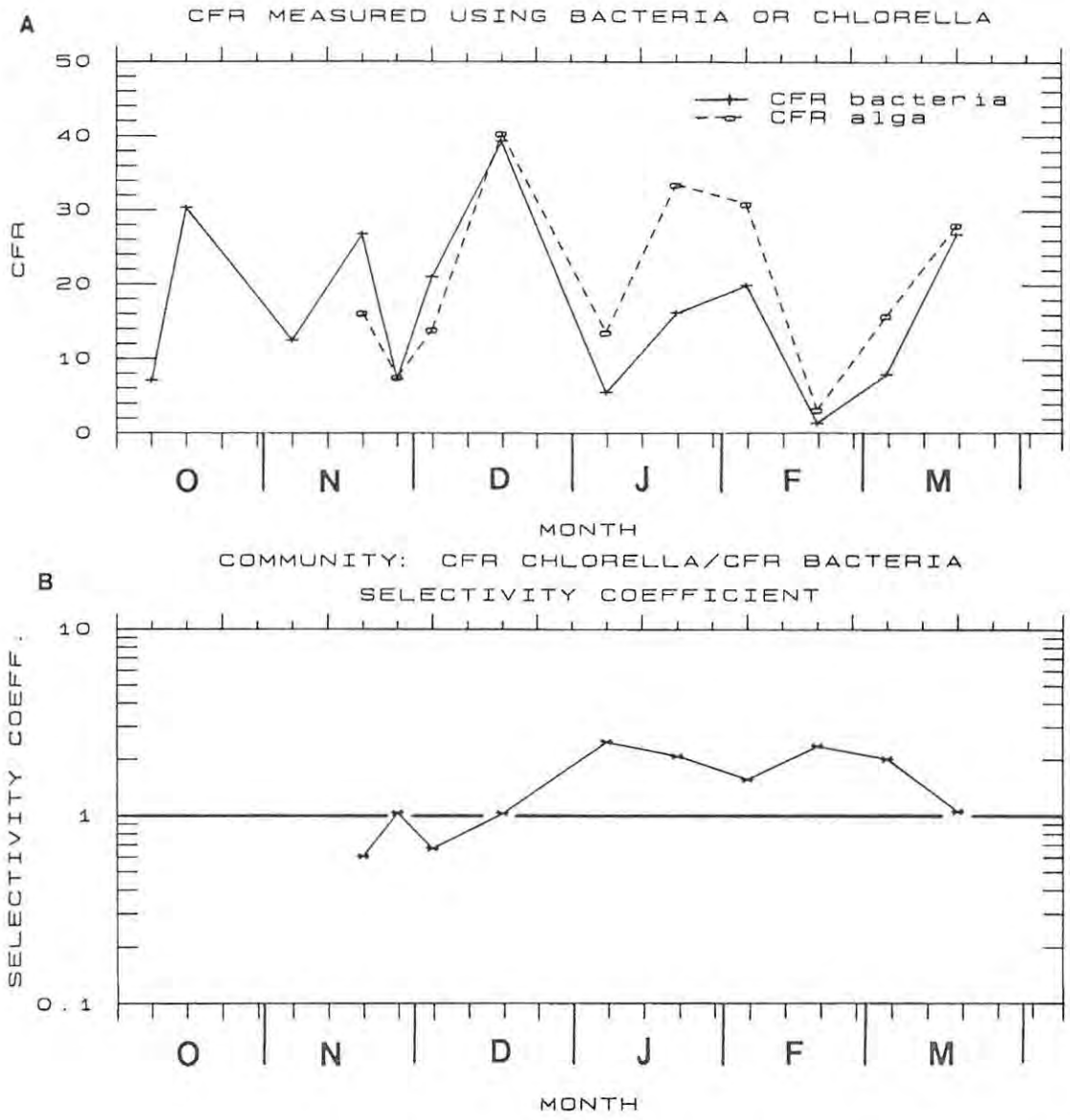


Figure 5.7: (a) Community filtration rates (mℓ h^{-1}) on *Chlorella* and natural bacteria and (b) community selectivity coefficients (CFR algae/CFR bacteria) from spring to late summer 1986 - 1987. Selectivity coefficient of 1 (no preference) shown as horizontal line, >1 = algal preference, <1 = bacterial preference.

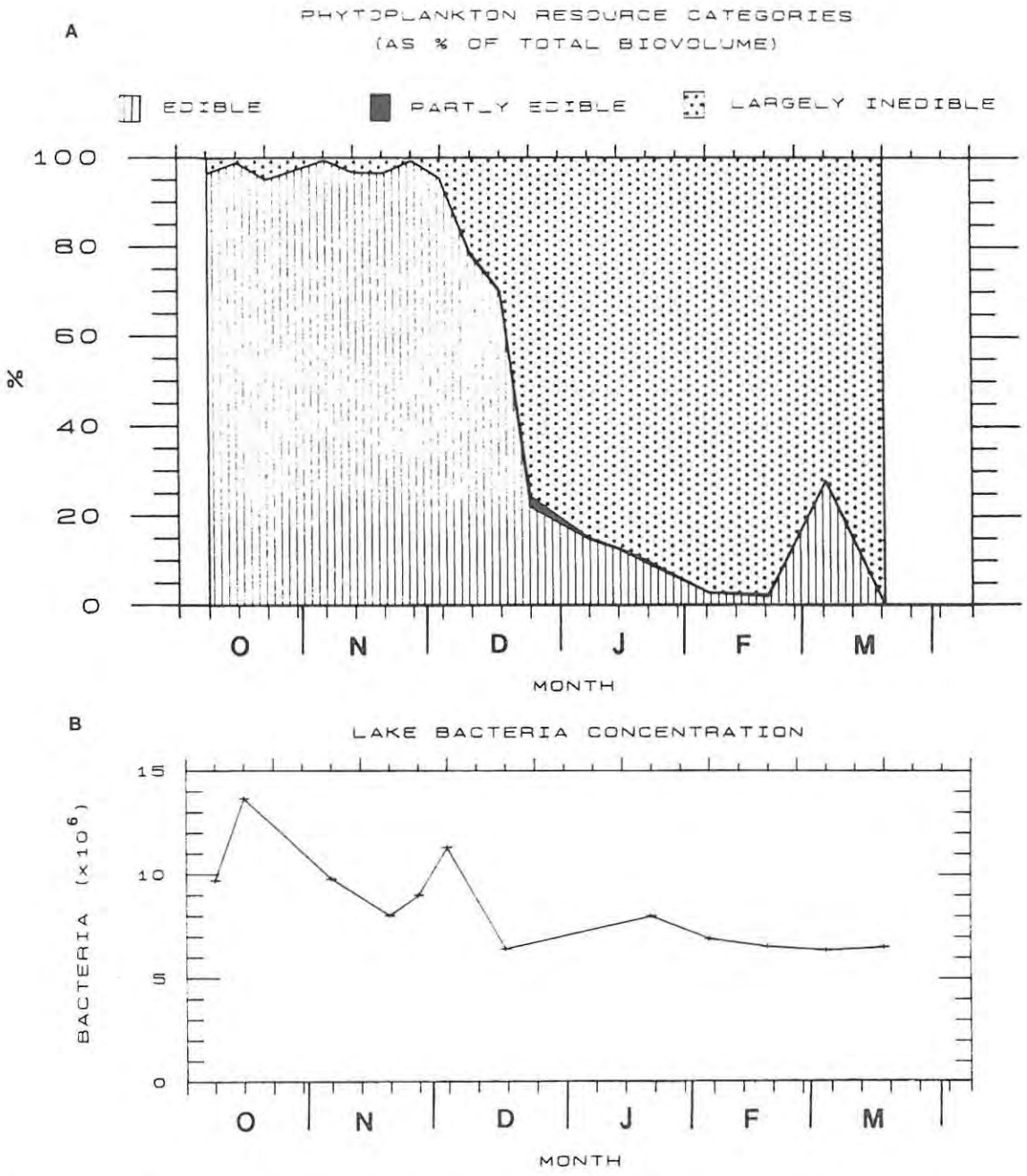


Figure 5.8: Food resources shown as percent biovolume of phytoplankton as categorized in Section 2, and bacteria number ($x10^6$ $m\ell^{-1}$) in Hartbeespoort Dam over the bacterioplankton grazing study period.

Table 5.5. Number of *in situ* measurements of zooplankton filtration rates using *Chlorella* and natural lake bacteria.

	Chlorella	Bacteria
Daphnia 1.75 - 2.25 mm	5	26
Daphnia 0.5 - 1.75 mm	73	181
Bosmina	19	29
Ceriodaphnia	72	119
Moina	31	34
Diaphanosoma	41	56
Thermodiaptomus	5	2
Brachionus	4	1
Total n	250	448
Grand Total		698

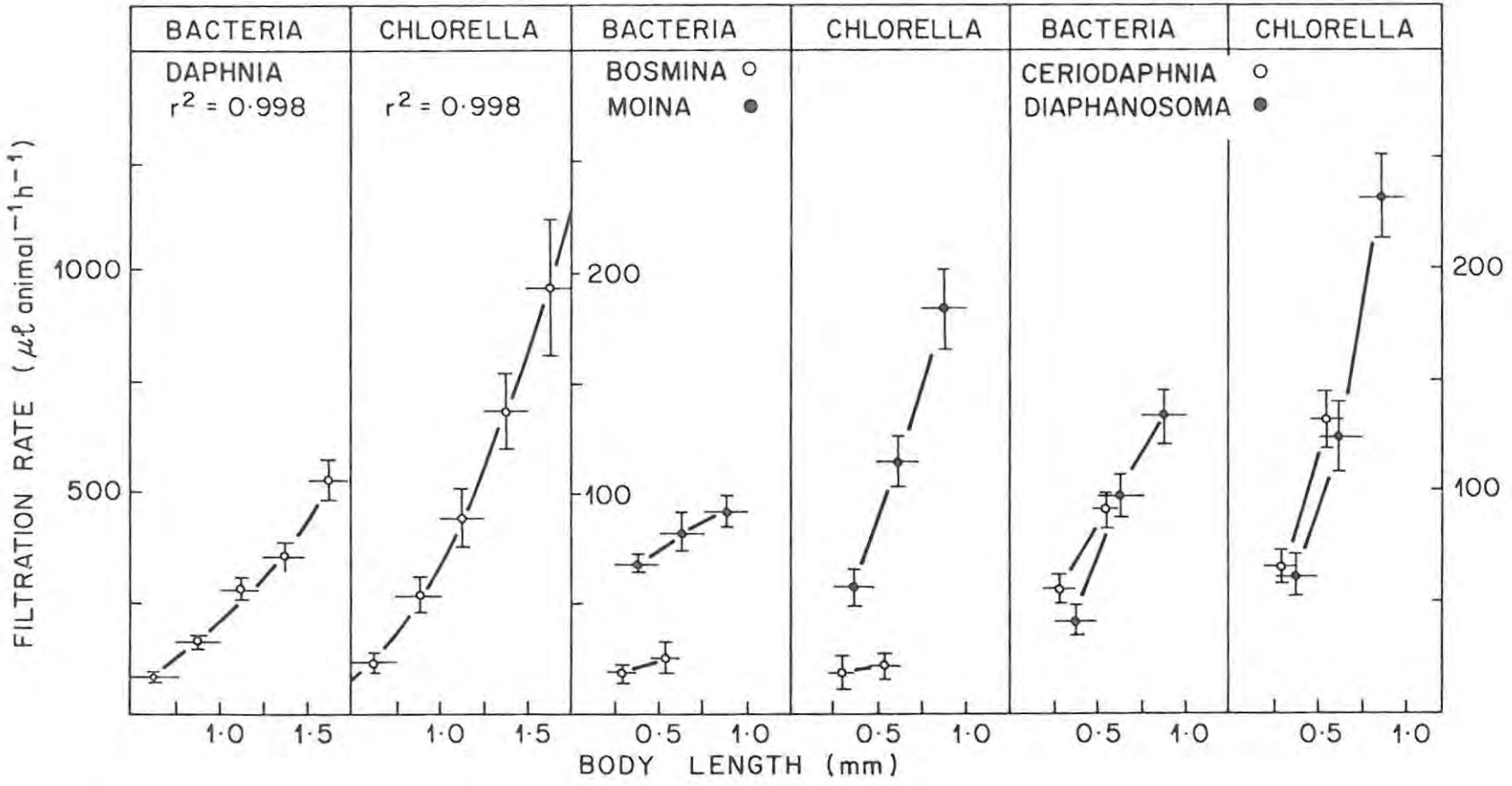


Figure 5.9: Mean filtration rates of body length-classes of the major cladocerans in Hartbeespoort Dam on natural bacteria and on *Chlorella*. Vertical bars indicate standard error.

