

**Manipulation of Zooplankton Communities  
in Waste Stabilization Lagoons, with a view  
to Optimizing Production for Potential  
Harvest.**

**By**

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## DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma by the University of Tasmania or any other institution, except by way of background information as duly acknowledged. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

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A handwritten signature in black ink, appearing to read 'Niall Doran', is written over a horizontal line.

Niall Doran.

May 1999.



## ABSTRACT

This project was developed as part of a program evaluating the potential coupling of biological waste treatment processes at the Werribee Treatment Complex (WTC) with the harvest of zooplankton material for commercial use. This work specifically aimed to manipulate the distribution of particular zooplankton species according to influent nutrient levels.

The project was conducted on a scale that is rare for such ecological manipulations, with the flow pattern of an operational twelve pond, 70.5 hectare sewage treatment lagoon system being drastically altered to provide two comparable halves composed of paired ponds of similar size, orientation, influent and capacity. The system was subsequently subjected to a target sewage inflow level of 12 million litres a day, with the level of flow experimentally divided equally and unequally between the halves.

Despite low and variable influent rates (due to unpredictable extrinsic factors), different and remarkably consistent patterns developed in the zooplankton communities for each flow regime. Zooplankton distribution and community structure changed markedly across the system following the alteration of flow from the original pattern. Under conditions of equal flow, zooplankton and various environmental concentrations - ammonia, nitrate, nitrite, phosphate and chlorophyll-a - synchronized well between corresponding ponds. Under unequal divisions of flow, both zooplankton and ammonia (representing a gross measure of overall nutrient loading) showed distinct differences between halves that directly corresponded with the new flow regimes, while patterns for nitrate, nitrite, phosphorus and chlorophyll-a reflected these changes by becoming increasingly disparate and chaotic.

It is suggested that these changes in distribution represent a strong nutrient-mediated successional pattern that is overlaid on normal zooplankton seasonal succession and short-term population cycles in such highly eutrophic environments. The unique layout of lagoon systems at WTC promotes the spatial expression of this pattern in contrast to similar temporal (and smaller-scale spatial) changes documented in previous studies. Manipulations of nutrient-mediated succession as conducted in this thesis appear to promote distinct and predictable changes in the character and composition of traditionally variable zooplankton assemblages. Combined with appropriate and adjustable harvesting regimes, these could provide the first major step towards an optimized and sustainable product.

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*"If sufficient control could be maintained on the lagoon system and operated for the maximum production of harvestable organisms such as cladocera, the possibility exists that sufficient biomass could be removed, together with phosphorus, nitrogen and organics that it contains, to provide a much improved effluent... In addition, the products have an economic value and this could be used to offset the cost of sewage treatment."*

(Hussainy 1979).

## 1. GENERAL INTRODUCTION

The treatment of domestic waste poses problems of cost, land use, and environmental impact. Increasing population pressures lead to greater treatment requirements, and greater stresses on the system. As a result, methods of treatment are constantly being evaluated, and methods of improving them are actively sought.

The Werribee Treatment Complex (WTC) provides a prime example of such increasing pressures. Situated on Port Philip Bay 35 km from Melbourne, the Complex is run by Melbourne Water. Covering nearly 11,000 hectares, it is one of the largest waste-water treatment plants in Australia and, arguably, the world. The Complex treats over half the city's wastewater by land filtration, grass filtration, and lagooning techniques (Section 3.1).

Melbourne Water is currently revising both its long and short term strategies on wastewater management, in order to meet growing treatment demands into the twenty-first century. Sewage inflows to WTC have been predicted to increase by over 50% in the next 50 years, to an expected dry weather flow of 730 million litres of wastewater per day by 2035 (WTC data). To cope with such an increased flow, WTC needs to increase its treatment capacity, whilst coping with restrictions on its available land due to increasing urbanisation of the Werribee area. Developments in the treatment processes need to be efficient, sustainable and economical; they need to combat the increasing demands and requirements of greater sewage inflow, maintain or improve the standard of outflow, and, if possible, provide income to offset the costs of treatment (Wong & Tam 1998).

At the same time, pressures are being felt in other industries. To ease the burden of overfishing, natural fisheries are being augmented with aquacultural produce. However, one problem for this growing industry is the source of food material for aquacultural stock, as fishmeal feed is currently produced from natural fish stocks. As conversion efficiency from such feeds is also low (De Silva & Anderson 1995), larger quantities of wild fish are required to produce given quantities of farmed fish. In this way, aquacultural production can

paradoxically increase the pressures on natural fisheries (Dr Kevin Williams, CSIRO 1997).

To increase the efficiency and effectiveness of fish farming, a new form of feed is needed to either supplement or augment the current feeds. Zooplankton-based feeds, whether live or preserved, offer such a potential alternative. In addition, zooplankton are in many ways superior to traditional artificial foodstuffs. Small fry normally feed on live zooplankton after hatching, and require material which is edible, digestible, nutritious, appropriately sized to match mouth aperture (yet variable enough to match diverse hatching sizes and initial growth rates), and, not least, is capable of movement to trigger critical first-feeding responses in the young (Pierce 1988). Harvested zooplankton represent an ideal and natural feed, particularly for fry that are too small to take pelleted food (Uhlmann 1980).

The nutritional value of rotifer zooplankton can be readily manipulated (Lubzens *et al.* 1989), and rotifers represent an important source of essential fatty acids that marine fish and larvae are unable to synthesize (Nichols *et al.* 1989, Watanabe 1991). Fatty acid and nutrient concentrations in crustacean zooplankton (particularly *Daphnia*) also reflect their diet and can be both high and relatively consistent between species (Sterner & Hessen 1994, Gulati & Demott 1997, Sekino *et al.* 1997, Vrede *et al.* 1999). Both rotifer and crustacean zooplankton provide a range of potential live and treated feeds for different developmental stages and nutritional requirements in fish rearing, ranging from intensive aquacultural production to domestic goldfish flakes. Increased and reliable supplies of zooplankton material are essential in the continued expansion of aquacultural finfish production (Yoshimura *et al.* 1996).

Improving sewage treatment is the topic of many research works and reviews, particularly in regard to waste stabilization ponds (e.g. Gloyna 1971, Caldwell Connell Engineers 1975, Mitchell 1980, Uhlmann 1980, Constable 1988, Inmuong 1993, DELM/DPIF 1996, Wong & Tam 1998, David Cartwright unpubl. data), and the opening quote by Hussainy (1979) represents one of the earliest suggestions that problems in these two very disparate industries may be solved, or at



least alleviated, through a common link. The successful coupling of sewage treatment and aquacultural activities is not new, however, particularly in Asian cultures (Mitchell 1980, Uhlmann 1980, Inmuong 1993). In Europe, treated sewage water of sufficiently low microbial levels may be used for irrigation (Uhlmann 1980), and Mitchell (1980) lists several potential food-related methods for exploiting sewage resources, some of which are already in effect.

WTC itself has successfully coupled treatment processes with cattle production for over a century. The Complex is comparable to the largest stations in Australia in terms of stock, running between 12,000 to 15,000 head of cattle for beef production per year (WTC information). An average of 14,000 to 16,000 sheep are also run on the property, although their numbers vary greatly according to season (e.g. from 6,000 over winter up to 25,000 and once even 75,000 in peak growing conditions).

Livestock grazing provides an essential component of land and grass filtration processes (Section 3.1), by removing the excess nutrient deposited by these treatment techniques. The meat is subsequently sold for human consumption under the provisions of the Health Act (1979) and the Abattoirs and Meat Inspection Act (1972); the stock meet Health Department guidelines once they are over 18 months old and are fed for a period "off-farm", by which time any parasites or pathogens they have carried will die.

The sale of livestock generates a significant financial return for WTC, which is directly used to offset the cost of sewage treatment. In 1991-2, income from this source was worth 3 million dollars, while total operational costs were 15.2 million dollars, balanced by a capital input of 12.5 million from Melbourne Water. Not surprisingly, Melbourne Water is keen to investigate any possibilities of increasing their income from such ventures, including parasite eradication programs that would enable the (highly profitable) sale of veal. At the same time, the potential of the lagoons for providing a similar, harvestable biotic product cannot be overlooked.

Lagooning treats over 110, 000 million litres (ML) of wastewater per year, or 60% of the annual inflow to WTC. It is the only one of the three treatment techniques to be used all year round, and is

even used to polish wastewater treated by the other means if required (Hussainy 1979). Although requiring greater amounts of land than other treatment processes, lagoons are relatively cheap to build, operate and maintain, provide highly polished effluent in terms of BOD and suspended solids, greatly reduce bacterial and viral numbers, smooth peak flows, and cope with seasonal changes in influent character (Mitchell 1980, Uhlmann 1980, Mezrioui & Oudra 1998). Lagoons are also ideal settings for additional and commercial biological treatments and manipulations. As Mitchell (1980) points out, such ponds are uniformly constructed and controllable, are easily accessible and obstruction-free, and already form an integral part of many treatment plants.

Sewage represents a wasted nutrient resource that currently acts as a pollutant (Caldwell Connell Engineers 1975, Kawai *et al.* 1987). Annual sewage production contains approximately 1 tonne of total phosphorus and 4 tonnes of total nitrogen per thousand people, amounts which represent high financial value in terms of fertiliser (Mitchell 1980). The incorporation of this material into biological processes provides a ready avenue for exploitation.

The planktonic biomass available in the WTC lagoons is immense, and while Mitchell (1980) found mixed results in the enhancement of water treatment afforded by zooplankton versus phytoplankton harvesting, the nutritional value of harvested material as a feed or fertiliser still represents a potential source of value-added income. A pilot (algal) harvesting plant has already produced encouraging results at Werribee, providing high protein feed or supplement that could be presented in either wet or dry form to cattle, sheep, pigs and poultry (Caldwell Connell Engineers 1975, Borowitzka 1998). Such algal material, however, is of less potential feed value than the zooplankton (Hussainy 1979, Canovas *et al.* 1995) and is technically more difficult to harvest (Kawai *et al.* 1987). In addition, zooplankton play a significant role in the operation of waste treatment systems and control of phytoplankton (Mitchell 1980, Uhlmann 1980, Groeneweg & Schluter 1981, Schluter & Groeneweg 1981, Berndorf 1990, Mezrioui & Oudra 1998, Pinto-Coelho 1998).

The annual net production of daphnid biomass in sewage lagoons is amongst the highest known for planktonic Crustacea (Mitchell 1980), and may contain high levels of phosphorus and nitrogen bound within the biological material (Baudouin & Ravera 1972, Hussainy 1979, Sterner & Hessen 1994, Vrede *et al.* 1999). Pilot studies of daphnid production for potential harvest from sewage lagoons have produced levels corresponding to 7 kg (dry weight) per thousand people per day (Kawai *et al.* 1987). Rotifers may also play a dominant role in annual secondary production, with turnover rates and numbers producing biomass equivalent to that of crustaceans (Orcutt & Pace (1984).

The Werribee Treatment Complex and its lagoons have been studied intensively in past studies (Caldwell Connell Engineers 1975, Hussainy 1979, Constable 1988, D. Cartwright, unpublished data). While previous investigations into potential zooplankton harvest have signalled difficulties with zooplankton collection methods (Mitchell 1980, Kawai *et al.* 1987), recent advances in equipment and techniques specifically suited to the layout of WTC have made planktonic harvesting a more viable and low-effort pursuit (Plate 3.2).

Plankton are frequently present in harvestable quantities within sewage treatment lagoons, but such blooms are not necessarily predictable or stable. The present project was conceived to investigate methods of manipulating zooplankton populations at WTC, and to indicate cost-effective ways of optimising the availability of zooplankton material for harvest. In turn, the study was planned to act as the ecological part (zooplankton manipulation) of a larger project, in which other researchers investigated harvesting techniques and efficiencies, methods of treating and storing harvested material, monitoring its nutritional value, and identifying potential difficulties posed by any contaminants (chemical and biological) that it may contain.

This project was established under an Australian Postgraduate Research Award (Industry), with support provided by Zootech Research Pty. Ltd., whose interest lay in the commercial harvest and use of zooplankton material in aquaculture, and by

Melbourne Water, who were interested in examining treatment enhancement options for WTC and in promoting possible financial return to offset treatment costs. The potential removal of significant amounts of nutrient from the waters, in the form of zooplankton biomass, was not seen as an alternative to the conventional treatment process, but additional to it, by exploiting populations that were already collecting nutrient from the system as they grew, but were returning it as they died. In short, representing the recycling of 'waste' nutrients rather than further exporting them into Port Philip Bay.

WTC itself provided an ideal large scale experimental environment for this study (Section 3.1). In addition to the work already conducted at the Complex, current studies by David Cartwright (Senior Biologist, WTC) were showing that the unique layout of the lagoon system seemed to promote unusual but consistent patterns in the distribution of zooplankton species through the lagoon systems. Cartwright's work was focussed on monitoring and recording these patterns, and relating them to water quality as a rapid biological indicator of treatment success. In turn, the direct aim of the present project was to dovetail with these studies by attempting to actively manipulate these patterns, and to determine whether they could be controlled in a predictable manner.

In short, this project specifically aimed to alter zooplankton community structure at WTC in a consistent and predictable manner by manipulating the spatial distribution/succession of particular zooplankton groups. This was attempted directly in the field by changing gross influent nutrient levels, controlled via flow rate (nutrient loading), throughout a full-scale, operational, 70.5 hectare system.

## **2. LITERATURE REVIEW**

### **FACTORS AFFECTING ZOOPLANKTON POPULATION DYNAMICS**

#### **2.1 Introduction**

One of the first steps in determining how to best manipulate zooplankton assemblages is to determine what is already understood about zooplankton population dynamics, and their responses to and effects on various physical, chemical, and biological or ecological factors. The aim of the following section is to review the available literature on these factors, with particular reference to wastewater lagoons.

#### **2.2 Physical factors**

##### **2.2.1 Temperature**

One of the primary physical factors influencing all living things is temperature. Temperature may vary with season, latitude, elevation, and thermal pollution (Nawaz & Kirk 1995). Higher water temperatures can increase microbial action, trigger phytoplankton blooms, and act as a major factor determining the occurrence of zooplankton (Mitchell 1980). Zooplankton are especially at the mercy of temperature variation, particularly when they are found in large shallow water bodies such as wastewater lagoons. Such lagoons possess very high surface area to volume ratios, and so temperature fluctuations can be great (Griggs 1993). Without the buffering effects of deeper pools, wastewater lagoons can display distinct diurnal and seasonal temperature cycles.

Despite their shallow depth, lagoons may have complicated temperature profiles that lead to stratification. Waste stabilization ponds (WSPs) may not completely mix as nearly all of the heat irradiation will be absorbed in less than the first metre of wastewater and may produce a steep vertical temperature gradient (Mitchell 1980, Uhlmann 1980). High levels of algae in the upper layers absorb the

majority of light, which can increase the temperature of the surface water (Mitchell 1980, Griggs 1993) and establish vertical gradients in oxidation/ reduction potential (Uhlmann 1980). Differences in water density become more pronounced per degree change of temperature, and provide resistance to mixing that may withstand moderate wind stress (Uhlmann 1980). Such effects may short-circuit retention times for WSPs (DELM/DPIF 1996), although large nocturnal drops in air temperature may produce convective overturn and mixing of the water body. Such daily stratification cycles are more important to shallow water bodies than seasonal ones (Mitchell 1980). While all non-aerated lagoons may stratify on occasion, most lagoons in Tasmania do not stratify consistently over winter months (DELM/DPIF 1996).

The likelihood of thermal stratification is influenced by weather conditions and the shape, dimensions, turbidity, and exposure of a lagoon (Mitchell 1980). Stratification may affect oxygen availability and other environmental factors, to the point where it may become self-maintaining. Differences in biochemical processes and the accumulation of compounds above and below the thermocline may develop into meromixis, and there may be a sharp decline in the bacterial kill-rate under stratified conditions (DELM/DPIF 1996).

At optimum temperatures, algal turnover may occur in hours (Mitchell 1980). Algal growth rates will increase as temperature approaches optimal, but will rapidly decline beyond it (Borowitzka 1998). Low temperatures may act against population booms of crustaceans such as *Daphnia* and *Moina* and rotifers such as *Brachionus* and *Filinia* (Uhlmann 1980), although species such as *Daphnia carinata* are also recorded as cold water forms, with high temperatures increasing mortality and reducing growth (Mitchell 1980). This may play a role in restricting this species from controlling phytoplankton blooms under high growth summer conditions.

Zooplankton grazing rates generally increase with temperature, until about 20-25°C (Hayward & Gallup 1976, Griggs 1993). This is due to an increase in the metabolic rate of the organisms, and a decrease in water viscosity (Horn 1981). Above these temperatures grazing rates may decline rapidly, as high metabolic rates lead to oxygen

deficiencies in the tissues (Hayward & Gallup 1976).

Densities of blue green algae (BGA) may be strongly controlled by water temperature (Havens *et al.* 1998). Zooplankton sensitivity to toxic BGA species may also increase with temperature (Claska & Gilbert 1998), as may the bioaccumulation of contaminants in general within zooplankton tissue (Nawaz & Kirk 1995). Unseasonal warm spells may affect overwintering success in zooplankton species by stimulating both the hatching of resting eggs and a switch from sexual to asexual reproductive modes at inopportune times (Chen & Folt 1996). Species of restricted thermal tolerance are also prone to the effects of such events, and all are risks posed by climate change.

### 2.2.2 Light

The effects of light on zooplankton are largely species dependent. In some species, grazing rates may increase as light intensity falls below 50 lux (Hanley & Hall 1975, Horn 1981). In others, grazing rates increase with light (Hayward & Gallup 1976, Horn 1981). Photoperiod may act as a cue to break diapause in crustacean zooplankton (Stross 1965, 1966), although whether this also occurs under the reduced light of turbid waste stabilization lagoons is unknown (Griggs 1993). The major effects of light on zooplankton are largely indirect, however, through their effects on phytoplankton.

Light, along with temperature and nutrient availability, is a major controlling influence on the seasonal occurrence, growth, and composition of phytoplankton communities (Mitchell 1980, Knowlton & Jones 1996). In eutrophic water bodies, high density algal surface layers may cause light deprivation and phytoplankton die-off at greater depths (Griggs 1993). Conversely, algae higher in the water column may be exposed to damaging or lethal levels of irradiance (Borowitzka 1998, Zagarese *et al.* 1998a, 1998b). Light penetration may be less than 0.5m under high phytoplankton blooms, which may promote a vertical gradient in oxidation/reduction potential (Uhlmann 1980). While temperature may act as an important trigger for phytoplankton blooms, self-shading and reduced oxygenation may be more important in

terminating them, and can lead to an increase in organic loading (Mitchell 1980).

Blue Green Algae (BGA) are able to operate at relatively low light levels, and in combination with their ability to fix atmospheric nitrogen and utilise carbon dioxide at high pH this may allow them to outcompete other primary producers (de Bernardi & Giussani 1990). Large BGA aggregations may form floating mats blocking light penetration and producing anaerobic conditions at greater depths in the water column (Mitchell 1980). Algal and BGA specific growth rates may be reduced under conditions of fluctuating light, as opposed to exposure to constant levels for the same length of time (Nicklisch 1998).

### **2.2.3 Substrate**

Substrate can be important for a variety of reasons, ranging from what it contains (Section 2.3.3) to acting as a storage bank for resting eggs, providing general grazing surfaces, and providing refuges. The development of refuges can significantly increase zooplankton abundance (Heisey & Porter 1977). Refuges may be highly important for predator avoidance (Section 2.4.6), and may be driven by light, temperature, oxygen concentration, physical factors such as macrophyte growth, behavioural or temporal factors, and predator inefficiency (Shapiro 1990). High phytoplankton levels may block light penetration, reducing macrophyte or littoral vegetation and the refuge it represents (Griggs 1993).

### **2.2.4 Circulation**

Agitation of waters, whether by artificial or natural means (e.g. wind: DELM/DPIF 1996), can serve to stop stratification (Section 2.2.1) and concomitant oxygen, temperature, pH, and toxin problems and deficiencies in the water column (Borowitzka 1998). Well-mixed waters can buffer against localised changes in parameters, such as changes in the partial pressure of carbon dioxide ( $p\text{CO}_2$ ) and resultant changes in pH (Morgan 1985).

Decreased water turbulence may lead to sedimentation of



algal material, and so decreases in apparent phytoplankton biomass (Dawidowicz 1990). Agitation may serve to stir up nutrients and algal sediments which have settled (Mitchell 1980), and this may have both positive and negative results. Agitation enables algae to remain suspended in the water column, exposes cells to light and increases exposure to nutrients and metabolites, but heightens the risk of mechanical damage (Borowitzka 1998) and exposure to damaging ultraviolet radiation (Zagarese *et al.* 1998a, 1998b), and may reduce specific growth rates through exposure to fluctuating levels of illuminance (Nicklisch 1998).

The success of agitation depends on the methods used and the prevailing conditions. Turbulence created by massive *Daphnia* swarms may be sufficient to maintain aerobic conditions in otherwise oxygen-depleted waters (Uhlmann 1980), while inflow may also cause sufficient turbulence in some situations (Mitchell 1980). In turn, high flushing rates may reduce biological activity and phytoplankton productivity (Mitchell 1980, Schluter & Groeneweg 1981). Increased circulation via wind or rain may increase pond turbidity, and this may decrease light penetration and enhance surface water warming (Hussainy 1967, Griggs 1993).

## **2.3 Chemical factors**

### **2.3.1 Oxygen concentration**

Dissolved oxygen concentration and percentage saturation will fluctuate with the photosynthetic activity of algae, and will tend to peak in afternoon hours when the temperature is high (Patil *et al.* 1975), before falling overnight to a minimum immediately preceding sunrise (DELM/DPIF 1996). Phytoplankton blooms may produce large diel variations in facultative ponds in particular (<1 to >30ppm: Mitchell 1980), with summer conditions and long daylight hours leading to supersaturated surface waters (Mitchell 1980).

Aside from diurnal patterns, the oxygen balance within lagoons may vary substantially over time, and is also influenced by

atmospheric diffusion, biological respiration, chemical oxidation of dissolved organic matter, and the balance of material at the sediment/water interface (Mitchell 1980, Uhlmann 1980). BOD levels exceeding 5 mg.l<sup>-1</sup> are indicative of poor quality in flowing waters (Mitchell 1980).

At oxygen concentrations under 2ppm, zooplankton display reduced egg production, growth and feeding rates, increased egg abortion rates, and altered feeding behaviour (Griggs 1993). Although tolerant of hypoxia, rotifers may be adversely affected under prolonged anoxic and very low oxygen conditions (Kawai *et al.* 1987), with reproduction ceasing and cultures dying at levels of 0.72 mg O<sub>2</sub>.l<sup>-1</sup> (Schluter & Groeneweg 1981). In contrast, there is no observed inhibition of reproduction at concentrations above 1.15 mg.l<sup>-1</sup> (Schluter & Groeneweg 1981), with increases in rotifer numbers matching rises in dissolved oxygen concentration in the field (Pudo 1978).

As well as physical factors such as temperature, light and pH, oxygen concentration directly affects feeding and respiration rates, and may often be a limiting factor for *Daphnia* species in nature (Kring & O'Brien 1976, Heisey & Porter 1977). Filtering and respiration rates may either decrease steadily in a linear dependence on oxygen concentration, or may remain high and constant until a threshold oxygen level is reached, after which rates may decline rapidly. Such differences in regulatory ability are species dependent, and are presumed to represent adaptive responses to environments where oxygen levels are typically low or variable (e.g. eutrophic temporary ponds versus saturated epilimnetic habitats in lakes: Heisey & Porter 1977).

Studies covering a range of aquatic organisms indicate that oxygen levels of 2 - 4 mg.l<sup>-1</sup> demark a consistent threshold below which blood pigments are required to allow normal physiological function. (Kring & O'Brien 1976). For *Daphnia*, 2.5 - 3.0 mg.l<sup>-1</sup> is generally the critical level in the first phase (Kring & O'Brien 1976, Heisey & Porter 1977). Crustacean zooplankton may then regain and even surpass their original filtering rates by facultative production of haemoglobin (Kring

& O'Brien 1976). This generally enhances filtering rate, egg production, general activity, and survivorship under low oxygen concentrations (Heisey & Porter 1977), but the process is energetically expensive and increases visibility to predators (Kring & O'Brien 1976).

Facultative haemoglobin production only begins after prolonged exposure to low oxygen levels (8-12 hours), which may represent a necessary physiological delay, or ensure responses do not occur to short-term oxygen fluctuations "such as diurnal fluctuations... in nutrient enriched ponds or daytime migrations into oxygen-poor hypolimnetic waters" (Kring & O'Brien 1976). Such lags between the onset of oxygen stress and haemoglobin production may limit the growth of *Daphnia* populations, although some species display a relatively greater tolerance of low oxygen concentrations (above threshold levels) by possessing higher basal levels of haemoglobin (Heisey & Porter 1977).

Visual predation may be avoided by vertical migration (Section 2.4.3) into deeper water where light levels are reduced (Zaret & Suffern 1976, Heisey & Porter 1977, Shapiro 1990). While species may be able to cope with low oxygen levels, this refuge from predation may be denied to them if the deeper water column becomes totally anoxic and, as a result, toxic.

Deoxygenation may result directly from natural and cyclic stratification processes, or may be enhanced by runoffs and pollutants from the surrounding land (Heisey & Porter 1977, Mitchell 1980). High BOD loads (over 5g/m<sup>3</sup>/d) may be deleterious to rotifer and crustacean zooplankton populations (Uhlmann 1980). Anoxia lower in the water column may lead to the production of toxic sulphides, enhanced by the continued sedimentation of organic matter from the increased phytoplankton crop above. Stratification may also produce marked effects in the protozoan and bacterial populations and distributions within lagoons (Patil *et al.* 1975).

The interplay of oxygen stratification and predation may heavily influence the seasonal abundances of specific species, and artificial aeration of anoxic depths in the face of trout predation has been reported to increase *Daphnia pulex* populations as much as 88-

fold (Heisey & Porter 1977). Interestingly, *Daphnia* swarms may greatly depress oxygen concentration peaks through respiration and by consuming most of the photosynthetic crop, particularly if such an event is coupled with calm and hot weather (Pinto-Coelho 1998); these same swarms may be large enough, however, to provide sufficient water turbulence to maintain aerobic conditions (Uhlmann 1980).

High dissolved oxygen concentrations signify an improvement in the condition of effluent and serve to eradicate anaerobic bacteria from better treated waters (Patil *et al.* 1975, DELM/DPIF 1996), although diurnal fluctuations in dissolved oxygen are less pronounced in waste-water lagoons towards the end of the treatment process (Mitchell 1980). Raised oxygen levels aid in the bacterial oxidation of organic compounds, and nitrogen removal increases when dissolved oxygen levels exceed  $1 \text{ mg.l}^{-1}$  (Constable 1988). Finally, at higher oxygen concentrations, zooplankton filtering rate becomes dependent upon food concentration (Kring & O'Brien 1976; Section 2.4.1).

### **2.3.2 pH and carbon dioxide concentration**

The pH of a water body can be significantly affected by redox reactions, acid production via decomposition, cation exchange with sediments, and various watershed reactions (Morgan 1985). pH fluctuations are strongly influenced by changes in the rate of carbon dioxide absorption by algae (Patil *et al.* 1975, Mitchell 1980, Schluter & Groeneweg 1981, DELM/DPIF 1996), and will vary diurnally and seasonally with photosynthetic fluctuations (Morgan 1985). pH increases with photosynthetic activity, and so tends to peak over summer and as algal levels increase through the course of a lagoon system (Mitchell 1980). Diurnal pH peaks occur around noon after rising gradually with increasing light intensity and temperature during the day (Patil *et al.* 1975), and may rise as high as 11 (Schluter & Groeneweg 1981). This is particularly the case in surface waters, and may influence behavioural activities such as vertical migration (see Section 2.4.3). Levels fall as carbon dioxide concentrations are

replenished by unmatched nocturnal respiration, and algae may be limited by carbon dioxide availability (Mitchell 1980).

Residential and agricultural development can lead to significantly elevated pH levels and more complicated pH profiles over time, primarily as the result of nitrate uptake due to enhanced primary productivity (Morgan 1985). These levels may drop at night or over winter, and such fluctuations may exert a regulating influence on zooplankton communities. pH decreases in WSPs may be due to influent septic or industrial wastes, algal population crashes, or sludge inversion (DELM/DPIF 1996).

Even though nutrient levels may remain consistently high, species composition and abundance may be more greatly influenced by the minimum pH values (Morgan 1985). Morgan suggested that the periodic and dramatic lowering of pH acted as a filter allowing only "acid tolerant" species to survive. O'Brien & de'Noyelles (1972) report a similar effect from photosynthetically elevated pH reaching levels that only alkaline tolerant species could survive. In poorly buffered waters, Morgan (1985) concludes, there may be a great difference between the ecologically effective pH and that measured in routine sampling.

Morgan (1985) stated that pH will rise with assimilation of nitrate/nitrite and with processes of denitrification and nitrate reduction, and will fall with ammonium assimilation and nitrification. The effect of ammonia, however, was limited in the systems he studied, as the ammonia levels were minimal, and so the pH level was essentially driven by nitrate uptake. pH will in turn exert an influence on the dynamics of nutrient cycles within a water body (Section 2.3.3). In stratified disturbed water systems, pH changes at the surface may not be reflected at depth, where light - and so photosynthetic activity - does not penetrate (Morgan 1985).

pHs in excess of 8-9 may be lethal depending on ammonium concentration (Schluter & Groeneweg 1981). Photosynthetic inhibition may occur if pH levels exceed 8.1, and levels over 9.0 may block carbonate uptake for photosynthesis or lead to algal flocculation (Borowitzka 1998). Different zooplankton species have different survival and optimal ranges, but at higher pH are prone to ammonia

toxicity (Hussainy 1979, Mitchell & Williams 1982, Gulyas & Fleit 1990). Cladocerans may persist in pH 9-10.5, but egg mortality may increase markedly even if adult and juvenile mortalities do not (Vijverberg *et al.* 1996). Ammonia toxicity increases with increasing pH, and longevity and rates of feeding in zooplankton may also be affected. pHs above 8.0 and ammonia concentrations over 34mg.l<sup>-1</sup> can inhibit photosynthesis and growth in phytoplankton (Mitchell 1980). In turn, ammonia may be lost to the atmosphere and phosphates may precipitate at pHs in excess of 9.0 (Mitchell 1980), although the latter will resolubilize when pH falls at night (Borowitzka 1998).

Population densities in *Brachionus* cultures will peak at pHs of 6-8 while abrupt declines will occur below pH 5 and above pH 9, with no rotifers surviving beyond pH 4.5 and 9.5 respectively (Schluter & Groeneweg 1981). This response to pH is believed to occur at the upper end of the scale even in the absence of ammonia and its toxic effects, and rotifer population increases can generally be expected under warm but cloudy conditions when photosynthesis does not elevate pH beyond these bounds.

Mitchell (1980) cites pHs of 9 as being the upper acceptable limit for domestic wastewater effluents in the US, but it is also worth noting that raised pH plays an important role in acting to disinfect treated waters, with the pH rise associated with algal activity helping to reduce the numbers of sulphur-using bacteria (Patil *et al.* 1975). In contrast, Blue Green Algae are highly efficient at utilizing carbon dioxide at high pH (de Bernardi & Giussani 1990), and elevated pH levels may also stimulate the release of phosphorus from the sediments.

### 2.3.3 Nutrient concentrations

Dissolved nutrients and organic substances can be utilized by several levels of the food web in WSPs, including bacteria and phytoplankton, and so a great deal of recycling occurs between different levels (Uhlmann 1980). Phosphorus and nitrogen are the two central elements in such systems, with the major pathway for the removal of

these nutrients being their incorporation into the phyto- and zooplankton (Mitchell 1980). The species present in WSPs are obviously able to tolerate and thrive in conditions of high nutrient loading.

Phosphorus loading is the single most important factor influencing the trophic status of a water body, with a critical level for eutrophication of  $0.015 \text{ mg.l}^{-1}$  (Schindler 1974, Mitchell 1980, Carpenter *et al.* 1995 & 1996). Biological processes rapidly reduce complex phosphate compounds to soluble orthophosphates during treatment processes. These move through a dynamic cycle, constantly being incorporated into organisms while at the same time being released by excretion and decay. Hence algae and bacteria may compete for phosphates, while at the same time providing these materials to one another (Mitchell 1980, Cottingham *et al.* 1997). Unlike nitrogen, which passes through a variety of forms and biological transformations, a large proportion of the total phosphorus pool may be organically bound and unavailable at any given time (Mitchell 1980).

Nutrients may also be released or deposited at the sediment/water interface (Uhlmann 1980). This exchange may act to strongly buffer shallow eutrophic waters from change (Moss *et al.* 1991), and is as a major mechanism in the aquatic phosphorus cycle (Mitchell 1980). Phosphates may precipitate at high pH, and the rate of exchange at the sediment/water interface is controlled by the concentration of oxygen at that point (release from the sediments is likely to be low in fully aerobic wastewater lagoons: Mitchell 1980).

The sediments are also a major store of nitrogen. Most (95-99%) nitrogen removed from the WTC ponds is removed as ammonia via desorption or concurrent nitrification/denitrification in the sediments (Constable 1988). Nitrification processes occur as bacteria convert organic, or protein based, nitrogen to ammonia, and then oxidise it to nitrite and nitrate (DELM/DPIF 1996). Denitrification occurs under deeper, anoxic conditions, when nitrate is facultatively reduced to (and released as) nitrogen gas. Exchange is again controlled by the presence of oxygen at the interface, with an oxidised surface layer preventing ammonium ion release (Mitchell 1980). Diurnal pH and

temperature variations may also influence this (Constable 1988).

Air drying and subsequent reflooding of sediments may cause massive ammonium release, increasing nitrification by an order of magnitude and potentially further promoting nitrogen loss through denitrification cycles (Song & McComb 1996). Sediments contain many forms of nitrogenous compounds, but as these are highly labile it is the total nitrogen pool that provides the best single indicator of pollution load, with a critical level for eutrophication of  $0.3 \text{ mg.l}^{-1}$  (Mitchell 1980).

As this suggests, the nitrogen cycle is also dynamic: nitrifying bacteria transform ammonia into nitrate, which in turn provides the main nitrogenous source for algae and larger plants, while ammonia is released once more from decomposing organic material and wastes produced higher in the food chain (Mitchell 1980). Nitrogen can become a limiting factor due to ammonia loss to the atmosphere at high pH (Mitchell 1980), and this may lead to favourable conditions for BGA. It is worth noting that while nitrite may be toxic to many aquatic animals, some species of *Brachionus* are tolerant of relatively high concentrations alongside other changes in nutrient and salinity conditions (Schluter & Groeneweg 1981).

In keeping with his findings on rises in pH in disturbed waters, Morgan (1985) found that there was a corresponding drop in nitrate levels over summer, when photosynthesis (and so nitrate uptake) was highest. Total phosphorus levels were also greatly elevated in the disturbed waters, but did not display obvious seasonal trends, while ammonia levels remained low throughout the year in that particular pond. Likewise, the concentrations and variations in all nutrients were barely detectable in undisturbed waters. In contrast, Mitchell (1980) found that, due to phytoplankton blooms, removal of both nitrate and phosphorus were highest over summer, while biochemical oxygen demand, suspended solids, total organic carbon, and organic nitrogen levels rose. The same author, however, did find that particulate phosphate levels were elevated in effluents. In Tasmania, both nitrogen and phosphorus removal from WSPs are optimal over summer and lower over winter (DELM/DPIF 1996).

With the exception of extremely polluted conditions (Fanuko



1984, Borowitzka 1998, Mezrioui & Oudra 1998), increased nutrient levels lead to significantly greater primary productivity and phytoplankton biomass (Morgan 1985, Carpenter *et al.* 1995, Blomqvist 1996 & 1997, Corkum 1996, Cottingham *et al.* 1998), and consequently a much greater abundance of zooplankton (Carpenter *et al.* 1996, Section 2.4.1). While phosphorus, ammonia, pH and temperature all appear to play a part (Mendes *et al.* 1995), phytoplankton community composition and abundance are ultimately a product of zooplankton grazing and light/nutrient availability (Benndorf 1990, Scharf 1997, Cottingham *et al.* 1998). If the latter is in excess, inedible species (such as BGA) are more likely to dominate. Patterns of zooplankton abundance may in turn be linked to the controlling effect of the basal pH (Morgan 1985, Section 2.3.2).

Decreases in phytoplankton biomass may match and exceed decreases in phosphorus loading (Blomqvist 1996). While phosphorus availability would appear to be the single-most important nutrient factor influencing or limiting phytoplankton growth (Benndorf 1990, Carpenter *et al.* 1995 & 1996, Diaz & Pedrozo 1996, Watson *et al.* 1997), algae in WSPs may become carbon limited under certain conditions, but this is unlikely to happen in natural water bodies (Mitchell 1990). Carbon limitation may result from the development of high algal standing crops due to increased retention times, leading to rapid depletion of nocturnally accumulated carbon dioxide on a diel basis. This would have a greater effect on green algae, and could again promote BGA dominance. Conversely, and unlike natural water bodies, nitrogen and phosphorus are unlikely to become truly limiting resources in WSPs (Mitchell 1980).

Some BGA are able utilize atmospheric nitrogen, however, and so have a competitive advantage in eutrophic waters with low nitrogen to phosphorus ratios (de Bernardi & Giussani 1990, Havens *et al.* 1998). Such conditions may be circumvented by nitrogen excretion and recycling in large daphnid populations, combined with differential phosphorus uptake rates in bacteria (Hargrave & Green 1968, MacKay & Elser 1998). Zooplankton may also promote a net downward movement of phosphorus through the settling of faecal pellets and

cast-off exuviae (Hargrave & Green 1968, Mitchell 1980, Wright & Shapiro 1984, Vrede *et al.* 1999). Although able to fix and store nitrogen, BGA species are poor competitors for phosphorus (MacKay & Elser 1998). Cladoceran zooplankton may themselves be more directly limited by variations in total phosphorus and inorganic nitrogen than by food availability/particulate carbon *per se* (Sterner & Hessen 1994, Gulati & Demott 1997, Van Donk & Lurling 1997, DeMott *Et al.* 1998, Pinto-Coelho 1998, Vrede *et al.* 1999).

#### 2.3.4 Toxins (chemical)

Raw sewage can also have varying toxic effects on different groups of plankton within the confines of a treatment plant, particularly when the treatment capacity of that plant is significantly exceeded. Algae are sensitive to a wide range of toxic domestic and industrial compounds in wastewater (Section 5.3), and different species may exhibit different levels of tolerance (Mezrioui & Oudra 1998). The accumulation of heavy metals and toxins within algal cells may provide a useful pathway for removing these materials from the water column (Borowitzka 1998).

Higher concentrations of toxic materials tend to select for smaller taxa and smaller individuals within taxa (Cattaneo *at al.* 1998). Contaminants such as DDT may accumulate in zooplankton through active water filtering, and passive diffusion into permeable cells (Nawaz & Kirk 1995). Marine planktonic groups such as stomatopod, gastropod, and chaetognath larvae are particularly sensitive to outfalling wastewaters; copepods, *Lucifer*, and decapod larvae are moderately tolerant, while *Acetes*, medusae, ctenophores and mysids are among the most resistant (Gajbhiye *et al.* 1987). Toxic events can produce marked responses in plankton communities at WTC (David Cartwright, pers. comm.), and the zooplankton of earlier WTC lagoons (i.e. those earlier in the treatment sequence) may accumulate heavy metals (Hussainy 1979).

## 2.4 Biological factors

### 2.4.1 Available food

Dissolved organic matter is taken up by bacteria, which in turn release carbon dioxide and stabilized nitrogen and phosphorus compounds which can be utilized by phytoplankton (Uhlmann 1973). Ciliates and protozoa may feed on bacteria (Mitchell 1980), while all of these groups (including phytoplankton) provide biomass for zooplankton consumption (Mitchell 1980, Uhlmann 1980, Marchessault & Mazumder 1997). Zooplankton release carbon, ammonia and phosphate wastes that may in turn be used by phytoplankton and bacteria, while phytoplankton release dissolved organic compounds that, irrespective of competition, may be of use to both bacteria and themselves (Uhlmann 1980, Hygum *et al.* 1997). The turnover in populations of these groups is higher over summer (Mitchell 1980).

Cladocera are filter feeders, and primarily feed on algae, protozoa, organic detritus and bacteria, although, as mentioned previously, at least some species may prefer young and actively dividing phytoplankton (Hussainy 1979). Diatoms are preferred over green algae because they can be better digested mechanically and enzymatically. In contrast, ungrazeable algal species (BGA and such forms as net algae) may benefit from competitive displacement and the benefits of zooplankton nutrient recycling.

