Effects of land use on pelagic food webs in a range of Otago wetlands

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Abstract

Inputs of nutrients and organic matter to wetlands from catchments influence water quality, which, in turn, determines the potential productivity of a wetland. While there are many studies of the effects of catchment development on water quality and aquatic communities in lakes, few studies include other wetlands. As these other wetlands are likely to be more affected by allochthonous inputs, littoral vegetation and autochthonous generation of organic matter, my aim was to determine the major influences on pelagic communities in a range of wetland systems.

Influences of catchment land use on water quality and the pelagic food web were examined in 45 wetlands representative of a range of wetland environments in Otago, including swamps and ponds, shallow lakes, riverine wetlands, estuaries, reservoirs and deep lakes. The pelagic zones of 40 wetland sites were sampled once in February-March 1999 (autumn), 15 were re-sampled in October 1999 (spring), when five additional sites were also sampled. Catchment variables included size and slope, wetland size and the percentage of land in the catchment in bare ground, indigenous forest, inland water, inland wetlands, planted forest, pasture, scrub, tussock, urban or urban open space. Water quality variables, or physicochemical measurements, included total phosphorus, total nitrogen, total dissolved phosphorus, total inorganic nitrogen, dissolved organic carbon, turbidity, Secchi depth, total suspended solids, water colour, chlorophyll *a*, pH, temperature and conductivity. The pelagic food web was sampled, including biomass, abundance, and identification to genus of phytoplankton, biomasses of picophytoplankton, heterotrophic bacteria and nanoflagellates, biomass and identification to genus or dominant group of ciliates, and abundance and identification to species of crustacean zooplankton. Relationships among catchment variables, water quality variables and the pelagic food web were determined using multivariate analysis and correlation analysis.

Increased development of pasture, exotic forestry and urbanisation in a catchment had negative effects on water quality, in comparison to unmodified catchments containing native vegetation communities. In turn, the biomass and composition of the pelagic community

related closely to catchment modification, via physicochemical attributes of the wetland. Deep lakes were the most oligotrophic wetlands and swamps and ponds were the most eutrophic.

Picophytoplankton and the cladoceran, *Bosmina meridionalis* were related positively to unmodified catchments, low trophic status of a wetland and deep lakes. Other components of the microbial food web, phytoplankton, copepods and *Daphnia carinata* were linked hierarchically to more intensive land use in the catchment, higher wetland trophy, swamps and ponds. A ciliate genus, *Urocentrum,* appeared to be detrimentally affected, and phytoplankton diversity reduced, by wetland catchment development and increases in wetland trophy.

Components of the pelagic food web were tightly correlated across adjacent trophic levels. Heterotrophic bacteria appeared to be a resource for heterotrophic nanoflagellates. Picophytoplankton populations might either be suppressed by ciliate grazing, or detrimentally affected by eutrophication. Small ciliates appear to consume other microbial food web components, while larger ciliates may depend more on phytoplankton. Copepods may be relying on consumption of ciliates. Cladocerans did not appear to depend on this resource to the same extent as copepods. Populations of *B. meridionalis* and *Ceriodaphnia dubia* were negatively related to the larger cladoceran, *D. carinata*.

Seasonal effects were apparent only at the level of zooplankton, the highest trophic level studied. *B. meridionalis* and *C. dubia* were more abundant in autumn than spring, while the reverse was true of *D. carinata*.

This study revealed relationships within pelagic food webs in a range of wetland systems. While resource supply appeared to be the foundation of relationships between aquatic organisms, top-down effects of predation in the food web could not be dismissed. The potential of organisms such as picophytoplankton, ciliates and phytoplankton to be indicators of aquatic ecosystem health has been revealed or strengthened by this study. This research provides evidence of the influence of land use and geographical features on water quality and pelagic communities of wetlands in Otago.

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List of Acronyms

Acronym	*Description*
ANOVA	Analysis of variance
CPUE	Catch per unit effort
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DRP	Dissolved reactive phosphorus
EP	Eukaryotic picophytoplankton
HNF	Heterotrophic nanoflagellates
PCA	Principal components analysis
PNF	Photosynthetic nanoflagellates
POM	Particulate organic matter
PP	Prokaryotic picophytoplankton
RDA	Redundancy analysis
TCCB	Total ciliate carbon biomass
TIN	Total inorganic nitrogen
TMCB	Total microbial carbon biomass
TN	Total nitrogen
TSS	Total suspended solids
TP	Total phosphorus

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Chapter 1 Introduction

Wetlands

Wetlands are important habitats, rich in wildlife and vegetation. Wetlands can improve water quality by removing pollutants from inflowing water. Important in preventing flooding, they may also act as carbon dioxide sinks and climate stabilisers. Recreation and education are also valuable roles of wetlands. However, wetlands are threatened by alteration by humans, pollution and climate change (Mitsch and Gosselink 2000).

As in the rest of the world, wetlands are among New Zealand's most threatened ecosystems, with little information available on their state, and subject to unsustainable management practices (Ward and Lambie 1998). New Zealand wetlands include rivers, bogs, swamps, estuaries, lagoons and lakes, with each type supporting unique aquatic and terrestrial wetland communities (Cromarty and Scott 1995). Wetland areas in New Zealand have been reduced by 85-90% since European settlement in the mid 19th century (Ogle and Cheyne 1981). Several thousand wetlands remain, but most are only a few hectares in size and many have been degraded by drainage, pollution, animal grazing and introduced plants (Stephenson 1983).

Threats to wetlands

Despite an increased understanding of the need to protect the high biodiversity and conservation values of wetlands in New Zealand, they are still under threat.

Modification

Wetlands are still being drained or modified in New Zealand to create land for farming and urban development, in hydro-electric schemes, by sand and gravel extraction for the construction industry, for flood control measures near rivers, in sewage treatment schemes, and, for developments for marine recreation (Elliot and Sorrell 2002, Cromarty and Scott 1995).

Pollution

Agriculture extends over half of New Zealand's land area and dominates middle and lower catchments of many streams and rivers. Runoff from land contains organic matter, nutrients and sediment, and runoff from agricultural land in particular includes faecal matter, nutrients from animal waste and fertilisers, and high sediment loads from catchment slopes that have been converted from native forest or tussock grasslands to pasture. Urban runoff contributes further nutrients and also heavy metals and toxic contaminants to wetlands. Point source pollution to wetlands is primarily from meat works and dairy sheds, but also industries, rubbish dumps and contaminated waste sites (Elliot and Sorrell 2002).

Exotic weeds

Introduced species such as broom, lupin, gorse, willow, and *Spartina* (seagrass) are also modifying wetland communities in New Zealand (Cromarty and Scott 1995), out competing native species, clogging waterways and destroying the beneficial functions of wetlands.

Climate change

Hydrologic parameters control wetland structure and function. The source, renewal rate and timing of the water regime directly control abiotic factors such as nutrient status, soil anaerobiosis, and salinity which will determine wetland ecosystem structure and function (Mitsch and Gosselink 1993). Temperature determines the rates of primary productivity, microbial productivity and evapotranspiration of wetlands (Öquist and Svensson 1997). The hydrology of a wetland is influenced by the topography, geology, soils and area of the catchment, and especially, by climate.

Climate change may have serious consequences for wetland systems in New Zealand in the coming decades. Rises in sea level may flood tidal wetlands with saline water and also increase salinity in non tidal wetlands. Coastal and inland wetlands alike will experience the alteration of hydrologic regimes by changes in precipitation, which will affect the biological, biogeochemical and hydrological functions of the wetland (Öquist and Svensson 1997). In New Zealand, climate change models predict that precipitation will increase in the west coast and decrease in the east coast of New Zealand, with warming of 0.5–2.0°C in inland Canterbury and Otago and 0.5–1.5°C elsewhere by 2030 (Watson *et al.* 1997).

The possible consequences of these threats to wetland communities need to be better understood to enable wetland managers to mitigate further damage.

Ecology of wetlands

Aquatic ecosystems are not closed, but are intimately linked to the adjacent terrestrial and atmospheric systems (Odum 1989). In order to understand aquatic systems, it is vital to appreciate the importance of the adjoining terrestrial and atmospheric systems.

The land surrounding a lake or wetland is its catchment (also known as watershed or drainage basin). Apart from direct precipitation to the water body, water inputs to a lake system are transported to the lake or wetland from the catchment via runoff, groundwater and streams. During the water's journey from the atmosphere to the streams the catchment will supply or subtract nutrients and matter (Moss 1988). The composition and quantity of elements in the water will be a function of the geological features, climate and land use of a catchment. Specifically, factors such as slope, precipitation, wind, temperature, erosion, vegetation and soil structure all play a role in the catchment water quality (Schindler 1997).

Inputs of nutrients and organic matter from the catchment will influence the physicochemical conditions in the wetland (Wetzel 1995, Thomas 1997), and nutrients and substrates will, in turn, determine the potential productivity of an aquatic system (Kitchell and Carpenter 1993).

Energy flow in aquatic systems

> "The extent to which physical-chemical or biotic factors influence community structure and ecosystem function continues as one of the fundamental issues of ecology."
>
> Kitchell and Carpenter (1993)

Ecologists and ecosystem managers need to predict the effects that human activities may have on a wetland system. They must define key features of ecosystems that enable them to understand, measure and explore their dynamic variation (Carpenter and Pace 1997). Determining the response of the food web to environmental variables is essential to understanding the functioning of aquatic systems.

Within aquatic systems, inorganic nutrients and carbon inputs set the potential productivity, and deviations from that potential are due to predator prey relationships in the food web (Kitchell and Carpenter 1993). Energy will flow upward through the food chain,

from inorganic nutrients and dissolved organic carbon at the base, while predator-prey interactions at the higher trophic levels will cascade downward and influence community structure at lower levels (Fig. 2.1).

The energy flow and trophic cascade functioning is apparent from many studies of the classical food chain, where inorganic nutrients and carbon supply determine the energy supplied to phytoplankton, which in turn will limit zooplankton growth. Alternatively, or simultaneously, we have the cascading effect of zooplankton predation limiting phytoplankton abundance. However, bacteria, flagellates and ciliates (the "microbial loop" or microbial food web) in the aquatic system may or may not account for a major portion of secondary production and nutrient remineralization (Pace 1991).

The microbial loop in aquatic systems may provide nutrients and carbon to higher trophic levels (acting as a source of resources), or alternatively, it may utilise nutrients otherwise used by, and thus limit resources for, the classical food chain, with production then lost to respiration and the detrital pool (acting as a sink for resources) (Sherr and Sherr 1991). The relative importance of the microbial portion of the community to energy transfer will vary among aquatic ecosystems (Gasol and Duarte 2000, Weisse 1991b, Pace and Funke 1991). To understand the impact on aquatic systems of a), trophic cascades from zooplankton predation and b), energy flow from inputs to systems, managers of aquatic systems must be able to determine the functioning of the microbial food web under various conditions.

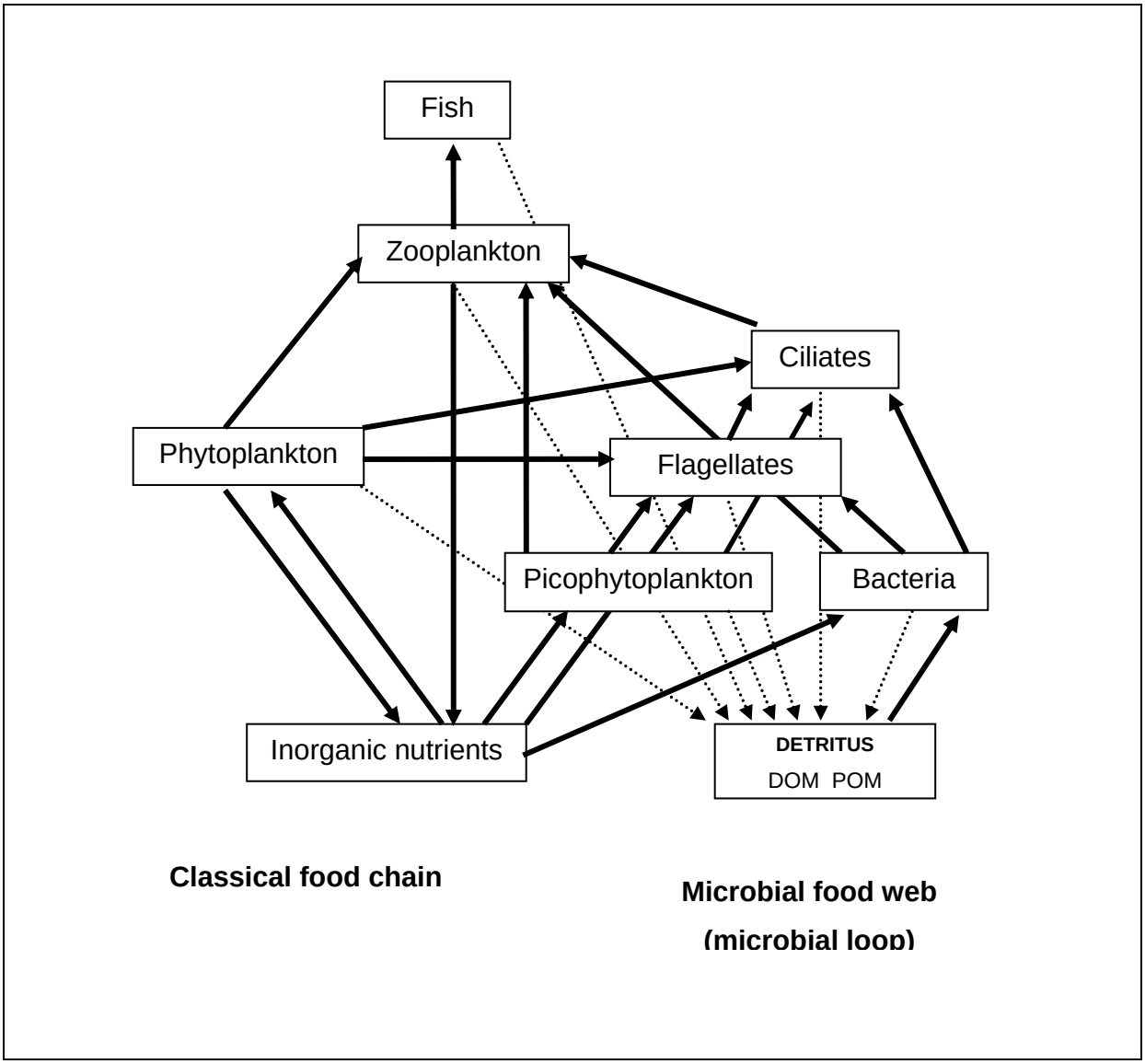


Figure 1.1 The classical food chain and the microbial food web and pathways of energy transfer. DOM is dissolved organic matter; POM is particulate organic matter. Solid lines indicate relationships of resource supply and consumption/predation, while dotted lines indicate the death-to-detritus pathways.

Microbial processes in wetlands

In aquatic systems, pelagic microbial food webs are complex, interacting communities that collectively account for primary production, nutrient regeneration and production to support zooplankton (Sherr and Sherr 1991). Understanding what influences microbial food web structure and functioning may help to detect and prevent environmental degradation of aquatic ecosystems (Munawar *et al* 1989).

The microbial food web paradigm was developed in the pelagic region of deep lakes and oceans. However, microbial food web structure in other wetlands differs from that in open water. Other wetlands have variable hydrological inputs, higher organic matter concentrations, and greater recycling and rates of utilisation of mineralised nutrients (Wetzel 1992). Whereas many studies of microbial food webs have been conducted in lakes, few studies focus on other wetlands (Prepas *et al.* 2001). There is a need to determine how aquatic microbial communities function in wetland systems.

Comparative studies

Whereas experimental studies to determine the functioning of microbial food web components in response to nutrients (bottom up) and predation (top down) allow us to determine potential productivity of the microbial food web under certain ranges of nutrients and predation, comparative studies sample aquatic systems to "capture" a wide range of nutrient and predation levels in order to determine the function of the microbial food web. In a comparative study, the sampling may be spatially defined (for example, sampling differing sites in the same system, or; different aquatic systems), or temporally defined (for example, sampling the same site over a period of time).

A comparative study is a 'snapshot' of the community, and measurement of the range of conditions at the time of sampling may not reflect the nutrient supply or predation level that resulted in the abundances of (fast growing) microbial food web components, and/or the response of the ecosystem to nutrients and predators, or spurious relationships may arise due to the relationship of both variables with a third variable not included in the study (Gasol and Duarte 2000).

Thus, the relationships revealed among elements of aquatic systems by single 'snapshot' population surveys cannot be statistically proven (Jager and Looman 1987). However, a comparative study may reveal relationships between the abundance of microbial

food web components and resources (or predation), and/or confirm that relationships determined by experimental studies are accurately describing the role of the microbial food web in the aquatic systems (Gasol and Duarte 2000, Kitchell and Carpenter 1993). While it is difficult to detect dynamics and infer responses from comparisons, they do reveal the range of ecosystem states possible, and relationships among key variables (Kitchell and Carpenter 1993).

Aim of this study

The overall aim of my study was to compare pelagic communities in a diverse range of wetlands, in catchments differing in geographic features and development, with the objective of relating community composition and biomass to land and water management practices and climatic effects.

My aim was to compare wetlands in Otago, New Zealand, by determining relationships between geographical variables (land cover, catchment size and slope wetland size), physicochemical variables (nutrients, substrates, temperature, conductivity and pH) and the biological community (the microbial food web, phytoplankton and zooplankton). The study included swamps and ponds, estuaries, riverine wetlands, reservoirs, shallow lakes and deep lakes. The diversity of types of wetland permitted a comparison of communities along gradients including catchment land use, wetland size, trophic state, season and salinity, to provide insight into the consequences for wetlands of land management practices and environmental perturbations.

Objectives

My study had five objectives:

1. To determine effects of geographical variables, wetland type and season on physicochemical variables in wetlands.

2. To determine effects of geographical variables, physicochemical variables, wetland type and season on the microbial food web in wetlands.

3. To determine effects of geographical variables, physicochemical variables, resource supply (the microbial food web and phytoplankton), predation (zooplankton), wetland type and season on ciliate community structure in wetlands.

4. To determine effects of geographical variables, physicochemical variables, predation (the microbial food web and zooplankton), wetland type and season on phytoplankton community structure in wetlands.

5. To determine effects of geographical variables, physicochemical variables, resource supply (the microbial food web and phytoplankton), wetland type and season on zooplankton community structure in wetlands.

Chapter 2 Methods

Sampling was conducted in 45 wetlands in Otago, New Zealand (Fig. 2.1) (Appendix I). The region sampled was approximately 32,000 km^2. Sampling was conducted in February-March 1999 (autumn) and October 1999 (spring), giving a total of 60 sampling sessions or sites. Water was sampled using a tube sampler to collect depth-integrated samples. Samples were collected at, or near, the centre of the water body between 0800 h and 1600 h. The entire water column was sampled in lakes up to 2 m deep, in deeper lakes the euphotic zone was sampled.

Wetland type

Wetland types included swamps, riverine wetlands, estuaries, reservoirs, shallow lakes and deep lakes (Table 2.1) (Appendix III). Wetland types were determined by several factors: "deep lakes" had a maximum depth greater than 5m; "estuaries" were primarily freshwater in terms of salinity, but had some physical connection to the sea; in "reservoirs" the outflows were manipulated; "riverine wetlands" were physically linked with an adjacent river, "shallow lakes" had a maximum depth less than 2 m; "swamps or ponds" were water bodies with a surface area of less than 1 ha. and/or emergent macrophytes across the bottom.

Table 2.1 Wetland type, and number of sites sampled of each wetland type.

Wetland Type	Number of sites
Deep lake	14
Estuarine	8
Reservoir	15
Riverine wetland	3
Shallow lake	10
Swamp/pond	10

Season

Season was determined by date of sampling (autumn or spring). Forty wetland sites were sampled once in February-March 1999 (autumn), 15 were re-sampled in October 1999 (spring), when five additional sites were also sampled (Table 2.2).

Table 2.2 Season, and number of sites sampled in each season

Season	Number of sites
Autumn	40
Spring	20

Land cover

The percentage of land cover in each catchment was divided into the following categories: bare ground, indigenous forest, inland water, inland wetlands, planted forest, pasture, scrub, tussock, urban and urban open space. Catchment land cover data was derived from a digital version of the Vegetative Cover of New Zealand (Newsome 1987) using Arcview GIS software. Catchments of interest catchments were digitised using 1:50,000 topographic data (primarily contour maps). The boundaries and land cover for smaller catchments were further defined and classified by referring to 1:25,000 scale colour aerial photographs. Once all layers had been checked for accuracy, catchments were used to calculate the percentage of land cover present per catchment.

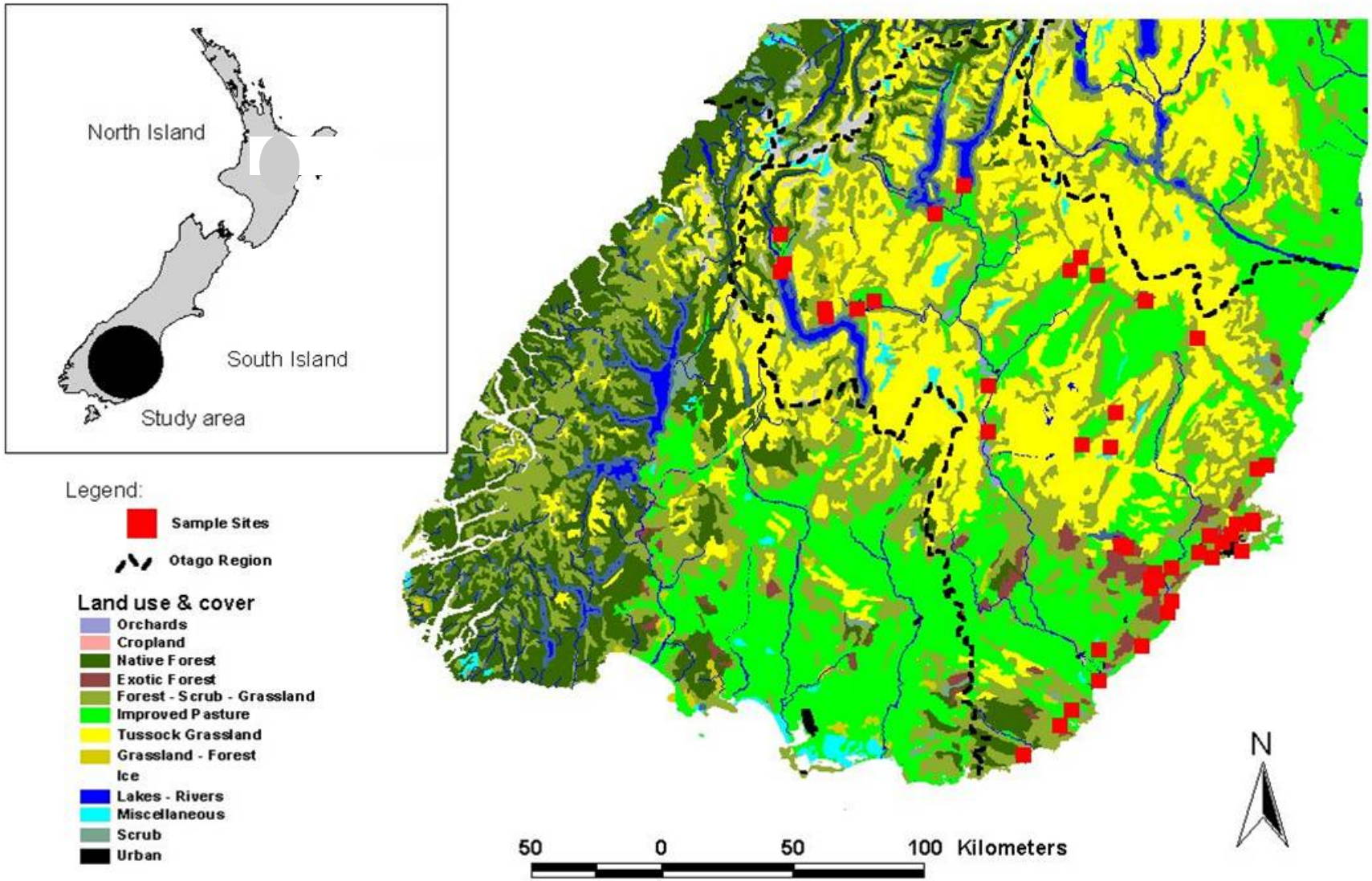


Figure 2.1 Map of Otago, New Zealand, showing study sites.

Temperature, oxygen, turbidity, water colour, total suspended solids, pH, Secchi depth, conductivity.

Temperature was measured 30 cm below the surface of the water body using a mercury thermometer (± 0.2^{o}C). Turbidity was measured using a Hach turbidimeter (model 2100A). Water colour (also known as gilvin or gelbstoff) was measured spectrophotometrically as absorbance at 440 nm on water samples filtered though 0.2 μm pore size pre-rinsed, Millipore mixed cellulose ester filters and converted to standard absorbance coefficient (Cuthbert and Del Giorgio 1992). Total suspended solids were measured gravimetrically, with samples filtered through pre-rinsed, pre-combusted Whatman GF/F glass-fibre filters, which were then re-weighed after drying for 24 h at 60^{o}C. pH was measured using a PHM 62 standard pH meter. A 20 cm diameter black and white disk was used to measure Secchi depth. Conductivity was measured with a HI 9033 multi-range conductivity meter.

Nutrients

Concentrations of phosphorus and nitrogen were determined spectrophotometrically in filtered samples using a Chemlab System 4 autoanalyser, as described in Wetzel and Likens (1991). Dissolved reactive phosphorus (DRP) was analysed using the antimony-ascorbate-molybdate method. Ammonium-N (NH_4-N) was measured by the phenol-hypochlorite method. Nitrate-N (NO_3-N) was measured after reduction to NO_2-N in a cadmium-copper column. Total nitrogen (TN) and total phosphorus (TP) were measured after oxidation in the presence of boric acid and sodium hydroxide (Valderra 1981). The detection limits for the nutrients were DRP - 0.5 mg m^{-3}; TP - 0.6 mg m^{-3}, NH_4-N - 0.6 mg m^{-3}; NO_3-N - 1.6 mg m^{-3}, TN - 6.0 mg m^{-3}.

Dissolved organic carbon

Samples for dissolved organic carbon (DOC) were collected, filtered using GF/F filters, and frozen until analysis by gas chromatography, using an adaptation of the persulphate oxidation method (Menzel and Vaccaro 1964). Samples of filtrate (15 ml) were added to vials containing 200 mg potassium persulfate and 0.75 ml of 3% phosphoric acid, and oxygen was bubbled through the sample for 5 minutes to remove any inorganic carbon. The glass vials were sealed with open topped screw caps fitted with teflon-lined silicone septa, and autoclaved for 40 minutes at 121-130^{o}C. The sample was removed from the vials using two 60 ml

syringes; 60 ml of nitrogen gas was inserted with one syringe, while the sample and headspace were removed with the other. The sample in the syringe was shaken for 1 minute and the gas from the syringe was injected into a Hewlett Packard 5890 Series II gas chromatograph, and the DOC was determined from glucose standards (Young and Huryn 1999).

Chlorophyll *a* and Phaeophytin

Water samples of up to 500 ml for each lake were collected for chlorophyll *a* analysis. Water was filtered through a 300 μm mesh to remove larger zooplankton which may ingest and degrade algae. Phytoplankton was collected on GF/F filters. The filters were kept desiccated and frozen in the dark at -15^{o}C until analysis. The samples were thawed in the dark for analysis and placed in a test tube with 10 ml of 90% alkaline acetone. They were left for 16-24 h in the dark. The samples were then analysed using a Sequoia-Turner fluorometer (Model 450) that was calibrated using a coproporphyrin secondary standard which was periodically calibrated to purified chlorophyll *a*. The fluorescence of each sample was measured both before and after the addition of two drops of 4N hydrochloric acid, to degrade the chlorophyll *a* to phaeophytin. Chlorophyll *a* and phaeophytin concentrations were calculated from these fluorescence values using the following equations:

$$[\text{chl}a\ (\mu g\ L^{-1})] = Kc * (R/(R-1)) * (Fb-Fa) * v/V \quad (1)$$

$$[\text{phaeo}\ (\mu g\ L^{-1})] = Kc * (R/(R-1)) * ((R * Fa-Fb) * v/V \quad (2)$$

where Kc is the chlorophyll calibration coefficient (1.329), R is the theoretical acidity ratio (12), Fb is the fluorescence before acidification, Fa is the fluorescence after acidification, v is the volume (ml) of acetone used in extraction, and V is volume (ml) of water filtered.

Bacteria

Water for bacteria samples was preserved with ice-cold 2% glutaraldehyde. Subsamples of 2 ml of water for bacteria samples were filtered on to black 0.2 μm pore size polycarbonate filters soon after sampling. The filters were then air-dried in a covered petri dish, in the dark. When ready to analyse, filters were rehydrated with 2 ml of filtered milliQ water, stained with DAPI (a DNA- specific fluorochrome stain) at 1 μg ml^{-1} final concentration. Filters were then mounted on microscopes slides with immersion oil. Fluorescence microscopy was used to count the bacteria with the ultraviolet excitation filter set (BP365/11/FT395/LP397). Bacteria

were counted at 1000x magnification with a Zeiss Axiophot epifluorescence microscope. Two replicate filters of each sample were counted. At least 400 cells were counted per filter (Appendix II).

Picophytoplankton

Water for picophytoplankton samples was preserved with ice-cold 2% glutaraldehyde. Within 48 h of collection subsamples of between 1.5 to 10 ml were filtered on to black 0.2 µm pore size polycarbonate filters. The filters were then frozen. Within 6 weeks they were thawed and the cells counted under 1000x magnification with a Zeiss Axiophot epifluorescence microscope. Fluorescence microscopy was used to count the cells with the green excitation filter set (BP546/12/FT580/LP590). The numbers of picophytoplankton per sample were determined by counting the cells in 20 microscope eyepiece grids, half grids, quarter grids or fields per slide. Up to 400 cells were counted per filter, or 20 fields in some low density samples. With the green filter set, prokaryotic (cyanobacterial) picophytoplankton (PP) fluoresced bright orange, and eukaryotic picophytoplankton (EP) fluoresced dull red (Weisse and Kenter 1991).

Flagellates

Water for flagellate samples was preserved with ice-cold 2% glutaraldehyde. Within 48 h of collection subsamples of 5 or 10 ml were filtered on to black 0.8 µm pore size polycarbonate filters, and stained with DAPI (1 µg ml^{-1} final concentration). The filters were then frozen. Within six weeks they were thawed and the cells were counted under 1000x magnification with a Zeiss Axiophot epifluorescence microscope. Fluorescence microscopy was used to count the cells with the ultraviolet excitation filter set (as for bacteria) for heterotrophic nanoflagellates (HNF) and the blue excitation (BP460-490/FT510/LP520) for photosynthetic nanoflagellates (PNF). The numbers of flagellates per sample were determined by counting the flagellates in 20 microscope eyepiece grids, half grids, quarter grids or fields per slide, with an average of 137 flagellates per slide counted.

Ciliates

Water samples of 500 ml were preserved with 1% Lugol's iodine for ciliates. Subsamples of measured volume were concentrated by sedimentation and the organisms were counted under

an inverted Nikon microscope at x200 magnification. Ciliate taxa were identified by reference to Streble and Krauter (1988) and Foissner and Berger (1996).

Phytoplankton

Water samples of 500 ml were preserved with 1% Lugol's iodine for phytoplankton. Subsamples of measured volume were concentrated by sedimentation and the organisms were counted under an inverted Nikon microscope at x400 magnification. Phytoplankton was identified to the genus level by reference to Streble and Krauter (1988), Canter-Lund and Lund (1995) and Moore (2000).

Zooplankton

Water samples for zooplankton were collected using the column sampler, passed through 150 μm mesh, back-rinsed using distilled water into a plastic container, and preserved with 4% buffered formalin. Zooplankton were counted using a dissecting microscope at 40x magnification. Each sample was made up to 200 ml with distilled water, mixed by shaking, and a 5 ml subsample was taken using a wide mouth pipette. The animals in up to 5 subsamples were counted, with the numbers of *Daphnia, Ceriodaphnia, Bosmina*, adult copepods, copepodites, and nauplii recorded. Zooplankton was identified to Order or Genus level only, owing to high taxonomic diversity of the samples (Chapman and Lewis 1976). Rotifers were not included in the study.

Biomass

Biomass of plankton was expressed in units of carbon (C, in $\mu g\ L^{-1}$) derived from chlorophyll *a* concentrations, cell densities and cell volumes of protozoa (based on measurements of cells and geometric formulae to determine volume (Hoehn *et al*. 1998)) and published conversion factors. These conversion factors were: phytoplankton = chlorophyll *a* ($\mu g\ L^{-1}$) x 50 (Strickland and Parsons 1972); heterotrophic bacteria = 20 fg C $cell^{-1}$ (Li *et al*. 1992); PP = 250 fg C $cell^{-1}$ (Li *et al*. 1992); flagellates = 0.4 pg dwt μm^{-3} (Borsheim and Bratbak 1987) and ciliates = 0.19 pg dwt μm^{-3} (Putt and Stoecker 1989).

Data analysis

$Log_{(10)}$ and $Log_{(10)}(x+1)$ transformations were used as the data were not normally distributed. SPSS (v. 10.1) software was used to perform statistical analyses of variance (ANOVA) and Pearson's correlation analysis. CANOCO (v. 4.0) software was used to conduct multivariate analyses.

Chapter 3 Land use

Introduction

Apart from direct precipitation to a water body, water inputs to a lake system are transported to the lake or wetland from the catchment via runoff, groundwater and streams. During the water's journey from the atmosphere to the streams the catchment will supply or subtract nutrients and matter (Moss 1988). The composition and quantity of elements in the water will be a function of the geological features, climate and land use of a catchment. Specifically, factors such as, catchment size, slope, precipitation, wind, temperature, erosion, vegetation and soil structure all play a role in the catchment water quality (Schindler 1997). Larger catchments will generally be associated with larger water bodies. Steep catchments will allow precipitation to reach the water body faster, meaning there is less time for water to dissolve nutrients and matter from the soil and, in addition, there is less chance of wetlands on steep catchments which could supply dissolved organic matter and nutrients to runoff (Rasmussen *et al*. 1989). From a data set of 337 North American lakes, Rasmussen *et al*. (1989) discovered that small, shallow lakes with larger, low sloped catchments containing wetlands are likely to be darker coloured than large, deep lakes with steep sloping, smaller catchments. Climatic factors affect the quantity and composition of elements within the water by processes such as precipitation input rate, erosion and evapotranspiration (Schindler 1997). Vegetation both provides elements to, and extracts elements from the water (Wetzel 1995), and vegetation (land cover) will be dependent on the land use (or land management) of the catchment. Land management of the catchment (agriculture, forestry, horticulture, conservation, industry and urban areas) influences the quality of water that enters the aquatic system (Moss 1988). Agricultural practices such as land clearance, irrigation, drainage, pesticide use, soil enrichment and animal waste will have consequences for the quality and quantity of water in the catchment (Elliott and Sorrell 2002, Moss 1988). Toxic substances are higher in runoff from urban and industrial areas than from undeveloped areas (Mitsch and Gosselink 1993). In 30 upland lakes in the UK, Maberly *et al.* (2003) found that lake water chemistry was strongly affected by land cover in the catchment, with phosphorus limitation positively related to

bracken cover, and negatively related to pasture cover, while nitrogen limitation was positively related to marsh, rough grass and woodland, and negatively related to rough pasture, shrub heath and bare ground. In 62 coastal and inland wetlands of the Great Lakes basin, Lougheed *et al*. (2001) determined that agricultural catchments contributed a higher proportion of inorganic sediment and nutrients than forested catchments. In New Zealand, Thompson and Townsend (1998) found that pastoral vegetation communities in Otago contributed higher concentrations of nitrogen and phosphorus to the water than native tussock grassland communities. Townsend *et al*. (2001) reported that concentrations of phosphate and nitrate were higher in streams with greater catchment development (sheep farming), than in streams in ungrazed tussock catchments. Fahey (1994) discovered that plantation forestry in New Zealand reduced evapotranspiration and water yield to wetlands compared to other land uses. Urbanisation of catchments was shown to increase runoff (McConchie 1992). Young and Huryn (1999) revealed that forested sites release more organic matter into New Zealand streams than pasture. Based on studies in the North Island of New Zealand, Elliott and Sorrell (2002) reported summaries of nitrogen and phosphorus export coefficients for catchments of varying land uses, which revealed that nitrogen supply was greatest from dairy farming, then hill pasture (sheep and cattle) and urban catchments, with a lower level of nitrogen supply from catchments of low intensity pastoral grazing, native bush and scrub, and pine forestry. Phosphorus supply was highest in catchments of hill pasture (due to greater erosion), then dairy farming, followed by low intensity pastoral grazing, urban development and forestry.

Inputs of nutrients and organic matter from the catchment will determine the physicochemical conditions in the wetland. Wetzel (1992) states that the drainage basin often provides the dominant loading of organic matter to streams and rivers, rather than that from phytoplankton within the system. However, the physicochemical conditions in the wetland will depend greatly on the size and type of wetland. Hydrological inputs, organic matter concentrations, and mineralised nutrient recycling and rates of utilisation will vary considerably among wetland types (Wetzel 1992). Small water bodies are much more affected by littoral vegetation and riparian influences than larger ones. Water residence time in the water body will also affect the concentrations of chemical variables via a number of biological processes such as synthesis or polymerisation from existing organic matter, degradation of organic matter and release from living and dead organisms (Thomas 1997). Precipitation directly to the lake surface will dilute concentrations of chemical variables and small, highly

productive wetlands may generate organic matter (Rasmussen *et al*. 1989). Thomas (1997) determined that the relative importance of allochthonous sources of dissolved organic matter to a system would decrease with increasing lake size. In turn, the inorganic nutrient and carbon concentrations within aquatic systems set the potential productivity of the system (Kitchell and Carpenter 1993). Via protozoa and zooplankton, this primary production provides energy for higher trophic levels (Porter 1995).

The aims of this component of my study were to measure physicochemical variables in 45 wetlands representative of a range of wetland environments in Otago in order to determine relationships between these variables and geographical variables (land cover, catchment area, water body area and slope).

I predicted that measures of physicochemical variables would correlate positively with: a) catchment modification (land cover greater in pasture, planted forest, scrub and urban areas, rather than bare ground, water, indigenous forest, and tussock); b) smaller catchments; c) less slope in the catchment; and d) smaller wetland area. I predicted also that mean measures of physicochemical variables would: e) increase along a gradient of wetland types ranging from deep lakes to swamps and ponds; and f) change between autumn and spring.

Methods

Refer to Methods (chapter 2, page 9)

Data analysis

Measures of physicochemical variables were $\log_{(10)}$ transformed as the data were not normally distributed. Several multivariate analyses were carried out: detrended correspondence analysis, redundancy analysis, and principal components analysis were performed using CANOCO (v. 4.0) software. Redundancy analysis was used as a detrended correspondence analysis determined that gradient lengths were less than 4 standard deviations (ter Braak and Šmilauer 1998). The influence of geographical variables on measures of physicochemical variables was investigated using redundancy analysis, with forward selection to assess the statistical significance of independent variables, and Pearson's correlation analysis to examine direct relationships. Differences in wetland types and seasons were examined using principal components analysis. ANOVA and Pearson's correlation analysis were performed with SPSS 10.1 software.

Results

Relationships among geographical variables

Multivariate Analysis

Principal components analysis determined that the percentage of bare ground in the catchment, slope and wetland size were positively related, and those variables related negatively to pasture development (Fig. 3.1).

Correlation Analysis

Correlation analysis showed that area of pasture was negatively correlated with areas of bare ground, indigenous forest, planted forest, scrub, tussock, wetland size and slope (Table 3.1). Area of bare ground was positively correlated with areas of tussock, catchment area, wetland size and slope. Area of inland wetlands correlated positively with area of planted forest. Catchment area correlated positively with area of tussock, and wetland size. Wetland size correlated positively with slope.

Table 3.1 Significant Pearson's correlation results (P <0.05, in parentheses) for correlations among geographical variables. The results in bold fit within the Bonferroni adjustment level of 0.00032. Shaded values are negative correlations.

	Bare ground	Indigenous forest	Inland water	Inland wetlands	Planted forest	Pasture	Scrub	Tussock	Urban	Urban open space	Catchment area	Wetland size	Slope
Bare ground								0.35 (0.0065)			**0.50 (0.0001)**	**0.77 (0.0000)**	**0.59 (0.0000)**
Indigenous forest						-0.39 (0.0022)							
Inland water													
Inland wetlands					0.27 (0.0380)								
Planted forest						-0.34 (0.0087)							
Pasture							-0.25 (0.0495)	**-0.51 (0.0000)**				-0.28 (0.0312)	-0.36 (0.0044)
Scrub													
Tussock											0.31 (0.0166)		
Urban													
Urban open space													
Catchment area												0.34 (0.0082)	
Wetland size													**0.53 (0.0000)**
Slope													

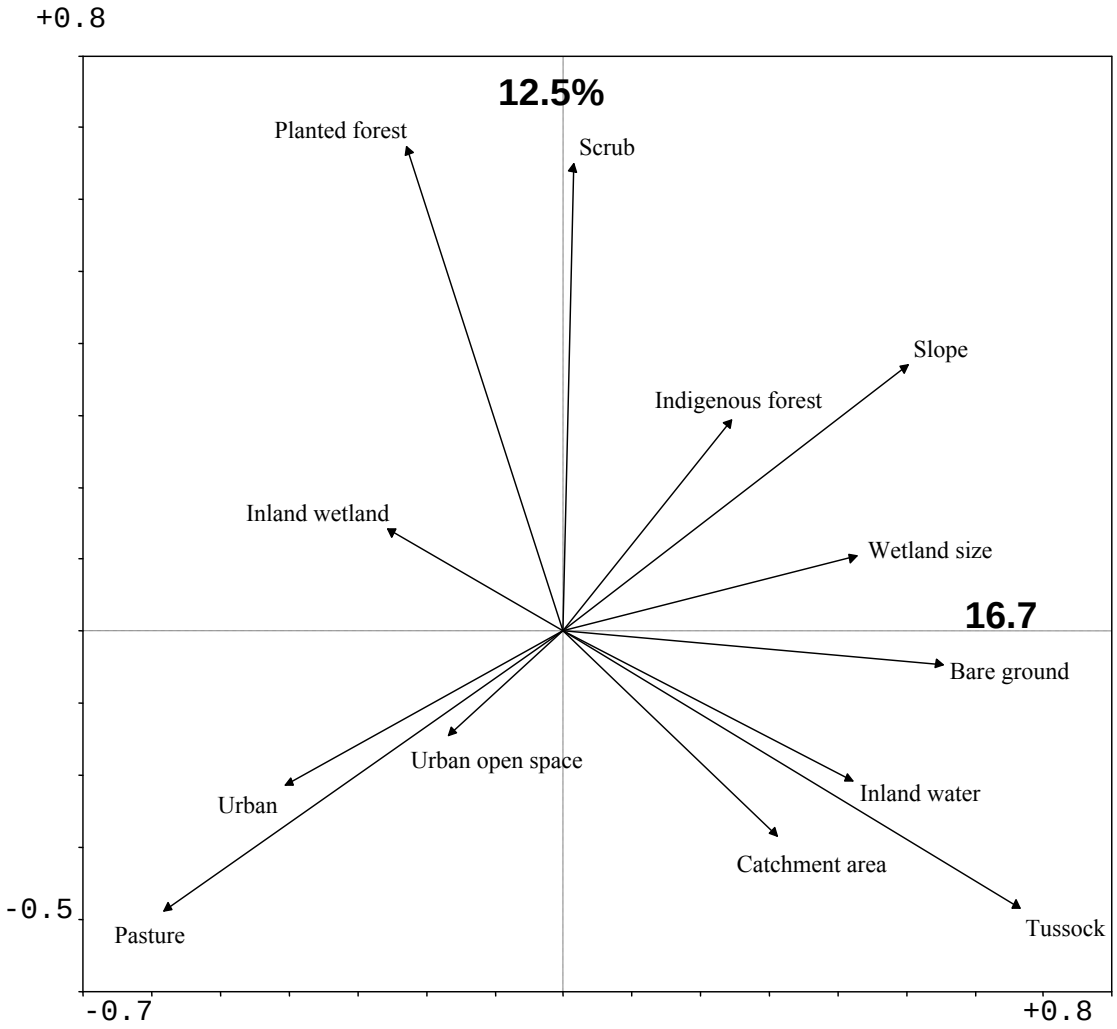


Figure 3.1 Ordination diagram for the principal components analysis of geographical variables. Arrows show the loading of each variable on the two canonical axes. Percentages refer to the percent variance explained by each axis.

Relationships between geographical variables and physicochemical variables

Multivariate Analysis

Redundancy analysis determined that the geographical variables explained a total of 35.9% of the variation in the physicochemical variables (Table 3.2). The first axis explained 21.9% of the physicochemical data. The geographical variables most strongly loaded with this axis were bare ground 12% (P=0.005), tussock 7% (P=0.005), slope 3% (P=0.03) and inland water 3% (P=0.03). The ordination shows that pasture, and to a lesser extent, planted forest, urban and urban open spaces, also loaded strongly, but negatively, with the first axis, (Fig. 3.2). The physicochemical variables most strongly loaded (negatively) with the first axis included TN, TP and DRP, while DOC, turbidity, TSS, water colour and chlorophyll *a* were also negatively loaded with this axis (Secchi depth was positively loaded). The first axis highlights the trophic state of the wetlands, with clear relationships emerging between the geographical and physicochemical variables, as more developed sites (pasture, urban and planted forest) correlated positively with physicochemical variables, while less developed sites (bare ground, tussock and indigenous forest) correlated negatively with physicochemical variables. Increased slope and greater wetland size were also related to lower measures of physicochemical variables.

The second axis explained a further 7.1% of the physicochemical data. The geographical variable most strongly positively loaded with this axis was catchment area 5% (P=0.02). The physicochemical variables most strongly loaded with the second axis were pH (negatively), and temperature and conductivity (positively). This indicates that the second axis defines a gradient of lowland to upland sites, with large, flat, lowland catchments correlating with higher temperatures due to the lower altitude, increased alkalinity and conductivity (due to the lowland, coastal, estuarine sites), whereas increased acidity is associated with upland catchments.

Table 3.2 Results of redundancy analysis of geographical variables and physicochemical variables.

Axes	1	2	3	4	Total variance
Eigenvalues :	0.219	0.071	0.041	0.027	1
Physicochemical-geographical correlations :	0.743	0.776	0.677	0.636	
Cumulative percentage variance					
of physicochemical data :	21.9	29	33.2	35.9	
of physicochemical-geographical relation:	54.6	72.5	82.8	89.7	
Sum of all unconstrained eigenvalues					1
Sum of all canonical eigenvalues					0.4

**** Summary of Monte Carlo test ****

Test of significance of first canonical axis: eigenvalue = .219
F-ratio = 12.875
P-value = .0050

Test of significance of all canonical axes : Trace = .400
F-ratio = 2.363
P-value = .0050

Forward Selection variable	LambdaA	P
Bare ground	0.12	0.005
Tussock	0.07	0.005
Catchment size	0.05	0.02
Slope	0.03	0.03
Inland water	0.03	0.03

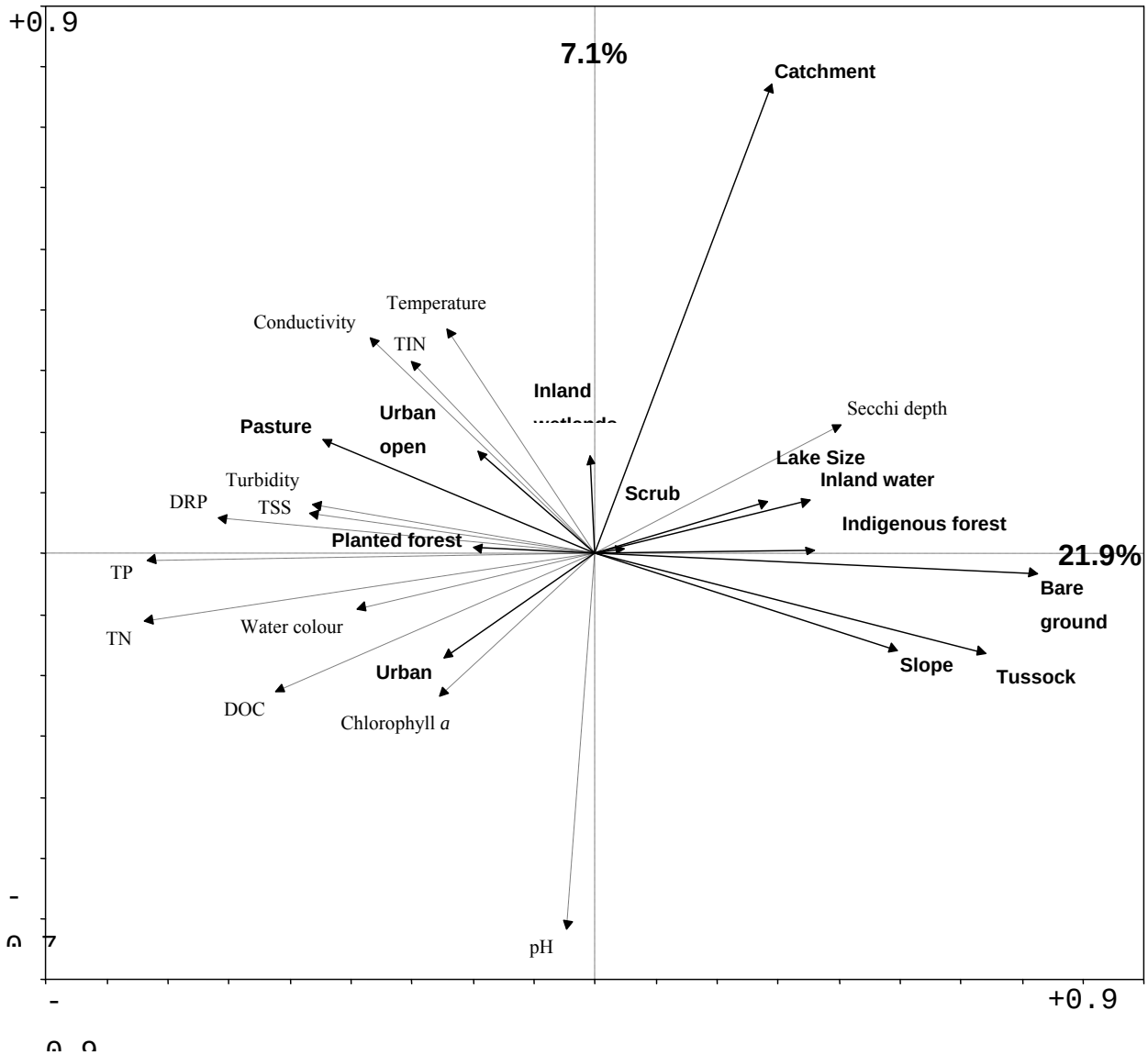


Figure 3.2 Ordination diagram for the redundancy analysis of geographical variables (solid lines) and physicochemical variables (dotted lines). Arrows show the loading of each variable on the two canonical axes. Percentages refer to the percent variance explained by each axis.

Correlation Analysis

Correlation analysis revealed that measures of TP, TN, DRP, DOC, turbidity, TSS, water colour and chlorophyll *a* correlated negatively, and water clarity as measured by Secchi depth correlated positively with area of bare ground. (Table 3.3). Levels of TP, TN, DRP, TIN, and water colour correlated negatively, and Secchi depth correlated positively with area of inland water. Levels of TP, TN, DRP, TIN, DOC, TSS, water colour, pH, temperature and conductivity correlated negatively with area of tussock. Turbidity correlated negatively with area of indigenous forest. Measures of TP, TN, DRP, DOC, turbidity, TSS, pH, temperature and conductivity correlated positively, and Secchi depth correlated negatively with area of pasture. The level of TIN correlated positively, and Secchi depth correlated negatively with area of scrub. Wetland pH correlated positively with urban area, while TIN concentration correlated positively with urban open space. The areas of inland wetland and planted forest were not correlated with any physicochemical variables.