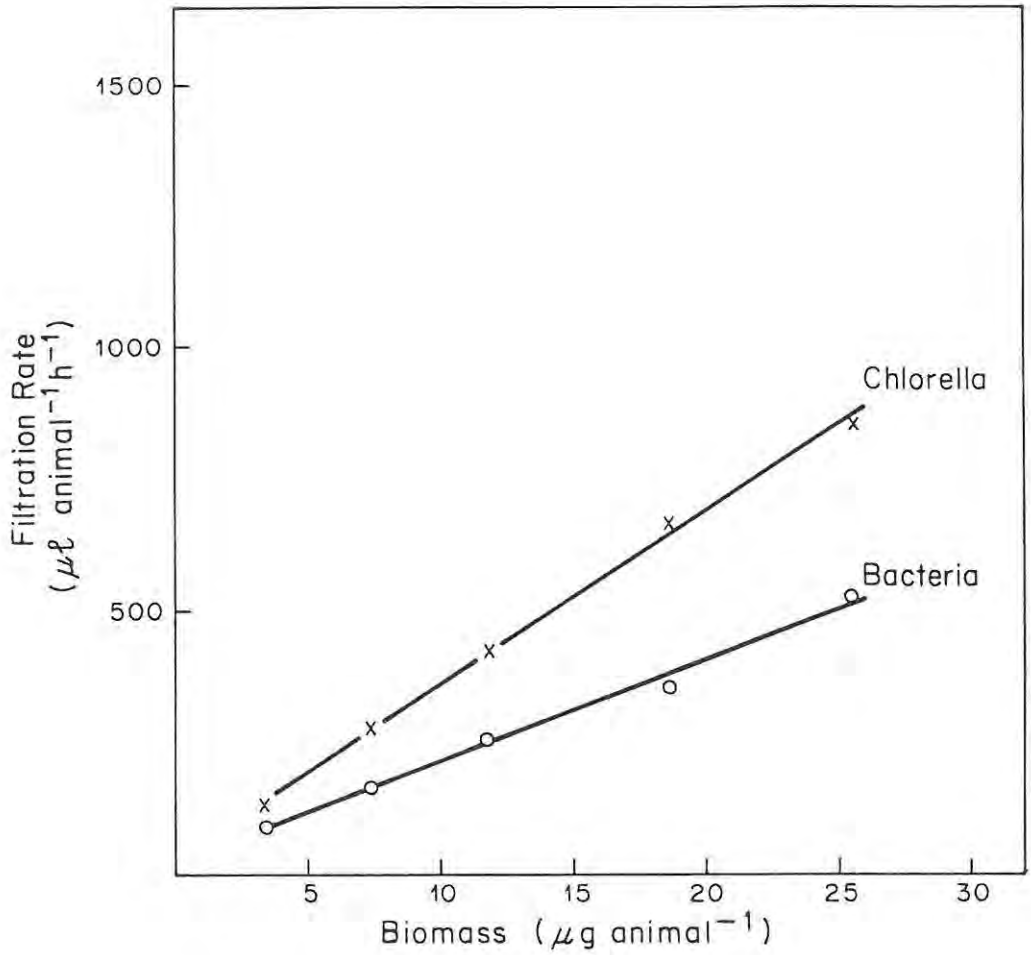


Figure 5.10: Relationship between *Daphnia* biomass and filtration rate on natural bacteria and on *Chlorella*.

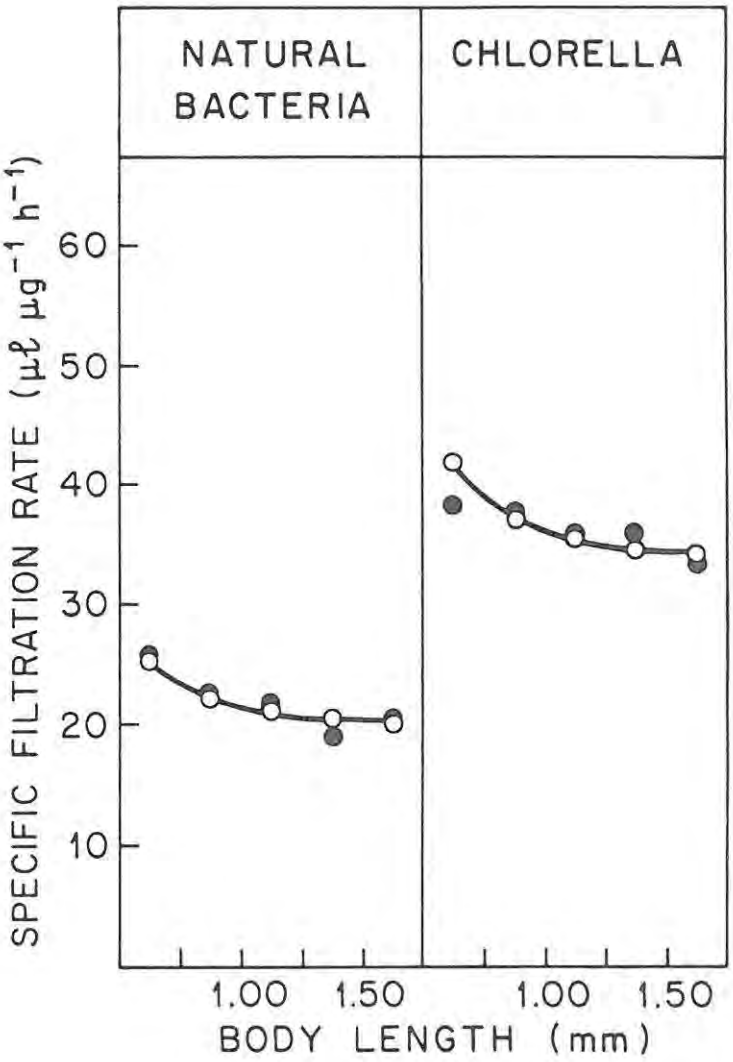


Figure 5.11: Closed circles: observed specific filtration rates of five length-classes of *Daphnia* on natural bacteria and on *Chlorella*. Open circles: specific filtration rates predicted from the relationships in Figure 5.10. Lines drawn by eye for predicted values.

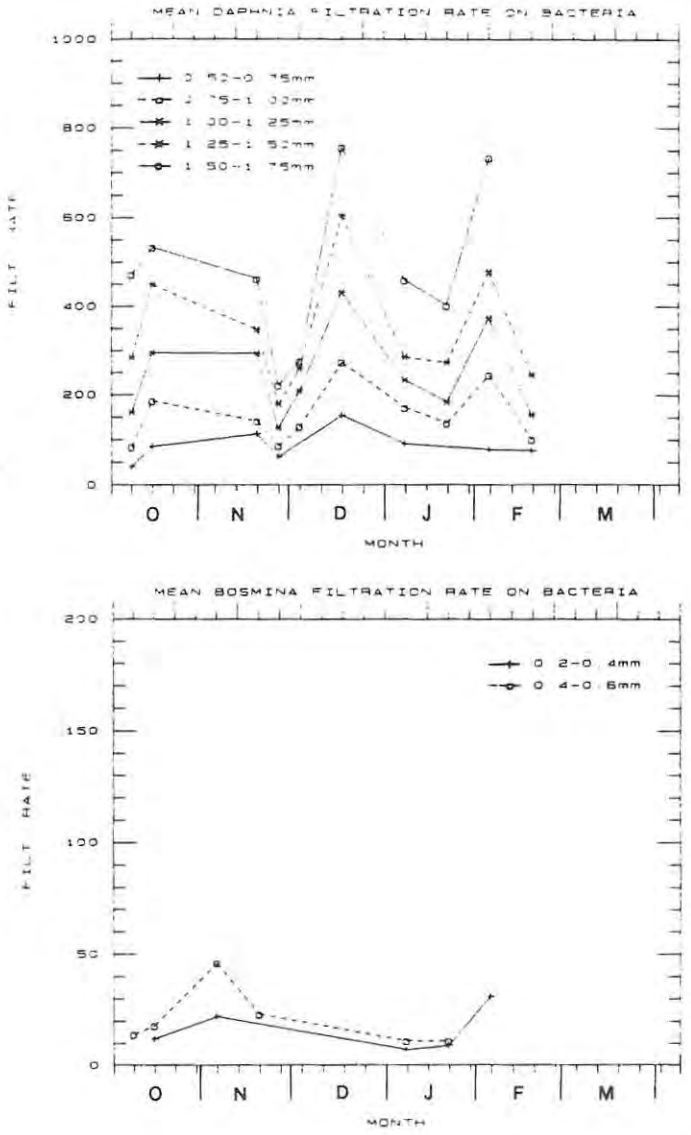


Figure 5.12: Seasonal changes in mean *in situ* filtration rates (µℓ animal^{-1} h^{-1}) of the major cladocerans on natural bacteria during spring-summer 1986-87.

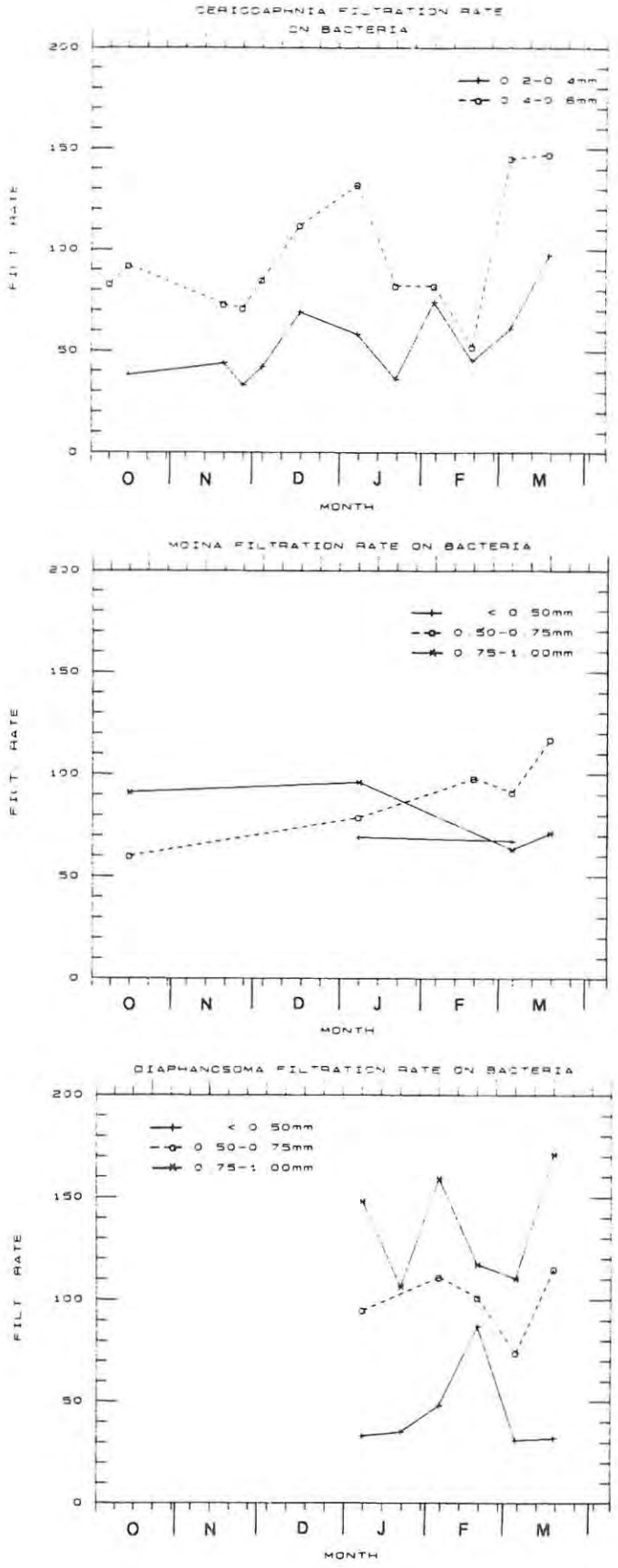


Figure 5.12: (continued).

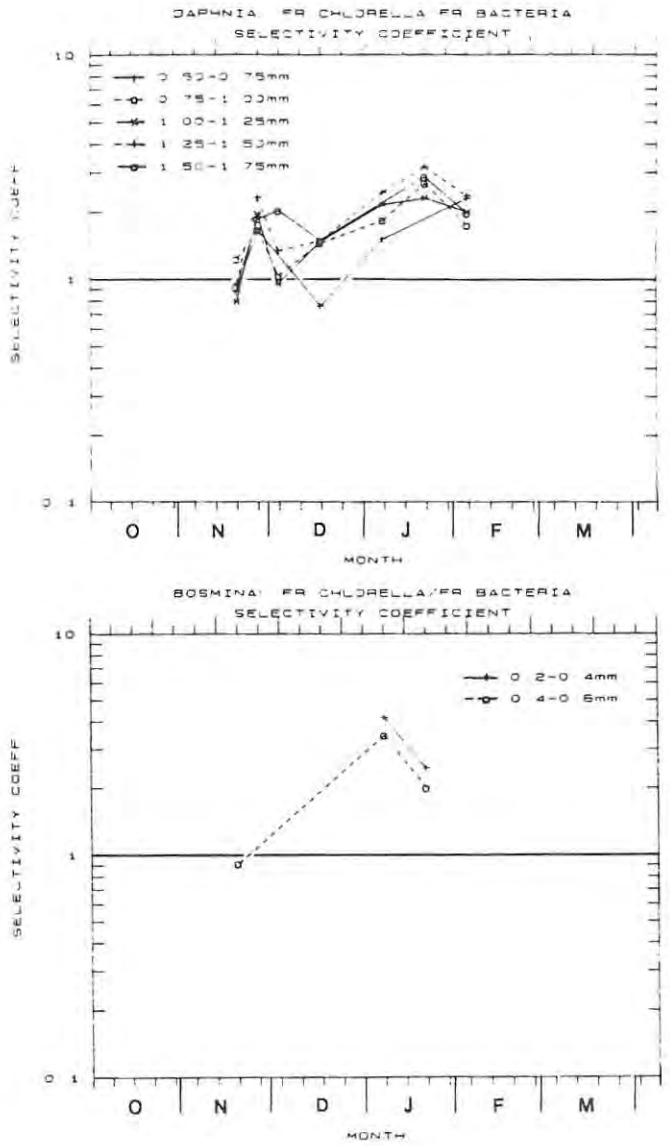


Figure 5.13: Mean selectivity coefficient (FR algae/FR bacteria) of the major cladocerans for each date over the spring to late summer study period 1986-1987. Selectivity coefficient of 1 (horizontal line) indicates no preference, >1 = algal preference, <1 = bacterial preference.