Spring phytoplankton blooms often lead to a rapid, exploitative growth of zooplankton populations (Geller 1986). Such zooplankton blooms have a great effect on the (palatable) phytoplankton assemblage, leading to a "clear water phase" if grazing outstrips algal growth for sufficient time (Mitchell 1980, Uhlmann 1980, Kawai *et al.* 1987, Benndorf 1990, Pinto-Coelho 1998). Similar decreases may also be seen in bacterial density due to grazing pressure, although this is less efficient than on algae (Gude 1988). Grazing may promote significant changes in the bacterial assemblage, but may also decrease overall grazing pressure and increase bacterial productivity and abundance by removing bacterivorous flagellates and releasing

previously inaccessible organic compounds from grazed algae (Gude 1988).

Zooplankton blooms follow those of phytoplankton then collapse as the latter do. This allows the phytoplankton to recover (Uhlmann 1980). Mitchell (1980) lists the major factors influencing algal productivity as being those that control reproductive rate (nutrients, light, temperature) and those that control mortality or removal rates (predation, flushing, sedimentation, and disease). Of these, light, temperature, and grazing pressures are believed to be the most influential in wastewater lagoons (White 1975), followed by carbon limitation and flushing rates (Mitchell 1980). Algal succession due to nutrient change affects the species and abundance of zooplankton, while different types of zooplankton can themselves affect the algal assemblage due to size and species specific grazing patterns (Pejler 1983, Green & Shiel 1992, Borowitzka 1998, Mezrioui & Oudra 1998).

Both cladocerans and rotifers have been recorded as reaching enormous numbers in lagoons at both WTC and elsewhere, before crashing as they consume all available food (Hussainy 1979; Uhlmann 1980). It is logical that detrital and bacterial food sources are particularly important during such collapses in phytoplankton numbers (Hussainy 1979, Sanders *et al.* 1989, Pinto-Coelho 1998), and facultative browsers such as *Daphnia carinata* have been recorded to ingest sediment at these times (Mitchell 1980). While some cladocerans may predominantly live on bacteria, they are a more important food source for rotifers depending on rotifer species and size (Ooms-Wilms 1997). Some zooplankton populations may be food limited at high densities (Mitchell 1980), and starvation is likely to be responsible for high zooplankton mortality rates in the field (Geller 1986).

Filtering and grazing rates depend on the quantity and quality of phytoplankton and other food. Grazing rate increases with food concentration until a limiting concentration - or incipient food level - is reached and then plateaus, while filtering decreases as food concentration increases and ingestion rates remain the same (Hayward & Gallup 1976, Kring & O'Brien 1976, Horn 1981, Pinto-Coelho 1991).

The amount of food ingested is limited by particle size and the maximum rate of filtering that is possible, while the relative length of daphnid filtering setae may vary according to prevailing food availability immediately preceding the last moult (Machacek 1998). This morphological response to low food may be suppressed under low oxygen concentrations, providing a synergistic deleterious effect between the two conditions (Hanazato 1996).

Feeding rates of *Daphnia* species can be adversely affected in high algal concentrations (Ryther 1954, Hussainy 1979). BGA can cause mechanical interference with filtering, can be toxic, and can interfere with the ability to assimilate food (Hayward & Gallup 1976, Griggs 1993). However, the suitability of BGA as food may vary greatly according to the biochemical properties of different species or strains of species, and the size and shape of the BGA cells and colonies (de Bernardi & Giussani 1990).

Mechanical interference may occur if BGA colonies are in filamentous form, cannot be broken into workable particle sizes, clog zooplankton filters and food grooves, or otherwise drastically increase handling time (Arnold 1971, Webster & Peters 1978, Porter & McDonough 1984, Infante & Abella 1985, de Bernardi & Giussani 1990). Under such conditions, large cladocerans may respond by producing smaller eggs and young (Moss *et al.* 1991). As with rotifers and other small zooplankton (Orcutt & Pace 1984), these are subsequently too small to be affected by the filaments.

BGA material of suitable size may be rejected on the basis of taste by both cladocerans and rotifers, which may show increased rejection frequencies in favour of other food types (de Bernardi & Giussani 1990). Such material may then represent energetic dead-ends within planktonic foodwebs (Blomqvist 1996). BGA materials that are eaten by zooplankton may be of low nutritional quality and may prove difficult to assimilate (Arnold 1971, Holm & Shapiro 1984). de Bernardi & Giussani (1990) report on earlier work showing the assimilation rate of one BGA species to be "half that of green algae", and for others to be "very low".

Despite this, species that show resistance to BGA toxins may be able to obtain some nutritional advantages from the material, either

by using it as a supplement to other foods, or as a main food that is itself supplemented from other sources, including bacteria. BGA potentially lack an essential compound that may be obtained from other sources, and in experiments providing a mixture of edible sized BGA and green algae, resultant assimilation rates were higher than for the green algae alone (de Bernardi & Giussani 1990).

While some zooplankton may show reduced fecundity on BGA diets (Arnold 1971), other rotifer and crustacean species may display levels of fecundity, somatic growth, reproduction and population increase comparable to those fed with green algae at a similar density (de Bernardi & Giussani 1990). In nature, BGA will rarely be found in axenic cultures with no supplemental material, and, provided they are in grazeable and non-toxic forms, may provide a usable resource for some zooplankton.

The food gathering abilities of rotifers and smaller cladocerans may not be influenced by BGA (de Bernardi & Giussani 1990), and these species may abound during BGA blooms. Algal filaments may not penetrate the filtering apparatus of smaller cladocerans, while greatly affecting larger species, and, consequently, their reproduction and population growth rates (Webster & Peters 1978, Gliwicz & Siedlar 1980, Porter & McDonough 1984, Dawidowicz 1990). Larger species, however, benefit in conditions of lower food. As a result, the zooplankton community composition may be directly influenced by the type and abundance of BGA, and de Bernardi & Giussani (1990) suggest that this may reflect some co-evolutionary mechanism or an adaptive process induced by grazing.

#### **2.4.2 Competition**

Freshwater zooplankton communities tend to be dominated by either large *or* small bodied species (Vanni 1986), with competition and predation both acting to structure zooplankton communities (Mitchell 1980). Planktivorous fish tend to remove the larger species, such as cladocerans, while the larger species are otherwise able to outcompete the other zooplankton, such as rotifers and copepods. The

competitive or predatory effects of larger zooplankton may be greater in eutrophic systems, where nutrient enrichment allows them to reach higher population densities (Vanni 1986). These possibilities have previously been presented in the competition-based size-efficiency hypothesis (Brooks and Dodson 1965) and its predatory alternative (Dodson 1974), and the two may effectively work in concert (Vanni 1986).

The outcome of competition between any two species of zooplankton may be greatly influenced by the other species present in the community, and is difficult to predict (Neill 1975, Vanni 1986). Ability to survive periods of starvation is likely to play a major role in competition between species in a water body and the resulting zooplankton community composition (Geller 1986).

Zooplankton species also show density-dependent behavioural and physiological modifications. Feeding rates may be inversely related to zooplankton density (Hayward & Gallup 1976, Horn 1981). Copepods may display increased development time and decreased survivorship, fecundity, and adult body size under higher densities (Ohno *et al.* 1990). Decreased survivorship may in part be due to density-dependent cannibalism between different life stages.

#### **2.4.3 Behavioural factors**

A major behavioural factor displayed by zooplankton is vertical migration in the water column (Moloney & Gibbons 1996). This is usually displayed by crustacean zooplankton, or mesozooplankton (Geller *et al.* 1992), and several different causes have been hypothesized for the behaviour. Vertical migration has been recorded in marine and freshwater environments, and in waters of different sizes, depths and trophic state (Geller 1986). Although it may provide an escape from physical factors such as excessive light intensity and heat (Cushing 1951), diurnal vertical migration appears to be less important in rotifer and ciliate microzooplankton (Geller *et al.* 1992), which are less sensitive to visual predation (Zaret & Suffern 1976). Vertical migration may be absent in larger zooplankton species when

predation pressure is not high or where water characteristics (e.g. turbidity) greatly restrict visual predation (Pinto-Coelho 1998).

However, predation avoidance does not fully explain vertical migration either, as not all species of the same genus will necessarily display the behaviour, migrating species will not necessarily display it consistently throughout the year, and species may or may not display the behaviour in systems of low or high predation pressure respectively (Geller 1986).

Summer/autumn patterns of vertical migration may represent a change from high to low and unpredictable levels of food supply, and a corresponding shift in the feeding strategy of some zooplankton from "exploitative" (of spring phytoplankton blooms) to "conservational". Some species may remain in the former category throughout the seasonal cycle, while others may jump between the two. By migrating deeper and acclimating to low temperature environments interspersed with short feeding bouts at higher temperatures, animals may decrease metabolic costs, in turn conserving energy and avoiding starvation (Geller 1986). The depth of migration may vary according to the vertical distance that must be travelled to acquire food, and by thermal stratification and oxygen concentration. Despite earlier predictions, the depth of migration does not appear to match consistent light intensities nor to exceed the sensory limits of visual predators.

Vertical migration will directly impose a diel rhythm on grazing activity (Geller *et al.* 1992), as it will tend to separate phyto- and zooplankton biomasses into the epi- and hypolimnion respectively. As a result, when vertical migration occurs, higher grazing rates will occur at night; position in the water column at various times will determine the type of food consumed and the rate of consumption. Vertical migration may also affect the relative nutrient concentration contained in the crustacean zooplankton of surface waters, as better conditioned animals may exhibit a greater tendency to vertically migrate (Hays *et al.* 1998).



#### **2.4.4 State of the animals/size of species**

Copepod body size may be affected by temperature (Section 2.2.1) and food abundance (Section 2.4.1), and in turn has a positive correlation with fecundity (Ohno 1990). General zooplankton grazing rates can be affected by sex, maturity, and body size and length (Griggs 1993). Larger zooplankton generally feed on larger particles at greater rates (Wright & Shapiro 1984). Filtering rate increases with zooplankton size (Burns & Rigler 1967, Burns 1969, Egloff & Palmer 1971), and is directly related to increasing weight (Schindler 1968, Kring & O'Brien 1976). The effects of factors such as oxygen concentration on filtering and respiration rates, however, are not necessarily size-specific within a species (Heisey & Porter 1977).

Larger cladoceran species may be more prone to filtering interference from filamentous BGA colonies (Webster & Peters 1978, Gliwicz & Siedlar 1980, Porter & McDonough 1984), while smaller zooplankton species may be too small to be affected (de Bernardi & Giussani 1990). The particle size and prey type that can be consumed by zooplankton is directly related to body size (Geller & Muller 1981, Ooms-Wilms 1997), and this directly controls the grazing effects they exert on phytoplankton assemblages (Dawidowicz 1990). The size of the dominant zooplankton species can have a great effect on the ecology of a water body (Section 2.4.6).

#### **2.4.5 Pathogens and toxins (biological)**

Pathogen levels are high early in the sewage treatment process (Mezrioui & Oudra 1998), but are reduced to very low numbers by the end (Mitchell 1980, Uhlmann 1980). Peters (1987) states that epidemiological studies on zooplankton are relatively rare, but that *Daphnia* are subject to infection by fungi, yeasts, bacteria and parasites. Microsporidian parasites may decrease birth rates and affect population growth.

Toxic substances may come from both chemical (Section 2.3.4) and biological sources. Toxins can be produced by bacteria under conditions of low oxygen concentration, such as those arising from

periods of stratification (Section 2.2.1), and which can be avoided by increased circulation and agitation (Section 2.2.4). Algae may produce antibacterial substances in interference competition with bacteria, and some macrophytes may produce antibiotic substances which in turn inhibit phytoplankton (Mitchell 1980, Borowitzka 1998).

The biochemical properties of some BGA may result in pronounced toxic effects on zooplankton. However, not all BGA species, and not all strains of the same species, may produce toxins (Nizan *et al.* 1986). Toxin production may not be continuous throughout BGA population growth; the exact cellular or extracellular mechanism of toxin production has not been isolated nor its evolutionary significance determined, and the actions of toxins on sensitive species are not fully known (de Bernardi & Giussani 1990). As a result, some cladoceran and rotifer species may be found thriving in high numbers amongst thick BGA blooms, and may show resistance to toxins that are present.

Species of *Daphnia* have been shown to be particularly sensitive to toxins from some strains of BGA (Nizan *et al.* 1986). BGA toxins may affect both survivorship and fecundity, influencing brood size and number, and the timing of reproductive events (Claska & Gilbert 1998). Juvenile zooplankton may be more sensitive to BGA toxins than adults, maturation rate may be slowed and the production of resting eggs may be triggered (Lauren-Maatta *et al.* 1997).

de Bernardi & Giussani (1990) report on work of other researchers that suggests BGA toxicity is related to the growth state of algae. Some of this work has indicated that young, rapidly dividing BGA cultures may be more toxic than older, stationary phase cultures; ironically, young and actively dividing phytoplankton may be a preferred food source for zooplankton in general (Hussainy 1979). In contrast, other work has shown aged BGA cultures to be more toxic than fresh ones (de Bernardi & Giussani 1990).

#### 2.4.6 Predation

Fish generally pose a major predation factor for zooplankton and, as discussed in Sections 2.4.2 and 2.4.3, can have a direct impact on zooplankton behaviour and community composition. Although fish predation may vary naturally in spatial or temporal terms (e.g. due to historical absences or winterkill: Vanni 1986), these effects provide the basis for biomanipulation and other “top-down” models (Section 2.5).

In systems where planktivorous fish are plentiful, *Daphnia* and other large zooplankton tend to be absent while smaller crustaceans (cladocerans and copepods) and rotifers dominate (Benndorf 1990, Marchessault & Mazumder 1997, Cottingham *et al.* 1998). The abundance of phytoplankton also tends to be high (White 1975, Carpenter *et al.* 1996). The removal or suppression of such predation pressure leads to minor increases in total zooplankton biomass but significant changes in the composition of that biomass, with larger species becoming much more prevalent and the mean sizes of other crustaceans (juvenile and adult) increasing by a similar degree (Benndorf 1990).<sup>1</sup>

Experimental manipulations involving an absence of planktivorous fish lead to increased zooplankton densities and biomass, which consequently affect the phytoplankton assemblages depending on the type and size of zooplankton involved (Dawidowicz 1990). Zooplankton may in turn affect each other via competition and/or predation (Brooks and Dodson 1965, Dodson 1974, Neill 1975 & 1984, Vanni 1986). Species may, however, compensate for invertebrate predation by increasing reproductive output (Vanni 1986), and so competitive interactions may be the more important of the two (Brooks and Dodson 1965, Neill 1984). Predatory effects may overlay the competitive reduction of smaller zooplankton, and promote their extinction in some situations (Vanni 1986).

Effects from reduced predation may have cyclic consequences on the whole food web, which may only be detectable in the long term

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<sup>1</sup> The exception to this rule occurs in some tropical situations, where cladoceran abundance may increase but community composition and body size does not, as large bodied species are absent in such regions regardless of trophic conditions or predation pressure (Crisman & Beaver 1990).

(Benndorf 1990). Reduced fish predation may enhance zooplankton populations which in turn undermine the phytoplankton stocks on which they feed (White 1975, Breen 1983). Increased zooplankton populations may then enhance waste nutrient levels for remaining phytoplankton, in both total amounts and availability per unit phytoplankton, and grazing may also enhance the water clarity and consequently photosynthetic activity of these individual plankters (Benndorf 1990).

Increased zooplankton grazing pressure may shift the composition of the bacterial community away from filamentous and aggregate growth forms towards smaller and evenly distributed single cells (Gude 1988). Grazing may also be beneficial to bacterial communities by reducing grazing pressure from flagellates that are also consumed, and by releasing organic carbon otherwise bound up in algal biomass. At the same time, changes in the dominant size class of zooplankton may influence the distribution and deposition of nutrients, whether by depressing pH by reducing photosynthetic activity, through the sedimentation of larger faecal materials, or by actively translocating nutrients during vertical migration (Wright & Shapiro 1984).

Greater piscivore predation on planktivorous fish may alter the available food resources, growth rates, and feeding habits of other planktivores. Numbers of large carnivorous invertebrates may be enhanced by the removal of planktivorous fish, and can provide an increased predation pressure of their own unless sufficient planktivorous fish remain to keep them in check. Moderate fish predation also promotes population stability among larger zooplankton by avoiding mass starvation and anoxia-driven mortality due to overpopulation. Consequently, 'minimum' and 'critical'/maximum planktivorous fish biomasses exist, between which large herbivorous zooplankton can thrive (Benndorf 1990).

Predation may play a major role in promoting behavioural activities such as vertical migration (Section 2.4.3) and may induce variable mating strategies in zooplankton (Maier 1996b). The type of predation (e.g. light dependent/visual) determines the relevant

importance of different types of refuge (Section 2.2.3). Invertebrate predation may also be highly important (Pinto-Coelho 1998), with predators ranging from water beetles and flatworms to cyclopoids and asplanchnid rotifers (Williamson 1983, Beisner *et al.* 1996, Pläßmann *et al.* 1997, Hampton & Starkweather 1998, Conde-Porcuna & Declerck 1998). *Asplanchna* spp. and predatory cyclopoids may regulate the abundance, density and fecundity of herbivorous rotifers, and may trigger morphological changes such as the development of protective spines (Beaton & Hebert 1997, Conde-Porcuna & Declerck 1998). Both fish and copepod predation may also directly target egg-carrying cladocerans.

## **2.5 Models of zooplankton responses and population dynamics: “bottom-up” and “top-down” approaches**

Planktonic community structure may be driven by the combined effects of bottom-up, top-down and lateral forces, such as light/nutrient availability, grazing/predation pressures, and competitive interactions, respectively (Blomqvist 1997).

It cannot be disputed that nutrient availability exerts a major controlling influence on the lower levels of the food chain, and these in turn influence each ensuing level (Section 2.3.3). Consequently, much work into eutrophication has focussed on resource limitation, or controlling the trophic pyramid from the bottom. Bottom-up mechanisms primarily appear to be controlled by phosphorus availability, and top-down manipulations in some systems may only be successful if total phosphorus levels are reduced (Benndorf 1990, Carpenter *et al.* 1996). In other systems, top-down control of general algal biomass may occur irrespective of phosphorus level, but may remain sensitive to phosphorus-driven blooms of BGA (Carpenter *et al.* 1995).

Control of phosphorus has been shown to be a crucial element in reducing or avoiding eutrophication (Schindler 1974, Mitchell 1980), and a variety of models have been constructed to

describe the phosphorus cycle, other nutrients and environmental variables, and their interplay with planktonic food webs (Sommer *et al.* 1986, Constable 1988, Moloney & Field 1989, 1991a, b & c, Richardson 1992, Davidson & Cunningham 1996, Moloney & Gibbons 1996, Flynn & Fasham 1997, Spencer & Ellis 1998, Baird & Emsley 1999). Just as bottom-up forces may influence top-down manipulations (Benndorf 1990, Carpenter *et al.* 1995 & 1996), however, bottom-up models may in turn be complicated or confounded by top-down effects (Scharf 1997).

In contrast, the general premise of top-down, or trophic-level, models is that the lower levels of the food-chain can be controlled by the higher levels, which are usually more easily manipulated. Wright & Shapiro (1984) and Vanni (1986) describe how profound an effect the presence or absence of planktivorous fish can have on the dominant zooplankton size class. Larger, more visible species (e.g. cladocerans) will be removed by size-selective predation to leave a dominance of smaller species (e.g. rotifers) when planktivorous fish are present, while smaller species will be displaced by dominant larger species when the fish are absent. Whether this displacement is also due to a top-down, invertebrate predation effect (Dodson 1974) or a competition effect in which resource depression introduces a bottom-up element (Brooks and Dodson 1965, Neill 1984) remains a subject of debate. Mitchell (1980) found both predation and competition to be highly important in structuring zooplankton communities, although the presence of omnivorous fish, rather than directly predatory ones, had no significant effect on planktonic populations.

Biomanipulation is the deliberate harnessing and directing of such top-down processes, or “cascading trophic interactions” (Crisman & Beaver 1990). The classic example is the removal of planktivorous fish from a water body in order to reduce and control algal/BGA levels. The removal or limitation of high-level planktivores leads to an increase in size and numbers of zooplankton, particularly cladoceran species, which in turn exert a greater grazing pressure on the phytoplankton (White 1975, Uhlmann 1980, Wright & Shapiro 1984, Vanni 1986, de Bernardi & Giussani 1990, Crisman & Beaver 1990, Dawidowicz 1990, Carpenter & Kitchell 1992, Carpenter *et al.* 1996). The

effectiveness of such methods has previously been the subject of debate (DeMelo *et al.* 1992, Carpenter & Kitchell 1992).

The removal or reduction of planktivores may be achieved in a variety of ways, from poisoning and fishing to the introduction of piscivores. Such methods appear to be more stable and reliable if they avoid poisoning and complete elimination of planktivorous fish, promote piscivore diversity, and are coupled with bottom-up methods - particularly as every top-down impact will automatically produce feedback responses in bottom-up mechanisms (Benndorf 1990: See Section 2.4.6).

The success of active biomanipulation relies on both the palatability of target phytoplankters to the zooplankton, and their ability to alter physical, chemical or biological conditions directly affecting the algal communities. In effect, all phytoplankton must be vulnerable to grazing, and grazing pressure must match the growth and reproductive rate of the algae. As de Bernardi & Giussani (1990) point out, an essential part of biomanipulation is the ability to predict the trophic consequences that it will cause, or else it may have the reverse effect of enhancing BGA crops.

Dawidowicz (1990) found that control of algal crops by smaller zooplankton may be limited, as they promote growth of larger phytoplankton by only removing the smaller algae, which out-compete larger species for limited nutrient resources, and they may excrete extra nutrients liberated from nanoplankton that would not otherwise be available. He concluded that larger zooplankton are required to keep such attempts at biomanipulation in balance, but problems may be encountered in BGA rich waters, which favour smaller plankton (Moss *et al.* 1991).

## 2.6 The scale of previous work

Despite the vast scope of material described in this review, most of this work has been conducted on the respective physical, chemical or biological factors in isolation, with little account taken of how they interact. Most studies have also been conducted on the theoretical or laboratory scale, with large scale field studies few and far between.

By necessity, work such as that described by Kring & O'Brien (1976) and Heisey & Porter (1977) on oxygen levels and filtration/respiration rates has been conducted in small aquaria in the laboratory, with the sole factor under investigation being tested. In their review of work on BGA/zooplankton interactions, de Bernardi & Giussani (1990) indicate that most of the work in this field has been conducted on laboratory cultures, and often considers single factors in isolation rather than in concert. Gajbhiye *et al.* (1987) studied the toxic effects of sewage on marine zooplankton within the laboratory, while Wright & Shapiro (1984) studied vertical translocation of nutrients in artificial water columns.

The importance of these experiments and their results cannot be questioned. However, they do not represent - nor claim to represent - the whole picture. All of the individual environmental factors studied combine in an inter-related mosaic in the real systems, and a change in one environmental parameter can significantly alter zooplankton responses to the others. Naturally, the effects of single factors such as light or temperature can become incredibly complex not just between days, but between seasons, and such complexity is impossible to capture in the laboratory. As Uhlmann (1980) indicated, the biotic structure of waste stabilization ponds is of much greater complexity than in artificial equivalents, including activated sludge tanks. Curiously, Uhlmann (*ibid*) demonstrated the complexity of planktonic interactions by using laboratory models under constant conditions to mimic community fluctuations that have otherwise been attributed to seasonal change.

Morgan (1985) recounts the scarcity of large scale work on the effects of photosynthesis on pH in comparison to laboratory



experiments on the same, before countering this with monitoring and analysis of two impounded ponds. Even this work, however, resorted to the use of laboratory work and 1m<sup>3</sup> enclosures within the ponds for actual manipulation experiments on the same. In testing the possibility of continuous mass production of rotifers from effluent, Schluter & Groeneweg and Groeneweg & Schluter (both 1981) used laboratory and small pond manipulations and experiments.

Enclosures provide a partial bridge between laboratory experiments and field data (Fussmann 1996), but are still more prone to edge effects and remain markedly simplified in comparison to the full scale systems they seek to reflect (Bloesch *et al.* 1988). Although working in a lake of 1888 hectares surface area and 8.1m mean depth, Dawidowicz (1990) used 3m<sup>3</sup> enclosures in studying the effectiveness of phytoplankton control by large and small bodied zooplankton. In work directly relevant to the current study, Kawai *et al.* (1987) decried the lack of full-scale, long-term studies on nutrient recycling strategies for WSP systems and the manipulation of the planktonic communities they contain. The same authors highlighted the shortfalls of earlier laboratory-scale works, and of the small-scale tanks used in their own field study.

Crisman & Beaver (1990) used enclosures to evaluate the efficacy of temperate zone biomanipulation techniques in the tropics, and commented that further work was required on the influence of specific fish taxa on phytoplankton community composition, nutrient cycling, and planktonic productivity before whole-lake manipulations could be conducted in tropical areas. Marchessault & Mazumder (1997) investigated ciliate population dynamics in a series of much larger (550m<sup>2</sup>) enclosures, while Blomqvist (1996 & 1997) used enclosures to gauge the interaction of nutrients and grazing on phytoplankton community growth. Potential predatory and competitive interactions between crustacean, rotifer and ciliate zooplankton have also been investigated in short-term bottle experiments (Gilbert 1989) and at the enclosure scale (Fussmann 1996, Plaßmann *et al.* 1997).

Vanni (1986) comments that many experimental field studies on zooplankton competition are short-term, limited in community

complexity, and conducted in small containers. While useful in assessing interactions among particular species, the same researcher points out that small-scale experiments miss the potentially crucial and complex species interactions that occur on the larger community scale. However, the necessity of reliably excluding fish meant that the enclosures used in his own experiments were again a fraction of the size (1.175 litres) of the lakes being studied, and, while workable, showed variations from the typical zooplankton assemblage of the surrounding waters. In contrast, Mitchell (1980) used enclosures to contain introduced fish, and also cited the lack of large scale research into seasonal cycles and population fluctuations in plankton. He highlighted the preponderance of short-term, mass-culture and laboratory-simulated studies, often run under ideal conditions.

In conducting experimental manipulations of nutrient loading and grazing pressure at a mesocosm scale, Cottingham *et al.* (1997) pointed out the utility of such systems in testing hypotheses under “controlled, replicated conditions”, but warned of their accuracy in relation to full-scale ecosystems. Extrapolating results from small scale enclosures to full scale systems can pose great problems (Carpenter & Kitchell 1992), especially when complex processes may be controlled by a variety of factors (Clymo 1995). As Berndorf (1990) states, the crucial role played by indirect effects, feedback mechanisms and time lags in top-down manipulated food webs means that the reliability of biomanipulative management tools can only be derived from whole-lake, long-term studies.

Large scale work has mostly consisted of monitoring, from general zooplankton community studies (Gannon *et al.* 1984, Orcutt & Pace 1984, Maier 1996a, Walsh 1996, Adrian 1997, Pinto-Coelho 1998) and the detailed studies on Lake Constance (Geller 1986, Gude 1988, Pinto-Coelho 1991, Geller *et al.* 1992) to work on sewage lagoons in general (Patil *et al.* 1975, Pudo 1978, Mitchell 1978 & 1980, Uhlmann 1980 (reviewing other work), Griggs 1993, Mendes *et al.* 1995, DELM/DPIF 1996) and the Werribee Treatment Complex itself (e.g. Hussainy 1979, Constable 1988, and the large volume of unpublished work by David Cartwright, WTC). Experimental work coupled with

these studies has again tended to be restricted to the laboratory, or, at best, enclosures.

Large-scale manipulative studies are rare (Fussmann 1996), and of those that have been carried out most are of crude design or intent (e.g. the mass nutrient loading of north American lakes: Schindler 1974) or focus directly on the manipulation of fish populations (work recounted by Wright & Shapiro 1984, Benndorf 1990). Consequently, detailed large-scale manipulations of plankton assemblages and the biotic and abiotic factors controlling them (e.g. Carpenter *et al.* 1995 & 1996, Christensen *et al.* 1996, Pace & Cole 1996, Cottingham *et al.* 1998) represent a valuable and largely unexplored avenue of future research. The present study therefore aimed to determine whether large-scale manipulations of zooplankton communities could be conducted in a consistent and predictable manner by controlling and varying the (flow-determined) nutrient loading of nutrient-rich environments.

### 3. GENERAL PROJECT RATIONALE & METHODS

#### 3.1 The study site

The Werribee Treatment Complex (WTC), Victoria, covers an area of 10,851 hectares<sup>1</sup>, and utilises approximately 6,336 of these for ground filtration, grass filtration, and lagoon treatment techniques. The remaining land is taken up by operational buildings and laboratories, plantations, roads, channel reserves and livestock grazing areas. In addition to waste treatment, the Complex acts as a major beef and sheep station, and is a wetland and wildlife sanctuary of international and national conservation significance.

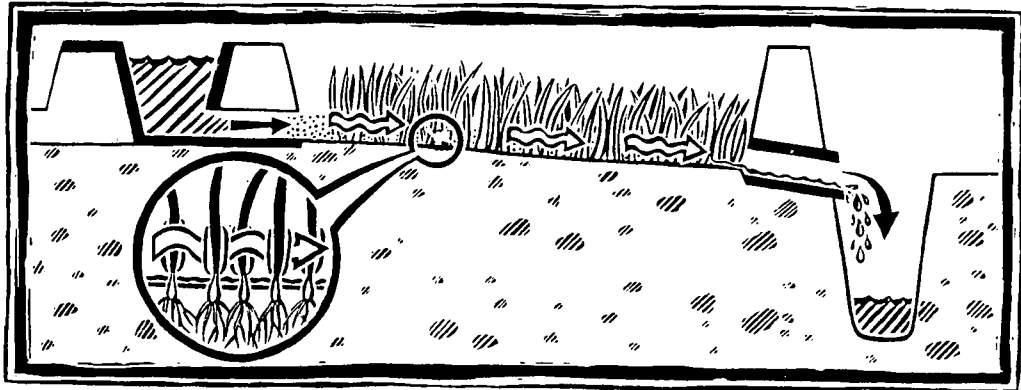
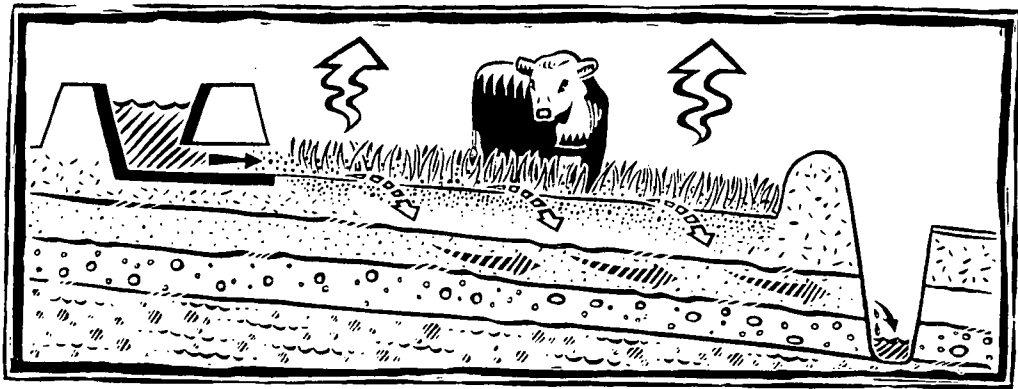
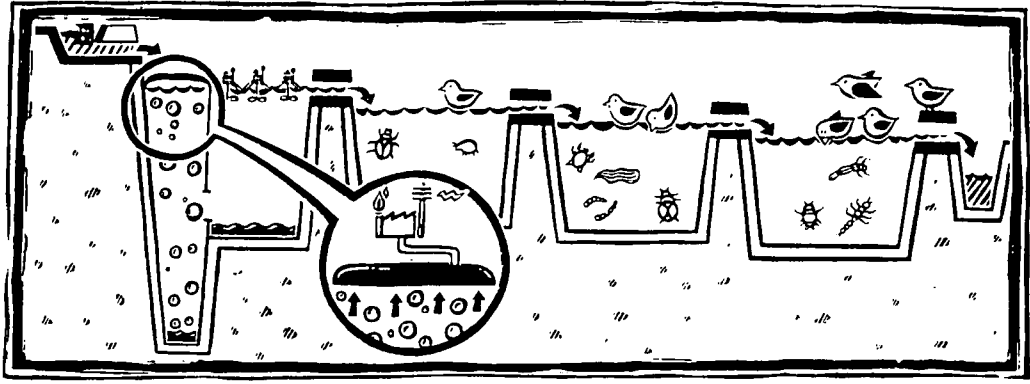
Low gradient, suitable soil type, high evaporation and low rainfall characteristics make the site ideal for the treatment processes. The complex treats 52% of Melbourne's domestic waste, and 90% of its agreed (non-toxic) trade waste, which amounts to approximately 520 million litres (ML) per day, or over 180,000 ML per year. Approximately half of this receives pretreatment in the form of primary sedimentation (Figure 3.1), which reduces the level of suspended solids to manageable proportions for grass filtration. After land, grass or lagoon treatment, water leaving the Complex is discharged by four main drains into Port Philip Bay. These discharges are regularly monitored and are of equal standard to secondary treated effluent from "conventional" treatment plants (WTC information).

Land filtration (Figure 3.1) has been used since the complex started operation in 1897, using 3,239 hectares of the more permeable soils along the Werribee River. Raw sewage is flooded over pasture land for one to two days, followed by five days of drying in which the water evaporates or filters through the soil, leaving the nutrients and contaminants behind. Most of the organic material accumulates as a rich humic layer, while other material is digested by biological activity in the soil. The enhanced pasture growth is then grazed by livestock for a further one to two weeks. Forty percent of the irrigated water is collected in sub-surface drains and flows into Port Philip Bay, while the

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<sup>1</sup> Based on 1991-1992 WTC figures and information.





rest is lost by evaporation and evapotranspiration. Land filtration is essentially a summer process, operating from October to April, when soil permeability, grass growth and evaporation are high and rainfall low. The process is repeated up to ten or more times per treatment season, and treats 15% of the flow, or 28, 000 ML, per year (WTC information).

Grass filtration (Figure 3.1), or overland flow, was introduced in 1930 to treat winter flows, and is carried out in about 1, 443 hectares of the heavier clays and loams on the western side of the Complex. This involves a slow, continuous flow of sedimented sewage through graded bays of dense Italian Rye grass. The vegetation traps suspended matter from the irrigant, while organic matter is actively removed by the biological films that develop on the plant stems and soil. Effluent is then collected by a network of drains and may be discharged directly or may pass through a lagoon for “final polishing” before passing into the Bay. More than 80% of the flow into the system, plus input from rainfall, appears as run-off at the end of the process, which takes three to four days. Grass filtration is a winter process, used from May to September. Between operational seasons, grass filtration areas are allowed to dry so that the grass goes to seed and cattle can feed on the dry vegetation. Rain and irrigation trigger the germination of fresh, unchannelled and unclogged grass at the start of the next operational period. Grass filtration treats an average of 46, 000 ML per year, or 25% of the annual flow (WTC information).

Lagooning (Figure 3.1) started in 1937, to treat daily peak flows and wet weather flows exceeding the capacity of the other treatment processes. About 1,654 hectares of the lower foreshore areas of the Complex are given over to lagooning, which provides arguably the most efficient form of treatment<sup>2</sup>. Lagoons provide simple and inexpensive operation, with high self-regulation potential and no additional energy requirements to meet basic aeration<sup>3</sup> or circulation needs (Uhlmann 1980, Mezrioui & Oudra 1998). Lagooning is used all

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<sup>2</sup> A recent and detailed review of waste stabilisation ponds is given by Inmuong (1993).

<sup>3</sup> Mechanical aeration, however, may greatly assist in removing odours from earlier parts of lagoon systems.

year round at WTC, and the lagoons receive approximately 60% of the annual inflow. They treat an average of 105, 000 to 110, 000 ML per year (WTC information).

The arrangement of lagoons at WTC is unique compared to almost anywhere else in the world, and is easily the most extensive lagoon development in Australia. Rather than having two or three closely linked small lagoons or small pond cells with medium to high retention time (e.g. Mitchell 1980, Griggs 1993, DELM/DPIF 1996), WTC boasts numerous complete systems composed of eight to twelve large (four to eight hectares or more) gravity fed lagoons in series. The ability to provide sufficient land to allow lagoon treatment, as well as land and grass filtration, on such a scale highlights the foresight of James Mansergh's recommendations for the site in 1890. It also highlights the success of such systems when managed within their operational limits<sup>4</sup>. Lagoons require a certain period for stabilization according to the load for which they are designed (Patil *et al* 1975), and reliable design procedures should be followed to ensure effluent quality meets a required standard (Uhlmann 1980).

The WTC lagoon systems vary in age, layout and size. Water is treated naturally by sedimentation, bacterial digestion, passive aeration, and algal/biological activity. Bacterial activity due to high organic loading and sedimentation leads to anaerobic conditions in the first lagoons of each system; this activity provides an initial breakdown of the wastewater, while sludge containing heavy metals and other undesirable chemicals tends to settle out. The anaerobic ponds are followed by facultative and aerobic lagoons as the wastewater progresses through the lagoon series. Algae grow on the breakdown products from the earlier lagoons, absorbing nutrients and trace elements from the

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<sup>4</sup> In 1996, local environment minister Peter Hodgeman claimed that sewage treatment lagoons in Tasmania were a failed experiment, calling them "a Queensland solution to a Tasmanian problem." The problem lies not with the lagoons' abilities to treat water, but with design and overloading problems that permit waste water to flow through these small systems before the organisms can act on it. Lagoons in temperate zones simply require longer treatment periods (Inmuong 1993). Such mismanagement and discharge of incompletely treated wastes is, however, an Australia-wide problem (Mitchell 1980). This is not the case at WTC, where lagoons are the most reliable treatment method despite a climate that is much closer to that of Tasmania than Queensland. Indeed, water mixing due to diurnal convective overturn means that sewage lagoons in temperate regions potentially hold better BOD removal efficiencies than those in the tropics (Uhlmann 1980). In late 1996 a report was produced announcing that 70% of Tasmania's sewage lagoon sites were underperforming, and offered an extensive list of problems, symptoms, causes and solutions relating to lagoon efficiency in the state (DELM/DPIF 1996).



water column. Nitrogen and phosphorus are removed by bacteria and phytoplankton, which are grazed on by zooplankton, which in turn may be fed on by carnivorous zooplankton and birds. Final effluents display markedly reduced BOD, SS, bacterial and viral levels (Mitchell 1980).

In older systems, pond sizes range between surface areas of four and eight hectares and depths of one to two metres. Pond sizes may vary between systems, but tend to be relatively consistent within a system. Sewage is retained for 40 to 90 days, with retention (and so treatment) time within each pond being deliberately varied to match the length (and so treatment capacity) of each system. In newer lagoon systems, pond sizes may be significantly larger and more variable (both within and between systems), and the first pond is partitioned to optimise treatment. This divides the initial pond into a small anaerobic reactor, and a larger mechanically aerated body. The newer lagoon series are typically ten ponds in length, with retention periods usually of 60 to 70 days.

Melbourne Water is currently in the process of revising its short and long term strategies on sewage treatment in order to meet its requirements for treating the city's wastewater in future years. An increase of over 50% has been projected for sewage inflows to WTC over the first half of the next century, with dry weather flows rising from 470 ML per day in 1988 to as high as 730 ML per day by 2035. Developments in the treatment processes need to be sustainable and economical; they need to combat the increasing demands and requirements of greater sewage inflow, maintain the standard of treatment it receives without jeopardising Port Philip Bay or the significance of the wetlands and, if possible, to provide further income to offset increased operating costs.

In the past, treatment through lagoon systems has been passive, with such processes as aeration being left to wind and photosynthetic activity from the algae. In more recent years, experimental modifications have been made to several newer lagoon systems, including deeper high rate anaerobic ponds, mechanical aeration of earlier lagoons, and the optimisation of later ponds for

**Plate 3.1 (facing): Lagoons at the Werribee Treatment Complex.**

*Top left:* pond 6 of the 115e system, facing south-west from directly above the centre outfall. At 2.3km, this single lagoon is longer than the entire 85wB system. *Top right:* the open supply carrier into the 85wB, showing the flow-measuring wheel at the start of the system. The original pond '2' of the 85wB (which was later cut out of the system) can be seen in the foreground, with pond 1 (the SL pond) beyond it. *Bottom right:* again showing the original pond '2', with pond 1 (SL) in the background on the right hand side and the junction of pond '2' with ponds 3 (1N) and 4 (SP) in the background towards the left. *Bottom left:* pond 4 (SP) of the 85wB system in the foreground, with pond 5 (1S) in the mid-ground and Port Philip Bay beyond it.