Levels of TP, TN, DRP, DOC, water colour and chlorophyll *a* correlated negatively, and Secchi depth correlated positively with catchment slope. Measures of TN, DRP, DOC, water colour and chlorophyll *a* correlated negatively, and Secchi depth correlated positively with wetland size. Levels of TN, DOC and chlorophyll *a* correlated negatively, and Secchi depth correlated positively with catchment area.

Thus, less developed catchments with more bare ground, inland water, tussock and indigenous forest were correlated with lower physicochemical concentrations and measurements. Increased catchment area, wetland size and slope also correlated with higher measures of physicochemical variables. Conversely, developed catchments with proportionally more land in pasture, scrub and urban development correlated with increases in measurements of physicochemical variables. The negative correlations of pH, temperature and conductivity with area of tussock in the catchment and their positive correlations with pasture development may be due to the extent of areas of pasture in lowland catchments and areas of tussock in upland catchments.

Table 3.3 Significant Pearson's correlation results (P<0.05, in parentheses) for correlations between geographical and physicochemical variables. The land cover variables inland wetland and planted forest were not correlated with any physicochemical variables. The results in bold fit within the Bonferroni adjustment level of 0.00032. Shaded values are negative correlations.

	Bare Ground	Indigenous Forest	Inland Water	Pasture	Scrub	Tussock	Urban	Urban Open Space	Catchment Area	Wetland Size	Slope
TP	-0.35 (0.0073)		-0.27 (0.0383)	**0.58 (0.0000)**		-0.31 (0.0175)					-0.34 (0.0110)
TN	-0.38 (0.0037)		-0.27 (0.0381)	**0.48 (0.0002)**		-0.28 (0.0355)			-0.27 (0.0387)	-0.26 (0.0460)	-0.35 (0.0080)
DRP	-0.29 (0.0253)		-0.44 (0.0005)	**0.49 (0.0001)**		-0.38 (0.0033)				-0.36 (0.0054)	-0.30 (0.0225)
TIN			-0.27 (0.0358)		0.33 (0.0101)	-0.28 (0.0282)		0.42 (0.0008)			
DOC	**-0.61 (0.0000)**			0.32 (0.0133)		-0.29 (0.0265)			-0.38 (0.0026)	-0.45 (0.0004)	**-0.47 (0.0002)**
Turbidity	**-0.47 (0.0002)**	-0.38 (0.0027)		0.35 (0.0059)							
Secchi depth	0.57 (0.0004)		0.42 (0.0112)	-0.37 (0.0303)	0.37 (0.0300)					0.46 (0.0049)	**0.61 (0.0001)**
TSS	-0.38 (0.0026)			0.42 (0.0008)		-0.30 (0.0200)					
Water colour	-0.41 (0.0013)		-0.32 (0.0140)			-0.27 (0.0355)				-0.31 (0.0166)	**-0.51 (0.0001)**
Chlorophyll *a*	-0.42 (0.0007)								-0.28 (0.0308)	-0.32 (0.0120)	-0.28 (0.0354)
pH				0.30 (0.0218)		-0.40 (0.0018)	0.35 (0.0073)				
Temperature				0.30 (0.0207)		-0.42 (0.0008)					
Conductivity				0.37 (0.0032)		-0.45 (0.0004)					

Differences in geographical variables among wetland types

Multivariate analysis

Principal components analysis shows the distribution of the samples for each wetland type and the direction of the geographical variables (Fig. 3.3). The ordinations reveal some separation among the wetland types, mainly for deep lakes. Deep lakes were positively aligned along the first axis, as were the geographical variables; bare ground and tussock cover, slope and wetland size. No other obvious groupings or relationships emerged for other wetland types.

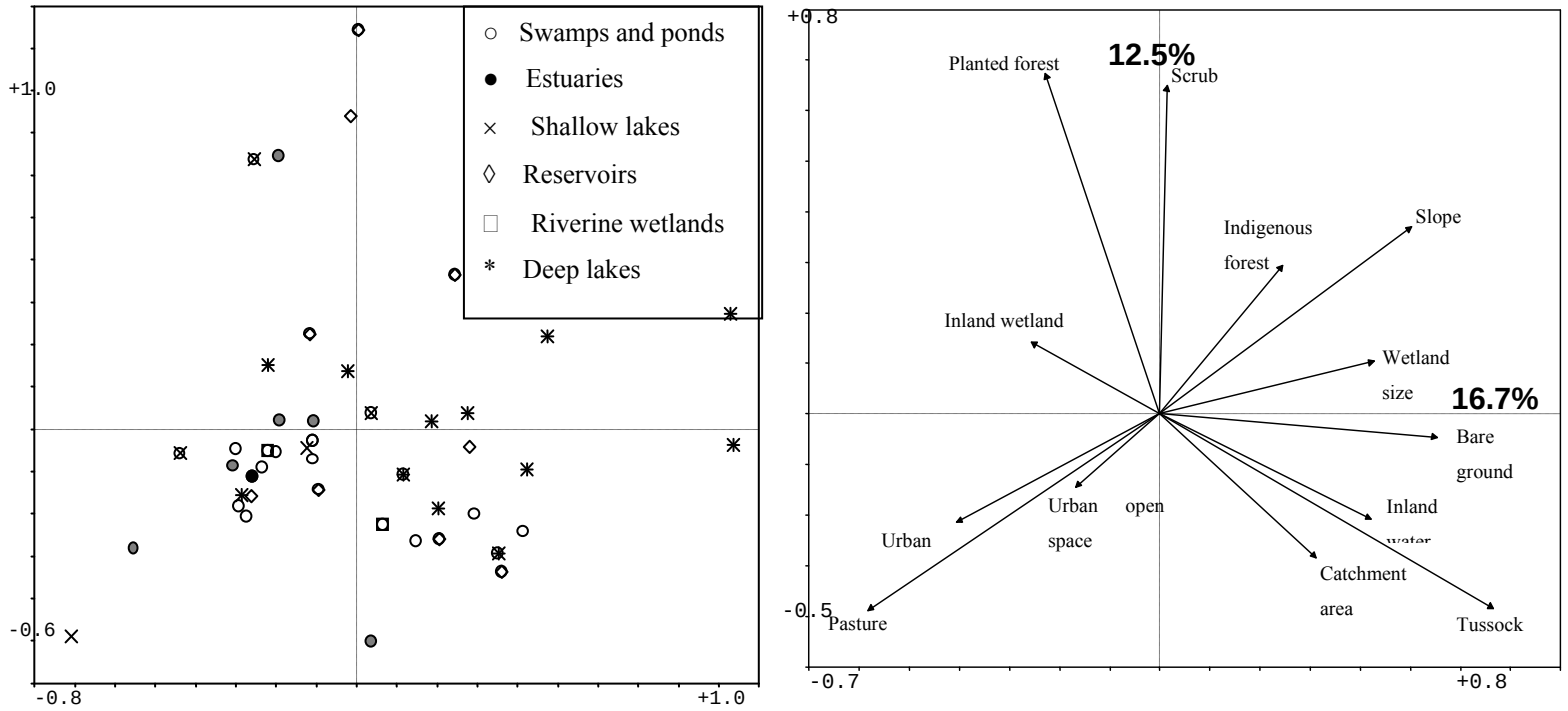


Figure 3.3 Ordination diagram of PCA of geographical variables showing sites by wetland. Left panel – wetland sites, with symbols denoting the different wetland types. Right panel – arrows indicate geographical variables relationships. Percentages refer to the percent variance explained by each axis.

Differences in physicochemical variables among wetland types

Overall, measures of water chemistry, colour and clarity ranged from highest in swamps and ponds, then estuaries, shallow lakes, reservoirs and riverine wetlands to the lowest concentrations in deep lakes (Table 3.4).

Table 3.4 Means (±1SE) of physicochemical variables for wetland types.

	Swamp/pond	Estuarine	Shallow lake	Reservoir	Riverine wetland	Deep lake
TP	139.8±40.46	84.9±20.78	60.1±10.94	29.8±2.99	33.1±8.09	17.5±1.69
TN	1813.0±379.66	841.5±196.76	951.0±237.50	464.6±62.93	300.9±80.51	265.4±50.18
DRP	49.9±17.79	24.3±4.38	13.5±3.95	3.8±0.63	6.1±2.26	1.2±0.30
TIN	128.0±57.40	132.6±38.39	60.8±9.30	70.2±27.62	37.7±6.61	39.8±5.08
DOC	10.7±2.80	9.5±3.45	10.0±3.03	4.4±0.58	4.3±2.11	2.2±0.67
Turbidity	8.1±2.13	7.4±1.07	5.9±1.42	3.3±0.95	2.3±0.27	2.5±0.51
pH	7.0±0.10	7.7±0.32	7.1±0.29	6.8±0.21	7.5±0.58	7.2±0.16
Temperature	12.8±1.78	17.1±1.51	16.4±1.31	13.8±1.01	19.2±2.17	16.1±0.65
Conductivity	283.6±104.30	12428.0±4736.04	3055.5±1477.50	83.1±11.99	4019.8±3357.48	79.8±12.03
Secchi	41.7±4.18	78.5±22.34	86.2±21.68	167.4±26.69	0.0±0.00	498.9±95.44
TSS	13.8±7.90	27.4±9.07	11.0±2.97	3.6±0.79	6.8±3.03	2.2±0.54
Water colour	0.03±0.01	0.02±0.01	0.03±0.01	0.01±0.00	0.00±0.00	0.0±0.00
Chlorophyll *a*	9.9±4.74	4.7±1.25	2.3±0.45	4.8±1.60	0.7±0.31	1.7±0.63

Multivariate analysis

Principal components analysis shows the distribution of wetland sites according to the characteristics of the samples that were colleted in them and the directional loading of the physicochemical variables (Fig. 3.4). The ordinations show clear separation in the wetland types. Swamps and ponds samples are closely related to the first axis, relating positively to the nutrient and organic matter measurements. Deep lakes are also clustered along the first axis, but relate negatively to the nutrient and organic matter measurements. Shallow lakes and reservoirs fall between the swamps and ponds and deep lakes clusters, with shallow lakes tending more to the swamps and ponds sites, whereas reservoirs tend more to the deep lake sites. So, a clear gradient is visible. Riverine wetlands fall between shallow lakes and deep lakes. Estuary samples are aligned with conductivity.

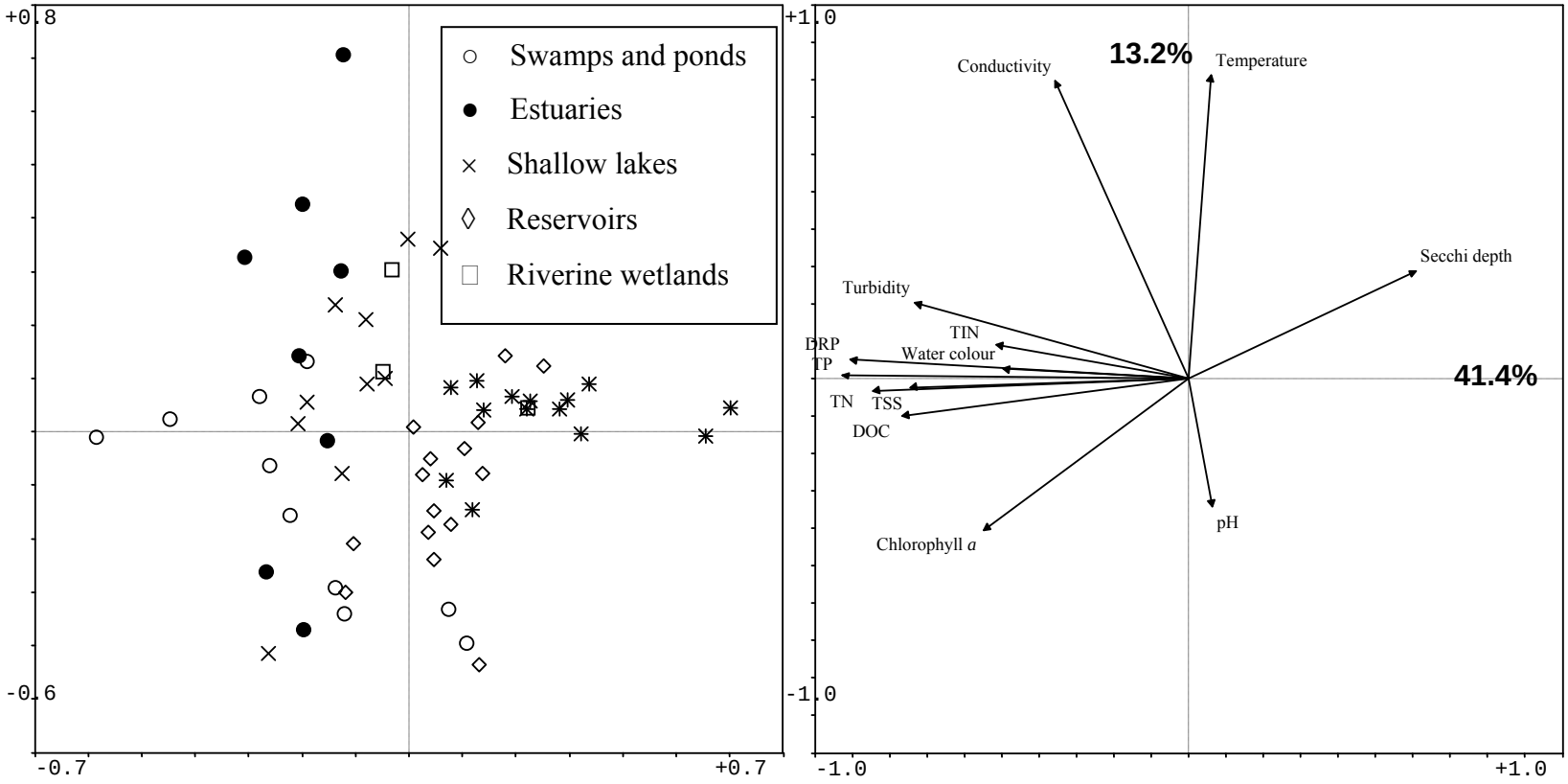


Figure 3.4 Ordination diagram of PCA of physicochemical variables showing sites by wetland. Left – wetland sites, with symbols denoting the different wetland types. Right – arrows indicate physicochemical variables relationships.

Differences in physicochemical variables between seasons

Principal components analysis shows the distribution of the samples for autumn and spring and the direction of the physicochemical variables (Fig. 3.5). The scatter of sites has arrows to show how the sites have changed between the seasons. There is a clear trend of the sites moving along the second axis. The only variable really associated with this axis is temperature, so the only finding is that water temperature decreased in spring from the temperature in autumn. As the sites did not appear to alter on the first axis, it appears that season does not greatly influence the physicochemical conditions in the wetland.

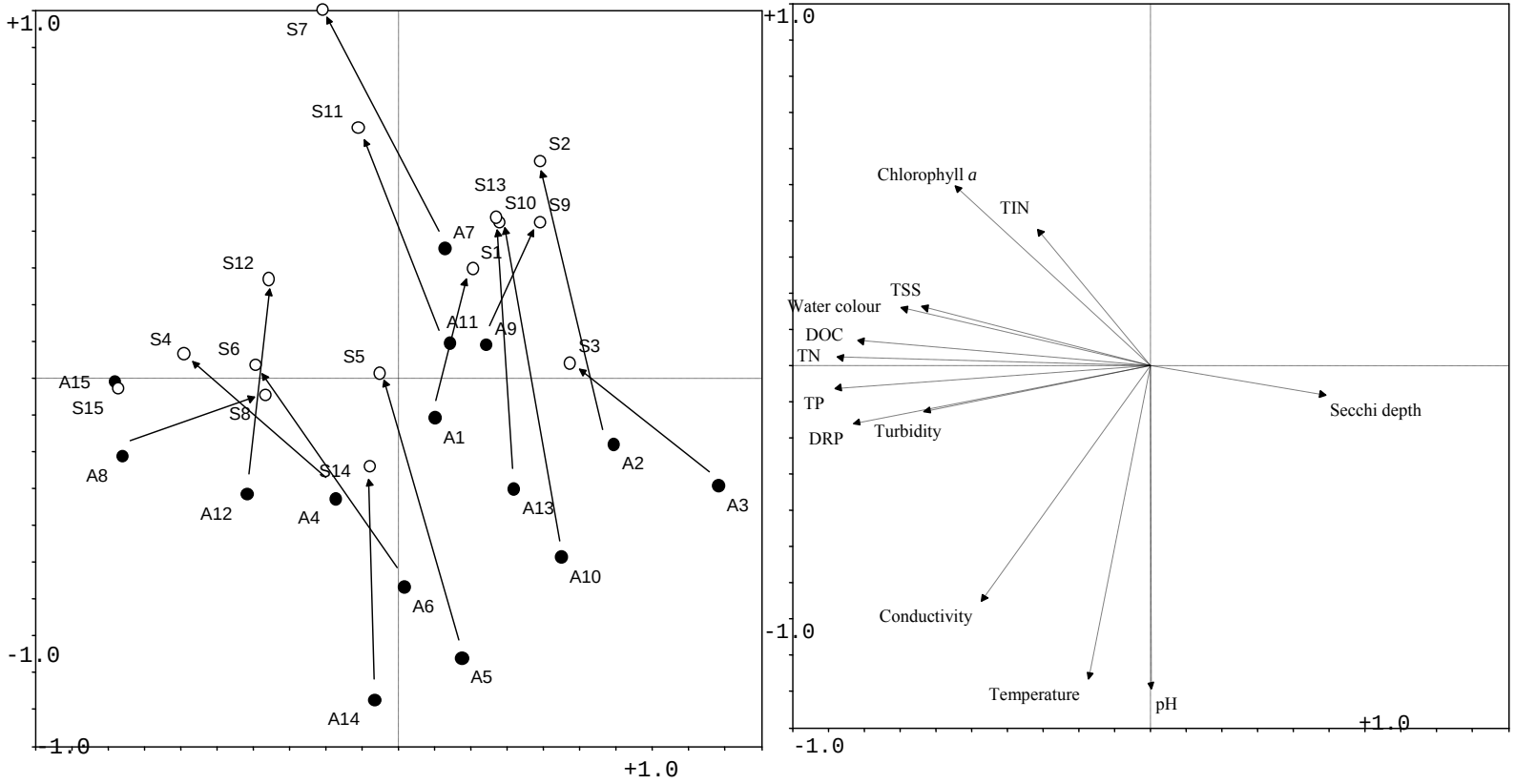


Figure 3.5 Ordination diagram of PCA of physicochemical variables showing sites by season. Left panel – A, black circle = autumn sites and S, open circle = spring sites. Numbers identify each wetland, arrows denote the change between autumn and spring for each wetland. Right panel – arrows indicate physicochemical variables relationships.

Discussion

Relationships among geographical variables

The positive relationships among bare ground, tussock and slope may be due to bare ground and tussock being features of steeper; "high country". Bare ground and wetland size were positively related as a result of the larger lakes in this study being generally at higher altitude than the smaller lakes. Larger catchments are also associated with larger water bodies. As farming is much more prevalent on low land of the study area, altitude also explains the negative relationship between pasture and many geographical measures (percentage of bare ground, indigenous forest, planted forest, scrub, tussock, wetland size and catchment slope).

Relationships between geographical variables and physicochemical variables

Land cover

My prediction that concentrations of nutrients and other components of water quality would correlate positively with the extent of catchment modification was confirmed. Physicochemical measures were positively related to the extent of modification in the catchment (a greater percentage of the catchment in pasture, planted forest, scrub and urban areas), and negatively related with lack of modification in the catchment (a greater percentage of the catchment in bare ground, water, indigenous forest, and tussock).

TP, TN, DRP, DOC and TSS concentrations and turbidity correlated positively and Secchi depth correlated negatively with the percentage of pasture in the catchment, which may be a result of nutrients added as commercial fertiliser in managed pasture catchments, in runoff from the dung and urine of stock, and increased sediment runoff from stock induced soil trampling (Elliott and Sorrell 2002).

Other studies confirm that physicochemical measures in surface water are positively related to catchment development, for example, in several studies in the U.S.A that compare agricultural and indigenous forested catchments (Wang *et al*. 1997, Loughheed *et al*. 2001, Sponseller *et al*. 2001, Bowen and Valiela 2001 and Tong and Chen 2002). My findings that physicochemical measures related positively to the percentage of pasture, and negatively to the percentage of indigenous forest, are similar to those of Loughheed *et al*. (2001) and Tong and Chen (2002). In 62 wetlands of the Great Lakes, U.S.A., sampled once in midsummer, measures of TN, TP, TSS, total inorganic suspended solids, chlorophyll *a* and turbidity were

related positively to agricultural land cover in the catchment and negatively to natural forest cover (Loughheed *et al*. 2001). In Ohio rivers, Tong and Chen (2002) reported N and P concentrations were positively correlated with agricultural, commercial and residential land cover in the catchment, and negatively correlated with natural forests. Sponseller *et al*. (2001) found total inorganic nitrogen was positively related to the proportion of non-forested land (agricultural and urban), compared to forested land, in streams in nine catchments in the Appalachian region, Virginia, U.S.A. My finding of increased nitrogen with increased percentage of pasture concurs with that of Bowen and Valiela (2001) who, by modelling the relative contribution of nitrogen from various sources in estuaries in Cape Cod, Massachusetts, U.S.A., compared to 52 years previous, demonstrated that an increase in nitrogen from increased agricultural development was the cause of eutrophication of the wetlands. However, Maberly *et al.* (2003) sampled 30 upland lakes in the UK, three times each lake, and discovered that whilst the differences in vegetation cover in the catchment were not due to development by humans, the area of pasture cover was still negatively related to phosphorus and nitrogen limitation in the catchment, while bracken and woodland was positively related to phosphorus and nitrogen limitation, thus, agriculture did not explain the increased nutrient export from greater areas of pasture.

In New Zealand, there are several examples of land use in the catchment determining the surface water quality. For example, in streams from catchments of low-intensity sheep farming, nitrogen and phosphorus concentrations were lower than in streams in catchments of high-intensity pasture and dairying, in the catchment of the Pomahaka River (Harding *et al*. 1999).

My study revealed also that whereas, overall, physicochemical measures were generally negatively related to the proportion of indigenous forest in the catchment, they were weakly positively related to the proportion of planted exotic forest. This result is consistent with collated data on nutrients contributed by catchments of varied land uses in New Zealand, which showed that nitrogen supply to surface waters was highest from catchments with dairy farms, then hill pasture (sheep and cattle grazing) and urban catchments, and lowest from catchments of low intensity pastoral grazing, native bush and scrub, and exotic pine forestry (Elliott and Sorrell 2002). Phosphorus supply to surface waters was highest in catchments of hill pasture, then dairy farming, followed by low intensity pastoral grazing, urban and forestry.

My findings of physicochemical measures correlating positively with the percentage of pasture cover in the catchment, and negatively with the percentage of indigenous forest cover in the catchment are consistent with those of Quinn and Stroud (2002) in Waikato streams draining catchments with pasture, pine forest, and native forest land cover. Quinn and Stroud (2002) reported that suspended solids and turbidity were lowest in streams from native forest catchments and highest in streams from pine forest catchments, Secchi depth was highest in streams from native forest catchments and lowest in streams from pine forest catchments, and DOC, TN, and TP were lowest in streams from native forest catchments and highest in streams from pasture catchments.

My results also concur with Williamson *et al*. (1996) in a study in the Lake Rotorua catchment in which grazing was excluded from riparian zones, erosion-prone slopes and native forest remnants. Measurements after excluding grazing in those areas found loads reduced by 85% for sediment, 27% for particulate P, 26% for soluble P and 40% for particulate N, and it was estimated TP loads to the lake were reduced by 20% overall.

Physicochemical measures (TP, TN, DRP, TIN, DOC, TSS, and water colour) were strongly negatively related to the percentage of native tussock grassland in the catchments in my study. The negative correlation between nutrient concentrations in wetlands and the percentage of tussock cover in the catchment may be due to an association between tussock grasslands and steep catchments which are not available for grazing. Alternatively, tussock grasslands have less productive and less palatable vegetation, and, thus, are either not grazed as intensively as pasture, or not grazed at all. If so, nutrient and substrate runoff via fertisers, stock effluent, and dislodging of sediments by ungulate trampling would be reduced. In addition, tussock is able to efficiently intercept water, and thus dissolved substrates and nutrients (Mark 1998b).

Negative relationships between physicochemical measures and tussock grasslands have been noted in other studies in Otago. Riley *et al*. (2003), in a study of 18 catchments in Otago, found that concentrations of phosphate and nitrate were higher with greater catchment development (sheep farming), than in ungrazed tussock catchments; Thompson and Townsend (1998) showed that pastoral vegetation communities contributed to higher concentrations of nitrogen and phosphorus in streams in the Taieri River catchment than native tussock grassland communities.

Catchment area, slope and wetland area

As I predicted, physicochemical variables correlated negatively with the geographical features of my study sites: catchment area, slope and wetland area. My findings that measurements of TP, TN, DRP, DOC, chlorophyll *a* and water colour correlated negatively, and Secchi depth correlated positively, with slope are consistent with those of other studies. For example, in 30 Canadian Shield lakes, Quebec, DOC, TP and chlorophyll *a* were negatively related to catchment slope (D'Arcy and Carignan 1997). However, D'Arcy and Carignan (1997) found that NO_3^- and NH_4^+ related positively to slope, possibly a result of high deposition of atmospheric nitrogen, a result of burning of fossil fuel in highly industrialised regions of North America (Bowen and Valiela 2001).

Measurements of TN, DRP, DOC, chlorophyll *a* and water colour were correlated negatively, and Secchi depth was correlated positively, with wetland area, and measurements of TN, DOC and chlorophyll *a* correlated negatively, and Secchi depth correlated positively with catchment area in my study. This was consistent with the findings of Rasmussen *et al*. (1989), who analysed of 337 lakes in North America and found that water colour correlated negatively with catchment slope and lake area and correlated positively with drainage ratio (drainage basin area ÷ lake volume).

My findings of physicochemical measures correlating negatively with wetland area concur with those of Prepas *et al*. (2001) who, in 26 headwater lakes in the Boreal Plain of Canada, reported measures of TP, chlorophyll *a* and water colour were positively related to the effective drainage basin area (EDBA) (Prepas *et al*. 2001). The apparent opposite finding of positive correlations in the Prepas *et al*. (2001) study compared to my negative correlations, is a result of their analysis using the EDBA = drainage basin area ÷ lake volume. For example, increased wetland size, in the same sized catchment correlated negatively with physicochemical measures, would be the equivalent of EDBA correlated positively with physicochemical measures. I did not analyse correlations between physicochemical variables and the EDBA in my study, as wetland depths were strongly related to wetland size, so volume increased with wetland size.

Differences in geographical variables among wetland types

The only relationship between wetland type and geographical factors in my study was of deep lakes associating with more bare ground and tussock cover, and greater slope. This was

expected, as deep lakes were generally in the steeper uplands, where tussock remains due to the unproductive (for agriculture) nature of the "high country", and the bare ground of the highest mountainous regions.

Differences in physicochemical variables among wetland types

I predicted that concentrations of nutrients and other components of water quality would increase along a gradient of wetland types ranging from deep lakes to swamps and ponds, and this was confirmed. Deep lakes were clearly the wetland type with the lowest mean measures of physicochemical variables, increasing to highest in swamps and ponds. Whereas other wetland types fell within the range of swamps/ponds and deep lakes, it was apparent that shallow lakes had greater mean measures of physicochemical variables than reservoirs. The difference in physicochemical measures among the wetland types could be a result of the close relationship between geographical variables and wetland type. However, deep lakes were the only wetland type to show any relationship with geographical variables (bare ground, tussock and slope).

Several other factors might explain the greater concentrations of physicochemical components in swamps and ponds: smaller water bodies are much more affected by littoral vegetation and riparian influences (Wetzel 1995); the relative importance of allochthonous sources of dissolved organic matter to a system increases with decreasing wetland size (Thomas 1997); precipitation directly to the (larger) surface of a lake will dilute concentrations of physicochemical components; and small, highly productive wetlands may generate organic matter via biological processes such as synthesis or polymerisation from existing organic matter, degradation of organic matter and release from living and dead organisms (Thomas 1997, Rasmussen *et al*. 1989).

Differences in physicochemical variables between seasons

Whereas I predicted that values of the measures of physicochemical variables would change between autumn and spring, measureable differences in physicochemical variables between seasons were not found. I expected the higher than average rainfall in Otago during the spring of 1998 to increase the nutrient loading to the wetlands via runoff (Schindler 1997), however, high precipitation directly into the wetlands may have simultaneously diluted concentrations of nutrient and substrates (Rasmussen *et al*. 1989).

Summary

This research provides evidence that land management will consistently influence the trophic state of wetlands in Otago. The land use and geographical features of a wetland's catchment appear to determine the physicochemical variables within the wetland, regardless of wetland type, which has implications for the wetland's biological community structure and productivity. Increased agriculture and plantation forestry in the catchment have been shown to affect stream water quality in Otago (Riley *et al*. 2003, Thompson and Townsend 1998) and my study confirms that they will also increase nutrient concentrations in lentic habitats.

Chapter 4 Microbial food web

Introduction

The microbial food web is regulated by predation (top-down), organic carbon and inorganic nutrients (bottom-up). Zooplankton graze on phytoplankton, protozoa and bacteria, and recycle nutrients through excretion (Kitchell and Carpenter 1993). Phytoplankton biomass and production are limited by phosphorus and nitrogen (Pace and Funke 1991, Pace 1991). Bacteria and picophytoplankton are effective competitors for phosphorus (Pace 1991), and are efficient at sequestering nutrients and dissolved organic carbon (Weisse 1991b). Bacteria have a large potential for growth, limited by the availability of nutrients and carbon supply from phytoplankton (Pace 1991) and other autochthonous and allochthonous sources. Therefore, bacterial abundance will be related to the phytoplankton biomass and productivity, and the trophic state of a system (Weisse 1991b). The relative significance of the microbial food web to carbon flux will be greater in nutrient limited oligotrophic systems (Porter 1988). Abundances of microbial food web components increase with increased nutrient loading and primary productivity, but the relative significance of the microbial food web to carbon flux decreases with increasing eutrophication, thus, the classic food chain becomes more dominant (Weisse 1991b). However, large carbon inputs from littoral and allochthonous sources will lessen the importance of phytoplankton to bacteria (Pace 1991).

There have been many comparative studies of aquatic community structure in aquatic systems recently, with varied findings. Auer *et al.* (2004) and Auer and Arndt (2001) compared the microbial food webs of 55 lakes in Germany. Auer *et al.* (2004) reported that the mean abundance and biomass of the microbial food web components increased with lake trophic status, and both bottom-up and top-down mechanisms regulated the community. Auer and Arndt (2001) found that increased lake productivity did not affect the taxonomic composition of the HNF community, but did affect the size distribution of the HNF, with large forms (>10µm) more abundant with nutrient enrichment. They found that cladocerans had a strong negative effect on HNF biomass. Seasonally, spring highs in HNF abundance and biomass were also observed (due to low predation pressure), as opposed to summer when high

cladoceran biomass reduced the HNF population. Larger and medium sized HNF were reduced in early summer, as these forms were more susceptible to predation.

Hwang and Heath (1997b) compared the microbial food web among sites in Lake Erie, USA, finding that the relative importance of HNF and ciliates as bacterial grazers varied with trophic status. Bacterial productivity was greater at coastal sites than at offshore sites, probably due to the higher productivity of coastal sites due to high nutrient loading and concentrations. At the oligotrophic offshore sites the protists consumed most of the bacterial productivity, while at the coastal sites a much smaller portion of bacterial productivity was grazed. The protistan bacterivory was also much more efficiently coupled with bacterial production than at coastal sites. Several reasons for this efficiency were proposed, including less top-down control of protists offshore, and less competition among protists for bacteria, and loss of bacterial cells due to binding with particles in the more turbid coastal zones.

Gasol *et al*. (1995) compared the abundances of HNF with the abundances of resources and predators, both among lakes of various trophic status, and within lakes. Among the lakes, resources (TP, phytoplankton and bacteria) were the best predictors of HNF and zooplankton abundance. Within lakes, grazer influence, with a seasonal effect, was a better predictor of abundance.

In New Zealand, Jeppesen *et al*. (2000) surveyed 25 shallow lakes to determine influences on the pelagic communities. They found that relationships between the microbial food web and predators were weak. *Ceriodaphnia* most often dominated in lakes of low TP and *Daphnia* and rotifers were more common at medium to high TP concentrations. However, top down effects from fish predation also had a major effect. Zooplankton grazing appeared to have only a slight effect on phytoplankton, but *Daphnia* were negatively related to ciliates. Abundances of calanoid copepods were not related to ciliates, and HNF were not related to any other variables.

Burns and Stockner (1991) examined autotrophic picoplankton abundance in six New Zealand lakes, in relation to season and trophic state. Picoplankton were negatively correlated with total chlorophyll *a* in all the lakes, consistent with the expectation that oligotrophic systems would be higher in total autotrophic picoplankton. Seasonal effects were also observed with higher picoplankton abundance in summer and autumn than in winter and spring, which the authors attributed to greater nutrient concentrations and/or light in summer and autumn.

In an experimental study to compare the consumer effects on protozoa in four lakes of different trophic status, Burns and Schallenberg (2001) recorded that the standing stock of total microplankton biomass was higher with increased lake trophic status (higher concentrations of chlorophyll *a* and nutrients). Both heterotrophic and autotrophic microplankton biomass were higher, but the relative contribution of protozoa to the total microplankton biomass decreased with more eutrophic conditions.

The aims of my study were to determine in 45 wetlands representative of a range of wetland environments in Otago the relationships between: 1) pelagic microbial food webs and phytoplankton biomass, and a) physicochemical variables, and b) geographical variables. A second aim, 2) was to determine in these wetlands the relative contribution of the microbial food web carbon to total microplankton carbon, and a) physicochemical variables, and b) geographical variables. In a third aim, 3), the microbial food web and phytoplankton biomass, and the relative contribution of the microbial food web carbon to total microplankton carbon among wetland types, were also determined.

I predicted that the abundance of zooplankton, and the percentage contribution of copepods to total zooplankton abundance would correlate positively with: a) measures of physicochemical variables; b) catchment modification (land cover greater in pasture, planted forest, scrub and urban areas, rather than bare ground, water, indigenous forest, and tussock; and c) biomass of microbial food web components and phytoplankton. I predicted also that the abundance of zooplankton and the percentage contribution of copepods to total zooplankton abundance would, d) increase, along a gradient of wetland types ranging from deep lakes to swamps and ponds, and e) change, between autumn and spring.

I expected that the biomass of microbial food web components would be strongly correlated between adjacent trophic levels. I predicted that the biomass of bacteria and heterotrophic microplankton would correlate positively, and picophytoplankton would correlate negatively, and the relative contribution of the microbial food web to total microplankton carbon would correlate negatively with: a) measures of physicochemical variables; b) catchment modification (land cover greater in pasture, planted forest, scrub and urban areas, rather than bare ground, water, indigenous forest, and tussock; and c) smaller catchments; d) less slope in the catchment; and e) smaller wetland area. In addition, I predicted that the microbial food web and phytoplankton biomass, and the relative contribution of the microbial food web to total microplankton carbon would, f) increase, along

a gradient of wetland types ranging from deep lakes to swamps and ponds; and g) change, between autumn and spring.

Methods

Refer to Methods (chapter 2, page 9)

Relative contribution of the microbial food web to total microplankton carbon

To determine the relative contributions of the classical food chain and the microbial food web to the total microplankton carbon, the proportion of phytoplankton (chlorophyll *a*) was analysed and these percentages of chlorophyll *a* were arcsine transformed.

Data analysis

Biomass of microbial food web components were log(10) transformed as the data were not normally distributed. Several multivariate analyses were carried out: detrended correspondence analysis, redundancy analysis, and principal components analysis were performed using CANOCO (v. 4.0) software. Redundancy analysis was used as a detrended correspondence analysis determined that gradient lengths were less than 4 standard deviations (ter Braak and Šmilauer 1998). The influence on the biomass of microbial food web components of measures of physicochemical variables, the abundance of zooplankton, and geographical variables, was investigated using redundancy analysis, with forward selection to assess the statistical significance of independent variables, and Pearson's correlation analysis to examine direct relationships. Differences in wetland types and seasons were examined using principal components analysis. ANOVA and Pearson's correlation analysis were performed with SPSS (v. 10.1) software.

Results

The contributions of autotrophs and heterotrophs to total microbial community carbon biomass varied substantially across the wetlands (Fig. 4.1). There was also a gradient of increasing total microplankton biomass in the wetlands, ranging from 21.7µg C L^{-1} in Lake Wakatipu in autumn, to 3061.1µg C L^{-1} in Waitepeka Swamp in autumn.

Relative contributions of microbial food web and phytoplankton to carbon biomass

Ranked in order of increasing relative contributions of phytoplankton, the wetlands showed a gradient from 4.4% in Ross Creek Reservoir in autumn, to 84.4% in Waitepeka Swamp in autumn (Fig. 4.2).

Relationships among the microbial food web and phytoplankton

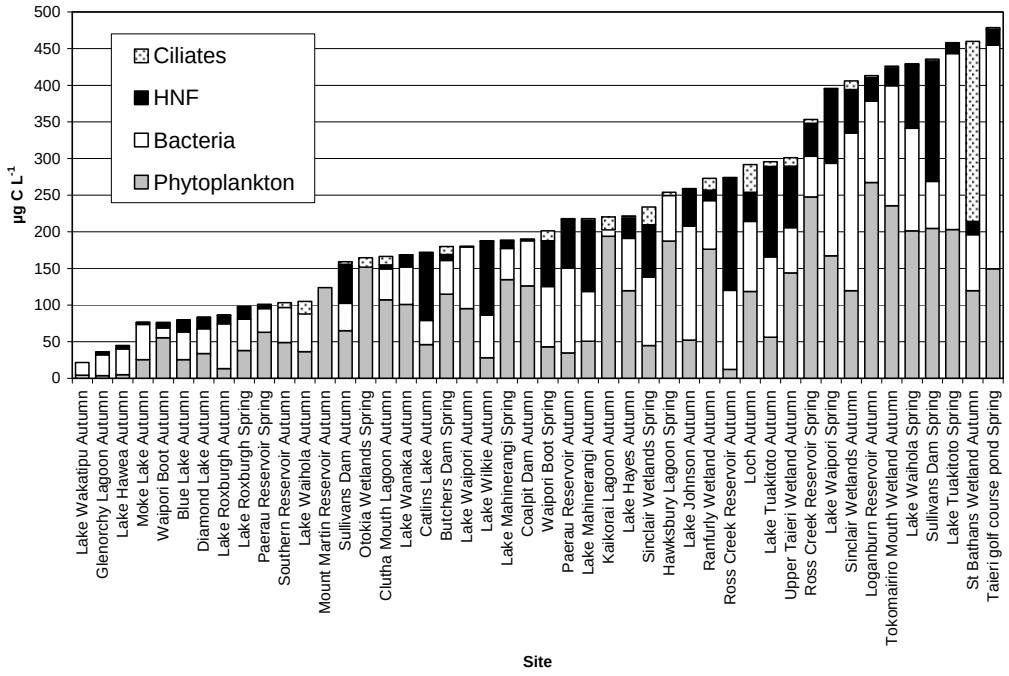
Multivariate analysis

Principal components analysis showed that bacteria and HNF have a very close association, and EP appears associated with both (Fig. 4.3). Phytoplankton biomass and ciliates are also closely related, and clearly negatively related to PP. Neither PP or phytoplankton and ciliate biomass appear to relate to bacterial and HNF biomass.

Correlations

Correlation analysis showed that bacterial biomass was positively correlated with biomass of HNF (Table 4.1). PP biomass was negatively correlated with ciliate biomass. Ciliate biomass was positively correlated with phytoplankton biomass.

a.



b.

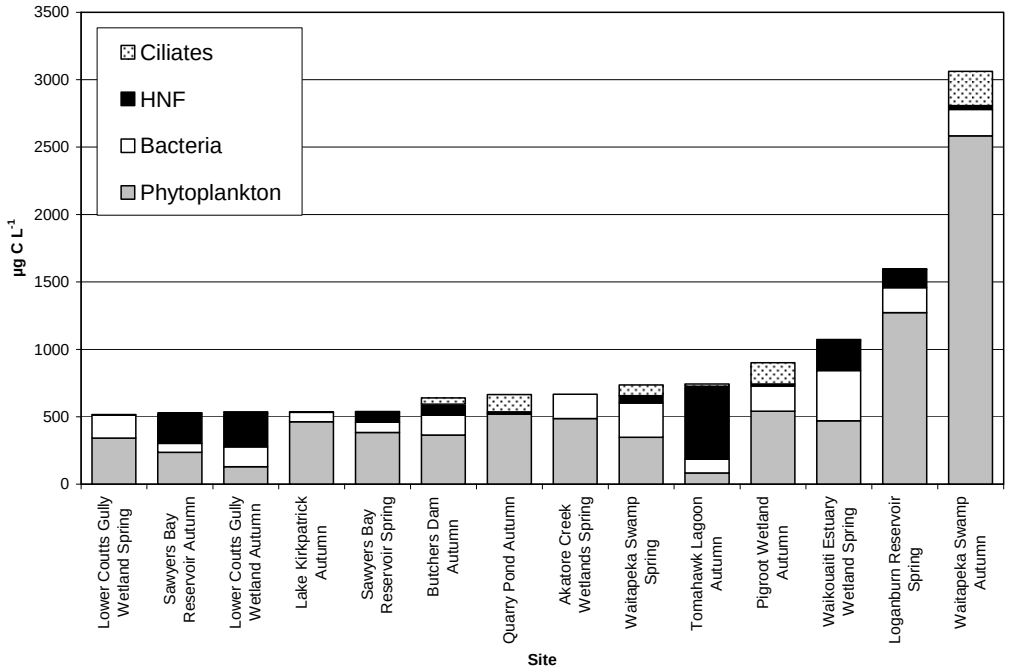


Figure 4.1 Microbial food web and phytoplankton biomass, expressed as µg C L^{-1} of autotrophs (phytoplankton) and the major heterotrophs (bacteria, HNF, ciliates). a. Sites with total biomass below 500 µg C L^{-1}. b. Sites with total biomass above 500 µg C L^{-1}.

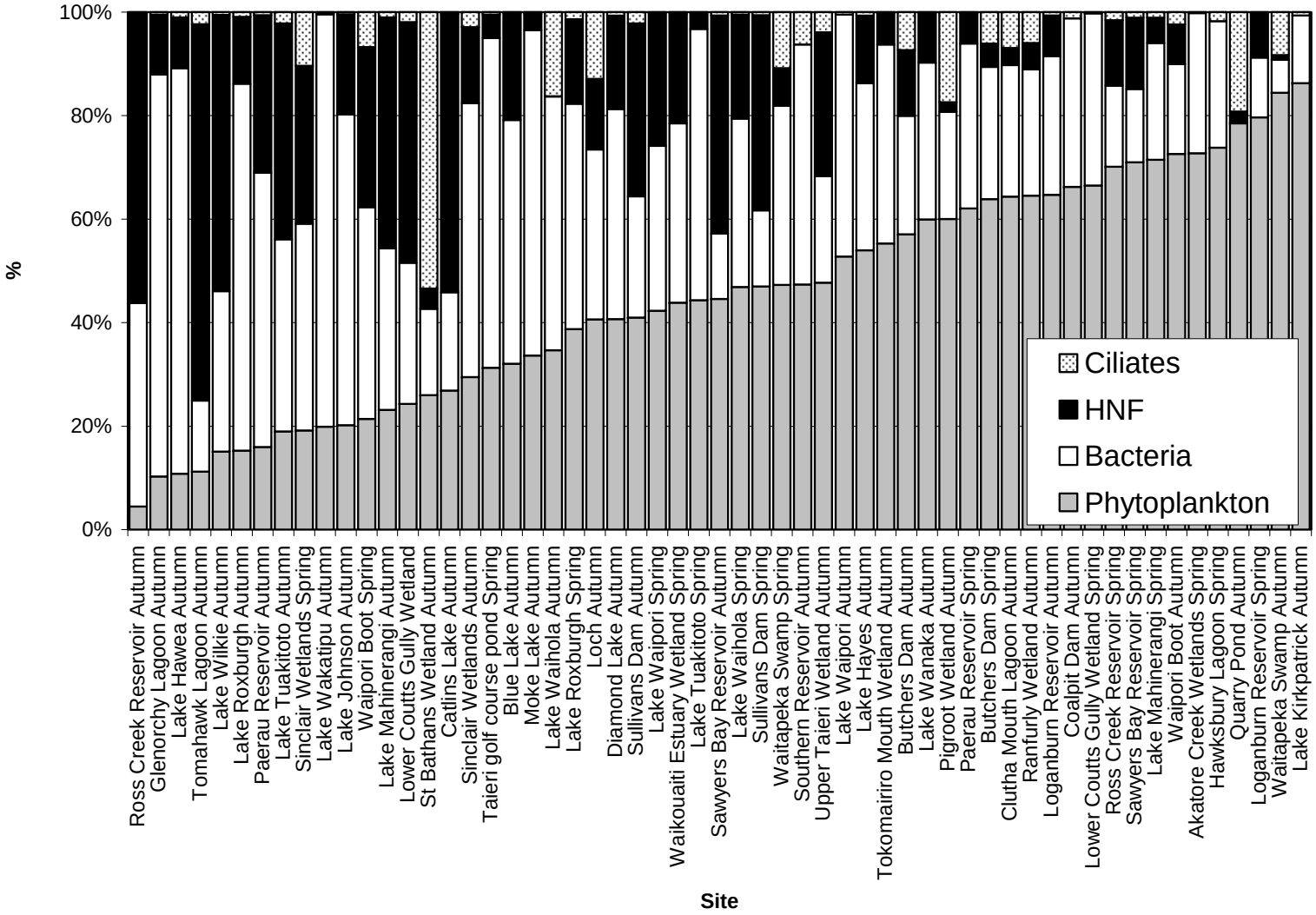


Figure 4.2 Percentage of contributions of autotrophs (chlorophyll *a*) and the major heterotrophs (bacteria, HNF, ciliates) to total carbon biomass of microplankton (<150µm) in the wetlands.

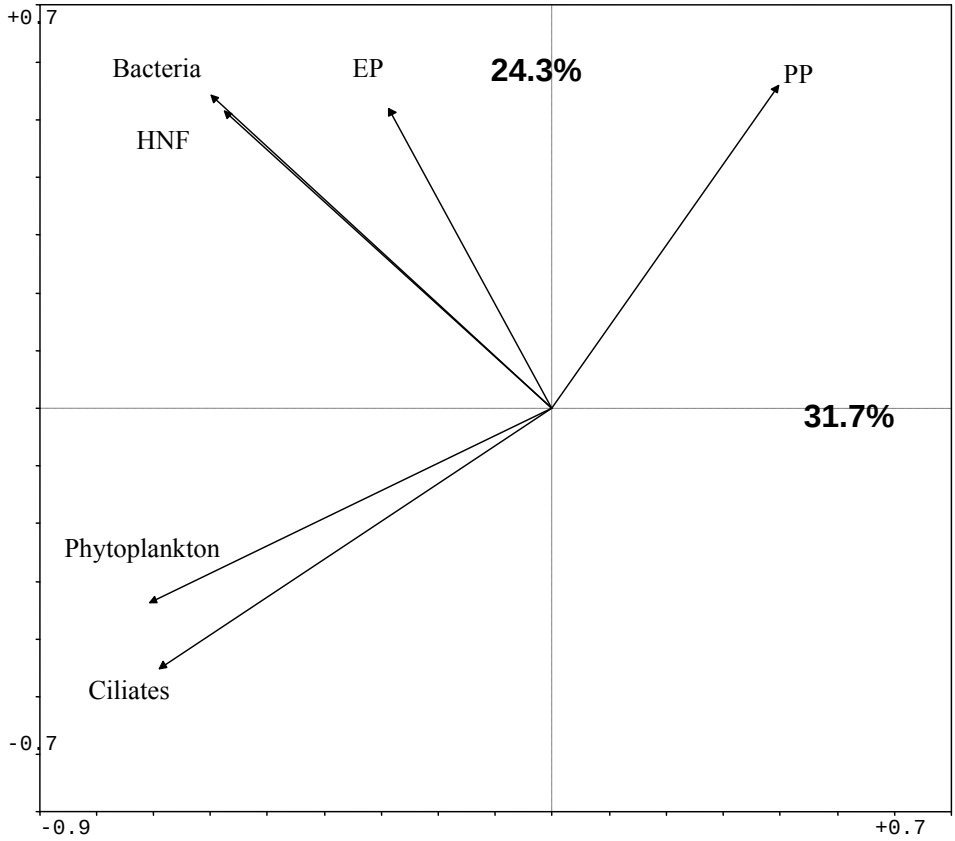


Figure 4.3 Ordination diagram for the principal components analysis of biomass of microbial food web and phytoplankton. Arrows show the loading of each variable on the two canonical axes. PP = prokaryotic picophytoplankton, EP = eukaryotic picophytoplankton, HNF = heterotrophic nanoflagellates. Percentages refer to the percent variance explained by each axis.

Table 4.1 Significant Pearson's correlation results (P<0.05, in parentheses) for correlations among microbial food web and phytoplankton biomass. The results in bold fit within the Bonferroni adjustment alpha level of 0.0017. Shaded values are negative correlations. PP = prokaryotic picophytoplankton, EP = eukaryotic picophytoplankton, HNF = heterotrophic nanoflagellates.

	Bacteria	PP	EP	HNF	Ciliates	Phytoplankton
Bacteria				**0.45 (0.0003)**		
PP					-0.33 (0.0106)	
EP						
HNF						
Ciliates						**0.49 (0.0001)**
Phytoplankton						

Relationships between the microbial food web and phytoplankton, and physicochemical variables

Multivariate analysis

Redundancy analysis showed that the physicochemical variables explained a total of 31% of the variation in the microbial community (Table 4.2). The first axis explained 16.5% of the microbial food web and phytoplankton data. The physicochemical variables most strongly loaded with this axis included DOC 12% (P=0.005), DRP 5% (P=0.02) and TP 3% (P=0.05) (Fig. 4.4). Other variables highly associated with this axis included TN, turbidity, Secchi depth and water colour. All variables, except Secchi depth, loaded negatively with this axis. The microbial food web and phytoplankton biomass most strongly loaded with the first axis included PP (negatively), ciliates and chlorophyll *a* (positively). Clearly, the first axis highlights that increases in concentrations of physicochemical variables are closely associated with increased phytoplankton. In turn, ciliate biomass appears closely linked to phytoplankton. PP declined with increases in concentrations of physicochemical variables.

The second axis explained a further 7.6% of the microbial food web and phytoplankton data. No physicochemical variables emerged to strongly load with this axis. Bacteria, EP and HNF were negatively associated with the second axis. Concentrations of bacteria, EP and HNF do not appear to be explained by any physicochemical variables. HNF appeared to be closely linked to bacteria and EP populations.

Correlation analysis

Correlation analysis did not show relationships between bacterial and EP biomass and concentrations of physicochemical variables (Table 4.3). PP was more common in clear, oligotrophic waters, as PP biomass correlated negatively with concentrations of DRP, DOC, TSS and conductivity, and correlated positively with increasing Secchi depth. HNF biomass correlated positively with concentrations of DOC and water colour, and correlated negatively with pH. Ciliate and phytoplankton biomass correlated positively with concentrations of TP, TN, DRP, DOC and measurements of turbidity and water colour and correlated negatively with Secchi depth. Phytoplankton biomass also correlated positively with TSS and correlated negatively with temperature.