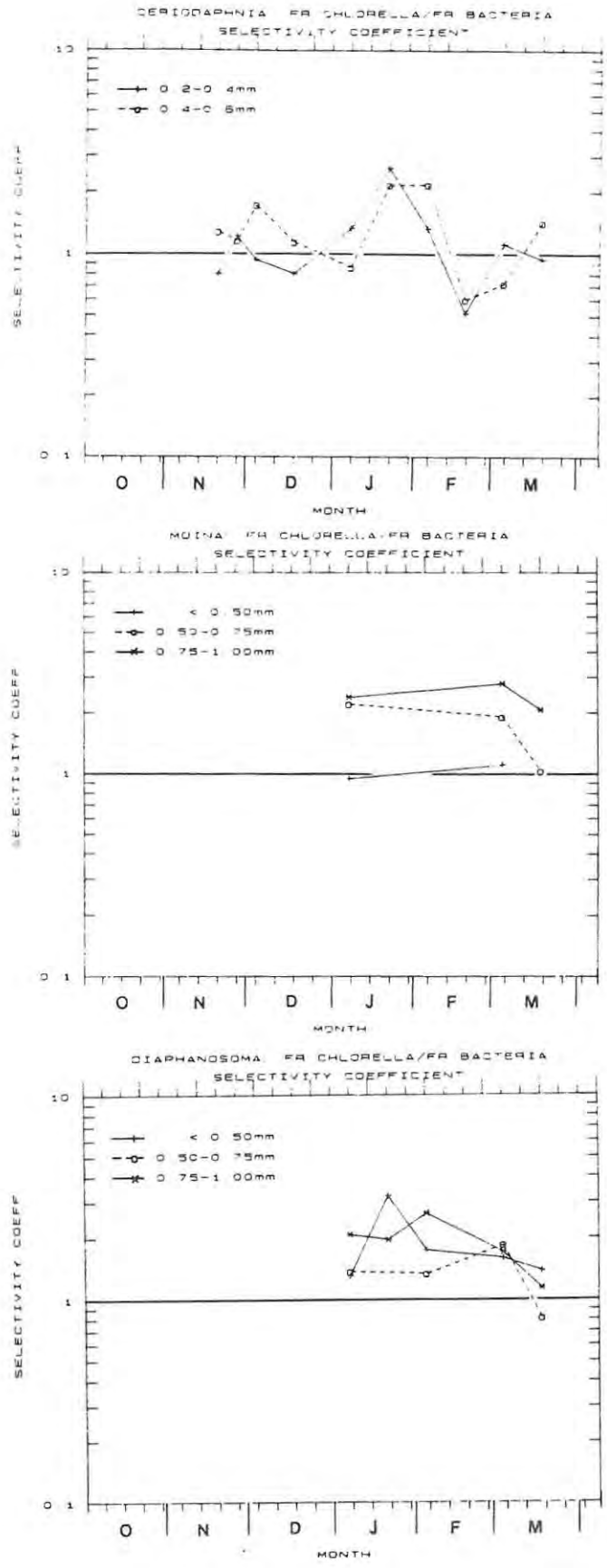


Figure 5.13: (continued).

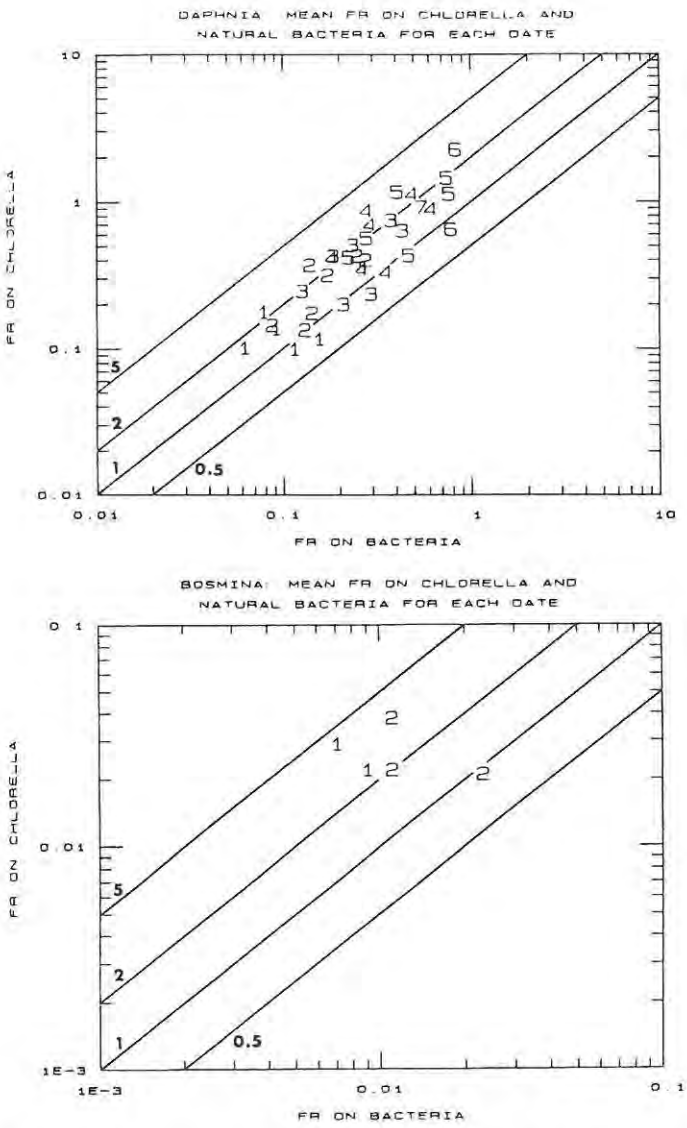


Figure 5.14: Mean filtration rates (mℓ animal^{-1} h^{-1}) of the major cladocerans on *Chlorella* and natural bacteria, on each date over the study period, plotted against selectivity isoclines of 0.5, 1, 2, 5 shown in bold (DeMott and Kerfoot 1982). Body length-classes numbered from the smallest to the largest (1-7).

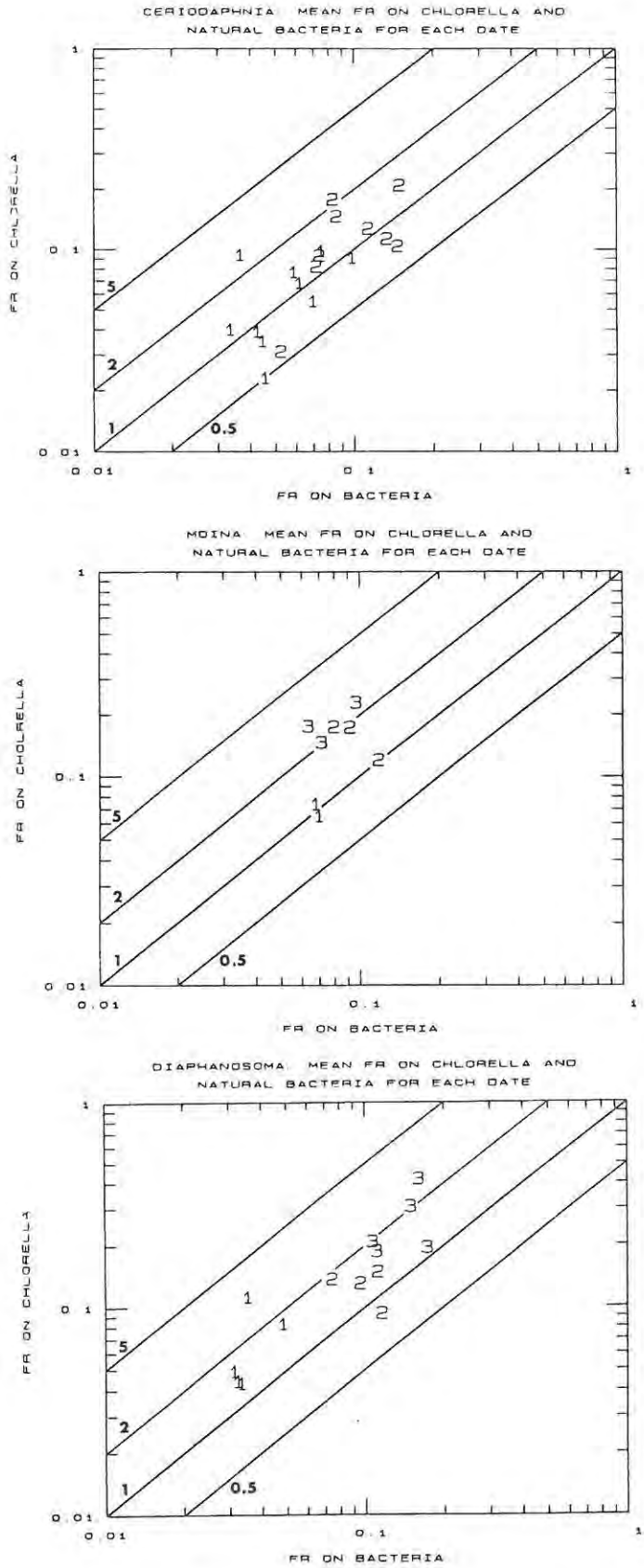


Figure 5.14: (continued).

All *Daphnia* length-classes filtered natural bacteria (~0.15 - 1.0 µm cocci diameter - rod length) at ~60% of their filtration rate on *Chlorella* (~8.5 µm cell diameter) with little variation in this relative feeding rate due to *Daphnia* body length. The average value ± standard error obtained for five length classes of *Daphnia* was 60.4% ± 2.3%. Transformation of *Daphnia* body length classes (X, mm) to biomass (Y, µg), using the power relationship $Y = 9.49X^{2.07}$ used in Section 4, shows the linear relationship between *Daphnia* biomass and filtration rate on natural bacteria or *Chlorella* (Figure 5.10). The slopes of this relationship on these food differed significantly ($p<0.01$). Measured biomass specific filtration rates of each *Daphnia* length class on natural bacteria and *Chlorella* plotted against predicted specific filtration rates calculated from the linear functions in Figure 5.10 are shown in Figure 5.11. These specific filtration rates also show no pronounced body size related influence upon filtration of either natural bacteria or *Chlorella* by *Daphnia*.

Of the smaller bodied cladocerans, *Bosmina longirostris* had the lowest mean filtration rates on both bacteria and *Chlorella*. Small *Moina micrura* (<0.5 mm length) filtered natural bacteria at a mean rate higher than that on *Chlorella* (Figure 5.9). This high relative filtration rate declined with increasing body length indicating that *Moina's* filtration efficiency on natural bacteria is related to body size (mean relative filtration rates of 117.2%, 71.9% and 50.3% for *Moina* <0.5 mm, 0.5-0.75 mm and 0.75-1.0 mm respectively). Similarly the mean relative filtration rate of small *Ceriodaphnia* on bacteria was higher than that of large *Ceriodaphnia* (0.2-0.4 mm 84.8%, 0.4-0.6 mm 69.7%). *Diaphanosoma excisum* did not exhibit consistent size related changes in mean relative filtration rate on natural bacteria (<0.5 mm 67.7%, 0.5-0.75 mm 79.0%, 0.75-1.00 mm 57.8%).

Figure 5.12 shows mean filtration rates on natural bacteria for the major herbivores present on each date over the study period. High *Daphnia* filtration rates on bacteria coincided with both the lowest recorded bacteria number and the occasion

of greatest change in phytoplankton species composition towards cyanophyte dominance (Figures 5.8 and 5.12). However, no consistent longer term or seasonal trends in individual filtration rates of either *Daphnia*, *Bosmina* or *Diaphanosoma* were apparent. Generally a slight increase in the filtration rates on bacteria of small *Ceriodaphnia* (0.2-0.4 mm length) and *Moina* of intermediate size (0.5-0.75 mm length) occurred over the study period whilst the filtration rate of large *Moina* (>0.75 mm) declined slightly. However, plotting seasonal changes in the selectivity coefficient for each species (DeMott and Kerfoot 1982) reveals an increase in selectivity for chlorophytes such as *Chlorella*, for all *Daphnia* length classes, after the change to cyanophyte dominance at midsummer (Figure 5.13). No pronounced or clear changes in the selectivity coefficients of *Ceriodaphnia*, *Moina* and *Diaphanosoma* were evident, and sparse data on the seasonal selectivity of *Bosmina* prevented conclusions being drawn.