**Plate 3.2 (overleaf): Harvesting at the Werribee Treatment Complex.**

*Top & bottom left:* active harvesting on the 115e system using Zootech's custom-built 'Baleen' harvester, on which selection of different screen sizes determines the fraction of zooplankton harvested. *Top & bottom right:* passive harvesting, and harvest collection, of large quantities of cladocerans (mostly *Daphnia* spp.) using outfall screens on pond 6 of the 115e system.







nitrogen removal via nitrification/denitrification bacterial processes (Constable 1988). Methane released from anaerobic reactors has been collected and burnt to reduce smell and Greenhouse emissions, recover heat for increased microbial digestion, and potentially generate power (this could be used to power aerators and other machinery at WTC, or sold for profit offsite). Recent research into lagoon design, biofilms, and plankton harvesting have been seen as potential pathways for improving nutrient removal (WTC information). Algal harvesting has been successfully trialed at the Complex (Caldwell Connell Engineers 1975), and trends in zooplankton populations have been monitored to develop a rapid biological measure of effluent quality (D. Cartwright, unpublished data). However, no attempts have previously been made to directly manipulate, or optimise for harvest, zooplankton populations themselves.

Zooplankton are present in harvestable quantities within the lagoons of the WTC at many times, and can be readily collected by both active and passive means (Plate 3.2). Such blooms, however, are not necessarily predictable or stable. To enable further research into this topic, WTC generously provided the 85wB lagoon system (Figure 3.2, Plate 3.1) for use and experimental modification in this project. The 85wB system is one of the older, smaller-to-medium sized systems at the Complex, consisting (prior to modification) of twelve lagoons connected in direct serpentine fashion. Individual ponds ranged from 3.76 to 10.97 hectares in surface area, and from 41.82 to 170.48 ML in capacity. The overall system was approximately 2 km long, covering a total area of 70.50 hectares with a total capacity of 926.93 ML (Figure 3.2).

### **3.2 The problem of experimental scale**

As described in Chapter 2, many studies have been conducted measuring the effect of various physical, chemical, and biological parameters on zooplankton populations. However, such studies have usually involved the use of enclosures or limnocorrals, or have been conducted in the laboratory. While such experimental systems provide

**Figure 3.2: Original flow pattern of the 85wB system, overlaid on the Melbourne Water map of the lagoons.  
The map includes the original pond numbering system plus imperial pond areas and capacities.  
Metric equivalents are given below.**

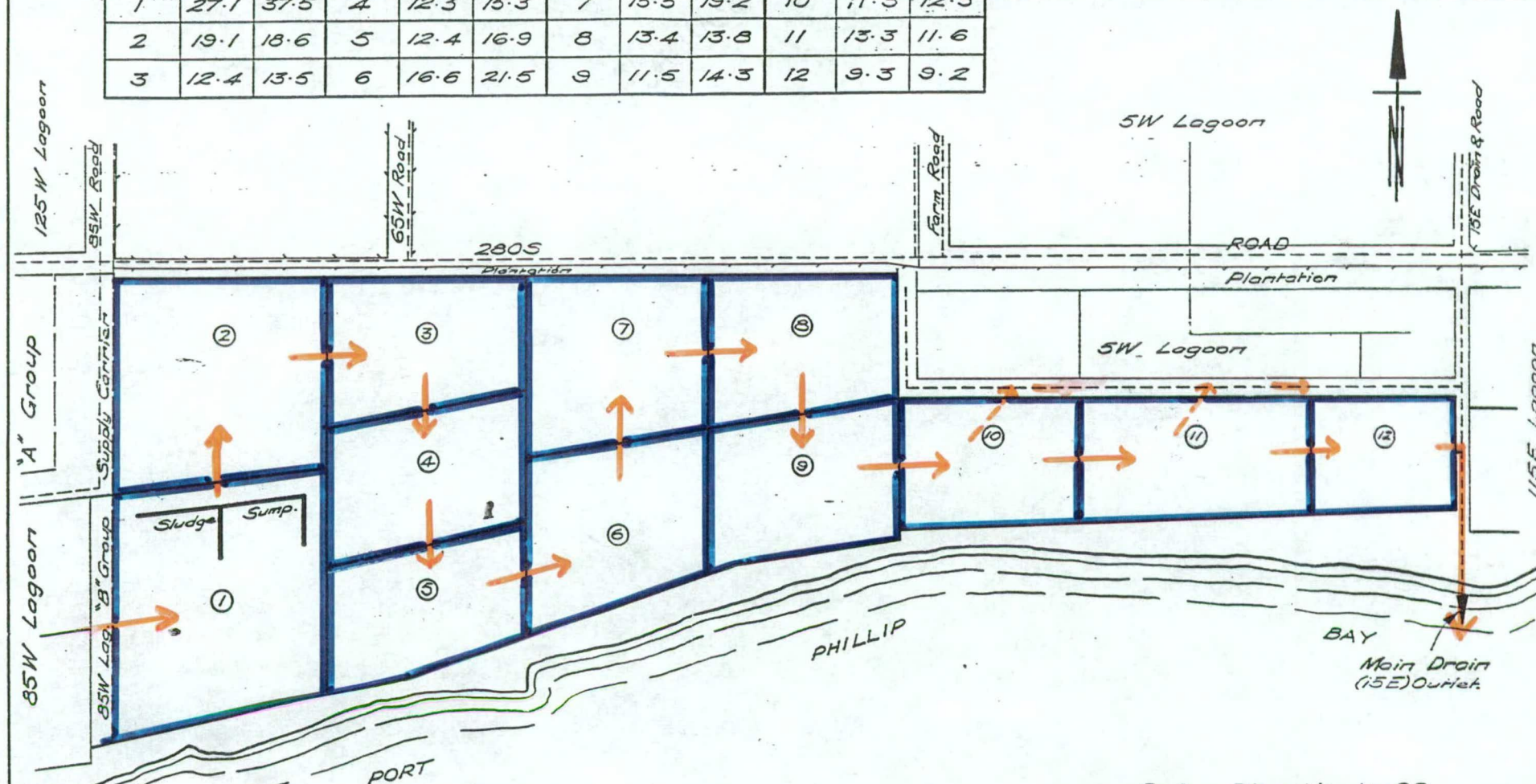
<u>Pond</u>	<u>Area (ha)</u>	<u>Capacity (ML)</u>	<u>Pond</u>	<u>Area (ha)</u>	<u>Capacity (ML)</u>
<b>1</b>	10.97	170.48	<b>7</b>	6.27	87.28
<b>2</b>	7.73	84.56	<b>8</b>	5.42	62.74
<b>3</b>	5.02	61.37	<b>9</b>	4.65	65.01
<b>4</b>	4.98	69.55	<b>10</b>	4.57	56.83
<b>5</b>	5.02	76.83	<b>11</b>	5.38	52.73
<b>6</b>	6.72	97.74	<b>12</b>	3.76	41.82

**Total area: 70.50 ha**  
**Total capacity: 926.93 ML**



Pond No.	Area Acs.	Cap'y. M.G.	Pond No.	Area Acs.	Cap'y. M.G.	Pond No.	Area Acs.	Cap'y. M.G.	Pond No.	Area Acs.	Cap'y. M.G.
1	27.1	37.5	4	12.3	15.3	7	15.5	19.2	10	11.3	12.5
2	19.1	18.6	5	12.4	16.9	8	13.4	13.8	11	13.3	11.6
3	12.4	13.5	6	16.6	21.5	9	11.5	14.3	12	9.3	9.2

**TOTALS : 174.2 Acs. , 203.9 M.G's.**



→ Direction of Flow

Approx. length of system = 2 km

BOARD OF WORKS FARM		Scale : Diagrammatic	
85W. LAGOON "B" GROUP		Passed:	Drawn: <i>Kab</i>
Areas & Capacities		<i>16/7/67</i>	Traced: <i>Kab</i>
		Checked: <i>ft.</i>	

**L-103**  
T-682

interesting results on the responses of zooplankton to specific sets of conditions, they are greatly simplified, are far more prone to edge effects, and cannot ensure sufficient support for extrapolation across the large scale environmental mosaic they seek to represent (Carpenter & Kitchell 1992). At best, the direct applicability of such studies to an entire lagoon system (e.g. Plate 3.1) is unknown, while, at worst, they may be unrealistic.

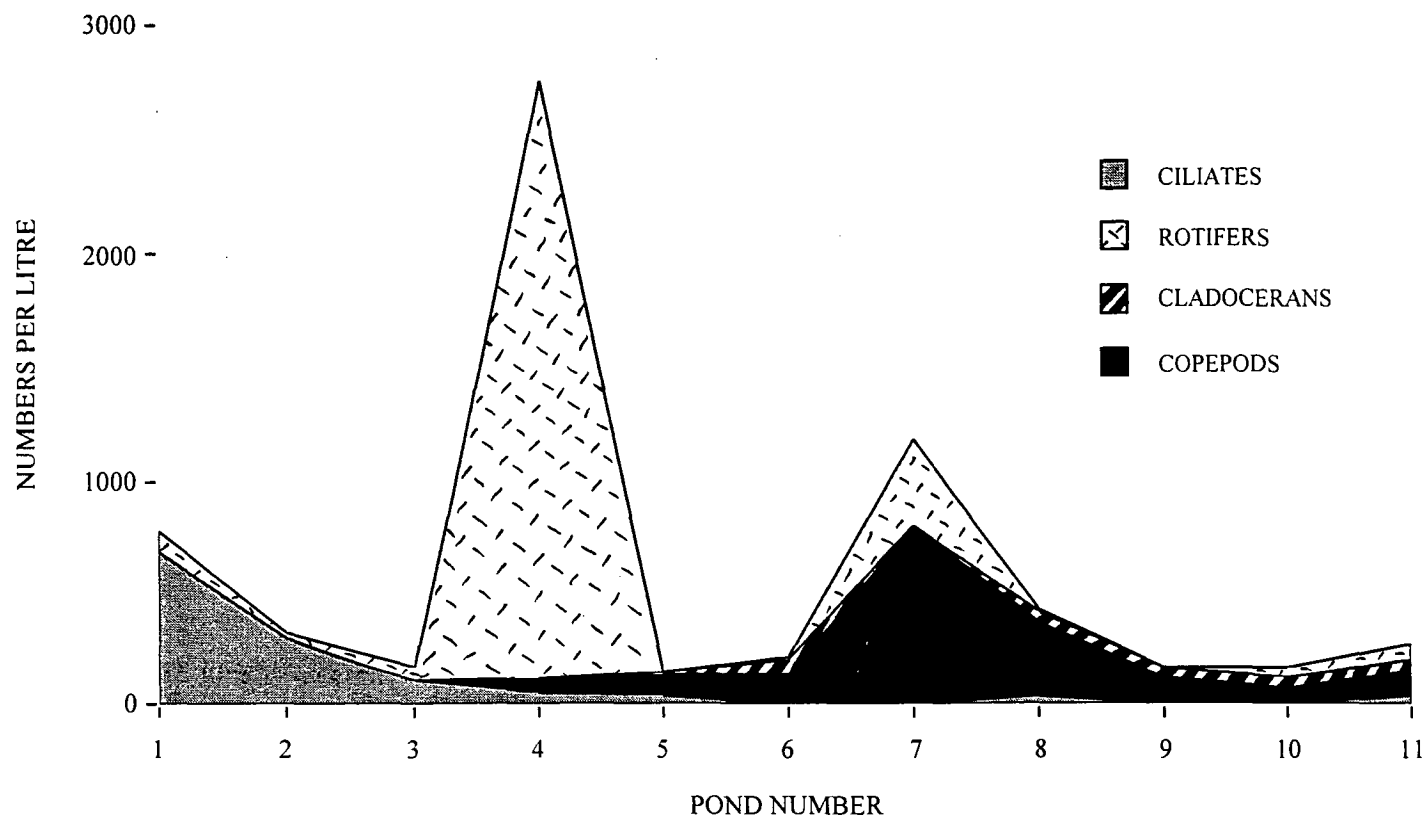
A second important factor is one of cost. Any successful method of optimised zooplankton production would need to be easily implemented at Werribee, to avoid excessive additional costs. With few exceptions, the factors previously discussed are too difficult or expensive to closely or reliably control on the large scale. Matters would be further complicated in a matrix of these conditions and their interactions, and so attempts to control most of them needed to be limited to the periphery of the main project, rather than forming its core.

Rather than investigate an 'ideal' regime that would prove impossible or prohibitively expensive to implement on the large scale, it was decided that this project would work directly with the entire 85wB lagoon system. This would serve to impose no edge effects or other reduced-scale effects other than those naturally and already present. While the scale of such an operation was expected to cause difficulties in both physical size and time, the resultant constraints on experimental design did not conflict with the intentions of the project.

Firstly, simplicity was in keeping with requirements of cost, and would also act as a potential buffer. The system would need to be durable despite the great degree of variation in many physical, chemical and biological factors, and it was hoped that such resilience would be achieved through its simplicity.

Secondly, with large scale ecological experiments, which are prone to the vagaries of field conditions and limitations in replicates, designs need to be relatively simple in order for the conclusions drawn from them to be valid. As stated by Hairston (1989): "Occasionally, more than one variable can be manipulated, but the number is severely





**Figure 3.3: 115e lagoon series, 10 February 1993, showing the typical gradation in zooplankton types across the system (graph derived courtesy of David Cartwright, unpublished data).**

limited... to overcome the [problem of natural variability] will limit the complexity of design and thus limit the sophistication of the kinds of questions that can be asked simultaneously." Simplicity of design does not imply overall simplicity to the project, however, as the amount of data that can be collected is great and much analysis and inference can be made. For ecological field experiments, environmental complexity replaces complexity of experimental design.

Finally, at least as far as the primary manipulations to the system were concerned, it appeared that a relatively simple and controllable factor was the most relevant. This factor was the flow rate through the lagoon systems, and presumably involved the nutrient loading/concentration of the water itself.

### **3.3 Prior data on WTC**

Unpublished studies by David Cartwright (biologist, WTC) have shown that the general pattern of zooplankton distribution at WTC closely matches the increase in water quality, or decrease in nutrient loading/concentration, as it progresses through the lagoons of each system (e.g Figure 3.3). In the first (anaerobic) and more nutrient loaded lagoons there are high numbers of ciliates, which then rapidly tail off. Rotifer numbers also peak early, usually after the anaerobic lagoon(s), persist for a number of ponds, and then decline to almost zero further in the system. At about this point, the microcrustacea - fractions of which have either been absent or in low levels up until now - begin to appear and dominate for the rest of the system, although in relatively lower overall numbers; numbers which in turn begin to tail off as the water reaches the end of the treatment process.

Noticeable changes in diversity and type also occur within these groups, and the location and extent of their distribution shifts according to both the pattern and level of flow through the particular lagoon system. In short, lagoon arrangement at WTC provides a unique situation where normal seasonal zooplankton succession is coupled with and overlayed by a distinct spatial succession.

The link between this spatial pattern and the nutrient loading of the water is further supported by lagoon systems that break from this general pattern. Two primary examples, again taken from David Cartwright's (unpublished) data, are the 115e and 55e systems.

In 1993, WTC Operations isolated lagoon 5 from the 115e system and reduced the flow into lagoon 6, in order to establish suitable conditions for a nitrification-denitrification microbial loop (as per Constable 1988). *Daphnia* spp. were then recorded in high numbers in the low flow/low nutrient loaded lagoon: a condition which put it out of sequence with the general zooplankton distribution described for the lagoons generally, and for the 115e system in particular. Shortly after this, a 'carbon-feed' (a euphemism for a small direct inflow link of raw sewage from lagoon 1) was introduced to pond 6, maintaining its low inflow level, but returning it to a high nutrient loading. While *Daphnia* levels remained high, a second *Brachionus* (rotifer) peak occurred in the pond. (This peak was markedly higher than the secondary peak shown in Figure 3.3, and was comparable to the earlier one).

A similar pattern was observed in the 55e system, with pond 5 receiving one-third of the flow through the system, and displaying high numbers of crustacean zooplankton. In 1993, to test the capacity of the (then new) system, the WTC engineers doubled the 55e flow rate from 30 to 60ML per day. This physically flushed the system free of its resident zooplankton, and only ciliates and rotifers returned to the system until nutrient levels started to return to normal.

It is worth noting that while the physical effects of flow rate may explain the increased persistence of early-pond species (such as rotifers) through a lagoon system, it does not explain the earlier occurrence of later-pond species under low flow conditions, their absence under high flow, nor the overall pattern observed under consistent flow (as per David Cartwright's data and that reported later in this thesis). As a result, nutrient loading combined with flow rate, rather than flow rate *per se*, is believed to be the crucial factor in zooplankton distribution at WTC.

The nutrient load entering a system is determined from the

following equation, and is effectively a product of flow rate (hydraulic loading) and the nutrient concentrations of the influent.

$$\text{Load (kg/d)} = (\text{Flow (kL/d)} \times \text{Pollutant Concentration (mg/l)}) / 1000$$

[DELM/DPIF 1996]

In reality, pond biota will be affected by the actual, relative and sequential concentrations of nutrients in their individual lagoons, or:

$$\text{Pollutant Concentration (mg/l)} = (1000 \times \text{Load (kg/d)}) / \text{Flow (kL/d)}.$$

This calculation therefore involves working backwards, as although nutrient concentration depends on the interplay of flow rate and nutrient loading, the latter can only be determined once those concentrations have been measured.

Although there is some variation in influent nutrient concentrations at WTC, they are surprisingly consistent on a daily, weekly, monthly and long-term basis (Constable 1988). It was therefore expected that the greatest degree of change in nutrient conditions per lagoon -- whether viewed as loading or concentration -- would occur through variation in flow rate.

### 3.4 Experimental design

This project aimed to dovetail the work of David Cartwright by testing the hypothesis that the spatial distribution of different zooplankton species/groups could be manipulated in a consistent and predictable manner by directly changing the flow rate (and hence the nutrient loading) through a system. In short, this project aimed to actively stimulate predictable distributional changes of the type that Cartwright was monitoring and recording.

To do this, major modifications were made to the 85wB system. The planned experimental design was to effectively divide the system into two comparable halves beyond the initial lagoons (Figure 3.4). Sewage would still enter the system via pond 1, but would then be directed into pond 4, where flow would be split between ponds 3 and 5.

Pond 3 would then empty in sequence through ponds 7, 8, and two ponds on the north side of the system that would be seconded to the 85wB. Pond 5 would empty into 6, 9, 10, and 11 in sequence on the southern side of the system (pond 12 was to be removed from the system by WTC due to work on the main drain system, and so was to be unavailable).

Melbourne Water provided earth moving equipment and operators to establish the altered flow patterns, by cutting the banks between lagoons that were not otherwise directly connected. Inflow to the 85wB was stopped to reduce water levels by 50% throughout the system (Plate 3.3). New channels (approximately two to three metres wide) were excavated between lagoons 1 & 4, 3 & 7, 6 & 9, and 8 & the two non-85wB lagoons positioned to the north of the end of the system. Lagoon 2 was completely cut out of the system (i.e. inlets and outlets sealed with boards), while outlets from lagoons 6 to 7 and 8 to 9 were also closed. For ponds 4, 5, 7, 9, and 10, which were to retain the same direction of outflow as in the original system, two of the three outflow points were sealed with only the central one remaining open. This was to provide as great a measure of similarity as possible to the flow patterns of the lagoons between which only single and central channels had been excavated. Similarly, the outer outlets of lagoon 3 were also sealed, although in this case the central one was to become the new inflow point for that pond. Fortunately, ponds 3 and 4 were effectively at the same height above sea level, and so the direction of flow between these ponds could be manipulated and reversed by adjusting the relative water levels between the two ponds.

To ease nomenclature for the revised system, the following labels were adopted for the lagoons (Figure 3.4):

pond 1 = SL (or 'sludge' lagoon);

pond 4 = SP (or 'split' lagoon) with outlets SPN and SPS to the north and south, respectively;

ponds 3, 7, 8, sequestered lagoon 1 and sequestered lagoon 2 =

1N, 2N, 3N, 4N and 5N ('northern side'), respectively;

ponds 5, 6, 9, 10, and 11 = 1S, 2S, 3S, 4S, and 5S ('southern side'), respectively; and

ponds 2 and 12 = cut out of system, so not renamed.

**Figure 3.4: Altered flow pattern of the 85wB system, as per Figure 3.2. The map includes the new pond nomenclature, and highlights the removal of lagoons ‘2’ and ‘12’ from the system alongside the incorporation of ‘4N’ and ‘5N’ to the system. Metric comparisons of paired pond surface areas and capacities are listed below.**

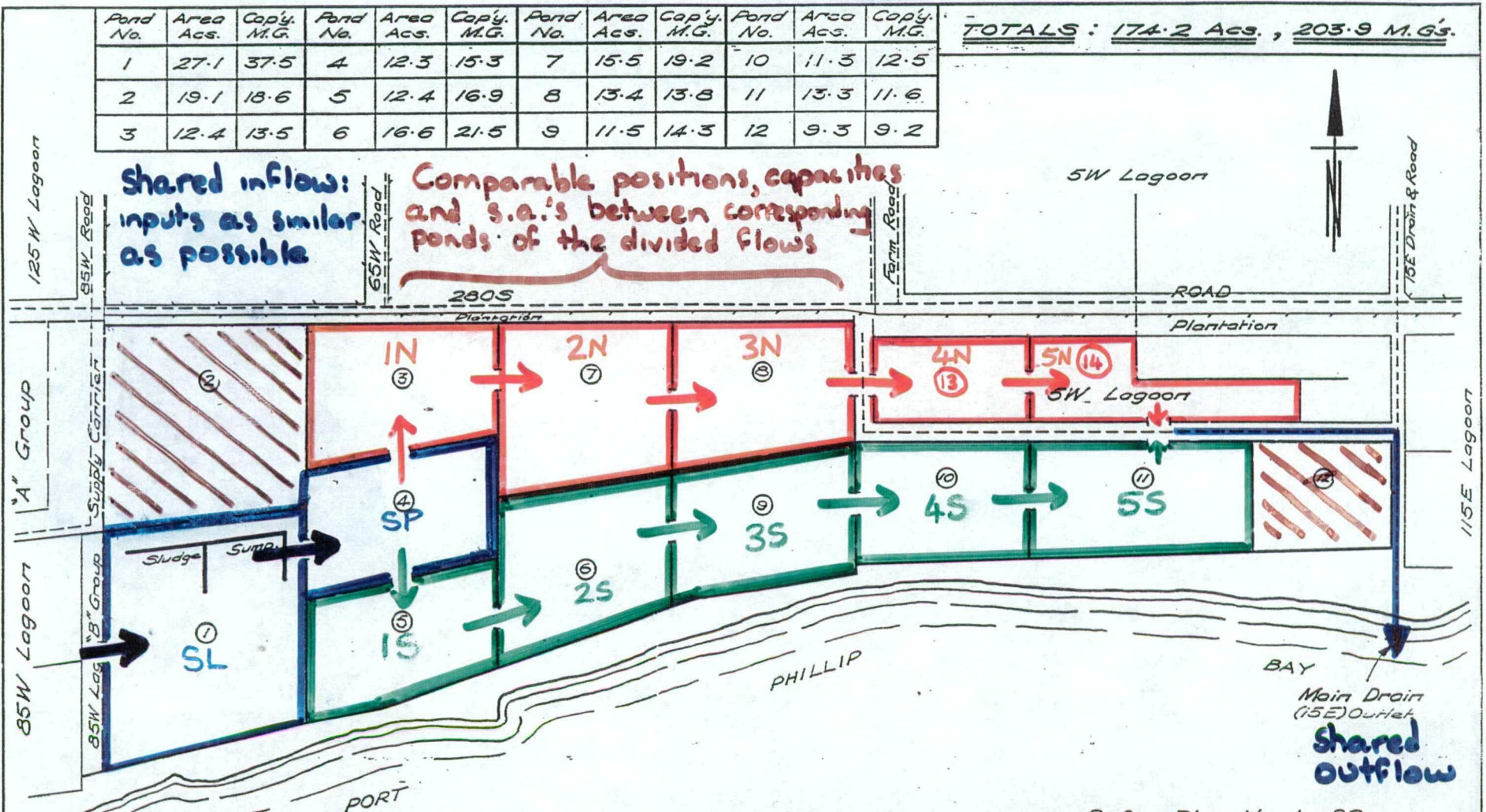
<u>Pond</u>	<u>Area (ha)</u>	<u>Capacity (ML)</u>
SL (1)	10.97	170.48
SP (4)	4.98	69.55
1N (3)	5.02	61.37
1S (5)	5.02	76.83
2N (7)	6.27	87.28
2S (6)	6.72	97.74
3N (8)	5.42	62.74
3S (9)	4.65	65.01
4N (-)	not surveyed	
4S (10)	4.57	56.83
5N (-)	not surveyed	
5S (11)	5.38	52.73

Pond No.	Area Acs.	Cap'y. M.G.	Pond No.	Area Acs.	Cap'y. M.G.	Pond No.	Area Acs.	Cap'y. M.G.	Pond No.	Area Acs.	Cap'y. M.G.
1	27.1	37.5	4	12.3	15.3	7	15.5	19.2	10	11.3	12.5
2	19.1	18.6	5	12.4	16.9	8	13.4	13.8	11	13.3	11.6
3	12.4	13.5	6	16.6	21.5	9	11.5	14.3	12	9.3	9.2

**TOTALS : 174.2 Acs. , 203.9 M.G's.**

**Shared inflow:**  
inputs as similar  
as possible

**Comparable positions, capacities  
and s.a.'s between corresponding  
ponds of the divided flows**



**— combined flow**  
**— divided flows (experimental  
and control systems)**

BOARD OF WORKS FARM

85W. LAGOON "B" GROUP  
— Areas & Capacities

Scale : Diagrammatic

Passed: *[Signature]*  
1/17/67  
Drawn: *[Signature]*  
Traced: *[Signature]*  
Checked: *[Signature]*

**L-103**

T-682



Beyond the initial ponds, the resultant system effectively provided two separate halves in which zooplankton responses to similar and different conditions could be tested and compared on the large scale. As originally conceived, one side could be used for experimental manipulation, while the other - of similar lagoon shape, position, orientation, capacity, and influent quality (Figure 3.4) - could act as a 'control'. In practice, however, the method of dividing flow rates between the halves of the system, and the wastewater volumes required, meant that neither side could be used as a formal control throughout the experimental period (under different divisions of flow, both were effectively 'treatments'). In this way, the system was more closely allied to (and treated as) an industrial processes, where trials can only be made over time. The way in which this was achieved will be discussed shortly.

While two completely separate systems would have been preferable for comparison, the system described above was the best option in terms of the ponds available. An alternative system, of using pond 2 as the first pond of an entirely separate northern system (with 4 cut out) was discounted, as pond 2 was not considered to be an ideal pair to the original pond 1. For several decades, pond 1 would have experienced significantly greater sedimentation than any other pond in the system, and the relative balance of materials at the sediment/water interface can greatly affect the oxygen budget and ecological stability of a pond (Uhlmann 1980). In addition, the presence of the sludge sump (absent from pond 2) could potentially make a great difference to the quality of effluent passing into the next pond, and a great difference existed in the relative area and volume of these two ponds (Figures 3.2 & 3.4). Such differences were identified as a major concern at the start of the system, with their potential to impact all of the ponds that followed. As the main interest of the project was the zooplankton which (theoretically) occurred later in the system, rather than the early dominance of ciliates, this alternative was dropped.

In contrast, the first three sets of paired ponds (1N+1S, 2N+2S, 3N+3S) were considered to be suitably well paired in capacity



**Plate 3.3:** Lowered water levels in the 85wB system, allowing the excavation of new channels between lagoons and modification of the existing flow pattern.



and history; these paired ponds were relatively close to each other in the original flow pattern and would not have experienced as great a disparity in sedimentation and effluent quality as that of the original pond 1. While pond sizes and histories between the new pairs 4N + 4S and 5N + 5S were not ideal, again it was a case of working with the available lagoons. As these ponds were at the end of the system, and so would not influence any following pond pairs, the couplings were considered adequate.

Concerns of pseudo-replication remained an ever-present consideration, both between halves of the system and over time. System manipulations were designed and conducted as carefully as possible under experimental constraints, and it was suggested (under formal statistical advice) that replication could be achieved over time rather than through large numbers of simultaneous trials. Essentially, *if* nutrient loading was highly important in determining the distribution and composition of zooplankton communities under different flow regimes (as hypothesized), shifts and changes in community composition should be apparent between the halves *irrespective* of previous events or treatments.

The effects of different flow rates and their resultant nutrient loadings were examined according to the general hypothesis presented in the equation:

$$G = AG + TE + FE + CE$$

where G = a measure of zooplankton growth, number, community composition or biomass;

AG = the 'average' growth, composition or biomass that would have occurred irrespective of other variation;

TE = time effects on growth, composition or biomass (such as seasonal changes in climate, effluent, *etc*);

FE = effects due to flow rate (nutrient loading); and

CE = chance effects.

Assuming TE, FE, and CE to be independent, both AG and TE would effectively nullify each other between sides of the system.

Providing these assumptions were kept in mind, comparison could then be made between different divisions of flow rate (change in flow), and *differences* in growth 'G' (rather than growth *per se*).

The new flow configuration of the 85wB was rated to be able to cope with and treat up to 12 ML per day, and this upper level was considered to be the best in order to maximise any differences produced by manipulations in flow/nutrient loading (FNR).

Delays in excavation and refilling of the lagoons delayed establishment of the new flow regime (at a 50:50 division of flow between the two halves) until January 1994, effectively in the middle of the first experimental/high growth season. Hussainy (1979) reports that more diverse plankton assemblages were found over the summer period, while David Cartwright (unpublished data) has shown that this difference is mainly divided into a six month period of lower productivity centred on winter with a corresponding six month period of higher productivity centred on summer.

As a result, it was decided that the system would be given a year from the start of excavation work to equilibrate to the new 50:50 flow division, and would be monitored continuously (according to the following sampling regime) over that time. In late August 1994, the flow rate would be divided to a 25:75% (north:south) division for three months, and then reversed to a 75:25% flow rate division between the two halves. Flow divisions were attained by partially blocking the active outlets of the SP pond with boards cut to the required sizes. The difference in flow was then automatically passed on to all ensuing ponds in sequence.

It was expected that zooplankton distribution per pond under the equilibrating system would undergo significant changes in comparison to the original flow pattern, but that the two halves of the changed system (as compared via paired outlets between the halves) would be similar. Unequal division of flow was then expected to produce shifts in the relative distribution of particular zooplankton groups between the halves. The similarity of zooplankton communities between paired outlets was expected to fall as the spatial distribution of zooplankton within each half of the system became less

similar under different flow conditions/nutrient loading. The direction and degree of this change was in turn expected to be reversible with a reversal of the division of flow.

In effect, the 'control' for the experiment was the equilibrium period of 50:50 flow, where differences were predicted to be minimised between corresponding ponds on each side, while the experimental 'treatments' were the ensuing periods of unequal flow division, which were expected to enhance the differences between the sides. In turn, both 'control' and 'treatment' data could also be compared to the baseline information collected from the original flow pattern prior to the alteration of the system.

To provide evidence of the predicted changes, sampling was conducted every two to three weeks (with David Cartwright's help until the sampling protocol was established) prior to excavations. Following the refilling of lagoons, samples were collected on a fortnightly basis, with more concentrated sampling taking place before and following each change in the division of flow. Sampling and analysis techniques were designed to fit the specific conditions of the 85wB system, while remaining as comparable as possible to previous studies (e.g. Orcutt & Pace 1984, Morgan 1985, Dawidowicz 1990, Griggs 1993). Samples were generally taken between 9 and 11am (at the same time of day as far as possible), and consisted of plankton, chlorophyll and water samples, as follows.

### *Zooplankton samples*

Zooplankton were collected from immediately prior to the outfall (a natural collecting point) within each lagoon. Sampling did not necessarily assume homogeneity in zooplankton assemblages across ponds, but specifically targetted the outlet end of ponds as: (i) given the size of lagoons, plankton assemblages were likely to change gradually across single ponds in keeping with the patterns of the spatial succession (e.g. the assemblages at the start and end of pond 2N could be expected to be less similar than those at the end of 1N and the start of 2N); (ii) whether this was the case or not, sampling was therefore better placed at discrete spatial intervals (outlets) to represent a specific, fixed point for that pond and which could be directly compared between ponds; and (iii) the project was specifically interested the types of

harvestable zooplankton seeding the next ponds in sequence (or, as in the terms of industrial processes, the zooplankton that represented the 'end product' of each lagoon and would pass through a harvestable point at the outlet). For this reason, it should be noted that throughout this thesis references to any pond and the conditions or assemblages it contains *specifically relate to the outlet of that pond and the 'end product' at that point.*

Zooplankton samples were taken from four litres of immediately subsurface (approx. 20-30cm deep) water collected separately in one litre batches using a dip sampler/bucket in preference to a net<sup>5</sup>, and filtered through 37µm mesh. Four separate samples were taken per pond to allow for patchiness in zooplankton distribution in the region of the outlet. These samples were then pooled on independent statistical advice, as the project sought to address variation *between* ponds and *over time*. (Maintaining separate samples would simply have increased precision in quantifying *within* pond variation, whereas this project was concerned with addressing questions at a larger scale by providing a 'bird's eye' view of what was happening between these ponds over time).

Pooled zooplankton were preserved in 4-10% formalin, and returned to the laboratory, where they were refiltered through 54µm mesh. The material was made up to 240ml with filtered water, agitated, and then subsampled by 2.4ml Stempel pipette. Zooplankton in the subsample were then identified and counted in a graded petri-dish under dissecting microscope. The number of ovigerous specimens<sup>5</sup> was also recorded for both rotifers and copepods but not for cladocerans, which tended to split and lose their eggs on preservation in formalin.

The degree of subsampling depended on the amount of plankton in the sample. Usually a tenth of the sample (10 x 2.4ml Stempel) would be taken; for species of excessively high abundance (greatly exceeding 300 individuals per sub-sample), smaller subsamples would be taken. The size of these depended on the relative abundance of the dominant species, and aimed to provide a subsample containing approximately 300-400 individuals, or as near to this number as possible. The results for those species counted in smaller subsamples were then combined with the results for those from the larger one.

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<sup>5</sup> Due to the inherent problems with volumetric estimates from nets (Bottrell *et al.* 1976).

Prior to and periodically throughout the enumeration of zooplankton samples, sub-sampling accuracy was checked against the coefficient of variance for a series of subsampled aliquots (Bottrell *et al.* 1976, Elliot 1977, R. Shiel pers. comm.). Subsamples were accepted with variances of less than 10%.

Rotifers and cladocerans were identified to genera, other Crustacea to order or lower if possible. The level of identification was determined by pragmatic constraint, depending on sample and specimen numbers, the degree of morphological variation within and between species, current levels of taxonomic knowledge and characteristics required for identification (Shiel 1995). Voucher specimens of identified animals were sent to the Murray-Darling Freshwater Research Centre (MDFRC) for confirmation and identification. MDFRC staff confirmed that difficulties in the identification of cyclopoid species precluded the practical identification of genera in the numbers involved. Identifications were made of the material they received, and all samples were identified to the level stated above.

### *Phytoplankton (chlorophyll) samples*

Phytoplankton samples were collected from the same point (outlet) and immediate subsurface depth (20-30cm) in each lagoon as the zooplankton samples. Twenty five millilitres of unfiltered lagoon water was extracted from a homogenised bucket sample collected from each lagoon for chlorophyll-a analysis, as a crude measure of phytoplankton biomass. High correlations have previously been found between densities of edible phytoplankton and chlorophyll-a concentration, with the latter providing a reasonable index of food quantity and, to a lesser degree, quality (Vanni 1986). While 25 ml was a small sample size, this was driven by the often extremely high phytoplankton concentrations in the lagoons and the consequent filtration problems. This was also in keeping with the standard sampling method arrived at by the Melbourne Water Laboratories in overcoming the same problem.

Samples were immediately placed in the dark, and were subsequently filtered and frozen as soon as possible. Analyses were

initially conducted at the WTC laboratories, and then at the University of Tasmania once the project was established.

Analysis followed the methanol-extraction methods of Walsh (1996). The water samples were filtered through Whatman GF C glass fibre filters, which were in turn cut into small strips to facilitate pigment extraction and stored in 10ml of 100% methanol under dark conditions at 4°C for 24 hours. Fine scissors and forceps used to cut filter papers were carefully washed down with the set volume of methanol to avoid losing pigment material from the analysis. Filtrates were subsequently centrifuged at 3,000 rpm for 15 minutes and the absorbance of the supernatant measured at wavelengths of 665 and 750nm, representing the absorption maximum of chlorophyll and approximate absorption levels of other coloured compounds plus turbidity, respectively. The concentration of chlorophyll-a was then determined by the equation:

$$\text{Chl a } (\mu\text{g.l}^{-1}) = (13.333 \times (E_{665} - E_{750}) \times EV) / 0.025$$

where 13.333 is constant;

$E_{665}$  and  $E_{750}$  = absorbance at the respective wavelengths;

EV = the extraction volume (methanol); and

0.025 is the volume (in litres) of the original sample.

If algae were exceptionally thick, smaller volumes of agitated sample were filtered, and calculations adjusted accorded to volume.

Although incorporated, correction factors based on absorbance at 750nm were mostly miniscule compared to the overall algal concentrations encountered in the 85wB. WTC calculations of chlorophyll-a levels based on other methods (including pre and post-treatment measurement at 6 separate wavelengths and specific corrections for phaeophytin and other degradation products) similarly showed only minor deviation from values calculated by the above method. At the high chlorophyll concentrations found in sewage lagoons generally, such methods have previously been found to be no more accurate than those based on single readings at 665nm (Mitchell 1980).

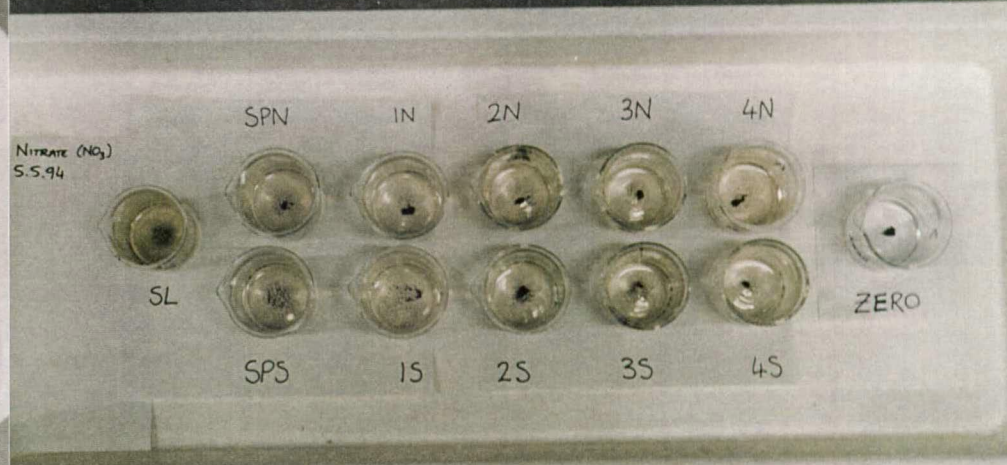
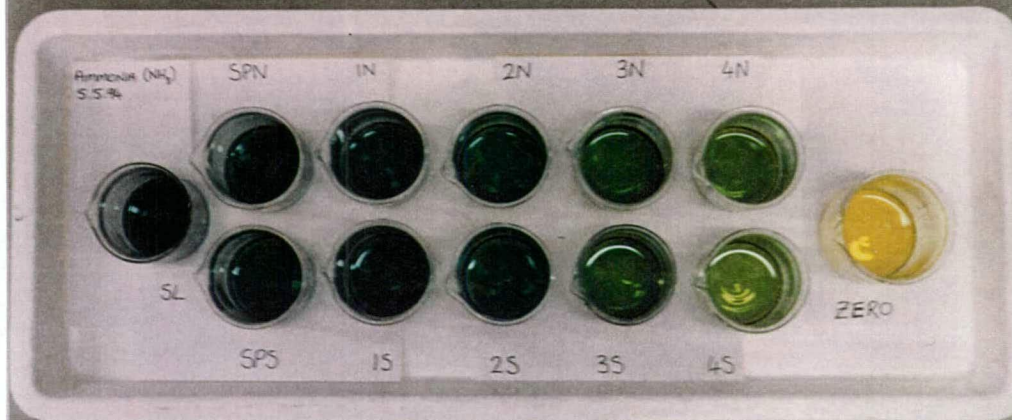


**Plate 3.4 (facing): Representative water samples from the 85wB system, showing the gradation in colour from earlier to later ponds.** *Top & bottom left:* field-filtered water samples from 21 April 1994 (some water already removed for analysis) and 5 May 1994, respectively. Both sets of samples are ordered from earlier to later ponds (SL, SPN, SPS, 1N, 1S, 2N, 2S, 3N, 3S, 4N, 4S), left to right. *Top & bottom right:* 200ml of water from each of the 5 May 1994 samples, pre- and post-settling of suspended materials, respectively. These are arranged in pairs from earlier to later ponds, left to right as labeled.