Table 4.2 Results of redundancy analysis of microbial food web and phytoplankton biomass and physicochemical variables

Axes	1	2	3	4	Total variance
Eigenvalues :	0.165	0.076	0.038	0.032	1
Species-physicochemical correlations :	0.758	0.57	0.553	0.527	
Cumulative percentage variance					
of species data :	16.5	24	27.8	31	
of species-physicochemical relation:	48.9	71.2	82.4	92	
Sum of all unconstrained eigenvalues					1
Sum of all canonical eigenvalues					0.337

**** Summary of Monte Carlo test ****

Test of significance of first canonical axis: P-value = .0050
Test of significance of all canonical axes: P-value = .0050

Forward Selection Variable	LambdaA	P
DOC	0.12	0.005
DRP	0.05	0.02
TP	0.03	0.05

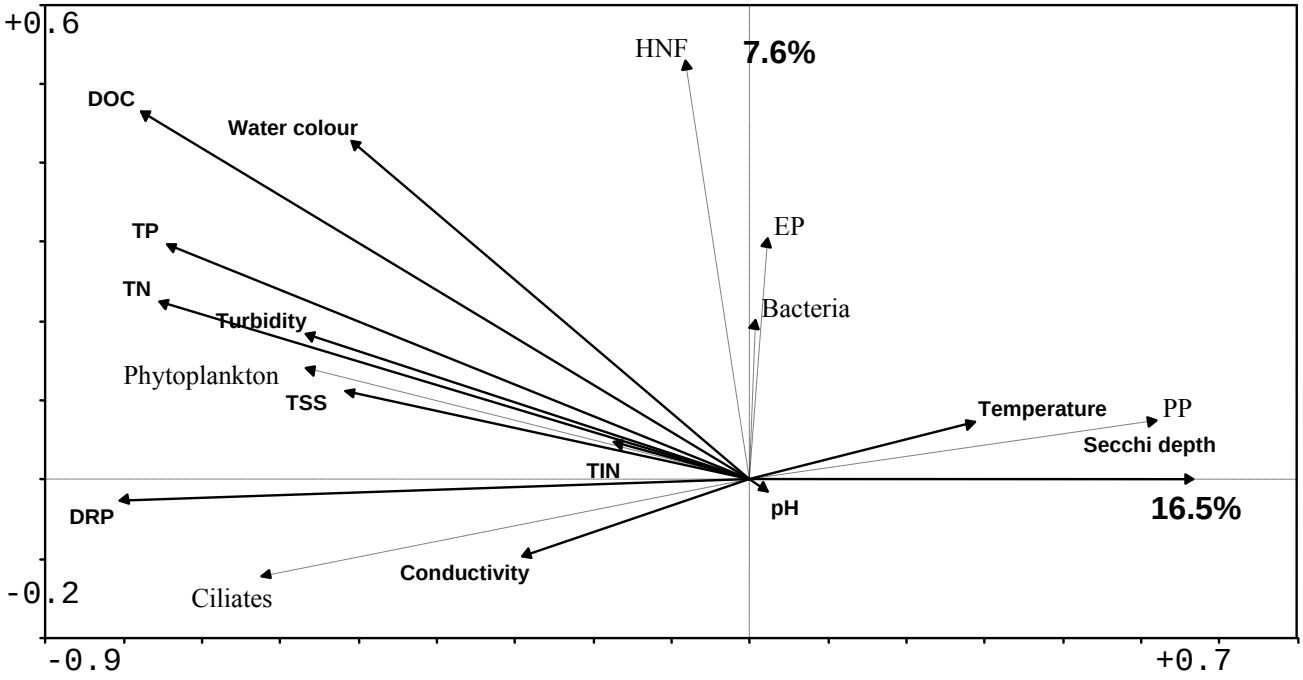


Figure 4.4 Ordination diagram for the redundancy analysis of microbial food web and phytoplankton biomass (dotted lines) and physicochemical variables (solid lines). Arrows show the loading of each variable on the two canonical axes. PP = prokaryotic picophytoplankton, EP = eukaryotic picophytoplankton, HNF = heterotrophic nanoflagellates. Percentages refer to the percent variance explained by each axis.

Table 4.3 Significant Pearson's correlation results (P<0.05, in parentheses) for correlations between biomasses of microbial food web components, phytoplankton and physicochemical variables. The results in bold fit within the Bonferroni adjustment level of 0.00069. Shaded values are negative correlations. PP = prokaryotic picophytoplankton, EP = eukaryotic picophytoplankton, HNF = heterotrophic nanoflagellates.

	TP	TN	DRP	TIN	DOC	Turbidity	pH	Temperature	Conductivity	Secchi depth	TSS	Water colour
Bacteria												
PP			**-0.43** (**0.0008**)		-0.40 (0.0014)				-0.37 (0.0036)	0.35 (0.0420)	-0.27 (0.0354)	
EP												
HNF					0.26 (0.0447)		-0.30 (0.0199)					0.28 (0.0323)
Ciliates	0.37 (0.0048)	**0.43** (**0.0007**)	0.40 (0.0018)		0.38 (0.0028)	0.27 (0.0357)				**-0.57** (**0.0004**)		0.29 (0.0256)
Phytoplankton	**0.47** (**0.0002**)	**0.52** (**0.0000**)	**0.48** (**0.0001**)		**0.54** (**0.0000**)	**0.45** (**0.0003**)		-0.26 (0.0445)		**-0.61** (**0.0001**)	**0.47** (**0.0002**)	0.35 (0.0059)

Relationships between the relative contribution of microbial food web and phytoplankton, and physicochemical variables

Correlation analysis showed the contribution of bacteria to the total microbial and phytoplankton carbon biomass correlated negatively with concentrations of the physicochemical variables; TP, TN, DRP, DOC (Bonferroni), turbidity and TSS, and correlated positively with Secchi depth (Table 4.4). The relative contribution of HNF was not correlated with any physicochemical variables, whereas that of ciliates correlated negatively with Secchi depth. The relative contribution of phytoplankton correlated positively with turbidity and TSS.

Table 4.4 Significant Pearson's correlation results (P<0.05, in parentheses) for correlations between the relative contribution of microbial food web components and phytoplankton to the total microplankton carbon biomass and physicochemical variables. The results in bold fit within the Bonferroni adjustment level of 0.00104. Shaded values are negative correlations.

	TP	TN	DRP	TIN	DOC	Turbidity	pH	Temperature	Conductivity	Secchi depth	TSS	Water colour
Bacteria	-0.29 (0.0274)	-0.36 (0.0062)	-0.41 (0.0014)		**-0.56 (0.0000)**	-0.36 (0.0047)				**0.62 (0.0001)**	-0.31 (0.0151)	
HNF												
Ciliates										-0.35 (0.0384)		
Phytoplankton						0.33 (0.0108)					0.28 (0.0300)	

Relationships between the microbial food web and phytoplankton, and geographical variables

Multivariate analysis

Redundancy analysis of the microbial food web data showed that the geographical variables explained a total of 25.4% of the variation (Table 4.5). The first axis explained 13.7% of the data. The catchment variable most strongly positively loaded with this axis was wetland size at 6% (P=0.015) (Fig. 4.5). The areas of bare ground and inland water, catchment size and slope loaded positively, while the area of pasture in the catchment loaded negatively, with the first axis. The microbial food web variables that loaded with the first axis were PP (positively), with ciliates and phytoplankton (negatively).

The second axis explained a further 6% of the microbial food web abundance data. No geographical variables were strongly loaded with this axis. Only inland wetlands (negatively) and urban (positively) were associated with this axis. The microplankton biomasses that loaded most strongly with this axis were bacteria and HNF, both negatively.

Correlation analysis

Bacterial biomass correlated positively with urban land cover in the catchment (Table 4.6). PP correlated positively with bare gound, tussock, catchment area and wetland size. HNF correlated negatively with bare ground, catchment area and slope. Ciliates correlated positively with pasture. Phytoplankton correlated negatively with bare ground. Both ciliate and phytoplankton biomasses correlated negatively with catchment area, slope and wetland size.

Table 4.5 Results of redundancy analysis of microbial food web and phytoplankton biomass and geographical variables

Axes	1	2	3	4	Total variance
Eigenvalues :	0.137	0.052	0.037	0.028	1
Species-physicochemical correlations :	0.689	0.461	0.508	0.488	
Cumulative percentage variance					
of species data :	13.7	18.9	22.6	25.4	
of species-physicochemical relation:	48	66.2	79	88.9	
Sum of all unconstrained eigenvalues					1
Sum of all canonical eigenvalues					0.286

**** Summary of Monte Carlo test ****

Test of significance of first canonical axis: P-value = .0250
Test of significance of all canonical axes : P-value = .0350

Forward Selection Variable	LambdaA	P
Wetland Size	0.06	0.015

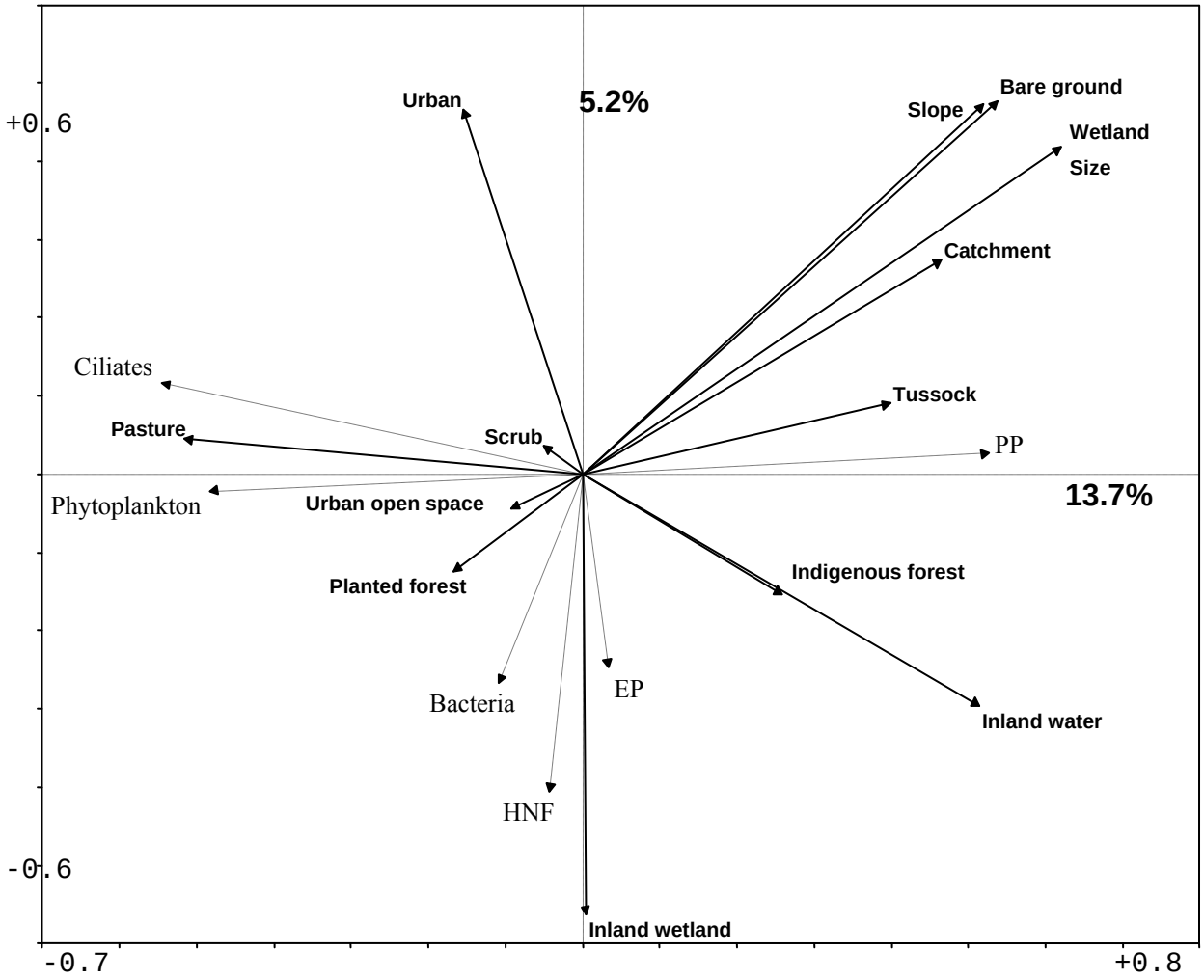


Figure 4.5 Ordination diagram for the redundancy analysis of biomasses of components of the microbial food web and phytoplankton (dotted lines) and geographical variables (solid lines). Arrows show the loading of each variable on the two canonical axes. PP = prokaryotic picophytoplankton, EP = eukaryotic picophytoplankton, HNF = heterotrophic nanoflagellates. Percentages refer to the percent variance explained by each axis.

Table 4.6 Significant Pearson's correlation results (P<0.05, in parentheses) for correlations between biomass of microbial food web components and phytoplankton and geographical variables. The results in bold fit within the Bonferroni adjustment level of 0.00064. Shaded values are negative correlations. Geographical variables not shown were not significantly correlated with microbial food web or phytoplankton biomass. PP = prokaryotic picophytoplankton, EP = eukaryotic picophytoplankton, HNF = heterotrophic nanoflagellates.

	Bare Ground	Pasture	Tussock	Urban	Catchment Area	Slope	Wetland Size
Bacteria				-0.27 (0.0391)			
PP	0.37 (0.0114)		0.35 (0.0171)		**0.50 (0.0004)**		0.47 (0.0009)
EP							
HNF	-0.34 (0.0130)				-0.29 (0.0393)	-0.30 (0.0315)	
Ciliates		0.35 (0.0066)			-0.29 (0.0275)	-0.34 (0.0084)	-0.33 (0.0095)
Phytoplankton	-0.42 (0.0007)				-0.28 (0.0308)	-0.28 (0.0354)	-0.32 (0.0120)

Relationships between the relative contribution of microbial food web and phytoplankton, and geographical variables

The contribution of bacteria to the total microbial food web and phytoplankton carbon biomass correlated positively with bare ground, wetland size, and marginally, with slope (Table 4.7). The relative contribution of HNF correlated positively with indigenous forest, whereas the relative contributions of ciliates and phytoplankton were not correlated with any geographical variables.

Table 4.7 Significant Pearson's correlation results (P<0.05, in parentheses) for correlations between the relative contribution of microbial food web components and phytoplankton to the total microplankton carbon biomass and geographical variables. No results fit within the Bonferroni adjustment level of 0.00096.

	Bare ground	Indigenous forest	Wetland size	Slope
Bacteria	0.38 (0.0028)		0.39 (0.0019)	0.26 (0.0452)
HNF		0.38 (0.0030)		
Ciliates				
Phytoplankton				

Differences among wetland types in the microbial food web and phytoplankton

Overall, total microplankton biomass ranged from highest in swamps and ponds, then estuaries, reservoirs, shallow lakes and deep lakes, to lowest in riverine wetlands (Table 4.8).

Table 4.8 Means of microplankton biomass (C μg L^{-1}±1SE) for wetlands. PP = prokaryotic picophytoplankton, EP = eukaryotic picophytoplankton, HNF = heterotrophic nanoflagellates.

Biomass	Deep lake	Estuarine	Reservoir	Riverine wetland	Shallow lake	Swamp/pond
Total biomass	169.6±36.07	511.8±112.82	390.4±97.12	104.7±49.64	294.6±38.49	746.6±266.79
Bacteria	59.3±9.13	132.0±41.32	76.9±12.39	41.3±20.99	118.0±20.50	131.2±33.30
PP	9.5±2.64	2.1±0.82	13.4±7.71	0.1±0.04	0.2±0.13	0.03±0.03
EP	7.3±6.69	0.9±0.47	0.7±0.34	0.2±0.12	1.5±0.64	0.01±0.01
HNF	21.9±6.99	139.4±68.06	69.6±18.10	24.1±19.06	55.7±15.22	27.5±7.62
Ciliates	3.7±2.62	8.0±2.57	6.3±2.98	5.2±4.22	6.9±2.61	90.8±31.65
Phytoplankton	84.7±31.48	232.5±62.30	237.6±79.82	34.1±15.59	114.0±22.54	497.1±237.11

Multivariate analysis

Principal components analysis shows the distribution of the samples for each wetland type and the direction of the microbial food web variables (Fig. 4.6). The ordinations indicate some separation in the wetland types. Swamps and ponds cluster positively with the ciliate and phytoplankton ordinations (arrows in Fig 4.6, Right panel), and strongly negatively with the PP ordination (arrow in Fig 4.6, Right panel), whereas deep lakes cluster strongly with the PP ordination and negatively with ciliate and phytoplankton ordinations. No clear distribution patterns are evident for the other wetland types.

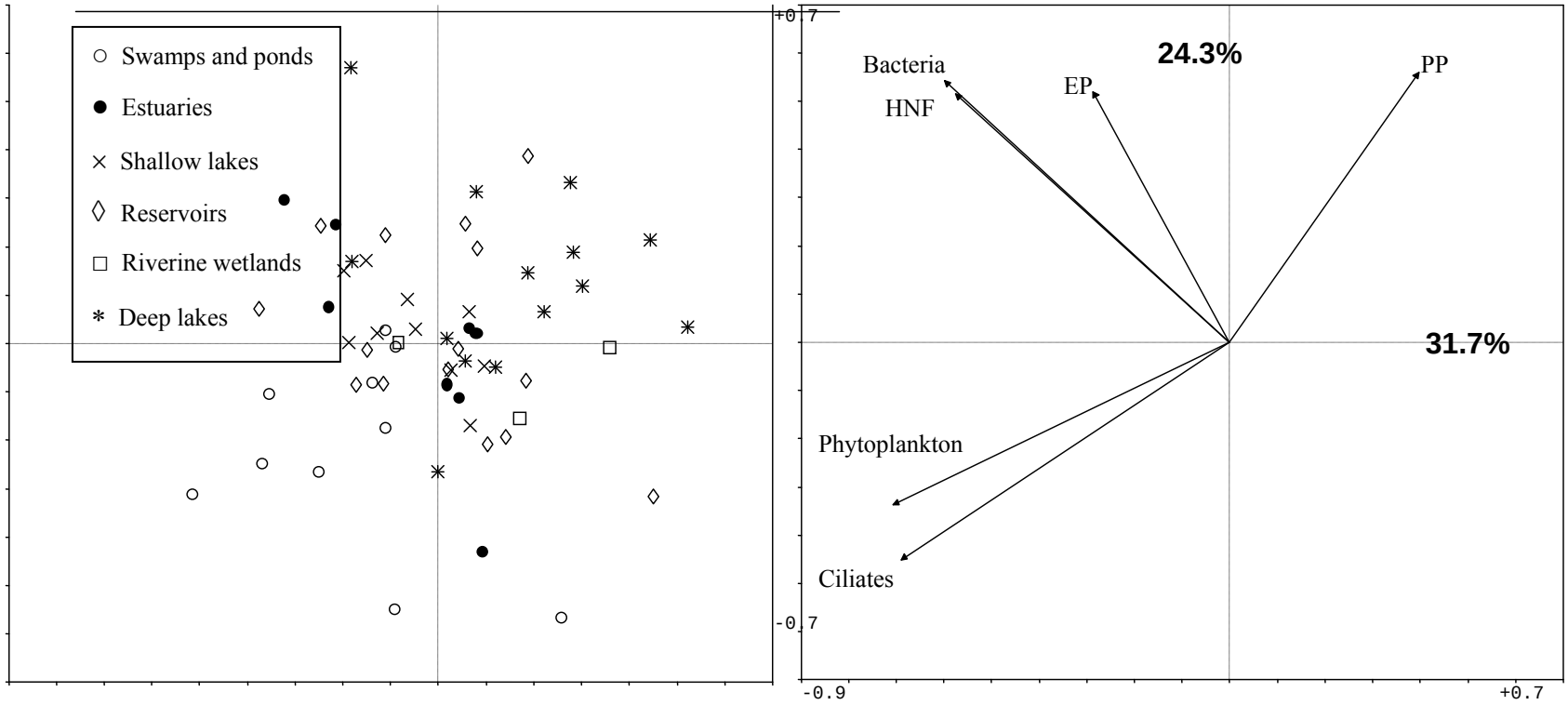


Figure 4.6 Ordination diagram of PCA of microbial food web and phytoplankton biomass showing sites by wetland. Left panel. Wetland sites, with symbols denoting the different wetland types. Right panel (Fig 4.3). Arrows indicate microbial food web and phytoplankton biomass relationship. Percentages refer to the percent variance explained by each axis. PP = prokaryotic picophytoplankton, EP = eukaryotic picophytoplankton, HNF = heterotrophic nanoflagellates.

Differences among wetland types in the relative contributions of the microbial food web and phytoplankton

The relative contribution of bacteria to the total microplankton carbon biomass was higher in deep lakes than in estuaries, reservoirs and swamps and ponds, and in riverine wetlands than in estuaries, reservoirs, shallow lakes and swamps and ponds (Table 4.9). Even shallow lakes had a higher proportion of bacterial biomass than in swamps and ponds. The contribution of HNF to the total microplankton carbon biomass was higher in estuaries than in swamps and ponds. Conversely, the contribution of ciliates to the total microplankton carbon biomass was higher in swamps and ponds than in all other wetland types. The relative contribution of phytoplankton was not different among wetland types.

Table 4.9 a. Relative contribution (mean %±1 SE) of microbial food web components and phytoplankton to the total microplankton carbon biomass for each wetland type. b. Results (P-values) of *post hoc* comparisons (LSD) among means of relative contributions for each wetland type, after ANOVA.

a.

	Deep lakes	Estuaries	Reservoirs	Riverine wetlands	Shallow lakes	Swamps and ponds
Bacteria	46.1%±5.64	23.0%±3.66	24.7%±3.67	45.3%±17.54	39.8%±3.17	22.6%±6.16
HNF	13.3%±3.01	24.7%±10.29	17.7%±4.57	16.7%±7.19	18.9%±5.99	5.9%±2.54
Ciliates	1.5%±0.88	2.5%±1.13	1.9%±0.64	3.2%±1.86	3.5%±1.72	12.8%±4.94
Phytoplankton	39.1%±5.91	49.7%±9.61	55.7%±6.16	34.8%±19.19	37.7%±5.74	58.7%±6.90

b.

	Bacteria	HNF	Ciliates	Phytoplankton
Deep lake - Estuarine	0.0031			
Deep lake - Reservoir	0.0012			
Deep lake - Swamp/pond	0.0017		0.0005	
Estuarine - Riverine wetland	0.0482			
Estuarine - Swamp/pond		0.0223	0.0045	
Reservoir - Riverine wetland	0.0499			
Reservoir - Shallow lake	0.0464			
Reservoir - Swamp/pond			0.0006	
Riverine wetland - Swamp/pond	0.0424			
Shallow lake - Swamp/pond	0.0419		0.0061	

Differences between seasons in the microbial food web and phytoplankton

The distribution of the samples (sites) for autumn and spring, and the direction of the microbial food web and phytoplankton biomass variables were analysed by principal components analysis (Fig. 4.7). The scatter of samples has arrows to show how the samples have changed between the seasons. There are no clear trends of change in the distribution of samples between seasons, either for components of the microbial food web or for phytoplankton biomass.

ANOVA did not reveal a difference in microplankton biomass between seasons.

Differences between seasons in the relative contributions of the microbial food web and phytoplankton

ANOVA did not reveal a difference between seasons in the relative contributions of the microbial food web and phytoplankton to total microplankton carbon biomass.

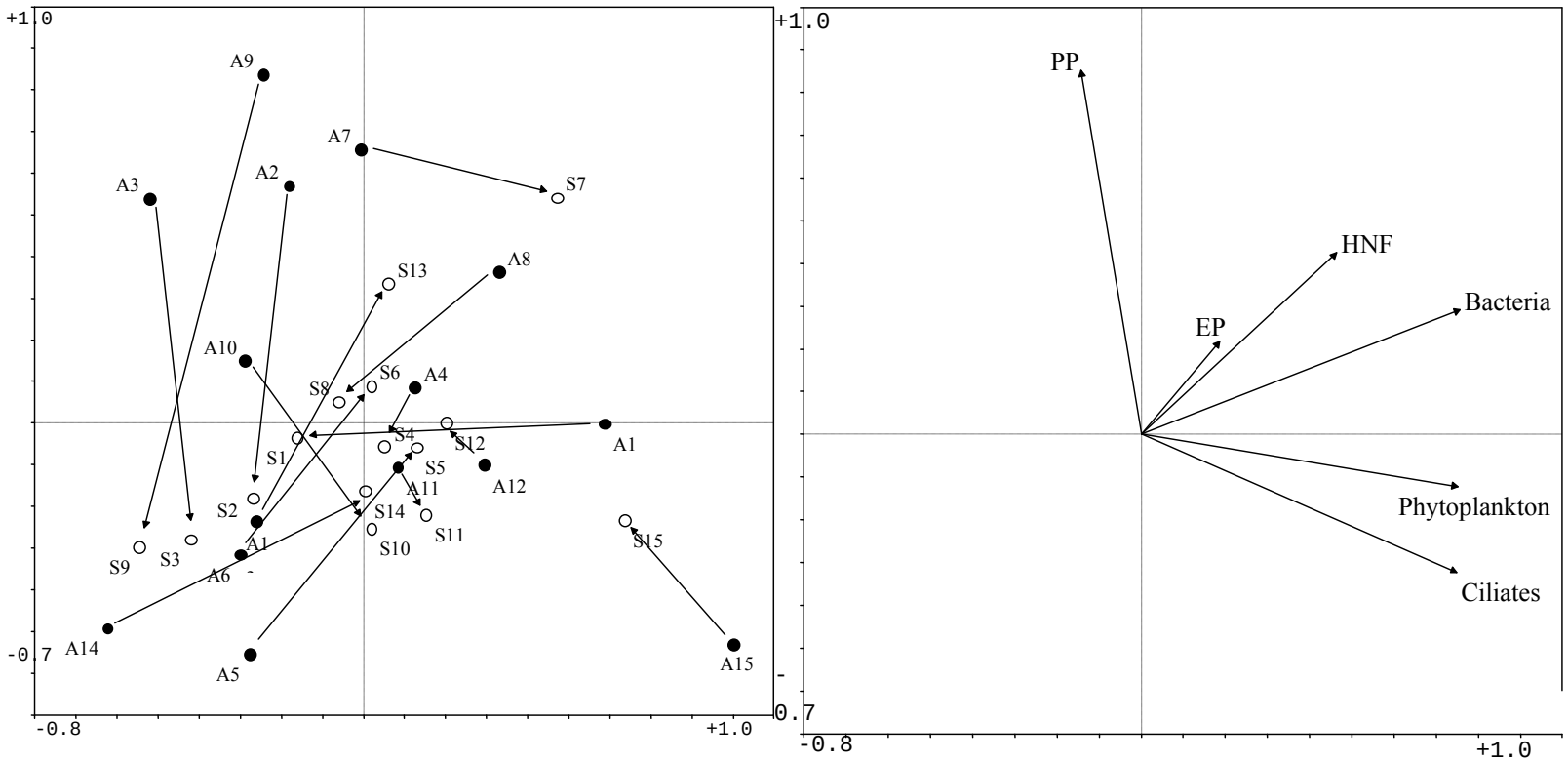


Figure 4.7 Ordination diagrams of PCA of microbial food web and phytoplankton biomass showing samples (sites). Left panel. A=autumn samples and S=spring samples, numbers identify each wetland; arrows denote the change between autumn and spring for each wetland. Right panel. Ordination diagram for the principal components analysis of biomass of microbial food web and phytoplankton for the 15 wetland sites that were sampled in both seasons. Arrows show the loading of each variable on the two canonical axes. PP = prokaryotic picophytoplankton, EP = eukaryotic picophytoplankton, HNF = heterotrophic nanoflagellates.

Discussion

Microbial food webs and phytoplankton

My prediction that the biomasses of components of microbial food webs would be tightly correlated with their potential resources and consumers in adjacent trophic levels was partially confirmed in that bacteria correlated positively with HNF biomass, ciliates correlated positively with phytoplankton, and ciliates and phytoplankton correlated negatively with PP across a diverse range of 45 wetlands. However, there were no correlations between ciliates and HNF, or ciliates and bacteria.

As HNF are capable of grazing substantially upon bacterial populations (Fenchel 1982, Bloem and Bär-Gilissen 1989), a negative correlation between bacteria and HNF might have been expected. For example, Hwang and Heath (1997a) observed that HNF abundance correlated negatively with bacterial abundance and productivity at oligotrophic offshore sites in Lake Erie; in *in situ* enclosure experiments in ultra-oligotrophic Lake Wakatipu, New Zealand, the abundance of heterotrophic bacteria was negatively related to flagellate abundance (Burns and Schallenberg 1998). However, in my study there was no indication that HNF biomass was related to lower concentrations of bacteria. Even when eutrophic wetlands were excluded, the relationship between bacteria and HNF in the remaining 32 wetlands (deep lakes, reservoirs and riverine wetlands) was still positive (Pearson correlation = 0.58, n=32, P=0.0004).

HNF depend upon resource supply (bacteria and phytoplankton), and are preyed upon by ciliates, rotifers and mesozooplankton (Riemann and Christoffersen 1993). The positive correlation between HNF biomass and biomass of heterotrophic bacteria, and lack of correlation with PP, EP or phytoplankton biomass in my study is consistent with bacteria being a major resource for HNF. These results support the generalisation that HNF biomass is determined primarily by abundance of heterotrophic bacteria, or that HNF and heterotrophic bacteria depend on the same, or a tightly coupled, resource, e.g. phytoplankton for HNF and exudates of phytoplankton for bacteria. Other studies have shown positive correlations between HNF and bacterial abundances and biomass across a range of wetlands (Berninger *et al*. 1991, Hwang and Heath 1997b, Auer and Arndt 2001, Auer *et al.* 2004). Berninger *et al*. (1991) analysed samples from 108 freshwater systems (lakes, ponds, rivers and bogs) worldwide, including New Zealand's volcanic lakes, and observed positive correlations

between the abundance of HNF and bacteria. Auer and Arndt (2001) and Auer *et al.* (2004) who sampled 55 north German lakes of varying trophic status, report that bacteria and HNF biomasses were positively correlated. In these lakes, HNF biomass was positively correlated also with ciliate biomass which they proposed was due to HNF and ciliates being controlled by the same factors, either predation or resource supply. Positive correlations between HNF and bacterial biomass, and HNF and ciliate biomass were also reported in offshore and coastal sites in Lake Erie, U.S.A. (Hwang and Heath 1997b). In contrast, in the Otago lakes of my study, HNF biomass was not related to ciliate biomass, either positively as in Lake Erie and in lakes in Germany (Hwang and Heath 1997b, Auer and Arndt 2001, Auer *et al.* 2004) or negatively, as might be expected from strong grazing pressure from ciliates, as reported by Weisse *et al*. (1991) in Lake Constance, Germany. Gasol *et al*. (1995) who sampled 16 lakes in Quebec monthly over the summer found no relationship between HNF and abundance of ciliates, and concluded that cladocerans (especially *Daphnia*) were suppressing HNF populations. Negative relationships between HNF and *Daphnia* may be due to the ability of this large cladoceran to feed directly on HNF, and potentially to suppress the HNF population through predation, as observed by Gasol *et al*. (1995) and Weisse (1991a) in Lake Constance, 1987-89. In most studies, HNF biomass is negatively related to cladocerans (Christoffersen *et al*. 1993, Jürgens and Stolpe 1995, Gasol *et al.* 1995, Mathes and Arndt 1994, Carrias *et al*. 1998, Auer and Arndt 2001, etc). For example, in a study of many lakes, Auer and Arndt (2001) and Auer *et al.* (2004) also reported that HNF biomass was negatively related to cladocerans. *Ceriodaphnia* had a negative effect on HNF growth in ultra-oligotrophic Lake Wakatipu (Burns and Schallenberg 1998), and *Daphnia* and *Ceriodaphnia* had negative effects on HNF populations in *in situ* enclosures in four lakes, ranging from ultra-oligotrophic to eutrophic in Otago, New Zealand (Burns and Schallenberg 2001). *Ceriodaphnia* is known to graze on heterotrophic bacteria (Thouvenot *et al*. 1999, Porter 1984), and PP (Porter *et al*. 1983). Grazing experiments by Toth and Kato (1997) demonstrated that *Bosmina longirostris* had a significant impact on the bacterial community, especially on larger, dividing and rod bacteria; in Mississipi, U.S.A., grazing by *Bosmina longirostris* on bacteria in Tuesday Lake was equivalent to grazing by *Daphnia pulex* in Paul Lake (Vaqué and Pace 1992). *Bosmina* and *Ceriodaphnia* may become the dominant zooplankton in systems where *Daphnia* are preyed upon by fish (Jeppesen *et al*. 1992) and, whereas the smaller cladocerans still prey on bacteria, HNF and ciliates, the predation does not keep pace with the production rate of their

prey (Riemann and Christoffersen 1993), accounting for the positive correlations between the predator and prey. However, in my study there were no significant relationships observed between HNF biomass and crustacean zooplankton (Chapter 7, Zooplankton).

PP biomass correlated negatively with ciliate biomass, which may suggest that predation by ciliates is suppressing the biomass of PP. In Lake Taupo, New Zealand, James *et al*. (1995) also observed negative correlations between PP and ciliate biomass, and confirmed, in grazing experiments, that PP was an important food source to ciliates. In other studies also, ciliates have been shown to significantly graze PP (Rassoulzadegan *et al*. 1988, Sherr *et al*. 1991). In ocean transects off the South Island, New Zealand, James and Hall (1995) found positive correlations between ciliate and PP abundance and suggested that PP was a resource for ciliates in the marine environment; there was no evidence of suppression of the standing stock of PP. My study included much more productive systems, however. As PP populations are suppressed by increases in nutrients and contaminants (Munawar and Munawar 1987, Munawar and Weisse 1989, Weisse 1991b), the negative correlation between ciliate biomass and PP biomass that I recorded may be a consequence of ciliates correlating positively, and PP correlating negatively, with nutrients. Burns and Stockner (1991) found negative correlations between PP abundance and chlorophyll *a* in six lakes in the South Island, New Zealand. In *in situ* enclosure experiments, Burns and Schallenberg (1996) showed that additions of nutrients (N and P) inhibited PP growth, and they recorded decreased PP growth rates in response to P addition (Burns and Schallenberg 2001).

Positive correlations of ciliate biomass and total phytoplankton biomass have been observed in previous studies (Pace 1986, Gasol *et al*. 1995, James and Hall 1995, Burns and Schallenberg 1996, Hwang and Heath 1997b, Jeppesen *et al*. 2000). Pace (1986) reported that ciliate biomass and abundance were highly positively correlated to chlorophyll *a* concentration in 12 sites, in 10 lakes of varying trophic status, Quebec. Gasol *et al*. (1995), James and Hall (1995) and Hwang and Heath (1997b) also observed positive correlations between ciliate abundance and chlorophyll *a* concentration. In three North Island and three South Island lakes, New Zealand, ciliate abundance was closely positively related to chlorophyll *a* concentration (r^2 = 0.87, P = 0.0046, Burns and Schallenberg 1996). Jeppesen *et al*. (2000) noted a positive correlation between ciliate abundance and chlorophyll *a* concentration in 25 shallow New

Zealand lakes, five of which were included in my study. The positive correlation may indicate that, by grazing heavily on PP, ciliates are releasing larger algae, which were a greater proportion of the total phytoplankton biomass in my study, from competition for nutrients and light. This outcome would be consistent with my observation of a negative relationship between PP and ciliates, and that of James *et al*. (1995) in Lake Taupo. The possibility that ciliate and phytoplankton biomasses are simultaneously suppressed by zooplankton grazing appears unlikely as neither correlated negatively with zooplankton abundance (Chapter 7, Zooplankton). Alternatively, ciliates may be grazing on larger phytoplankton, but not suppressing phytoplankton biomass due to growth rates of phytoplankton exceeding the predation rate of ciliates. In addition, phytoplankton release exudates which stimulate bacterial growth, in turn stimulating the growth of HNF (which may also graze directly upon PP and release larger phytoplankton). Ciliates feed upon bacteria, HNF and algae (Fenchel 1987); therefore, ciliates may also be grazing upon the increased bacteria and HNF, thereby reducing suppression on phytoplankton biomass. Neither biomass of bacteria nor HNF correlated with ciliate biomass in my study, due possibly to an inability of ciliates to suppress rapidly growing bacterial or HNF biomass, and the diversity of their prey. Gasol *et al*. (1995) also found that while ciliate abundance correlated positively with chlorophyll *a* concentration, ciliate abundance did not correlate with HNF. Alternatively, James and Hall (1995) and Hwang and Heath (1997b) did observe correlations between ciliate and bacterial abundance and between ciliate and HNF abundance in addition to positive correlations between ciliate abundance and chlorophyll *a* concentration.

Physicochemical variables

My predictions that biomasses of the microbial food web components and phytoplankton would correlate positively with concentrations of nutrients, and picophytoplankton would correlate negatively, were partially confirmed. Ciliate and phytoplankton biomass associated positively with measurements of DOC, DRP, TP, TN, turbidity, and water colour, and negatively with Secchi depth, and PP biomass associated negatively with measurements of DOC, DRP, TP, TN, turbidity, and water colour, and positively with Secchi depth. My prediction of a positive correlation between bacteria and their nutrients/substrates was not confirmed. However, the positive correlation between HNF biomass and DOC concentrations and water colour, concurs with the suggestion that substrates are stimulating the "bacterial-to-

HNF" pathway. Although this was not supported by correlations between bacteria and substrates, the increase in HNF may be a result of their consuming increased bacterial production, without affecting the bacterial standing stock across the wetlands (Bird and Kalff 1984).

Two possible explanations for relationships between microbial food web components and phytoplankton and physicochemical variables are: either, increases in concentrations of nutrients may have stimulated phytoplankton growth, which, in turn, stimulated the biomass of ciliates that consume phytoplankton, with ciliate communities thriving by bypassing the microbial food web in the more productive systems, or, bacterial production increases in response to increased concentrations of nutrients/substrates and phytoplankton, but is immediately grazed by HNF (and/or ciliates and zooplankton), which, in turn, are grazed by ciliates, and the microbial food web is contributing to higher trophic levels. However, these explanations are not mutually exclusive: both may be operating in a single lake or, one or the other may dominate in different lakes or, there may be seasonal changes in the dominance of one of the explanations.

Negative correlations between PP biomass and physicochemical variables may be due to a detrimental effect of high concentrations of nutrients (Weisse 1991b, Burns and Schallenberg 1996, 2001).

Relationships between the relative contributions of the microbial food web and phytoplankton and physicochemical variables

As I predicted, the relative contribution of the microbial food web components (bacteria, HNF and ciliates) as a percentage of total microplankton (microbial food web and phytoplankton) expressed as carbon biomass (TMCB) correlated negatively with concentrations of some physicochemical variables. The proportion of heterotrophic bacteria was the microbial food web component that correlated strongest, negatively, with concentrations of physicochemical variables (TP, TN, DRP, DOC, turbidity and TSS). Thus, in more oligotrophic conditions bacteria formed a larger percentage of the TMCB than in more eutrophic wetlands. The percentage of HNF and ciliates to TMCB were not correlated with any physicochemical variables in my study, and a possible explanation for the lack of a negative relationship (due to the lower proportion of bacteria) is that HNF and ciliate communities may be compensating by grazing more heavily on phytoplankton in more productive systems. Further taxonomic

analysis of the HNF and ciliate community might support this proposal, i.e., the relative proportion of those protozoa which are known to consume phytoplankton might correlate positively with nutrients/substrates. In this study most ciliate genera correlated positively with measures of physicochemical variables (Chapter 5, Ciliates).

Stockner and Porter (1988) and Weisse (1991b) suggest that the microbial food web as a source of carbon to higher trophic levels will be more important in oligotrophic systems than in eutrophic systems. Oligotrophic systems have lower primary productivity, with higher proportions of picoplankton rather than algae, requiring the protozoa and microzooplankton trophic levels to enable carbon transfer from picoplankton to crustacean zooplankton. In more eutrophic systems primary productivity is higher, with a greater proportion of algae providing the source of carbon directly to crustacean zooplankton. My finding of a greater proportion of bacteria in oligotrophic wetlands and a greater proportion of phytoplankton in eutrophic wetlands is consistent with these proposals. However, the lack of relationships between percentages of HNF and physicochemical variables and percentages of ciliates and physicochemical variables implies that whether the microbial food web is more, or less, important is not relevant, because the food web structure simply adapts, with protozoa still contributing to higher levels even in eutrophic systems. This may occur by protozoa consuming different resources, and/or variations in protozoan community structure across a gradient of wetland trophy.

My finding of a negative correlation between the bacterial proportion of carbon biomass and physicochemical variables needs to be interpreted cautiously, as the ratio of bacterial production (to total production) may actually increase with increased lake trophy, while predation pressure increases simultaneously, thus leaving the standing biomass of bacteria the same (Bird and Kalff 1984). Production rates of bacteria need to be measured to interpret the importance to carbon transfer of either the microbial food web or the classical food chain. For example, in seasonal measurements over 2 years in Lake Kinneret, Israel, the ratio of bacterial productivity to chlorophyll *a* productivity increased with primary production, but grazing maintained bacteria biomass at the same level throughout (Hart *et al*. 2000).

Geographical variables

Land use and geographical features influenced the physicochemical variables in the wetlands (Chapter 3, Land use). There was strong support for my prediction that the influence of land use and geographical features would be evident in the aquatic community, with increased

catchment development, and decreased wetland area, catchment area and slope resulting in increased growth of heterotrophic microplankton and phytoplankton, and declines in PP.

Relationships between the relative contribution of the microbial food web and phytoplankton and geographical variables

There was support for the prediction that the importance of the microbial food web pathway would increase in wetlands in unmodified catchments, as the percentage of bacterial carbon of the TMCB correlated positively with the percentage of bare ground in the catchment. Also, the relative contribution of HNF correlated positively with percentage of indigenous forest in the catchment. However, there were no relationships to provide evidence that the microbial food web is less important in carbon flow in more modified catchments or with varying geographical features.

Wetland types

As I predicted, the biomasses of ciliates and phytoplankton were highest in swamps and ponds, and lowest in deep lakes, while the opposite was true of PP biomass. This is probably a consequence of swamps and ponds being the most eutrophic wetland types, whereas deep lakes were the most oligotrophic (Chapter 3, Land use). However, no clear differences in biomass of the microbial food web or phytoplankton were observed for other wetland types that fell within the extreme trophic states of deep lakes and swamps/ponds.

Differences among wetland types in the relative contributions of the microbial food web and phytoplankton

The bacterial carbon percentage of the TMCB was highest in the oligotrophic deep lakes and riverine wetlands than other wetland types, consistent with the prediction that in deep lakes the relative contribution of the microbial food web to TMCB would be the greatest. The high percentage of bacterial carbon in riverine wetlands, despite these wetlands not being as oligotrophic as deep lakes, might be due to unsuitable conditions for phytoplankton in riverine wetlands (thus increasing the bacterial proportion of the TMCB). This suggestion is supported by the observation that the mean bacterial biomass in riverine wetlands was 70% of that in deep lakes, whereas the mean phytoplankton biomass in riverine wetlands was only 40% of that in deep lakes. Contrary to my predictions, the relative contribution of phytoplankton was

not different among wetland types, however the structure of the phytoplankton community differed between seasons (Chapter 6, Phytoplankton).

Seasonality

Contrary to my predictions, there was no detectable difference in the biomass of microbial food web components and phytoplankton between seasons. In the northern hemisphere, there is often a spring phytoplankton bloom, which is followed by a late spring peak in the microbial food web, as the warmer weather and phytoplankton exudates allow for optimal bacterial growth (Laybourn-Parry 1992). Grazing pressure from metazooplankton is usually still low in spring, but begins to affect the microbial food web and phytoplankton biomass in early summer, leading to the "clear water phase". However, in New Zealand, the phytoplankton standing biomass does not necessarily reach a peak in spring. New Zealand has a temperate and oceanic climate, without sustained extreme temperatures, and with high average wind speeds, leading to an absence of thermal stratification in many lakes. Often, year-round growth of phytoplankton supports over-wintering zooplankton populations, which may quickly consume any rapidly growing phytoplankton in spring (Malthus and Mitchell 1990).

In my study, sampling was conducted in late summer when temperatures were highest, and in mid-spring. Phytoplankton biomass correlated negatively with temperature, which is consistent with the possibility of increased growth of phytoplankton in spring, even though no difference was observed in phytoplankton biomass between seasons. It is possible that a spring phytoplankton pulse had not yet reached its peak during the sampling period.

The biomass of the microbial food web components was also not higher in spring than summer. I might have expected the peak biomass of the microbial food web to follow a peak biomass of phytoplankton, due to the growth of bacteria on phytoplankton exudates, and the consumption of phytoplankton by HNF and ciliates. However, if the phytoplankton biomass had not yet peaked during the sampling period, an increase in the biomass of the microbial food web would not yet have occurred.

Chapter 5 Ciliates

Introduction

Ciliates can play a major role in energy and matter transfer in pelagic food webs (Simek *et al*. 1995). With their rapid growth potential they may play an important role as grazers of bacteria and flagellates, providing a link to higher trophic levels (Stockner and Porter 1988). Resource availability will determine the growth potential of the ciliate community. In addition to ciliate communities being determined by resource supply (bottom-up), they may also have an effect upon their prey (top-down). Smaller ciliates, especially, consume picoplankton (bacteria and picophytoplankton), while larger ciliates may also feed upon algae, flagellates and other ciliates (Sherr and Sherr 1987, Laybourn-Parry 1992, Reimann and Christoffersen 1993). Ciliates may affect lower trophic levels directly via grazing, and indirectly by controlling populations that graze on lower trophic levels. For example, ciliates prey upon heterotrophic flagellates, and as heterotrophic flagellates graze on picoplankton, picoplankton populations are released from grazing pressure. Ciliates also consume algae, which contribute to the dissolved organic carbon pool that bacteria utilise for growth (Laybourn-Parry 1992), thus bacteria populations may potentially be indirectly suppressed by ciliate grazing.

Monthly sampling of Lake Oglethorpe, Georgia, USA, in 1979, revealed positive correlations between oligotrich ciliates and bacteria, and scuticociliates (15x30µm) and bacteria, while larger ciliate taxa correlated positively with chlorophyll *a,* suggesting that the smaller ciliates consumed bacteria, larger ciliates were consuming algae, but neither limited the resource (Pace 1982). James *et al*. (1995) compared ciliate communities in Lake Taupo and Lake Okaro, North Island, New Zealand, based on monthly sampling for 1 year 1990-1991. The results revealed that the abundance of ciliates was positively correlated with bacteria, and negatively with flagellates, in both lakes, from which it can be inferred that the ciliates consumed and limited the flagellate population, which in turn released the bacterial growth.

The abundance and biomass of ciliates has been shown to increase with lake trophy, in the USA (Pace 1982), Germany (Pfiister *et al*. 2001) and New Zealand (James *et al*. 1995,

Jeppesen *et al*. 2000, Burns and Schallenberg 2001). Burns and Schallenberg (2001) investigated consumer effects on protozoa on four lakes of different trophic status, ultra-oligotrophic Lake Wakatipu, oligotrophic Lake Manapouri, mesotrophic Lake Mahinerangi and eutrophic Lake Hayes, Otago, New Zealand, and found that the biomass of ciliates increased with increased lake trophy. In 25 lakes, South Island, New Zealand, Jeppesen *et al*. (2000) reported that total ciliates correlated positively with chlorophyll *a*. Pfiister *et al*. (2001) studied the abundance, biomass and community structure of ciliates quarterly, 1996-1997, in 58 lakes differing in morphology, water chemistry and trophy in north Germany, including shallow and deep lakes, brackish coastal lakes and peat ponds, ranging from mesotrophic to hypertrophic. They observed the highest ciliate abundance, biomass and number of species in eutrophic conditions, with the lowest in mesotrophic lakes. Lake type also influenced the community with the lowest ciliate biomass in deep, freshwater lakes, and the highest ciliate biomass in peat ponds due to their high primary productivity.

Community structure may also depend on lake trophy. In two lakes in New Zealand of contrasting trophic state, ciliate abundance in eutrophic Lake Okaro was dominated by the oligotrichs, *Halteria, Strombidium* and *Strobilidium* whereas in oligotrophic Lake Taupo ciliate abundance was dominated by small ciliates less than 20µm: *Strobilidium, Urotricha* and *Pseudobalanion* (James *et al*. 1995).

Additionally, because metazooplankton consume ciliates, top-down effects may influence the growth and structure of the ciliate community. Jeppesen *et al*. (2000) reported that total ciliate abundance correlated negatively with the abundance of *Daphnia*. In *in situ* enclosures in mesotrophic Lake Mahinerangi, New Zealand, *Daphnia* and *Boeckella* had negative effects on total ciliate growth (Burns and Schallenberg 1996). The effect of *Boeckella* was stronger than that of *Daphnia,* and *Boeckella* had a greater effect on large oligotrichs (>20µm) than small oligotrichs (<20µm).

More information is needed on ciliate community structure in New Zealand across a wide range of wetland types and trophic states. The aims of my study were to determine, in 45 wetlands representative of a range of wetland environments in Otago, the relationships between ciliate biomass and community structure and, 1) microbial food web biomass, 2) physicochemical variables, and 3) geographical variables. Ciliate biomass and community structure among wetland types were also determined.

I predicted that ciliate biomass and the number of taxa would correlate positively with, a) biomass of heterotrophic bacteria, flagellates and phytoplankton; b) concentrations of nutrients and substrate; c) increasing modification of the catchment (proportion of land cover greater in pasture, planted forest, scrub and urban areas, rather than in bare ground, water, indigenous forest, and tussock), and with decreasing catchment area, slope, and wetland area; d) a gradient of wetland types from deep lakes to swamps and ponds. Because some zooplankton prey on ciliates I predicted that the ciliate biomass and number of taxa would correlate negatively with crustacean zooplankton abundance. Because of the dominance of relatively large ciliates in eutrophic Lake Okaro and small ciliates in oligotrophic Lake Taupo (James *et al*. 1995), I predicted that the percentage of small ciliates to the total ciliate carbon biomass would correlate negatively with: i) concentrations of nutrients and substrate; ii) catchment modification, and with decreasing catchment area, slope and wetland area; iii) a gradient of wetland types from deep lakes to swamps and ponds. I predicted also that ciliate biomass and community structure will change between autumn and spring.

Methods

Refer to Methods (chapter 2, page 9)

As the genera *Strobilidium, Strombidium* and *Halteria* were counted separately, they are not included in the biomass of "oligotrichs", which refers to other oligotrichs that were not identified to genera.

Table 5.1 Sizes (μm) of ciliate taxa from all the wetlands included in the study.

Small prostomatids	Large prostomatids	Very small oligotrichs	Small oligotrichs	Large oligotrichs	Very large oligotrichs	*Strobilidium*	*Strombidium*
<20	>20	<10	10-20	20-40	>40	21 x 17	25 x 17
						25 x 15	32 x 17
						32 x 21	42 x 21
						46 x 21	53 x 32
							63 x 42
Halteria	Peritrichs	Hypotrichs	*Litonotus*	Gymnostomatids	*Urocentrum*	Heterotrichs	Unidentified ciliates
15 x 15	13 x 13	21 x 17	25 x 21	25 x 8	25 x 21	116 x 42	25 x 21
17 x 17	15 x 15	21 x 21	53 x 21	42 x 42	32 x 21		32 x 25
21 x 21	17 x 8	32 x 17		63 x 63	42 x 21		42 x 42
30 x 30	19 x 19	32 x 32			42 x 32		84 x 42
32 x 32	21 x 21	74 x 42					
	25 x 17						
	25 x 21						
	32 x 32						
	42 x 11						
	63 x 63						

Data analysis

Ciliate biomasses were $\log_{(10)}$ transformed as the data were not normally distributed. Several multivariate analyses were carried out: detrended correspondence analysis, redundancy analysis, and principal components analysis were performed using CANOCO (v. 4.0) software. Redundancy analysis was used as a detrended correspondence analysis determined that gradient lengths were less than 4 standard deviations (ter Braak and Šmilauer 1998). The influence on ciliate biomass of: measures of physicochemical variables, biomass of biological variables (heterotrophic bacteria, picophytoplankton, flagellates and phytoplankton), abundance of zooplankton, and geographical variables, were investigated using redundancy analysis, with forward selection to assess the statistical significance of independent variables. Pearson's correlation analysis was used to examine direct relationships. Differences in wetland types and seasons were examined using principal components analysis. ANOVA and Pearson's correlation analysis were performed with SPSS (v. 10.1) software.