Relationships between the feeding 'preferences' on *Chlorella* or bacteria of each length class of the main filter-feeders in Hartbeespoort Dam were further examined by plotting their mean filtration rates (mℓ animal^{-1} h^{-1}) on these foods on each date in relation to selectivity isoclines (DeMott and Kerfoot 1982). Figure 5.14 shows that no body size related variation in selectivity coefficients were evident for *Daphnia*, *Bosmina* and *Diaphanosoma*. Selectivity coefficients for *Daphnia* lay between 0.75 and 3.18. Coefficients for *Ceriodaphnia* ranged from 0.51 to 2.61 with animals of 0.4 - 0.6 mm length showing a slightly greater 'preference' for *Chlorella*. Selectivity coefficients of ∿1 (indicating no food preference) were recorded for small *Moina* (<0.5 mm length) whereas larger animals showed a stronger selectivity for *Chlorella* (coefficients up to 2.78). *Diaphanosoma* and *Bosmina* both exhibited strong preferences for *Chlorella* (coefficients up to 3.20 and 4.14 for *Diaphanosoma* and *Bosmina* respectively) when compared to filtration rates on natural bacteria, with one exceptional value (coefficient <1) for both species.

5.3.3 A cladoceran filtration rate - body length model for natural lake bacteria

Regression analysis of combined data for all species-specific filtration rates on natural bacteria were carried out to enable development of zooplankton community and cladoceran FR:L models on this food resource (Table 5.6, Figure 5.15). For all zooplankton species the FR:L relationship (ln transformed and converted to mℓ animal^{-1} d^{-1}) when feeding on natural bacteria was:

$$FR = 4.429L^{1.408} \qquad (5.1)$$

($r^2 = 0.663$, $n = 448$, $p<0.001$)

A paucity of data on the filtration rates of *Thermodiaptomus* and *Brachionus* (n of 2 and 1 respectively), due to their virtual absence during the study period using natural bacteria, largely limited this model to five cladoceran species:

$$FR = 4.486L^{1.420} \qquad (5.2)$$

($r^2 = 0.674$, $n = 445$, $p<0.001$)

Visual examination of residual variance scatter-plots indicated that the ln transformation was suitable and the error variance was stable.

This FR:L relationship for cladocerans was analyzed using stepwise multiple regression to examine influences associated with temperature and grazer species (species entering the model as binary coded 'dummy' variables; Kim and Kohout 1975). In addition to cladoceran body length, stepwise inclusion of these variables increased explained variance from 67.4% to 78.6% (Table 5.7). Model entry of each variable significantly reduced unexplained variance ($p<0.01$). The combined influence of cladoceran species accounted for 11% of the variance, whilst temperature entered the model last and reduced unexplained variance by only 0.2%. *Bosmina* had the greatest

Table 5.6. Regression model parameters of ln transformed zooplankton FR:L relationships on natural bacteria measured *in situ* in Hartbeespoort Dam. Regression equation: ln FR = ln a + b ln L, where FR = filtration rate in $\mu\ell$ animal^{-1} h^{-1}, L = body length in mm. Slopes significant at $p<0.001$. Cladoceran model excludes data from the rotifer *Brachionus* and copepod *Thermodiaptomus*.

		All zooplankton	Cladoceran
	a	5.218	5.231
	b	1.408	1.420
	r^2	0.663	0.674
	n	448	445
Sample mean	X	-0.370	-0.371
	Y	4.697	4.704
Sample variance	X	0.349	0.349
	Y	1.043	1.042

Table 5.7. Stepwise multiple regression of cladoceran filtration rates measured *in situ* on natural lake bacteria. Variance in ln filtration rate explained as a function of ln body length, cladoceran species and temperature in order of entry into the model. Species entered as binary coded 'dummy' variables with *Daphnia* as the reference category (Kim and Kohout 1975) and therefore not represented. ($p<0.01$ in all cases).

Variable	Coefficient	R^2	ΔR^2	F ratio
Constant	4.927			
Ln length	1.477	0.674	0.674	436.26
Bosmina	-1.022	0.754	0.080	70.59
Moina	-0.352	0.765	0.011	12.96
Ceriodaphnia	0.318	0.782	0.017	10.37
Diaphanosoma	-0.235	0.784	0.002	6.68
Temperature	0.017	0.786	0.002	3.13

Degrees of freedom: Model 6, Residual 438

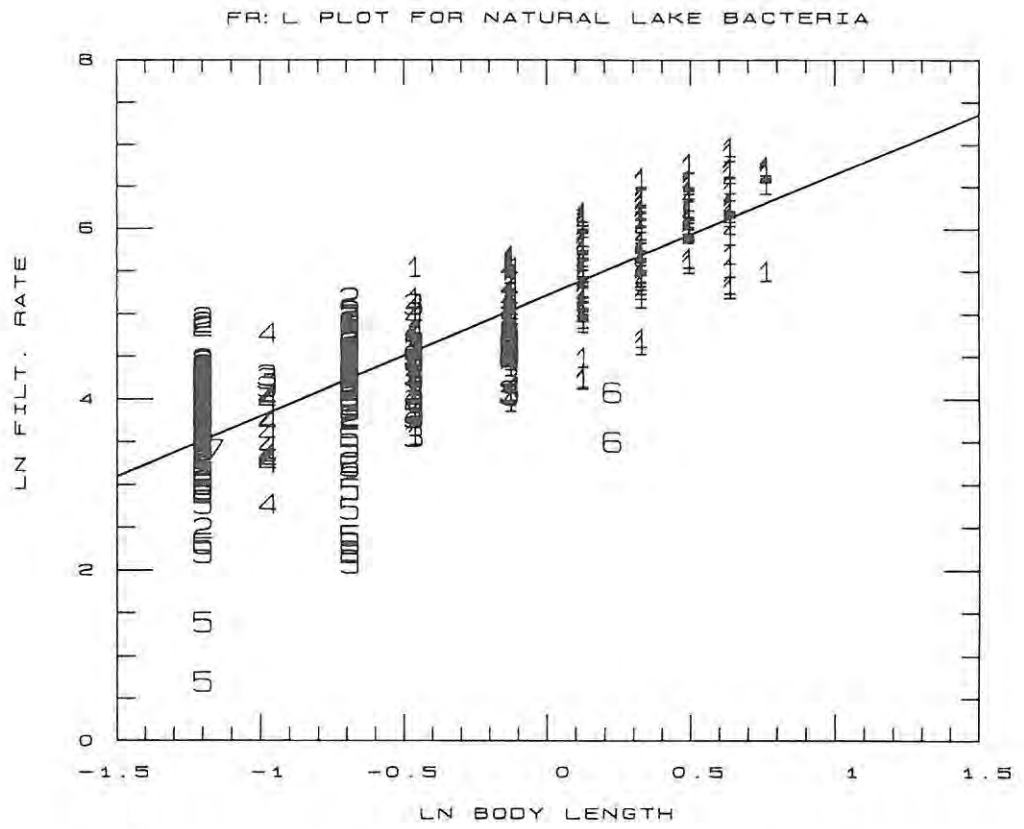
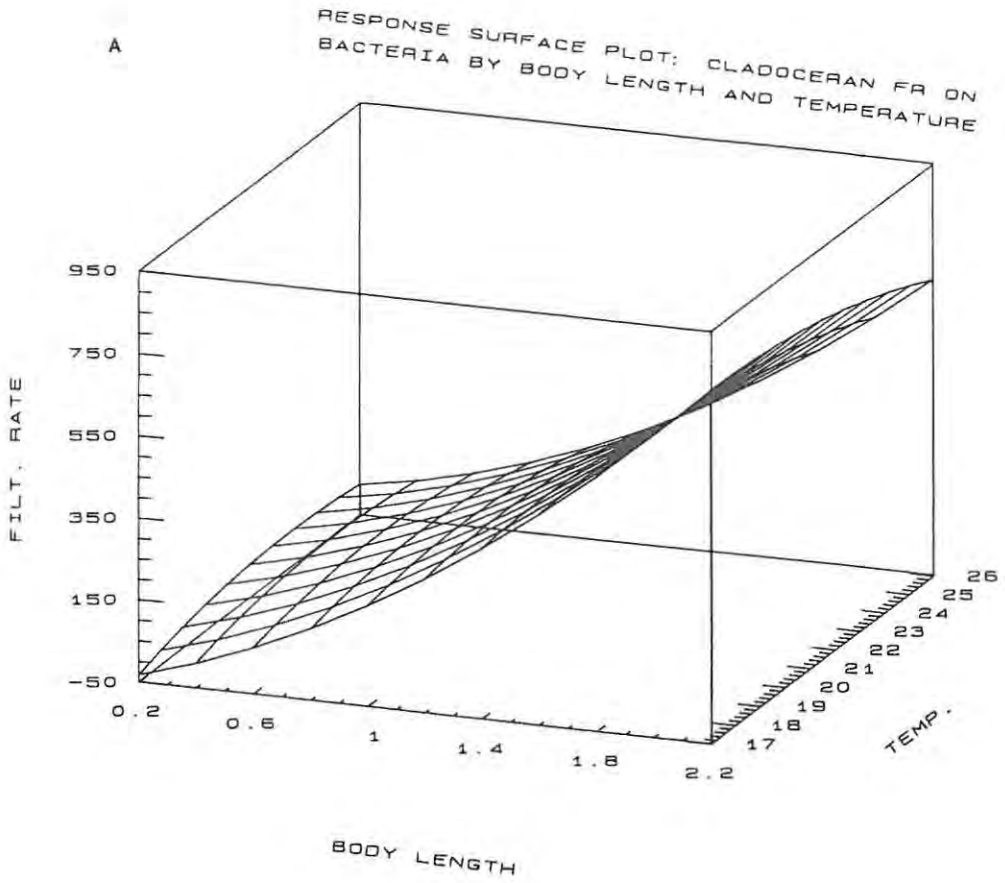


Figure 5.15: Natural logarithm transformed scatter-plot of individual zooplankton filtration rate ($\mu\ell$ animal^{-1} h^{-1}) against animal body length (mm) when feeding *in situ* on natural bacteria. Regression line drawn from data on cladoceran filtration rates only. Species coded as : *Daphnia* 1; *Ceriodaphnia* 2; *Moina* 3; *Diaphanosoma* 4; *Bosmina* 5; *Thermodiaptomus* 6 and *Brachionus* 7.



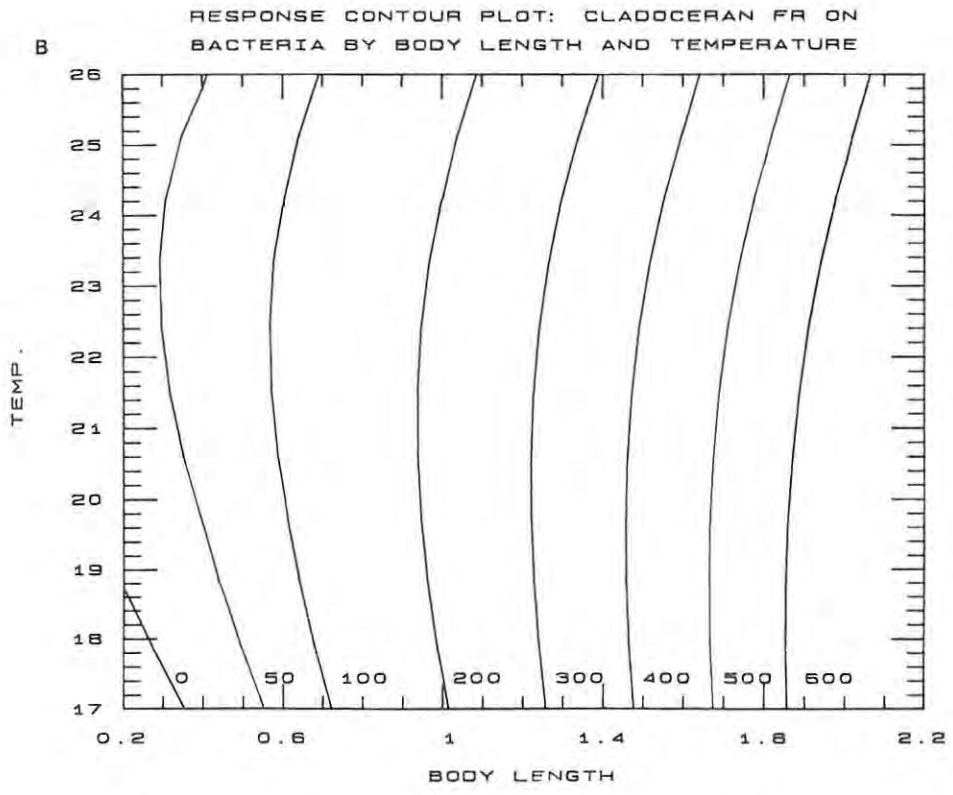


Figure 5.16: Response surface plot of cladoceran filtration rate (µℓ animal^{-1} h^{-1}) on natural bacteria in relation to the interactions of body length (mm) and temperature (°C) expressed in their polynomial form; (a) three-dimensional and (b) contour plot.

influence of the small cladoceran species on this bacteria-model due to its very low filtration rate on natural bacteria and accounted for 8% of the model variance (*Daphnia* was the reference species, so its influence was already incorporated in the model).