**Plate 3.5 (overleaf): Representative water samples after nutrient analysis.** Samples from 5 May 1994 arranged in pairs from earlier to later ponds, left to right as labeled. *Top and bottom left:* analysis for ammonia and reactive phosphate, respectively, using 25ml of sample diluted at 1:20. *Top and bottom right:* analysis for nitrate and nitrite, respectively, using 25ml of undiluted sample. *For all tests:* increased colour signified higher concentrations of the respective nutrient, and the much darker (green) ammonia samples from the earlier ponds required further dilution. [The zero samples for both ammonia and nitrate were straight reagent blanks using distilled water. For both phosphate and nitrite, zero samples were equal volumes of untreated water from the corresponding ponds (as per Plate 3.4, bottom right). For phosphate these were diluted in keeping with the treated sample, and so were more consistent with the 'typical' zero pictured. Phosphate reactions also required a reagent blank measurement per batch of reagent].







### *Water samples for nutrient analysis*

One litre of 37 $\mu$ m filtered water was collected from each lagoon outlet for nutrient analysis. Samples were collected in opaque acid-washed bottles (Plate 3.4), and were placed in the dark and frozen before being shipped back to Hobart for storage at -18°C or lower. Samples were analysed for ammonia, nitrate, nitrite and reactive phosphorus (orthophosphate) concentrations (Plate 3.5), as well as pH, salinity and conductivity.

Nutrient concentrations were determined using a Hach DR-2000 portable spectrophotometer, while pH, salinity and conductivity were measured using corresponding and standard probes. All nutrient analysis methods used were as per Hach (1992). Ammonia nitrogen was determined according to the Salicylate Method (method 8155, giving mg.l<sup>-1</sup> N as total NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup>), nitrate nitrogen according to the Cadmium Reduction Method (method 8171, giving mg.l<sup>-1</sup> N as NO<sub>3</sub>), nitrite nitrogen according to the Diazotization Method (method 8507, giving mg.l<sup>-1</sup> N as NO<sub>2</sub>), and reactive phosphate according to the PhosVer 3/Ascorbic Acid Method (method 8048, giving mg.l<sup>-1</sup> PO<sub>4</sub>). All analyses used the reagent powder pillows for the corresponding methods. Samples (mostly those for ammonia and phosphate analysis) were diluted as required, and calculated concentrations were adjusted according to dilution factor.

Independent testing at WTC has previously shown the results of Hach nitrate analysis to be variable and, as noted by Hach (1992), highly dependent on subjective elements of analysis technique. While care was taken to limit these variations, nitrate levels within this thesis are therefore reported with caution. WTC tests have confirmed the accuracy and precision of the other tests used here, however, with both the Diazotization Method and the PhosVer 3/Ascorbic Acid Method also USEPA approved.

Biological treatment rapidly hydrolyses complex polyphosphates into soluble orthophosphates (Mitchell 1980), and so analysis for these provides a good measure of phosphorus levels within a lagoon. In contrast, the lability of nitrogen compounds means that pollution levels are better indicated by the total nitrogen pool rather than any single nitrogenous form (Mitchell 1980). Unfortunately, determination of total Kjeldahl nitrogen (TKN), alongside total organic

carbon (TOC), was prohibitively expensive and fell beyond the resources of this project, particularly given the sampling protocol required. High influent concentrations of ammonia were therefore considered to give the best rule-of-thumb indication of the prevailing nitrogen loading per pond. Similarly, the concentrations and levels of all of the above-measured parameters were intended to indicate the patterns in physico-chemical conditions for each lagoon, if not necessarily providing a comprehensive environmental mosaic to which plankton dynamics could be directly linked.

#### *Flow rate and other data recorded*

The flow rate into the system was measured via a flow-wheel installed at the start of the 85wB system (Plate 3.1), and notes taken on sampling time, wind direction, and general weather conditions. In contrast to plankton and water sample collection, these data were collected weekly.

Supplementary work, including further monitoring and preliminary harvesting experiments, were conducted in April 1995 and March 1996 (Appendix 3), although the results of this work are not presented in the body of this thesis.

### **3.5 Analysis**

Data analysis was conducted using version 4.0 of the PRIMER multivariate statistical package from the Plymouth Marine Laboratory, specifically the CLUSTER, SIMPER, and MDS sub-routines (Carr 1996). In keeping with the general principles of the equation on zooplankton growth/abundance listed on page 3.12, it was hoped to identify specific and consistent changes in the pattern of zooplankton distribution that could be directly attributed to manipulations of flow rate/nutrient loading and their effects on the prevailing conditions within individual ponds. It was hoped that this would help provide at least a preliminary indication of how these may be used to reliably manipulate zooplankton populations at the Complex.

## 4. RESULTS

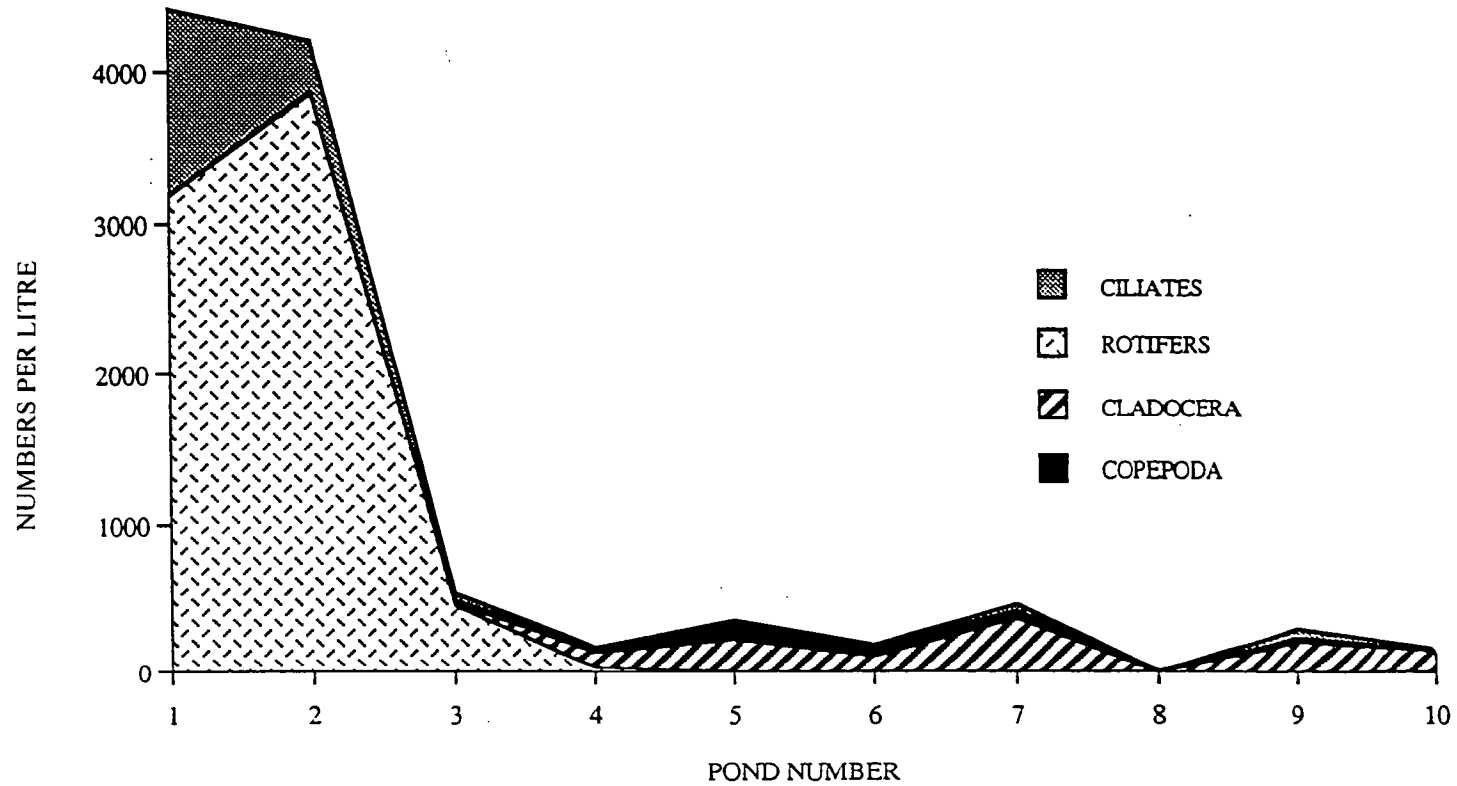
### 4.1 Fitting the pattern

The initial pattern of zooplankton distribution in the 85wB system prior to the modification of flow was consistent with the typical spatial pattern identified in Section 3.3 (Figure 4.1). Ciliate numbers were highest at the start of the system, rotifers peaked shortly afterwards, and the high numbers of both rapidly diminished to give way to increasing crustacean numbers. The crustaceans dominated for the remainder of the system (at much lower overall numbers), with copepods peaking and declining earlier than the cladocerans.

Identifications of taxa present throughout the experimental period were confirmed with voucher specimens sent to Ms Jackie Griggs and Dr Russ Shiel at the Murray Darling Freshwater Research Centre (MDFRC) in Albury, NSW, who were also able to provide identifications to lower taxonomic levels for specific specimens.

As can be seen in the raw data presented in Appendix 2, the rotifer assemblage was dominated by extremely high numbers of *Brachionus*, *Polyarthra* and *Filinia* spp, with *Asplanchna* also quite common. Amongst these, MDFRC staff identified *Brachionus calyciflorus* (Plate 4.1 a-c), *Polyarthra dolichoptera* (Plate 4.2a), *Filinia longiseta* (Plate 4.2b), and *Asplanchna* cf. *sieboldi* (not quite like the nominate species: Plate 4.2c). While these species-level identifications are by no way a complete listing of the species that are likely to be present in the lagoons, they do provide an indication of those present amongst the predominant taxa [as outlined in Section 3.4, analysis was run at higher taxonomic levels].

Copepods were also heavily represented in the lagoons, primarily by cyclopoids. The genera *Australocyclops* (Plate 4.3 a-c), *Paracyclops*, and *Thermocyclops* were all identified from amongst the voucher specimens, and it is likely that the lagoons contained many more. Various forms of cyclopoid juveniles (Plate 4.4a) and nauplii (Plate 4.4b-d) were present in very high numbers in the relevant



**Figure 4.1: 85wB lagoon series, 28 July 1993, showing the typical pattern of zooplankton distribution prior to alteration of the system.**

lagoons, and again would appear to represent a variety of species. Calanoid copepods and nauplii (Plate 4.5a-c) were also present in some lagoons at particular times, although these were less common, while harpacticoid copepods (Plate 4.5d) were extremely rare.

The other dominant group amongst the zooplankton was the Cladocera, primarily represented by species of both *Daphnia* (*D. carinata*: Plate 4.6a) and *Moina* (*M. micrura*: Plate 4.6 b-c). Less common and more sporadic crustacean taxa included the chydorids *Alona* sp. (Plate 4.7a), *Leydigia* sp. (Plate 4.7b), and *Pleuroxus inermis* (Plate 4.7c), various unidentified ostracods (Plate 4.7d), and miscellaneous material such as eggs and resting eggs (e.g. Plate 4.6d, most likely from a cladoceran).

The presence and prevalence of these taxa is consistent with previous studies and observations at WTC. Hussainy (1979) noted that the zooplankton at the Complex were dominated by "*Filinia longesita*, *Brachionus* spp., *Daphnia carinata*, and [the cyclopoid] *Mesocyclops leuckartii*." David Cartwright (unpubl. data) has recorded large numbers of *Brachionus*, *Polyarthra*, *Filinia*, *Aplanchna*, *Daphnia*, *Moina*, and cyclopoids, as well the less common forms listed above. Again, he has recorded *Mesocyclops* from among the cyclopoids, and while Hussainy (1979) states that no calanoids are found at WTC, Cartwright has recorded calanoids of the genus *Boeckella* from several specific lagoons, albeit less common than the ubiquitous cyclopoids.

## 4.2 Experimental timing and raw data

For all parameters, the data are presented on a timescale with 26 May 1993 designated Day 1. This date represents the first day on which zooplankton samples were collected in order to determine the initial (unaltered) pattern of zooplankton through the original 85wB lagoons prior to the alteration of flow. Subsequent pre-alteration samples were taken on 9 June, 23 June, 14 July, and 28 July of the same year. Following this, there was a hiatus in sampling corresponding to the emptying and excavation of lagoon channels and the time taken for



the lagoons to refill and stabilise. While samples were still taken at times during this period, regular zooplankton sampling in line with the experimental protocol (Section 3.4) resumed in April 1994. Unrelated drain construction work by Melbourne Water meant that pond 5S was cut out of the system and unavailable for sampling from 2 June to 28 July 1994 inclusive.

Non-zooplankton parameters are plotted against the same "Day 1" time scale to avoid confusion, although these parameters were not measured from that day (not all analyses were available and some ponds were not even in existence at this point). In most cases, measurement of non-zooplankton parameters began either at the start of 1994 or with the onset of regular plankton sampling in April of that year.

Starting from 26 May 1993 means that seasonal changes over the main experimental period are as follows:

Days 1-6 = autumn 1993 (end of)

Days 7-98 = winter 1993

Days 99-189 = spring 1993

Days 190-279 = summer 1993/4

Days 280-371 = autumn 1994

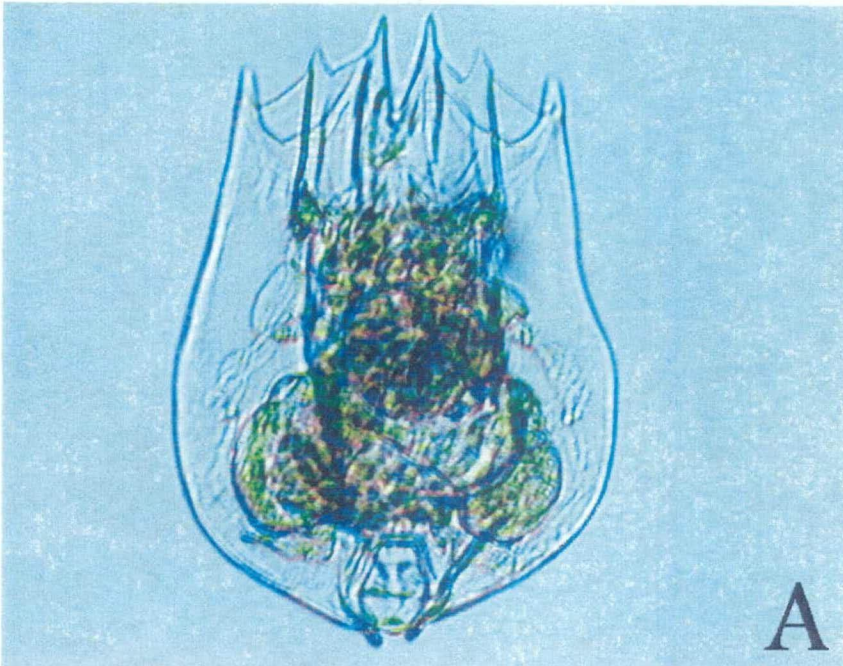
Days 372-463 = winter 1994

Days 464-554 = spring 1994

Days 555-644 = summer 1994/5

Supplementary experimental work, not reported in the body of this thesis, was conducted over Days 678-687 and 1023-1037, representing mid autumn (April) 1995 and early autumn (March) 1996, respectively.

Raw data for chlorophyll and physical and chemical parameters are given in Appendix 1. Raw zooplankton data are given in Appendix 2; tables of supplementary data not presented in the main text are given in Appendix 3.

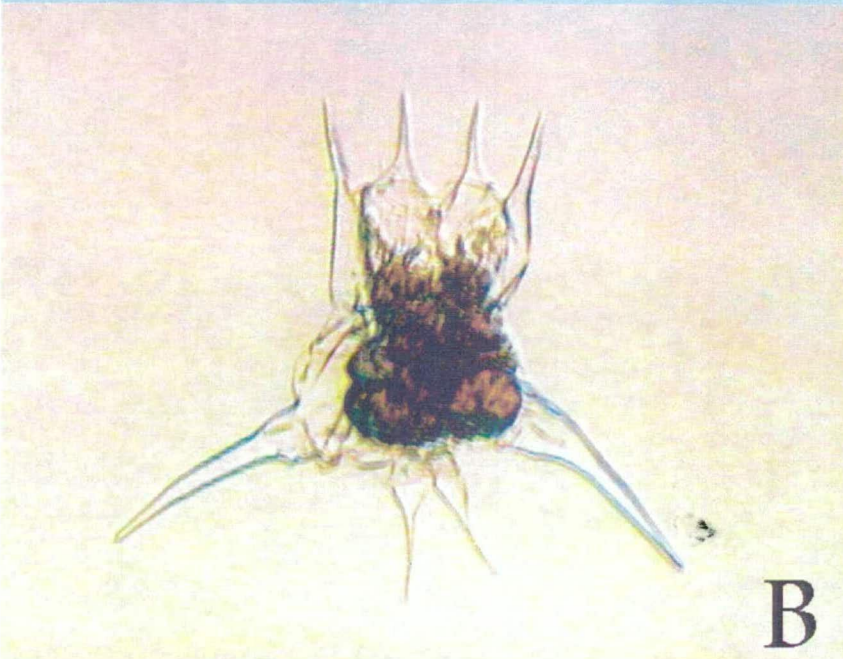


A

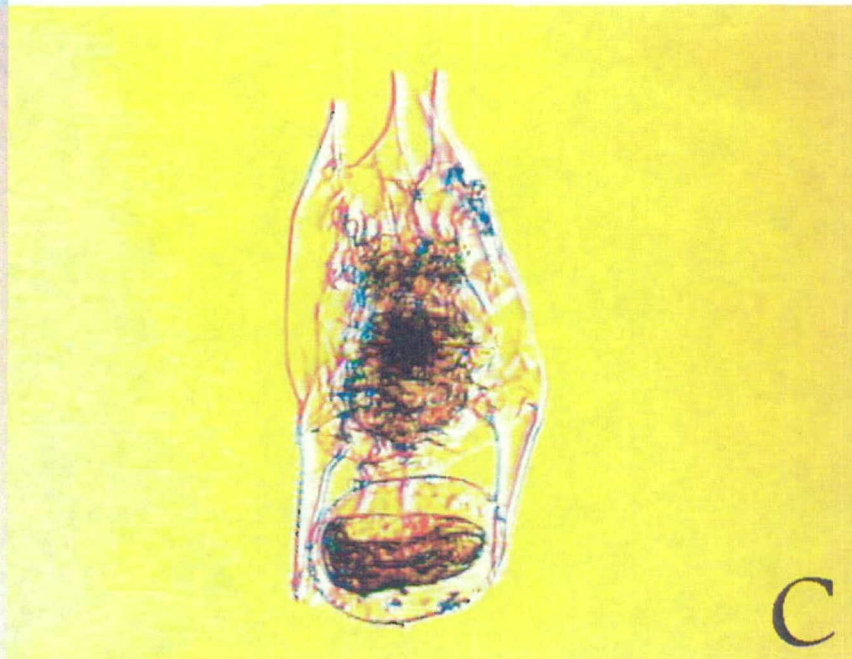
**Plate 4.1**

*Brachionus calyciflorus* (rotifer),  
showing the variation in spination.

- (a) Short spines, 180 $\mu$ m from top mid-spine to base.
- (b) Long spines, 370 $\mu$ m from top mid-spine to bottom mid (not outer) spine.
- (c) Long spines, with egg. 408 $\mu$ m from top mid-spine to bottom of egg.

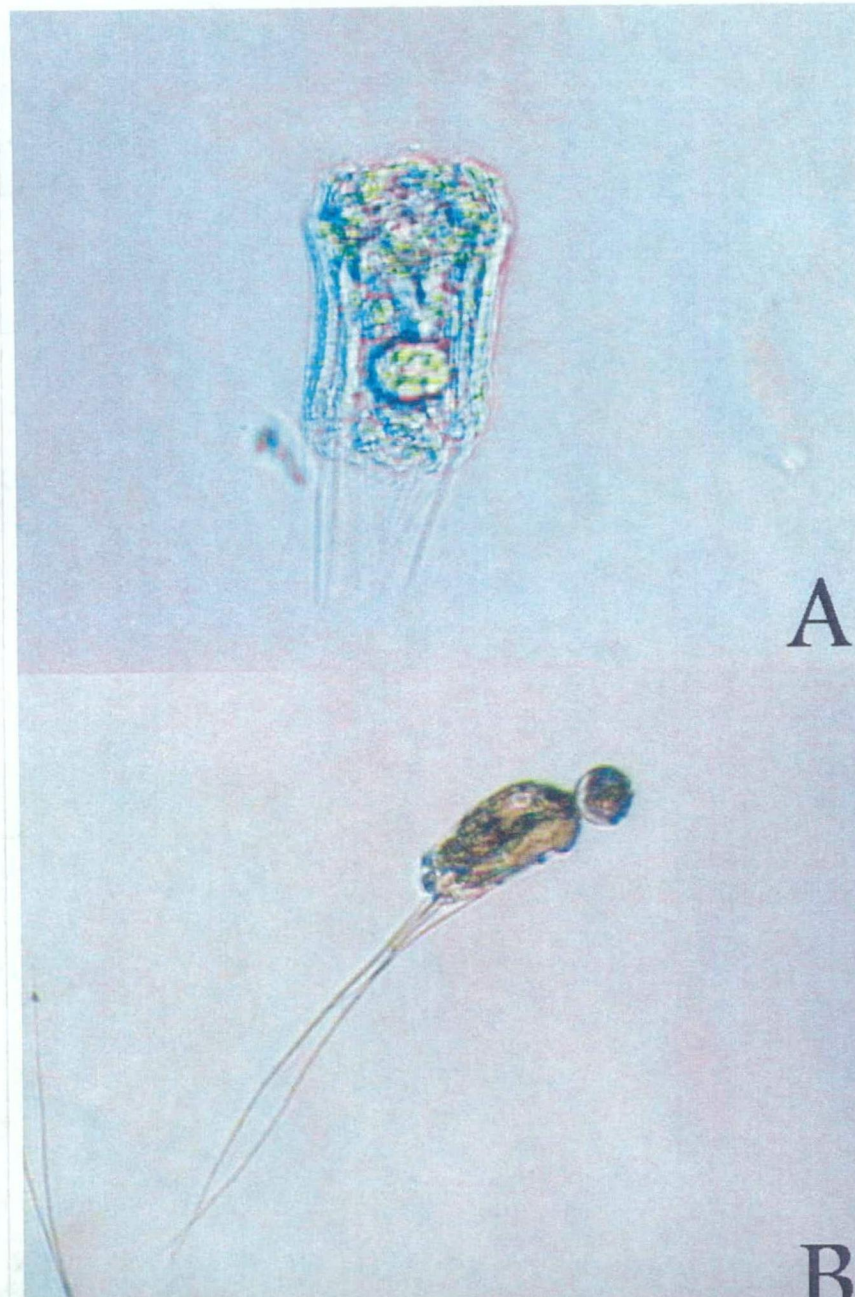


B



C

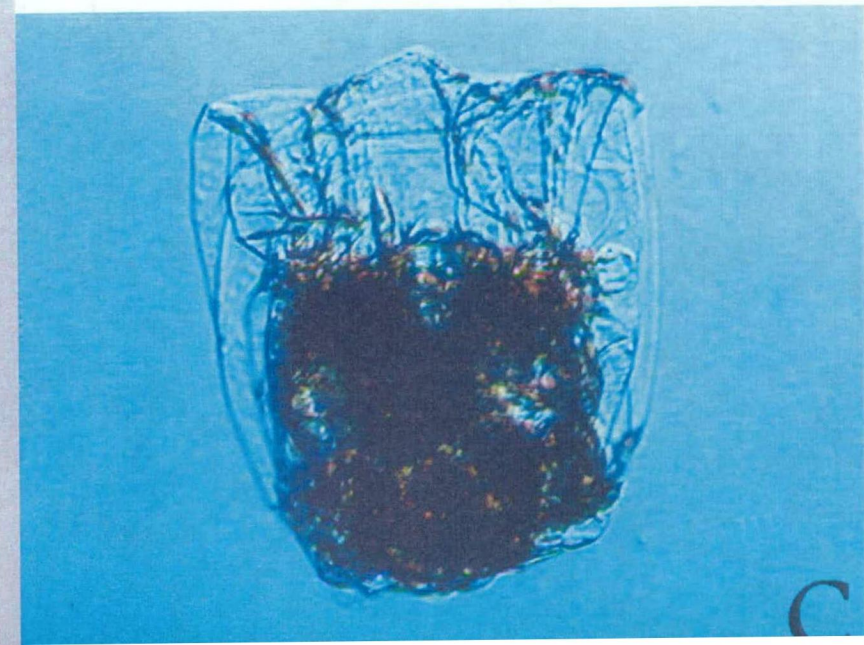




#### Plate 4.2

The other main rotifer genera, 85wB system:

- (a) *Polyarthra dolichoptera*, 370 $\mu$ m from top to bottom (excluding spines).
- (b) *Filinia longiseta*, body length 133 $\mu$ m (excluding egg and spines).
- (c) *Asplanchna* cf. *sieboldi*, body length 397 $\mu$ m.



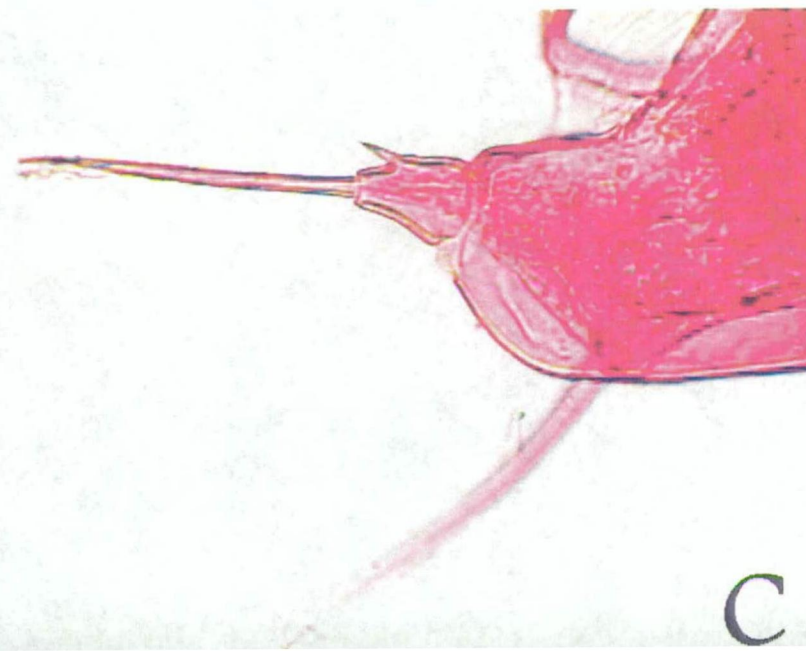




A



B



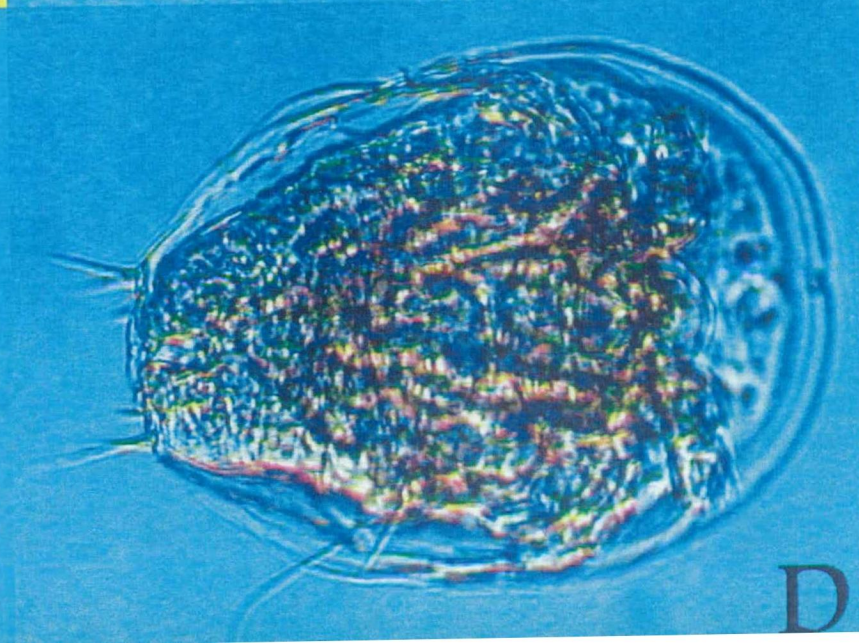
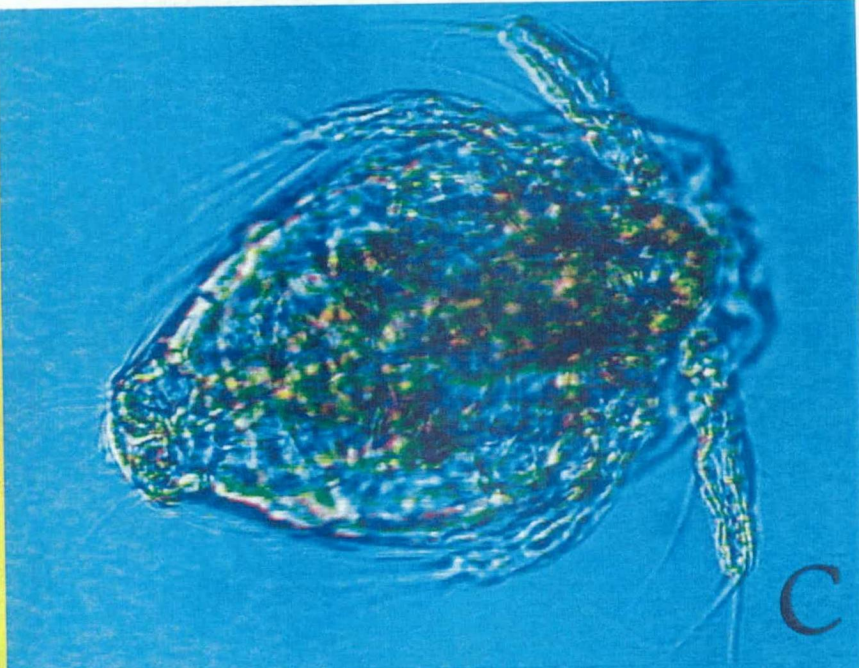
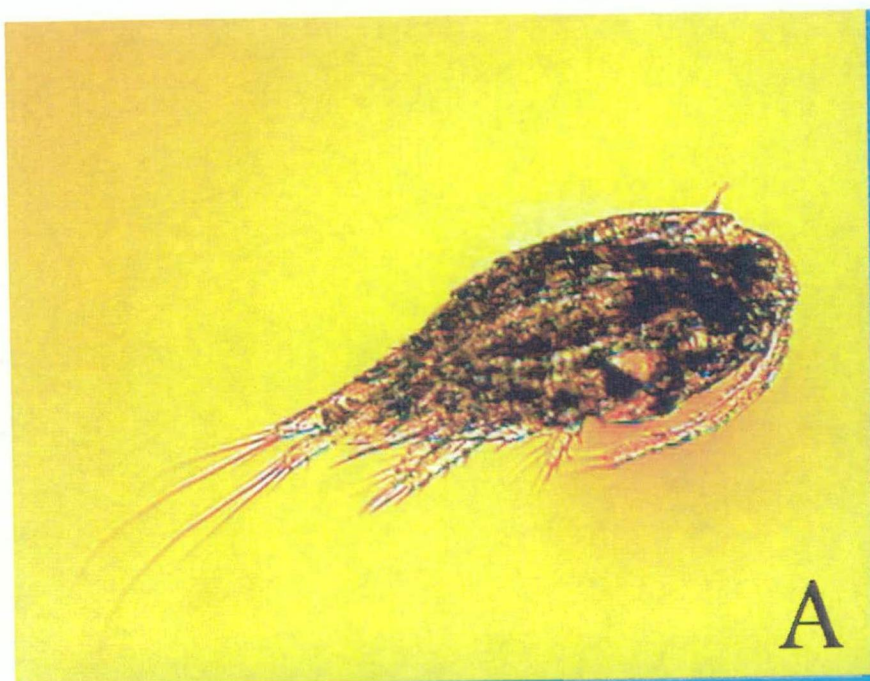
C

### Plate 4.3

Cyclopoid copepods, 85wB:

- (a) *Australocyclops* sp., ovigerous female.
- (b) *Australocyclops* sp., male.
- (c) Taxonomic feature: P5s (5<sup>th</sup> pair of swimming legs) of *Australocyclops* sp.



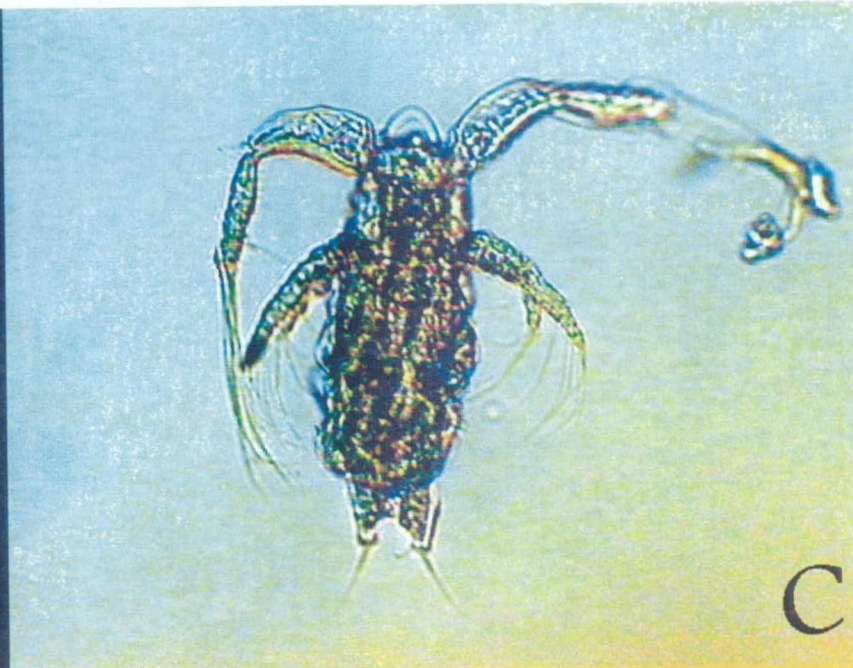
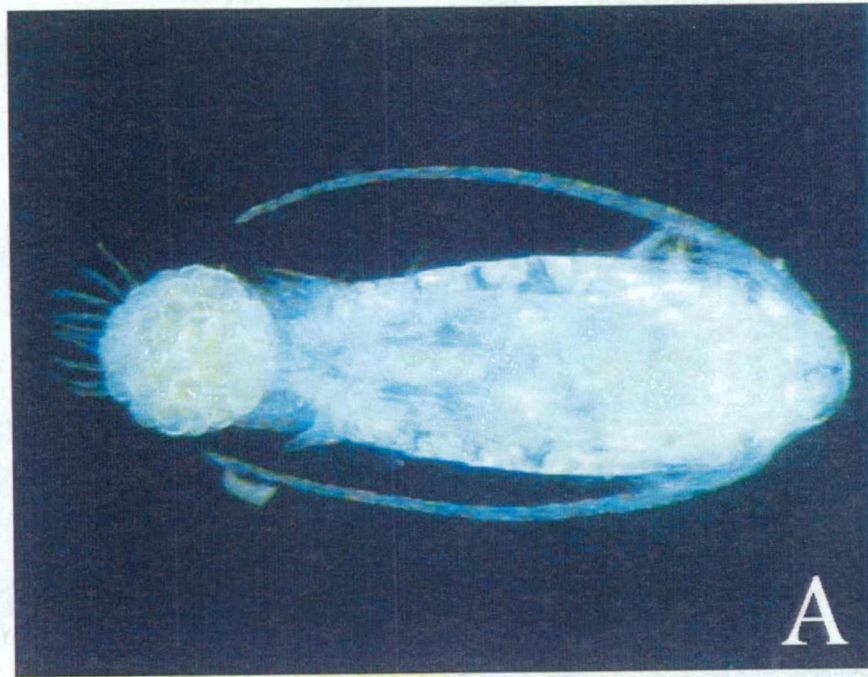


**Plate 4.4**

Immature cyclopoid  
copepods, 85wB:

- (a) Juvenile cyclopoid,  
body length 621  $\mu\text{m}$ .
- (b) Cyclopoid nauplius,  
body length 201  $\mu\text{m}$ .
- (c) Cyclopoid nauplius,  
different to (b),  
body length 207  $\mu\text{m}$ .
- (d) Cyclopoid nauplius,  
different to (b)+(c),  
body length 212  $\mu\text{m}$ .



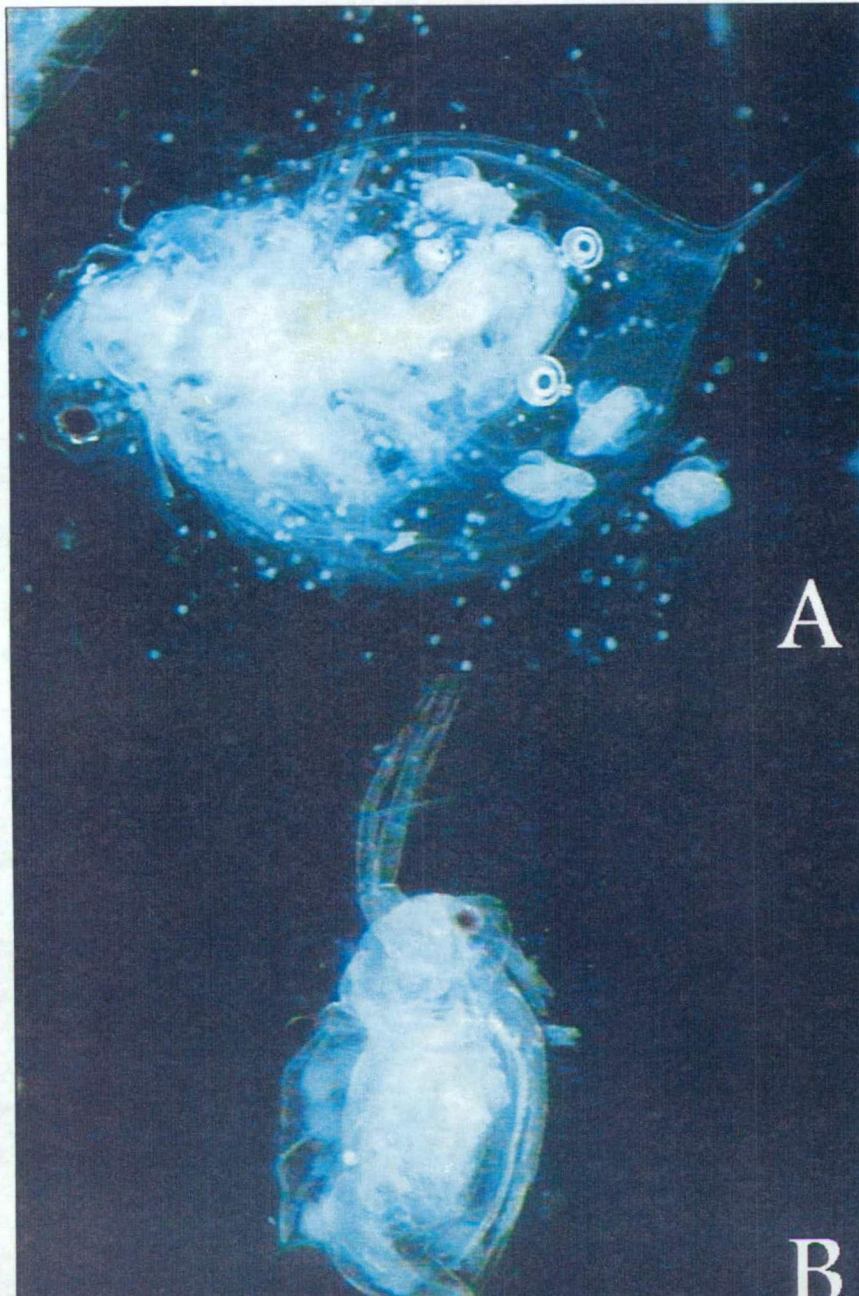


#### Plate 4.5

Non-cyclopoid copepods,  
85wB:

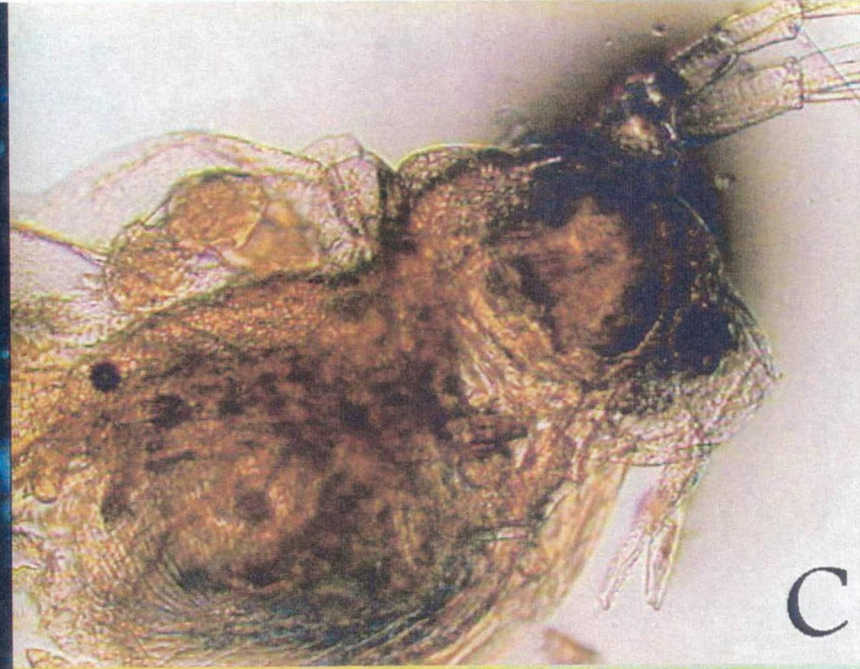
- (a) Female calanoid with eggs, 2185 $\mu$ m long (excluding setae on tail).
- (b) Juvenile calanoid, 555 $\mu$ m long (excl. setae on tail).
- (c) Calanoid nauplius, 190 $\mu$ m long.
- (d) Harpacticoid (rare), 300 $\mu$ m long.