Results

The contributions of ciliate taxa to total ciliate carbon biomass varied considerably across the wetlands (Fig. 5.1). There was also a gradient of increasing total ciliate carbon biomass in the wetlands, ranging from 0.041µg C L^{-1} in Mount Martin Reservoir in autumn, to 254.0µg C L^{-1} in Waitepeka Swamp in autumn.

Relative contribution of ciliate taxa to total ciliate carbon biomass

Within the ciliate community, oligotrichs were the dominant contributors to total ciliate carbon biomass (Fig. 5.2). Prostomatids, *Strobilidium, Strombidium,* peritrichs and hypotrichs were also dominant in some wetlands.

Relationships among ciliate taxa

Multivariate analysis

Principal components analysis showed that 54.9% of the variance in the ciliate data could be explained by relationships among ciliate taxa (Fig. 5.3). All size classes of prostomatids, most size classes of oligotrichs (except larger than 40µm), peritrichs, *Halteria,* and *Litonotus* were positively related, and were most strongly associated with the first axis. *Strombidium* and *Strobilidium,* other oligotrichs (larger than 40µm) and unidentified ciliates were positively related, and negatively related to *Urocentrum* and hypotrichs, which were positively related to each other. Heterotrichs and gymnostomatids were not significantly associated with any other ciliate taxa.

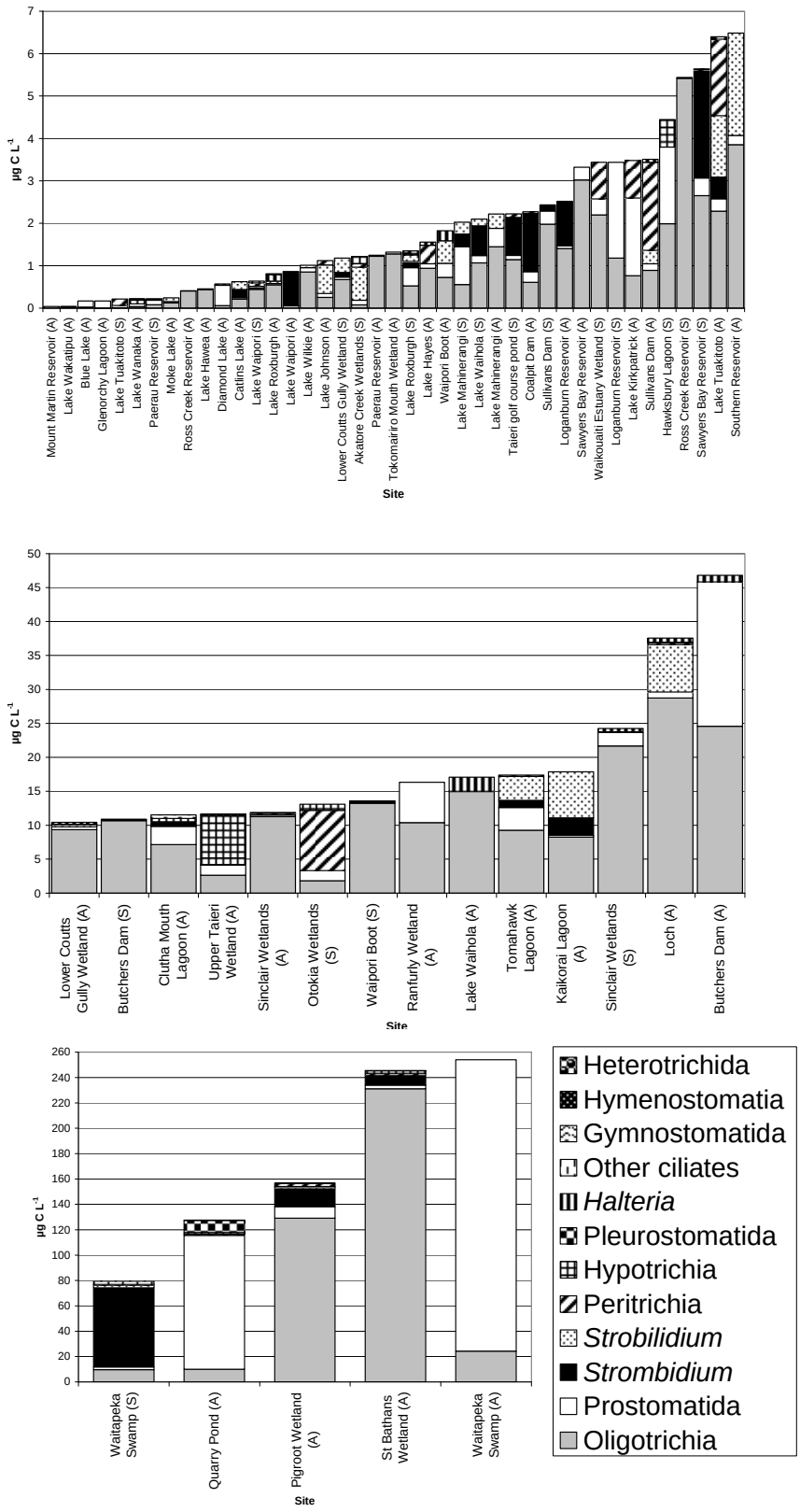


Figure 5.1 Ciliate biomass, expressed as µg C L^{-1}. Sites with total biomass: a. below 10 µg C L^{-1}, b. below 50 µg C L^{-1}, c. Sites with total biomass above 50 µg C L^{-1}.

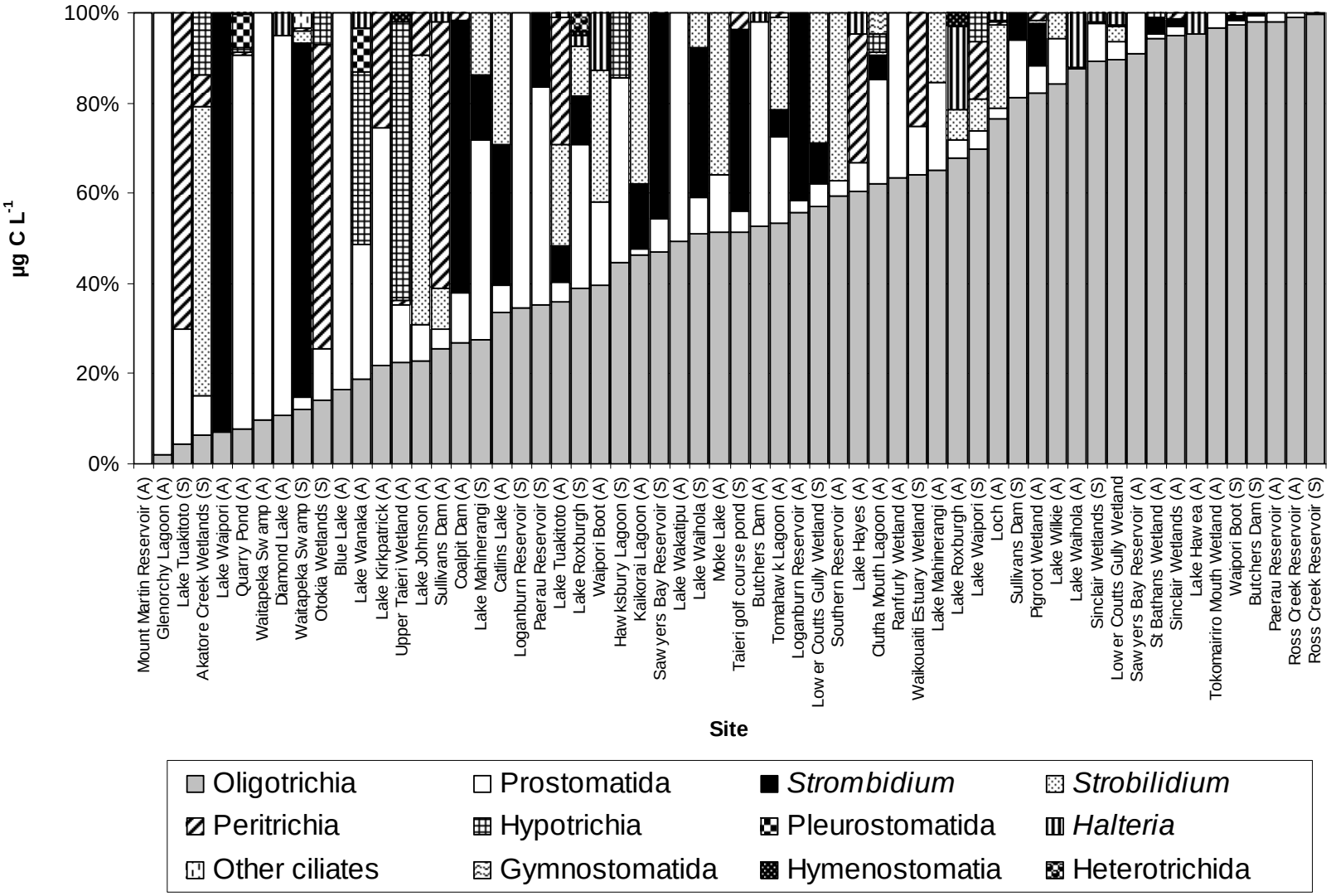


Figure 5.2 Percentage of contributions of ciliate taxa to total ciliate carbon biomass in the wetlands.

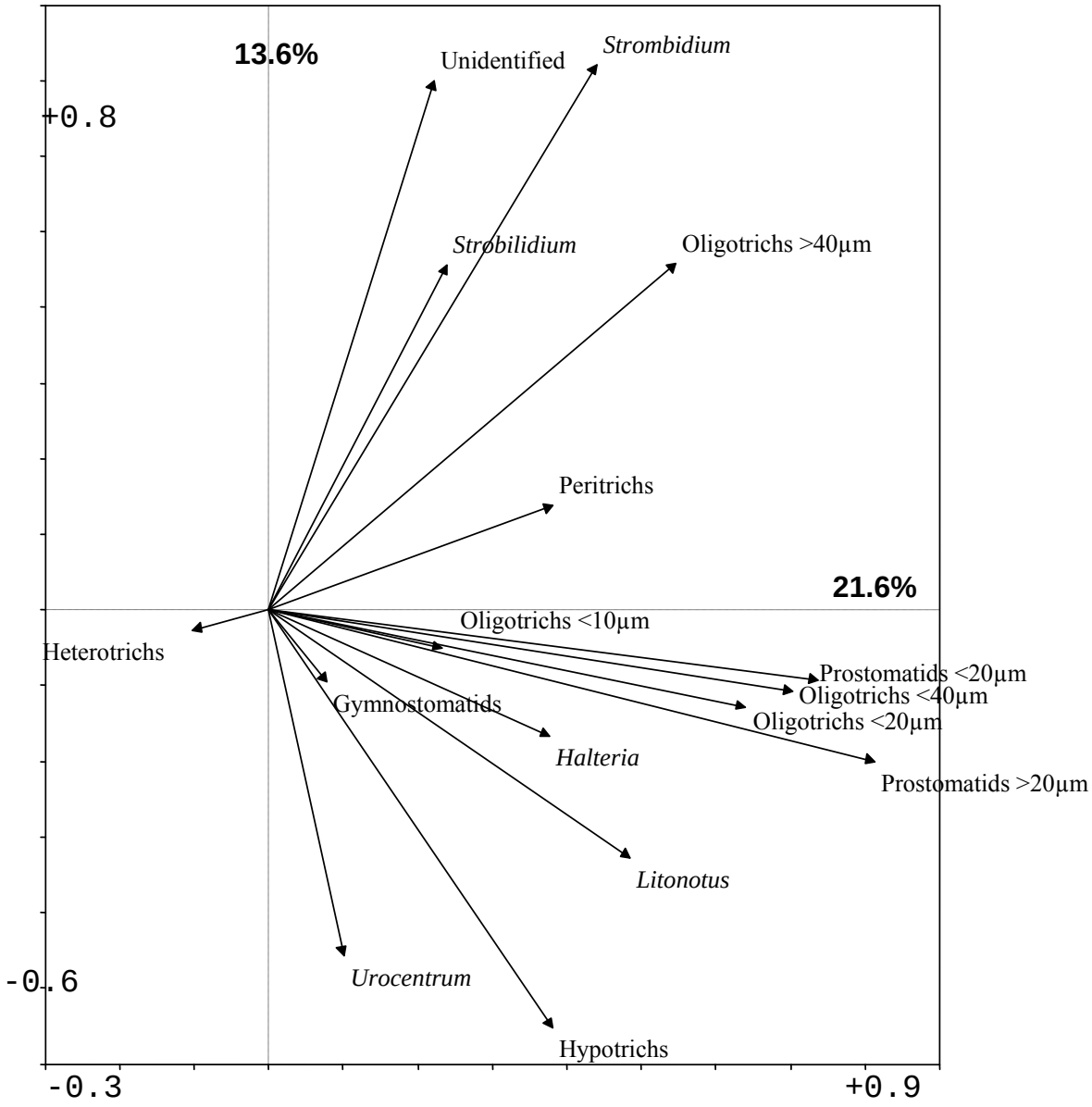


Figure 5.3 Ordination diagram for the principal components analysis of biomass of ciliate taxa. Arrows show the loading of each variable on the two canonical axes. Percentages refer to the percent variance explained by each axis.

Correlation analysis

Among ciliate taxa significant correlations in biomass were all positive (Table 5.2). All correlations were between individual taxa. The biomasses of heterotrichs and gymnostomatids were not correlated with any other ciliate taxa. The biomasses of both size classes of prostomatids correlated with those of oligotrichs (except oligotrichs less than 10μm), hypotrichs, and *Litonotus*. Oligotrich biomasses correlated with biomasses of prostomatids, *Halteria, Strobilidium, Strombidium,* and peritrichs. *Strobilidium* biomass correlated with *Strombidium* biomass. *Strombidium* biomass correlated with peritrichs biomass. Biomass of hypotrichs correlated with those of *Litonotus* and *Urocentrum*.

Table 5.2 Significant Pearson's correlation results (P<0.05, in parentheses) for correlations among ciliate taxa. The results in bold fit within the Bonferroni adjustment alpha level of 0.00018.

Biomass	Prostomatids <20µm	Prostomatids >20µm	Total Prostomatids	Oligotrichs <10µm	Oligotrichs 10-20µm	Oligotrichs 20-40µm	Oligotrichs >40µm	Total Oligotrichs	*Halteria*	*Strobilidium*	*Strombidium*	Peritrichs	Hypotrichs	*Litonotus*	*Urocentrum*	Unidentified	Total ciliates
Prostomatids <20µm		**0.76** (**0.0000**)	**0.86** (**0.0000**)	0.38 (0.0029)	0.41 (0.0013)	0.26 (0.0443)	0.40 (0.0017)	0.42 (0.0008)						0.39 (0.0020)			**0.62** (**0.0000**)
Prostomatids >20µm			**0.98** (**0.0000**)		0.43 (0.0006)	**0.47** (**0.0001**)	0.42 (0.0008)	**0.53** (**0.0000**)					0.25 (0.0499)	**0.55** (**0.0000**)			**0.71** (**0.0000**)
Total Prostomatids					**0.47** (**0.0001**)	0.46 (0.0002)	0.42 (0.0008)	**0.55** (**0.0000**)					0.27 (0.0348)	**0.52** (**0.0000**)			**0.74** (**0.0000**)
Oligotrichs <10µm																	
Oligotrichs 10-20µm						**0.57** (**0.0000**)		**0.71** (**0.0000**)	0.36 (0.0052)								**0.66** (**0.0000**)
Oligotrichs 20-40µm								**0.89** (**0.0000**)	**0.49** (**0.0001**)		0.28 (0.0300)		0.29 (0.0247)				**0.79** (**0.0000**)
Oligotrichs >40µm								**0.54** (**0.0000**)			**0.50** (**0.0001**)					0.32 (0.0127)	**0.55** (**0.0000**)
Total Oligotrichs									0.45 (0.0003)	0.29 (0.0252)	0.45 (0.0003)	0.26 (0.0410)					**0.93** (**0.0000**)
Halteria																	0.38 (0.0024)
Strobilidium											0.35 (0.0067)						0.31 (0.0170)
Strombidium												0.30 (0.0214)				**0.70** (**0.0000**)	**0.49** (**0.0001**)
Peritrichs													0.27 (0.0376)				0.38 (0.0028)
Hypotrichs														0.31 (0.0165)	**0.78** (**0.0000**)		0.33 (0.0111)
Litonotus																	0.32 (0.0118)
Urocentrum																	
Unidentified																	
Total ciliates																	

Relationships between ciliates, the microbial food web and phytoplankton

Multivariate analysis

Redundancy analysis showed that other components of the microbial food web and phytoplankton biomass explained a total of 14.7% of the variation in the ciliate taxa data (Table 5.3). The first axis explained 9.2% of the variation in ciliate taxa (P=0.025). The variable most strongly positively loaded with this axis was phytoplankton 6% (P=0.005) (Fig. 5.4). PP biomass was negatively associated with this axis. The ciliate taxa most strongly positively loaded with the first axis included prostomatids, oligotrichs, peritrichs, hypotrichs and *Litonotus*.

The second axis only explained a further 3.7% of the variation in the ciliate data. Bacterial biomass was the variable that loaded most strongly with this axis, at 5% (P=0.085), negatively. *Strombidium* was the ciliate taxon most strongly associated with this axis, also negatively.

Thus, the analysis clearly shows that the strongest relationship amongst ciliate taxa and other components of the microbial food web and phytoplankton is the biomass of oligotrichs which was closely positively related to phytoplankton biomass, and closely negatively related to PP. No ciliate taxa appeared to relate to the biomass of heterotrophic bacteria. *Strombidium* biomass was closely associated with HNF biomass.

Correlations

Correlation analysis showed that biomasses of peritrichs and *Litonotus* were negatively correlated with bacterial biomass (Table 5.4). Total ciliate biomass was negatively correlated with PP biomass and *Halteria* biomass was positively correlated with EP biomass. Biomass of oligotrichs (less than 10µm) correlated positively with that of HNF (less than 5µm), and PNF. Oligotrichs biomass (10-20µm) and *Strobilidium* biomass correlated positively with HNF biomass (5-8µm).

Prostomatids (all sizes), oligotrichs biomass (less than 20µm) and total ciliates were positively correlated with phytoplankton biomass.

The number of ciliate taxa in the wetlands did not correlate with the biomass of any other microbial food web components or phytoplankton.

Table 5.3 Results of redundancy analysis of biomass of ciliate taxa and microbial food web and phytoplankton biomasses.

Axes	1	2	3	4	Total variance
Eigenvalues :	0.092	0.032	0.019	0.005	1
Species-environment correlations :	0.598	0.502	0.378	0.199	
Cumulative percentage variance					
of ciliate taxa data :	9.2	12.3	14.2	14.7	
of ciliate-mfw relation:	61.1	82.2	94.8	97.8	
Sum of all unconstrained eigenvalues					1
Sum of all canonical eigenvalues					0.15

**** Summary of Monte Carlo test ****

Test of significance of first canonical axis: eigenvalue = .092
F-ratio = 5.452
P-value = .0250

Test of significance of all canonical axes : Trace = .150
F-ratio = 1.908
P-value = .0250

Forward selection variable	LambdaA	P	F
Phytoplankton	0.06	0.005	3.68
Bacteria	0.05	0.085	2.9

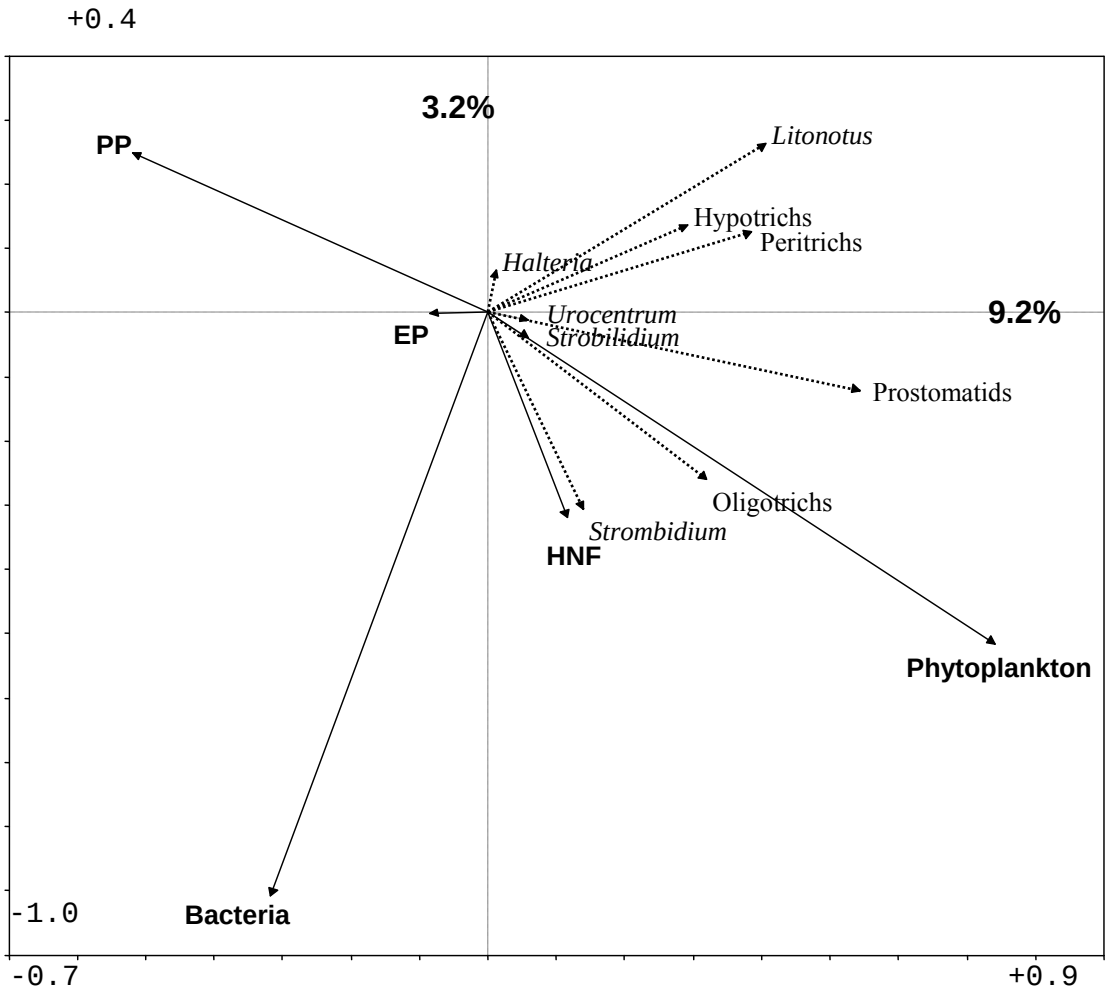


Figure 5.4 Ordination diagram for the redundancy analysis of biomass of ciliate taxa (dotted lines), microbial food web components and phytoplankton (solid lines). Arrows show the loading of each variable on the two canonical axes. Percentages refer to the percent variance explained by each axis.

Table 5.4 Significant Pearson's correlation results (P<0.05, in parentheses) for correlations between biomasses of ciliate taxa, microbial food web components and phytoplankton. The results in bold fit within the Bonferroni adjustment alpha level of 0.00035. Shaded values are negative correlations. PP = prokaryotic picophytoplankton, EP = eukaryotic picophytoplankton, HNF = heterotrophic nanoflagellates.

Biomass	Bacteria	PP	EP	HNF <5µm	HNF 5-8µm	PNF <5µm	PNF 5-8µm	Phytoplankton
Prostomatids <20µm								**0.50 (0.0000)**
Prostomatids >20µm								**0.46 (0.0002)**
Total Prostomatids								**0.51 (0.0000)**
Oligotrichs <10µm				**0.49 (0.0001)**		**0.78 (0.0000)**	**0.56 (0.0000)**	0.30 (0.0202)
Oligotrichs 10-20µm					0.29 (0.0233)			0.27 (0.0369)
Oligotrichs 20-40µm								
Oligotrichs >40µm								
Total Oligotrichs								0.35 (0.0060)
Halteria			0.28 (0.0287)					
Strobilidium					0.28 (0.0318)			
Strombidium								
Peritrichs	-0.26 (0.0473)							
Hypotrichs								
Litonotus	-0.36 (0.0043)							
Gymnostomatids								
Urocentrum								
Heterotrichs								
Unidentified								
Total ciliates		-0.33 (0.0106)						**0.49 (0.0001)**
Number of ciliate taxa								

Relationships between ciliates and zooplankton

Multivariate analysis

Redundancy analysis of ciliate biomasses and zooplankton abundance data was not significant.

Correlation analysis

The biomass of peritrichs correlated positively with *Daphnia* abundance, and that of oligotrichs 10-20µm correlated positively with *Ceriodaphnia* abundance (Table 5.5). The biomasses of hypotrichs and total ciliates correlated positively with adult copepods; those of prostomatids, oligotrichs 10-20µm, and total ciliates correlated positively with copepodite abundance. The biomasses of oligotrichs 10-20µm, and greater than 40µm, *Strombidium* and total ciliates correlated positively with nauplii abundance. The biomass of prostomatids greater than 20µm, total prostomatids, oligotrichs greater than 10µm, *Strombidium* and total ciliates correlated positively with total copepod abundance; that of oligotrichs 10-20µm correlated positively with *Bosmina* abundance.

The number of ciliate taxa in the wetlands did not correlate with the abundance of any zooplankton taxa.

Table 5.5 Significant Pearson's correlation results (P<0.05, in parentheses) for correlations between biomass of ciliate taxa and zooplankton abundance. Results in bold fit within the Bonferroni adjustment level of 0.00033.

	Daphnia	*Ceriodaphnia*	Adult copepods	Copepodite	Nauplii	Total copepods	*Bosmina*	Total Zooplankton
Prostomatids <20µm				0.27 (0.0369)				
Prostomatids >20µm				0.39 (0.0022)		0.26 (0.0440)		0.30 (0.0181)
Total prostomatids				0.40 (0.0015)		0.27 (0.0379)		0.29 (0.0259)
Oligotrichs <10µm								
Oligotrichs 10-20µm		0.27 (0.0383)		0.26 (0.0409)	0.26 (0.0442)	0.29 (0.0257)	0.28 (0.0314)	0.30 (0.0207)
Oligotrichs 20-40µm						0.29 (0.0239)		0.26 (0.0446)
Oligotrichs >40µm					0.33 (0.0100)	0.28 (0.0280)		
Total oligotrichs				0.27 (0.0378)	0.37 (0.0037)	0.38 (0.0026)		0.31 (0.0159)
Halteria								
Strobilidium								
Strombidium					0.33 (0.0096)	0.27 (0.0337)		
Peritrichs	0.30 (0.0202)							0.30 (0.0215)
Hypotrichs			0.27 (0.0373)					
Litonotus		0.35 (0.0065)						
Gymnostomatids								
Urocentrum								
Heterotrichs								
Unidentified								
Total ciliates			0.27 (0.0385)	0.39 (0.0018)	0.40 (0.0016)	**0.45 (0.0003)**		0.40 (0.0014)

Relationships between ciliates and physicochemical variables

Multivariate analysis

Redundancy analysis showed that the physicochemical variables explained a total of 26.9% of the variation in the ciliate taxa data (Table 5.6). The first axis explained 16.8% of the ciliate taxa. The physicochemical variable most strongly positively loaded with this axis was DRP 5% (P=0.015) (Fig. 5.5). Other variables positively associated with this axis included: TN, TP, DOC, turbidity and TSS. The ciliate taxa most strongly loaded with the first axis included prostomatids, oligotrichs, *Strombidium*, peritrichs, hypotrichs and *Litonotus*.

The second axis explained a further 6.1% of the ciliate data. The physicochemical variable most strongly loaded with this axis was water colour 3% (P=0.035), negatively. Conductivity was also negatively loaded with this axis. *Halteria* (negatively) and *Urocentrum* (positively) were the ciliate taxa most strongly associated with this axis.

Therefore, peritrichs, *Litonotus, Strombidium* and prostomatids appear to be the ciliate taxa strongest positively influenced by nutrient status of the wetland, while oligotrichs appear to be strongly positively related to dissolved organic carbon.

Table 5.6 Results of redundancy analysis of physicochemical variables and biomass of ciliate taxa.

Axes	1	2	3	4	Total variance
Eigenvalues :	0.168	0.061	0.022	0.018	1
Ciliate-physicochemical correlations :	0.781	0.566	0.539	0.401	
Cumulative percentage variance					
of ciliate data :	16.8	22.9	25.1	26.9	
of ciliate-physicochemical relation:	57.3	78.1	85.6	91.8	
Sum of all unconstrained eigenvalues					1
Sum of all canonical eigenvalues					0.293

**** Summary of Monte Carlo test ****

Test of significance of first canonical axis: P-value = .0100
Test of significance of all canonical axes : P-value = .0300

Forward Selection Variable	LambdaA	P
Temperature	0.06	0.005
TIN	0.06	0.005
DRP	0.05	0.015
Water colour	0.03	0.035

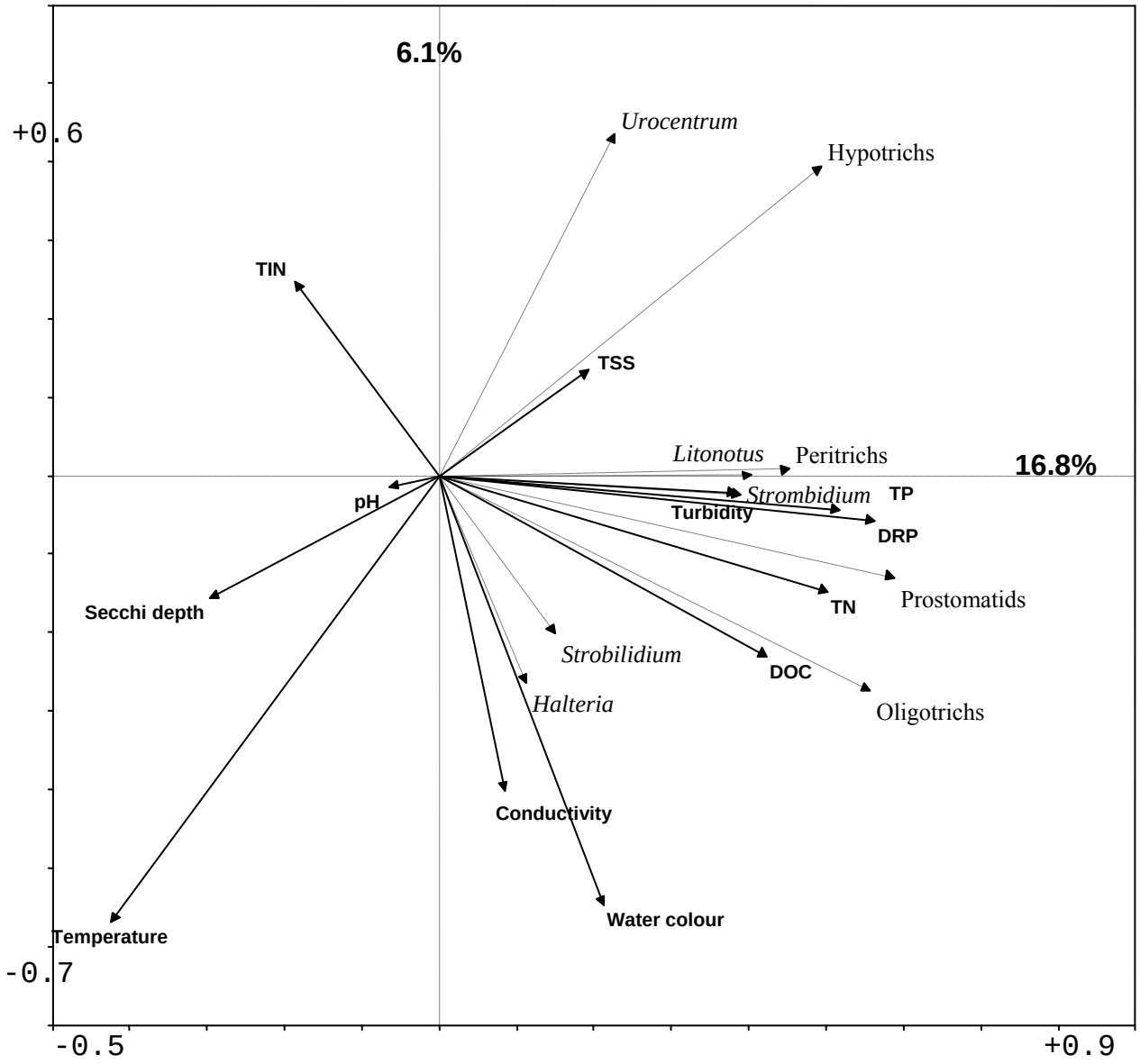


Figure 5.5 Ordination diagram for the redundancy analysis of biomass of ciliate taxa (dotted lines) and physicochemical variables (solid lines). Arrows show the loading of each variable on the two canonical axes. Percentages refer to the percent variance explained by each axis.

Correlation analysis

Correlation analysis showed that biomass of total ciliates correlated positively with measures of TP, TN, DRP, DOC, turbidity and water colour, and correlated negatively with Secchi depth (Table 5.7). Biomass of prostomatids less than 20µm correlated positively with measures of TN, DRP, DOC, and correlated negatively with Secchi depth. Biomass of prostomatids greater than 20µm correlated positively with measures of TN, DRP and TSS, and correlated negatively with Secchi depth. Total prostomatids biomass correlated positively with measures of TP, TN, DRP, DOC and, and correlated negatively with Secchi depth. Biomass of oligotrichs <10µm correlated negatively with measures of pH and temperature. Biomass of oligotrichs 10-20µm correlated positively with measures of DOC and water colour, and correlated negatively with Secchi depth. Biomass of oligotrichs greater than 40µm correlated positively with measures of DRP. Total oligotrich biomass correlated positively with measures of TN, DRP, DOC and water colour, and correlated negatively with Secchi depth. *Strobilidium* biomass correlated positively with temperature. *Strombidium* biomass correlated positively with measures of TN. Peritrichs biomass correlated positively with measures of TP, TN, DRP, DOC and turbidity. Hypotrichs and *Urocentrum* biomass correlated negatively with temperature, and *Urocentrum* also correlated negatively with water colour. Gymnostomatids biomass correlated positively with conductivity. Unidentified ciliates correlated positively with measures of TP and TN. *Halteria*, *Litonotus* and heterotrichs biomass were not significantly correlated with any of the physicochemical variables.

The number of ciliate taxa in the wetlands did not correlate with the measurements of any physicochemical variables.

Table 5.7 Significant Pearson's correlation results (P<0.05, in parentheses) for correlations between biomass of ciliate taxa and physicochemical variables. The results in bold fit within the Bonferroni adjustment of 0.00023. Shaded values are negative correlations.

Ciliate taxa	TP	TN	DRP	TIN	DOC	Turbidity	pH	Temperature	Conductivity	Secchi depth	TSS	Water colour
Prostomatids <20µm		0.29 (0.0252)	0.28 (0.0304)		0.27 (0.0399)					-0.48 (0.0034)		
Prostomatids >20µm		0.33 (0.0103)	0.29 (0.0274)							-0.42 (0.0115)	0.03 (0.8141)	
Total Prostomatids	0.27 (0.0429)	0.36 (0.0061)	0.31 (0.0187)		0.28 (0.0276)					-0.46 (0.0053)		
Oligotrichs <10µm							-0.37 (0.0035)	-0.28 (0.0329)				
Oligotrichs 10-20µm					0.27 (0.0372)					-0.38 (0.0237)		0.31 (0.0162)
Oligotrichs 20-40µm										-0.46 (0.0054)		
Oligotrichs >40µm			0.26 (0.0461)									
Total Oligotrichs		0.30 (0.0235)	0.31 (0.0194)		0.30 (0.0212)					-0.51 (0.0019)		0.30 (0.0183)
Halteria												
Strobilidium								0.29 (0.0239)				
Strombidium		0.30 (0.0202)										
Peritrichs	0.37 (0.0041)	0.33 (0.0104)	0.32 (0.0147)		0.30 (0.0206)	0.29 (0.0224)						
Hypotrichs								**-0.46 (0.0002)**				
Litonotus												
Gymnostomatids									0.31 (0.0171)			
Urocentrum								-0.29 (0.0230)				-0.29 (0.0267)
Heterotrichs												
Unidentified	0.31 (0.0198)	0.27 (0.0424)										
Total ciliates	0.37 (0.0048)	0.43 (0.0007)	0.40 (0.0018)		0.38 (0.0028)	0.27 (0.0357)				-0.57 (0.0004)		0.29 (0.0256)
Number of ciliate taxa												

Relationships between the relative contribution of ciliates, and physicochemical variables

Correlation analysis showed the percentage of prostomatids of the total ciliate carbon biomass (TCCB) greater than 20µm correlated negatively with temperature (Table 5.8). The percentage of oligotrichs greater than 20µm and the percentage of *Halteria* correlated positively with conductivity. The proportion of *Strombidium* correlated positively with concentrations of TP and TN. The percentage of peritrichs correlated positively with concentrations of TP, TN and DRP. The hypotrich percentage correlated negatively with temperature. The percentage of gymnostomatids correlated positively with conductivity. The proportion of *Urocentrum* correlated negatively with temperature and water colour. The percentage of unidentified ciliates correlated positively with concentrations of TP and TN.

Table 5.8 Significant Pearson's correlation results (P<0.05, in parentheses) for correlations between the relative contribution of ciliate taxa to the total ciliate carbon biomass and physicochemical variables. No results fit within the Bonferroni adjustment level of 0.00035. Shaded values are negative correlations.

	TP	TN	DRP	TIN	DOC	Turbidity	PH	Temperature	Conductivity	Secchi (cm)	TSS	Water colour
Prostomatids <20µm												
Prostomatids >20µm								-0.36 (0.0050)				
Oligotrichs <10µm												
Oligotrichs 10-20µm												
Oligotrichs 20-40µm									0.26 (0.0416)			
Oligotrichs >40µm									0.30 (0.0192)			
Halteria									0.29 (0.0240)			
Strobilidium								0.35 (0.0060)				
Strombidium	0.26 (0.0493)	0.28 (0.0343)										
Peritrichs	0.32 (0.0128)	0.27 (0.0399)	0.27 (0.0407)									
Hypotrichs								-0.39 (0.0022)				
Litonotus												
Gymnostomatids									0.31 (0.0171)			
Urocentrum								-0.28 (0.0314)				-0.31 (0.0166)
Heterotrichs												
Unidentified	0.31 (0.0198)	0.27 (0.0424)										

Relationships between ciliates and geographical variables

Multivariate analysis

Redundancy analysis performed on the ciliate taxa data and geographical variables was not significant.

Correlation analysis

Total ciliate biomass correlated negatively with catchment area, wetland area and slope, and correlated positively with the percentage of pasture in the catchment. Amongst ciliate taxa, prostomatids greater than 20µm were correlated negatively with the percentage of inland water and correlated positively with the percentage of pasture (Table 5.9). Total prostomatids were correlated negatively with the percentage of wetland area and slope. The biomass of oligotrichs 10-20µm correlated positively with the percentage of planted forest, and that of oligotrichs 20-40µm correlated negatively with the percentage of inland water. The biomass of oligotrichs greater than 40µm correlated negatively with catchment slope, while that of total oligotrichs correlated negatively with wetland area. *Strobilidium* biomass correlated negatively with catchment slope. *Urocentrum* biomass correlated positively with the percentage of tussock and catchment area. Heterotrichs correlated positively with catchment area. *Halteria*, *Strombidium*, peritrichs, hypotrichs, *Litonotus*, gymnostomatids and unidentified ciliates biomass were not significantly correlated with any of the geographical variables.

The number of ciliate taxa in the wetlands did not correlate with any geographical variables.

Table 5.9 Significant Pearson's correlation results (P<0.05, in parentheses) for correlations between ciliate taxa and geographical variables. No results fall within the Bonferroni adjustment level of 0.00023. Shaded values are negative correlations. Geographical variables not shown were not significantly correlated with ciliate taxa.

Ciliate taxa	Inland water	Planted forest	Pasture	Tussock	Catchment Area	Wetland area	Slope
Prostomatids <20µm							
Prostomatids >20µm	-0.27 (0.0353)		0.32 (0.0133)				
Total Prostomatids						-0.27 (0.0344)	-0.28 (0.0304)
Oligotrichs <10µm							
Oligotrichs 10-20µm		0.36 (0.0045)					
Oligotrichs 20-40µm	-0.33 (0.0107)						
Oligotrichs >40µm							-0.33 (0.0099)
Total Oligotrichs						-0.28 (0.0284)	-0.34 (0.0070)
Halteria							
Strobilidium							-0.35 (0.0058)
Strombidium							
Peritrichs							
Hypotrichs							
Litonotus							
Gymnostomatids							
Urocentrum				0.33 (0.0112)	0.31 (0.0172)		
Heterotrichs					0.40 (0.0014)		
Unidentified							
Total ciliates			0.35 (0.0066)		-0.29 (0.0275)	-0.33 (0.0095)	-0.34 (0.0084)
Number of ciliate taxa							

Relationships between the relative contribution of ciliates, and geographical variables

Correlation analysis showed the percentage of prostomatids greater than 20µm of the TCCB correlated negatively with the percentage of inland water in the catchment (Table 5.10). The percentage of oligotrichs 10-20µm correlated positively with the percentage of planted forest, whereas that of oligotrichs greater than 40µm correlated positively with the percentage of inland water, and negatively with catchment slope. The proportion of *Strobilidium* correlated positively with the percentage of urban area and negatively with catchment slope. The percentage of gymnostomatids correlated positively with catchment area. The relative contribution of *Urocentrum* correlated positively with the percentage of tussock. The percentage of heterotrichs correlated positively with catchment area.

Table 5.10 Significant Pearson's correlation results (P<0.05, in parentheses) for correlations between the relative contribution of ciliate taxa to the total ciliate carbon biomass and geographical variables. The results in bold fit within the Bonferroni adjustment level of 0.00026. Shaded values are negative correlations.

	Inland water	Planted forest	Tussock	Urban	Catchment area	Slope
Prostomatids <20µm						
Prostomatids >20µm	-0.31 (0.0148)					
Oligotrichs <10µm						
Oligotrichs 10-20µm		0.40 (0.0018)				
Oligotrichs 20-40µm						
Oligotrichs >40µm	0.35 (0.0063)					-0.29 (0.0233)
Halteria						
Strobilidium				**0.57 (0.0000)**		-0.26 (0.0435)
Strombidium						
Peritrichs						
Hypotrichs						
Litonotus						
Gymnostomatids					**0.65 (0.0000)**	
Urocentrum			0.28 (0.0274)			
Heterotrichs					0.40 (0.0014)	
Unidentified						

Differences among wetland types in ciliates

Total ciliate biomass ranged from highest in swamps and ponds, then estuaries, reservoirs, shallow lakes and deep lakes to lowest in riverine wetlands (Table 5.11).

Multivariate analysis

No clear relationships were determined between wetland type and the biomass of ciliate taxa using principal components analysis.

ANOVA

The mean biomass of prostomatids in swamps and ponds (35.8µg C L^{-1}) was higher than in all other wetland types. Oligotrichs 20-40µm mean biomass in swamps and ponds was higher than in riverine wetlands, reservoirs and deep lakes. The mean biomass of oligotrichs greater than 40µm in swamps and ponds was higher than in shallow lakes, reservoirs, estuaries and deep lakes. *Strobilidium* mean biomass in estuaries was higher than in shallow lakes and reservoirs. *Strombidium* mean biomass in swamps and ponds was higher than in shallow lakes, estuaries, reservoirs and deep lakes. The mean biomasses of peritrichs and hypotrichs in swamps and ponds were higher than in shallow lakes, estuaries, reservoirs and deep lakes. Total ciliate mean biomass in swamps and ponds was higher than in shallow lakes, estuaries and reservoirs. Gymnostomatids were only found in estuaries. Unidentified ciliates were only found in swamps and ponds.

The number of ciliate taxa in the wetlands was not different among wetland types.

Differences between seasons in ciliates

No clear relationships between seasons and the biomass of ciliate taxa were determined using principal components analysis or ANOVA.

Table 5.11 a. Means and standard errors of ciliate biomass (C µg L^{-1}) for wetlands. b. P-values of LSD *post hoc* tests of significance of means between wetland types, significant results shown only (P<0.05).

a.

Biomass (C µg L^{-1})	Deep lakes n=14	Estuaries n=8	Reservoirs n=15	Riverine wetlands n=3	Shallow lakes n=10	Swamps/ponds n=10
Prostomatids <20µm	0.06±0.024	0.15±0.067	0.07±0.034	0.05±0.036	0.04±0.028	0.34±0.111
Prostomatids >20µm	0.07±0.026	0.10±0.057	0.11±0.088	0.03±0.018	0.10±0.049	0.74±0.258
Total prostomatids	0.39±0.138	0.91±0.466	1.70±1.403	0.21±0.064	0.47±0.239	35.82±23.849
Oligotrichs <10µm	0.00±0.001	0.01±0.014	0.04±0.022	0	0.01±0.008	0.01±0.009
Oligotrichs 10-20µm	0.13±0.054	0.25±0.096	0.22±0.067	0.11±0.090	0.26±0.125	0.31±0.084
Oligotrichs 20-40µm	0.16±0.098	0.48±0.157	0.35±0.099	0.04±0.030	0.38±0.124	0.78±0.218
Oligotrichs >40µm	0	0	0.01±0.013	0.44±0.344	0.12±0.084	0.46±0.227
Total oligotrichs	2.46±2.023	4.65±1.494	3.86±1.640	4.65±4.293	5.46±2.435	42.16±24.327
Halteria	0.07±0.044	0.04±0.035	0.07±0.064	0.08±0.079	0.25±0.206	0.07±0.047
Strobilidium	0.61±0.494	1.50±0.854	0.18±0.161	0.19±0.174	0.18±0.142	0.31±0.218
Strombidium	0.03±0.023	0.57±0.318	0.35±0.191	0.03±0.034	0.23±0.101	8.61±6.179
Peritrichs	0.12±0.068	0.14±0.106	0.14±0.138	0	0.22±0.177	1.45±0.860
Hypotrichs	0.01±0.006	0.08±0.059	0	0	0.07±0.064	1.14±0.709
Litonotus	0.01±0.007	0	0	0.03±0.034	0.01±0.006	0.96±0.937
Gymnostomatids	0	0.07±0.067	0	0	0	0
Urocentrum	0.00±0.002	0	0	0	0	0.02±0.024
Heterotrichs	0.00±0.004	0	0	0	0	0
Unidentified	0	0	0	0	0	0.26±0.258
Total ciliates	3.70±2.618	7.95±2.573	6.31±2.983	5.19±4.220	6.89±2.611	90.80±31.647
Number of ciliate taxa	5.7±0.53	6.0±0.63	4.6±0.40	5.7±1.45	5.8±0.59	6.9±0.72

b.

	Swamp/pond - Shallow lake	Swamp/pond - Riverine wetland	Swamp/pond - Reservoir	Swamp/pond - Estuarine	Swamp/pond - Deep lake	Shallow lake - Estuarine	Riverine wetland - Deep lake	Reservoir - Estuarine	Estuarine - Deep lake
Prostomatids <20µm	0.0006	0.0212	0.0008	0.0344	0.0006				
Prostomatids >20µm	0.0005	0.0074	0.0002	0.0010	0.0001				
Total prostomatids	0.0134		0.0092	0.0209	0.0077				
Oligotrichs 20-40µm		0.0154	0.0218		0.0015				
Oligotrichs >40µm	0.0262		0.0018	0.0054	0.0016		0.0454		
Total oligotrichs	0.0132		0.0050	0.0167	0.0041				
Strobilidium						0.0423		0.0288	
Strombidium	0.0227		0.0142	0.0385	0.0122				
Peritrichs	0.0229		0.0087	0.0225	0.0084				
Hypotrichs	0.0121		0.0038	0.0192	0.0045				
Gymnostomatids				0.0432		0.0432		0.0290	0.0310
Total ciliates	0.0000		0.0000	0.0001					

Discussion

Ciliate taxa

The biomasses of most ciliate taxa were positively correlated with each other, implying that various ciliate taxa are increasing or decreasing in response to common factors, such as shared resources (for example, bacteria or algae), or similar environmental tolerances, or predators in common. However, the biomasses of *Urocentrum* and hypotrichs were negatively associated with other taxa, but positively correlated with each other, which may be explained by both taxa being negatively correlated to temperature in the wetland, unlike the other taxa sampled.

Microbial food web and phytoplankton

My prediction that ciliate biomass would correlate positively with the biomass of microbial food web components and phytoplankton was confirmed. There were positive relationships between the biomass of ciliates and flagellates, and between the biomass of ciliates and phytoplankton. However, the biomass of peritrichs and *Litonotus* (both taxa were usually less than 25µm in length) was negatively correlated with the biomass of bacteria suggesting that these ciliates may consume bacteria and effectively suppress bacterial populations. Many species of peritrichs are bacterivores (Foissner and Berger 1996). Only a few species of *Litonotus* feed on bacteria (Foissner and Berger 1996), however, *Litonotus* populations may have increased by preying on increased populations of other bacterivorous ciliates or flagellates, which may account for the negative correlation between *Litonotus* and bacteria. Subsequent increases in bacteria as they are released from predation by bacterivorous ciliates or flagellates either might not be detected during the infrequent sampling regime of my study, or *Litonotus* predation on other ciliate or flagellate populations might not have suppressed these populations. Support for the latter situation in my study includes the absence of negative relationships between *Litonotus* and other ciliates or between *Litonotus* and flagellates, to indicate suppression by *Litonotus*, and a report by Foissner and Berger (1996) of a highly significant correlation between bacterivorous prostomatids and *Litonotus.*

Peritrich consumption of bacteria was also the explanation that James *et al*. (1995) provided for the positive correlations between bacteria and peritrichs observed in monthly sampling for 1 year in two North Island, New Zealand, lakes. They found that the abundance of oligotrichs 20-30µm was positively correlated with bacteria in oligotrophic Lake Taupo, and the abundance of *Vorticella* (20-40µm peritrich) was positively correlated with bacteria in

eutrophic Lake Okaro. *Vorticella* feed on bacteria (James *et al*. 1995, Foissner and Berger 1996), thus *Vorticella* in Lake Okaro were probably consuming, while not limiting, bacterial population growth. Pace (1982) sampled Lake Oglethorpe, Georgia, U.S.A., monthly and found clear positive correlations between small and large oligotrichs and bacteria, and scuticociliates (15x30µm) and bacteria. Pace surmised that the relationships were likely to be a result of the ciliates consuming bacteria (while not suppressing bacterial growth).

In my study, biomass of oligotrichs (less than 10µm) correlated positively with HNF (less than 5µm) biomass and PNF biomass, and biomass of oligotrichs (10-20µm) and *Strobilidium* (mostly 20-25µm length) correlated positively with HNF biomass (5-8µm). It is possible that the small oligotrichs depend upon the nanoflagellates as a resource, but do not limit the nanoflagellate growth. However, it is also possible that the small oligotrichs and HNF are both consuming bacteria. Foissner and Berger (1996) did not describe any oligotrich species as feeding on protozoa, while several are known to graze on bacteria. Grazing experiments by Sherr and Sherr (1987) showed that small ciliates were very effective at consuming bacteria.