Further multiple regression analysis of the interactive influence of body length and temperature on cladoceran filtration rates on natural bacteria was carried out to allow comparisons to be made with results of similar analysis of CFRs on *Chlorella* (Section 3). The polynomial expression of body length-temperature interactions on the bacterial filtration rate of cladocerans was:

$$FR = -10.90LT + 333.55L + 113.92L^2 + 83.02T - 1.73T^2 - 977.87 \quad (5.3)$$

$$(R^2 = 0.741;\ df\ 5,\ 439;\ p<0.001)$$

where FR = filtration rate ($\mu\ell$ animal^{-1} h^{-1}), L = body length (mm) and T = temperature (°C) .

Response surface plots of this equation are shown in Figure 5.16. The influence of temperature on bacterial filtration rates was generally negative at greater cladoceran body lengths. As observed previously from similar analyses of biomass-temperature interrelations on community filtration rates on *Chlorella* (Section 3), the influence of temperature on filtration rates of small cladocerans on natural bacteria was positive up to an optimum of 22-24 °C, becoming negative again with higher temperatures.

5.4 Discussion

5.4.1 Species-specific filtration on natural bacteria

Change in the selectivity coefficient of the spring-summer zooplankton community of Hartbeespoort Dam, from little or no feeding 'preference' on bacteria to higher selectivity

coefficients (a preference for *Chlorella*), occurred in response to both the species composition of the grazer community and temporal changes in species selectivities on their foods. When *Daphnia* was numerous its increasing selectivity coefficient had a marked influence on community selectivity (Figures 5.7b and 5.13). Thereafter, with the change in zooplankton community composition, the feeding preferences for *Chlorella* of *Diaphanosoma* and *Moina* >0.5 mm maintained a community selectivity of >1.

The composition of the phytoplankton community present had a marked influence on the *Chlorella*/bacteria selectivity coefficient of *Daphnia*. The selectivity coefficient of all length classes of *Daphnia* increased after mid-December, coinciding with the maximum change in phytoplankton composition towards *Microcystis* dominance and the greatest decrease in bacteria number in Hartbeespoort Dam (Figures 5.8 and 5.13). The mean selectivity coefficient of all *Daphnia* length-classes up to mid-December was 1.37 ± 0.10 (minimum 0.80, maximum 2.32, n = 21). This indicated no pronounced preference by *Daphnia* for either *Chlorella* or natural bacteria during *in situ* experiments. However, from mid-December to March, all length classes of *Daphnia* exhibited a constant preference for *Chlorella* (mean coeff. 2.29 ± 0.13 minimum 1.51, maximum 3.18, n = 14). In contrast to the effect of this shift in both phytoplankton species composition and bacteria number upon the selectivity coefficient of *Daphnia*, it did not influence the feeding selectivity of *Ceriodaphnia* which was present over the same period.

The abundance of *Daphnia* and concomitant high community grazing rates during the spring edible-phytoplankton phase in Hartbeespoort Dam (Section 3), and *Daphnia*'s undifferentiated filtration on edible algae and natural bacteria before the mid-summer shift in phytoplankton composition, together indicate that zooplankton community grazing on bacteria is also very high in spring-early summer in Hartbeespoort Dam. Robarts and Sephton (1984) and Robarts *et al.* (1986) noted an annual decrease in bacterial number during January and February

each year. The suggestion that this occurs directly in response to high grazing pressure on bacteria by macrozooplankton unfortunately cannot either be confirmed or refuted. Data presented here indicate that grazing on bacteria can be very intense at this time if *Ceriodaphnia* is numerous, but during this study community selectivity for *Chlorella* increased after mid-summer. The measurement of other factors that may influence bacterial production or losses was beyond the scope of this study. Data on changes in heterotrophic activity and production occurring in association with phytoplankton successional events, changes in dissolved organic carbon, temperature and the abundance of micro-zooplankton grazers (mainly protozoans) is not available for the summer of 1986-87 in Hartbeespoort Dam. Previously bacterial number has been shown to increase with temperature, heterotrophic activity (Robarts and Sephton 1984) and extra-cellular dissolved organic carbon produced by algae (Robarts and Sephton 1987), but examination over three years shows that these trends, although significant, tend to be very variable (Robarts 1987). This annual mid-summer reduction in bacteria number also occurs in association with the increase in the *Ceriodaphnia* population, which has high size-specific filtration rates on bacteria compared to other cladocerans (Figures 5.12 and 5.15). However, cause and effect indicated between the annual decline in bacteria and high bacterioplankton grazing by *Ceriodaphnia* cannot be confirmed by the data presented here.

Length-specific selectivity was not evident within the *Daphnia* population. This was unexpected in the light of Lampert's (1974) results and given that the filter mesh size of individuals can increase with animal growth (Geller and Müller 1981). If the filter mesh aperture of both juvenile and adult animals is smaller than the food particle size then length-specific selection of food particles would not occur. However, the size range (cell diameters or widths) of natural rods and cocci in Hartbeespoort Dam (up to 0.71 µm, but as low as ∿0.09 µm) extends below the reported values for filter mesh sizes in *Daphnia pulex* and *D. longispina* (animals ∿1.9 mm length, mesh aperture 0.38 - 0.40 µm, Brendelberger 1985).

Although the filter mesh size of juvenile *Daphnia pulex* is not reported, it is assumed to be smaller and hence some length-specific differences in relative filtration rate of *Daphnia* on natural bacteria compared to unicellular algae (Porter *et al.* 1983) was expected. Absence of this phenomenon shown in Figures 5.10 and 5.11 and by the ∿60% relative filtration rate of bacteria compared to *Chlorella* for all *Daphnia* length-classes suggests firstly that this cladoceran is able to collect food particles smaller than its minimum filter mesh size, or secondly that all *Daphnia* filter only bacteria above the same undetermined cell size (e.g. ∿0.40 µm diameter) with equal efficiency. Assuming that within a species the individual filter mesh size can increase with body size (Geller and Müller 1981, Brendelberger 1985) the latter suggestion cannot be supported. The former suggestion (possible collection of particles smaller than the filter mesh size) lends some support to the filter-feeding mechanism postulated by Gerristen and Porter (1982) that surface charge and the wettability allows collection of particles smaller than the minimum mesh size of zooplankton filtering appendages, or that filtration occurs by mechanisms other than interception and sieving (Rubenstein and Koehl 1977).

The selectivity coefficients of *Daphnia pulex* in Hartbeespoort Dam (Figure 5.13) are very similar to those reported for *D. rosea* and *D. pulicaria* by DeMott (1982; 0.89 ± 0.05 to 1.8 ± 0.15) and DeMott and Kerfoot (1982; 1.91 ± 0.17 to 3.06 ± 0.12) measured *in situ* using natural algae and a cultured bacterium (*Aerobacter aerogenes*, 0.3 μm^3). Selectivity coefficients for a species of *Diaphanosoma (D. brachyurum)* and for *Bosmina longirostris* were also reported by DeMott (1982) and DeMott and Kerfoot (1982). As in Hartbeespoort Dam, these studies showed that *Bosmina* exhibited a pronounced preference for unicellular algae over bacteria. Using natural algae and cultured bacteria DeMott and Kerfoot (1982) measured selectivity coefficients of up to 15.7, higher than coefficients for *Bosmina* in Hartbeespoort Dam, which peaked at 4.14 (Figures 5.13 and 5.14). *Bosmina*'s selectivity coefficient measured by DeMott and Kerfoot (1982) also fluctuated between

a slight preference for bacteria when 'resistant' algae dominated (82% of total algal cell counts) to a strong algal preference when flagellated algae were abundant. Unfortunately examination of similar food-mediated changes in *Bosmina*'s seasonal feeding selectivity was not possible in Hartbeespoort Dam due to the limited and irregular presence of *Bosmina* in *in situ* experiments.

Strong similarity also exists between the *Chlorella*/bacteria selectivity of *Diaphanosoma excisum* in Hartbeespoort Dam (Figures 5.13 and 5.14) with coefficients measured for *D. brachyurum* by DeMott and Kerfoot (1982; their Figure 10). In Hartbeespoort Dam, *D. excisum* had body length-specific filtration rates similar to those of *Daphnia* on both *Chlorella* and natural bacteria, while *Diaphanosoma*'s selectivity coefficient (which generally ranged between 1 and 2) also demonstrated its preference for the unicellular alga.

Filtration rates for the genus *Moina* on natural bacteria are not well represented in the literature. In Hartbeespoort Dam body size related selectivity of *Moina* against natural bacteria was pronounced although based upon only limited measurements. Algal/bacterial selectivity coefficients showed juvenile *Moina* to filter these foods non-selectively (Figure 5.13) whilst the absolute filtration rates of larger *Moina* on bacteria were low in comparison with those of *Daphnia* and *Diaphanosoma* of similar size and with those of smaller *Ceriodaphnia* adults (Figure 5.9).

Filtration rates of *Ceriodaphnia* on natural bacteria in Hartbeespoort Dam were high in relation to its small body size (Figures 5.12 and 5.15) and support its classification by Geller and Müller (1981) as a 'high efficiency bacteria feeder'. Filtration rates of both length-classes of *C. reticulata* in Hartbeespoort Dam were slightly lower than the rates measured by Porter *et al.* (1983) for marginally larger *C. lacustris*. The absence of a strong feeding preference for algae over bacteria in both large and small *Ceriodaphnia* (selectivity coefficients generally close to 1.0, Figures 5.13 and 5.14)

has also been noted elsewhere. Ganf and Shiel (1985) found no feeding preference for *C. quadrangula* on particles 3-12 µm in diameter. Using natural bacteria (<1 µm) Pace *et al.* (1983) found biomass-specific filtration rates to be similar between *Daphnia* and *Ceriodaphnia* while results of their life table experiments, in which the survivorship of *Ceriodaphnia* was good on low bacterial food concentrations, showed the better utilization of bacteria by this small cladoceran than the larger *Daphnia*. The data on bacterial filtration from Hartbeespoort Dam and results of other studies point to the importance of bacteria as a summer food resource for *Ceriodaphnia*.

Zooplankton species-specific filtration rates on natural bacteria and their *Chlorella*/bacteria selectivity coefficients, when considered in the light of other studies on bacteria or small particle grazing, aid in the interpretation of resource-mediated successional events within the zooplankton community of hypertrophic Hartbeespoort Dam. Geller and Müller (1981) categorized some filter-feeding zooplankton species as either high or low efficiency bacteria feeders based on the minimum size of the filter mesh on their feeding appendages. Data on species filtration rates of natural bacteria in Hartbeespoort Dam generally supports this categorization, but not in the order (congeneric species) as listed by filter mesh size (Geller and Müller 1981, in their Table 1). The high filtration rate of *Ceriodaphnia* on natural bacteria in relation to body length and its low algal preference compared to other cladocerans places *Ceriodaphnia* as the most efficient filter-feeder of natural bacteria in Hartbeespoort Dam. The filtration efficiencies of *Daphnia* and *Diaphanosoma* on bacteria were similar. Differences between the length-specific filtration rates of *Daphnia* and *Diaphanosoma* were slight and both cladocerans also had similar selectivity coefficients. However, the very high population densities attainable by *Daphnia* in spring and its large body size imply that *Daphnia* has a greater potential grazing impact than *Diaphanosoma* on the bacteria of Hartbeespoort Dam. *Moina*'s filtration efficiency on bacteria, although high in

the case of individuals <0.5 mm, was generally low following both from the low filtration rates and high algal preference of large *Moina*. Geller and Müller's (1981) classification of *Bosmina* as a low efficiency bacteria feeder is supported by data from Hartbeespoort Dam.

Brendelberger (1985) suggested that there is a competitive advantage for those filter-feeders able to feed efficiently on particles <1.0 µm diameter when bacterial numbers are high. This advantage increases both with increasing eutrophication and with seasonal increases in temperature. In combination with *Ceriodaphnia*'s high survivorship on natural bacteria (Pace *et al.* 1983), its high size-related filtration rates on bacteria in Hartbeespoort Dam further support the postulations made in Sections 3 and 4 that the summer *Ceriodaphnia* population is, to a large degree, supported by bacterioplankton when edible algal biovolume is low. The largely non-selective filter-feeding of *Daphnia*, possibly even on particles smaller than its filter mesh size, and its large body size and high filtration rate on bacteria, indicate potentially intense competition between *Daphnia* and *Ceriodaphnia* on bacteria at mid-summer. Interference to *Daphnia*'s non-selective collection of food particles by *Microcystis* colonies (clogging and frequent particle rejection; Webster and Peters 1978, Porter and McDonough 1984) and reduction in its filtration efficiency (Section 4) due to the presence of *Microcystis* colonies (e.g. narrowing of its carapace gape; Gliwicz and Siedler 1980) have already been suggested as factors leading to the mid-summer decline in *Daphnia* (Section 4). Furthermore the better competitive advantage of *Ceriodaphnia* over *Daphnia* when feeding on bacteria (better survivorship and feeding efficiency at low bacteria concentrations; Pace *et al.* 1983) and its higher size-specific filtration rates on natural bacteria are also factors, which in combination with those mentioned above, further reduce the competitive ability of *Daphnia* at mid-summer in Hartbeespoort Dam.