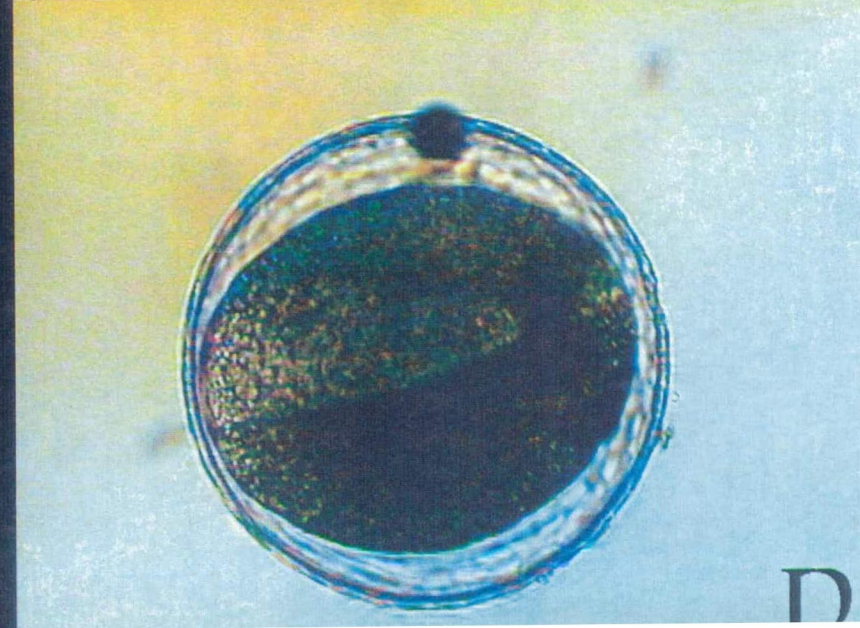


A

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C



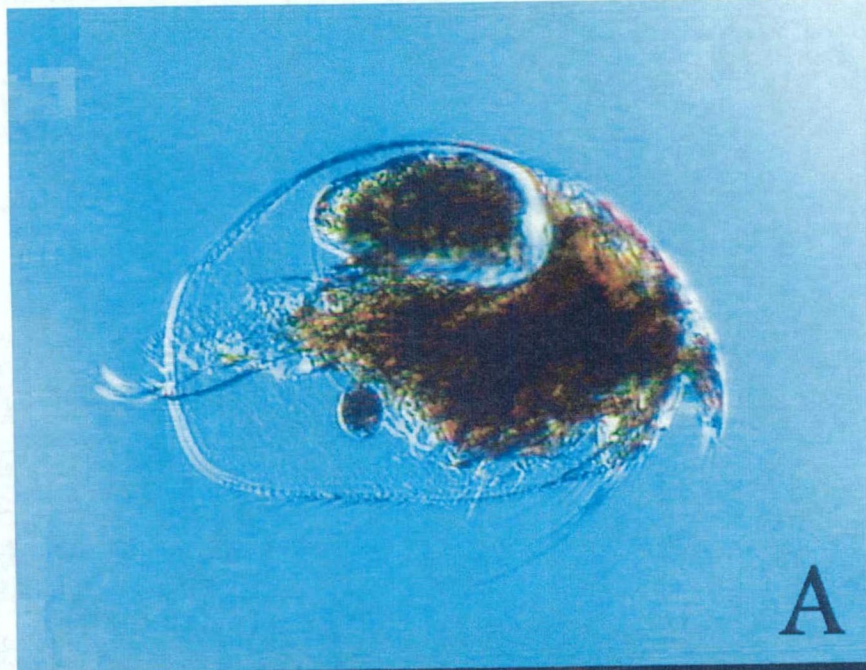
D

#### Plate 4.6

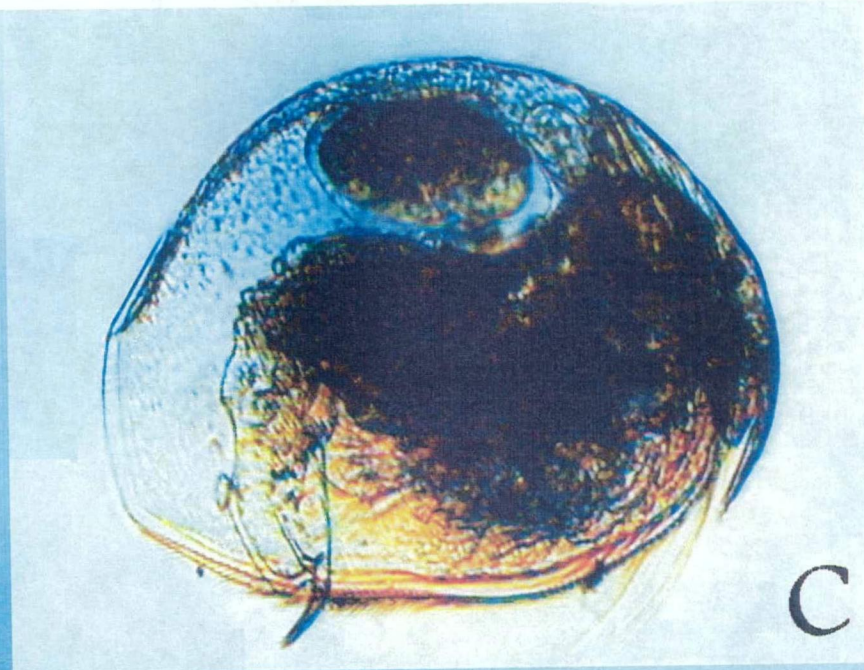
Cladocerans, 85wB:

- (a) *Daphnia carinata*,  
with developing  
young visible within  
the carapace,  
3604 $\mu$ m (3.6mm)  
long.
- (b) *Moina micrura*,  
1076 $\mu$ m long.
- (c) *Moina micrura*,  
same animal as (b),  
showing close-up of  
head (identification  
point).
- (d) Resting egg,  
probably cladoceran.

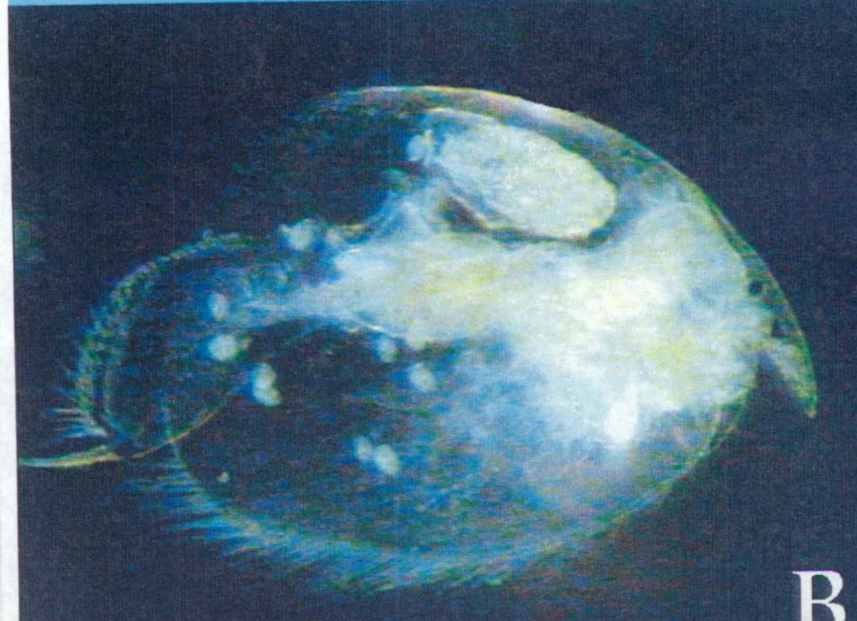




A



C



B



D

**Plate 4.7**

Chydorids (a-c) and ostracods (d), 85wB:

- (a) *Alona* sp., 413 $\mu$ m long.
- (b) *Leydigia* sp., 1075 $\mu$ m long.
- (c) *Pleuroxus inermis*, 516 $\mu$ m long.
- (d) Ostracod, 593 $\mu$ m long.

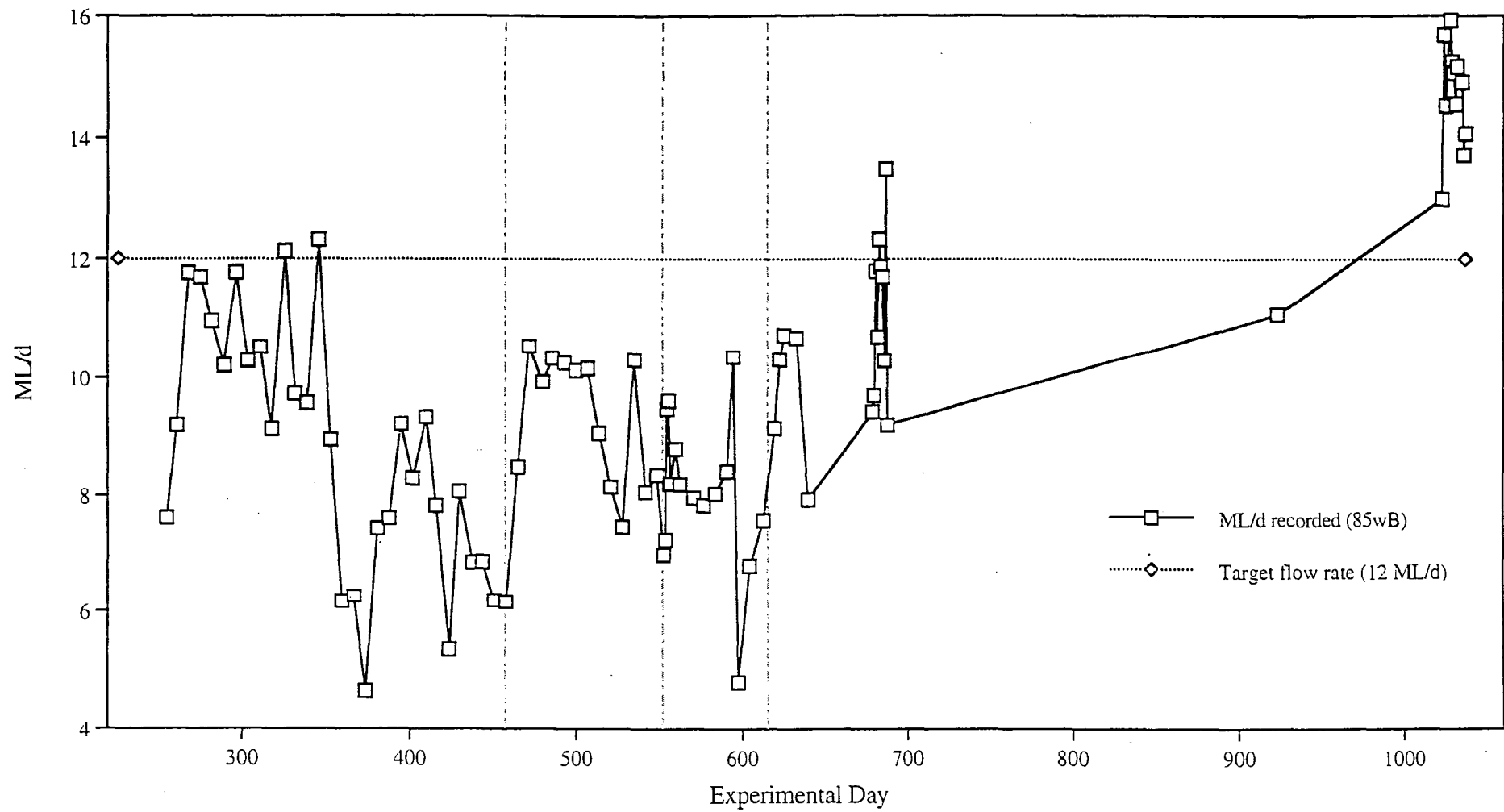


### 4.3 Flow rates

Flow rates for the volume of influent entering the start of the 85wB system were measured from the start of 1994, and are given in ML/d in Figure 4.2. Although providing the system with 12 ML/d had initially posed no difficulties to Melbourne Water, drought conditions struck Victoria in 1994 and had a severe impact on the amount of influent into WTC as a whole. This effect was compounded by the introduction of a 'user-pays' water system for the state, with the result that domestic wastewater levels dropped dramatically.

Given their need to provide adequate flow to maintain the major lagoon systems, Melbourne Water could no longer provide sufficient flow to maintain a 12 ML/d level to the 85wB, and could no longer guarantee that the level of flow received by this system would be consistent. This remained the case for the duration of the main experimental period (although it improved for the second and final supplemental experimental period in 1996). As a result, it was recognised that differences due to divisions of flow/nutrient loading between paired lagoons may not be maximised on any given date, and that the degree of difference may be variable between dates.

Figure 4.2 shows the variation in flow rate for the 85wB system over the course of experimental work, with the ideal or target level of 12 ML/d marked by the horizontal line. Flow rate rarely reached this level until the second period of supplemental work in March 1996 (Days 1023-1037). At the end of the 1993/4 summer (Days 255-279), flow averaged 10.1 ML/d with a standard deviation (SD) of 2.0. This continued at an average 9.8 ML/d (SD 1.9) for Autumn (Days 280-371), before falling to 7.2 ML/d (SD 1.4) for winter (Days 372-463). Averages for spring (Days 464-554) and summer 1994/5 (Days 555-644) rose to 9.1 ML/d (SD 1.2) and 8.5 ML/d (SD 1.5), respectively. For supplemental experimental work in autumn 1995 (Days 678-687), the flow rate rose to 11.0 ML/d (SD 1.4), while a single reading in December 1995 (Day 923) indicated that the average flow rate over the intervening period continued at 11.1 ML/d (no SD can be calculated). Flow rate rose substantially by the time the second supplemental experimental period



**Figure 4.2:** Flow rate per day over the course of experimental work. Vertical lines represent changes in the division of flow (as per text).

began (Days 1023-1037, Autumn 1996), reaching an average of 14.7 ML/d with an SD of 0.9 ML/d. The highest flow rate was recorded in this period (15.9 ML/d, Day 1028), while the lowest recorded flows were in the main experimental period, at 4.6 and 4.8 ML/d (Days 373 and 597, respectively).

Reduced flow rates and exceptionally hot and sunny summer days in December and January 1994/5 combined to further confound the experimental protocol. Under these conditions, evaporation across the large surface areas of the lagoons was high, and on the low-flow (south) side the accumulated water loss exceeded the inflow levels for ponds 3S, 4S and 5S. Outlet levels began to drop: in early January flow stopped completely between the 4S and 5S lagoons, and in mid-January it stopped between the 3S and 4S lagoons. Water levels continued to drop below these outfalls (although flowing the week before, the water level was nearly 4cm below the 4S outfall on 9 January, and on 19 January levels were 4cm and 15cm below the 3S and 4S outfalls, respectively). Flow even reversed from the outfall pond (original pond 12, or 6S) into the 5S pond. During this period, flow remained high through the northern side of the system.

Although the original experimental plan had been to divide the flow at percentages of 25N:75S and 75N:25S for three months each in order to monitor changes and reversals between halves over an equal period of time, it was decided that the loss of flow from the southern side was excessive, potentially harmful to the treatment capacity of the lagoon system, and would increasingly invalidate comparisons between the sides of the system on the basis of flow. It was therefore decided to reverse the flow after two months instead of three, and establish a second 25N:75S division for the remaining month of the experimental period. While this would not allow the southern side to complete the same theoretical three month transition to low flow as the northern side (a transition that was in jeopardy from the loss of flow in any case), it did allow the southern side to refill and permitted observations to be made of the effects of more frequent reversals in flow division on the entire system. In Figure 4.2 and subsequent graphs, the three broken vertical lines represent these changes in flow

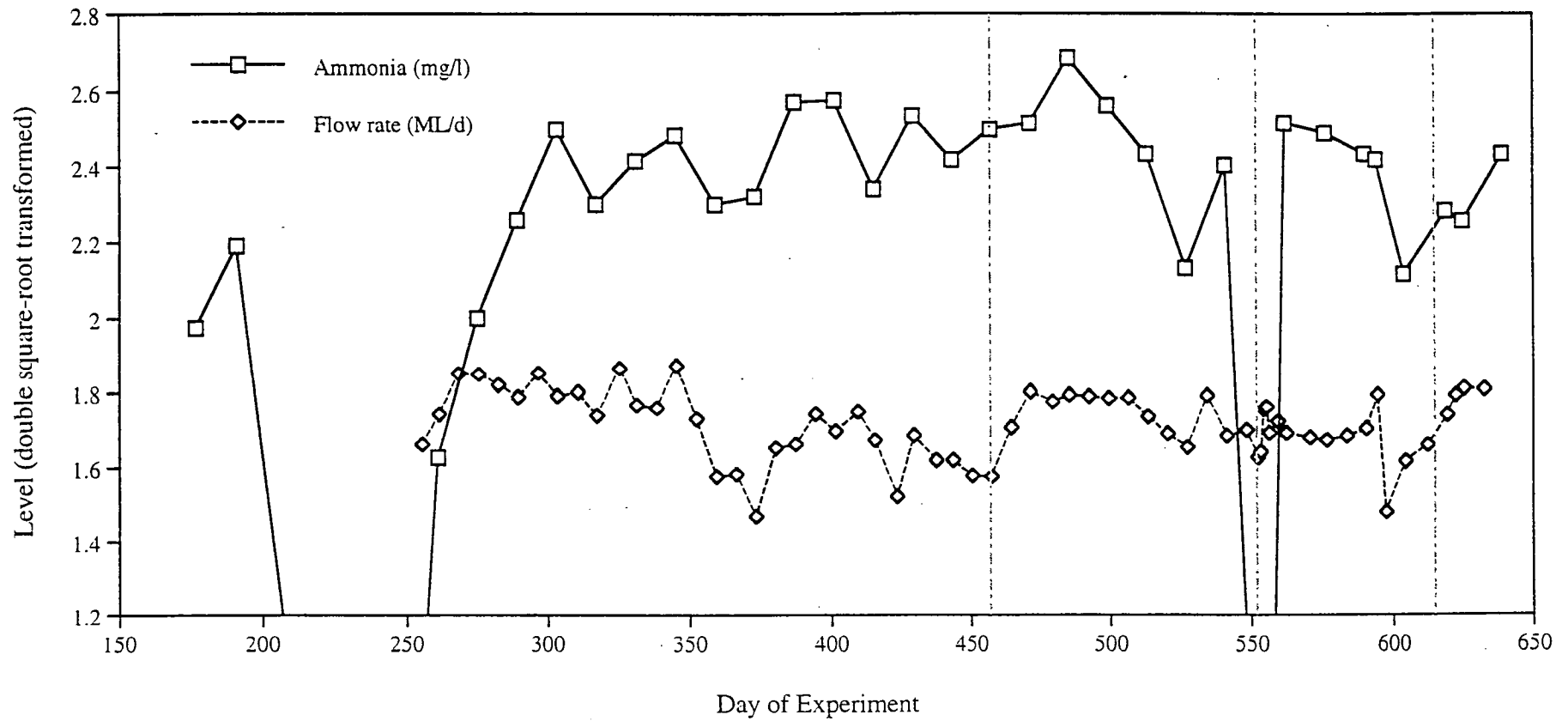


Figure 4.3: Comparison of ammonia levels and flow rate (double square-root transformed) in the SL pond (85wB) over the main experimental period.

division between the two sides of the system (50N-50S to 25N-75S, 25N-75S to 75N-25S, and 75N-25S to 25N-75S, respectively).

Finally, at various times the divided system was found to have sprung leaks or the boards covering closed outlets were found to have worked themselves free (some of these are noted in Appendix 2). Leaks and unintentional diversions of flow also occurred further up the drains and channels through which WTC supplied waste-water to the 85wB system. While all such problems were rectified as soon as possible, the duration and effect these events on flow and the 85wB could not always be determined.

#### **4.4 Chemical and physical data**

##### **4.4.1 Ammonia**

Ammonia concentration was considered to be the chemical factor of potentially greatest importance, given the high levels of this compound that occur in the lagoons, the levels of nitrogen they represent, and its potential toxicity at high pH. Ammonia has also been identified as the chemical factor providing the best correlation with zooplankton populations at WTC (David Cartwright, unpublished data).

Figure 4.3 shows the flow rate entering the system and the level of ammonia at the outlet of the SL pond over the course of the experimental period. As the first lagoon in series, the SL pond represented the lowest level of treatment within the system, and so the closest match to influent ammonia levels. This match was not exact, however, as the influent fully mixed with the lagoon water prior to reaching the outlet (it is this mixing that allowed fluctuations in flow rate to be detected chemically as it was represented by a mixture of lagoon and influent concentrations, rather than simply as material of the same concentration flowing at a faster rate).

Figure 4.3 shows that there was a good match in general

between flow rate and ammonia levels at the SL stage in the lagoon system, with peaks and troughs occurring in approximately the same pattern if not to the same magnitude. The flow rate graph is more finely detailed, as the rate was recorded weekly rather than fortnightly. It should also be noted that corresponding features on the two graph lines coincide closer in time than would actually have been the case, as calculations of flow rate were based on the period between two wheel readings (i.e. the values represent the rate *preceding* the time they were recorded) whereas nutrient values represent direct determination of the concentrations present *at* the time of collection.

It is possible that there may also have been seasonal variation in the ammonia concentration of inflowing waste-water, but any such trends (or seasonal averages) would have been masked and confused by the high degree of variation in flow rate between and within seasons (Figure 4.2), and the subsequent rate of mixing of this material within the body of the lagoons. Influent ammonia and other nutrient levels at WTC have previously been found to remain relatively consistent over a daily, weekly, monthly and long-term basis (Constable 1988).

The actual levels of ammonia present in the SL and subsequent lagoons of the 85wB system are given in Figure 4.4 (a-f). Again, the earlier ponds (SPN+S and 1N+S) tended to display the same general pattern as in the SL lagoon, with this pattern becoming more exaggerated and corrupted further into the system (2N+S to 4N+S). General levels of ammonia declined as the water moved through the system, and a distinct seasonal peak (ca. days 325 to 525) appeared to emerge above the general fluctuations for ponds 2N+S to 4N+S. This peak was centred on winter 1994 and ranged from mid autumn to mid/late spring, in keeping with the winter/summer extremes at WTC identified by David Cartwright (unpublished data). Both the fall and change in pattern of general ammonia levels most likely represented the effects of biological action and the treatment process as the waste-water moved through the system (Plates 3.4 & 3.5).

As before, the divisions of flow are marked by the vertical lines in Figure 4.4 (a-f). The graphs themselves show a marked response in ammonia levels, directly corresponding to the prevailing flow conditions. In general, ammonia values were noticeably different between pond pairs immediately following construction of the new

outlets and the beginning of refilling and equilibration (Day 191). During the equilibration period (50:50 flow) the ammonia levels drew steadily closer between pond pairs, and, with occasional variations, began to mirror each other quite closely. Following the first flow change on Day 457, there was a noticeable increase in the levels of the southern (high flow) lagoons relative to the northern ones in each pond pair. This was dramatically reversed following the second flow change (Day 552), with levels in the southern side falling and those in the northern side rising, and the north now representing the higher flow and higher concentration of ammonia. This was again reversed with the reversal of flow on Day 619. Apart from the increases and decreases in ammonia concentration immediately following the changes in flow, and apart from the consequent differences in levels between corresponding pairs, the patterns of change in ammonia concentration otherwise remained similar between each pond pair.

The changes described above were particularly noticeable over pond pairs 1-4, although the beginnings of these trends could be seen between the SPN and SPS pair, despite these outlets belonging to opposite sides of the same pond. This may represent the two sides of the SP pond starting to operate, at least partially, as two separate bodies of water. The differences in ammonia concentration were not as pronounced between these two outlets as between the true pond pairs, although one outlet (albeit the low-flow SPN!) remained consistently higher than the other after the first and third flow changes, with a very erratic pattern of difference between the two following the second flow change.

The changes in ammonia concentration between pond pairs appeared to occur with very little lag time for even the later ponds. Although the direct effects of a higher nutrient loading at one end of the 85wB would take some time to reach later ponds due to the retention time of the system, the influence of increased or decreased flow could be expected to manifest itself quickly through all the pond pairs. Increased inflow would rapidly lead to increased overflow (spillage) at the outlet at other end of a pond, and so while newly introduced waters and nutrients would not penetrate the later ponds as quickly, these lagoons would still be receiving an increased volume of these materials for mixing from the preceding ponds.

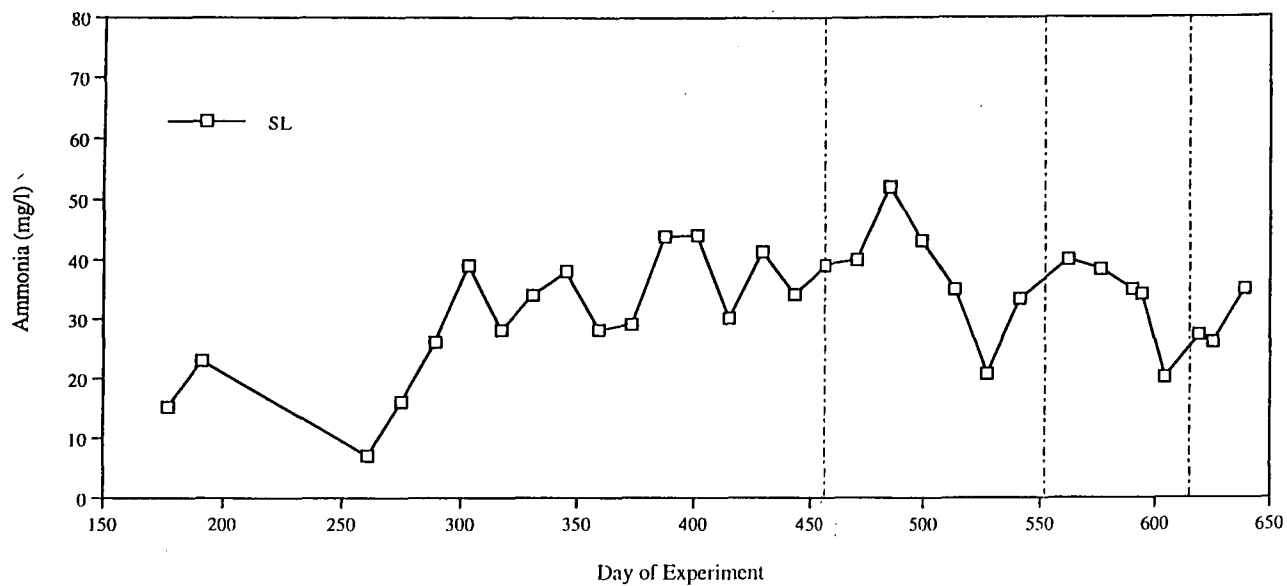


Figure 4.4(a): Ammonia levels in the SL pond (85wB) over the main experimental period.

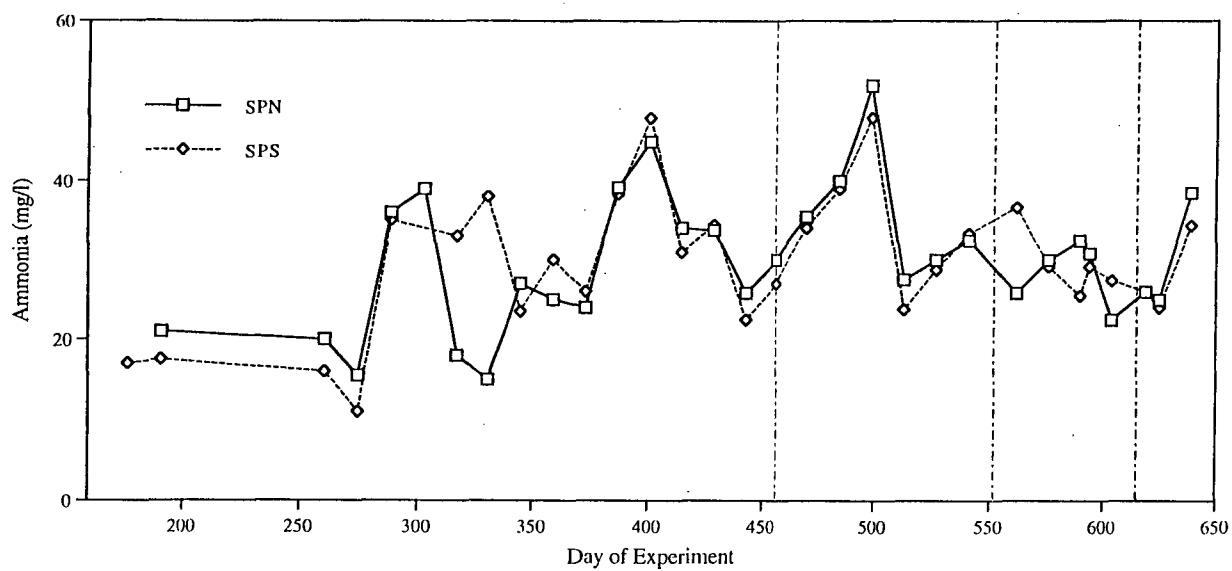


Figure 4.4(b): Ammonia levels in the SPN+ SPS ponds (85wB) over the main experimental period.

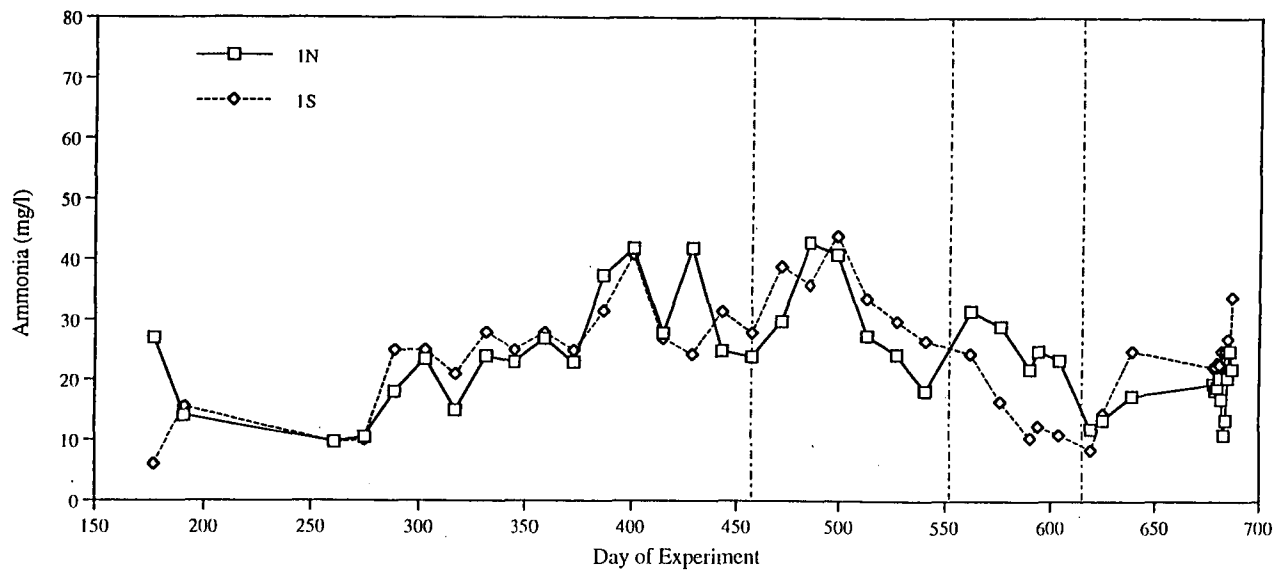


Figure 4.4(c): Ammonia levels in the 1N+ 1S ponds (85wB) over the main experimental period.



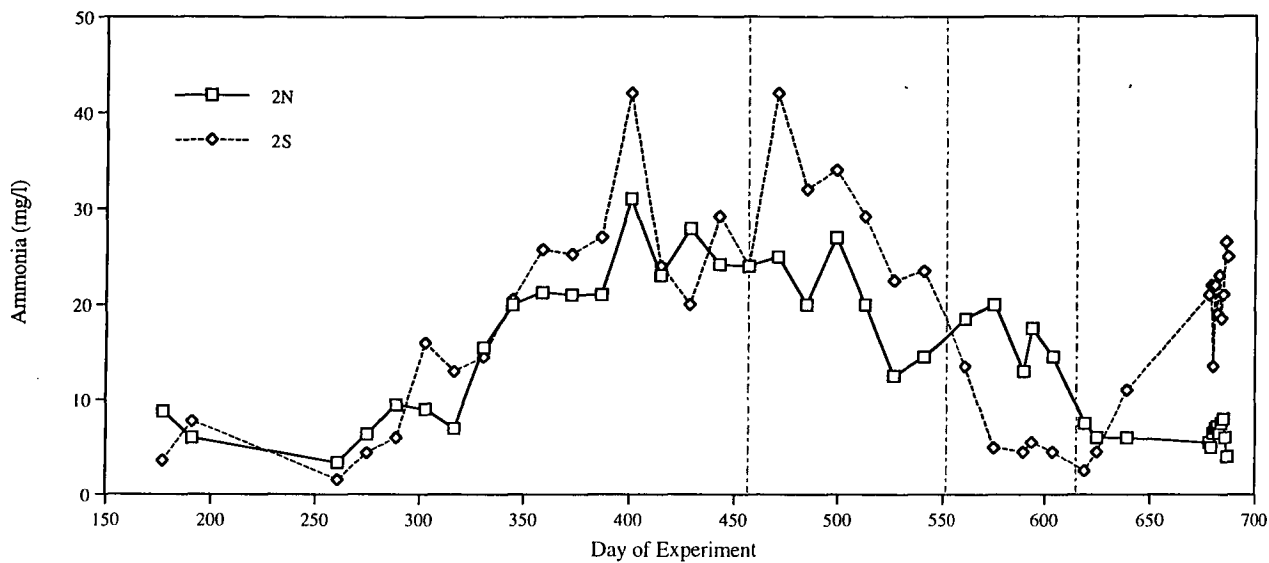


Figure 4.4(d): Ammonia levels in the 2N+ 2S ponds (85wB) over the main experimental period.

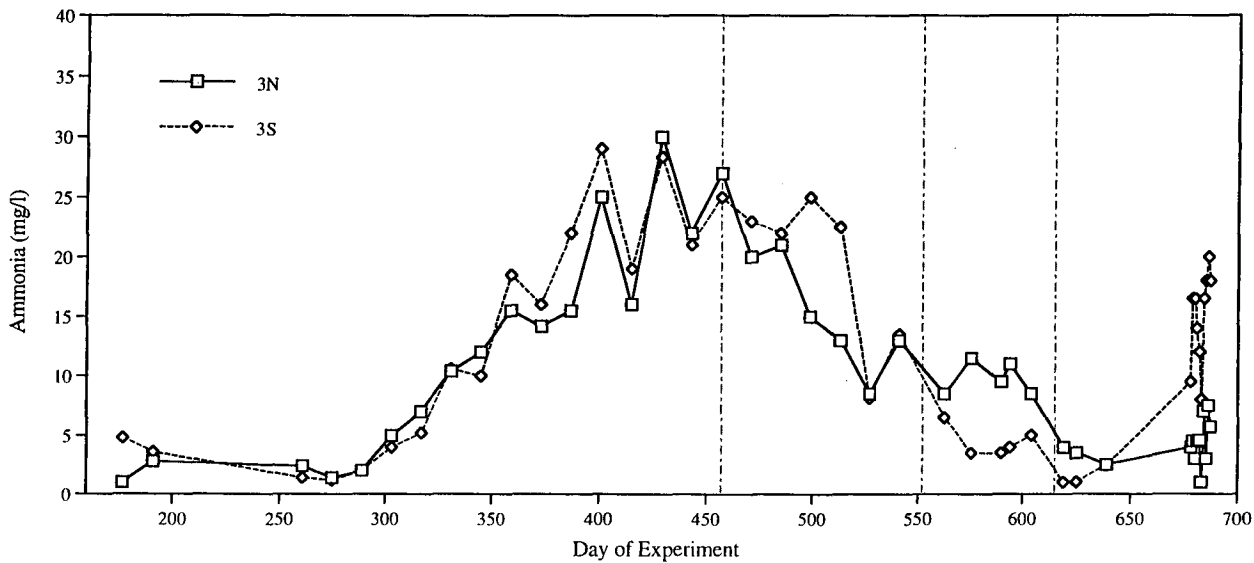


Figure 4.4(e): Ammonia levels in the 3N+ 3S ponds (85wB) over the main experimental period.

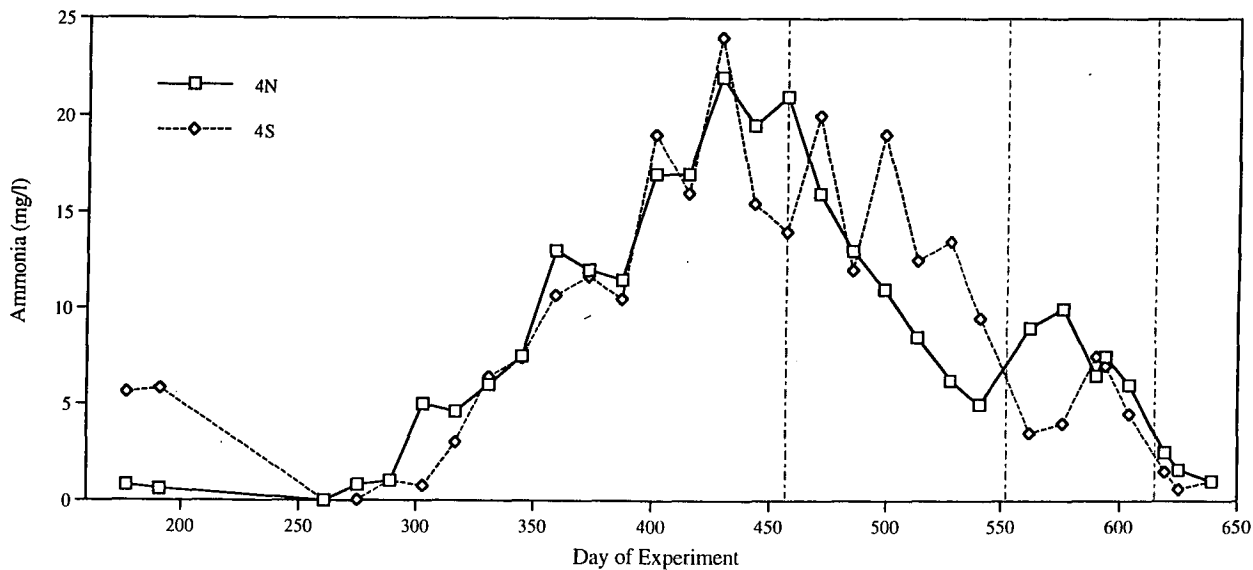


Figure 4.4(f): Ammonia levels in the 4N+ 4S ponds (85wB) over the main experimental period.

#### 4.4.2 Nitrate and nitrite

Other chemical factors were predicted to be less well defined indicators of the changes in flow than ammonia, given their relatively lower levels and the direct interplay between these factors and biological activity within the lagoons. While changing chemical conditions *per se* may have been expected to occur in a consistent and predictable manner following each division, these levels would, in turn, be increased, reduced, or converted by the bacterial, phytoplankton and zooplankton communities that were themselves responding to these changes (Section 2.3.3). Without the buffer of a large standing pool (as per ammonia), the effects of flow divisions on these factors could be expected to be far more complicated. It was predicted that pond pairs would therefore exhibit similarities in these factors over the equilibration period, but would then display increasingly disparate, inconsistent, unpredictable and possibly compounding differences following the divisions in flow.