Other studies also report positive correlations between ciliates and flagellates. For example, James *et al*. (1995) found that abundances of *Spirostomum* (325x32µm heterotrich) were positively correlated with flagellates in eutrophic Lake Okaro, while *Strombidium* (47-51x37-43µm oligotrich) abundance was positively correlated with flagellates in oligotrophic Lake Taupo.

The biomasses of prostomatids, oligotrichs, and total ciliates were positively related to phytoplankton biomass. However, the relationships between ciliates and phytoplankton may be direct or indirect, i.e., the positive relationships between algae and the algivorous ciliates may be due to direct grazing, while bacterivorous ciliates may still be grazing upon bacterial populations that are enhanced by DOC exudates from algae. Bacterial biomass and biomass of peritrichs and *Litonotus* were negatively correlated, which may confirm that bacteria populations are being suppressed by ciliate grazing. My findings of strong positive relationships between phytoplankton biomass and ciliate biomass in New Zealand lakes concur with those of Burns and Schallenberg (1996) and Jeppesen *et al*. (2000). In three North Island and three South Island lakes, New Zealand, ciliate abundance was closely positively related to chlorophyll *a* concentration (r^2 = 0.87, P = 0.0046, Burns and Schallenberg 1996), while Jeppesen *et al*. (2000) found that the abundance of total ciliates

correlated positively with chlorophyll *a* in single samples over summer from 25 shallow, South Island, New Zealand, lakes. Pace (1986) reported that ciliate biomass and abundance were highly positively correlated to chlorophyll *a* concentration in 12 sites, in 10 lakes of varying trophic status in Quebec. Mathes and Arndt (1994) observed a positive correlation between ciliate biomass and chlorophyll *a* concentration in 19 north German lakes.

In studies within lakes, positive correlations between chlorophyll *a* and ciliates have been recorded. In Lake Oglethorpe, U.S.A., Pace (1982) reported that while *Lembadion magnum* and gymnostomes correlated with chlorophyll *a* alone, *Coleps* sp. (a prosotomatid 31x42µm), and scuticociliates correlated positively with both chlorophyll *a* and bacteria. Even though scuticociliates are bacterivorous, they correlate also with chlorophyll *a,* due to the close positive relationship between bacteria and chlorophyll *a.* Conversely, *Coleps* sp. feed on algae, which are also correlated with bacteria. However, in contrast to my findings, James *et al*. (1995) found that chlorophyll *a* was negatively correlated with abundance of *Askenasia,* total ciliate abundance and total ciliate biomass in Lake Taupo, but the authors concluded that this finding was due to chlorophyll *a* concentration peaking in winter when ciliate abundance was low.

Prokaryotic picophytoplankton biomass was negatively correlated with the biomass of total ciliates. While PP biomass did not correlate with those of particular ciliate taxa, multivariate analysis revealed that biomasses of oligotrichs were the most closely negatively related to PP biomass, thus oligotrichs may be capable of suppressing PP populations via grazing. Simek *et al*. (1996) investigated ciliate planktivory with grazing experiments in two different freshwater systems in the Czech Republic, one eutrophic and one oligo- to mesotrophic, with abundant autotrophic picoplankton. Whereas oligotrichs consumed both bacteria and PP, Simek *et al*. (1996) calculated that they were capable of meeting their energy requirements on PP alone. James *et al*. (1995) observed that the abundance of total ciliates, small ciliates (<20µm) and *Askenasia* (31µm long) correlated positively with picophytoplankton in Lake Taupo, and determined that picophytoplankton were an important food source for small ciliates in Lake Taupo. The positive correlation that James *et al*. (1995) reported, in contrast to my finding of a negative relationship, may have been due to small ciliates using picophytoplankton as a resource without suppressing picophytoplankton growth in Lake Taupo, whereas ciliates in my study suppressed PP growth across a range of wetlands. In ocean transects off South Island, New Zealand, James and Hall (1995) found positive

correlations between ciliate and PP abundance, and suggested that PP was a resource for ciliates in the marine environment; there was no evidence of suppression of the standing stock of PP. My study included much more productive systems, however. As PP populations are suppressed by increases in nutrients and contaminants (Munawar and Munawar 1987, Munawar and Weisse 1989, Weisse 1991b), the negative correlation between ciliate biomass and PP biomass that I recorded may be a consequence of ciliates correlating positively, and PP correlating negatively, with nutrients.

Zooplankton

My prediction that ciliate biomass would correlate negatively with crustacean zooplankton abundance was not confirmed, as correlations between ciliates and crustacean zooplankton were all positive. These positive correlations may have been due to resources in common, or a result of zooplankton grazing on ciliates without suppressing ciliate growth. The strongest relationship was between the biomass of oligotrichs and copepod abundance. The biomasses of prostomatids greater than 20µm, total prostomatids, *Strombidium,* hypotrichs and total ciliates all correlated positively with copepods. As copepod abundance correlated positively with phytoplankton biomass, and ciliate biomass correlated positively with phytoplankton biomass, it is possible that the positive correlation between ciliates and copepods is due to both ciliates and copepods consuming phytoplankton, rather than copepods preying directly on ciliates However, Burns and Gilbert (1993) observed that calanoid copepods fed efficiently on oligotrichs, and concluded that the copepods would be capable of suppressing populations of some ciliates. In *in situ* enclosure experiments in four South Island lakes, Burns and Schallenberg (1996, 2001) reported that grazing by *Boeckella* on ciliates had negative effects on total ciliate growth. Auer *et al.* (2004) suggested that ciliates were directly affected by grazing pressure from copepods in their survey of 55 north German lakes, when they observed that copepod biomass was negatively correlated to ciliate biomass. Thus, the positive correlations between biomass of ciliates and crustacean zooplankton abundance that I recorded could be a result of the zooplankton consuming ciliates, with my infrequent sampling missing the subsequent declines in ciliate biomass which may occur.

While the biomass of total ciliates in my study did not correlate with *Daphnia* abundance, peritrich biomass correlated positively with *Daphnia* abundance. The positive correlation may suggest that both organisms share a resource, without any negative effects of

competition occurring. Peritrichs are more likely to occur in more eutrophic environments (Foissner and Berger 1996), as is *Daphnia* in this study (Chapter 7 Zooplankton). However, other studies have found that *Daphnia* has suppressed ciliate growth (Porter *et al*. 1979, Pace and Funke 1991, Burns and Schallenberg 1996), either by predation, interference or competition for resources (Jack and Gilbert 1994). Burns and Schallenberg (1996) found negative effects of *Daphnia* on ciliate growth due to grazing. Thus, the positive correlations between *Daphnia* abundance and peritrich biomass in my study may be a consequence of increased growth of *Daphnia* by preying on peritrichs, with a decline in the peritrich population later as the *Daphnia* population increases.

The biomass of oligotrichs 10-20µm correlated positively with abundances of both *Ceriodaphnia* and *Bosmina*. These small cladocerans may be consuming the oligotrichs, without suppressing the population of ciliates. In *in situ* enclosure experiments in Lake Wakatipu, New Zealand, *Ceriodaphnia dubia* had a negative effect on ciliate growth rates (Burns and Schallenberg 1998). While I observed a positive correlation between oligotrichs and *Ceriodaphnia* in my study, again, the infrequent sampling period may have missed subsequent declines in ciliate biomass.

Burns and Schallenberg (1996) reported that *Boeckella* had a higher negative effect on ciliate growth than *Daphnia,* which is consistent with my findings of stronger correlations between copepods and ciliates than between *Daphnia* and ciliates. In other *in situ* enclosure experiments in South Island lakes, the rates of clearance of ciliates by copepods were higher than those of *Daphnia* (Burns and Schallenberg 2001). Burns and Schallenberg (2001) found that, as lakes became more eutrophic, copepods become more effective at exploiting ciliates as a food resource, and cladocerans become less effective at consuming ciliates. As suspension feeding cladocerans feed automatically, the cladoceran's food collection systems may become clogged by increased particles (e.g. phytoplankton) in the more eutrophic systems and, when the animal clears the particles away, ciliates are also rejected. In contrast, copepods are able to select ciliates from among other particles (Burns and Gilbert 1993). This may be due to cladocerans consuming *Strombidium,* and suppressing the growth of the ciliate, while copepods are more likely to be consuming phytoplankton, sharing a resource with *Strombidium,* rather than preying on the ciliate. My finding of a negative relationship between ciliates and cladocerans concurs with those of Jeppesen *et al*. (2000) which reported that densities of total ciliates correlated negatively with those of *Daphnia* in 25 shallow South

Island lakes, some of which were also included in my study. Jeppesen *et al*. (2000) did not observe any relationships between ciliates and copepods.

Physicochemical variables

The biomass of total ciliates, and most ciliate taxa, apart from *Halteria*, *Litonotus, Urocentrum* and heterotrichs, correlated positively with measures of TP, TN, DRP, DOC, turbidity and water colour, and correlated negatively with Secchi depth, which confirmed my prediction that ciliate biomass would correlate positively with measurements of nutrients and substrate. Increased concentrations of nutrients and substrates stimulated phytoplankton growth (Chapter 4, Microbial food web), thereby providing resources for ciliate growth. My findings were consistent with those of Burns and Schallenberg (2001) that the biomass of ciliates correlated positively with lake trophy in four lakes of different trophic status, one ultra-oligotrophic, two oligotrophic and one mesotrophic, in Otago, New Zealand. Pace (1982) reports that ciliate abundance increased with lake productivity in Lake Oglethorpe. In *in situ* enclosure experiments in Lake Mahinerangi, New Zealand, Burns and Schallenberg (1996) found total ciliate abundance did not change in response to nutrient enrichment, even though phytoplankton and bacterial abundance increased. However, HNF abundance had also not increased by the end of their four-day experiments, and it may be that the HNF and ciliate community had not yet responded to the enrichment.

Correlations between ciliate biomass and measures of physicochemical variables may be due to either increased concentrations of nutrients that stimulate phytoplankton growth, which, in turn, stimulate the biomass of ciliates that consume phytoplankton, with ciliate communities thriving by bypassing the microbial food web in the more productive systems, or, increased bacterial production in response to increased concentrations of nutrients/substrates and phytoplankton, that is immediately grazed by HNF (and/or ciliates and zooplankton), which, in turn, are grazed by ciliates. However, these explanations are not mutually exclusive: both may be operating in a single lake, or one or the other may dominate in different lakes, or there may be seasonal changes in dominance of one of the explanations.

Relationships between the relative contribution of ciliates and physicochemical variables

My prediction that the percentage of small ciliates to the TCCB would correlate negatively with measures of physicochemical variables, similar to the findings of James *et al*. (1995) that relatively large ciliates dominated in eutrophic Lake Okaro and small ciliates dominated in

oligotrophic Lake Taupo, was not confirmed. Jeppesen *et al*. (2000) also did not observe any correlation between the relative contribution of small ciliates and measures of physicochemical variables, reporting that the abundance of small oligotrichs (10-20µm) dominated the protozoa in almost all of the 25 shallow South Island lakes sampled, ranging from oligotrophic to hypertrophic. In my study, oligotrichs were most often the dominant contributors to total ciliate carbon biomass, but prostomatids, peritrichs and hypotrichs were also dominant in some wetlands.

My finding that the percentage of *Strombidium* correlated positively with concentrations of TP and TN is similar to that of Jeppesen *et al*. (2000) that *Strombidium* composed 84% of the abundance of protozoa in the most hypertrophic lake sampled, where trophy was defined by TP concentration.

Geographical variables

Land use and geographical features influenced measures of physicochemical variables in the wetlands (Chapter 3, Land use), and resulting relationships between physicochemical variables and phytoplankton were observed (Chapter 4, Microbial food web). Via physicochemical variables and phytoplankton, the influence of land use and geographical features were evident in the ciliate community, with increased catchment development, and decreased wetland area, catchment area and slope correlating with increased biomass of total ciliates, prostomatids, oligotrichs and *Strobilidium*. This correlation confirmed my prediction that the land use and geographic features would influence the ciliate community, indirectly. However, contrary to my predictions, *Urocentrum* biomass correlated positively with the percentage of tussock and catchment area, suggesting that *Urocentrum* biomass may be higher in undeveloped catchments.

Relationships between the relative contribution of ciliates and geographical variables

My prediction that the percentage of smaller ciliates correlated negatively, and thus larger ciliates correlate positively, with catchment modification was only partially confirmed. There were no significant relationships between the percentage contribution of smaller ciliates and land use. However, for the percentage of larger ciliates (oligotrichs 10-20µm, *Strobilidium*) correlated positively with catchment modification, whereas, contrary to my predictions, the percentage of *Urocentrum* correlated positively with the percentage of tussock. This is probably a consequence of these taxa having relationships with trophic state of the wetlands.

Wetland types

My prediction that the biomass of ciliate taxa would vary among wetland types was shown with swamps and ponds having greater mean biomass of total ciliates, including prostomatids, oligotrichs, *Strombidium,* peritrichs and hypotrichs, than most other wetland types. This outcome is likely to be a result of swamps and ponds being the most eutrophic wetland types, whereas deep lakes were the most oligotrophic (Chapter 3, Land use). This finding was consistent with those in 58 north German wetlands that included shallow and deep lakes, brackish coastal lakes and peat ponds, ranging from mesotrophic to hypertrophic, which were sampled quarterly (Pfiister *et al*. 2001). In these wetlands, the lowest ciliate biomass was recorded in deep, freshwater lakes, and the highest ciliate biomass was in peat ponds due to their high primary productivity.

Strobilidium and gymnostomatids were more abundant in estuaries than in other wetland types, which may indicate these taxa have a higher salinity tolerance than other ciliate taxa.

Seasonality

My prediction that ciliate biomass and community structure would change between autumn and spring was not confirmed. There was no difference in the mean biomass of ciliate taxa between seasons. Contrary to my results, Pfiister *et al*. (2001) recorded a strong influence of season, with lower abundance, biomass and numbers of species in early summer and higher in spring and autumn in north German lakes. However, northern Germany has more sustained extreme temperatures than New Zealand, with most lakes thermally stratified seasonally. A spring phytoplankton bloom in northern hemisphere temperate lakes is followed by a late spring peak in ciliates (Laybourn-Parry 1992). In New Zealand, there is often year-round growth of phytoplankton (Malthus and Mitchell 1990), to support both algivorous and bacterivorous ciliates over winter, as bacteria are also supported by phytoplankton exudates.

In my study, sampling was conducted in late summer when temperatures were highest, and in mid-spring. Despite not finding any differences in biomass of ciliate taxa between seasons, *Strobilidium* biomass correlated positively with temperature, suggesting that this genus reaches its peak population density in late summer, and biomasses of hypotrichs and *Urocentrum* correlated negatively with temperature, suggesting these taxa were more abundant

in spring. The percentage contribution of prostomatids greater than 20μm, hypotrichs and *Urocentrum* to the TCCB correlated negatively with temperature, implying that these taxa are more dominant in spring when temperatures are lower than in autumn.

Chapter 6 Phytoplankton

Introduction

Primary production is the basis of food webs, and in the open-water regions of wetlands phytoplankton (cyanobacteria and algae) is the dominant primary producer (Laybourn-Parry 1992). Therefore, phytoplankton productivity and community composition determines the structure and productivity of higher trophic levels in wetlands. In addition, excessive phytoplankton production (blooms), especially blooms of cyanobacteria which can produce toxins, may affect water quality. Phytoplankton biomass and community structure is determined by physicochemical conditions in the wetland (nutrients, carbon dioxide, light and temperature), and biological parameters (e.g. predation by zooplankton). In wetland systems, the main influences on phytoplankton community composition are attributed to nutrient status in oligotrophic wetlands, zooplankton grazing in mesotrophic wetlands, and light and carbon availability in eutrophic wetlands (Naselli-Flores 2000). Zooplankton graze selectively on phytoplankton, for example, *Daphnia* sp. consume picoalgae as well as phytoplankton, whereas copepods prefer larger particles (Reimann and Christoffersen 1993). Thus, whether the zooplankton community is dominated by cladocerans or copepods will affect the size and species composition of the phytoplankton community.

Understanding the influences on phytoplankton community structure and the functioning of wetlands in New Zealand is vital to detect and prevent their degradation. The potential of phytoplankton communities to provide information on wetland health has been studied by Cottingham and Carpenter (1998) who investigated the responses of freshwater phytoplankton populations (species), communities (genera, Divisions, size classes) and ecosystems (productivity, chlorophyll and biomass) to nutrient enrichment of lakes in Michigan, USA, and determined that the responses of some phytoplankton variates to nutrient enrichment could be used as environmental indicators of ecosystem conditions. Reynolds (2000) proposes functional group classifications of phytoplankton species or genera that may occur in a certain habitat, or within particular environmental parameters.

In New Zealand, Viner and White (1987) summarised data on phytoplankton community structure from studies of several North and South Island lakes, and made the

generalisations that the chrysophyte *Dinobryon* and diatoms *(Melosira* in the North Island lakes, and *Cyclotella* and *Synedra* in the South Island lakes) were the most widespread phytoplankters, occurring in a range of trophic states, but these were less common when cyanobacteria, mostly *Anabaena,* were present. Seasonal changes in biomass and abundance of phytoplankton have been observed in New Zealand lakes, with examples of North Island lakes showing chlorophyll peaks in winter, whereas phytoplankton biomass in South Island lakes often climaxes in summer, however, there was much variation in these patterns among lakes, and between years in single lakes (Viner and White 1987). Changes in phytoplankton community species composition in response to season were also variable among lakes and between years in single lakes, with no clear patterns in seasonal succession observed (Viner and White 1987).

The aims of my study were to determine in 45 wetlands representative of a range of wetland environments in Otago, the relationships between the abundance, biomass and community composition (genera and Divisions) of phytoplankton and: 1) physicochemical variables, 2) microbial food web biomass, 3) crustacean zooplankton abundance, and the relative contribution of copepods to total crustacean zooplankton abundance, and 4) geographical variables. The abundance, biomass and community composition of phytoplankton among wetland types were also determined (5).

I predicted that the abundance and biomass of phytoplankton genera and Divisions would relate positively to: a) measures of physicochemical variables, b) biomasses of microbial food web components, c) catchment modification (land cover greater in pasture, planted forest, scrub and urban areas, rather than bare ground, water, indigenous forest, and tussock), d) smaller catchments, e) less slope in the catchment, f) smaller wetland area, and g) along a gradient of wetland types ranging from deep lakes to swamps and ponds. I predicted that the abundance and biomass of phytoplankton genera and Divisions would relate negatively with, h) abundance of crustacean zooplankton, and the percentage of copepods of total crustacean zooplankton abundance. I predicted also that the abundance and biomass of phytoplankton genera and Divisions would be greater in autumn than in spring.

Methods

Refer to Methods (chapter 2, page 9)

Data analysis

Phytoplankton abundances were $\log_{(10)}$ transformed as the data were not normally distributed. Several multivariate analyses were carried out: detrended correspondence analysis, redundancy analysis, and principal components analysis were performed using CANOCO (v. 4.0) software. Redundancy analysis was used as a detrended correspondence analysis determined that gradient lengths were less than 4 standard deviations (ter Braak and Šmilauer 1998). The influence on phytoplankton abundance of: measures of physicochemical variables, the biomass of microbial food web components, the abundance of zooplankton, and geographical variables, was investigated using redundancy analysis, with forward selection to assess the statistical significance of independent variables. Pearson's correlation analysis was used to examine direct relationships. Differences in wetland types and seasons were examined using principal components analysis. ANOVA and Pearson's correlation analysis were performed with SPSS (v. 10.1) software.

Results

In the wetlands, 39 genera of phytoplankton were counted, encompassing the Divisions; bacillariophytes, chlorophytes, chrysophytes, cryptophytes, dinophytes, and euglenophytes (Table 6.1). There was a huge gradient of increasing phytoplankton biomass in the wetlands, ranging from 3.7µg C L^{-1} in Glenorchy Lagoon in autumn, to 2584.3µg C L^{-1}.in Waitepeka Swamp in autumn (Fig. 6.1). The number of phytoplankton genera counted ranged from 6 in Akatore Creek Wetlands in spring, Blue Lake in autumn and Butchers Dam in autumn, to 22 genera in Waitepeka Swamp in spring (Fig. 6.2).

Relationships among phytoplankton

Phytoplankton genera

PCA analysis revealed two distinct groupings of phytoplankton genera (Fig. 6.3). Abundance of the chlorophytes, *Staurastrum, Cosmarium, Tetrastrum, Tetraedron, Coelastrum, Schizochlamys* and *Scenedesmus,* and the chrysophyte, *Synura,* were positively associated with the first axis. Abundance of the euglenophytes, *Euglena* sp. 2 and *Phacus,* and the diatoms, *Asterionella* (colonies) and *Cyclotella,* were positively associated with the second axis.

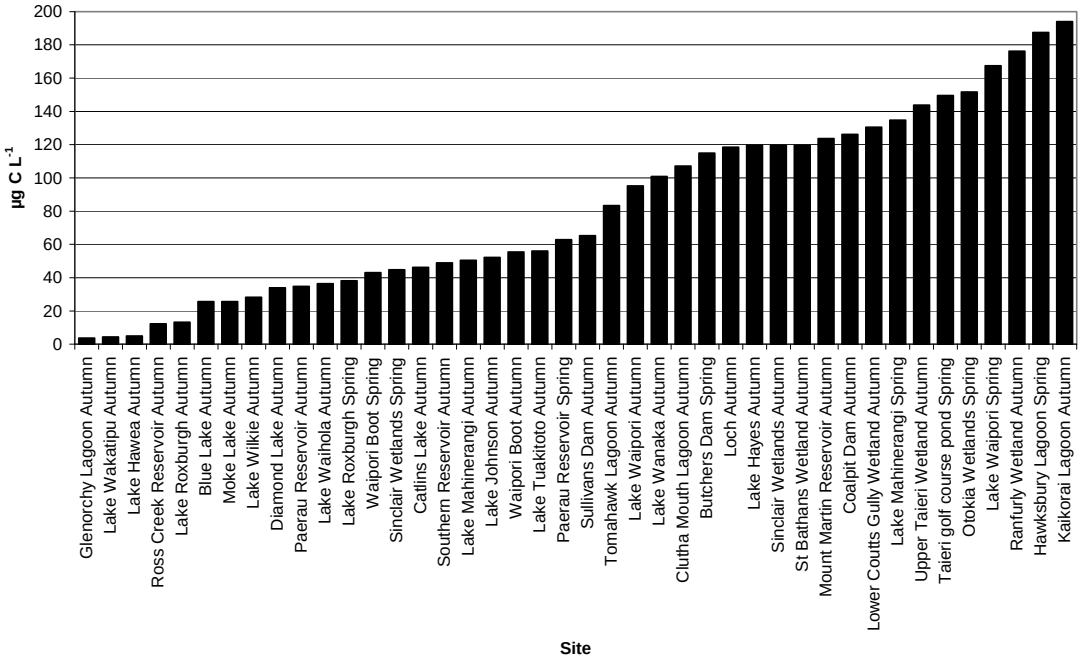
Phytoplankton Divisions

PCA analysis of phytoplankton Divisions explained 79.7% of the variance (Fig. 6.4). Abundance of diatoms, chlorophytes, cryptophytes and euglenophytes were positively associated with the first axis. Abundance of chrysophytes and dinophytes were negatively associated with the second axis, while the abundance of cyanobacteria was positively associated with the second axis.

Table 6.1 Phytoplankton taxa occurrences and abundance (cells ml^{-1}, and range) in wetlands.

Division	Description	Genera	Cells ml^{-1} Mean±SE	Cells ml^{-1} Range	No. of wetlands occurring
Bacillariophytes	Diatom Pennales	*Cymatopleura*	2±2.0	(3.89 - 121.55)	3
		Cymbella	7±2.2	(0.49 - 77.79)	23
		Fragilaria	10±4.6	(0.65 - 218.79)	20
		Gomphonema	11±4.1	(0.49 - 218.79)	31
		Gyrosigma	2±1.0	(1.94 - 48.62)	9
		Navicula	56±19.7	(0.49 - 904.33)	40
		Nitzschia	5±2.3	(0.97 - 121.55)	13
		Stauroneis	2±1.0	(1.94 - 58.34)	7
		Surirella	8±6.4	(2.43 - 384.10)	9
		Tabellaria	4±1.5	(0.97 - 60.32)	15
	Diatom Golden Brown Algae Pennales	*Asterionella*	5±3.1	(0.49 - 135.16)	10
		Asterionella colonies	1±0.4	(0.23 - 24.31)	7
	Diatom Unicellular Centrales	*Cyclotella*	211±133.0	(0.97 - 7815.65)	55
Chlorophytes	Green Algae	*Tetraedron*	14±13.4	(0.97 - 802.23)	8
		Tetrastrum	85±49.4	(1.94 - 2391.29)	11
		Westella	9±4.5	(0.97 - 245.26)	11
	Green Algae Colonial	*Coelastrum*	49±21.6	(0.97 - 797.10)	18
		Kirchneriella	41±26.8	(0.49 - 1604.46)	19
		Oocystis	4±1.7	(4.86 - 72.93)	8
		Pediastrum	0±0.3	(0.51 - 19.45)	4
		Scenedesmus	29±14.4	(0.49 - 777.92)	21
		Volvox	90±89.9	(5396.81 - 5396.81)	1
	Green Algae Gelatinous	*Schizochlamys*	27±13.4	(0.24 - 705.12)	16
	Green Algae Unicellular	*Ankistrodesmus*	499±432.4	(0.49 - 25982.31)	37
		Ankistrodesmus colonies	280±255.7	(0.97 - 15328.80)	9
		Chlamydomonas	651±216.6	(1.94 - 10044.95)	60
		Staurastrum	25±10.7	(0.24 - 459.86)	18
		Staurodesmus	0±0.3	(1.94 - 19.45)	3
	Green Algae Unicellular Desmid	*Closterium*	66±29.6	(0.24 - 1473.18)	33
		Cosmarium	3489±3088.6	(0.97 - 185199.75)	38
		Netrium	12±6.0	(0.97 - 282.00)	17
Chrysophytes	Golden Brown Algae	*Dinobryon*	12±6.3	(0.06 - 277.13)	15
		Synura	21±17.5	(1.94 - 1050.43)	10
Cryptophytes	Brown	*Cryptomonas*	187±63.8	(0.97 - 3111.67)	56
Cyanobacteria	Blue Green Algae	*Anabaena*	0±0.3	(0.97 - 14.59)	4
Dinophytes	Dinoflagellate	*Peridinium*	36±18.8	(0.97 - 960.44)	27
Euglenophytes	Euglenoids	*Euglena* sp. 1	131±82.8	(0.97 - 4223.08)	21
		Euglena sp. 2	17±10.3	(0.97 - 571.28)	13
		Phacus	5±3.8	(1.94 - 194.48)	4
Phytoplankton biomass µg C L^{-1}			214±47.8		

a.



b.

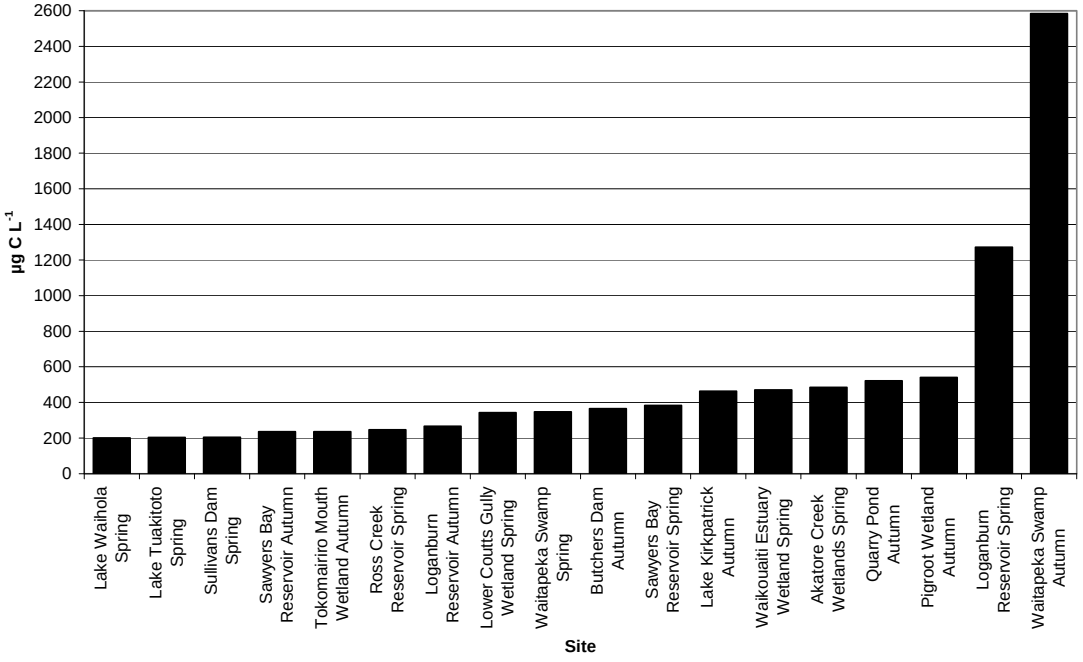


Figure 6.1 Phytoplankton biomass a. Sites with biomass less than 200 µg C L^{-1}. b. Sites with biomass greater than 200 µg C L^{-1}

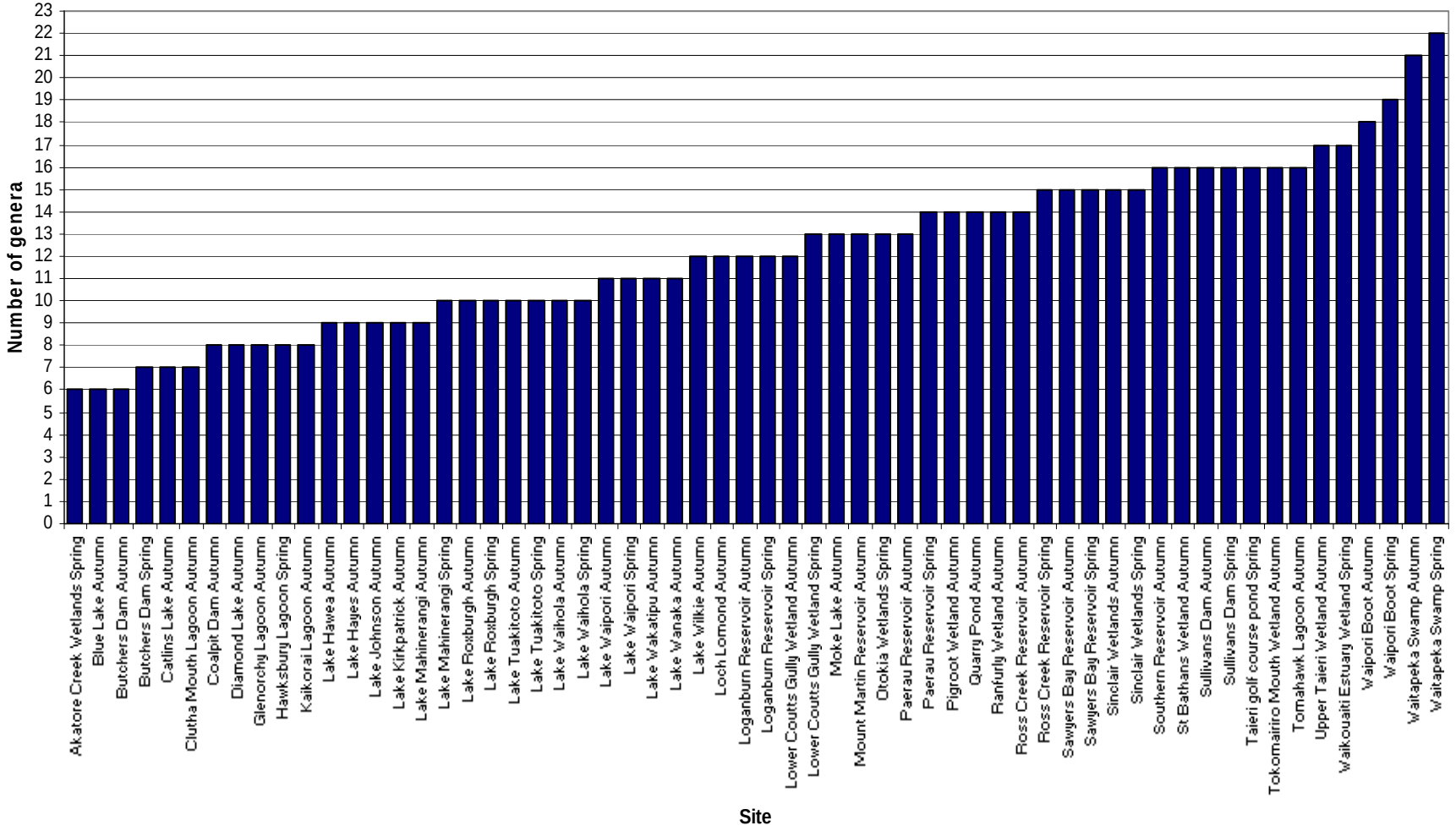


Figure 6.2 Number of phytoplankton genera counted for each wetland

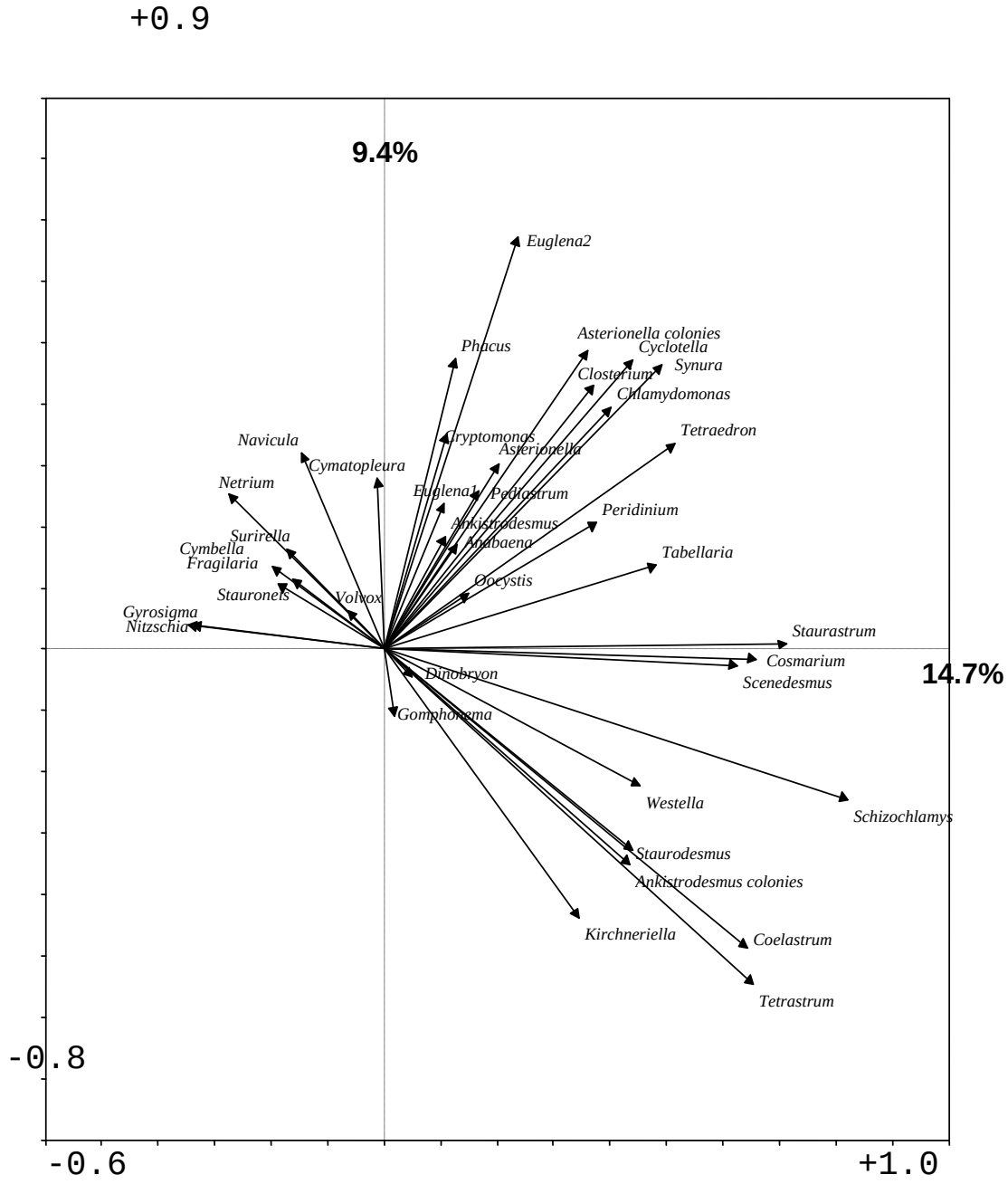


Figure 6.3 Ordination diagram of PCA analysis of phytoplankton genera abundance data. Arrows denote the relationship of each genus with the axes. Percentages refer to the percent variance explained by each axis.

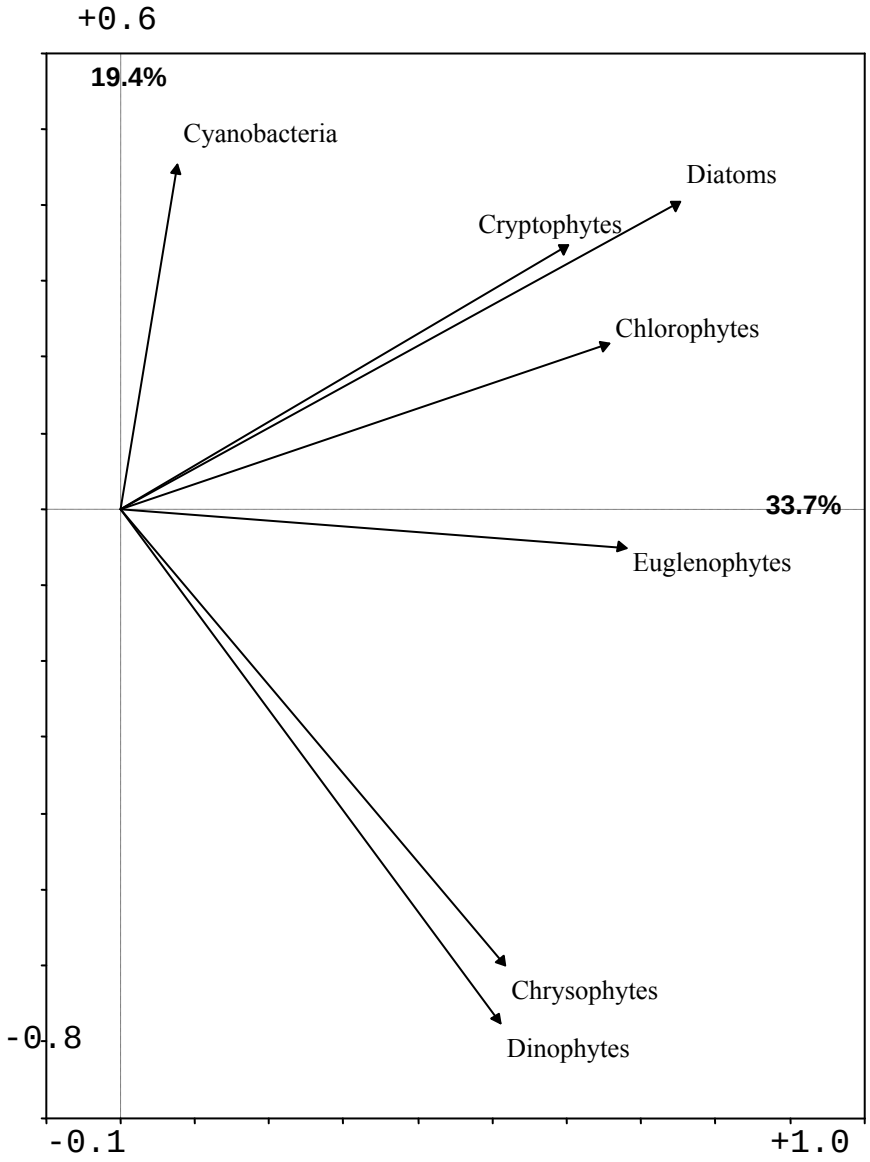


Figure 6.4 Ordination diagram of PCA analysis of phytoplankton Divisions abundance data. Arrows denote the relationship of each Division with the axes. Percentages refer to the percent variance explained by each axis.

Relationships between phytoplankton and physicochemical variables

Multivariate analysis

Phytoplankton genera

Redundancy analysis showed measures of physicochemical variables explained a total of 18.3% of the variation in the phytoplankton genera (P=0.030) (Table 6.2a). The first axis explained 7.8% of the phytoplankton genera data (P=0.020). The variables most strongly negatively loaded with this axis were measures of TIN 3% (P=0.045) and TSS, whereas Secchi depth was positively loaded (Fig. 6.5). Abundance of the chlorophyte genera, *Staurastrum, Scenedesmus, Cosmarium, Schizochlamys* and *Coelastrum* were most strongly positively loaded with the first axis, whereas abundance of the diatom, *Nitzschia,* was negatively loaded.

The second axis explained a further 5.9% of the phytoplankton genera data. Measures of DRP 6% (P=0.005), turbidity 3% (P=0.01) water colour 2% (P=0.02), TP, TN and DOC were the physicochemical variables most strongly loaded with this axis. Abundance of the chlorophyte genera, *Chlamydomonas* and *Netrium,* the cryptophyte, *Cryptomonas,* the chrysophyte, *Synura,* and the euglenophyte, *Euglena* sp. 2, were most strongly positively loaded with the second axis, while the abundance of the chlorophyte, *Kirchneriella,* was negatively loaded with the second axis.

Thus, *Staurastrum, Scenedesmus, Cosmarium, Schizochlamys*, *Coelastrum Chlamydomonas Netrium, Cryptomonas, Synura* and *Euglena* sp. 2 were the genera most likely to increase with increased measures of physicochemical variables. The diatom, *Nitzschia,* was the only genus that appeared to decrease with increased measures of physicochemical variables.

Phytoplankton Divisions

Redundancy analysis showed measures of physicochemical variables explained a total of 28.5% of the variation in the phytoplankton Divisions (P=0.01) (Table 6.2b). The first axis explained 15.5% of the phytoplankton Division data (P=0.005). The variables most strongly negatively loaded with this axis were measures of DOC 10% (P=0.005), TN and turbidity (Fig. 6.6). Abundance of the Divisions, cryptophytes, chlorophytes and diatoms were strongly negatively loaded with the first axis.

The second axis explained a further 9.2% of the phytoplankton Division data. Measures of TP 4% (P=0.06), TSS, DRP and TIN were the physicochemical variables most strongly negatively loaded with this axis. The abundance of dinophytes was strongly positively loaded with the second axis.

In summary, the phytoplankton Divisions most strongly related to measures of physicochemical variables were cryptophytes, chlorophytes and diatoms (positively) and dinophytes (negatively).

Correlation of total phytoplankton biomass and measures of physicochemical variables

Correlation analysis revealed that phytoplankton biomass correlated positively with measures of TP, TN, DRP, DOC, TSS, turbidity and water colour, and correlated negatively with Secchi depth and temperature (Table 6.3). The number of phytoplankton genera identified per sample correlated negatively with measures of TP, DRP, TIN and TSS.

Table 6.2 Results of redundancy analysis of physicochemical variables with a. phytoplankton genera data and b. phytoplankton Division data.

a.

Axes	1	2	3	4	Total variance
Eigenvalues :	0.078	0.059	0.025	0.021	1
Phyto-physicochemical correlations :	0.772	0.849	0.632	0.729	
Cumulative percentage variance					
of phyto genera data :	7.8	13.7	16.2	18.3	
of phyto genera-physicochemical relation:	31.1	54.4	64.3	72.7	
Sum of all unconstrained eigenvalues					1
Sum of all canonical eigenvalues					0.252

**** Summary of Monte Carlo test ****

Test of significance of first canonical axis: P-value = .0200

Test of significance of all canonical axes : P-value = .0300

Forward Selection Variable	LambdaA	P	F
DRP	0.06	0.005	3.76
Turbidity	0.03	0.01	1.88
Water colour	0.02	0.02	1.7
TIN	0.03	0.045	1.69
Temperature	0.03	0.045	1.57

b.

Axes	1	2	3	4	Total variance
Eigenvalues :	0.155	0.092	0.021	0.017	1
Species-environment correlations :	0.697	0.694	0.454	0.447	
Cumulative percentage variance					
of phyto Division data :	15.5	24.6	26.8	28.5	
of phyto Division-physicochemical relation:	49.9	79.4	86.2	91.9	
Sum of all unconstrained eigenvalues					1
Sum of all canonical eigenvalues					0.31

**** Summary of Monte Carlo test ****

Test of significance of first canonical axis: F-ratio = 8.615

P-value = .0050

Test of significance of all canonical axes : F-ratio = 1.764

P-value = .0100

Forward selection variable	LambdaA	P	F
DOC	0.1	0.005	6.73
Temperature	0.03	0.055	2
TP	0.04	0.06	2.46

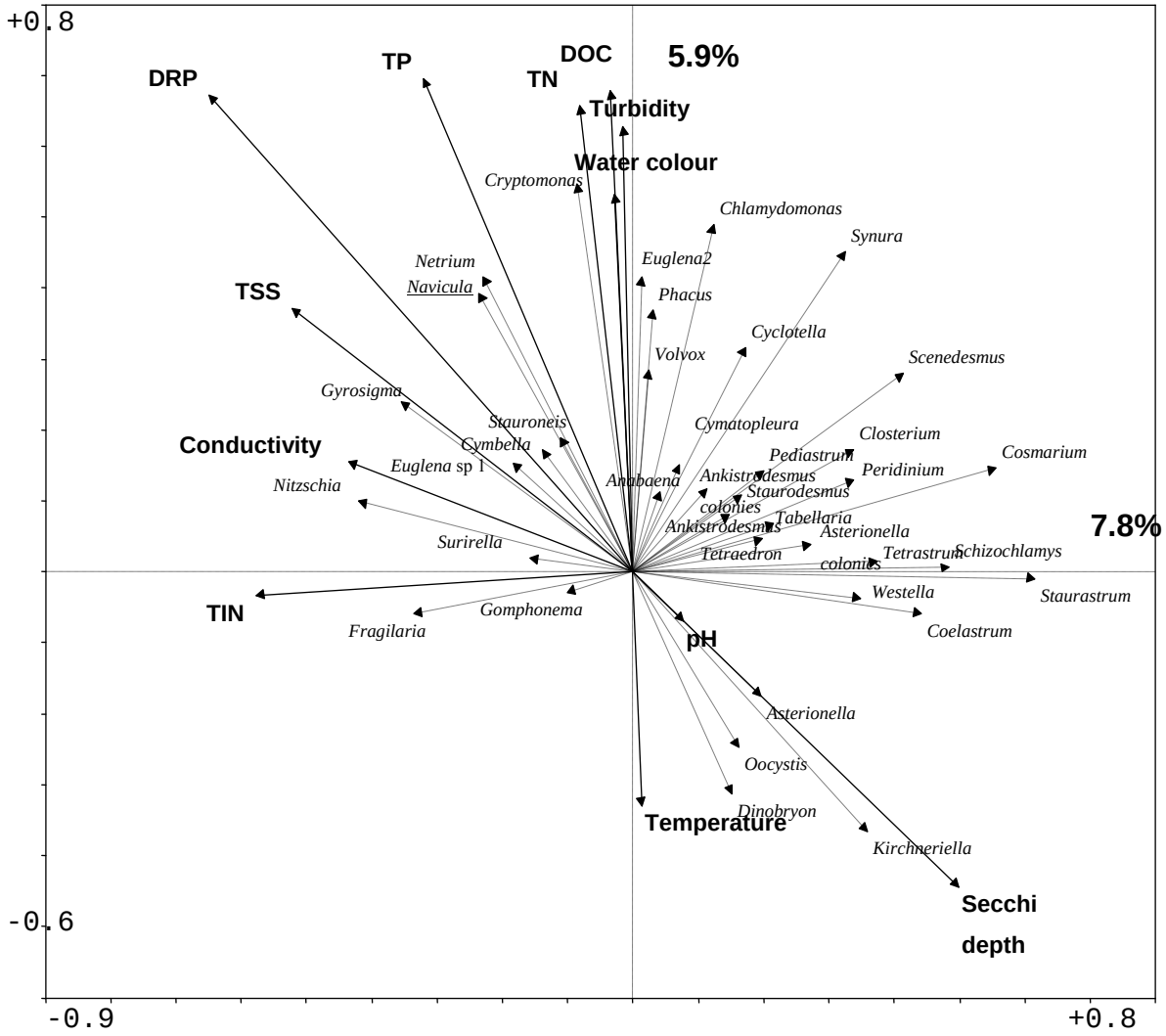


Figure 6.5 Ordination diagram for the redundancy analysis of phytoplankton genera abundance (dotted lines) and physicochemical variables (solid lines). Percentages refer to the percent variance explained by each axis.

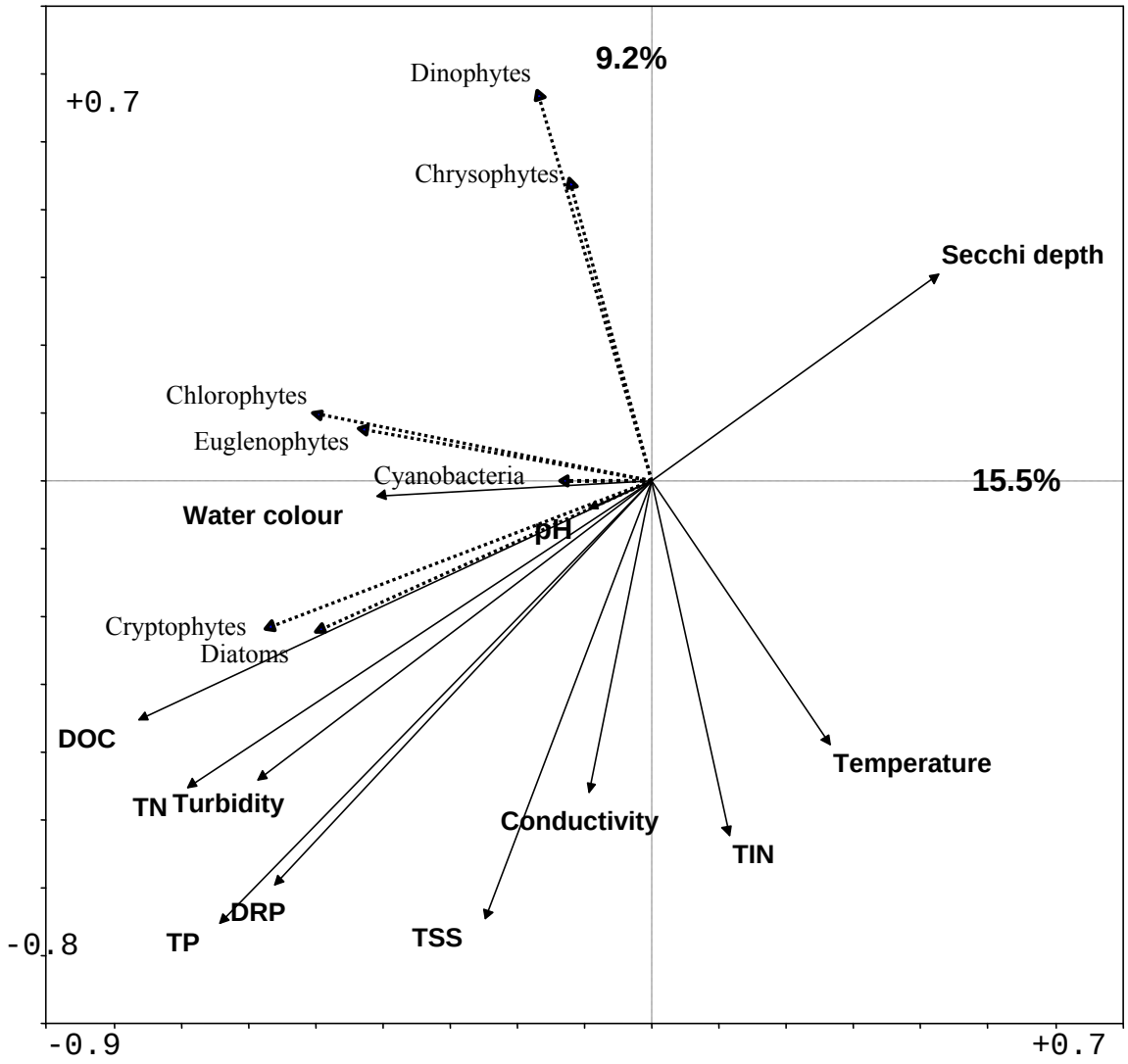


Figure 6.6 Ordination diagram for the redundancy analysis of phytoplankton Divisions abundance (dotted lines) and physicochemical variables (solid lines). Percentages refer to the percent variance explained by each axis.