5.4.2 Filtration rate-body length models on bacteria

As anticipated from results of FR:L measurements using *Chlor-*

ella and *Microcystis* (Section 4), cladoceran filtration rate on natural bacteria was best predicted by a power function of animal body length (Equation 5.2). The influences of grazer species and water temperature, while of much lesser importance, remained significant variables (Table 5.7). Of interest was the strong influence of *Bosmina* on the FR:L relationship on bacteria ($\Delta R^2 = 0.080$). In similar analyses on other foods (Section 4) *Bosmina* contributed little to the reduction of unexplained variance (Tables 4.3-4.5). The scatter-plot of ln transformed filtration rates on bacteria against body length (Figure 5.15) shows the low length-specific filtration rates of *Bosmina* which influence the FR:L model on natural bacteria (Table 5.7, negative regression coefficient) and the opposing influence of *Ceriodaphnia* (positive regression coefficient) which had high length-specific filtration rates. Both *Moina* and *Diaphanosoma* also had negative regression coefficients. The very slight influence of *Diaphanosoma* on the FR:L model (0.2% of model variance) again shows the similarity in length-specific filtration rates between *Diaphanosoma* and *Daphnia* (*Daphnia* used as the reference category for species coded variables). The three non-cladoceran data points are also shown in Figure 5.15. Conclusions cannot be drawn from such limited data on the length-specific filtration rates of *Brachionus* and *Thermodiaptomus*. The filtration rates of *Thermodiaptomus* on bacteria do not comply with the FR:L cladoceran model, its length specific rates being low, as also noted for the *Chlorella* and *Microcystis* FR:L models in Section 4.

Relationships between zooplankton body lengths and their filtration rates on bacteria have not been examined in as much detail as for algal foods used in feeding studies. Furthermore those studies describing zooplankton FR:L relations on bacteria have varied in the type of bacteria used (natural community or monospecific culture), in the zooplankton grazers examined, and in the experimental conditions (laboratory or *in situ*).

DeMott (1982) described the filtration rates of both *Daphnia rosea* and *Bosmina longirostris* on cultured bacteria as a power

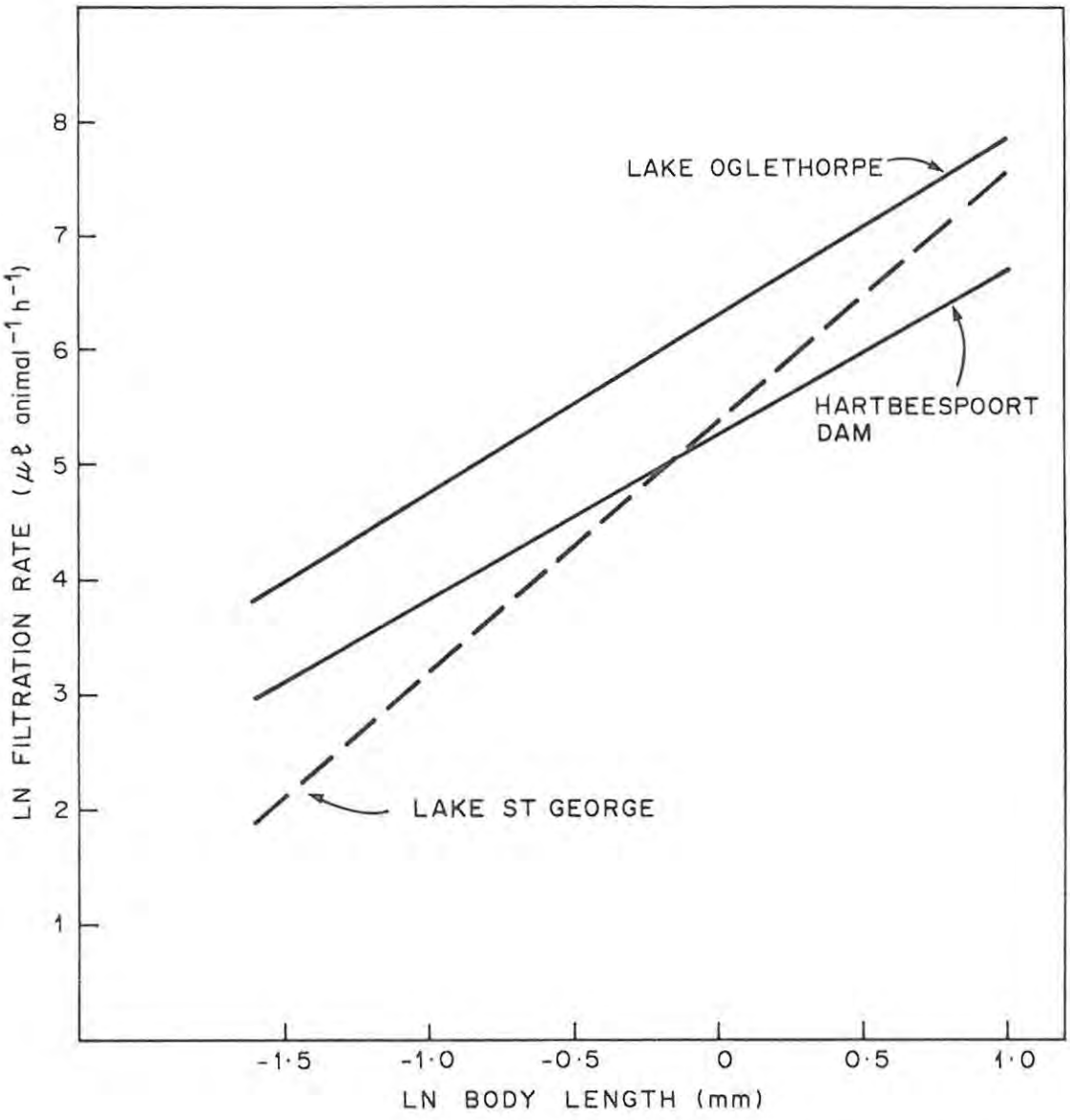


Figure 5.17: Comparison of the cladoceran FR:L model on natural bacteria measured *in situ* in Hartbeespoort Dam with the models of Porter *et al.* (1983) from Lake Oglethorpe (using natural bacteria in laboratory experiments) and of Knoechel and Holtby (1986b) from Lake St George (using cultured bacteria in *in situ* experiments.

function of body length. The slopes of these relationships for *Daphnia* were greater than those obtained for *Bosmina* on bacteria, algae and food mixtures. Schoenberg and Maccubbin (1985) also examined the FR:L relationship of species individually, but used natural bacteria of three size fractions. *Ceriodaphnia reticulata* was found to have a slight preference for free-bacteria compared to attached bacteria and had a lower size threshold than the other cladocerans examined. They noted that filtration rates of cladocerans in the Okefenokee Swamp (*Eubosmina, Chydorus, Acantholeberis, Ceriodaphnia* and *Pseudosida*) were generally not related to body size across species when feeding on free-bacteria, but this relationship was significant for attached bacteria. Contrary to the results of Schoenberg and Maccubbin (1985), Porter *et al.* (1983) and Knoechel and Holtby (1986b) in their regression models for cladocerans found body length to account for 88% and 87% respectively of the variance in filtration rate on bacteria. The markedly different slopes between the FR:L models of Porter *et al.* (1983, eutrophic Lake Oglethorpe) and Knoechel and Holtby 1986b, mesotrophic Lake St. George) may be attributed to differences in experimental conditions and foods (laboratory experiments with natural bacteria, or *in situ* with cultured bacteria respectively).

The above cladoceran FR:L models on bacteria are shown in Figure 5.17 compared to the relationship observed in Hartbeespoort Dam using natural bacteria (Equation 5.2). In both studies using natural bacteria the very similar slopes of the FR:L models (b = 1.545, Porter *et al.* 1983; b = 1.420, this study) show an almost identical length-specific filtration rate response of cladocerans on this generally very small food. The cultured bacterium (*Flavobacterium aquatile*, ~1.2 µm) used by Knoechel and Holtby (1986b) was filtered at comparatively higher rates by large cladocerans and at lower rates by small individuals. This difference further highlights the problem of extrapolation from filtration rates measured using cultured bacteria to estimate rates *in situ* on natural bacteria which are generally of a smaller size. The differences in intercepts between the models of Porter *et al.*

(1983) and Hartbeespoort Dam may be attributed to the different methods used to measure filtration rates (laboratory, direct cell count, corrected for cell growth; *in situ*, radiolabel technique, corrected for adsorption and isotope loss) and to the different cladoceran species included in each model. The remarkably close similarity in FR:L model exponents derived using natural bacteria shows not only that body size can be used as a fairly reliable predictor of cladoceran filtration rate on bacteria, but also that this FR:L relationship is generally similar for limnetic cladoceran communities, at least in eutrophic lakes, although model exponents and intercepts are likely to vary depending upon community composition (high or low efficiency bacterial feeding species).

6.0 GENERAL DISCUSSION

Species succession within the plankton community, seasonal variations in community grazing and biomass-specific grazing rates, and the species-specific filtration rates on *Chlorella*, *Microcystis* and bacteria, when viewed together confirm the suggestion that food quality (food type and particle size) rather than food quantity alone (chlorophyll *a* concentration) is of paramount importance in the regulation of zooplankton community structure and biomass under hypertrophic conditions.

A number of fairly predictable successional events that occur annually in Hartbeespoort Dam support this conclusion. Positive correlation between food quantity (in terms of chlorophyll *a* concentrations) and zooplankton community biomass over each of the six years studied was either absent or, when lagged to include any delayed response in the development of community biomass, generally revealed a negative association between stocks of producers and consumers in the plankton of Hartbeespoort Dam. The mid-summer shift in phytoplankton community composition, from the edible chlorophyte-cryptophyte phase in spring to the largely inedible *Microcystis* phase from summer to mid-winter, was identified as the most influential event associated with the concomitant shift in zooplankton community composition from the high biomass-high grazing rate '*Daphnia* phase' to the low biomass-low grazing rate '*Ceriodaphnia* phase'. In the absence of strong size-selective predation by fish in Hartbeespoort Dam, various aspects of the feeding ecology of the principal herbivores must be considered together when describing the events and processes that occur within the plankton of this hypertrophic impoundment. These processes include niche overlap and food resource partitioning, food limitations, fecundity, feeding efficiencies on different foods, and the exploitation of alternative food resources (e.g. bacteria).

Data presented here for Hartbeespoort Dam show that, at the onset of rapid algal growth in spring, the phytoplankton is

is composed mainly of edible unicellular chlorophytes and cryptophytes. Both herbivore biomass (principally *Daphnia* and *Bosmina*) and community grazing rates increase rapidly at this time and can peak at very high values (integrated herbivore biomass of up to 1.64 mg dry wt ℓ^{-1} and ΣCGR of up to 260% d^{-1} over the aerobic water column). This edible phytoplankton phase is often characterized by low chlorophyll *a* concentrations and low phytoplankton standing stocks. This occurs when water temperature, daylength and euphotic depth increase. Consequently primary production and growth of edible phytoplankton species in Hartbeespoort Dam, in the absence of nutrient limitation, have the potential to lead to the development of extremely high phytoplankton densities and chlorophyll *a* concentrations similar to those measured when *Microcystis* dominates in summer-autumn (T. Zohary pers. comm.).

The failure of the edible phytoplankton community to achieve their potentially very high standing stocks in spring, concomitant with the aforementioned intense grazing pressure of the zooplankton community (*Daphnia* phase), strongly support the conclusions that filter-feeding by zooplankton is able to limit the biomass of the phytoplankton community. Thus chlorophyll *a* concentrations during spring in this hypertrophic impoundment can be limited by zooplankton grazing.

The ability of the zooplankton community to limit phytoplankton standing stocks in spring is further supported by evidence of food resource limitations to the population growth of *Daphnia*. Marked decreases in *Daphnia* fecundity, brood size and the percentage of gravid adults occur during the spring edible phytoplankton phase (Figures 2.7 and 2.8). Regular decreases in all three of these population growth parameters occur during the spring and are good indicators of the level of food resources available to cladoceran populations (Threlkeld 1985).

A further indication of the potential for high zooplankton biomass and grazing rates to limit phytoplankton biomass in

spring is also evident from the increased euphotic depth in Hartbeespoort Dam (Robarts and Zohary 1984, NIWR 1985) when both edible phytoplankton forms and high *Daphnia* numbers occur together (spring 'clear-water' phase). The biomass of zooplankton or 'critical concentration of zooplankton' present, at times when high losses of particles attributed to intense grazing occur in a variety of lakes, has been examined by Lampert (1985). From oligotrophic to mesotrophic lakes this critical concentration was estimated by Lampert (1985) to be 1.0 - 3.6 g dry wt m^{-2}, and 1.5 - 4.5 g dry wt m^{-2} for eutrophic lakes. Identification of dates when the first pronounced increase in Secchi depth occurred during the winter-early spring part of the *Daphnia* phase (NIWR unpublished data) enabled the corresponding 'critical concentrations of zooplankton' to be estimated in Hartbeespoort Dam (after Lampert 1985) for each year of this study (Table 6.0). With the exception of the winter-spring of 1981 these critical concentrations of zooplankton from 1982-86 in hypertrophic Hartbeespoort Dam are considerably higher than those estimated by Lampert (1985) for eutrophic lakes (Table 6.0).