Nitrate<sup>1</sup> and nitrite (Plate 3.5) were less abundant forms of nitrogen in the 85wB system, and were potentially far more variable than ammonia due to biological activity and conversion between the two forms throughout the system. The levels of nitrate and nitrite determined for corresponding pond pairs during the main experimental period are given in Figures 4.5 (a-f) and 4.6 (a-f). Again, the pond pairs reflected similar patterns to those shown in the SL pond for both factors - particularly with emerging winter- and spring-based seasonal highs, respectively - but these patterns were less well defined than for ammonia. Levels tended to increase following the SL pond (dramatically so for nitrite), reflecting the increasing biological utilisation of (and decrease in) ammonia, and the increase in nitrification/denitrification activity (Section 2.3.3).

Pond pairs 2 and 4 showed a noticeable initial difference in nitrate levels between corresponding lagoons, and minor differences also existed between nitrite levels in lagoon pairs. As with ammonia, all pairs then began to match each other quite closely through the equilibration period. This was particularly so with pairs 1 and 3, and

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<sup>1</sup> As noted in Section 3.4, nitrate results are reported with caution.

while some variation did occur in magnitude for pairs 3 and 4, these still followed each other closely in pattern.

With the timing of the first flow division, however, the similarities between both the levels and patterns of nitrate and nitrite in the pond pairs became less apparent. The beginnings of this trend could again be seen with the outlets of the SP pond, before becoming more pronounced in the pond pairs proper. Peaks and patterns became more separated, and while some marked rises or falls in nutrient levels immediately followed changes in flow division, many did not, with the north and south halves of the system swapping in dominance in a seemingly random manner.

The changes in flow would therefore appear to have been having a definite effect on relative nitrate and nitrite levels between the pond pairs, but the specifics of this effect are not immediately apparent. Although initial nitrate and nitrite levels may have directly reflected flow changes, it is again more than likely that they were being complicated and masked by biological responses to the same factor.

#### **4.4.3 Phosphate**

While occurring at much higher levels than nitrate or nitrite, phosphate is also directly involved in the uptake and release of nutrients from biological cycles, and is of critical importance to such systems (Section 2.3.3). It also appears to have provided a more complicated response to changes in flow conditions than those seen for ammonia (Figure 4.7 a-f).

As would be expected, phosphate followed the trend set by the previous nutrients, with initial differences in paired lagoons giving way to generally similar levels and patterns over the equilibration period. Some degree of disruption then followed the first division of flow, and continued (and in some cases magnified) through the remaining divisions. This effect was more sporadic than with nitrate and nitrite, however, with only one major variation occurring between the SP outlets (possibly due to contamination of the sample), and with no marked variation between the 1N and 1S ponds until the second flow division.

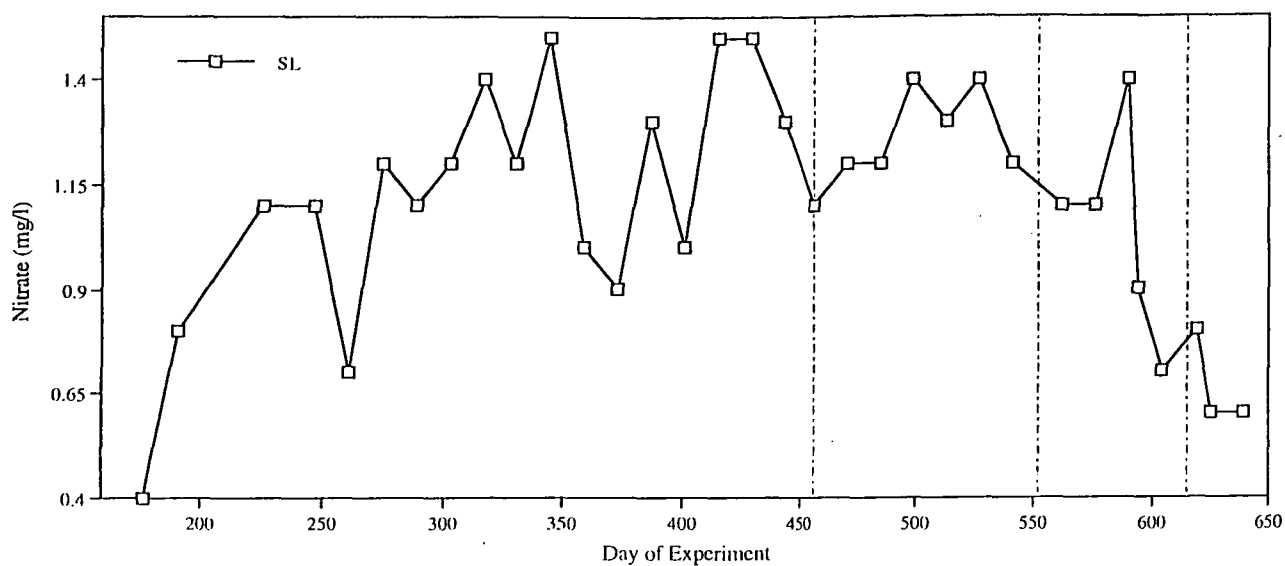


Figure 4.5(a): Nitrate levels in the SL pond (85wB) over the main experimental period.

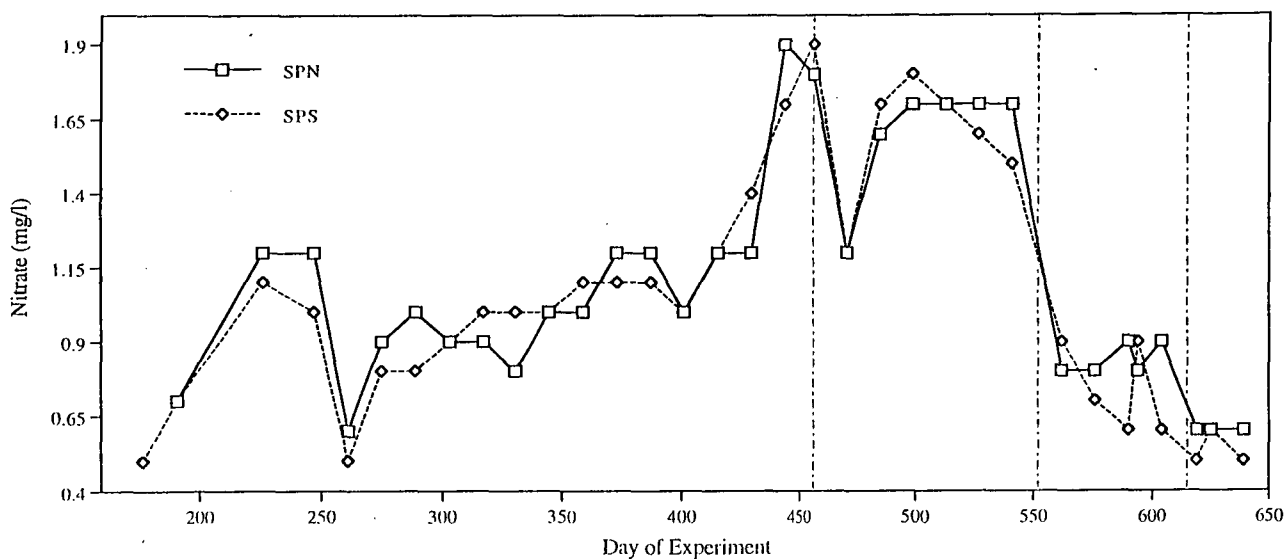


Figure 4.5(b): Nitrate levels in the SPN+ SPS ponds (85wB) over the main experimental period.

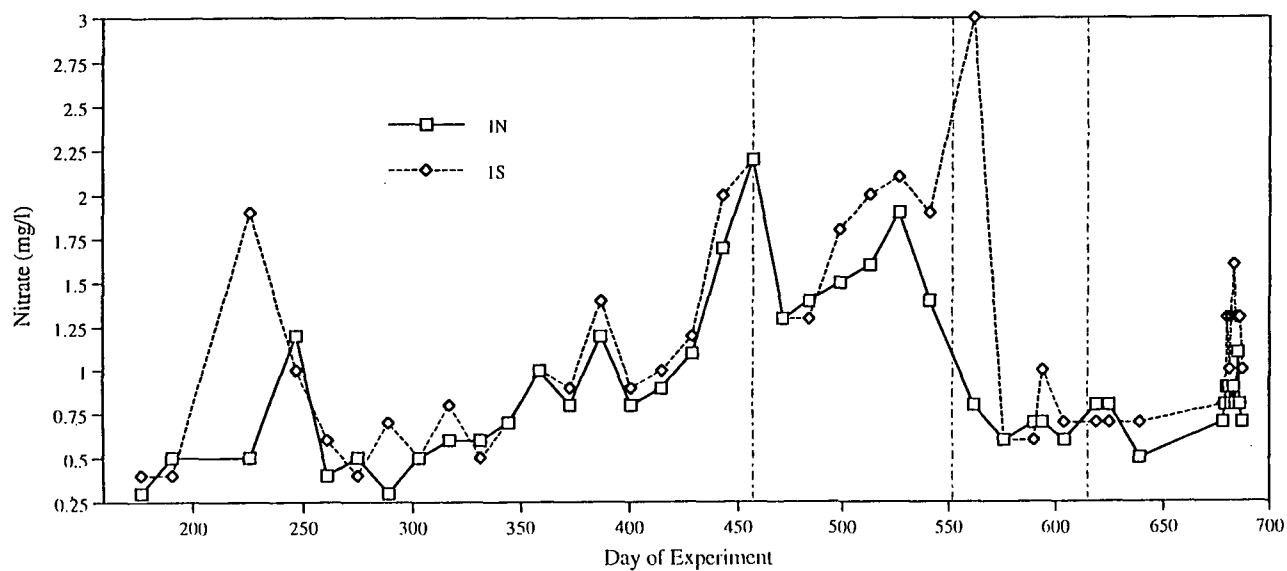


Figure 4.5(c): Nitrate levels in the 1N+ 1S ponds (85wB) over the main experimental period.

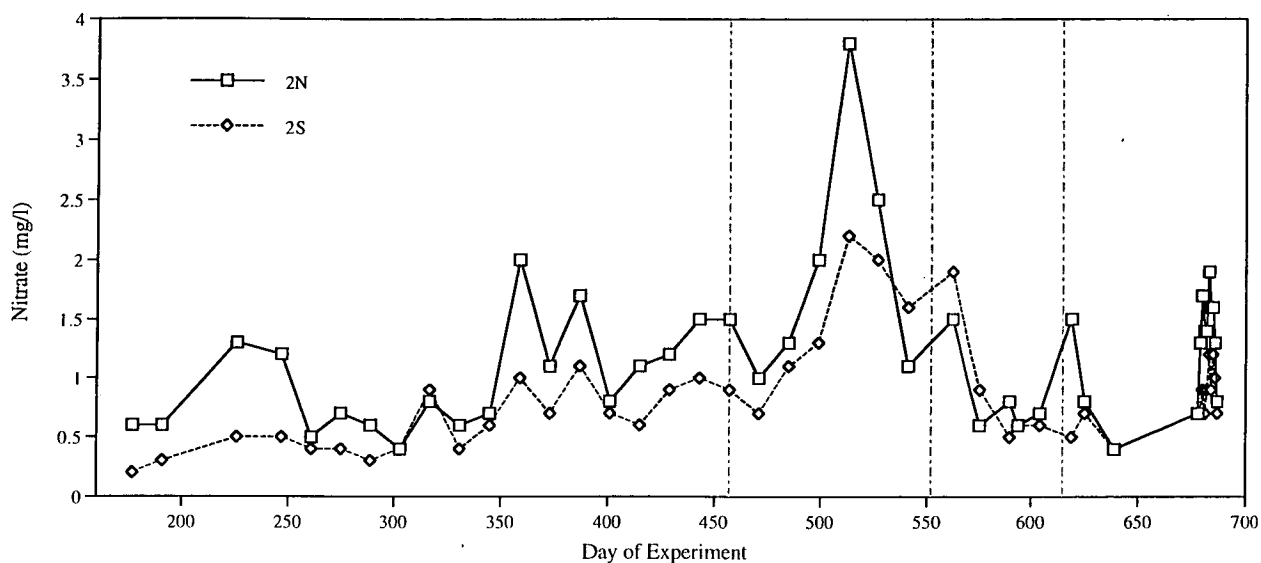


Figure 4.5(d): Nitrate levels in the 2N+2S ponds (85wB) over the main experimental period.

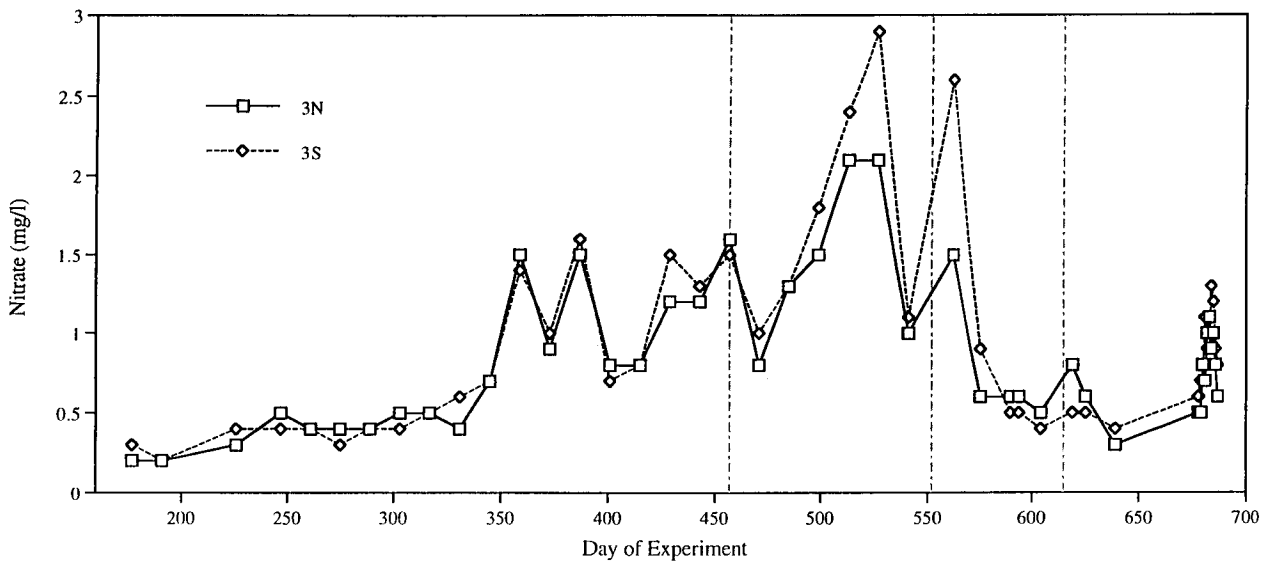


Figure 4.5(e): Nitrate levels in the 3N+3S ponds (85wB) over the main experimental period.

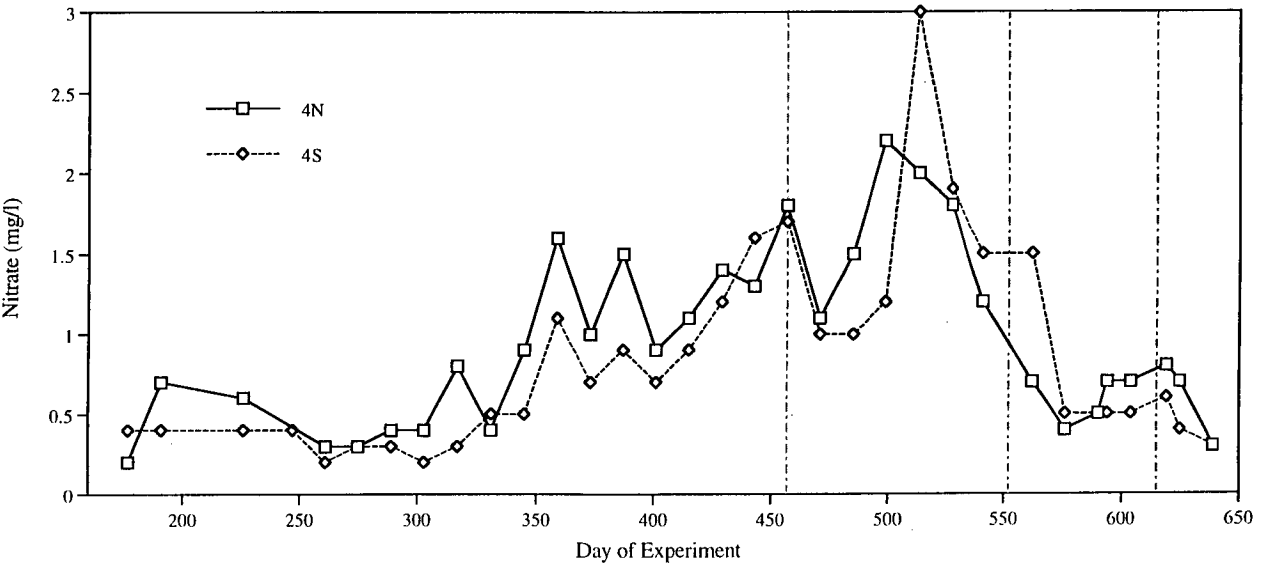


Figure 4.5(f): Nitrate levels in the 4N+4S ponds (85wB) over the main experimental period.

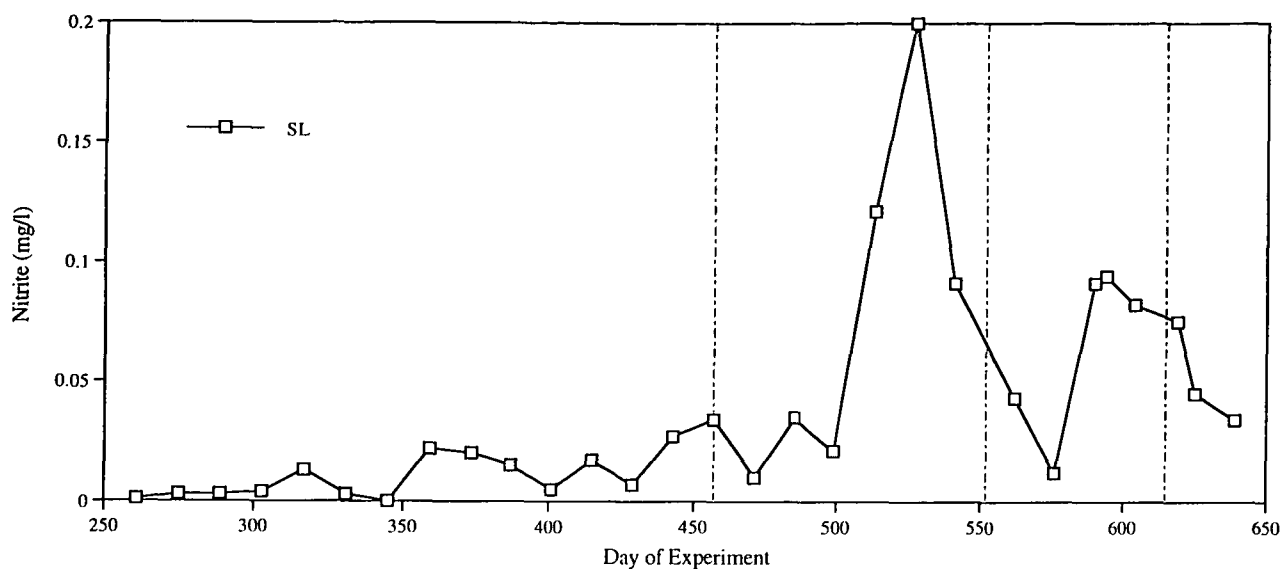


Figure 4.6(a): Nitrite levels in the SL pond (85wB) over the main experimental period.

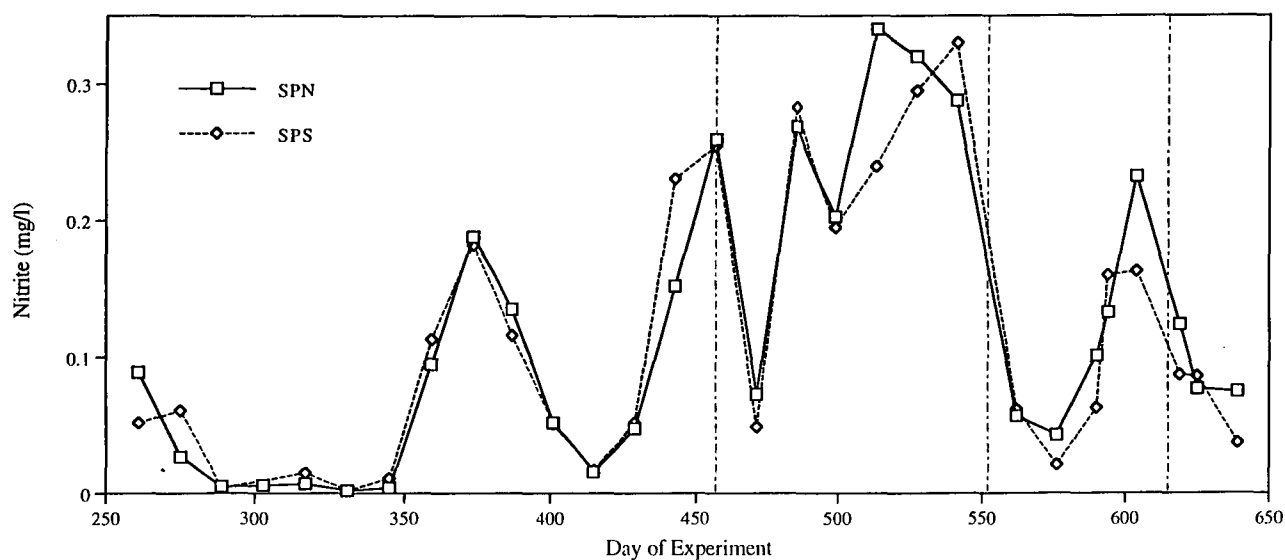


Figure 4.6(b): Nitrite levels in the SPN + SPS ponds (85wB) over the main experimental period.

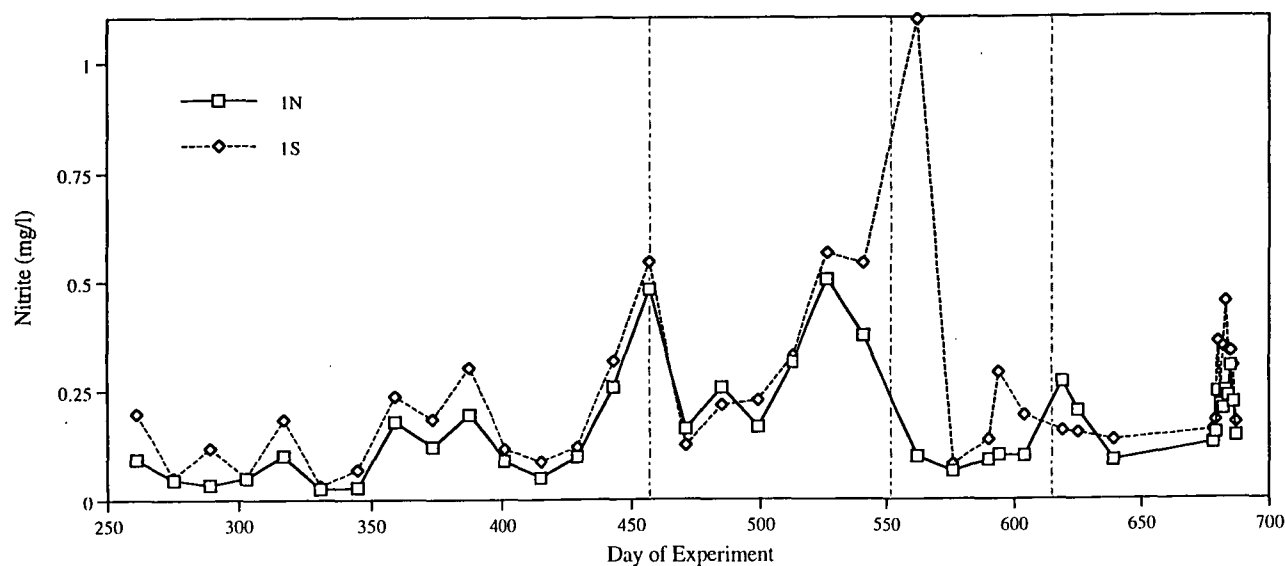


Figure 4.6(c): Nitrite levels in the 1N + 1S ponds (85wB) over the main experimental period.

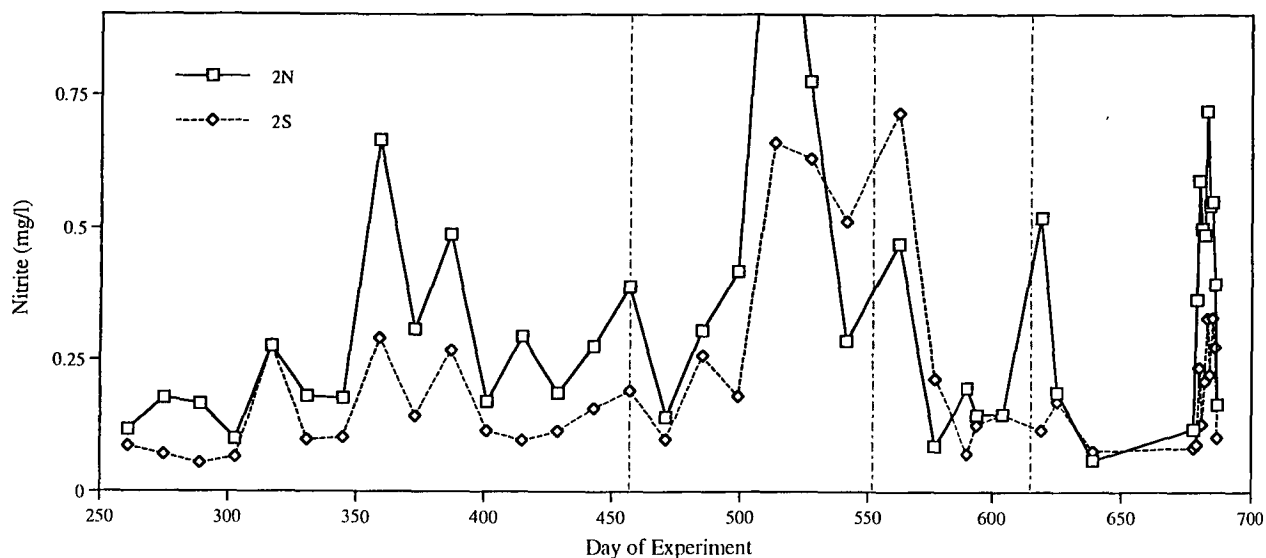


Figure 4.6(d): Nitrite levels in the 2N + 2S ponds (85wB) over the main experimental period.

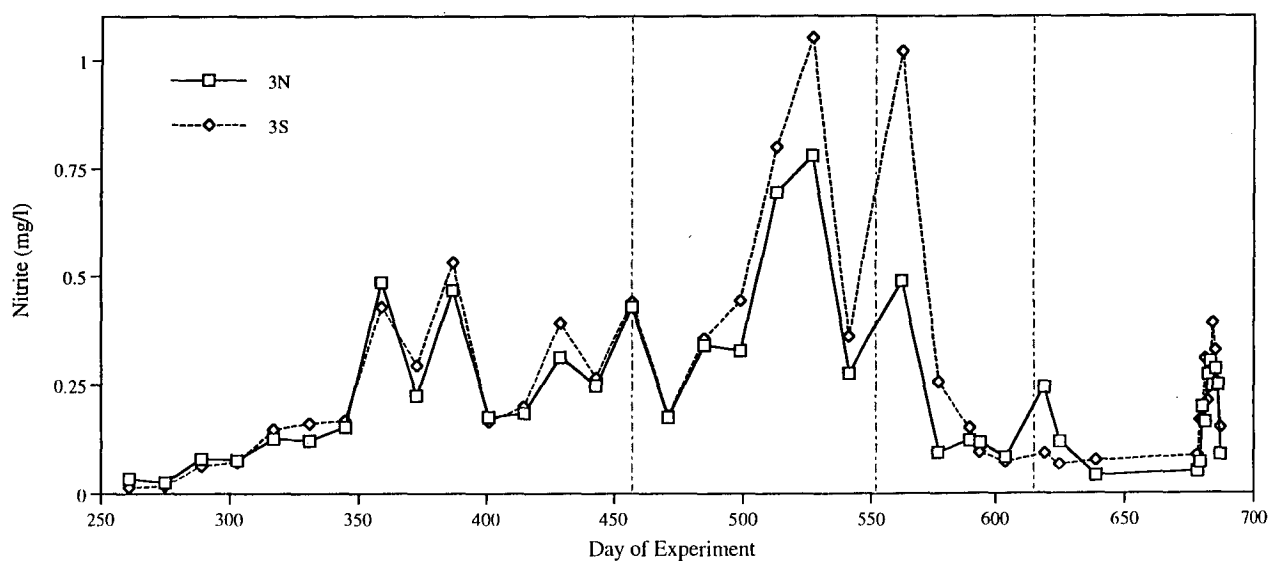


Figure 4.6(e): Nitrite levels in the 3N + 3S ponds (85wB) over the main experimental period.

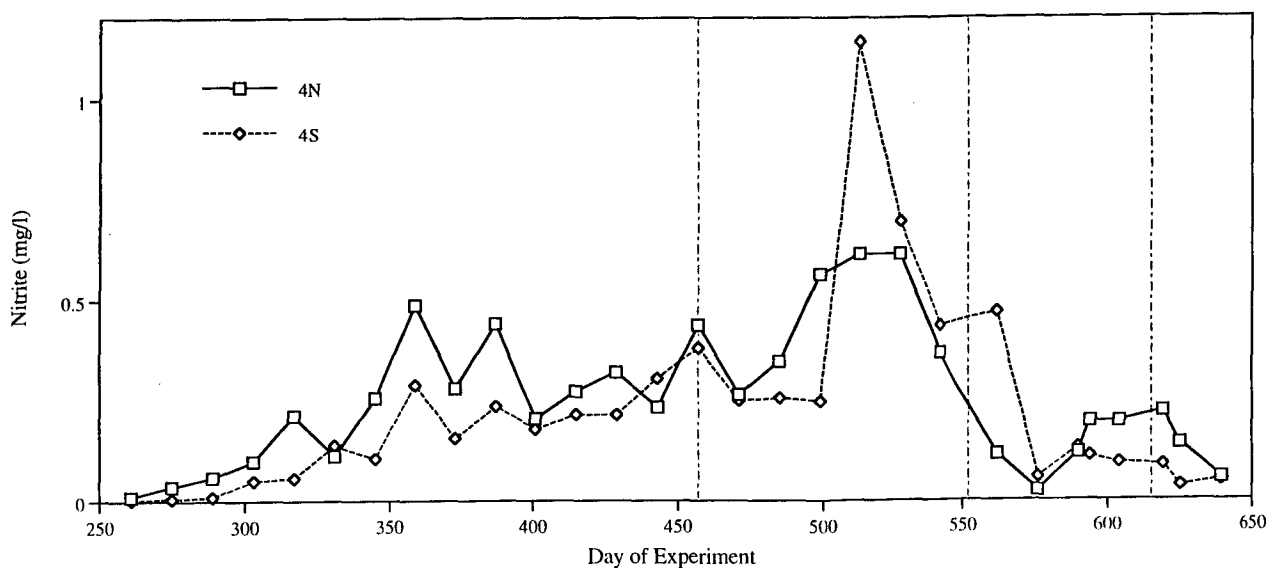


Figure 4.6(f): Nitrite levels in the 4N + 4S ponds (85wB) over the main experimental period.

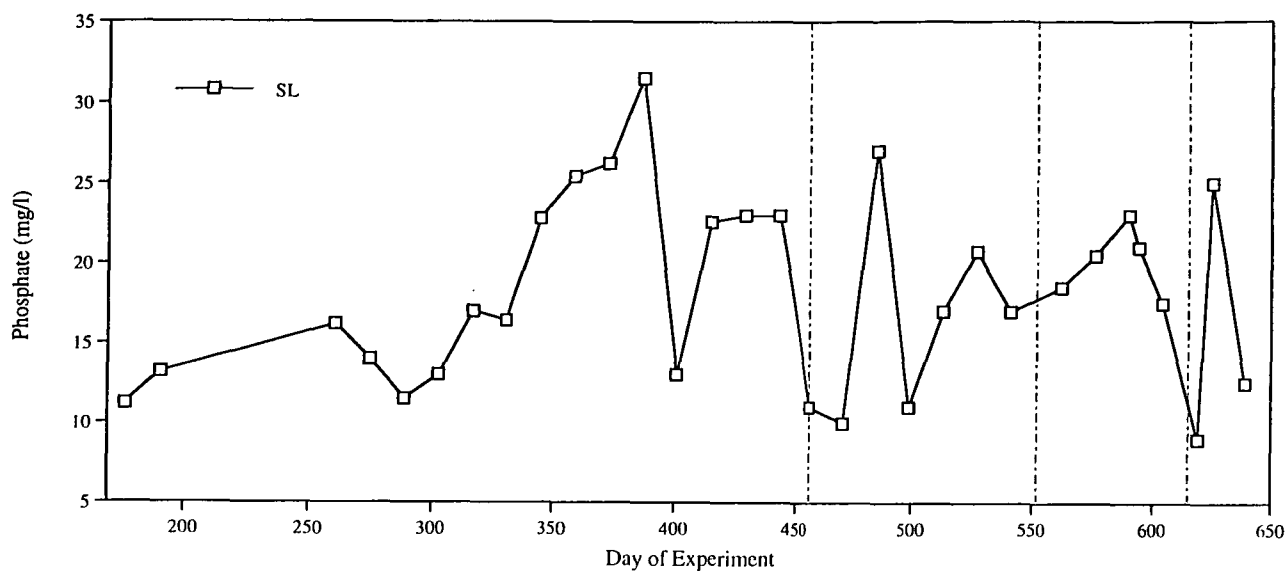


Figure 4.7(a): Phosphate levels in the SL pond (85wB) over the main experimental period.

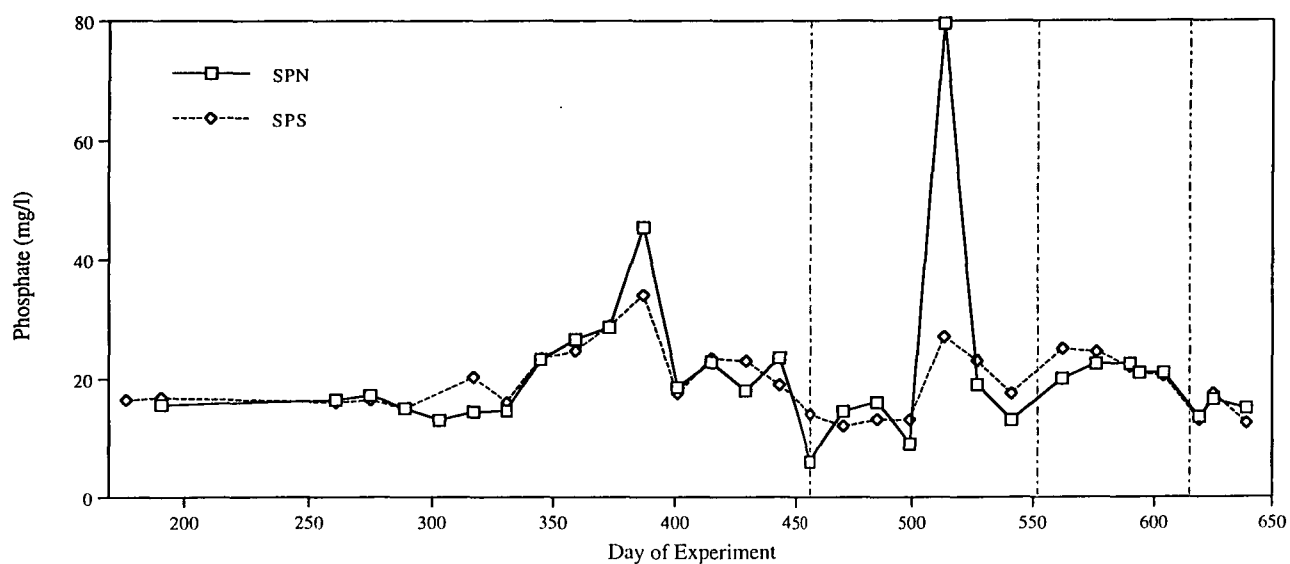


Figure 4.7(b): Phosphate levels in the SPN + SPS pond (85wB) over the main experimental period.

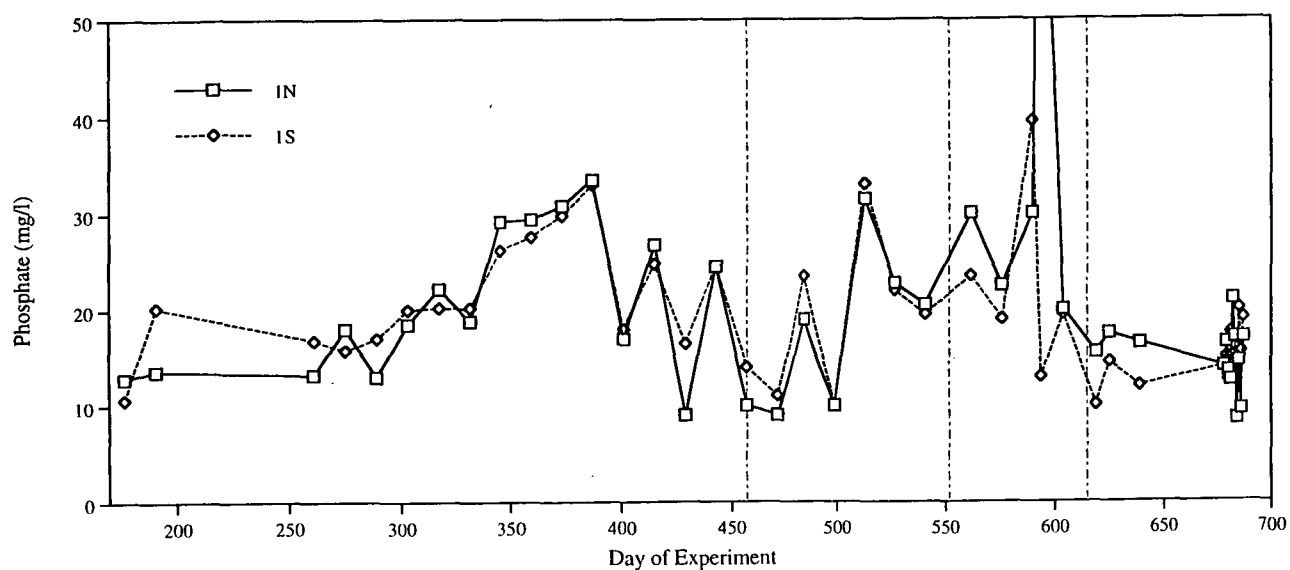


Figure 4.7(c): Phosphate levels in the 1N + 1S pond (85wB) over the main experimental period.



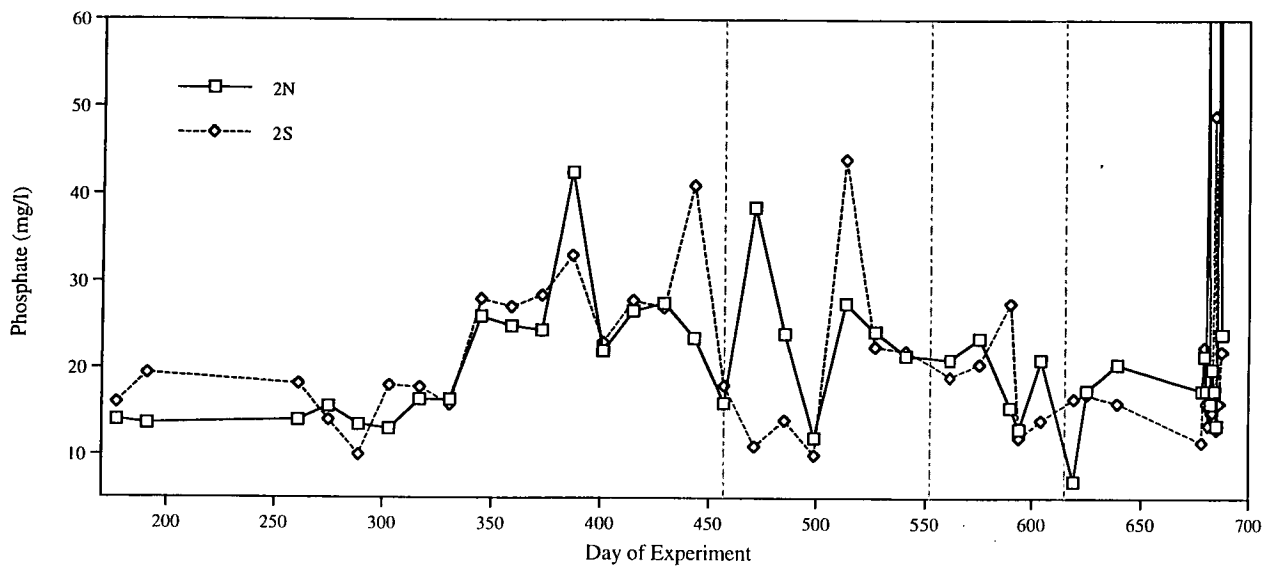


Figure 4.7(d): Phosphate levels in the 2N + 2S pond (85wB) over the main experimental period.