Table 6.3 Significant Pearson's correlation results (P<0.05, in parentheses) for correlations between phytoplankton biomass and measures of physicochemical variables, and the number of phytoplankton genera and measures of physicochemical variables. Shaded values are negative correlations.

	Total Phytoplankton Biomass	Number of genera
TP	0.47 (0.0002)	-0.37±0.0047
TN	0.52 (0.0000)	
DRP	0.48 (0.0001)	-0.43±0.0007
TIN		-0.31±0.0167
DOC	0.54 (0.0000)	
Turbidity	0.45 (0.0003)	
pH		
Temperature	-0.26 (0.0445)	
Conductivity		
Secchi depth	-0.61 (0.0001)	
TSS	0.47 (0.0002)	-0.44±0.0004
Water colour	0.35 (0.0059)	

Relationships between phytoplankton and the microbial food web

Multivariate analysis

Phytoplankton genera

Redundancy analysis showed microbial food web components explained a total of 13.8% of the variation in the phytoplankton genera data (P=0.0050) (Table 6.4a). The first axis explained 6.1% of the phytoplankton genera data (P=0.0050). The microbial food web components most strongly positively loaded with this axis was ciliate biomass 5% (P=0.005) (Fig. 6.7). Abundance of the cryptophyte genera, *Cryptomonas,* the diatom, *Cyclotella,* the chrysophyte, *Synura,* and the euglenophyte, *Euglena* sp. 2 were the genera most strongly positively loaded with the first axis.

The second axis explained a further 4.3% of the phytoplankton genera data. PP biomass 5% (P=0.005) was strongly positively loaded with this axis. Abundance of the chlorophyte genus, *Coelastrum* was positively associated with this axis.

Therefore, ciliates were the only heterotrophic microbial food web component that correlated with phytoplankton genera. Relationships between ciliates and *Cryptomonas,* ciliates and *Cyclotella,* ciliates and *Synura,* and ciliates and *Euglena* sp. 2 were all positive, as was the relationship between PP and *Coelastrum.*

Phytoplankton Divisions

Redundancy analysis showed microbial food web components explained a total of 23.8% of the variation in the phytoplankton Division data (P=0.005) (Table 6.4b). The first axis explained 18.1% of the phytoplankton Division data (P=0.005). The microbial food web component most strongly positively loaded with this axis was ciliate biomass 16% (P=0.005) (Fig. 6.8). Abundance of diatoms and euglenophytes were the phytoplankton Divisions most strongly positively loaded with the first axis.

The second axis explained a further 3.2% of the phytoplankton data. HNF biomass 3% (P=0.045) was strongly negatively loaded with this axis, however, no phytoplankton Divisions were associated with this axis.

Thus, the multivariate analysis revealed that ciliate biomass was most strongly positively related to the abundance of diatoms and euglenophytes.

Correlations of total phytoplankton biomass and biomass of microbial food web components

Correlation analysis showed that phytoplankton biomass was positively correlated with ciliate biomass (Table 6.5). The number of phytoplankton genera was not correlated with the biomass of any microbial food web component.

Table 6.4 Results of redundancy analysis of biomass of microbial food web components with a. phytoplankton genera data, and b. phytoplankton Division data.

a.

Axes	1	2	3	4	Total variance
Eigenvalues :	0.061	0.043	0.02	0.014	1
Phyto-mfw correlations :	0.84	0.603	0.731	0.534	
Cumulative percentage variance					
of phyto genera data :	6.1	10.3	12.3	13.8	
of phyto genera-mfw relation:	40.9	69.8	83.2	92.8	
Sum of all unconstrained eigenvalues					1
Sum of all canonical eigenvalues					0.148

**** Summary of Monte Carlo test ****
Test of significance of first canonical axis:
F-ratio = 3.484
P-value = .0050
Test of significance of all canonical axes :
F-ratio = 1.881
P-value = .0050

Forward Selection Variable	LambdaA	P	F
Ciliates	0.05	0.005	3.33
PP	0.05	0.005	2.7

b.

Axes	1	2	3	4	Total variance
Eigenvalues :	0.181	0.032	0.013	0.011	1
Species-environment correlations :	0.753	0.517	0.289	0.299	
Cumulative percentage variance					
of phyto Division data :	18.1	21.3	22.7	23.8	
of phyto Division-mfw relation:	75.5	88.8	94.4	99	
Sum of all unconstrained eigenvalues					1
Sum of all canonical eigenvalues					0.24

**** Summary of Monte Carlo test ****
Test of significance of first canonical axis:
F-ratio = 11.952
P-value = .0050
Test of significance of all canonical axes :
F-ratio = 3.410
P-value = .0050

Forward Selection Variable	LambdaA	P	F
Ciliates	0.16	0.005	11.17
HNF	0.03	0.045	2.06

a.

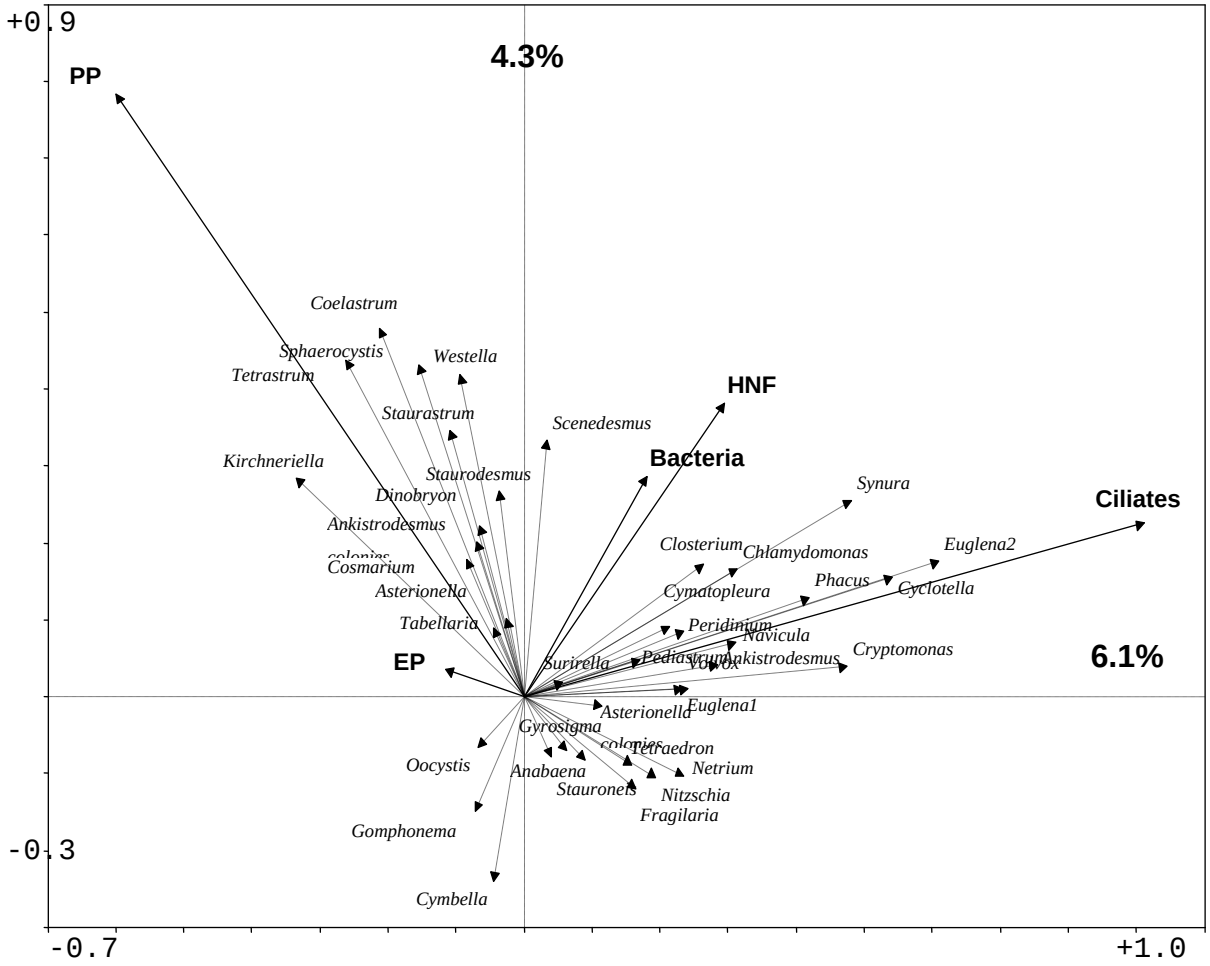


Figure 6.7 Ordination diagram for the redundancy analysis of phytoplankton genera abundance (dotted lines) and microbial food web components (solid lines). Percentages refer to the percent variance explained by each axis.

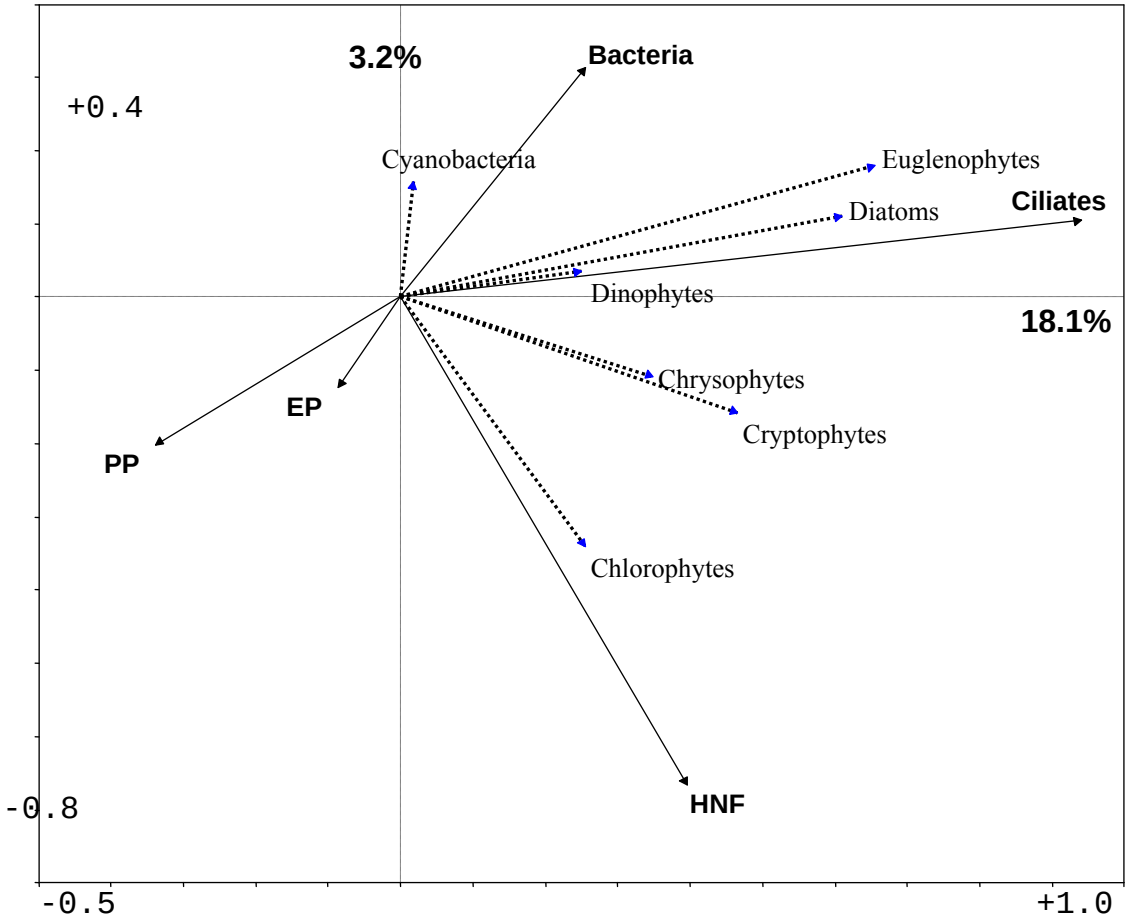


Figure 6.8 Ordination diagram for the redundancy analysis of phytoplankton Divisions abundance (dotted lines) and microbial food web components (solid lines). Percentages refer to the percent variance explained by each axis.

Table 6.5 Significant Pearson's correlation results ($P<0.05$, in parentheses) for correlations between phytoplankton biomass and biomass of microbial food web components. The number of phytoplankton genera and biomass of microbial food web components were not correlated. PP = prokaryotic picophytoplankton, EP = eukaryotic picophytoplankton, HNF = heterotrophic nanoflagellates.

	Total Phytoplankton Biomass
Bacteria	
PP	
EP	
HNF	
Ciliates	0.49 (0.0001)

Relationships between phytoplankton and ciliates

Phytoplankton genera

Redundancy analysis showed that ciliate data explained a total of 18.8% of the variation in the phytoplankton genera data (P=0.0350) (Table 6.6a). The first axis explained 7.4% of the phytoplankton genera data (P=0.0550). The ciliate taxa most strongly positively loaded with this axis were biomass of prostomatids 5% (P=0.015), *Strombidium* 4% (P=0.04), and oligotrichs (Fig. 6.9). Abundance of the cryptophyte, *Cryptomonas,* the diatom, *Cyclotella*, the chrysophyte, *Synura,* and the euglenophytes, *Euglena* sp. 2, and *Phacus,* and were the phytoplankton genera most strongly positively loaded with the first axis.

The second axis explained a further 4.6% of the phytoplankton genera data. The ciliate taxon most strongly positively loaded with this axis was *Urocentrum* biomass. Abundance of the diatom, *Navicula,* was strongly positively loaded with this axis.

Therefore, *Cryptomonas, Cyclotella*, *Synura, Euglena* sp. 2 and *Phacus* abundances were positively related to biomasses of prostomatids, *Strombidium* and oligotrichs, while *Navicula* abundance was positively related to *Urocentrum* biomass.

Phytoplankton Divisions

Redundancy analysis showed ciliate data explained a total of 32.3% of the variation in the phytoplankton Division data (P=0.01) (Table 6.6b). The first axis explained 23.5% of the phytoplankton data (P=0.005). The ciliate taxa most strongly positively loaded with this axis were biomass of prostomatids 15% (P=0.005) and oligotrichs 5% (P=0.005) (Fig. 6.10). Abundance of diatoms and euglenophytes were the phytoplankton Divisions most strongly positively loaded with the first axis.

The second axis explained a further 3.9% of the phytoplankton data, however, no ciliate taxa or phytoplankton Divisions were strongly loaded with this axis.

Thus, the phytoplankton Divisions that were the most associated with ciliates were diatoms and euglenophytes, which were positively related to prostomatids and oligotrichs.

Correlation of total phytoplankton biomass and ciliate biomass

Correlation analysis showed that phytoplankton biomass was positively correlated with biomasses of prostomatids (all sizes), oligotrichs (less than 20µm) and total ciliates (Table

6.7). The number of phytoplankton genera was not correlated with the biomass of any ciliate taxa.

Table 6.6 Results of redundancy analysis of biomass of ciliate taxa with a. phytoplankton genera data, and b. phytoplankton Division data.

a.

Axes	1	2	3	4	Total variance
Eigenvalues :	0.074	0.044	0.039	0.031	1
Phytoplankton genera -ciliate correlations :	0.872	0.774	0.825	0.89	
Cumulative percentage variance					
of phytoplankton genera data :	7.4	11.8	15.6	18.8	
of phytoplankton genera -ciliate relation:	28.7	45.8	61	73.1	
Sum of all unconstrained eigenvalues					1
Sum of all canonical eigenvalues					0.257

**** Summary of Monte Carlo test ****
Test of significance of first canonical axis: eigenvalue = .074
F-ratio = 3.900
P-value = .0550
Test of significance of all canonical axes : Trace = .257
F-ratio = 1.691
P-value = .0050

Forward selection variable	LambdaA	P	F
Prostomatids	0.05	0.015	3.15
Pleurostomatids	0.04	0.035	2.46
Strombidium	0.04	0.04	2.29

b.

Axes	1	2	3	4	Total variance
Eigenvalues :	0.235	0.039	0.029	0.019	1
Phytoplankton Division-ciliate correlations :	0.858	0.496	0.449	0.415	
Cumulative percentage variance					
of phytoplankton Division data :	23.5	27.5	30.4	32.3	
of phytoplankton Division-ciliate relation:	66.6	77.8	86.1	91.6	
Sum of all unconstrained eigenvalues					1
Sum of all canonical eigenvalues					0.353

**** Summary of Monte Carlo test ****
Test of significance of first canonical axis:
F-ratio = 14.452
P-value = .0050
Test of significance of all canonical axes :
F-ratio = 2.138
P-value = .0100

Forward selection variable	LambdaA	P	F
Prostomatids	0.15	0.005	10
Oligotrichs	0.05	0.005	3.88

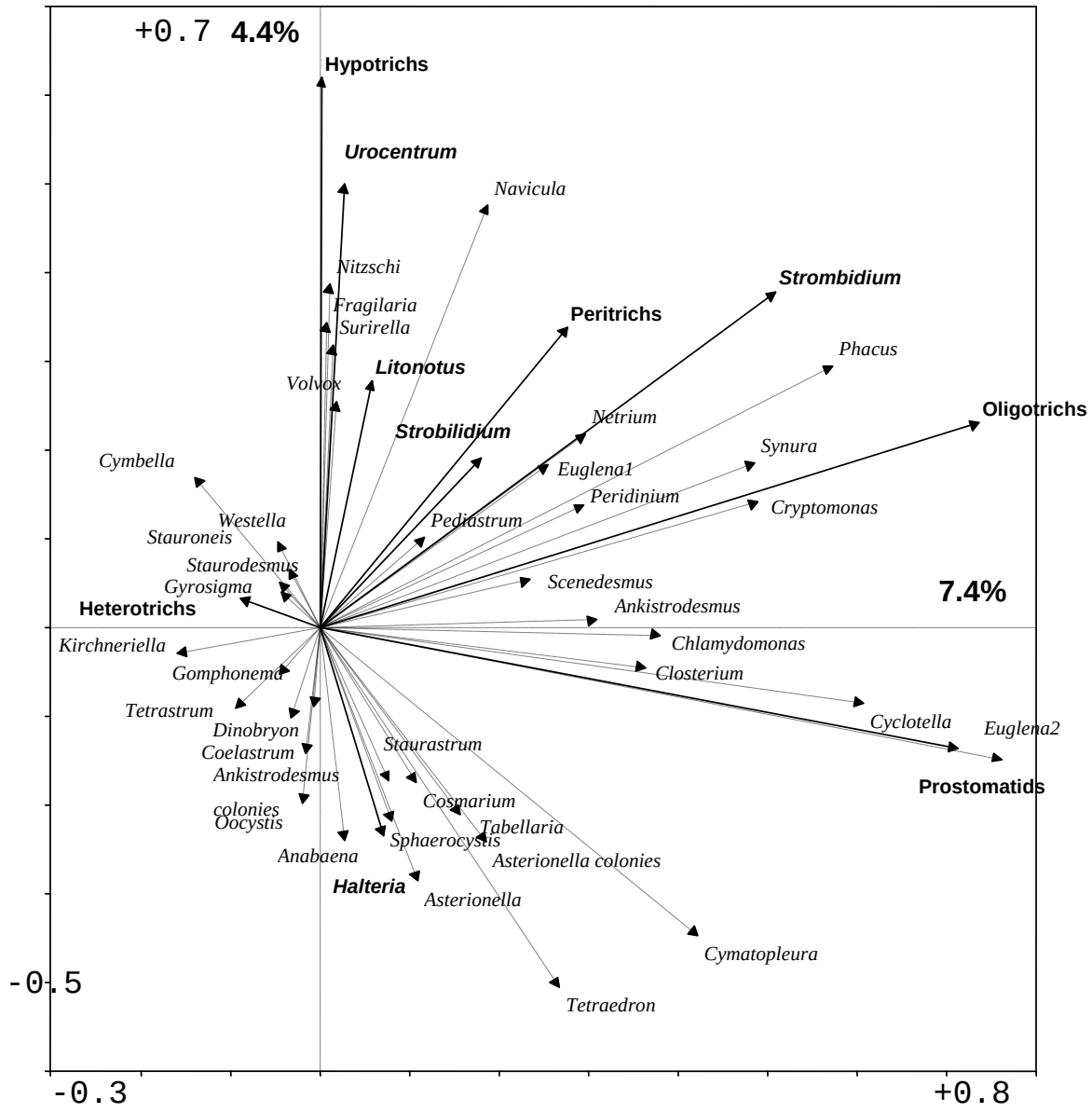


Figure 6.9 Ordination diagram for the redundancy analysis of phytoplankton genera abundance (dotted lines) and ciliate taxa (solid lines). Percentages refer to the percent variance explained by each axis.

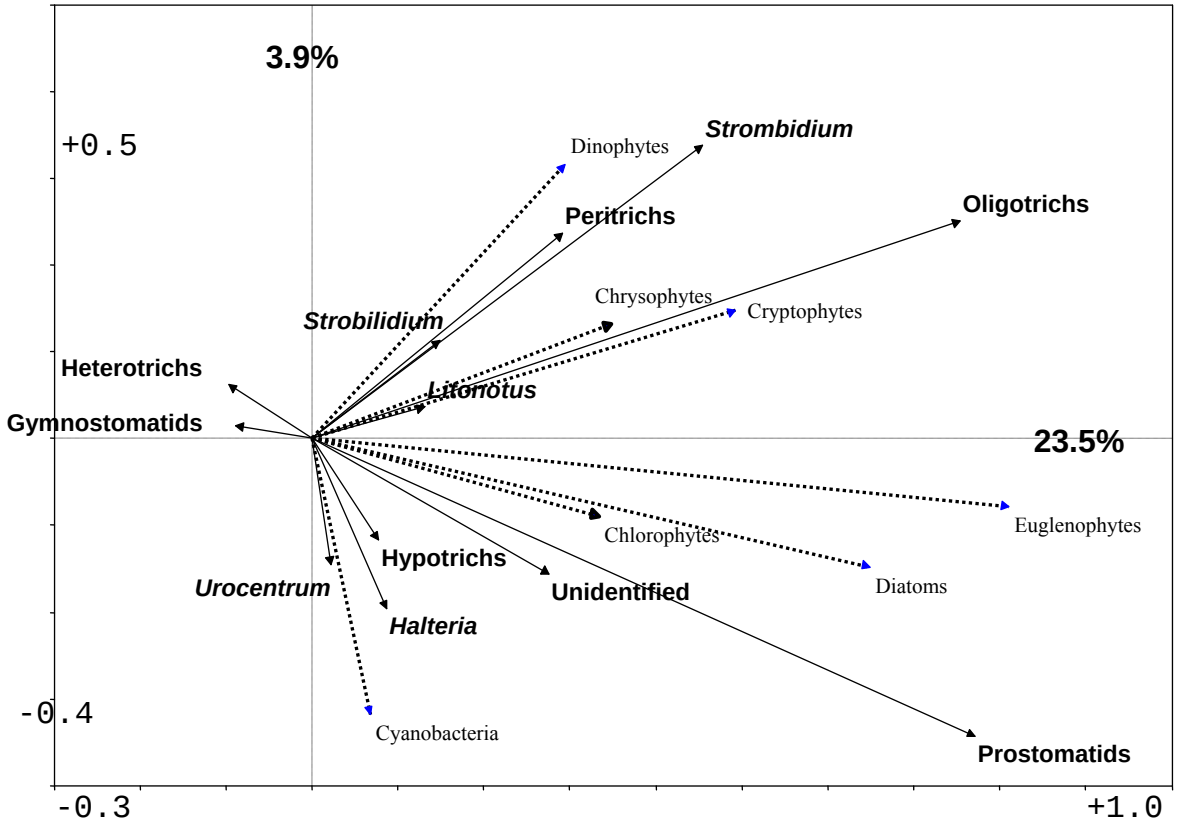


Figure 6.10 Ordination diagram for the redundancy analysis of phytoplankton Divisions abundance (dotted lines) and ciliate taxa (solid lines). Percentages refer to the percent variance explained by each axis.

Table 6.7 Significant Pearson's correlation results (P<0.05, in parentheses) for correlations between phytoplankton biomass and ciliate biomass. The number of phytoplankton genera did not correlate with ciliate biomass.

Ciliate biomass	Total phytoplankton biomass
Prostomatids <20µm	0.50 (0.0000)
Prostomatids >20µm	0.46 (0.0002)
Total Prostomatids	0.51 (0.0000)
Oligotrichs <10µm	0.30 (0.0202)
Oligotrichs 10-20µm	0.27 (0.0369)
Oligotrichs 20-40µm	
Oligotrichs >40µm	
Total Oligotrichs	0.35 (0.0060)
Halteria	
Strobilidium	
Strombidium	
Peritrichs	
Hypotrichs	
Litonotus	
Gymnostomatids	
Urocentrum	
Heterotrichs	
Unidentified	
Total ciliates	0.49 (0.0001)
Number of ciliate taxa	

Relationships between phytoplankton and crustacean zooplankton

Multivariate analysis

Phytoplankton genera

Redundancy analysis showed abundance of crustacean zooplankton taxa explained a total of 15.9% of the variation in the phytoplankton genera data (P=0.005) (Table 6.8a). The first axis explained 6.5% of the phytoplankton genera data (P=0.025). The crustacean zooplankton taxa most strongly positively loaded with this axis was the abundance of *Bosmina* 6% (P=0.01) (Fig. 6.11). The abundance of the chlorophytes, *Scenedesmus, Cosmarium, Pediastrum* and *Staurastrum,* colonies of the diatom, *Asterionella,* the euglenophyte, *Phacus*, and the chrysophyte, *Synura*, were the phytoplankton genera most strongly positively loaded with the first axis.

The second axis explained a further 4.3% of the phytoplankton genera data. Abundance of nauplii 4% (P=0.02) was the crustacean zooplankton taxon most strongly positively loaded with this axis. Abundance of the euglenophyte, *Euglena sp. 2*, and the diatoms, *Cyclotella, and Cymatopleura* were the genera most positively associated with this axis.

Therefore, abundances of *Scenedesmus, Cosmarium, Pediastrum* and *Staurastrum,* colonies of *Asterionella, Phacus*, and *Synura*, were the phytoplankton genera most strongly positively related to *Bosmina* abundances, while *Euglena sp. 2*, *Cyclotella, and Cymatopleura* were positively related to copepod nauplii abundance.

Phytoplankton Divisions

Redundancy analysis showed the crustacean zooplankton abundance data explained a total of 15.5% of the variation in the phytoplankton Division data (P=0.065) (Table 6.8b). The first axis explained 8.6% of the phytoplankton data. The crustacean zooplankton taxon most strongly positively loaded with this axis was the abundance of copepodites 7% (P=0.005) (Fig. 6.12). Abundance of diatoms and euglenophytes were the phytoplankton Divisions most strongly positively loaded with the first axis.

The second axis explained a further 3.2% of the phytoplankton Division data. Adult copepods and *Ceriodaphnia* were the crustacean zooplankton taxon loaded with this axis, with cyanobacteria the phytoplankton Division most strongly positively loaded with this axis.

Therefore, the strongest relationship among the phytoplankton Divisions and crustacean zooplankton was the positive relationship between abundances of diatoms and euglenophytes and copepodites, while adult copepods and *Ceriodaphnia* were related positively to cyanobacteria.

Correlations of total phytoplankton biomass and zooplankton abundance

Correlation analysis showed that phytoplankton biomass correlated positively with abundances of *Daphnia* and copepods (adults, copepodites and nauplii) (Table 6.9). The number of phytoplankton genera was negatively correlated with the abundance of *Daphnia,* and positively correlated with the abundance of *Ceriodaphnia* and *Bosmina.*

Table 6.8 Results of redundancy analysis of crustacean zooplankton with a. phytoplankton genera data, and b. phytoplankton Division data.

a.

Axes	1	2	3	4	Total variance
Eigenvalues :	0.065	0.043	0.032	0.019	1
Phytoplankton genera-zooplankton correlations :	0.812	0.827	0.747	0.549	
Cumulative percentage variance					
of Phytoplankton genera data :	6.5	10.8	14	15.9	
of Phytoplankton genera-zooplankton relation:	36.2	60	78	88.3	
Sum of all unconstrained eigenvalues					1
Sum of all canonical eigenvalues					0.18

**** Summary of Monte Carlo test ****

Test of significance of first canonical axis: eigenvalue = .065
F-ratio = 3.685
P-value = .0250

Test of significance of all canonical axes : Trace = .180
F-ratio = 1.936
P-value = .0050

Variable	LambdaA	P	F
Bosmina	0.06	0.01	3.65
Nauplii	0.04	0.02	2.51

b

Axes	1	2	3	4	Total variance
Eigenvalues :	0.086	0.032	0.026	0.01	1
Phytoplankton Division-zooplankton correlations :	0.581	0.459	0.382	0.257	
Cumulative percentage variance					
of Phytoplankton Division data :	8.6	11.8	14.4	15.5	
of Phytoplankton Division-zooplankton relation:	53.7	73.4	89.9	96.3	
Sum of all unconstrained eigenvalues					1
Sum of all canonical eigenvalues					0.16

**** Summary of Monte Carlo test ****

Test of significance of first canonical axis: eigenvalue = .086
F-ratio = 5.000
P-value = .1000

Test of significance of all canonical axes : Trace = .160
F-ratio = 1.688
P-value = .0650

Variable	LambdaA	P	F
Copepodites	0.07	0.005	4.46

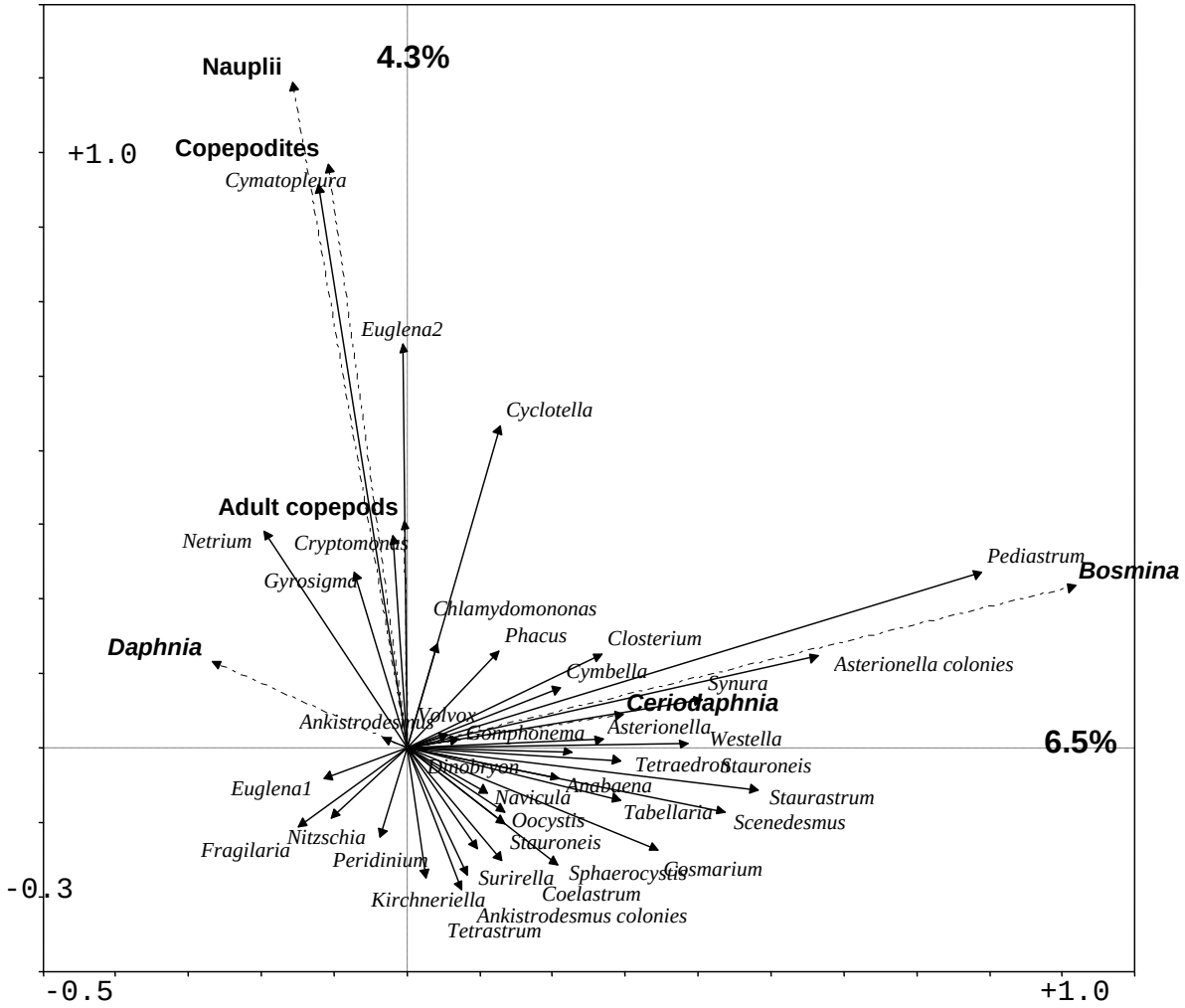


Figure 6.11 Ordination diagram for the redundancy analysis of phytoplankton genera abundance (solid lines) and crustacean zooplankton abundance (dotted lines). Percentages refer to the percent variance explained by each axis.

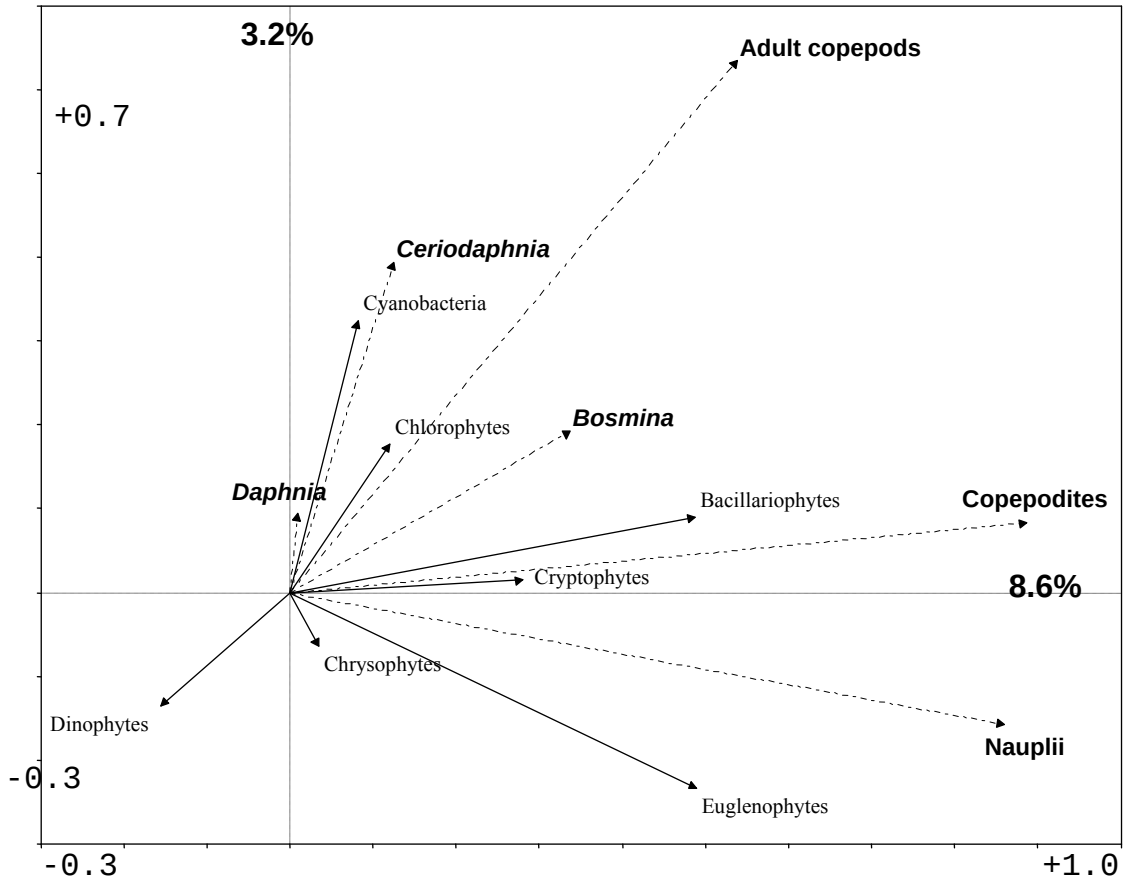


Figure 6.12 Ordination diagram for the redundancy analysis of phytoplankton Divisions abundance (solid lines) and crustacean zooplankton abundance (dotted lines). Percentages refer to the percent variance explained by each axis.

Table 6.9 Significant Pearson's correlations (P<0.05, in parentheses) between phytoplankton biomass and zooplankton abundance, and the number of phytoplankton genera and zooplankton abundance. Shaded values are negative correlations.

Zooplankton abundance biomass	Total phytoplankton biomass	Number of genera
Daphnia	0.30 (0.0211)	-0.27±0.0341
Ceriodaphnia		0.36±0.0049
Adult copepods	0.43 (0.0007)	
Copepodite	0.51 (0.0000)	
Nauplii	0.47 (0.0002)	
Total copepods	0.52 (0.0000)	
Bosmina		0.46±0.0002
Total Zooplankton	0.52 (0.0000)	

Copepods versus Cladocerans

The abundance of the diatom, *Nitzschia* correlated positively with the proportion of copepods and, hence, negatively with the proportion of cladocerans (Table 6.10). The abundance of the chlorophytes, *Coelastrum, Kirchneriella, Schizochlamys, Staurastrum, Tetraedron* and *Westella* correlated negatively with the proportion of copepods and, hence, positively with the proportion of cladocerans.

Table 6.10 Significant Pearson's correlation results (P<0.05, in parentheses) for correlations between abundance of phytoplankton genera and the percentage of copepods in the total crustacean zooplankton abundance. No results fit within the Bonferroni adjustment alpha level of 0.00125. Shaded values are negative correlations.

Division	Genera	Copepod%
Bacillariophytes	*Nitzschia*	0.26 (0.0495)
Chlorophytes	*Coelastrum*	-0.30 (0.0230)
	Kirchneriella	-0.34 (0.0086)
	Schizochlamys	-0.40 (0.0016)
	Staurastrum	-0.36 (0.0054)
	Tetraedron	-0.34 (0.0088)
	Westella	-0.27 (0.0405)

Relationships between phytoplankton and geographical variables

Multivariate analysis

Redundancy analysis of geographical variables and the phytoplankton genera and Division data was not significant.

Correlation analysis

Total phytoplankton biomass was not correlated with percentage of land cover in the catchment or any geographical variables. The number of phytoplankton genera was negatively correlated with percentage of pasture in the catchment and positively correlated with the percentage of tussock (Table 6.11).

Table 6.11 Significant Pearson's correlations (P<0.05, in parentheses) between the number of phytoplankton genera and geographical variables. There were no correlations between phytoplankton biomass and geographical variables Shaded values are negative correlations.

	Number of genera
Bare ground	
Indigenous forest	
Inland water	
Inland wetlands	
Planted forest	
Pasture	-0.40 (0.0018)
Scrub	
Tussock	0.30 (0.0179)
Urban	
Urban open space	
Catchment Area	
Wetland size	
Slope	

Differences among wetland type

Multivariate analysis

Phytoplankton genera

Phytoplankton genera and sites are shown on the ordination (Fig. 6.13). Abundance of most genera of phytoplankton are negatively associated with deep lakes, estuaries and reservoirs, and positively associated with swamps and ponds. However, the abundance of genera such as the chlorophytes, *Kirchneriella, Tetrastrum, Coelastrum, Staurodesmus* and colonies of *Ankistrodesmus* are positively related with deep lakes and reservoirs, and negatively with swamps and ponds. Abundance of the chlorophytes, *Chlamydomonas, Closterium* and *Tetraedron,* the dinophyte, *Peridinium,* the diatoms, *Asterionella, Cyclotella* and *Tabellaria*, the euglenophytes, *Euglena* sp. 2 and *Phacus* and the chrysophyte, *Synura* are negatively associated with deep lakes and positively associated with swamps and ponds.

Phytoplankton Divisions

Phytoplankton Divisions and sites are shown on the ordination (Fig. 6.14). Abundance of diatoms, chlorophytes, cryptophytes and euglenophytes were positively associated with swamps and ponds and negatively associated with deep lakes. The abundance of diatoms was lower in deep lakes than in estuaries, reservoirs and swamps and ponds, and the abundance of diatoms was lower in shallow lakes than in estuaries and swamps and ponds, and lower in reservoirs than in swamps and ponds. Chlorophytes, chrysophytes and cyanobacteria were not significantly different among wetland types. The abundance of cryptophytes was higher in estuaries than in deep lakes, higher in swamps and ponds than in deep lakes and reservoirs and lower in riverine wetlands than in estuaries, reservoirs, shallow lakes and swamps and ponds. The abundance of dinophytes was higher in swamps and ponds than in estuaries. The abundance of euglenophytes was higher in swamps and ponds than in all other wetland types.

ANOVA

The number of phytoplankton genera was lower in estuaries than in deep lakes, reservoirs and shallow lakes (Table 6.12).

Table 6.12 a. Mean number (±1SE) of phytoplankton genera in six types of wetland. b. Results of *post-hoc* comparisons of mean number of phytoplankton genera between wetland types. c. Mean number (ml^{-1}±1SE) of phytoplankton grouped by Division, for wetlands. d. Results of *post-hoc* comparisons of mean phytoplankton Division abundances between wetland types. n=number of sites sampled.

a.

	Deep lake	Estuary	Reservoir	Riverine	Shallow lake	Swamp/Pond
	n=14	n=8	n=15	n=3	n=10	n=10
No. of genera	13.1±0.66	8.6±0.86	13.3±0.89	13.0±1.73	12.8±1.70	11.5±1.19

b.

Post-hoc comparison – no of genera	P-values
Deep lake - Estuarine	0.0070
Estuarine - Reservoir	0.0040
Estuarine - Shallow lake	0.0172

c.

Phytoplankton Division	Deep lakes	Estuaries	Reservoirs	Riverine wetlands	Shallow lakes	Swamps and ponds
	n=14	n=8	n=15	n=3	n=10	n=10
Bacillariophytes	91.5±61.39	327.2±143.63	228.9±129.73	126.9±53.48	84.6±27.81	1081.9±767.85
	13745.7±	4423.3±	3660.4±	5196.5±	323.3±	2067.9±
Chlorophytes	13463.84	3348.01	1098.28	5158.55	89.58	1044.93
Chrysophytes	41.9±23.81	0	12.6±10.00	0.5±0.49	6.5±4.35	117.5±103.81
Cryptophytes	27.4±10.05	783.1±417.47	86.2±24.64	9.9±8.70	67.0±15.55	256.1±97.77
Cyanobacteria	0	0	0.6±0.42	0	1.5±1.46	0
Dinophytes	7.0±3.34	0.3±0.30	10.2±6.75	3.2±3.24	5.8±4.29	181.5±104.51
Euglenophytes	0.0±0.00	3.4±3.40	1.1±0.78	0.6±0.65	1.3±0.72	126.0±61.25

d.

Post-hoc comparisons – phytoplankton Divisions	Bacillariophytes	Chlorophytes	Chrysophytes	Cryptophytes	Cyanobacteria	Dinophytes	Euglenophytes
Deep lake - Estuarine	0.0005			0.0077			
Deep lake - Reservoir	0.0043						
Deep lake - Swamp/pond	0.0000			0.0008			0.0000
Estuarine - Riverine wetland				0.0066			
Estuarine - Shallow lake	0.0470						
Estuarine - Swamp/pond						0.0330	0.0023
Reservoir - Riverine wetland				0.0416			
Reservoir - Swamp/pond	0.0366			0.0467			0.0003
Riverine wetland - Shallow lake				0.0273			
Riverine wetland - Swamp/pond				0.0019			0.0212
Shallow lake - Swamp/pond	0.0054						0.0019

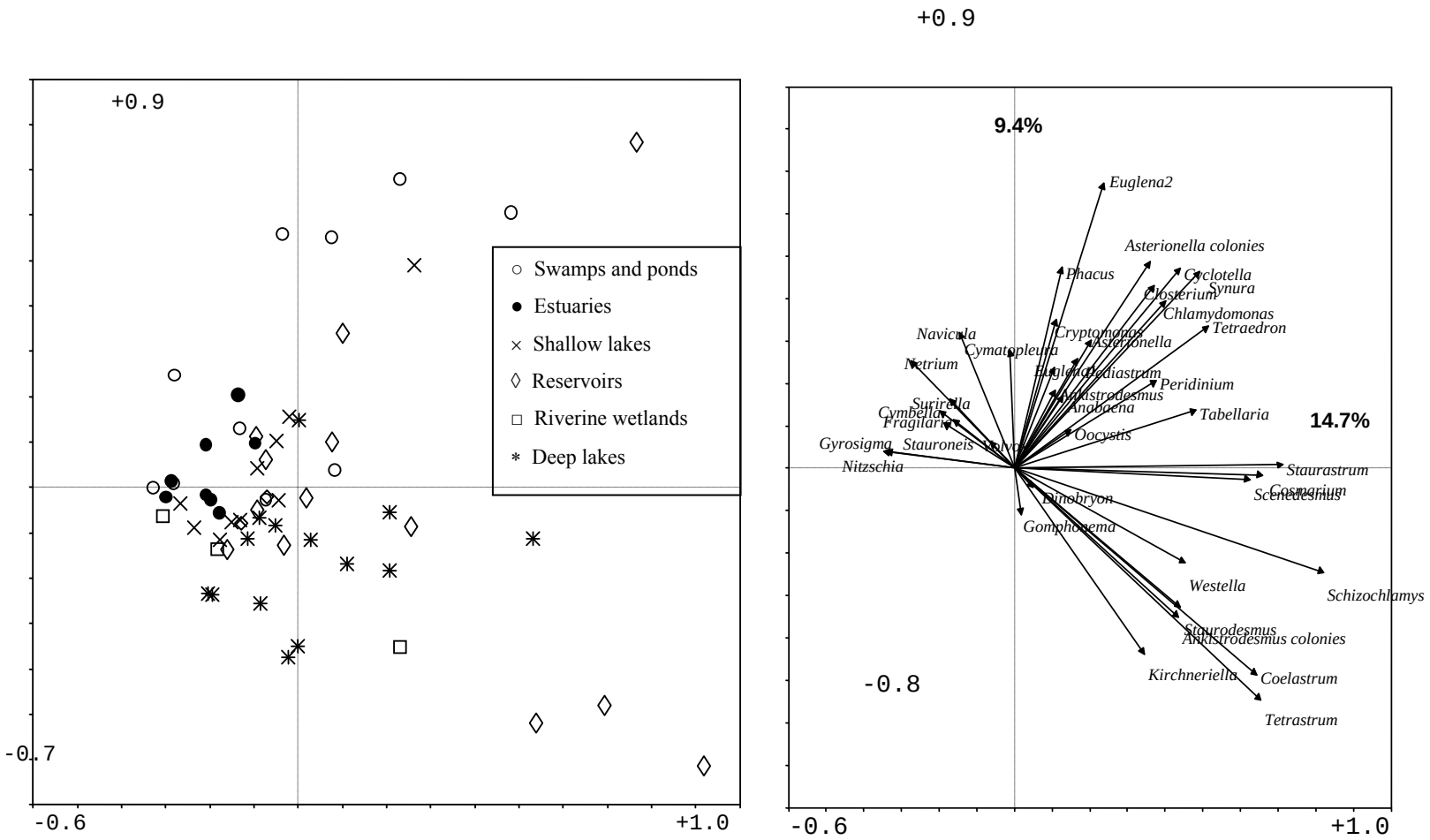


Figure 6.13 Ordination diagram of PCA of phytoplankton genera abundance showing sites by wetland. Left panel: wetland sites. Right panel: arrows indicate the phytoplankton genera variation. Percentages refer to the percent variance explained by each axis.

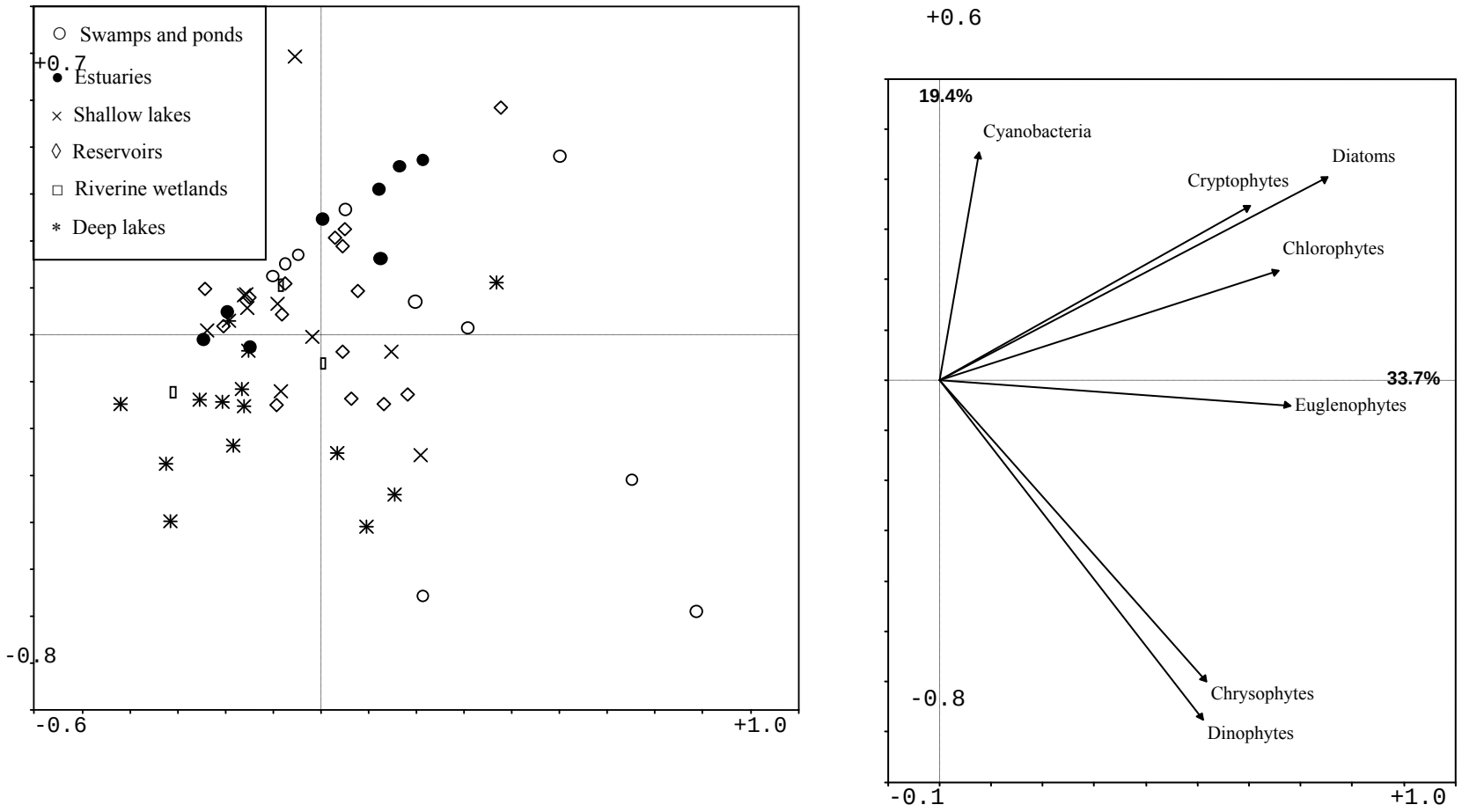


Figure 6.14 Ordination diagram of PCA of phytoplankton Division abundance showing sites by wetland. Left panel: wetland sites. Right panel: arrows indicate the phytoplankton Division variation. Percentages refer to the percent variance explained by each axis.