A pronounced decrease in total phytoplankton density in spring as a result of the corresponding extremely high herbivore biomass observed in many lakes (Lampert 1978, Lampert *et al.* 1986) was anticipated in Hartbeespoort Dam. However, this was expected to be less pronounced due to the very high solar radiation and nutrient concentrations available for algal growth in this impoundment (Robarts 1984, Zohary and Robarts in prep.). Grazing by zooplankton to the point where phytoplankton standing stock can be markedly limited requires the development of not only high zooplankton biomass, but also requires a community composed of grazer species that have high size-specific grazing rates, such as *Daphnia*. These events occur annually in Hartbeespoort Dam.

Whether this high spring grazing pressure on edible chlorophyte and cryptophyte forms plays a major role in promoting the rapid return to dominance of *Microcystis* by mid-summer cannot be confirmed. Conditions in Hartbeespoort Dam such as

Table 6.0. Estimates of 'critical concentrations of zooplankton' in Hartbeespoort Dam from 1981-86 at times when the first marked increase in Secchi depth occurred during the edible phytoplankton phase in spring. The start of this clear-water phase shown here may be attributed to high grazing rates of the 'high biomass - *Daphnia* phase' zooplankton community. Data summarised by Lampert (1985; his Table 1) from eutrophic lakes is included for comparison (marked as *). Estimates of the total biomass of herbivores only, calculated for Hartbeespoort Dam.

Date	Secchi depth (m)	Spring maximum Secchi depth (m)	'Critical' concentration of zooplankton (g dry wt.m^{-2})
18.8.81	2.00	3.35	3.87
22.6.82	2.90	2.90	9.54
16.8.83	5.90	5.90	12.60
11.9.84	1.80	2.50	7.72
15.7.85	1.75	2.60	10.48
19.8.86	2.50	2.50	9.84
April - June	-	-	1.5 - 4.5*

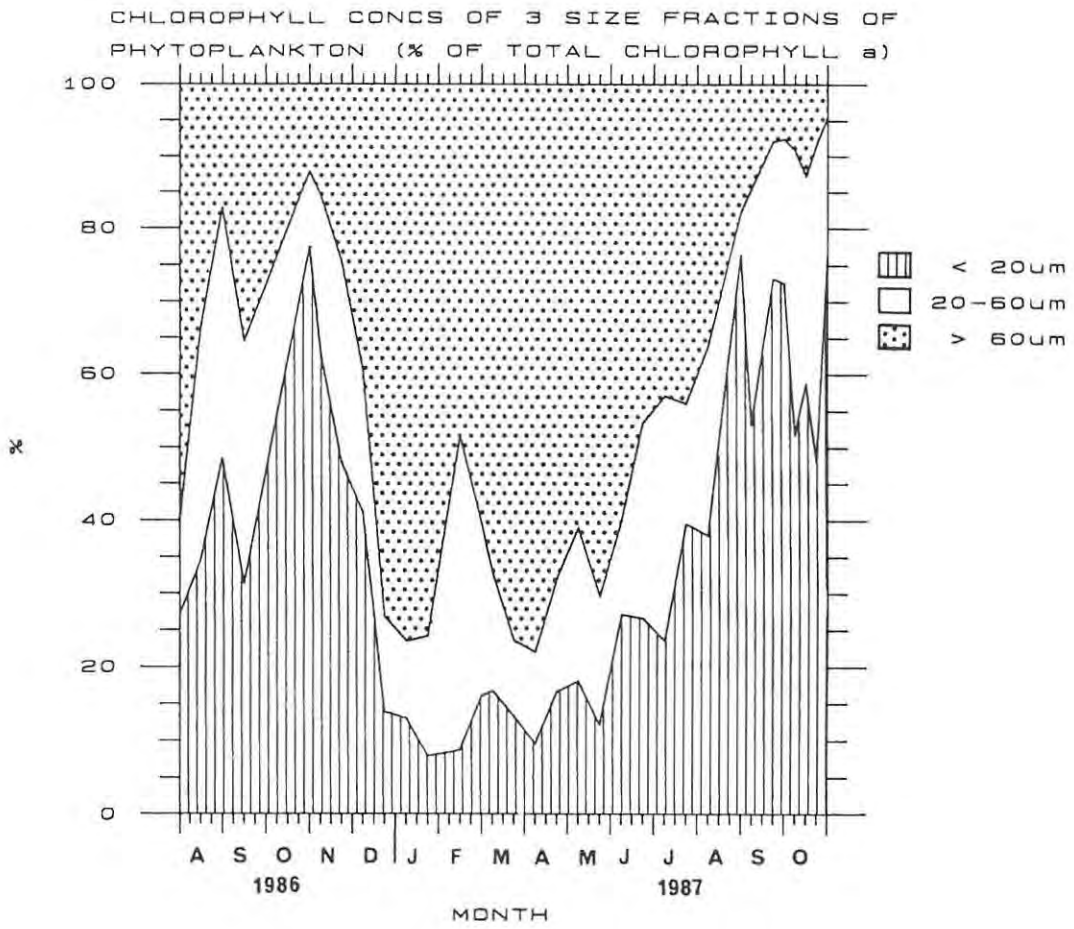


Figure 6.0: Chlorophyll *a* concentrations of 3 size fractions of phytoplankton in Hartbeespoort Dam (1986-87) expressed as % of total chlorophyll *a* concentration.

high nutrient concentrations, very low N:P ratio, generally low wind speeds and little turbulent mixing, and light limitation to primary production rates (Robarts 1984, Robarts and Zohary 1984, NIWR 1985) together indicate that buoyant cyanophytes such as *Microcystis* are likely to dominate the phytoplankton irrespective of grazing influences. However, both the brevity of the spring clear-water phase and the very rapid and marked shift between the edible and largely inedible phytoplankton phases, following November-December peaks in zooplankton biomass, are likely to be enhanced by the high zooplankton grazing rates recorded in Hartbeespoort Dam. Lampert (1985) summarised this process when examining data on the biomass of phytoplankton fractions above and below 35 µm . The biomass of the phytoplankton fraction >35 µm increased markedly after the spring clear-water phase, whilst that of the <35 µm fraction remained low throughout the late summer, even as the zooplankton biomass declined (Lampert 1985; his Figures 1a and 1c). High phytoplankton biomass in summer was mainly due to the growth of large particles whilst the biomass of small particles remained suppressed by zooplankton grazing. Takamura *et al.* (1986) described similar events in hypertrophic Lake Kasumigaura when *Microcystis* blooms occurred. The biomass, photosynthetic rate and primary production of nanoplankton (<20 µm) declined during the bloom, whilst *Microcystis* dominated both the large and small phytoplankton fractions (small colonies <20 µm).

The occurrence of these events can also be observed in Hartbeespoort Dam. Based upon results obtained on filter-feeding on different colony size fractions of *Microcystis* (Section 4), data was gathered on the chlorophyll *a* concentrations of three size fractions of phytoplankton from integrated sampling in Hartbeespoort Dam (<20 µm, 20-60 µm and >60 µm fractions). Seasonal variations in the chlorophyll *a* concentration of these three phytoplankton fractions during 1986-1987 are presented in Figure 6.0. A pronounced decrease in chlorophyll *a* representing the smallest particle fraction, and an associated increase in chlorophyll *a* representing the largest particle fraction (Figure 6.0) and total chlorophyll *a* concentration

Figure 2.3) occurs in mid-summer. This is another indication that the process of phytoplankton succession occurring annually in this hypertrophic impoundment is influenced by size-selective grazing on food-particles by zooplankton, as described above in less enriched lakes by Lampert (1985).

In addition to data on phytoplankton resource categories (Figures 2.2 and 2.3) Figure 6.0 further highlights both the magnitude of the change in the size of food particles that occurs at mid-summer and the paucity of food in the form of small particles that are available to the summer herbivore community (e.g. chlorophytes, cryptophytes and unicellular or very small *Microcystis* colonies). The sudden reduction in the filtration efficiency of *Daphnia* in the presence of the large cyanophyte particles, to filtration rates only 25-36% of those recorded before this shift towards phytoplankton dominance by *Microcystis* of colonial morphology, was reported in Section 4 and is regarded as a major factor contributing to the mid-summer decline of the *Daphnia* population. Thereafter, in summer it is suggested that, based upon the low filtration rates of the small-bodied cladocerans on small *Microcystis* particles (Figure 4.1), the summer grazer community depends upon both the low concentrations of edible algal particles <20 μm and bacteria (see also conclusions in Sections 2 and 3).

Coexistence of the two major herbivores *Daphnia* and *Ceriodaphnia* at mid-summer is usually brief and accompanied by vertical separation of their population maxima in the water column. Based on feeding studies using *Chlorella*, *Microcystis* and bacteria, interspecific competition between these cladocerans rather than food resource partitioning is indicated. The food particle size-spectrum of *Daphnia* was wide, ranging from natural free-living bacteria up to *Microcystis* colonies of 60-100 μm diameter, and overlapped the particle size-spectra of all other filter-feeding cladocerans. All of the small-bodied, summer cladoceran species (*Ceriodaphnia*, *Moina* and *Diaphanosoma*) had low filtration rates on the smallest *Microcystis* fraction (5-20 μm) relative to rates on *Chlorella*

and essentially did not feed upon larger *Microcystis* fractions (Figure 4.1). *Ceriodaphnia* in particular had high size-specific filtration rates on both the palatable chlorophyte *Chlorella* and on natural bacteria but did not significantly utilize *Microcystis*.

The general feeding strategies of the major cladocerans can therefore be summarized according to their filtration rates on different types and size fractions of foods. *Daphnia* is a fairly non-selective filter-feeder able to reach high population densities when unicellular chlorophytes and cryptophytes are dominant in spring. Thereafter, in combination with subsequent food limitation and promotion of the rapid development of *Microcystis* of colonial morphology, *Daphnia* feeds upon both the 'nutritionally poor' (Arnold 1971, Lampert 1977b, 1977c) cyanophyte food in summer and undergoes a marked decline in filtration efficiency in the presence of abundant large particles. Associated with this change in the food particle-sizes available and decline in edible algal resources the essentially non-selective filter-feeding of *Daphnia* on bacteria compared to *Chlorella* undergoes a slight shift towards an algal preference. Therefore a combination of these events and feeding responses together lead to the mid-summer decline in the *Daphnia* population observed in Hartbeespoort Dam.

The small-bodied cladocerans *Ceriodaphnia*, *Moina*, *Diaphanosoma* and *Bosmina* are more selective feeders than *Daphnia*. In relation to body size *Bosmina*, in particular, was able to feed upon very large particles (*Microcystis* colonies up to 60-100 µm diameter; Figure 4.1). This cladoceran also had very low filtration rates on bacteria (high selectivity coefficients indicating a strong preference for *Chlorella*; Figures 5.13 and 5.14). This is consistent with the foraging feeding strategy of *Bosmina* described by DeMott (1982) and DeMott and Kerfoot (1982).

Both *Moina* and *Diaphanosoma* essentially did not utilize *Microcystis* colonies and exhibited a preference for *Chlorella*

compared to natural bacteria. Their size-specific filtration rates on *Chlorella* were, however, slightly lower than the rates of *Daphnia* (Figure 4.0). Therefore their absence during the spring chlorophyte - cryptophyte phase may be partly due to competitive exclusion by *Daphnia* based on size-specific feeding rates. Filtration rates of *Moina* and *Diaphanosoma* appear not to be depressed in the presence of abundant *Microcystis* colonies (Figure 4.4 and 4.5). Hence their population growth and survival in summer depends largely upon the low concentrations of edible algal particles (edible phytoplankton <20 µm; Figures 4.5 and 6.0).

Similarly, *Ceriodaphnia* seems to be largely dependent on small edible particles whilst its feeding efficiency is unaffected by the presence of abundant large *Microcystis* colonies during summer and autumn. Although *Ceriodaphnia* exhibits no clear preference for *Chlorella* or bacteria (Figures 5.13 and 5.14), this absence of a strong algal preference indicates *Ceriodaphnia*'s potentially greater dependence on bacteria than the other cladoceran species studied. However, no seasonal change in *Ceriodaphnia*'s algal/bacterial selectivity occurred as the algal resources available decreased with increasing *Microcystis* dominance.

When the combined influences of the algal/bacterial feeding preferences of the summer grazer community (mainly *Ceriodaphnia*, *Moina* and *Diaphanosoma*), and the seasonal increase in algal preference by *Daphnia*, are viewed in the form of total community selectivity coefficients, an increase in algal preference (*Chlorella*) is clearly evident (Figure 5.7b). This was unexpected and questions the established view that shifts to bacteria utilization occur during the summer cyanophyte phase of eutrophic lakes (e.g. Gliwicz 1969a, 1969b, 1977, Pace *et al.* 1983).

The absence of a summer shift towards the selection and utilization of bacterial food resources in Hartbeespoort Dam raised the question - to what extent is the bacterioplankton, present during the summer period of low edible algal-resource

levels, an important alternative food resource for the small bodied cladoceran community present? Data on bacterial number were therefore supplemented by data on bacterial production (R.D. Robarts unpublished data) in order to allow estimation of both bacterial standing stocks, the cell numbers produced and thus bacterial doubling times (Table 6.1). These values were calculated to provide insight into the growth rate of the bacterial food resource and the potential macrozooplankton grazer (>60 µm) impact on the numerically abundant, yet very small cell size and low biomass, bacterioplankton population present in this enriched system (Robarts 1987). Zooplankton ΣCGRs on bacteria were estimated using the ΣCGR values measured on *Chlorella* (Figure 3.12) adjusted by the relative CGRs measured on bacteria (Figure 5.7a).