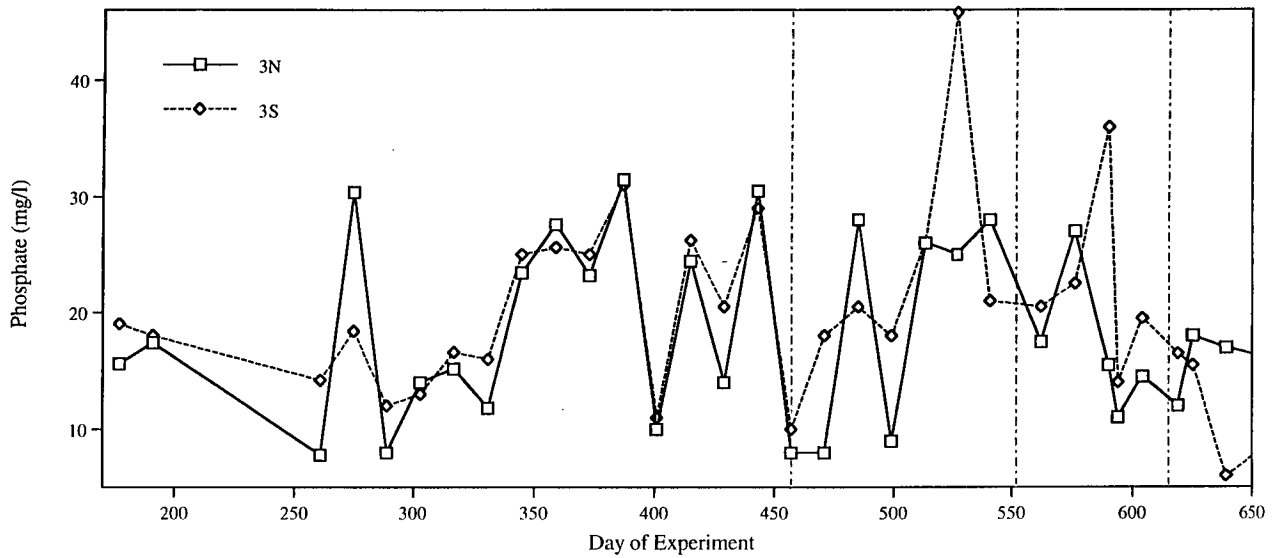


Figure 4.7(e): Phosphate levels in the 3N + 3S pond (85wB) over the main experimental period.

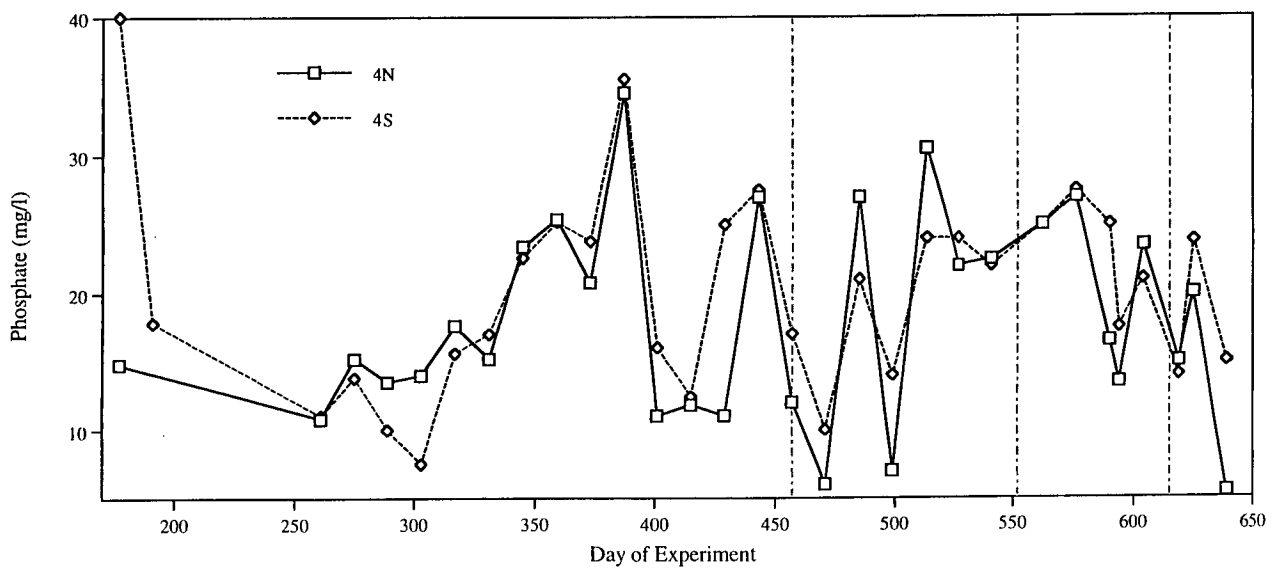


Figure 4.7(f): Phosphate levels in the 4N + 4S pond (85wB) over the main experimental period.

#### 4.4.4 pH, conductivity and salinity

Differences in pH, conductivity, and salinity between the pond pairs are shown in figures Figures 4.8 (a-f), 4.9 (a-f), and 4.10 (a-f), respectively. pHs over the main experimental period ranged from 6.79 to 9.41, but rarely fell below 7.00 or rose above the (theoretically toxic) level of 9.00. Although there was an initial difference recorded between ponds 2N and 2S early in 1994, pond pairs otherwise remained relatively similar in pH levels, with little effect following the divisions in flow. The only deviations of note occurred on days 429 and 471. On the former day (equal flow), pH levels dropped markedly in ponds 1S, 2S, 3S & 4S, but this drop was only matched on the northern side of the system in pond 2N. On Day 471 (first flow division), marked drops in pH level were recorded for ponds SPN and 2N (low flow) and 3S (high flow). These falls in pH did not correspond with any similar patterns in ammonia, nitrate, nitrite, phosphate, or chlorophyll-a levels for these lagoons, although ammonia levels were depressed in ponds 1S and 2S on Day 429.

Conductivities ranged from 941 to 2350  $\mu\text{S}/\text{cm}$  over the main experimental period, and also showed little pattern between the earlier pond pairs. Pond pairs 3 and 4, however, displayed the familiar pattern of large initial differences between the northern and southern ponds, followed by similar levels over the remainder of the equilibration period, and increased variability during the periods of flow division. Some of these variations also coincided with the changes in flow division, although the start of variation between ponds 3N and 3S preceded the end of equilibration.

As would be expected, salinity in the system was low, ranging from 0.4 to 1.3 salinity units. As with the previous factors there is little pattern immediately apparent, apart from pond pairs 3 and 4, which, not surprisingly, closely mirrored the conductivity patterns described above. Curiously, however, the second flow division brought salinity levels in the 3N and 3S ponds to a very close match, while the other flow divisions saw them draw apart.

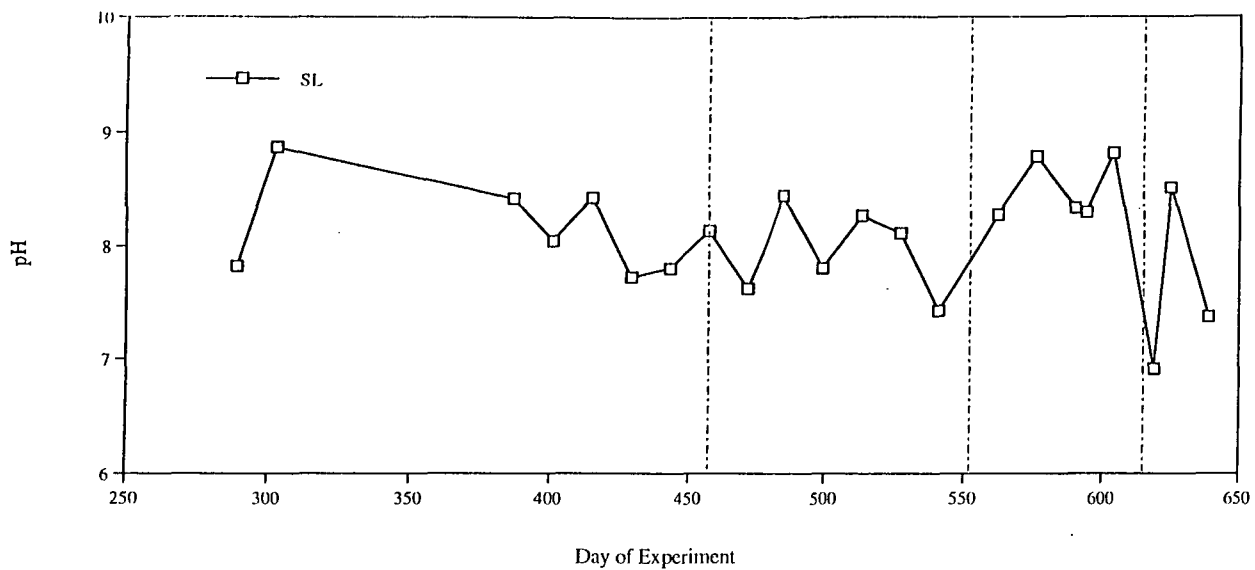


Figure 4.8(a): pH levels in the SL pond (85wB) over the main experimental period.

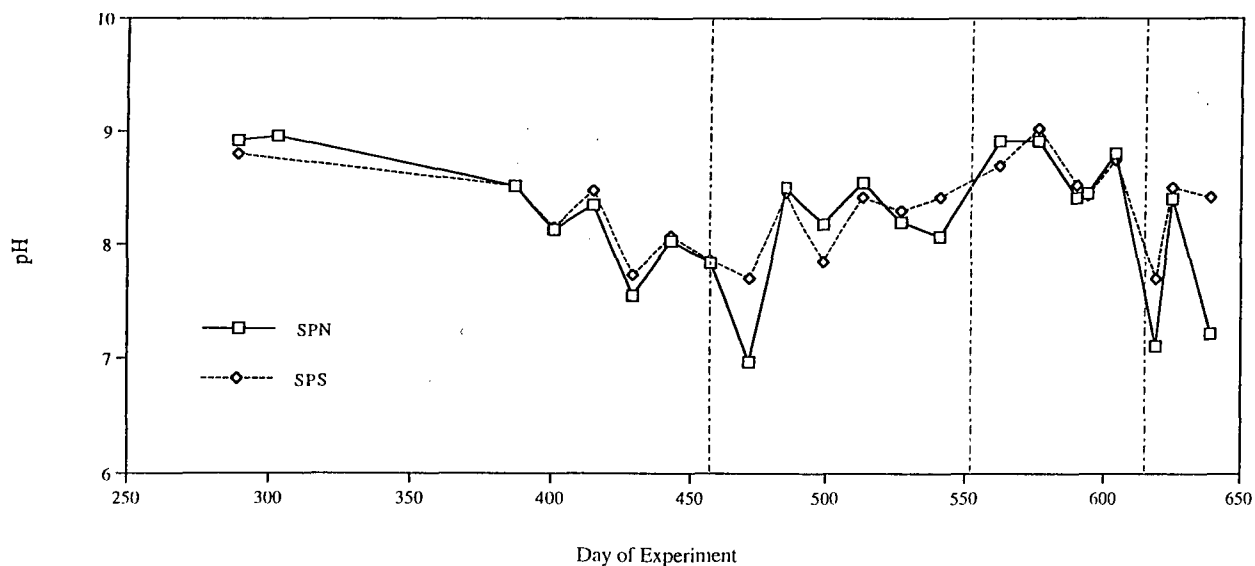


Figure 4.8(b): pH levels in the SPN+SPS ponds (85wB) over the main experimental period.

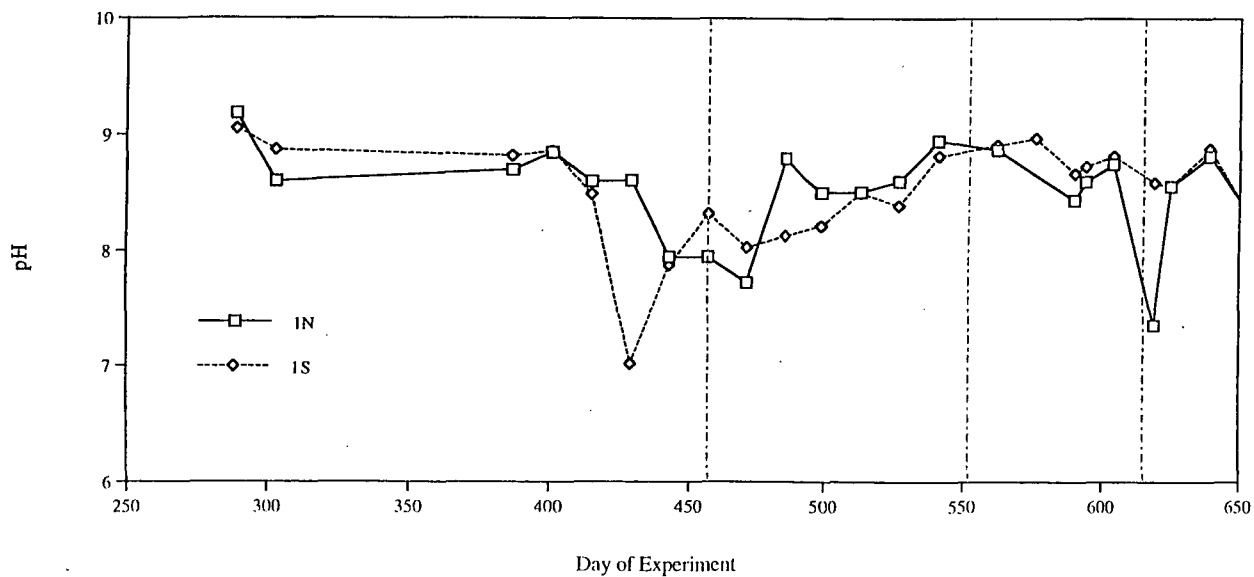


Figure 4.8(c): pH levels in the 1N+1S ponds (85wB) over the main experimental period.

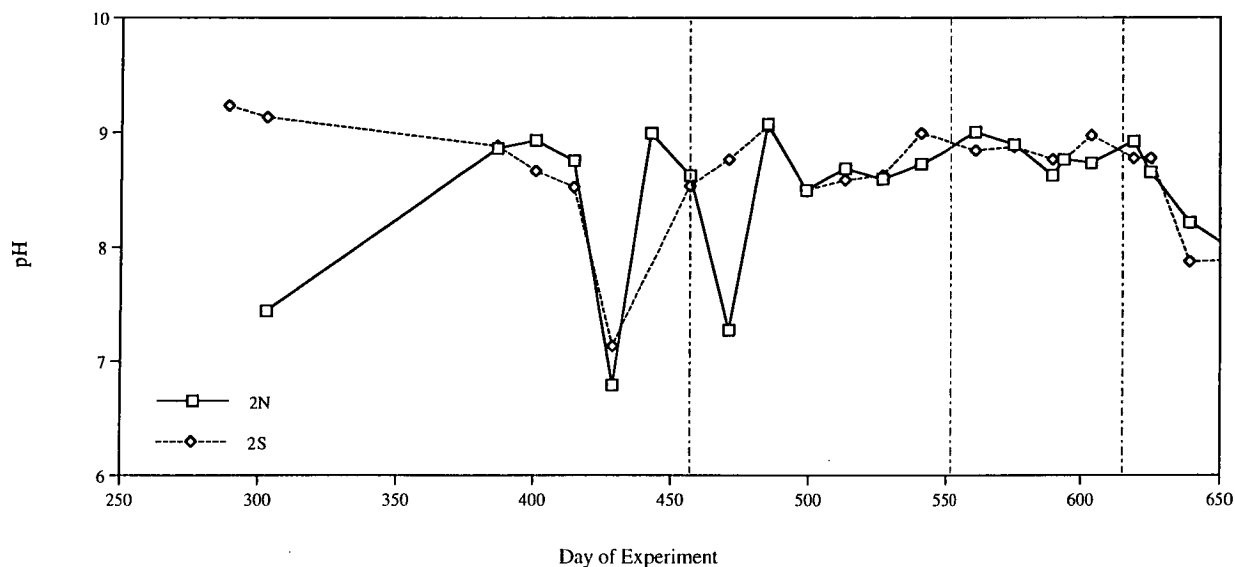


Figure 4.8(d): pH levels in the 2N+2S ponds (85wB) over the main experimental period.

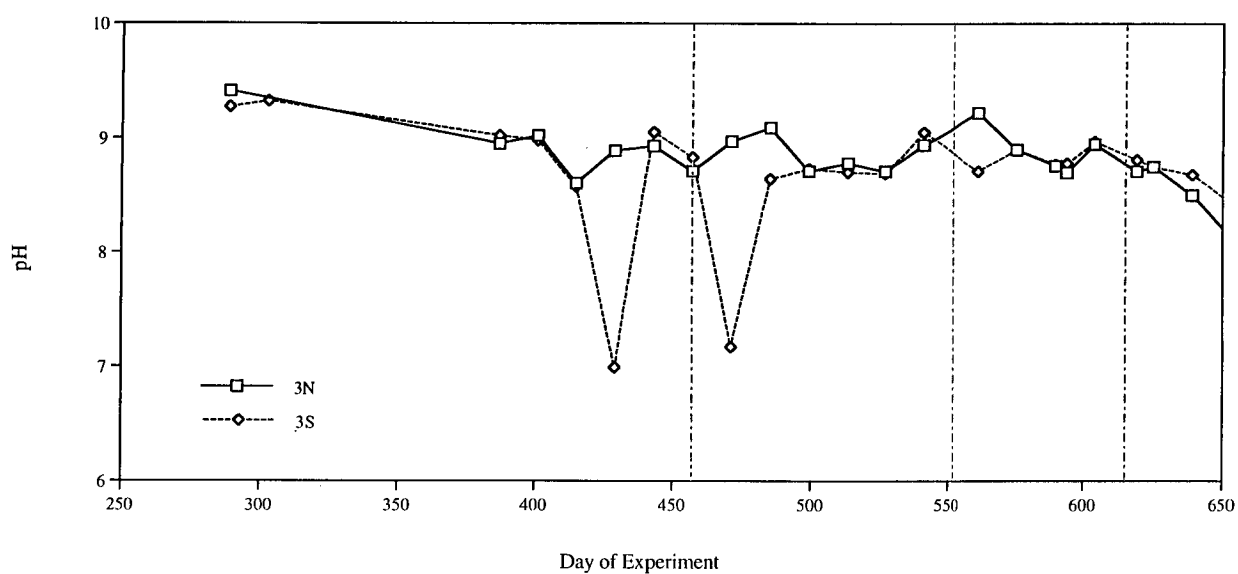


Figure 4.8(e): pH levels in the 3N+3S ponds (85wB) over the main experimental period.

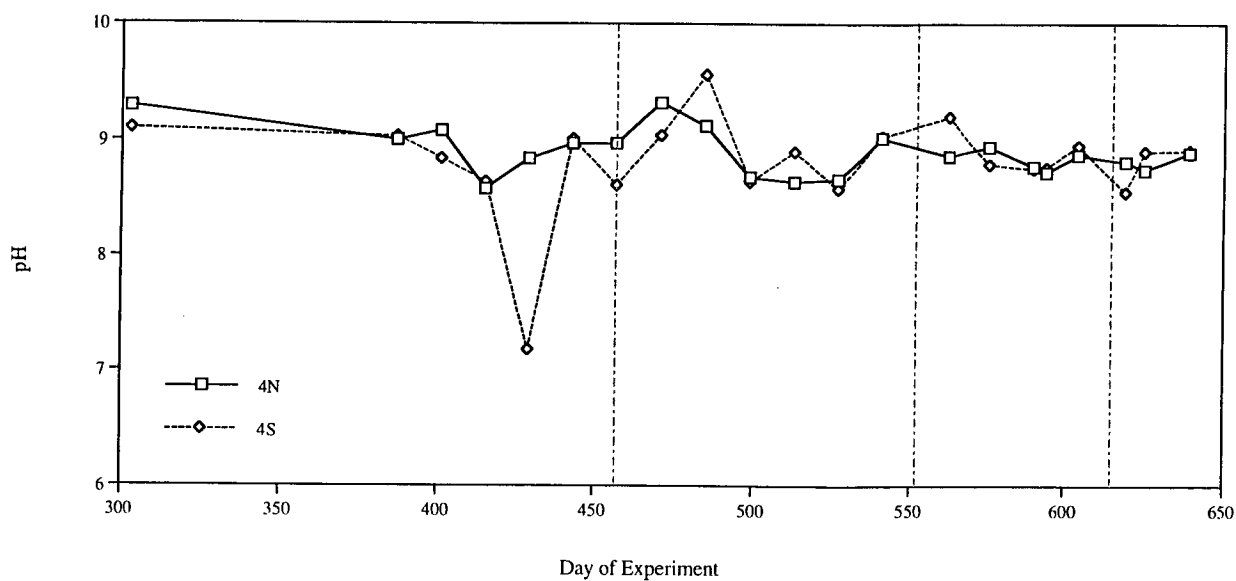


Figure 4.8(f): pH levels in the 4N+4S ponds (85wB) over the main experimental period.

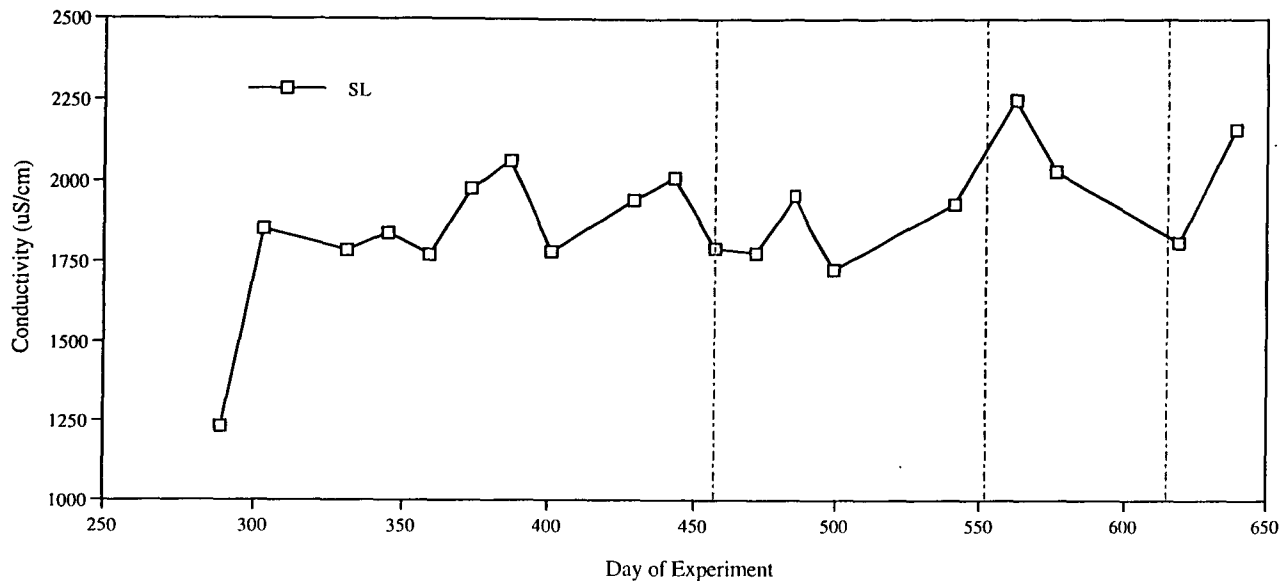


Figure 4.9(a): Conductivity in the SL pond (85wB) over the main experimental period.

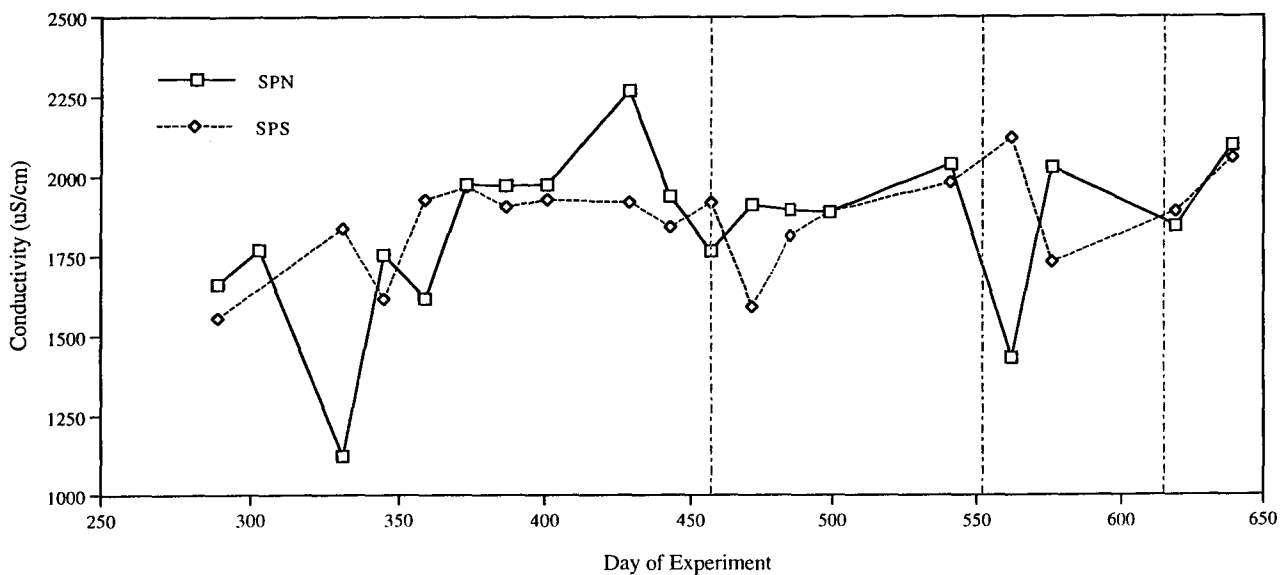


Figure 4.9(b): Conductivity in the SPN + SPS ponds (85wB) over the main experimental period.

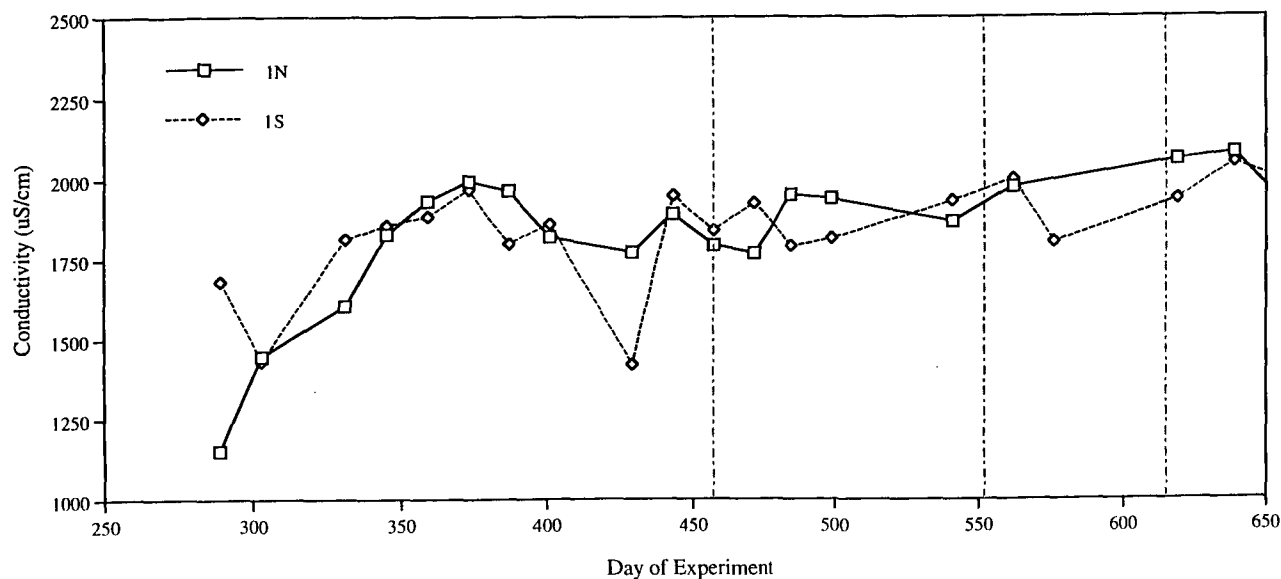


Figure 4.9(c): Conductivity in the 1N + 1S ponds (85wB) over the main experimental period.

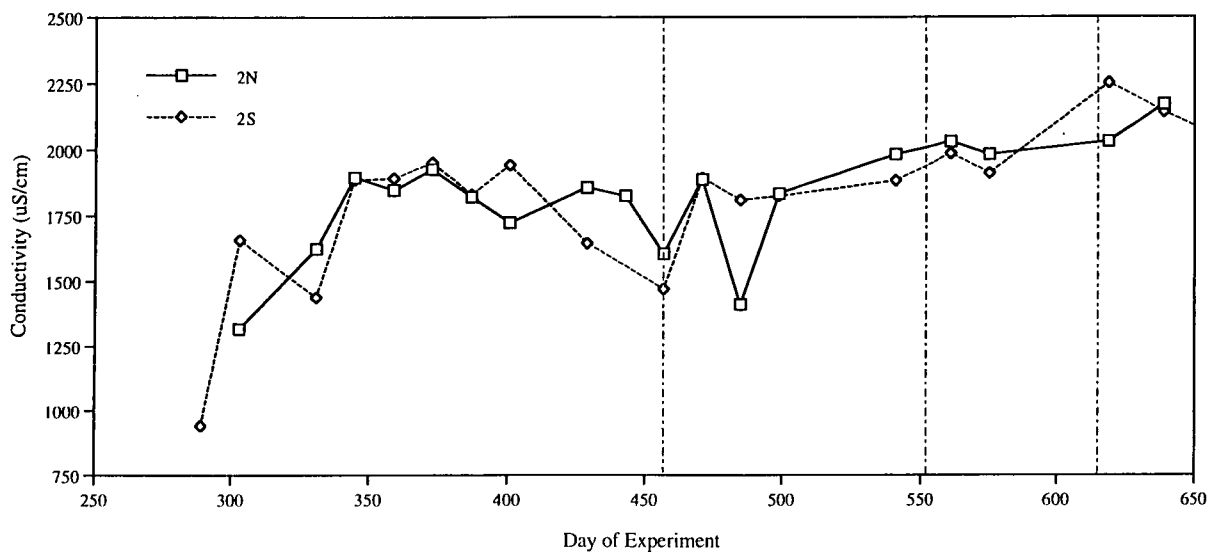


Figure 4.9(d): Conductivity in the 2N + 2S ponds (85wB) over the main experimental period.

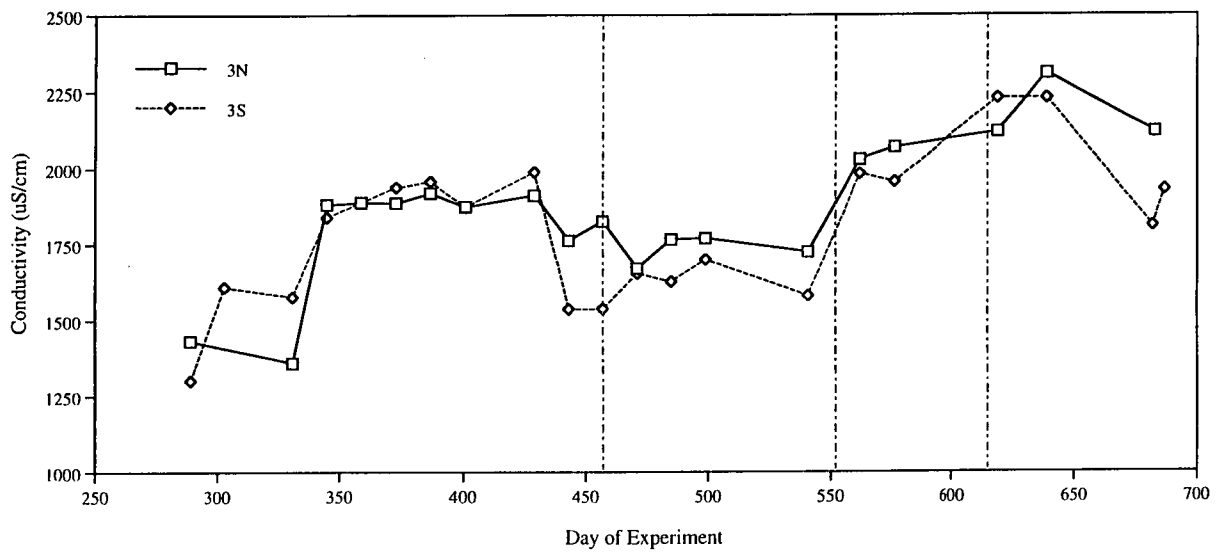


Figure 4.9(e): Conductivity in the 3N + 3S ponds (85wB) over the main experimental period.

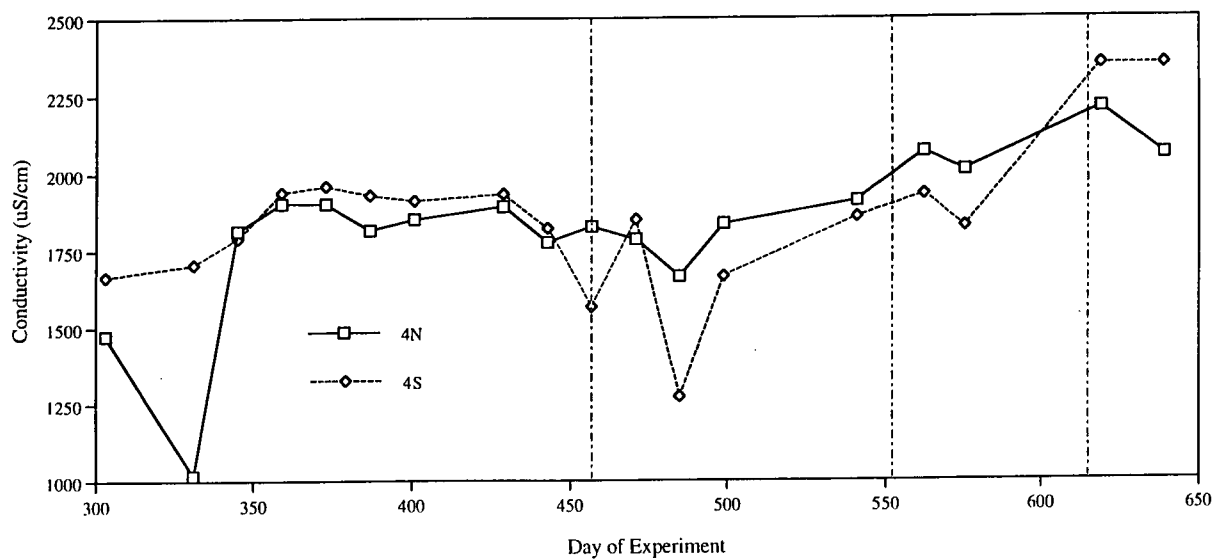


Figure 4.9(f): Conductivity in the 4N + 4S ponds (85wB) over the main experimental period.

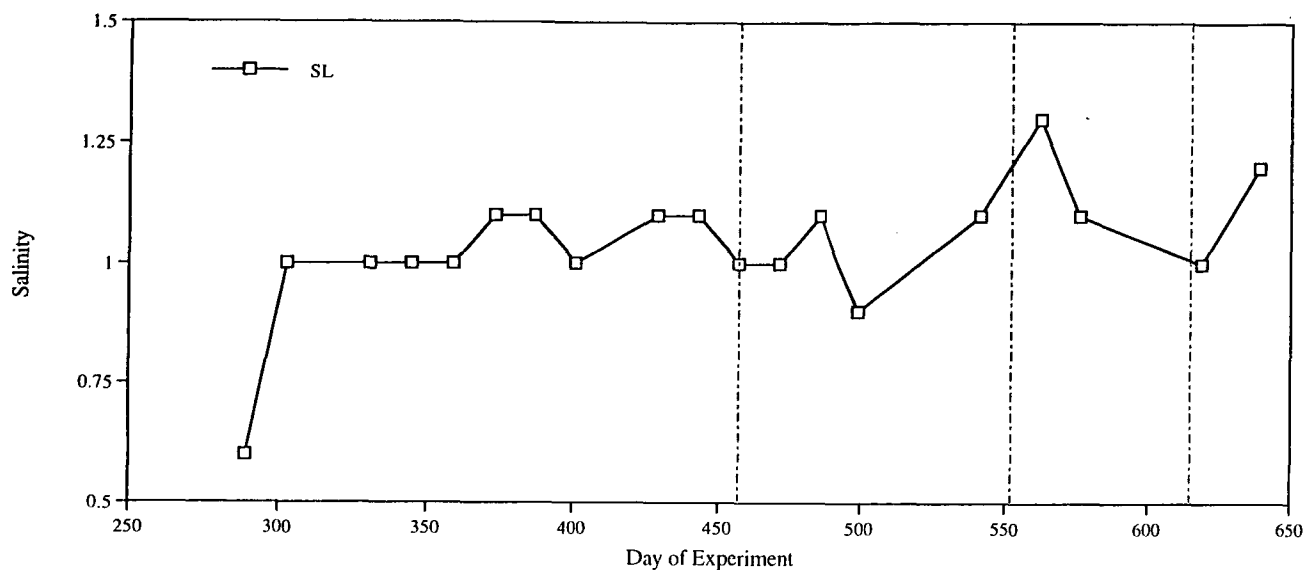


Figure 4.10(a): Salinity levels in the SL pond (85wB) over the main experimental period.

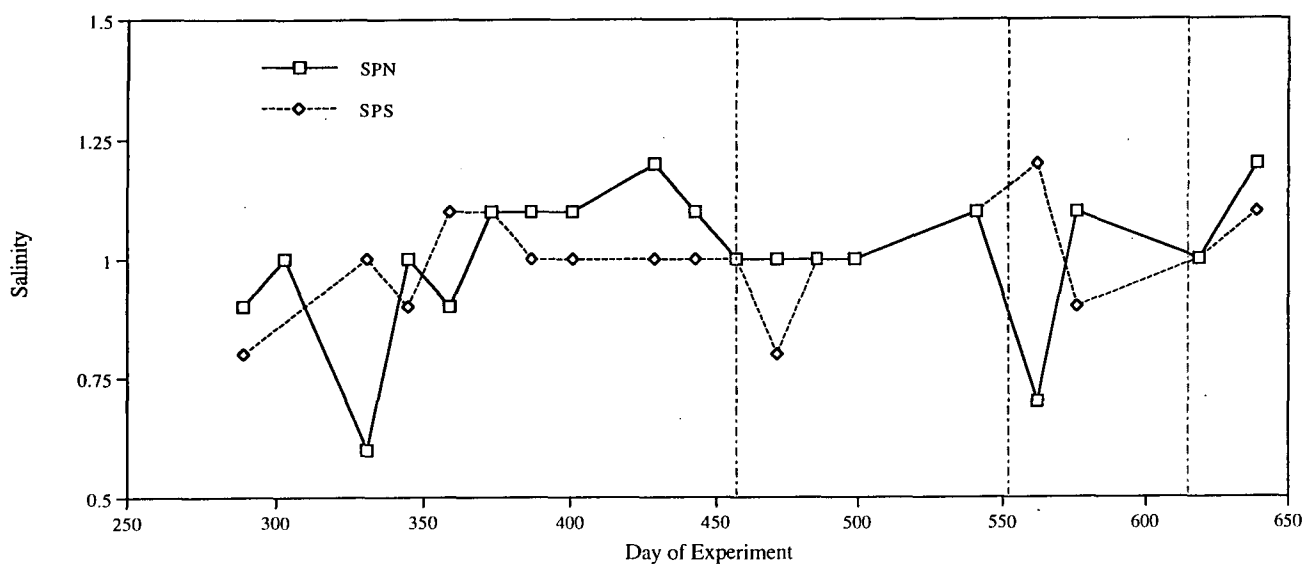


Figure 4.10(b): Salinity levels in the SPN+SPS ponds (85wB) over the main experimental period.