Differences between autumn and spring

Multivariate analysis

Phytoplankton genera

The PCA did not reveal any seasonal trends in the phytoplankton genera data.

Phytoplankton Divisions

The PCA appeared to show some trends in the phytoplankton data when grouped into Divisions (Fig. 6.15). In some wetlands arrows have moved to the left on the first axis from autumn to spring, indicating a decline in the dominance of diatoms and Cyanobacteria from autumn to spring. In other wetlands, the arrows have moved both up and down on the second axis from autumn to spring, indicating a decline in the dominance of dinophytes from autumn to spring in some wetlands and an increase in the dominance of dinophytes in other wetlands.

Analysis of variance

Phytoplankton genera

ANOVA showed that abundances of the diatom, *Cyclotella,* and the chlorophytes, *Staurastrum, Schizochlamys, Kirchneriella* and *Westella* were greater in autumn. Abundance of diatoms, *Fragilaria* and *Nitzschia,* and the chlorophyte, *Ankistrodesmus,* were higher in spring (Table 6.13). The number of phytoplankton genera was slightly lower in spring than in autumn, (P=0.0611) (Table 6.14a).

Phytoplankton Divisions

ANOVA showed that the abundance of dinophytes was slightly higher in autumn than in spring (P=0.0558) (Table 6.14b).

Table 6.13 Mean phytoplankton concentrations (cells ml^{-1}±1SE) in autumn and spring. Significant differences between seasons are shown (righthand column).

Division	Genera	Autumn	Spring	P-value
Bacillariophytes	*Asterionella*	10±8.8	1±0.3	
	Asterionella colonies	2±1.6	0±0.4	
	Cyclotella	689±522.8	35±12.6	0.0153
	Cymatopleura	8±8.1	0±0.3	
	Cymbella	3±1.9	2±1.2	
	Fragilaria	1±0.3	22±14.5	0.0457
	Gomphonema	5±2.0	6±2.2	
	Gyrosigma	0±0.1	1±0.4	
	Navicula	17±7.6	21±8.0	
	Nitzschia	0±0.0	7±3.2	0.0152
	Stauroneis	1±0.8	1±0.8	
	Surirella	3±1.6	2±1.7	
	Tabellaria	2±1.7	7±3.5	
Chlorophytes	*Ankistrodesmus*	50±24.7	142±72.0	0.0616
	Ankistrodesmus colonies	1049±1020.3	65±64.8	
	Chlamydomonas	375±122.0	871±482.0	
	Closterium	52±26.0	24±17.3	
	Coelastrum	105±66.8	48±45.2	
	Cosmarium	320±251.1	473±449.0	
	Kirchneriella	23±11.7	1±0.6	0.0511
	Netrium	4±3.2	1±0.5	
	Oocystis	5±4.9	1±1.0	
	Pediastrum	2±1.3	0±0.0	
	Scenedesmus	43±23.8	55±51.7	
	Schizochlamys	82±49.6	17±16.2	0.0754
	Staurastrum	75±38.4	1±0.3	0.0773
	Staurodesmus	2±1.3	0±0.0	
	Tetraedron	54±53.4	0±0.1	
	Tetrastrum	230±169.2	103±100.4	
	Volvox	0±0.0	0±0.0	na
	Westella	31±17.1	2±1.9	0.0628
Chrysophytes	*Dinobryon*	19±18.5	12±10.1	
	Synura	4±2.4	1±0.8	
Cryptophytes	*Cryptomonas*	96±34.4	71±20.5	
Cyanobacteria	*Anabaena*	1±0.4	0±0.0	
Dinophytes	*Peridinium*	4±1.5	10±7.2	
Euglenophytes	*Euglena* sp. 1	301±280.8	208±178.6	
	Euglena sp. 2	41±37.9	8±8.1	
	Phacus	0±0.3	13±13.0	
Phytoplankton biomass		275±167.1	254±78.4	

Table 6.14 Mean phytoplankton concentrations (cells ml^{-1}±1SE) and the results of ANOVA between seasons. a. the number of phytoplankton genera, and b. cell abundance in phytoplankton Divisions between seasons.

a.

	Autumn n=40	Spring n=20	F-value	P-value
No. of genera	12.9±0.59	11.0±0.81	3.65	0.0611

b.

Phytoplankton Division	Autumn n=40	Spring n=20	F value	P value
Bacillariophytes	407.7±202.56	153.4±50.80		
Chlorophytes	7301.4±4730.71	1508.9±667.02		
Chrysophytes	45.4±27.21	10.0±7.62		
Cryptophytes	229.7±93.86	100.7±34.79		
Cyanobacteria	0.2±0.16	0.7±0.73		
Dinophytes	49.7±27.94	7.4±5.42	3.81	0.0558
Euglenophytes	25.0±15.46	16.0±15.79		

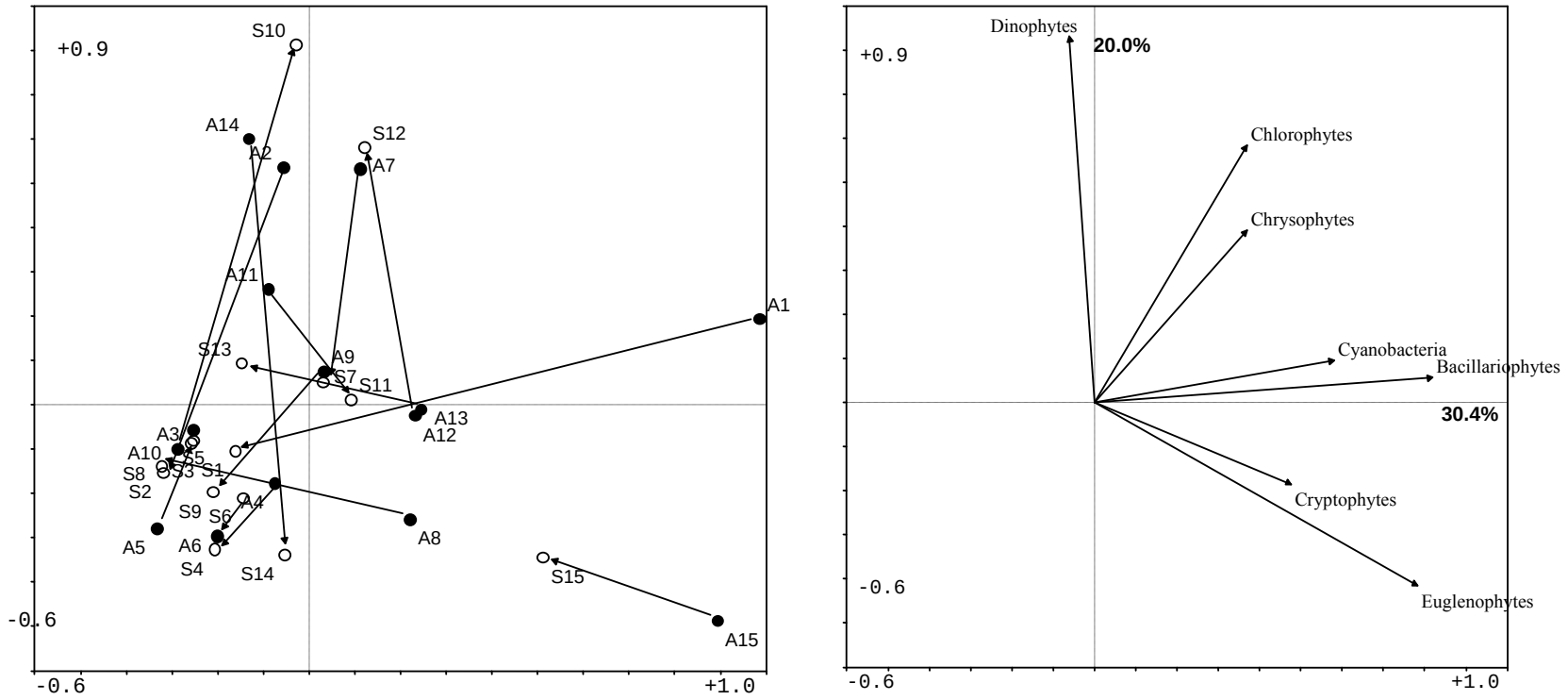


Figure 6.15 Ordination diagrams of PCA of abundance of phytoplankton Divisions showing samples (sites). Left panel. A=autumn samples and S=spring samples, numbers identify each wetland; arrows denote the change between autumn and spring for each wetland. Right panel. Ordination diagram for the principal components analysis of abundance of phytoplankton Divisions for the 15 wetland sites that were sampled in both seasons. Arrows show the loading of each variable on the two canonical axes. Percentages refer to the percent variance explained by each axis.

Discussion

Phytoplankton

The most prevalent phytoplankton genera were *Chlamydomonas, Cryptomonas, Cyclotella* and *Navicula,* found in 60, 56, 55 and 40 samples respectively. *Cyclotella* is frequently the dominant phytoplankton genus in South Island, New Zealand, lakes (Viner and White 1987).

Abundance of the chlorophytes, *Staurastrum, Cosmarium, Tetrastrum, Tetraedron, Coelastrum, Schizochlamys* and *Scenedesmus,* and the chrysophyte, *Synura,* were related. Abundance of the euglenophytes, *Euglena* sp. 2 and *Phacus,* and the diatoms, *Asterionella* (colonies) and *Cyclotella* were related. My finding that *Coelastrum* and *Scenedesmus* were associated is in agreement with that of Reynolds *et al*. (2002) who stated that *Coelastrum* and *Scenedesmus* share a habitat of shallow, highly enriched systems. My observation that *Asterionella* (colonies) and *Cyclotella* are related concurs with that of Viner and White (1987) who observed that *Asterionella* and *Cyclotella* were together year round in four South Island lakes, and during winter in one North Island lake, Lake Taupo.

Physicochemical variables

My prediction that the abundance and biomass of phytoplankton would relate positively with measures of physicochemical variables was confirmed. Abundance of the chlorophytes, *Staurastrum, Scenedesmus, Cosmarium, Schizochlamys* and *Coelastrum* were the genera most strongly positively related to measures of TIN and TSS, and negatively related to Secchi depth. In contrast, the abundance of the diatom, *Nitzschia* was negatively related to measures of TIN, TSS and conductivity, and positively related to Secchi depth.

Abundance of the chlorophytes, *Chlamydomonas* and *Netrium,* the cryptophyte, *Cryptomonas,* the chrysophyte, *Synura,* and the euglenophyte, *Euglena* sp. 2, were the genera most strongly positively related to measures of DRP, turbidity, water colour, TP, TN and DOC while the abundance of the chlorophyte, *Kirchneriella,* was negatively related to the same variables.

My findings concur with those of other New Zealand studies, where *Scenedesmus, Coelastrum, Cryptomonas* were observed in eutrophic lakes, however, genera such as *Staurastrum* and *Cosmarium* were found in both oligotrophic and eutrophic lakes (Viner and White 1987). The increased abundance of phytoplankton genera with increased measures of physicochemical variables that I observed agrees with the finding of Cottingham and

Carpenter (1998) that the abundance of *Staurastrum, Cryptomonas* and *Scenedesmus* increased in response to nutrient enrichment in Peter, Paul and Long Lakes, Michigan, USA. My finding of *Euglena* positively related to measures of physicochemical variables concurs with Rojo *et al.* (2000) who report that *Euglena* was found in the most hypertrophic of five ponds sampled in Spain. In Spanish reservoirs, Dasi *et al.* (1998) described *Scenedesmus* and *Chlamydomonas* as eutrophy indicators, and *Staurastrum*, *Cosmarium, Cryptomonas* and *Coelastrum* were also important in eutrophic waters, which agrees with my findings of the same genera positively related to lake trophy.

Abundance of the Divisions, cryptophytes, chlorophytes and diatoms were positively related to measures of DOC, TN and turbidity, while the abundance of dinophytes was negatively related to measures of TP, TSS, DRP and TIN. My findings are similar to those of Cottingham and Carpenter (1998), who also determined that cryptomonads (cryptophytes) increased in response to nutrient enrichment, while dinoflagellates (dinophytes) decreased. In a survey of 55 north German lakes Auer *et al.* (2004) also reported that the biomass of chlorophytes and diatoms increased, and dinophyte biomass decreased, with nutrients.

Total phytoplankton biomass correlated positively with measurements of DOC, DRP, TP, TN, turbidity, and water colour, and negatively with Secchi depth, probably due to the increased concentrations of nutrients stimulating phytoplankton growth. While increased DOC, turbidity and water colour may be detrimental to phytoplankton growth, by blocking light needed by phytoplankton (Carignan and Planas 1994), no negative correlation was observed between these variables and total phytoplankton biomass in my study. Phytoplankton have been shown to photo-adapt relatively rapidly and these adaptations may possibly have been a feature of phytoplankton communities in Lake Wakatipu and Lake Dunstan, Otago, New Zealand (Schallenberg and Burns 1997). However, it is possible that variables were not sufficiently high to limit phytoplankton growth by limiting light penetration in my study sites. Schallenberg and Burns (2003) found that negative effects on planktonic primary productivity in Lake Waihola (one of my study sites) by wind-induced sediment resuspension, due to restriction of light penetration to the wetland, was rare, and relationships between sediment resuspension and phytoplankton were more often positive.

The number of phytoplankton genera counted correlated negatively with measures of TP, DRP, TIN and TSS, suggesting that genera richness declines in more eutrophic systems.

This concurs with the finding of Cottingham and Carpenter (1998) that genera richness declined with lake enrichment.

The microbial food web

My prediction that the abundance and biomass of phytoplankton would relate positively to the biomass of microbial food web components was confirmed. Abundance of *Cryptomonas, Cyclotella, Synura,* and *Euglena* sp. 2 were the genera most strongly positively related to total ciliate biomass. Amongst phytoplankton Divisions, the abundance of diatoms and euglenophytes were positively related to ciliates biomass. These findings may indicate that ciliates in the wetlands consume these phytoplankton genera and Divisions, while not suppressing their populations. Alternatively, the ciliate populations and these phytoplankton genera and Divisions may share a zooplankton predator, explaining their apparent relationship. Ciliate growth may be stimulated by consuming other genera and Divisions which have been suppressed by ciliate grazing, without the negative relationship being revealed by my study.

As phytoplankton genera, or Divisions, and heterotrophic flagellates were not correlated, there is no evidence of flagellates grazing on phytoplankton populations.

Total phytoplankton biomass was positively correlated to ciliate biomass as in previous studies (Pace 1986, Gasol *et al*. 1995, James and Hall 1995, Burns and Schallenberg 1996, Hwang and Heath 1997b, Jeppesen *et al*. 2000). The positive correlation may indicate that, by grazing heavily on PP, ciliates released larger algae, which were a greater proportion of the total phytoplankton biomass than PP in my study, from competition for nutrients and light. This scenario would be consistent with my observation of a negative relationship between PP and ciliates (Chapter 4 Microbial food web). The possibility that ciliate and phytoplankton biomasses are simultaneously suppressed by zooplankton grazing appears unlikely as neither correlated negatively with zooplankton abundance (Chapter 7, Zooplankton). Alternatively, ciliates may be grazing on larger phytoplankton, but not suppressing phytoplankton biomass due to growth rates of phytoplankton exceeding the predation rate of ciliates. In addition, exudates of phytoplankton stimulate bacterial growth, in turn stimulating the growth of HNF (which may also graze directly on PP and release larger phytoplankton). Ciliates feed on bacteria, HNF and algae (Fenchel 1987); therefore, ciliates may also graze on the increased bacteria and HNF, thereby reducing their suppression of phytoplankton biomass. Neither biomass of bacteria nor HNF correlated with ciliate biomass in my study (Chapter 4 Microbial

food web), due possibly to an inability of ciliates to suppress rapidly growing bacterial or HNF biomass, and the diversity of their prey. Gasol *et al*. (1995) also found that while ciliate abundance correlated positively with chlorophyll *a* concentration, it did not correlate with HNF. In contrast, James and Hall (1995) and Hwang and Heath (1997b) observed correlations between ciliate and bacterial abundance, and between ciliate and HNF abundance, in addition to positive correlations between ciliate abundance and chlorophyll *a* concentration.

Ciliates

Abundances of *Cryptomonas, Cyclotella, Synura, Euglena* sp. 2, and *Phacus* were the genera most strongly positively related to the biomass of prostomatids, *Strombidium* and oligotrichs, and the abundance of the diatom, *Navicula* was strongly positively related to the biomass of *Urocentrum*. The abundance of diatoms and euglenophytes were the phytoplankton Divisions most strongly positively related to the biomass of prostomatids and oligotrichs. Again, contrasting factors may explain these relationships: prostomatids, *Strombidium*, oligotrichs and *Urocentrum* in the wetlands may directly consume these phytoplankton genera and Divisions, while not suppressing populations of the genera or Divisions, or, the ciliate populations and these phytoplankton genera and Divisions may share a zooplankton predator, and be suppressed at the same rate.

Phytoplankton biomass was positively related to the biomass of total ciliates, prostomatids, oligotrichs, peritrichs, hypotrichs and *Litonotus*, indicating that a wide variety of ciliate taxa and sizes will consume algae. Alternatively, the relationship between algae and the larger ciliates may be due to direct grazing, while the smaller ciliates may still be grazing on bacterial populations that are enhanced by DOC exudates from algae. While there were no relationships between bacteria and oligotrichs, bacteria and prostomatids and bacteria and hypotrichs (usually greater than 30µm in length), the negative correlations between bacterial biomass and biomass of peritrichs and *Litonotus* (both taxa were usually less than 25µm in length) may confirm that suggestion. In Lake Oglethorpe, Pace (1982) reported that three taxa of larger ciliates, *Lembadion magnum* (a hymenostomatid 50x100µm), *Coleps* sp. (a prosotomatid 31x42µm), and gymnostomes (43x53µm) correlated positively with chlorophyll *a*, implying that the larger ciliates were consuming algae. The same ciliates that correlated to bacteria in Lake Oglethorpe were also correlated positively to phaeopigments, implying that those taxa were feeding on dead or dying algae and/or the bacteria associated with the dying

algae. However, James *et al*. (1995) found that chlorophyll *a* was negatively correlated with abundance of *Askenasia,* total ciliate abundance and total ciliate biomass in Lake Taupo, but a possible explanation for this was that chlorophyll *a* concentration peaked in winter when ciliate abundance was low.

Zooplankton

I predicted that the abundance and biomass of phytoplankton would relate negatively with the abundance of crustacean zooplankton, however, the relationships between the abundances of phytoplankton and crustacean zooplankton were all positive. The abundances of *Scenedesmus, Cosmarium, Pediastrum* and *Staurastrum,* colonies of *Asterionella, Phacus*, and *Synura* were positively related to *Bosmina* abundances, while *Euglena sp. 2*, *Cyclotella, and Cymatopleura* were positively related to nauplii abundance. The abundances of the phytoplankton Divisions: diatoms and euglenophytes were positively related to copepodites, while cyanobacteria were related positively to adult copepods and *Ceriodaphnia*. Several factors may explain the relationships between the phytoplankton genera and Divisions and zooplankton: the zooplankter may directly consume the genera and Divisions to which it is positively related, while not suppressing the phytoplankton population. Burns (1979) also observed diatoms, among other phytoplankters, in the gut contents of the copepod *Boeckella dilatata* from Lake Hayes, one of my study sites. Euglenophytes were not detected, but as they are soft bodied, without the siliceous frustule of the diatoms, they are readily digested by the zooplankton and rarely detectable in gut contents. Alternatively, the zooplankton population may increase by preying on a component of the microbial food web that directly consumes the phytoplankton genera or Division. It may also be possible that a positive relationship between zooplankton taxa and phytoplankton genera or Divisions is a result of zooplankton thriving by grazing the edible algae during a peak in phytoplankton population growth, while the inedible algae remain and become the dominant alga in the phytoplankton community. This latter explanation may be supported by my finding that cyanobacteria *(Anabaena)* were positively related to adult copepods and *Ceriodaphnia,* as toxic effects or rejection by predators of *Anabaena* have previously been reported in New Zealand lakes (Viner and White 1987).

<u>Copepods versus Cladocerans</u>

I predicted that the abundance and biomass of phytoplankton would relate negatively with the percentage of copepods of total crustacean zooplankton abundance. The abundance of the chlorophytes, *Coelastrum, Kirchneriella, Schizochlamys, Staurastrum, Tetraedron* and *Westella* correlated negatively with the proportion of copepods and positively with the proportion of cladocerans, while the opposite was true of the abundance of the diatom, *Nitzschia*. This may be due to copepods selectively feeding on larger particles, while cladocerans are relatively unselective in the size of food they consume, which may range from bacteria to algae (Stockner and Porter 1988).

Geographical variables

The number of phytoplankton genera was negatively correlated with percentage of pasture in the catchment and positively correlated with the percentage of tussock, providing support for my prediction that the influence of land use and geographical features would be evident in phytoplankton variates. Land use will affect the phytoplankton community via physicochemical variables, i.e. the percentage of pasture correlated positively with measures of physicochemical variables, while the percentage of tussock correlated negatively with measures of physicochemical variables in my study (Chapter 3 Land use). Thus, the negative relationship between phytoplankton genera richness and catchment modification was consistent with my finding of phytoplankton genera richness correlated negatively with measures of physicochemical variables.

Wetland type

I predicted that the abundance and biomass of phytoplankton would relate positively along a gradient of wetland types ranging from deep lakes to swamps and ponds. Abundance of the chlorophytes, *Kirchneriella, Tetrastrum, Coelastrum, Staurodesmus* and colonies of *Ankistrodesmus* were more commonly associated with deep lakes and reservoirs than with swamps and ponds. Abundance of the chlorophytes, *Chlamydomonas, Closterium* and *Tetraedron,* the dinophyte, *Peridinium,* the diatoms, *Asterionella, Cyclotella* and *Tabellaria*, the euglenophytes, *Euglena* sp. 2 and *Phacus* and the chrysophyte, *Synura* were more commonly associated with swamps and ponds than with deep lakes and reservoirs. The number of phytoplankton genera was lower in estuaries than in deep lakes, reservoirs and shallow lakes.

My findings of *Coelastrum* and *Staurodesmus* associated with deep lakes and reservoirs and *Cyclotella* associated with swamps and ponds, contrasts with those of Reynolds *et al*. (2002) who determined *Coelastrum* belonged in the group characteristic of shallow enriched lakes, ponds and rivers, *Staurodesmus* in the group typical of mesotrophic epilimnia, and *Cyclotella* in the group characteristic of clear, well mixed lakes. However, my observations that *Closterium, Peridinium, Asterionella, Cyclotella* and *Tabellaria*, the euglenophytes, *Euglena* sp. 2 and *Phacus* and the chrysophyte, *Synura* were associated with swamps and ponds agree closely with those of Reynolds *et al*. (2002) who placed *Closterium, Peridinium, Asterionella* and *Tabellaria* in eutrophic and mesotrophic classes, and euglenoids (euglenophytes) and *Synura* in the group characteristic of small organic ponds.

The higher abundance of euglenophytes in swamps and ponds than in all other wetland types was, again, consistent with Reynolds *et al*. (2002) finding of euglenoids (euglenophytes) characteristic of small organic ponds.

Seasonality

I predicted that the abundance and biomass of phytoplankton would change between autumn and spring. The abundances of the diatom, *Cyclotella,* and the chlorophytes, *Staurastrum, Schizochlamys, Kirchneriella* and *Westella* were greater in autumn, which had warmer temperatures than in spring during my study. Abundances of the diatoms, *Fragilaria* and *Nitzschia,* and the chlorophyte, *Ankistrodesmus,* were higher in spring. Viner and White (1987) report that *Fragilaria* and *Ankistrodesmus* either occurred year-round or in winter in New Zealand lakes, while *Kirchneriella* occurred during summer, and these findings are consistent with mine. In Lakes Hayes and Johnson, two of my study sites, Burns and Mitchell (1974) observed *Staurastrum* during summer, and *Fragilaria* year-round (in Lake Johnson only), which is similar to my findings, however, *Cyclotella* occurred in winter. I found *Cyclotella* more abundant (for the whole dataset) in the warmer autumn than spring, but my study did not include winter sampling. I sampled Lakes Hayes and Johnson only in autumn, with *Cyclotella* a very small part of the total phytoplankton abundances (0.2% and 0.1% respectively), so there was no discrepancy between my results and those of Burns and Mitchell (1974).

Among the phytoplankton Divisions, there was a decline in the dominance of diatoms and cyanobacteria from autumn to spring (over winter), suggesting that a late summer/autumn

peak of diatoms and cyanobacteria occurs. A peak in *Anabaena* sp. in New Zealand lakes in summer is common (Viner and White 1987).

Chapter 7 Zooplankton

Introduction

Zooplankton are the link from primary production to higher trophic levels in the aquatic food web (Stockner and Porter 1988). In addition, by grazing on protozoa, zooplankton link the classical food chain with the microbial food web by grazing on protozoa (Porter 1995).

The species composition of the zooplankton community is a result of resource supply (bottom-up effect), predation by planktivores (top-down effect), environmental conditions (bottom-up and top-down effects) and other factors. The supply of food will determine the potential for growth of zooplankton population numbers. Predation by planktivorous fish and invertebrates will limit zooplankton population growth. Selective predation will alter size and species composition. Environmental conditions in the wetland will also determine the zooplankton community via a number of mechanisms: if the population growth of phytoplankton or microbial food web components is limited by nutrient levels, light or temperature, zooplankton population growth will subsequently be affected (bottom-up effect), or, optimal conditions for the growth of macrophytes may ensure refugia for zooplankton from planktivores (top-down effect), or, zooplankton species may only tolerate a certain range of salinity, temperature (Hall and Burns 2002a, 2003) or toxic substances in the wetland (Galbraith and Burns 1997) (bottom-up effects).

Differences in the structure of the zooplankton community will have implications for lower trophic levels, via top-down control. For example, cladocerans have been shown to consume at all levels of the microbial community (Stockner and Porter 1988), behaving as a keystone species on the microbial food web (picoplankton, protozoa) and phytoplankton through which carbon flows (Porter ***et al.*** 1988). Copepods do not appear to consume prey at the picoplankton level (Scavia and Laird 1987) but they may be more effective consumers of protozoa than cladocerans (Burns and Schallenberg 2001). Therefore, whether the zooplankton community is dominated by cladocerans or copepods may influence the top-down effects of the zooplankton on lower trophic levels (Reimann and Christoffersen 1993).

The aims of my study were to determine in 45 wetlands representative of a range of wetland environments in Otago, the relationships between: 1) the abundance of zooplankton and, a) physicochemical variables, b) geographical variables, c) microbial food web and phytoplankton biomass; and 2) the relative contribution of copepods to total zooplankton abundance and, a) physicochemical variables, b) geographical variables, c) microbial food web and phytoplankton biomass. 3) The abundance of zooplankton and the relative contribution of copepods to total zooplankton abundance among wetland types were also determined.

I predicted that the abundance of zooplankton and the percentage of copepods of total zooplankton abundance would correlate positively with: a) measures of physicochemical variables; b) biomass of microbial food web components and phytoplankton; c) catchment modification (higher percentage of land cover in pasture, planted forest, scrub and urban areas, rather than bare ground, water, indigenous forest, and tussock; d) smaller catchments; e) less slope in the catchment; f) smaller wetland area and; g) along a gradient of wetland types ranging from deep lakes to swamps and ponds. I predicted that the abundance of zooplankton and the percentage of copepods of total zooplankton abundance would change between autumn and spring.

Methods

Refer to Methods (chapter 2, page 9)

Data analysis

Abundances of zooplankton were $\log_{(10)}$ transformed as the data were not normally distributed. Several multivariate analyses were carried out: detrended correspondence analysis, redundancy analysis and principal components analysis were performed using CANOCO (v. 4.0) software. Redundancy analysis was used as a detrended correspondence analysis determined gradient lengths were less than 4 standard deviations (ter Braak and Šmilauer 1998). The influence on the abundance of zooplankton of measures of physicochemical variables, biomass of the microbial food web and phytoplankton, and geographical variables, were investigated using redundancy analysis, with forward selection to assess the statistical significance of independent variables. Pearson's correlation analysis was used to examine direct relationships. Differences in wetland types and seasons were examined using principal components analysis. ANOVA and Pearson's correlation analysis were performed with SPSS (v. 10.1) software.

Results

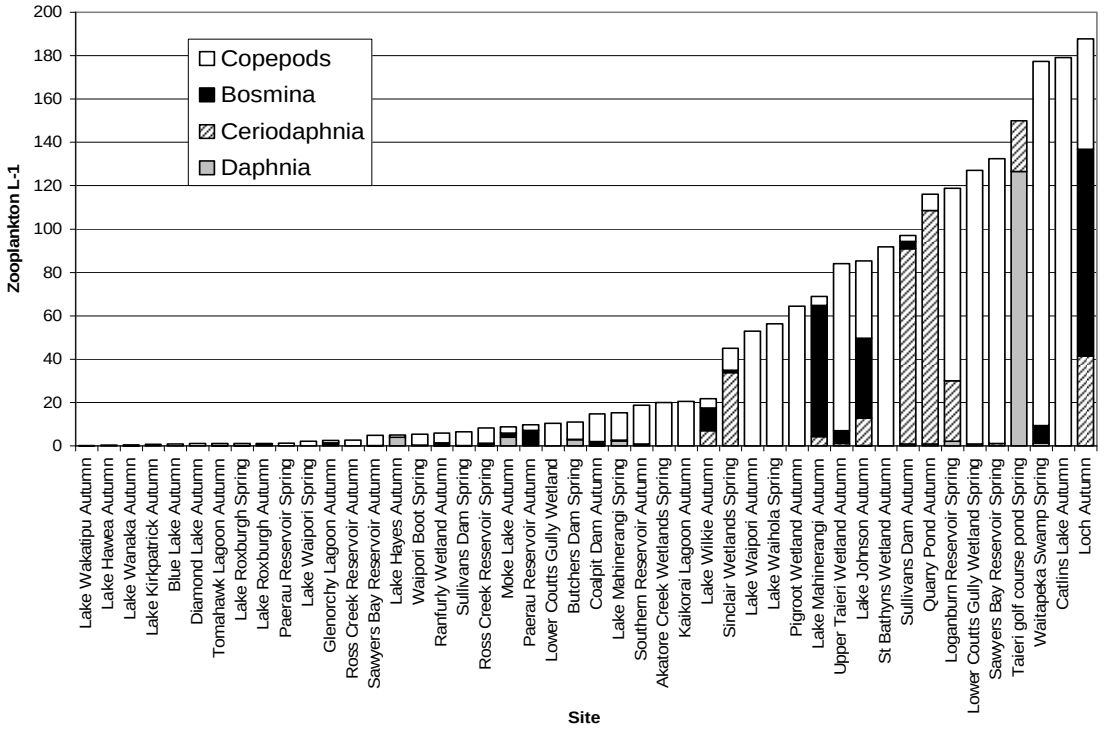
The taxa found in the wetlands sampled included *Daphnia carinata* King, *Ceriodaphnia dubia* Richard, *Bosmina meridionalis* Sars and copepod spp (Table 7.1). Copepod genera were primarily calanoid copepods in the genus *Boeckella.* In addition, harpacticoid and cyclopoid copepods occurred in Hawksbury Lagoon in spring, and Upper Taieri Wetland and Waitapeka Swamp in autumn, with harpacticoids also present in Southern Reservoir in autumn.

Table 7.1 Median, mean±SE and range of the abundance of zooplankton in the wetlands, and the number of wetlands in which the zooplankton taxa occurred.

		Zooplankton (L-1)			
Taxa		Median	Mean±SE	Range	No. of samples with taxon present (total 60)
Daphnia carinata		0.00	18.29±8.66	(0 - 345.00)	24
Ceriodaphnia dubia		0.00	10.88±4.21	(0 - 195.20)	25
Bosmina meridionalis		0.13	26.15±13.00	(0 - 683.81)	32
Copepods					
	Total	10.26	77.87±26.34	(0 - 1384.83)	53
	Adult	1.01	7.98±1.92	(0 - 60.69)	45
	Copepodite	0.73	17.90±8.67	(0 - 433.10)	44
	Nauplii	5.38	51.99±17.71	(0 - 891.03)	51
Total zooplankton		33.37	133.19±32.03	(0.11 – 1431.72)	

The relative contribution of the major crustacean zooplankton taxa to total crustacean zooplankton abundance in the wetlands is shown in Figure 7.1. There was a vast gradient of increasing total crustacean zooplankton abundance in the wetlands, ranging from 0.11 zooplankton L^{-1} in Lake Wakatipu in autumn, to 1431.72 zooplankton L^{-1} in Waitepeka Swamp in autumn. As the figure shows, the composition of the zooplankton community did not remain constant as total zooplankton abundance increased.

a.



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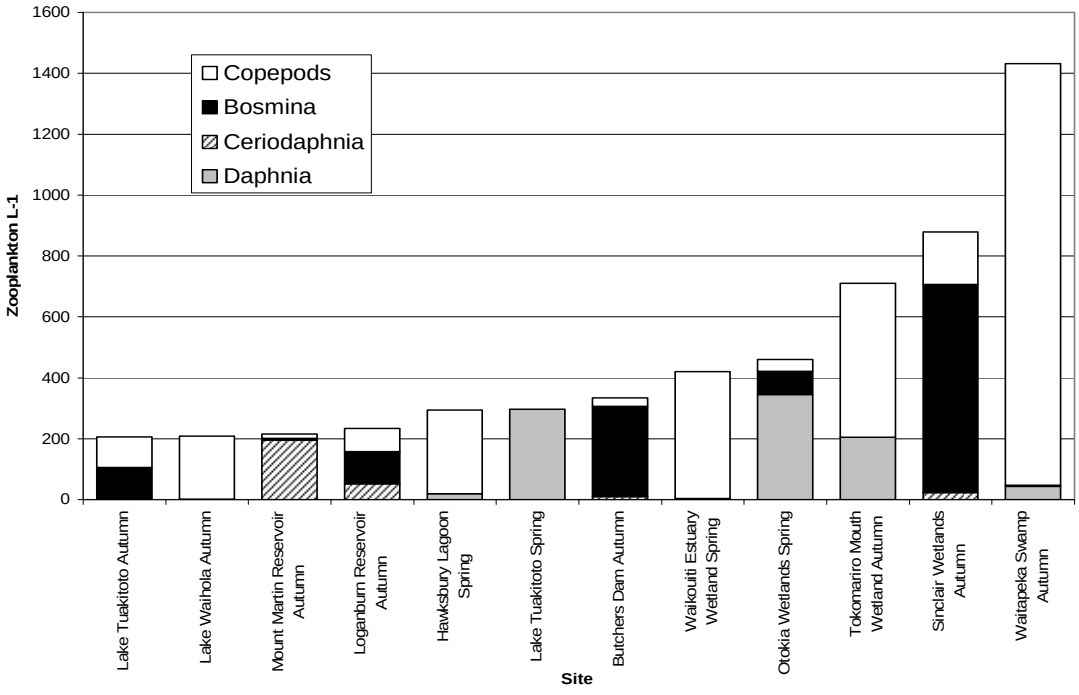

Figure 7.1 Zooplankton abundance a. Sites with total abundance less than 200 zooplankton L^{-1}. b. Sites with total abundance greater than 200 zooplankton L^{-1}.

Relationships among zooplankton

Multivariate analysis

Principal components analysis shows that copepods were weakly positively associated with *D. carinata,* but not with *C. dubia* or *B. meridionalis* (Fig. 7.2). *C. dubia* and *B. meridionalis* were clearly closely positively related and they were both negatively related to *D. carinata.*

Correlation analysis

Within the zooplankton community, *D. carinata* and copepod abundance (adults and copepodites) were positively correlated (Table 7.2). *C. dubia* and *B. meridionalis* abundance were positively correlated. *B. meridionalis* abundance correlated positively with copepodite abundance. As expected, the abundances of adult copepods, copepodites and nauplii were all highly positively correlated among life stages.

Table 7.2 Significant Pearson's correlation results (P <0.05, in parentheses) for correlations among zooplankton taxa. The results in bold fit within the Bonferroni adjustment alpha level of 0.0008.

	Daphnia carinata	*Ceriodaphnia dubia*	*Bosmina meridionalis*	Adult copepods	Copepodite	Nauplii
Daphnia carinata				0.29 (0.0240)	0.31 (0.0171)	
Ceriodaphnia dubia			**0.47 (0.0002)**			
Bosmina meridionalis					0.35 (0.0069)	
Adult copepods					**0.76 (0.0000)**	**0.64 (0.0000)**
Copepodite						**0.71 (0.0000)**
Nauplii						

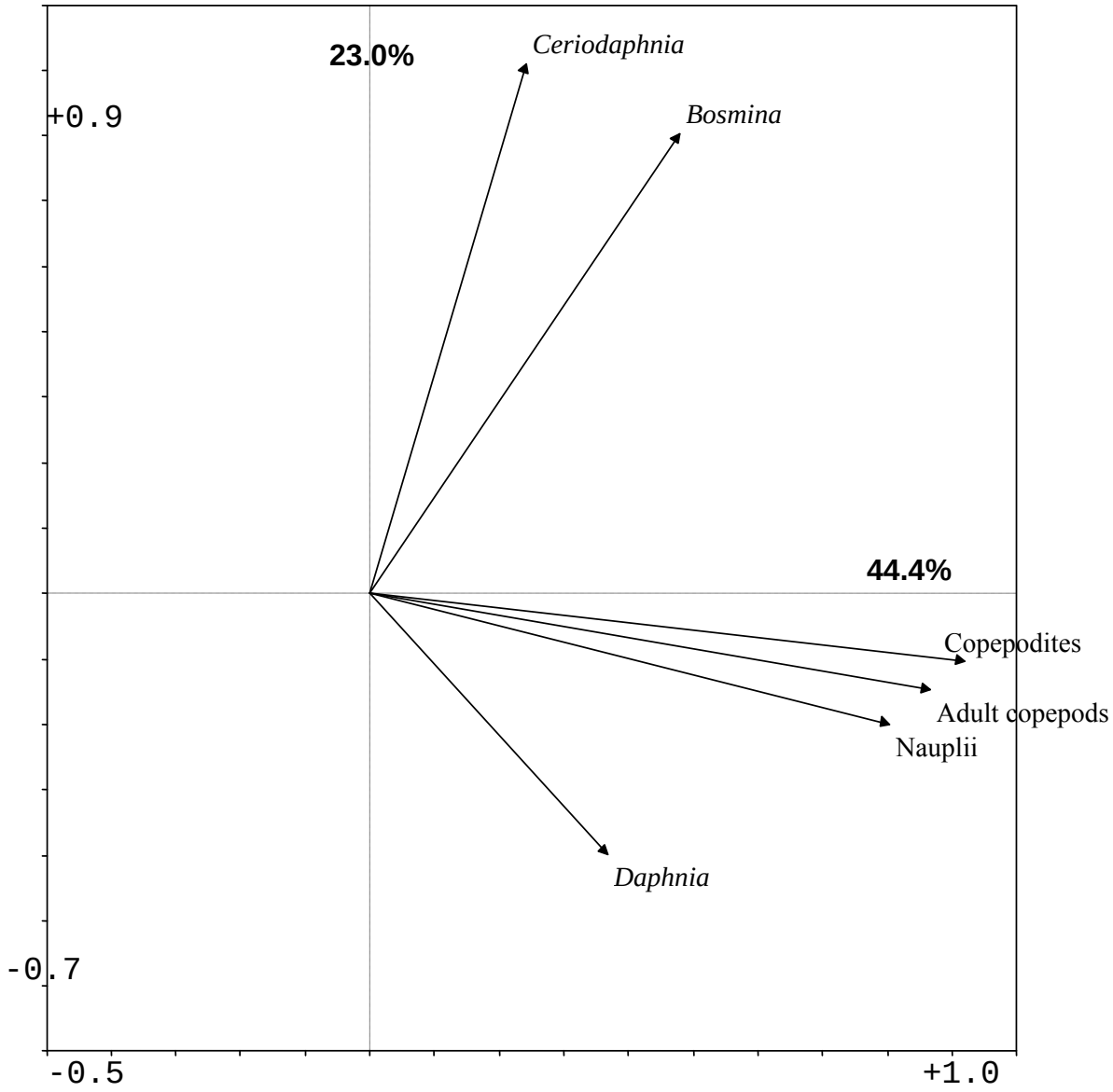

Figure 7.2 Ordination diagram of principal components analysis of zooplankton taxa. Arrows denote the relationships between each zooplankton group. Percentages refer to the percent variance explained by each axis.

Relationships between zooplankton and physicochemical variables

Multivariate Analysis

Redundancy analysis showed physicochemical variables explained a total of 27.8% of the variation in the crustacean zooplankton community (Table 7.3). The first axis explained 15.3% of the zooplankton data. The measures of physicochemical variables most strongly positively loaded with this axis were TP 4% (P=0.03), TN, DRP, TSS and water colour, while TIN 4% (P=0.04) and increased Secchi depth were strongly negatively loaded with this axis (Fig. 7.3). Copepod (adults, copepodites and nauplii) abundance was most strongly positively loaded with the first axis, and *D. carinata* was also associated with this axis. Copepods were the zooplankton taxa to correspond most closely with trophic state, increasing with increasing concentrations of physicochemical variables.

The second axis explained a further 8.0% of the zooplankton data. Conductivity 7% (P=0.015), temperature and pH were the physicochemical variables most strongly negatively loaded with this axis. *B. meridionalis* abundance was most strongly positively loaded with the second axis, and *C. dubia* abundance was also positively associated with this axis.

Correlations

The correlation analysis showed that *D. carinata* abundance correlated positively with conductivity (Table 7.4). *C. dubia* abundance correlated positively with water colour. *B. meridionalis* abundance correlated negatively with pH and conductivity. Copepod abundance correlated positively with TP (copepodites and nauplii), TN (nauplii), DRP (nauplii), TSS (copepodites and nauplii), water colour (nauplii) and conductivity (nauplii) and negatively with Secchi depth (nauplii). Overall, total zooplankton abundance correlated positively with all variables (negatively with Secchi depth) except TIN, temperature and pH. TIN and temperature did not correlate with the abundance of any zooplankton taxa.

Table 7.3 Results of redundancy analysis of zooplankton and physicochemical variables.

Axes	1	2	3	4	Total variance
Eigenvalues :	0.153	0.08	0.029	0.016	1
Species-environment correlations :	0.591	0.592	0.557	0.357	
Cumulative percentage variance					
of species data :	15.3	23.3	26.3	27.8	
of species-environment relation:	52.8	80.4	90.5	95.9	
Sum of all unconstrained eigenvalues					1
Sum of all canonical eigenvalues					0.29

**** Summary of Monte Carlo test ****

Test of significance of first canonical axis: eigenvalue = .153

F-ratio = 8.316

P-value = .0850

Test of significance of all canonical axes : Trace = .290

F-ratio = 1.567

P-value = .0150

Forward Selection Variable	LambdaA	P	F
Conductivity	0.07	0.015	4.14
TP	0.04	0.03	2.89
TIN	0.04	0.04	2.56

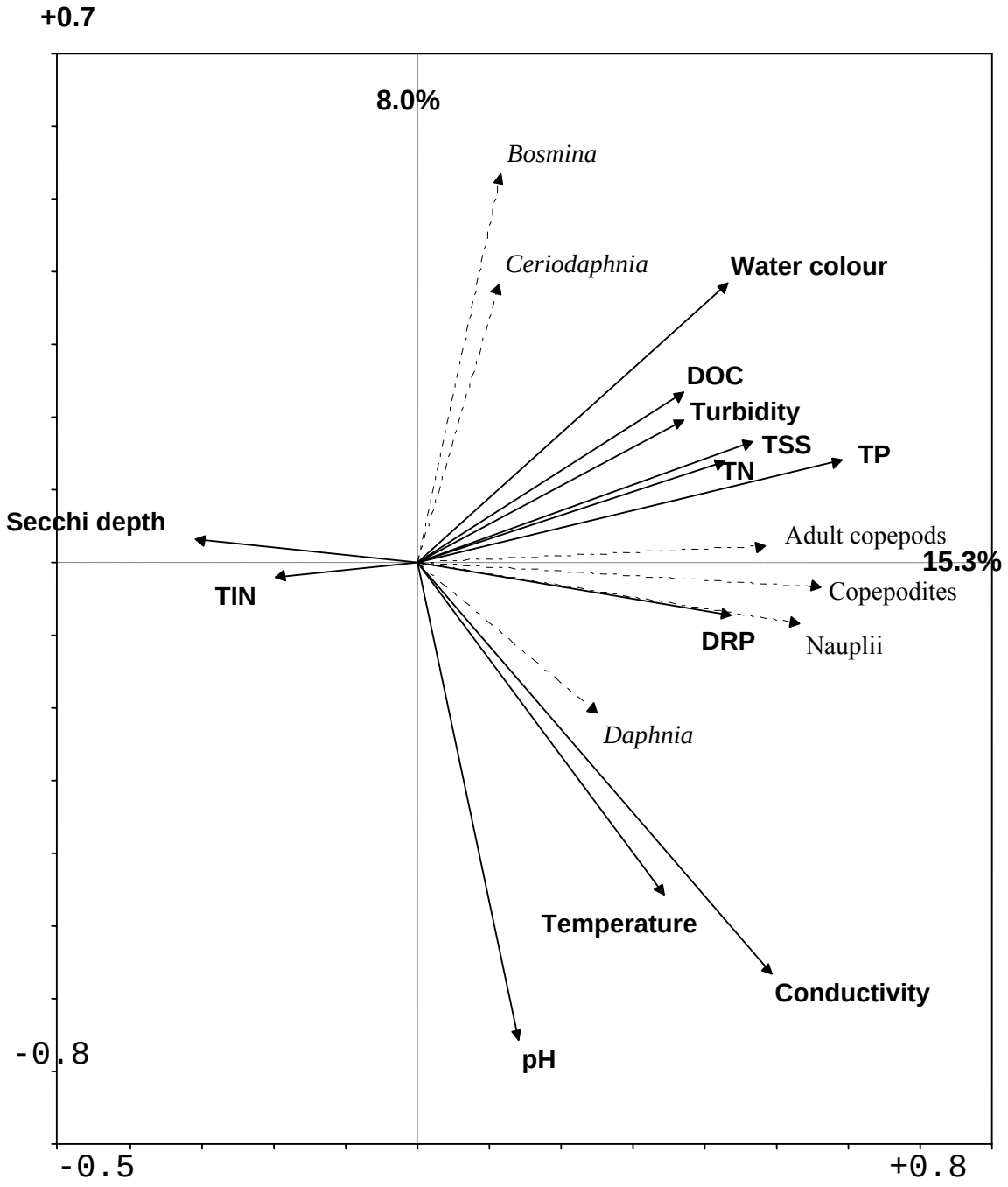

Figure 7.3 Ordination diagram for the redundancy analysis of zooplankton abundance (dotted lines) and physicochemical variables (solid lines). Percentages refer to the percent variance explained by each axis.

Table 7.4 Significant Pearson's correlation results (P <0.05, in parentheses) for correlations between zooplankton abundance and physicochemical variables. No results fit within the Bonferroni adjustment alpha level of 0.0005. Shaded values are negative correlations. TIN and temperature were not correlated with zooplankton taxa.

	TP	TN	DRP	TIN	DOC	Turbidity	TSS	Water colour	Secchi depth	Temperautre	pH	Conductivity
Daphnia carinata												0.27 (0.0417)
Ceriodaphnia dubia								0.27 (0.0426)				
Bosmina meridionalis											-0.30 (0.0192)	-0.29 (0.0257)
Adult copepods												
Copepodite	0.31 (0.0209)						0.27 (0.0410)					
Nauplii	0.36 (0.0054)	0.31 (0.0193)	0.33 (0.0125)				0.30 (0.0197)	0.30 (0.0241)	-0.36 (0.0368)			0.41 (0.0014)
Total Zooplankton	0.36 (0.0057)	0.28 (0.0372)	0.33 (0.0119)		0.28 (0.0317)	0.31 (0.0159)	0.31 (0.0180)	0.38 (0.0029)	-0.35 (0.0408)			0.31 (0.0153)

Relationships between zooplankton and the microbial food web and phytoplankton

Multivariate analysis

Redundancy analysis of zooplankton abundance data and the biomasses of microbial food web components and phytoplankton was not significant.

Correlations

Abundance of *D. carinata* correlated positively with the biomass of phytoplankton (Table 7.5). *B. meridionalis* abundance correlated positively with the biomass of EP. Adult copepod abundance correlated positively with the biomasses of ciliates and phytoplankton. Copepodite abundance correlated positively with the biomasses of EP, ciliates and phytoplankton whereas that of nauplii correlated positively with the biomasses of heterotrophic bacteria, ciliates and phytoplankton.

The number of ciliate taxa in the wetlands did not correlate with the abundance of any zooplankton.

Table 7.5 Significant Pearson's correlation results (P <0.05, in parentheses) for correlations between zooplankton abundance and biomass of microbial food web components and phytoplankton. Results in bold fit within the Bonferroni adjustment level of 0.00063. PP = prokaryotic picophytoplankton, EP = eukaryotic picophytoplankton, HNF = heterotrophic nanoflagellates, PNF = photosynthetic nanoflagellates.

	Bacteria	PP	EP	HNF	PNF	Ciliates	Phytoplankton
Daphnia carinata							0.30 (0.0211)
Ceriodaphnia dubia							
Bosmina meridionalis			0.31 (0.0144)				
Adult copepods						0.27 (0.0385)	0.43 (0.0007)
Copepodite			0.28 (0.0314)			0.39 (0.0018)	**0.51 (0.0000)**
Nauplii	0.32 (0.0130)					0.40 (0.0016)	**0.47 (0.0002)**
Total Zooplankton						0.40 (0.0014)	**0.52 (0.0000)**

Relationships between zooplankton and geographical variables

Multivariate analysis

Redundancy analysis of geographical variables and the zooplankton data was not significant.

Correlations

The correlation analysis showed that *D. carinata* abundance correlated positively with urban cover (Table 7.6). *C. dubia* did not correlate with any geographical variables. *B. meridionalis* abundance correlated positively with tussock cover. Copepod abundance correlated positively with urban (adults and copepodites) and pasture (copepodites).

Table 7.6 Significant Pearson's correlation results (P <0.05, in parentheses) for correlations between zooplankton abundance and geographical variables. There were no results within the Bonferroni adjustment alpha level of 0.0005. Bare ground, inland wetlands, indigenous forest, inland water, planted forest, scrub, urban open space, catchment area, wetland size and slope were not correlated with zooplankton taxa.