Table 6.1 shows that the grazer-bacteria turnover times (clearance time by zooplankton) were less than the bacterioplankton doubling times. This implies that the bacteria in Hartbeespoort Dam experience high 'grazing pressure', and thus that bacterial standing stocks may be limited by macrozooplankton grazing. Confirmation of the latter conclusions is, however, not possible based only on the data presented here. This conclusion may be true with regard to large rods or attached bacteria and could account for their rare occurrence in relation to small cocci in the bacterial community of Hartbeespoort Dam (R.D. Robarts pers. comm.). However, the high numbers of generally very small cells present (Tables 5.1 and 6.1) indicate that, regardless of the potentially very high grazing rates on bacteria, the bacterial population is able to remain abundant. Therefore it is suggested that, in response to these high grazing rates (low turnover times) and associated size-selective grazing (selection for large cells), the bacterial community structure in Hartbeespoort Dam is influenced by macrozooplankton grazers which promote bacterioplankton dominance by very small sized free-living cells. Any further contribution by the microzooplankton (ciliates and flagellates) to grazing rates on natural bacteria and their influence on bacterial community structure unfortunately remains unknown.

Table 6.1. Estimates of bacterial doubling times, integrated zooplankton grazing rates on bacteria and grazer-bacteria turnover times during the summer period of maximum *Microcystis* dominance. Unpublished data from R.D. Robarts on bacterial production and number in 1987 were used to calculate bacterial cells produced using a conversion factor of thymidine (Tdr) incorporation rate to cells dividing of 2×10^{18} cells $mole^{-1}$ (Moriarty in press). Bacterial doubling time = (bacteria number $\times 10^6$/cell production $\times 10^6$ $m\ell^{-1}$ h^{-1})/24 hours. Mean monthly zooplankton ΣCGRs from January - March (1983 - 85) were converted using monthly bacteria CFR/Chlorella CFR efficiencies (January - March 1987) to give estimates of ΣCGR on bacteria and the grazer-bacteria turnover time.

Month	Bacterial production pM Tdr ℓ^{-1} h^{-1} (mean of top 10 m)	Bacteria number $\times 10^6$ $m\ell^{-1}$ (mean of top 10 m)	Bacteria cells produced $\times 10^4$ $m\ell^{-1}$ h^{-1}	Bacteria doubling time (days)	Zooplankton ΣCGR on bacteria (% d^{-1})	Grazer-bacteria turnover time (days)
Jan.	33.8	9.75	6.76	6.0	25.6	3.9
Feb.	10.3	9.39	2.06	19.0	30.7	3.3
March	29.6	7.99	5.92	5.6	48.4	2.1

Table 6.2. Bacterial carbon consumed, expressed as % of zooplankton as carbon present during the summer period of maximum *Microcystis* abundance. Bacterial carbon was calculated from bacterial numbers (Table 6.1), mean cell volume (Table 5.1) and the cell volume to carbon conversion factor of 1.2×10^{-13} g C μm^{-3} (Watson *et al.* 1977). Mean monthly ΣSFRs from January - March (1983-85) were converted using monthly bacteria CFR/*Chlorella* CFR efficiencies (January to March 1987) assuming a carbon content of 40% (zooplankton dry weight; Lampert 1985) and expressed as daily rates. ΣSFR was used to calculate bacterial carbon consumption assuming that all bacteria $m\ell^{-1}$ filtered were consumed.

Month	Bacterial Carbon $\mu g\ C\ m\ell^{-1}$	ΣSFR on bacteria $m\ell\ mg^{-1}$ zoop. $C\ d^{-1}$	Bacteria C consumed as % of zoop. C
Jan.	0.01521	640.8	1.0
Feb.	0.01465	1143.4	1.7
March	0.01246	2173.0	2.7

Whilst the number of cocci in Hartbeespoort Dam is high their total biomass remains low due to their small size. Therefore the contribution to the daily carbon requirements of the zooplankton community by free-living bacteria during summer is also small (Table 6.2). Assuming that 100% of the bacterial carbon consumed by the zooplankton is assimilated, then grazing on bacterioplankton (ΣSFR on bacteria) contributes only 1-2.7% of zooplankton body carbon per day. Daily zooplankton carbon incorporation has been estimated to be about 30% body C d^{-1} (14.4-32.5% body C d^{-1} for *Daphnia pulex*; Bell and Ward 1970) and the respiratory carbon loss of herbivorous zooplankton species has been found to vary around 22% body C d^{-1} at 20 °C (Lampert 1984). These estimates of the daily carbon requirements of zooplankton show that the contribution by bacteria is minor in Hartbeespoort Dam (Table 6.2). Therefore, while the bacterioplankton of Hartbeespoort Dam may be regarded as a supplementary food resource to some summer cladocerans (e.g. *Ceriodaphnia*; Pace *et al.* 1983), in general it is not an important food resource. Therefore the summer cladoceran community must depend mainly upon the low concentrations of edible algal food resources (chlorophytes and cryptophytes) present during the period of *Microcystis* dominance (Figure 4.5).

The successional events described here for a hypertrophic lake extend the pattern of events described by Geller and Müller (1981; their Figure 6). This pattern can be summarized as a general progression from 'macrofiltrator' dominance in oligotrophic lakes to dominance by 'high efficiency bacteria feeders in eutrophic lakes. Geller and Müller's winter period dominated by macrofiltrators (typically coarse filter-mesh copepods) in eutrophic lakes does not occur in Hartbeespoort Dam, and the summer period of high efficiency bacteria feeders (characterised by *Ceriodaphnia* in Hartbeespoort Dam; Figure 2.1) usually extends throughout the autumn.

Further comparison of the successional events in Hartbeespoort Dam with the most usual and important events in other lakes listed in the PEG-model (Sommer *et al.* 1986) reveals a gener-

ally close agreement. Considering that Hartbeespoort Dam is hypertrophic (a level of lake enrichment not represented in the PEG-model) and subtropical (a climatic zone only represented by oligotrophic lakes Sibaya and Le Roux), this similarity in successional events between Hartbeespoort Dam and the PEG-model show that most of the descriptive statements making up this model remain valid for warm, highly enriched lakes. However, under these hypertrophic conditions, statements in the PEG-model describing processes that are partially driven by nutrient limitation do not apply. For example, in most lakes both nutrient availability and increased light are regarded as the major stimuli for unlimited phytoplankton growth early in spring (Sommer *et al.* 1986). However, Robarts (1984) and Robarts and Zohary (1984) have shown that increased light and to some degree increased turbulent mixing by wind, in combination with reduced buoyancy of *Microcystis* in winter (T. Zohary pers. comm.) are the most important stimuli for phytoplankton growth in Hartbeespoort Dam. Thereafter, the processes described in the PEG-model leading to a grazer-mediated clear-water phase in spring apply to Hartbeespoort Dam.

With regard to the dichotomy in the PEG-model sequences at mid-summer between lakes with or without an abundant growth of phytoplankton, Hartbeespoort Dam clearly falls into the former category typical of eutrophic lakes that develop high phytoplankton densities without the depletion of phosphorus (NIWR 1985). Zooplankton populations (raptors) able to feed upon the large 'canopy' algae and cyanophytes (e.g. *Microcystis*) do not develop in Hartbeespoort Dam and the small herbivores largely depend on edible 'undergrowth' algal species (Sommer *et al.* 1986). Further growth of the large herbivore population (*Daphnia*) is partly suppressed by cyanophyte colony interference to efficient food collection. An autumn peak in zooplankton biomass composed mainly of small herbivore species occurs in Hartbeespoort Dam (Figures 2.0b and 2.1) as described by the PEG-model. However, a pronounced or prolonged winter period of low food resources, low zooplankton fecundity and diapause does not occur in this subtropical impoundment.

The seasonal succession of zooplankton viewed both in relation to food resources and the *in situ* feeding studies using chlorophyte and cyanophyte foods, provide insight into the likely outcome of biomanipulation strategies in Hartbeespoort Dam. A primary goal of any biomanipulation programme in this hypertrophic impoundment would be a reduction in the frequency and magnitude of phytoplankton blooms; a symptom of excessive enrichment most often causing concern to a variety of lakeshore and water users.

Biomanipulation studies have indicated that phytoplankton control by increased zooplankton grazing can be achieved by reducing or eliminating zooplanktivorous fish stocks (directly via poisoning or indirectly via piscivorous fish; Shapiro 1980a, 1980b, Lynch and Shapiro 1980, Shapiro and Wright 1984). Recently, Carlson and Schoenberg (1983) and Schoenberg and Carlson (1984) have suggested that both direct grazing by large-bodied herbivores, such as *Daphnia*, and the indirect effects of intensive grazing on abiotic factors (pH, transparency and nutrients) can control phytoplankton levels and even cyanophyte blooms in a hypertrophic lake. They cited conflicting evidence from a number of studies using various foods to support the view that zooplankton can graze effectively on colonial cyanophytes such as *Microcystis*. Whilst Schoenberg and Carlson (1984) reported that *Daphnia* could ingest *Microcystis* colonies >20 µm they also noted a slight increase in the mean diameter of remaining colonies, indicating selection for small colonies in their laboratory experiments. In combination with indirect effects of grazing on the phytoplankton, Schoenberg and Carlson (1984) suggested that large herbivores such as *Daphnia* could retard the development of cyanophyte dominance. However, these authors expressed caution regarding the extrapolation of small scale enclosure and laboratory experiments to reliably represent responses within an entire lake.

Data from Hartbeespoort Dam indicate that predation by zooplanktivorous fish is not of paramount importance in controlling zooplankton standing stocks and so has less potential for

successful use in biomanipulation within this impoundment. The *in situ* feeding study on *Microcystis* colonies in Hartbeespoort Dam extends the results of Schoenberg and Carlson (1984) and others (see Section 4) and identifies upper colony size limits to grazing by a number of zooplankton species. But, when viewed against the successional events occurring in Hartbeespoort Dam, the results do not support the hypothesis that zooplankton grazing can control cyanophyte blooms. As stated above, very high grazing rates in spring have the potential to limit the phytoplankton standing crop (clear-water phase) to the level where some food limitation to fecundity and growth of the dominant grazer becomes evident. Nevertheless, the generally low filtration rates and filtration efficiencies of most cladocerans on both large and small *Microcystis* colonies, and the suppressed filtration rates of *Daphnia* in the presence of abundant large *Microcystis* colonies, show that zooplankton grazing is not able to suppress or control *Microcystis* blooms in hypertrophic Hartbeespoort Dam.

An additional aspect of the feeding study on Hartbeespoort Dam that is of potential value to any future lake restoration programme is the impact that high zooplankton densities in spring can have on the phytoplankton, if this is composed mainly of edible algal species. Lake restoration strategies that are able to produce a pronounced and prolonged shift in phytoplankton species composition away from conditions favouring *Microcystis* and towards forms more palatable to the zooplankton community (e.g. combination of artificial destratification and vertical mixing, nutrient load reduction and N:P ratio manipulations) can lead, through intensive zooplankton grazing pressure, to a marked lowering of algal biomass. But, since the majority of filter-feeders select particles <20 µm in diameter, the zooplankton cannot completely remove all particles from the water. Therefore, the extent to which this process of selective feeding on preferred algal foods can promote the return of more 'resistant' algae or cyanophytes, is not easy to assess. The information presented here on feeding rates under hypertrophic conditions, the impact of zooplankton grazers on the phytoplankton and *vice versa*,

cannot accurately predict plankton producer-consumer levels if used in isolation, but can contribute to the validity of such predictions if used as part of larger lake ecosystem models.

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8. APPENDIX

Published manuscripts arising from this study (up to December 1987):

(i) Manuscripts directly related to this thesis:

Jarvis, A.C. (1986) Zooplankton community grazing in a hypertrophic lake (Hartbeespoort Dam, South Africa). J. Plankton Res. 8: 1065-1078.

Jarvis, A.C., Hart, R.C. and Combrink, S. (1987) Zooplankton feeding on size fractionated *Microcystis* colonies and *Chlorella* in a hypertrophic lake (Hartbeespoort Dam, South Africa): implications to resource utilization and zooplankton succession. J. Plankton Res. 9: 1231-1249.

Jarvis, A.C., Hart, R.C. and Combrink, S. Cladoceran filtration rate-body length relations: model improvements developed for a *Microcystis* dominated hypertrophic reservoir. J. Plankton Res. 10 (in press).

(ii) Manuscripts partially related to this thesis, using limited data, information or methods described herein:

Cochrane, K.L., Ashton, P.J., Jarvis, A.C., Twinch, A.J. and Zohary, T. (1987). An ecosystem model of phosphorus cycling in a warm monomictic hypertrophic impoundment. Ecol. Modelling 37: 207-233.

Grobbelaar, J.U., Jarvis, A.C., Robarts, R.D., Sephton, L.M., Steenkamp, M. and Cawood, M.E. (1987) A diel study of carbon flow in the pelagic zone of a small lava-lakelet on Marion Island (Sub-Antarctic). Polar Biol. 7: 115-124.

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Thornton, J.A., Cochrane, K.L., Jarvis, A.C., Zohary, T., Robarts, R.D. and Chutter, F.M. (1986). An evaluation of management aspects of a hypertrophic African impoundment. Water Res. 20: 413-419.