	Pasture	Tussock	Urban
Daphnia carinata			0.26 (0.0465)
Ceriodaphnia dubia			
Bosmina meridionalis		0.27 (0.0379)	
Adult copepods			0.27 (0.0370)
Copepodite	0.26 (0.0475)		0.26 (0.0476)
Nauplii			
Total Zooplankton			

Relative contributions of zooplankton species to total zooplankton abundance

The composition of the zooplankton community varied substantially across the wetlands, ranging from cladoceran dominated, as in Lake Wakatipu in autumn, to copepod dominated, such as Catlins Lake in autumn (Fig. 7.4).

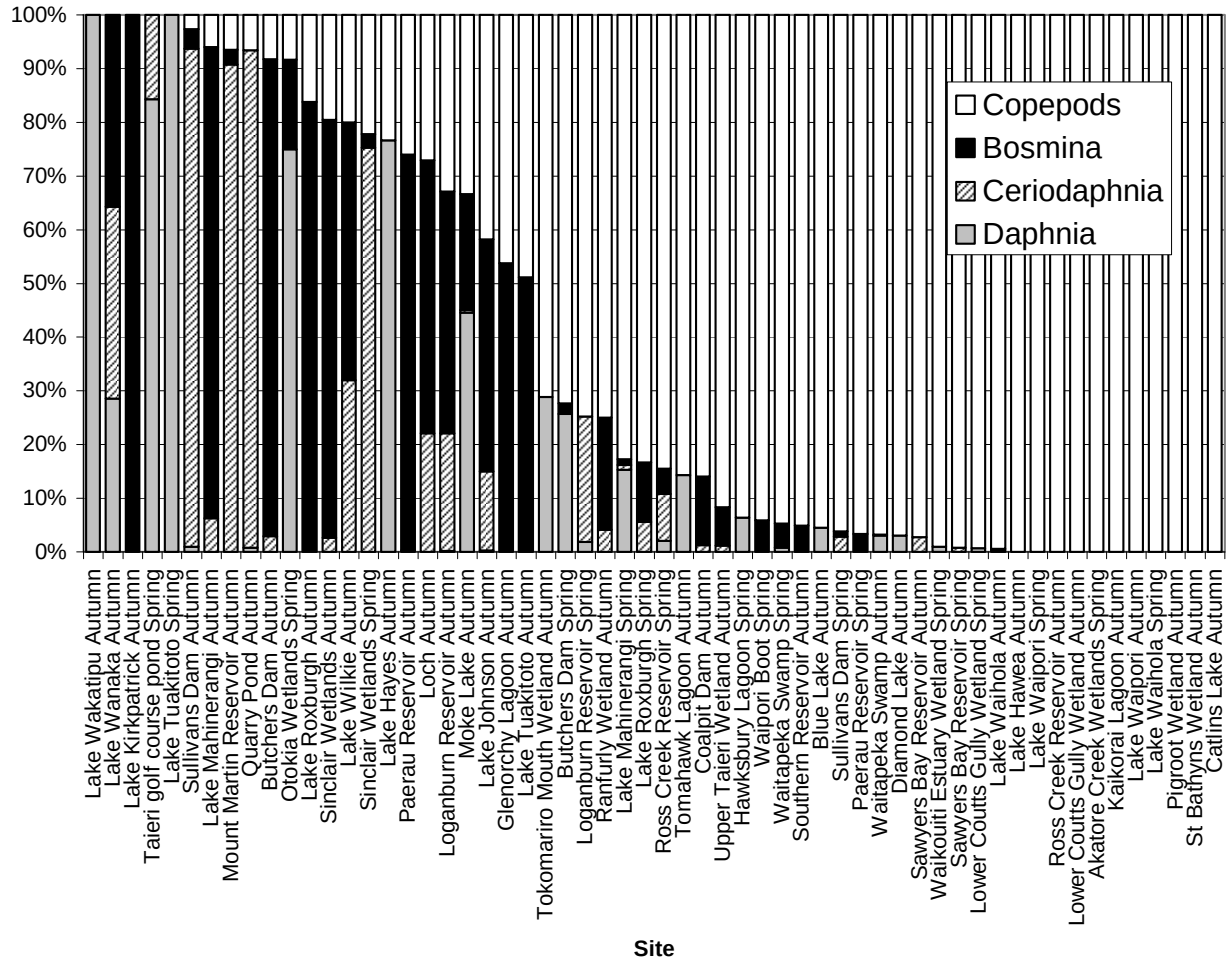

Figure 7.4 Percentage contributions of main crustacean zooplankton taxa to total abundance of these taxa in the wetlands. Percentages refer to the percent variance explained by each axis.

Relative contribution of cladocerans and copepods to the total zooplankton abundance

The relative contribution of copepods and cladocerans to the total zooplankton abundance is shown in Figure 7.5. Copepods dominated in 35 of the 60 wetland samples, and were the only mesozooplankters observed in 11 wetlands.

Relationships between the relative contribution of cladocerans and copepods and physicochemical variables

The relative contribution of copepods to the total zooplankton abundance correlated positively and cladocerans correlated negatively with TSS concentrations (Table 7.7) (As cladocerans and copepods were the only crustacean groups, their percentage contributions were complementary so one will be positive and the other negative, and the correlation coefficients will be the same, with inverse signs).

Table 7.7 Significant Pearson's correlation results (P <0.05, in parentheses) for correlations between proportions of cladocerans and copepods and physicochemical variables. No results fit within the Bonferroni adjustment level of P<0.0042. Shaded values are negative correlations.

	TP	TN	DRP	TIN	DOC	Turbidity	Secchi depth	TSS	Water colour	pH	Temperature	Conductivity
Copepod%								0.30 (0.0208)				
Cladoceran%								-0.30 (0.0208)				

Relationships between the relative contribution of cladocerans and copepods and the microbial food web and phytoplankton

The relative contribution of copepods and cladocerans were not correlated with biomasses of the microbial food web or phytoplankton.

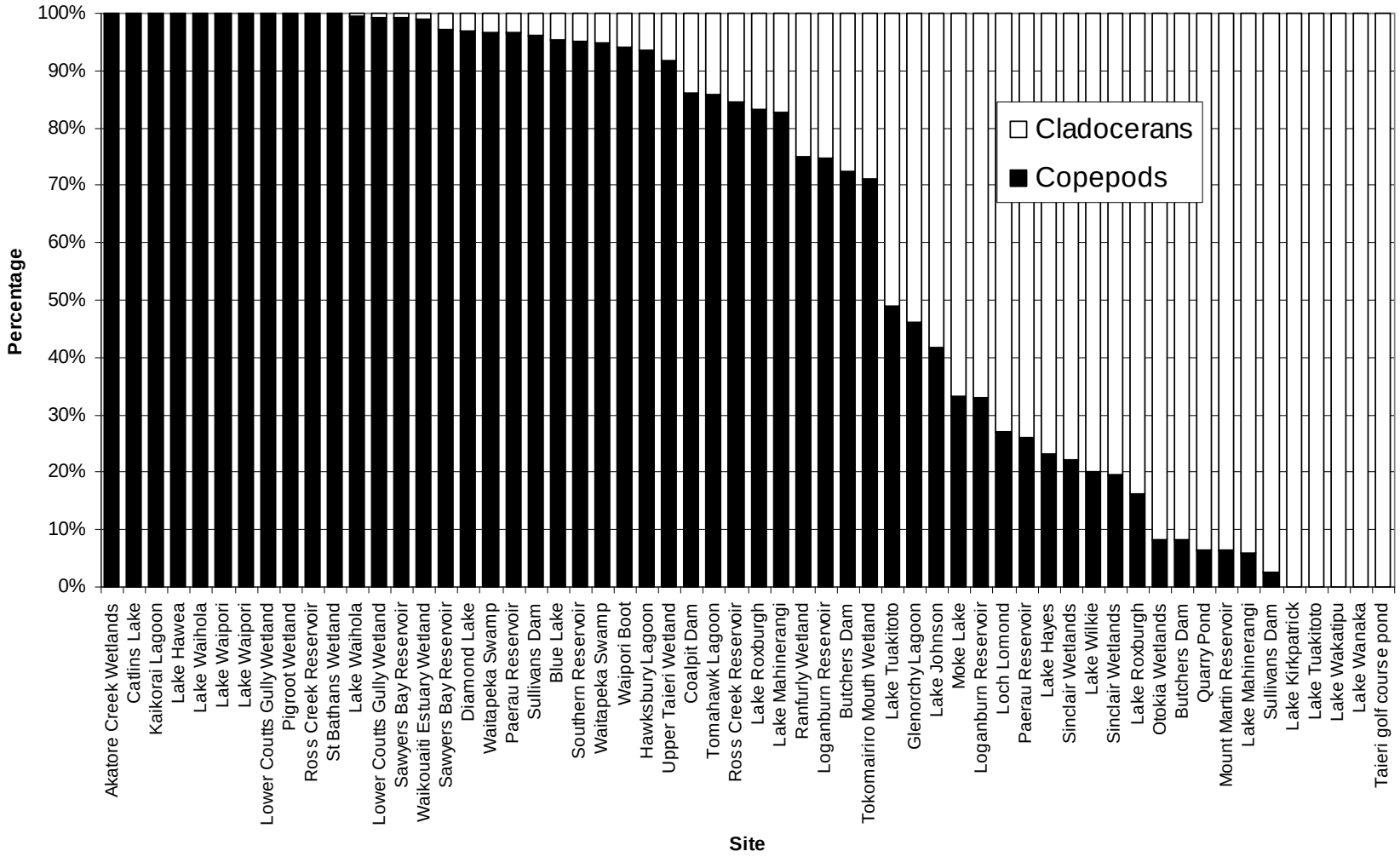

Figure 7.5 Copepod and cladoceran proportion in each wetland as a percentage of total zooplankton abundance.

Relationships between the relative contribution of cladocerans and copepods and geographical variables

The relative contribution of copepods and cladocerans to the total zooplankton abundance was not correlated with geographical variables.

Relative contribution of cladocerans and copepods among wetland types

The relative contribution of copepods to the total zooplankton abundance was higher than that of cladocerans in estuaries and reservoirs (Table 7.8). Deep lakes were the only wetland types where cladocerans dominated; however, this was not significant.

Table 7.8 Relative contribution of cladocerans and copepods among wetland types. Mean percentages (±1SE, N=60) and results of ANOVA (P≤0.05).

	Copepod%	Cladoceran%	P-value
Deep Lakes	40±11	60±11	
Estuaries	10±2	0±2	0.0000
Reservoirs	70±10	30±10	0.0375
Riverine Wetlands	70±24	30±24	
Shallow Lakes	60±13	40±13	
Swamps and Ponds	60±13	40±13	

Differences in relative contribution of cladocerans and copepods between seasons

The relative contribution of copepods to total zooplankton abundance was higher than that of cladocerans in spring, but not significantly in autumn (Table 7.9).

Table 7.9 Percentage contribution (±1SE) of cladocerans and copepods to total zooplankton abundance in autumn and spring.

	Copepod%	Cladoceran%	P-value
Autumn	0.6±0.10	0.4±0.10	
Spring	0.7±0.10	0.3±0.10	0.0025

Differences among wetland types

Multivariate analysis

Zooplankton species and sites are shown on the ordination diagram (Figure 7.6). Copepods appear to be positively associated with swamps and ponds, and negatively associated with deep lakes. No other relationships are clear.

Analysis of variance

ANOVA confirmed that *D. carinata* was more abundant in swamps and ponds than in all other wetland types (Table 7.10). *C. dubia* abundance was higher in reservoirs than in estuaries. *B. meridionalis* was not significantly different among wetland types. Abundances of copepods were lower in deep lakes and riverine wetlands than in shallow lakes and swamps and ponds; nauplii were also less abundant in deep lakes than in estuaries. The proportions of cladocerans were lower, and those of copepods were higher, in estuaries than in deep and shallow lakes.

Table 7.10 Zooplankton abundance among wetland types. a. Mean abundance (No. L^{-1} ± 1SE). b. ANOVA results. Abundance of *B. meridionalis* was not significantly different among wetland types.

a.

	Deep Lakes	Estuaries	Reservoirs	Riverine Wetlands	Shallow Lakes	Swamps and Ponds
Daphnia carinata	0.8±0.39	0.7±0.56	0.4±0.23	0.0±0.00	31.6±29.56	72.3±37.52
Ceriodaphnia dubia	4.2±3.00	0.0±0.00	25.1±13.84	0.0±0.00	6.4±3.82	13.2±10.74
Bosmina meridionalis	14.1±7.90	0.0±0.00	28.1±20.39	0.6±0.42	80.2±67.86	9.5±7.52
Adult copepods	1.9±1.33	11.6±6.73	6.3±3.21	0.1±0.09	10.6±5.38	15.4±6.45
Copepodite	1.0±0.47	42.5±36.47	3.7±1.51	0.1±0.11	16.8±8.40	50.2±42.60
Nauplii	5.0±2.81	56.4±25.56	16.9±8.52	1.9±1.60	60.6±19.87	168.6±92.26
Total Zooplankton	26.9±14.35	111.2±57.54	80.6±27.34	2.7±1.56	206.2±82.67	329.2±140.22
Copepod%	0.4±0.11	1.0±0.02	0.7±0.10	0.7±0.24	0.6±0.13	0.6±0.13
Cladoceran%	0.6±0.11	0.0±0.02	0.3±0.10	0.3±0.24	0.4±0.13	0.4±0.13

b.

						Cladocerans:	
	Daphnia	*Ceriodaphnia*	Adult copepods	Copepodites	Nauplii	Copepods	Total zooplankton
Deep lake - Estuarine					0.0119	0.0030	0.0190
Deep lake - Reservoir							0.0157
Deep lake - Shallow lake			0.0175	0.0332	0.0053		0.0002
Deep lake - Swamp/pond	0.0028		0.0037	0.0068	0.0033		0.0000
Estuarine - Reservoir		0.0404					
Estuarine - Riverine wetland							0.0273
Estuarine - Shallow lake						0.0498	
Estuarine - Swamp/pond	0.0104						
Reservoir - Riverine wetland							0.0343
Reservoir - Swamp/pond	0.0014						0.0147
Riverine wetland - Shallow lake			0.0481				0.0024
Riverine - Swamp/pond	0.0219		0.0203	0.0356	0.0401		0.0006
Shallow lake - Swamp/pond	0.0442						

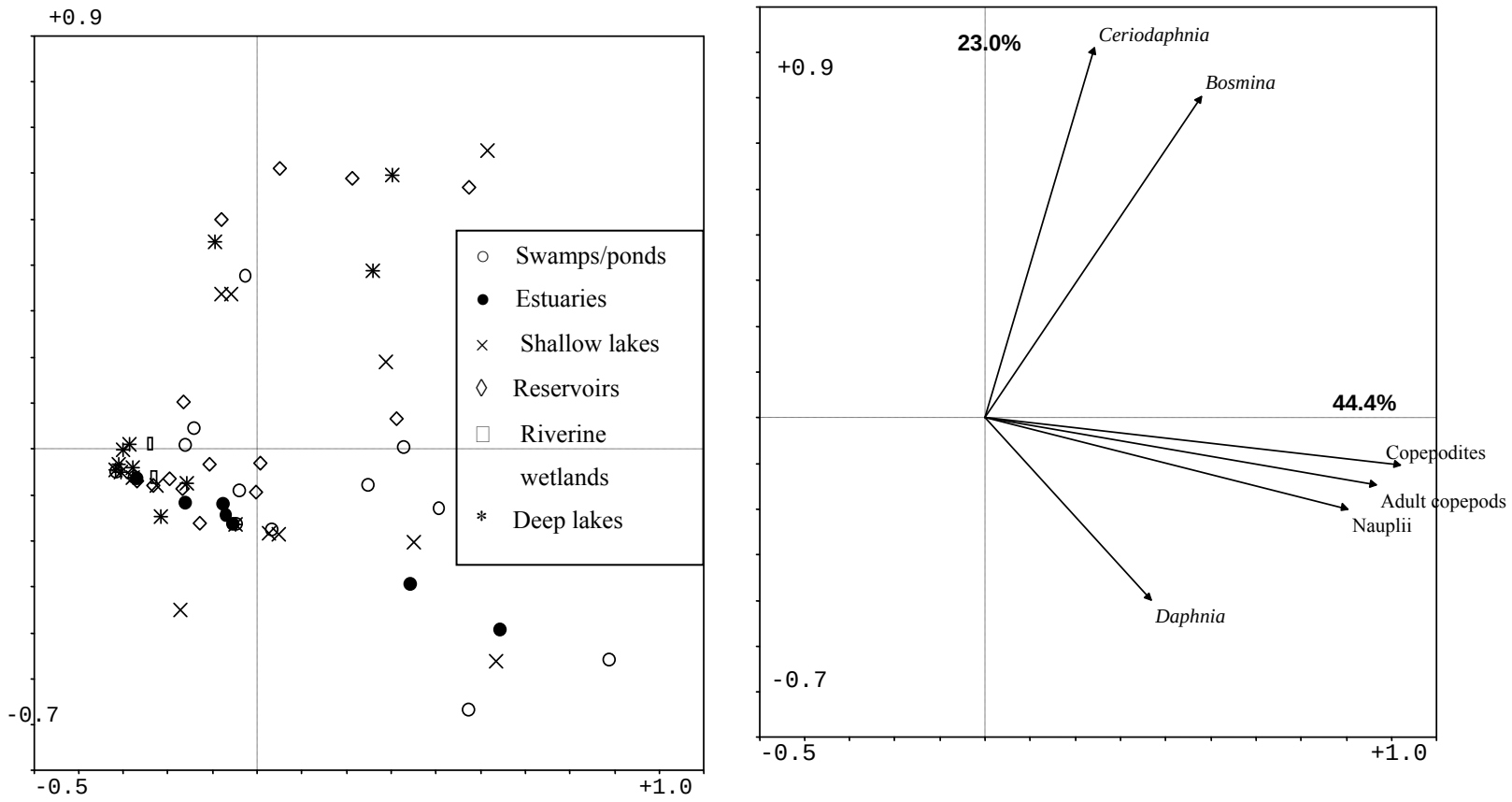

Figure 7.6 Ordination diagram of PCA of zooplankton taxa showing sites by wetland. Left panel – wetland sites, with symbols denoting the different wetland types. Right panel – arrows indicate microbial food web components relationships. Percentages refer to the percent variance explained by each axis.

Differences between autumn and spring

Multivariate analysis

The ordination shows sites by season, with arrows showing the change between autumn and spring (Figure 7.7). Some sites show a trend to move along the second axis, suggesting there may be some seasonal influence on the zooplankton abundance related to cladoceran abundance. *B. meridionalis* and *C. dubia* have a stronger relationship with autumn samples, while *D. carinata* is strongly related to spring samples. Copepods did not show a relationship with season.

Analysis of variance

ANOVA showed that *B. meridionalis* was the only crustacean zooplankter that differed in abundance between seasons, and was much more abundant in autumn than in spring (Table 7.11).

Table 7.11 Zooplankton abundance between spring and autumn. a. Mean abundance (No. L^{-1} ± 1SE). b. ANOVA results.

	Autumn	Spring	P-value
Daphnia carinata	3.0±2.94	20.5±19.76	
Ceriodaphnia dubia	11.9±6.63	4.3±2.80	
Bosmina meridionalis	84.5±47.53	0.7±0.53	0.0056
Adult copepods	9.5±4.54	5.7±3.19	
Copepodite	37.3±28.42	4.1±2.03	
Nauplii	89.8±58.73	31.8±12.54	
Total zooplankton	235.9±103.62	67.0±22.45	
Copepod %	60±10	70±10	
Cladoceran%	40±10	30±10	

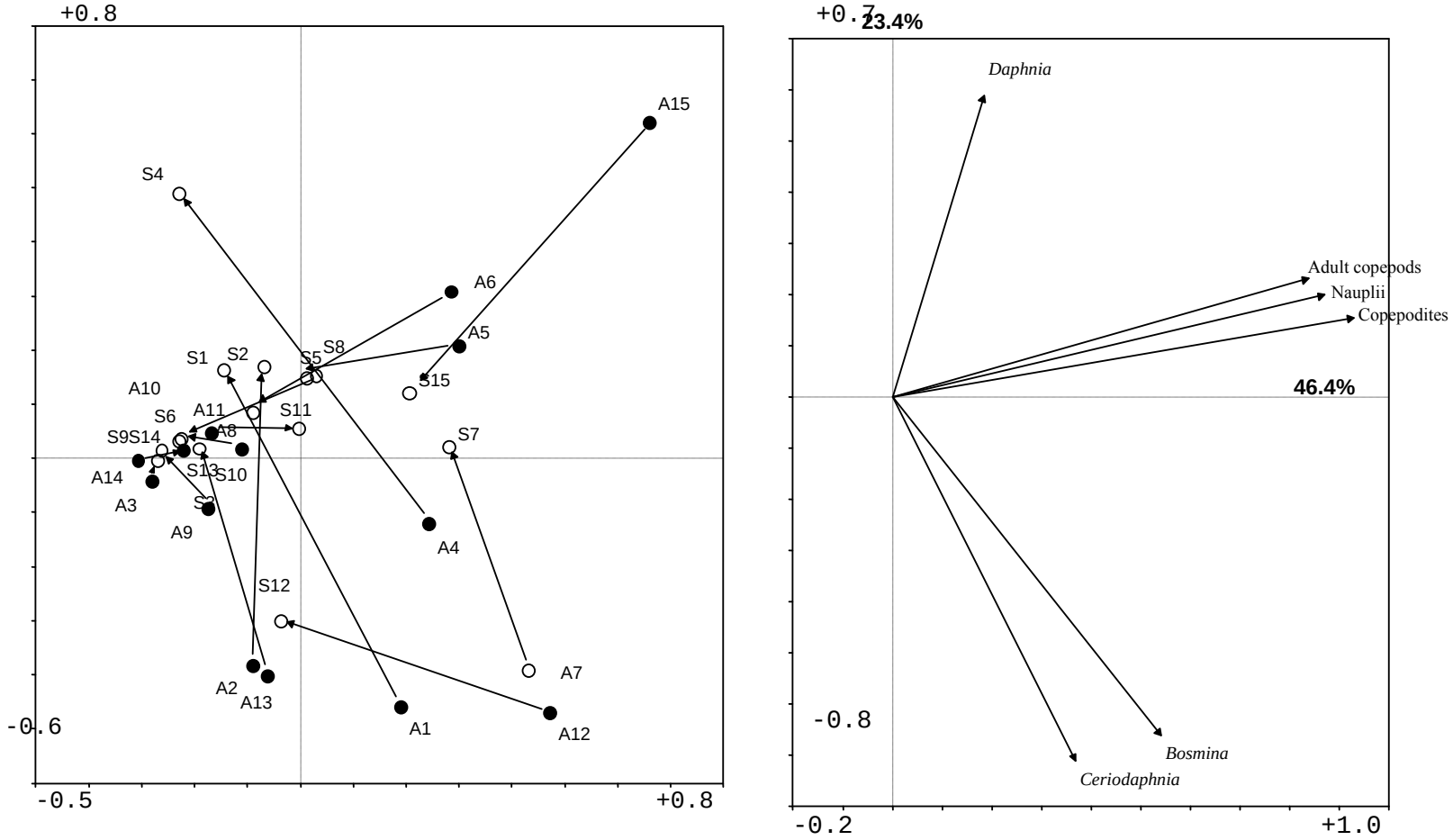

Figure 7.7 Ordination diagrams of PCA of zooplankton taxa showing sites by season. Left –A=autumn sites and S=spring sites, numbers identify each wetland, arrows denote the change between autumn and spring for each wetland. Right – arrows indicate zooplankton relationships. Percentages refer to the percent variance explained by each axis.

Discussion

Zooplankton

My finding of a positive association between abundances of copepods and *D. carinata* abundances is probably due to both these crustacean zooplankton taxa sharing a resource. The abundances of both copepods and *D. carinata* were positively correlated to phytoplankton biomass, so consuming phytoplankton may be stimulating populations of the crustaceans. *C. dubia* and *B. meridionalis* abundances were closely positively related, which may be attributed to both these cladoceran species being very common in New Zealand lakes (Chapman *et al.* 1985, Chapman and Green 1987), however the abundances of both were positively related to picophytoplankton biomass, which may indicate that they have a resource in common. James (1987) found that the peak biomasses of *C. dubia* and *B. meridionalis* did not coincide in New Zealand lakes, and he suggested that these two cladocerans compete for resources or space. Chapman and Green (1987) determined that *C. dubia* and *B. meridionalis* had inverse relationships in abundance in many lakes, which they propose may be either due to coincidence or competitive interactions between the species. Although my sampling was not frequent enough to determine whether *C. dubia* and *B. meridionalis* populations peaked at different times, my findings support the suggestion that they are sharing a resource.

There may be several reasons for the negative relationship between the abundances of both *C. dubia* and *B. meridionalis* and that of *D. carinata*. These cladocerans may compete for resources and space to the detriment of one species, or, *D. carinata* may be more capable than the other two, smaller species, of consuming large phytoplankton that are more abundant in eutrophic systems. The smaller *C. dubia* and *B. meridionalis* cannot consume the larger algae (abundances of *C. dubia* and *B. meridionalis* were not related to phytoplankton biomass) but showed a positive relationship with picophytoplankton biomass in my study. Fish may prey on the large bodied *D. carinata* allowing the smaller *C. dubia* and *B. meridionalis* to fill the niche left by *D. carinata,* the size-selection hypothesis (Brooks and Dodson 1965). Support for the latter explanation is the study by Jeppesen *et al.* (1992) who observed that *Bosmina* replaced *Daphnia* when fish predation pressure increased, and Jeppesen *et al.* (2000) recorded that the contribution of *D. carinata* to the total cladoceran biomass was negatively related to fish CPUE (catch per unit effort) in 25 South Island, New Zealand, lakes, four of which were included in my study.

Physicochemical variables

My prediction that the abundance of zooplankton would correlate positively with measures of physicochemical variables was confirmed for abundances of copepods and *D. carinata.* Copepods were positively related to measurements of TP, TN, DRP, DOC, TSS, turbidity and water colour, and *D. carinata* abundance was positively related to measurements of DRP. Phytoplankton and ciliate biomasses were also correlated with these measures of physicochemical variables (Chapter 4, Microbial food web). As *D. carinata* abundance correlated with the biomass of phytoplankton, and copepod abundance correlated with the biomasses of ciliates and phytoplankton, measures of physicochemical variables affect zooplankton abundance via increased primary production, both directly and via subsequent increases in ciliate population growth. An increase in *D. carinata* abundance after an increase in trophic status in Lake Mahinerangi, New Zealand, was attributed to the large cladoceran's ability to utilize increased phytoplankton production (Mitchell 1975). My findings concur with those of Jeppesen *et al.* (2000) who found that total zooplankton biomass, and *D. carinata* biomass was positively related to TN, during their one-off sampling over summer in 25 shallow New Zealand lakes, four of which were included in my study. Auer *et al.* (2004) report that copepod and cladoceran biomass and abundance increased with lake trophic status in their survey of 55 north German lakes, however, cladoceran population increases were lower than those of copepods and may have been curbed by fish predation pressure and/or increases in inedible cyanobacteria at higher trophic levels.

However, contrary to my predictions of positive correlations between zooplankton abundance and measures of physicochemical variables, *C. dubia* and *B. meridionalis* abundances were negatively related to TIN and positively related to Secchi depth. A possible explanation for the negative relationships between *B. meridionalis* abundance and TIN concentration may be that the abundance of this species was positively related to EP, which might indicate that it consumes picoplankton. Picocyanobacteria have been found in the guts of *B. meridionalis* in six New Zealand lakes, four of which were included my study, across a range of trophic states (Burns and Stockner 1991). As picophytoplankton are more abundant in oligotrophic wetlands (Chapter 4, Microbial food web), this might explain the higher abundance of these small cladocerans in more oligotrophic wetlands. Alternatively, *D. carinata* may be

more capable than *B. meridionalis* of consuming larger phytoplankton that are more abundant in eutrophic systems, and so out compete *B. meridionalis* in eutrophic conditions.

The positive correlations between copepod abundance and conductivity, and *D. carinata* abundance and conductivity, were most likely due to measures of conductivity correlating positively with the concentration of DRP (Pearson correlation = 0.37, P-value = 0.0038), and DRP concentration, in turn, correlating positively with the biomass of phytoplankton (Chapter 4, Microbial food web), rather than directly due to increased conductivity. Hall and Burns (2002) reported that *D. carinata* survival was reduced at higher salinity and, after monthly sampling for a two year period in Lake Waihola (one of the lakes of my study), they observed that with increased conductivity the abundance of *D. carinata* and the copepod *Boeckella hamata* declined, and the crustacean dominance of these species declined in favour of an estuarine copepod species (Hall and Burns 2003). Schallenberg *et al.* (2003) found that the salinity optima and tolerances of *Boeckella hamata* and *D. carinata* were low (tolerances of 0-0.6 psu and 0-0.3 psu respectively) in a coastal wetland, Lake Waihola (one of the wetlands of my study). However, Hall and Burns (2003) findings of negative effects of conductivity on cladocerans concur with mine of the abundance of *B. meridionalis* correlated negatively with conductivity, as does the finding of Schallenberg *et al.* (2003), that *B. meridionalis* has a low salinity optima and tolerance (tolerance of 0-0.5 psu).

The microbial food web and phytoplankton

Positive correlations were found between *D. carinata* abundance and phytoplankton biomass, and between copepod abundance and biomasses of phytoplankton, ciliates and bacteria, confirming my prediction that the abundance of crustacean zooplankton would relate positively to the biomasses of microbial food web components and phytoplankton.

The positive correlation between *D. carinata* abundance and phytoplankton biomass is probably an outcome of *D. carinata* consuming phytoplankton without suppressing phytoplankton growth. In short-term experiments in two of the study lakes, grazing by *D. carinata* had a negative effect on chlorophyll *a* concentration, (Burns and Schallenberg 1996, Hall and Burns 2002b). It is possible that in my study, subsequent declines in phytoplankton biomass due to grazing by *D. carinata* were not observed due to the one-off sampling procedure.

Positive correlations between copepod abundances and the biomasses of phytoplankton and ciliates may imply either that copepods are consuming both phytoplankton and ciliates,

without suppressing the growth of either, or that copepods and ciliates have a resource in common, with both copepods and ciliates consuming phytoplankton. However, these explanations are not mutually exclusive and both may be operating in a single lake or one, or the other, may dominate in different lakes, or there may be seasonal changes in the dominance of one or the explanations. Auer *et al.* (2004) suggested that ciliates were directly affected by grazing pressure from copepods in their survey of 55 lakes, when they observed that copepod biomass was negatively correlated to ciliate biomass, contrary to my findings of a positive correlation.

The positive correlation between nauplii abundance and bacteria biomass may be evidence of the ability of nauplii to graze effectively on bacteria. Auer *et al.* (2004) also observed a positive correlation between copepod and bacteria biomasses.

Abundances of *B. meridionalis* correlated positively with EP biomasses. As picocyanobacteria were found in the guts of *B. meridionalis* and *C. dubia* in six New Zealand lakes across a range of trophic states (Burns and Stockner 1991), the increased abundance of *Bosmina* may be due to their consuming picophytoplankton.

Geographical variables

The influences of land use and geographical features were evident in the zooplankton community, via physicochemical variables, primary production and the microbial food web, with increased abundance of *D. carinata,* copepod adults and copepodites correlating with increased urban land use, and increased pasture correlating with increased abundance of copepodites. Thus, my prediction that the abundance of zooplankton would correlate positively with catchment modification was partially confirmed. Contrary to my predictions, *B. meridionalis* abundance correlated positively with tussock cover, however, this was consistent with my finding that *B. meridionalis* abundance was negatively related to measures of physicochemical variables.

Wetland type

My prediction that zooplankton would vary among wetland types was confirmed with swamps and ponds having greater mean abundance of *D. carinata* and copepods than other wetland types. The higher abundance of these taxa in swamps and ponds than in other wetland types is likely to be a consequence of swamps and ponds being the most eutrophic wetland types (Chapter 3, Land use), and thus having the highest primary productivity leading to enhanced

D. carinata growth. In their review of New Zealand crustacean zooplankton studies, Chapman and Green (1987) also observed that *D. carinata* was found more frequently in ponds than in lakes, which did not appear to be linked to lower predation by fish in the ponds, and thus was more likely to be a result of *D. carinata* thriving on increased phytoplankton production of ponds.

Seasonality

My prediction that there would be seasonal differences in zooplankton abundance was confirmed as *B. meridionalis* and *C. dubia* were more abundant in autumn than spring, while the reverse was true of *D. carinata*. This appears to contrast with the findings of Chapman *et al.* (1985) who observed both *B. meridionalis* and *C. dubia* year round in seven Rotorua, New Zealand, lakes, without any seasonal patterns in population fluctuations. In eutrophic Lake Rotongaio, the biomass of *B. meridionalis* peaked both in November and April, in contrast to my finding, and the biomass of *C. dubia* peaked in winter (James 1987). However, my findings of higher abundances of *B. meridionalis* and *C. dubia* in autumn than in spring concurs with James (1987) finding that *B. meridionalis* biomass in oligotrophic Lake Taupo peaked in summer, and the biomass of *C. dubia* increased over the summer, peaking in late autumn. The author concluded that temperature and food supply were both important variables in seasonal differences in zooplankton biomass.

My finding of possibly greater abundance of *D. carinata* in spring than autumn may be due to an increase in food supply, as I found phytoplankton increased with cooler temperatures, which occurred during spring (Chapter 4, Microbial food web). The explanation for the inverse relationship between *D. carinata* and the smaller cladocerans may be the size-selective hypothesis (Brooks and Dodson 1965), whereby populations of the larger cladocerans are selectively preyed on by juvenile fish in the spring, and subsequently suppressed. *B. meridionalis* and *C. dubia* populations are then able to thrive without, or with less, competition for food than occurs when the more efficient feeders, the larger bodied *D. carinata,* are present. This mechanism may explain the higher abundances of *B. meridionalis* and *C. dubia* in autumn than in spring. This rationale is supported by the finding of Jeppesen *et al.* (2000), as mentioned previously, that the contribution of *D. carinata* to cladoceran biomass decreased with increasing fish catch per unit effort (CPUE) in South Island lakes (including four of my study).

Contrary to my prediction, copepods did not show a relationship with season, and this contrasted with the findings of James (1987) that copepod abundance and biomass peaked in both oligotrophic Lake Taupo and eutrophic Lake Rotongaio in summer and were at a minimum in April (late autumn). However, my findings concur with those of Chapman *et al.* (1985) who did not observe seasonal differences in total numbers of copepods in seven North Island, New Zealand, lakes.

Relationships between the relative contribution of cladocerans and copepods and physicochemical variables

As relative contribution of copepods correlated positively, and that of cladocerans correlated negatively, only with TSS concentrations, and not with measures of any other physicochemical variable, there was little support for my prediction that the percentage of copepods of total zooplankton abundance would correlate positively with measures of physicochemical variables. Neither did Jeppesen *et al.* (2000) detect any clear changes in the relative contributions of copepods and cladocerans along a nutrient gradient.

Relationships between the relative contribution of cladocerans and copepods and the microbial food web and phytoplankton

Contrary to my predictions, the percentage of copepods and cladocerans of total zooplankton abundance did not correlate with the biomasses of microbial food web components or phytoplankton. Thus, resource supply was not shown to influence the proportion of copepods or cladocerans in my study. However, with the one-off sampling regime of my study, subsequent responses in the proportion of copepods or cladocerans to grazing on increased populations of various components of the microbial food web or phytoplankton, might not have been revealed. Alternatively, instead of the predicted switch in dominance from cladocerans to copepods in response to measurements of physicochemical variables, the cladocerans appear to maintain their proportion across the range of wetlands due possibly to their interspecific environmental tolerances, with the abundance of *D. carinata* correlated positively, and abundances of *C. dubia* and *B. meridionalis* negatively, with measurements of physicochemical variables.

Relationships between the relative contribution of cladocerans and copepods and geographical variables

Contrary to my prediction the percentage of copepods or cladocerans of total zooplankton abundance did not correlate positively with catchment modification, catchment area, slope or wetland area. However, as with the relationship between the relative proportion of copepods and cladocerans and physicochemical variables, this lack of a relationship might have been due to the proportion of cladocerans being composed of all three cladoceran species: *D. carinata, C. dubia,* and *B. meridionalis.* The abundance of *D. carinata* correlated positively with catchment modification, with the opposite true of *B. meridionalis* abundance, so that replacement in dominance of one cladoceran species by another in response to geographical variables might have masked the predicted switch in dominance from cladoceran species to copepod species.

Differences in relative contribution of cladocerans and copepods among wetland types

The relative contribution of copepods to total zooplankton abundance was higher than that of cladocerans in estuaries and reservoirs. My finding of higher copepod proportions in estuaries is consistent with findings of Hall and Burns (2002) and Schallenberg *et al.* (2003) that cladoceran species populations were less tolerant of raised salinity in Lake Waihola and were replaced by an estuarine copepod.

Differences in relative contribution of cladocerans and copepods between seasons

The relative contribution of copepods to total zooplankton abundance was higher than that of cladocerans in spring, but not significantly higher in autumn. This contrasts with the findings from 55 German lakes, where the cladoceran contribution to the total metazooplankton biomass was higher in spring (up to 33%), than in all other seasons (Auer *et al.* 2004). Again, as *B. meridionalis* and *C. dubia* were more abundant in autumn than spring, while the reverse was true of *D. carinata,* cladocerans appear to maintain their proportion of total zooplankton abundance due to an inverse relationship among the cladoceran species, either due to size-selective grazing by fish, temperature tolerances or food supply.

Chapter 8 General discussion

Introduction

This comparison of pelagic communities along gradients of catchment land use, wetland size and trophic state, has provided insight into the consequences for wetlands of land management practices and environmental perturbations. Linkages between catchments, water quality and the pelagic food web have been examined from both a bottom-up (resource supply) and a top-down (predation) perspective. While the effects of catchment features, via nutrient and substrate runoff, were evident in the abundance and structure of the aquatic community, the impact of wetland type was also apparent.

Geographical factors

My prediction of higher nutrient and substrate loads contained in runoff from modified catchments was confirmed. The percentage of pasture in a catchment related positively to levels of physicochemical variables in the wetland, confirmation that agricultural use of land negatively affects water quality. Conversely, the percentage of the catchment covered in native tussock was negatively related to physicochemical measures, supporting the premise that the native vegetation may protect the water supply from detrimental effects by intercepting water, and thus dissolved substrates and nutrients. However, the enhancement in water quality in tussock catchments may be due to tussock grasslands being more commonly found in steep catchments which are not available for grazing, or the less productive and less palatable tussock vegetation is not grazed, hence the tussock community is not subject to the effects of fertiliser applications, stock effluent and stock trampling. The percentage of the catchment in plantation forest cover was related to wetlands of higher trophy, while the opposite was true of indigenous forests, evidence that conversion of catchments to exotic forests may have consequences for wetland communities. An increasing proportion of a catchment in urban development was also observed to be detrimental to wetland water quality.

Physicochemical variables correlated negatively with the geographical features of the study sites: catchment area, slope and wetland area. Many factors may explain the relationships: larger catchments are associated with wetlands of larger volume which will

dilute concentrations of nutrients and substrates and, steep catchments will deliver water to the wetland faster than gently sloping catchments, allowing less time for water to accumulate nutrients and matter from the soil.

Further, the influence of land use and geographical features on water quality was evident in the aquatic community, with increased catchment modification, and decreased wetland area, catchment area and slope stimulating populations of microbial food web components and phytoplankton, and suppressing biomass of picophytoplankton. This provides evidence that enables managers of water supplies, wetland conservators and those responsible for land management, to foresee the effects of activities in catchments on the pelagic food web.

In addition, the influence of land use and geographical features were evident in the ciliate community, with increased catchment development, and decreased wetland area, catchment area and slope correlating with increased biomass of prostomatids, oligotrichs and *Strobilidium*. However, *Urocentrum* biomass correlated positively with the percentage of tussock and catchment area, suggesting that *Urocentrum* biomass might be higher in wetlands in undeveloped catchments. The finding that a ciliate genus may respond to activities in the catchment raises the potential of ciliate taxa to be indicators of water quality. Identifying ciliates to the species level in comparative studies such as mine might reveal certain species that would be useful for this purpose. Further testing of the environmental tolerances of such species, using *in situ* experiments, may provide managers with a valuable tool for testing aquatic ecosystem health.

Land use affected the highest trophic levels of the pelagic food webs that I studied, in that increased catchment modification was clearly positively related to copepod and *D. carinata* abundance, whereas natural tussock cover related to *B. meridionalis* abundance. Thus, it appears that land use activities resonate in the aquatic community.

Land use development that leads to enrichment in wetlands appears to reduce phytoplankton diversity, as the number of phytoplankton genera in the wetlands were negatively related to percentage of pasture and positively related to the percentage of native tussock grassland in the catchment.

Physicochemical variables

Increasing trophic status of the wetlands was associated with enhanced populations of microbial food web components and phytoplankton, as I predicted. However, the mechanism that caused the enhancement in population growth is not obvious from my

study. Indeed, a variety of mechanisms may be operating to stimulate plankton growth in the wetlands. Increases in concentrations of nutrients may stimulate phytoplankton growth, which, in turn, stimulates the biomass of ciliates that consume phytoplankton, with ciliate communities thriving by bypassing the other heterotrophic components of the microbial food web in the more productive systems. Increased bacterial production in response to increased concentrations of nutrients/substrates and phytoplankton, may be immediately grazed by heterotrophic nanoflagellates (and/or ciliates and zooplankton), which, in turn, are grazed by ciliates, meaning the microbial food web contributes to higher trophic levels.

By manipulating the physicochemical variables in *in situ* experiments in these wetlands, to replicate the range of environmental tolerances of the microbial food web components that I observed, the true mechanisms that govern the niches of the pelagic aquatic organisms might be revealed.

A decline in picophytoplankton biomass with increased measures of physicochemical variables supported the proposition that high concentrations of nutrients result in an unfavourable environment for these organisms. Researchers have proposed that picophytoplankton may be useful indicators of aquatic ecosystem health in lakes (Munawar and Weisse 1989, Schallenberg and Burns 2001). My findings suggest that the use of picophytoplankton as indicator organisms may extend more widely to cover a range of wetlands. *In situ* experiments of picophytoplankton growth in response to nutrients, with the exclusion of predators, would further knowledge in this area.

My findings of a greater proportion of bacteria in oligotrophic wetlands and a greater proportion of phytoplankton in eutrophic wetlands are consistent with the concept that the microbial food web as a source of carbon to higher trophic levels will be more important in oligotrophic systems than in eutrophic systems. However, the lack of relationships between percentages of heterotrophic nanoflagellates and physicochemical variables, and between percentages of ciliates and physicochemical variables led to the conclusion that, whether the microbial food web is more, or less, important is not relevant, because the food web structure simply adapted, with protozoa still contributing to higher levels even in eutrophic systems. This may occur by protozoa consuming different resources, and/or variations in protozoan community structure across a gradient of wetland trophy.

The biomasses of most ciliate taxa increased with measures of physicochemical variables. However, the biomass of *Urocentrum* contrasted with the majority of ciliate taxa by not responding to increasing trophic status, and relating negatively to water colour. This

response is consistent with the positive relationship between this ciliate and tussock grassland catchments, noted before, and further supports the potential of this genus to be an indicator organism of aquatic health. The relative proportion of *Strombidium* to total ciliate biomass correlated positively with nutrient levels, providing some evidence of variation in ciliate community composition in response to lake trophy. The identification of ciliates in my study was at a high level, with only some taxa identified to genus and most taxa grouped into Divisions. Further investigation of the response of the ciliate community to physicochemical variables at genus, or ideally, species level might reveal that ciliate community composition does, indeed, respond strongly to wetland eutrophication.

Abundances of copepods and *D. carinata* responded positively to increases in measures of physicochemical variables, through consuming phytoplankton or components of the microbial food web. Both pathways were likely to have been operating among wetland types and even within the same wetland, but the contributions of one or the other is likely to vary seasonally or, with dominance of zooplankton species. This study could not distinguish which pathway was contributing the most to zooplankton population growth, and only direct examination of the relationships between each component of the pelagic food web by experimentation will allow certainty of the mechanisms operating in each system.

C. dubia and *B. meridionalis* abundances were negatively related to water quality variables, and it may be worthwhile to examine the causes of the decline in these species with trophic state. These cladocerans may rely on picophytoplankton, which are more likely in the oligotrophic system or, *D. carinata* may be more capable than the smaller cladocerans of consuming larger phytoplankton that are more abundant in eutrophic systems.

The diversity of the phytoplankton community in wetlands may be sensitive to enrichment. The number of phytoplankton genera in the wetlands was negatively related to measures of physicochemical variables, thus enrichment of wetlands appears to reduce phytoplankton diversity. In addition, my findings that abundances of phytoplankton Divisions, cryptophytes, chlorophytes and diatoms were positively related, whereas dinophyte abundance was negatively related, to measures of physicochemical variables, reveals that there are distinct shifts in community composition in response to trophy of these wetlands.

The aquatic community

Across the diverse range of 45 wetlands, the biomasses of components of microbial food webs were tightly correlated with their potential resources and some components were coupled with consumers in adjacent trophic levels. Heterotrophic bacteria appeared to be a

major resource for heterotrophic nanoflagellates or, these flagellates and heterotrophic bacteria may depend on the same, or a tightly coupled, resource, e.g. phytoplankton for heterotrophic nanoflagellates, and exudates of phytoplankton for bacteria. Picophytoplankton biomass may have been suppressed by ciliate predation, unless ciliate growth was stimulated, via phytoplankton grazing, by the same nutrients which suppress picophytoplankton populations. If ciliates were grazing heavily on picophytoplankton, they may have released larger algae from competition for nutrients and light or, they may have grazed on larger phytoplankton without suppressing phytoplankton biomass. In addition, phytoplankton exudates may have stimulated bacterial growth, in turn stimulating the growth of heterotrophic nanoflagellates (which may have grazed directly upon picophytoplankton and released larger phytoplankton). Ciliates may also have grazed on the increased bacteria and heterotrophic nanoflagellates, thereby reducing suppression on phytoplankton biomass. Thus, while relationships among trophic levels in the wetlands have been revealed, the mechanisms that gave rise to the relationships now need to be explored. Further "snapshot" comparison studies may reveal these mechanisms if they include samples from the same wetland over consecutive days, to encompass several generations of the micro-organisms.

Examination of relationships between ciliate taxa and other components of the microbial food web, and between ciliate taxa and phytoplankton suggests potential mechanisms of trophic transfer in aquatic communities. Positive relationships between relatively small ciliate taxa and bacteria and flagellates (peritrichs and bacteria, *Litonotus* and bacteria, *Strobilidium* and nanoflagellates, and other small oligotrichs and nanoflagellates) suggest pathways of carbon flow up via the heterotrophic microbial food web. Groups of other larger ciliates (prostomatids and oligotrichs) were related to phytoplankton biomass, thus the ciliate community may utilise algae for growth. While my comparative study implies these mechanisms are in use, experimentation is needed to exclude the other factors which may give rise to the relationships, for example, shared resources, or a common predator.

Picophytoplankton biomass was negatively related with oligotrich biomass, implying that oligotrichs are the ciliate taxa that may suppress picophytoplankton populations via grazing, if the negative relationship is direct, and not a result of opposing reactions to nutrient enrichment. The picophytoplankton experiments suggested previously would also be useful for examining this relationship.

Ciliates and crustacean zooplankton were positively related, either due to resources in common or zooplankton grazing on ciliates. Whereas the biomasses of oligotrichs, prostomatids, *Strombidium* and hypotrichs were related to copepod abundance, oligotrichs and copepods had the strongest relationship. While the positive relationships between ciliate biomass and copepods may be due to both groups consuming phytoplankton, it could also have been a result of the zooplankton feeding directly on ciliates, with my infrequent sampling missing the subsequent declines in ciliate biomass which may occur. The same issues arise when considering the positive relationship between the biomass of the ciliate group of peritrichs, and *D. carinata* abundance. If sampling of the same wetland over consecutive days revealed subsequent declines in ciliate biomass, then grazing is more likely to be the cause of the relationship than shared resources. Grazing experiments to determine if ciliates are a major part of the copepod diet in these wetlands may also shed light on the interactions. The positive relationship between the biomass of small oligotrichs and abundances of both *C. dubia* and *B. meridionalis* again raises additional questions. These small cladocerans may be consuming the small oligotrichs, or they may share a resource. The shared resource is less likely to be phytoplankton, than with the ciliate, copepod and *D. carinata* relationship, as abundances of *C. dubia* and *B. meridionalis* did not relate to phytoplankton biomass. Therefore, further experiments would be useful to investigate whether the small cladocerans and ciliates share a resource other than phytoplankton (picoplankton or nanoflagellates) or, whether grazing is occurring.

Positive associations between copepod and *D. carinata* abundances may have been due to both these crustacean zooplankton taxa sharing a resource (phytoplankton), to which they were both positively related. Close positive relationships between *C. dubia* and *B. meridionalis* abundances suggest these species also shared a resource, but the resource was more likely to be picophytoplankton than algae. Grazing experiments to determine if picophytoplankton is effectively utilised as a food resource by *C. dubia* and *B. meridionalis* would be of interest to verify the grazing relationship between the groups that is implied by my study. These experiments would also illuminate the interactions that are occurring that give rise to the negative relationships between *C. dubia* and *B. meridionalis* and that of *D. carinata*, i.e., whether *D. carinata* consume phytoplankton while the smaller *C. dubia* and *B. meridionalis* cannot consume the larger algae and instead feed on pico- or nano-plankton size organisms. Alternative explanations for the relationships could be competition for resources and space is detrimental to either *D. carinata* or the smaller cladocerans or, fish may prey on the larger bodied *D. carinata* releasing the smaller cladocerans.

Wetland type

Deep lakes were the wetland type most commonly located in steeper upland catchments which had a higher percentage of bare ground and tussock cover than lowland catchments. The lack of development in the catchment may partially explain the lowest measurements of physicochemical variables being recorded for deep lakes, while the highest measures were recorded in swamps and ponds. However, physical factors are likely to influence the trophic state of the wetland: the large volume of water in deep lakes, and the flushing effect of throughflow of water in reservoirs, allows more dilution of nutrients and substrates flowing in the wetland than is possible for other wetland types, whereas swamps, ponds and shallow lakes are likely to be much more affected by allochthonous inputs, littoral vegetation, and autochthonous generation of organic matter.

Wetlands at the ends of the trophic gradient, oligotrophic deep lakes and the eutrophic swamps/ponds showed clear differences in aquatic communities, with the biomasses or abundances of ciliates, phytoplankton, *D. carinata* and copepods highest in swamps and ponds, and lowest in deep lakes, whereas picophytoplankton biomass showed the opposite pattern of distribution. The relationships revealed by my study emphasise that the physical features of a wetland are an important consideration when managing catchments for protection of water quality.

Seasonality

Measurements of water quality and the biomass of the microbial food web and phytoplankton did not differ between autumn and spring in this study. Therefore, it was difficult to attribute any seasonal changes in the aquatic community to resource supply. However, the cladoceran zooplankton species, *B. meridionalis* and *C. dubia,* were more abundant in autumn than spring, while the reverse was true of *D. carinata*. The cause of this difference was unclear from my study, either size-selective grazing by fish on the large cladoceran species in spring or variation in temperature tolerance, may be occurring. However, the finding shows that it would be worthwhile investigating seasonal aspects of the zooplankton community in South Island lakes more thoroughly; tracking the growth of zooplankton populations through frequent sampling over a one to two year period in one or a few wetlands may detect strong seasonal influences.

This research provides evidence of the influence of land use and geographical features on water quality and pelagic communities of wetlands in Otago. Some of this evidence might be used by managers of aquatic systems to improve water quality. The potential of organisms such as picophytoplankton, ciliates and phytoplankton to be indicators of aquatic ecosystem health has been revealed or strengthened by this study. This study also revealed relationships within pelagic food webs in a range of wetland systems. The mechanisms that govern these relationships might be answered in future studies that entail manipulating the food web in controlled conditions, or more focused comparative studies of wetlands.

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