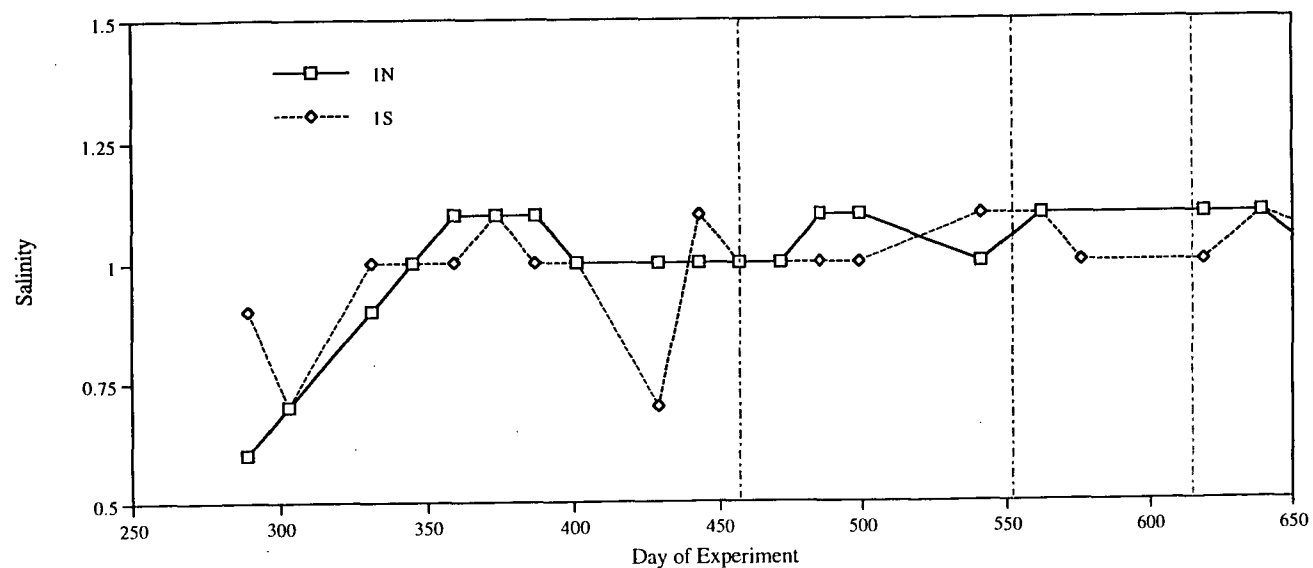


Figure 4.10(c): Salinity levels in the 1N + 1S ponds (85wB) over the main experimental period.

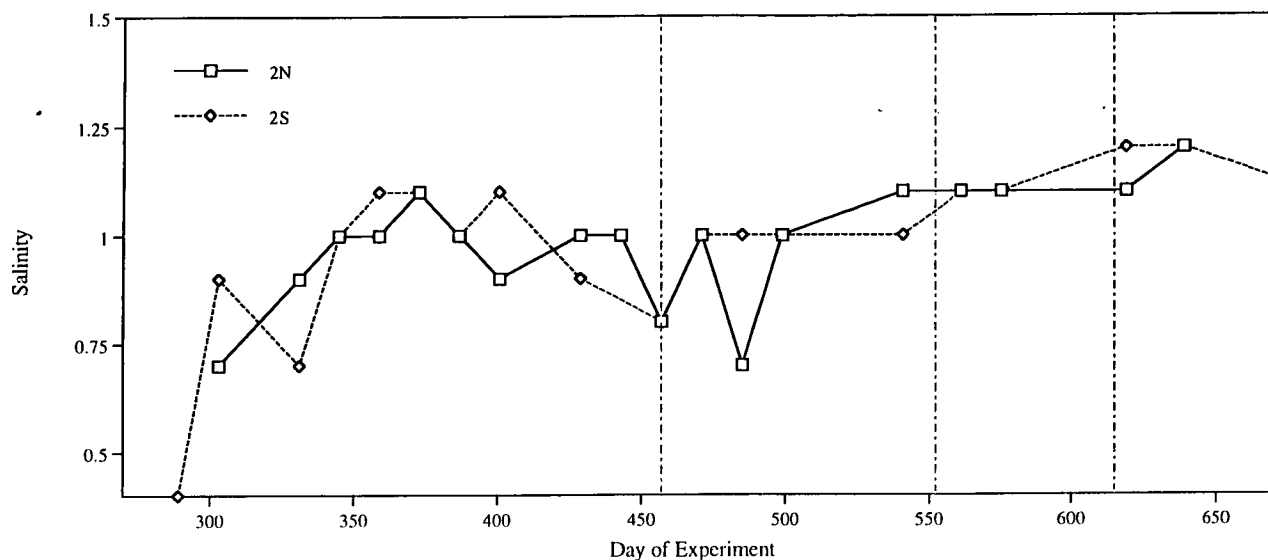


Figure 4.10(d): Salinity levels in the 2N + 2S ponds (85wB) over the main experimental period.

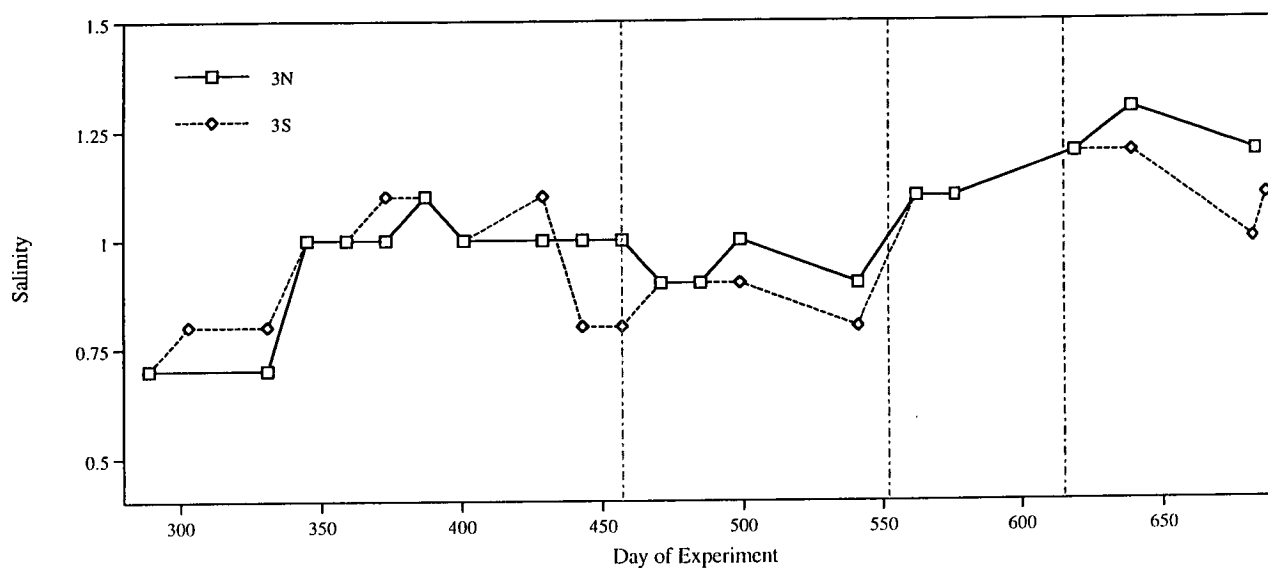


Figure 4.10(e): Salinity levels in the 3N + 3S ponds (85wB) over the main experimental period.

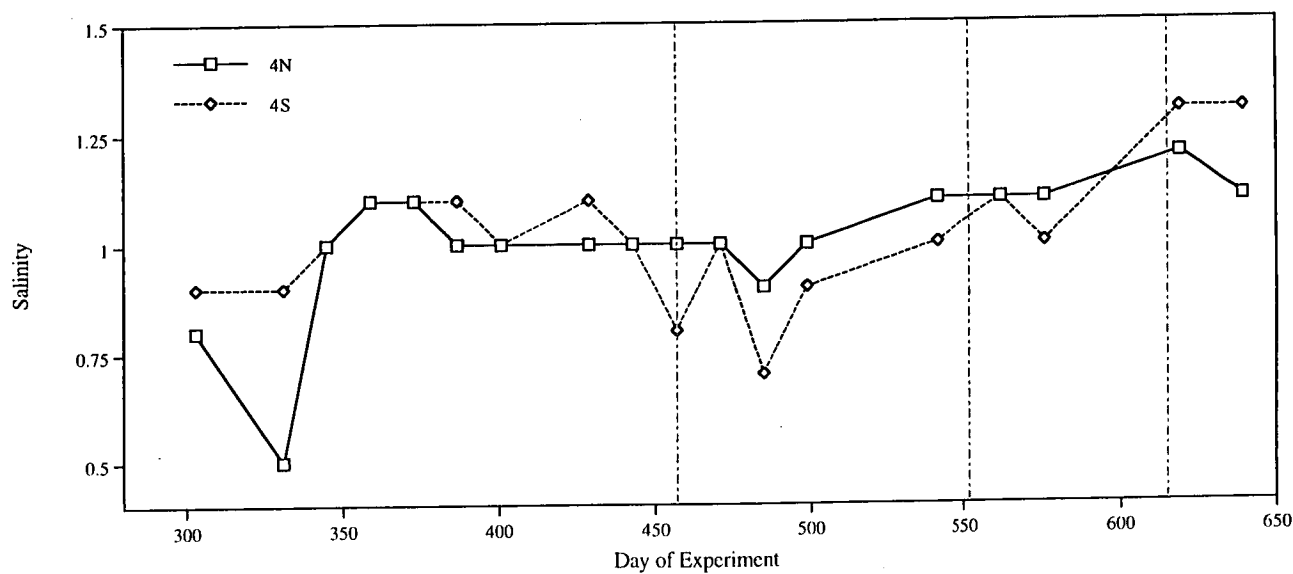


Figure 4.10(f): Salinity levels in the 4N + 4S ponds (85wB) over the main experimental period.



## 4.5 Biological data

### 4.5.1 chlorophyll-a

The phytoplankton biomass in the lagoons would also be expected to display a complicated response, or series of responses, to variations in the division of flow between the halves of the 85wB system. This response was predicted to be at least as complicated as that displayed by the non-ammonia compounds listed above, if not more so due to the addition of top-down grazing effects complicating any nutrient-related patterns and cycles.

The levels of chlorophyll-a through the 85wB system over the main experimental period are shown in Figure 4.11(a-g), and are listed in Appendix 1. As would be expected in such a nutrient rich, eutrophic environment, chlorophyll levels were usually exceptionally high. While clearwater phases did occur ( $0 \mu\text{g.l}^{-1}$ ), chlorophyll-a frequently reached levels in excess of  $1000 \mu\text{g.l}^{-1}$ , with maximums of  $2660 \mu\text{g.l}^{-1}$  (or  $2.66 \text{ mg.l}^{-1}$ ) recorded in pond 4N on Day 303 and  $2731 \mu\text{g.l}^{-1}$  (or  $2.731 \text{ mg.l}^{-1}$ ) recorded in pond 5N on Day 594 (early autumn and mid-summer, respectively).

Curiously, the peak in phytoplankton levels in the SL pond occurred between Days 400-500, or winter to early spring 1994. This pattern was repeated through the SP outlets and ponds 1N and 1S (where the peak was much thinner), but the reverse was true for pond pairs 2 to 5, where the previously secondary peaks of early autumn (ca. Day 300) and summer (ca. Day 600) progressively came to dominance and the winter peak disappeared. It is possible that this distinct seasonal difference in chlorophyll peaks between the earlier and later parts of the system represents the times of year at which conditions in these areas are optimal for phytoplankton, in keeping with the characteristics of the spatial succession discussed in Section 3.3. Interestingly, this peak in chlorophyll-a early in the system occurred at a point where phytoplankton numbers were usually low, and coincided with the period over which overall flow rate (and so nutrient loading) to the

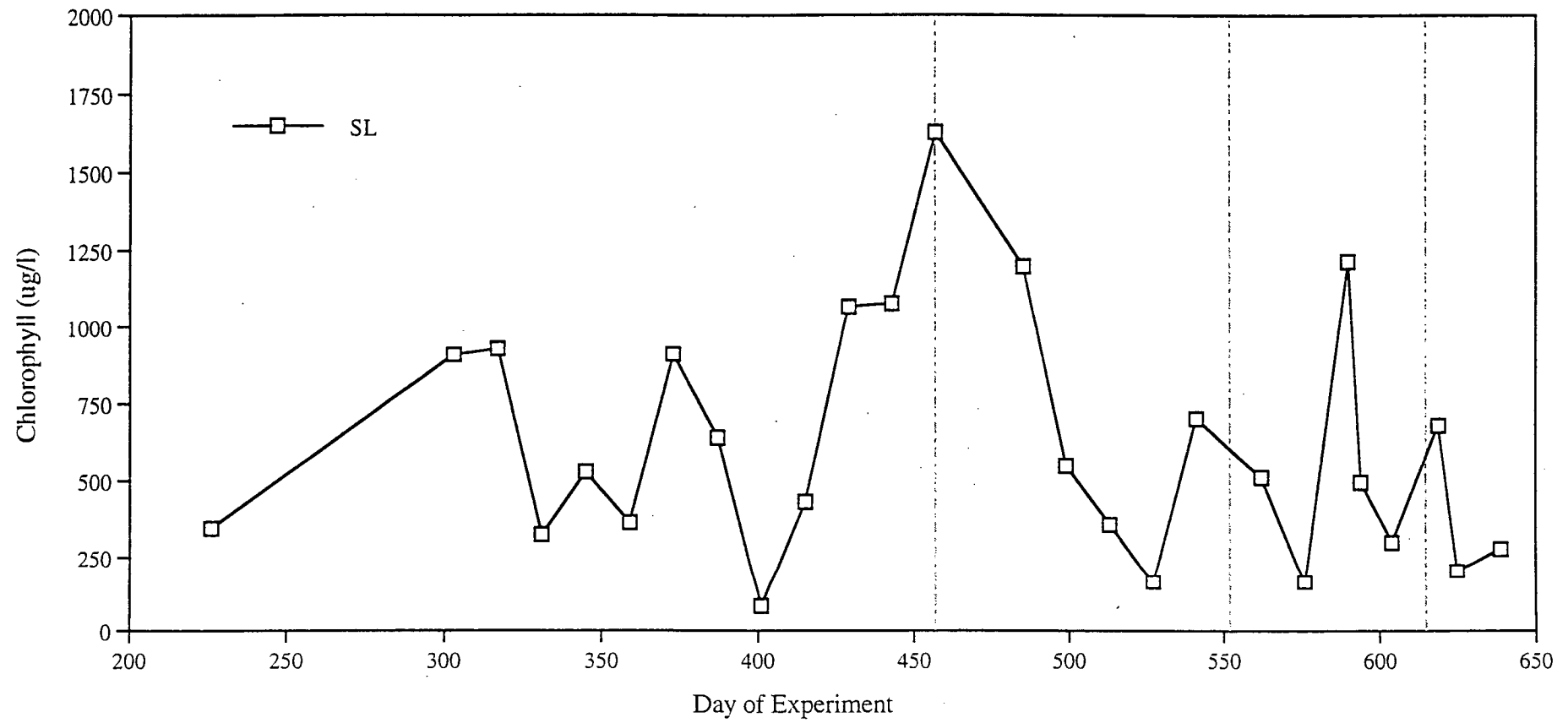


Figure 4.11(a): Chlorophyll levels in the SL pond (85wB) over the main experimental period.

85wB system was lowest (Section 4.3).

The response of chlorophyll-a levels to the experimental (as opposed to operational) changes in flow was also interesting. Again, all pond pairs displayed a marked early difference in chlorophyll-a levels, which steadily gave way to similar general patterns and levels between pond pairs for the remainder of the equilibration period. While some small degree of variation occurred between the earlier pond pairs following the first division of flow, these differences were much more marked in the later pond pairs, and magnified with time. While seasonal effects may certainly have played a role in the levels of growth exhibited here, it is the *difference* in levels and patterns of growth that indicate the effects of the different flow divisions on the phytoplankton in the 85wB.

As with nitrate, nitrite and phosphate levels, the divergence of these patterns between paired lagoons can be seen as the result of the changes in flow/nutrient loading, and was again likely to have been complicated and altered from a single pattern of response by the differing cycles becoming established within those lagoons. This is well illustrated by the dramatically out-of-phase peaks in lagoons 3N and 3S after Day 500, followed by the relatively large drop in levels for the (low-flow) 3S during the second drought-affected flow division (Section 4.3) and its subsequent recovery into the third. Only pond pair 5 exhibited a pattern that can be interpreted as directly reflecting the changes in flow, with 5N displaying noticeably higher chlorophyll levels than 5S during the first and third flow divisions, but more similar levels for at least the start of the second.

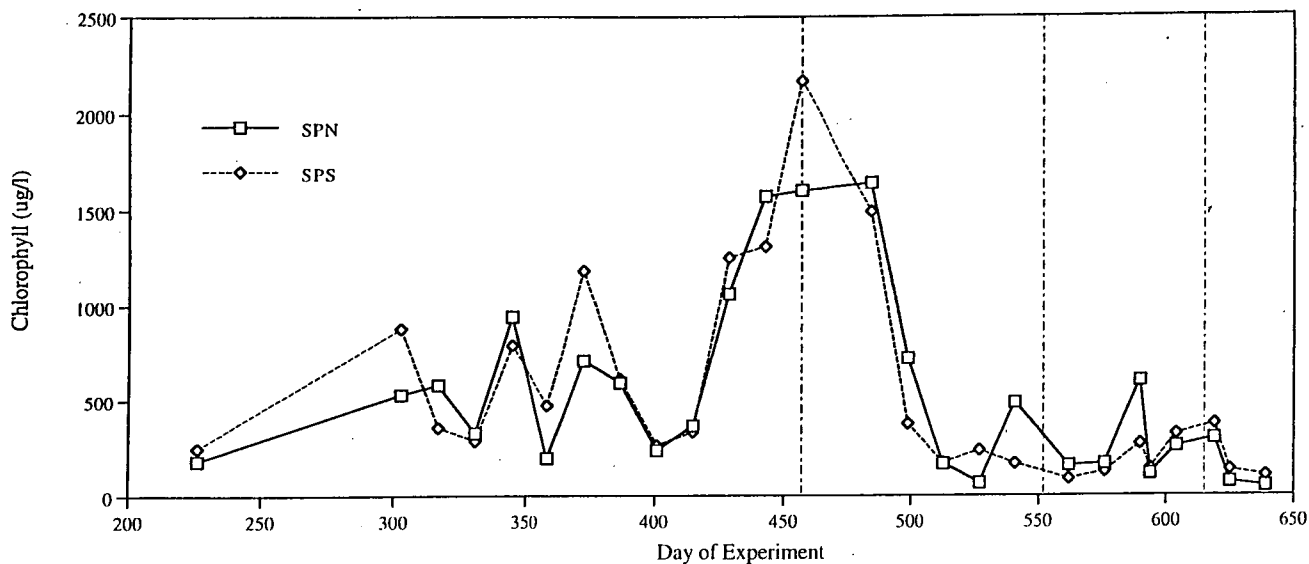


Figure 4.11(b): Chlorophyll levels in the SPN and SPS ponds (85wB) over the main experimental period.

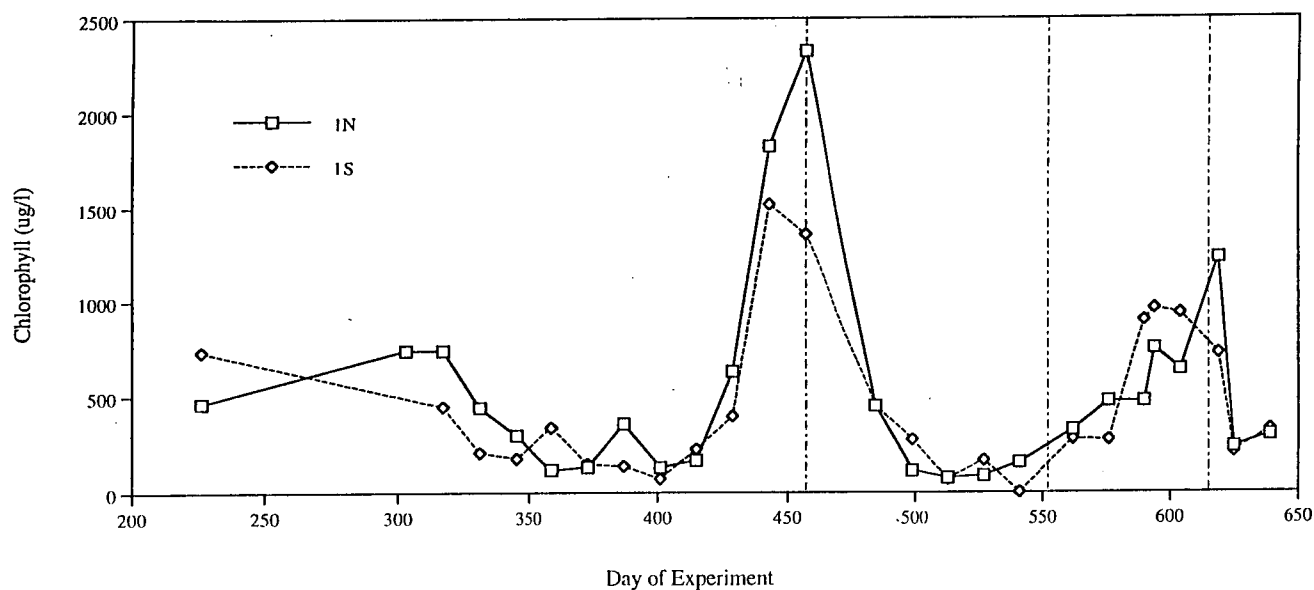


Figure 4.11(c): Chlorophyll levels in the 1N and 1S ponds (85wB) over the main experimental period.

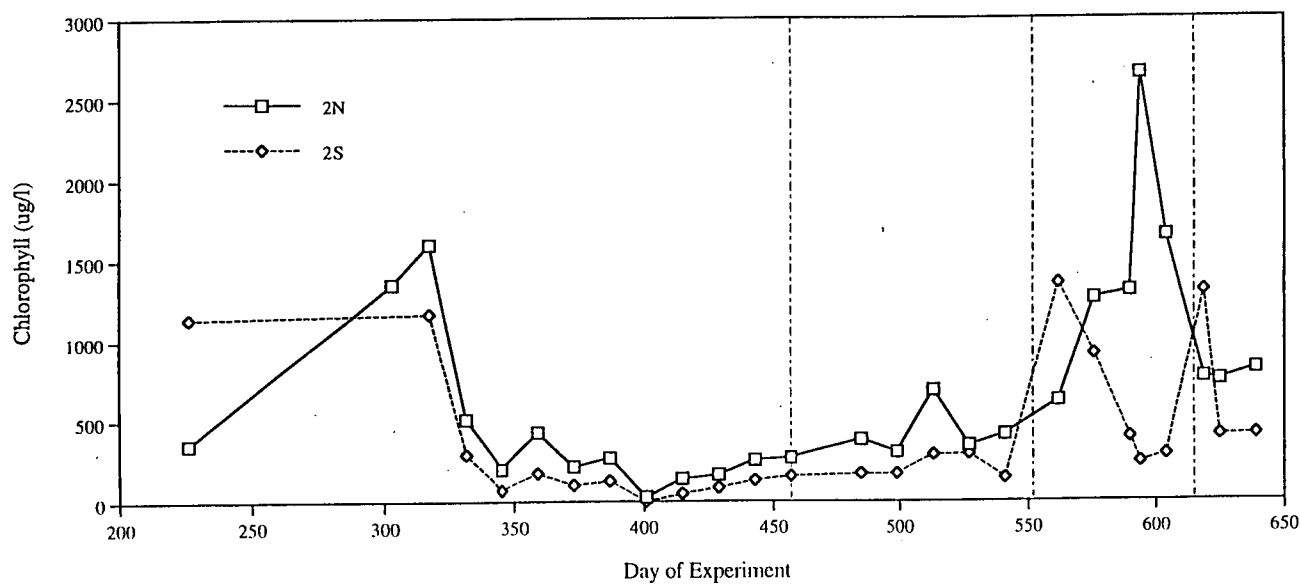


Figure 4.11(d): Chlorophyll levels in the 2N and 2S ponds (85wB) over the main experimental period.

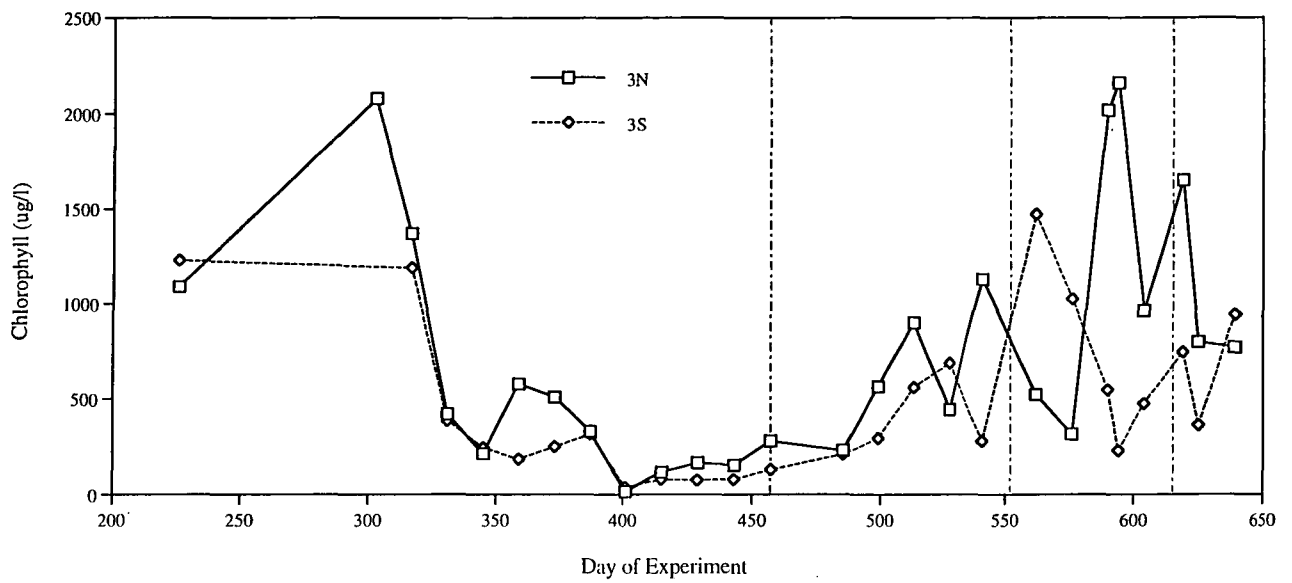


Figure 4.11(e): Chlorophyll levels in the 3N and 3S ponds (85wB) over the main experimental period.

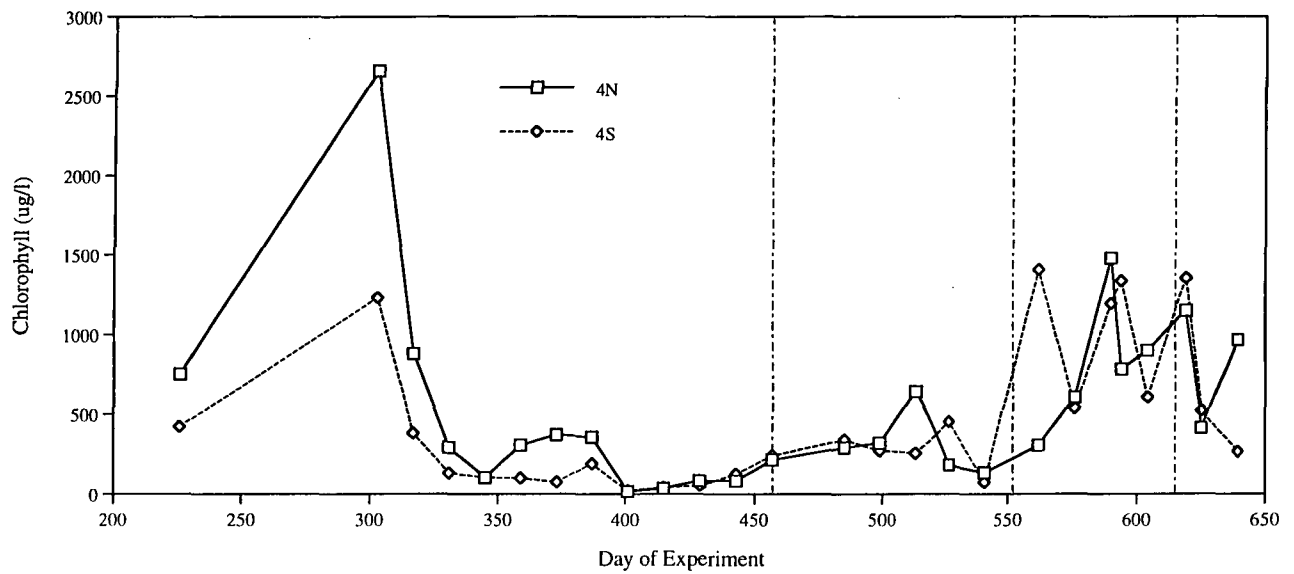


Figure 4.11(f): Chlorophyll levels in the 4N and 4S ponds (85wB) over the main experimental period.

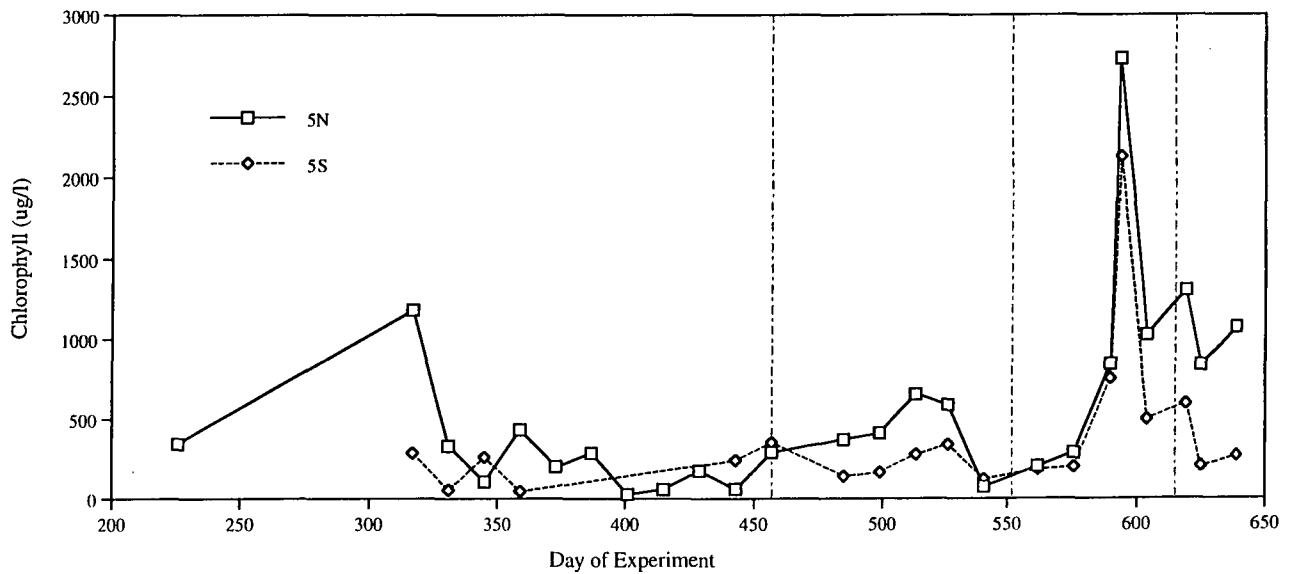


Figure 4.11(g): Chlorophyll levels in the 5N and 5S ponds (85wB) over the main experimental period.

**Table 4.1: Taxa absences.** R = rotifers, C = crustaceans, NA = sample not available.

[illegible]

#### 4.5.2 Zooplankton - general trends

The general trends in zooplankton abundance are shown in Figures 4.13-4.22, and include the taxa that are subjected to more rigorous analysis in Section 4.5.3. Recorded values (individuals per litre) have been double square-root transformed to allow more workable comparisons between taxa.

##### *Rotifers*

The total abundance of rotifer species is shown in Figure 4.13(a-g). In general, rotifer numbers tended to be most numerous at the start of the system, usually peaking in the SP pond rather than SL, and then progressively declining through the subsequent ponds. Ponds from which rotifers were completely absent all occurred towards the end of the lagoon system (Table 4.1), and this overall pattern is in keeping with that identified for the system as a whole (Figure 4.1).

Total rotifer numbers in the SL pond tailed off into winter, before rising again through spring to peak in summer. Two particular peaks at the start and towards the end of the second flow division correspond to points at which flow rate and ammonia levels were low (Figure 4.3). Total rotifer numbers varied from this pattern in the following ponds, but remained relatively consistent with each other. In these ponds, rotifer numbers still fell over the winter period, but not to as low a level. Total numbers also tended to peak earlier (in spring) and then tailed off during summer. The exception to this was the final pond pair (5N+5S), where rotifer numbers tended to remain constant and low, with the exception of a high 5S peak at the start of the third flow division.

Although partly seasonal, the SP pond showed a marked jump in total rotifer numbers following its promotion in sequence between the original flow pattern and the equilibrium period. Similarly, pair 1N & 1S, which displayed a marked initial difference in total rotifer numbers under the original flow regime, became more similar with the alteration of flow pattern and establishment of equal

flow between the halves. In contrast, pairs 2N+2S and 3N+3S, which had been directly connected in sequence, showed much less variation under the original flow pattern than under the equal division of flow.

Overall, corresponding ponds paired relatively well during the equilibrium period, but rarely presented perfect matches. Sufficient variability existed between these ponds that differences resulting from unequal divisions of flow were not particularly pronounced and, without further analysis (as per Section 4.5.3), are not of themselves convincing. However, some patterns do emerge from the noise.

As with nutrient concentrations, the SP outlets remained closely paired in terms of total rotifer numbers throughout the three flow divisions, despite the differences in flow across the two halves of the pond. Although SPN and SPS dominated in total rotifer numbers at the start of the second and third flow divisions, respectively, there was little overall pattern in difference or dominance between the two sides at this stage in the system. A slightly more defined pattern developed with ponds 1N+1S, however. These ponds remained very closely paired at the start of the first flow division before 1S (high flow) came to dominate in terms of total rotifer numbers. Pond 1N then dominated for the duration of the second flow division, before 1S returned to dominance for the third. This general pattern was followed by subsequent pond pairs, with some degree of cycling. The pattern became less consistent with pond pairs 4 and 5, where nutrient levels were much lower and pond dimensions less comparable, and also showed some variation towards the end of the third flow division.

These system-based, seasonal and flow-related patterns for total rotifer numbers largely resulted from the combined dynamics of *Brachionus* and *Polyarthra* species (Figures 4.14(a-g) & 4.15(a-g), respectively). *Brachionus* numbers were similar in both the SL and SP lagoons, while *Polyarthra* peaked in the latter, and numbers of both taxa decreased across the system. In earlier ponds, *Brachionus* numbers were high in late autumn, fell over winter before peaking in spring, and fell again over summer. Later in the system and as overall numbers fell, the secondary peak in late autumn disappeared, and winter numbers fell to zero, while, in the final pond pair, the peak



shifted into summer. In contrast, *Polyarthra* numbers tended to be high over early winter, fell briefly before peaking in spring, and generally fell again over summer. *Polyarthra* numbers again fell dramatically across the system, and in later ponds the genus was exclusively found in winter and early spring.

In combination, *Brachionus* and *Polyarthra* contributed heavily to the shape of the total rotifer graphs. In later ponds these patterns tended to be driven by *Polyarthra* over the winter period and by *Brachionus* over spring and summer. The SP pond showed a marked difference in *Brachionus* numbers between the original flow pattern and the equilibration period (again incorporating a seasonal component), and the initially 1N and 1S ponds became markedly more similar in numbers of this genera. While *Brachionus* did exhibit some similarity to the pattern of total rotifers over the three flow divisions, *Polyarthra* matched it particularly well for most pond pairs, with the respective high flow sides tending to dominate in numbers.

*Filinia* numbers (Figure 4.16) also followed similar patterns. This genus was far more numerous in the SP pond than in SL, and numbers progressively fell across subsequent ponds in the system. Two particularly high peaks in the SL pond occurred at the start and towards the end of the second flow division and, as with similar but less pronounced peaks in *Brachionus* and *Polyarthra* numbers for that pond, these corresponded to reduced flow rate and ammonia levels at those times.

Across the system, *Filinia* displayed two distinct seasonal peaks, with one in late autumn and the second in early summer. In all lagoons, *Filinia* showed a more dramatic winter decrease than the preceding taxa, falling to zero abundance in all cases. In later ponds (pairs 3-5), the genus was only found at times corresponding to its seasonal peaks.

Whether due to seasonal differences or altered flow, *Filinia* numbers markedly increased in the SP pond between the original flow pattern and the equilibrium period, although ponds 1N and 1S displayed little initial difference in *Filinia* numbers, and general pairings showed some degree of variation. Despite this, *Filinia*

numbers, when present, strongly reflected the high flow side in each of the flow divisions beyond the SP pond. This was demonstrated by dominance of numbers on the respective high flow sides within each period, by the markedly higher peaks that dominance entailed, and the occurrence of several solitary peaks on the high flow side when *Filinia* were absent from the corresponding low flow ponds.

Numbers of *Asplanchna* (Figure 4.17(a-g)), a predator, were generally lower across the system than were the preceding genera. When present, numbers were highest the SP pond and pair 1, and relatively constant throughout the rest of the system. The genus peaked around late winter, and may have persisted in the SL lagoon due to the availability of ciliate prey. Asplanchnid numbers otherwise followed a more erratic pattern over spring and summer across all ponds. Beyond this, the genus showed little by way of meaningful results for the change from the original flow pattern to the equilibration period, and no readily apparent pattern matching the divisions of flow.

### *Crustaceans*

The total abundance of crustacean species (double square-root transformed) is shown in Figure 4.18(a-g). Crustacean numbers were generally absent or low earlier in the system, before dominating at lower overall but more consistent numbers than the rotifers. Although the bulk of crustaceans appeared after the SP pond rotifer peak, total crustacean numbers showed some overlap with rotifers, particularly in pond pairs 1 and 2. Crustacea were only completely absent from ponds at the start of the 85wB (Table 4.1), and this overall pattern is in keeping with that identified for the system as a whole (Figure 4.1). Notably, total crustacean numbers were relatively consistent within the SP pond over the winter prior to the alteration of flow pattern (when the pond was situated fourth in series), but were mostly absent over the corresponding season during the equilibration period (when the pond was second in series).

Total crustacean numbers tended to build towards a summer peak in all ponds, including SL and SPS. Minimum crustacean levels

occurred over winter. These levels represented zero abundance in the most nutrient enriched ponds (SL and SP), became less dramatic in the intermediate ponds (pairs 1, 2 and 3), and started to become more pronounced again (but did not fall to zero) in the least nutrient enriched ponds (pairs 4 and 5). These troughs were matched by corresponding peaks in *Asplanchna* numbers in the lagoons (Figure 4.17).

As with total rotifers, pairings between corresponding ponds were variable, and undoubtedly complicated by population and other biological cycles within the lagoons. Additional complications with the Crustacea may also have included potentially longer response times in these taxa, their broader ecological range, and, in regard to the graphs of individual groups that follow, the lower level of taxonomic resolution available in the data. Without the desired (higher) level of flow rate into the 85wB promoting greater change (Section 4.3), differences resulting from the unequal divisions of flow were again largely masked by this noise, and the results again inconclusive without more rigorous analysis (Section 4.5.3). Keeping this in mind, however, some general trends can be identified from the graphs of total crustacean numbers and the individual groups they contain.

Although inconsistencies occurred, total crustacean numbers tended to be higher on the low flow side for the SP pond and pairs 1 & 2. This was reversed for pair 3, with total crustacean numbers tending to be dominant on the high flow side. For pairs 1 and 3, however, the respective changes in dominance preceded changes in flow, so that high and low flow ponds, respectively, were dominant at the end of each of the first two flow divisions. This pattern was repeated in pair 4, where - as with pair 3 - high flow numbers were initially dominant for the first and second flow divisions, before being exceeded their low flow counterparts. This swap in dominance occurred earlier in pair 4 than in the preceding pairs. The pattern repeated again in pair 5, but by this stage was becoming more variable. (This pattern was largely driven by, and can be more clearly seen in, the copepod nauplii fraction of the crustacean zooplankton, as described below).

Within the Crustacea, *Daphnia* (Figure 4.19(a-g)) was only

rarely collected from the SL pond, and only then over summer. Daphnid numbers were also low in the SP pond, and (post flow-change) restricted to the same time of year, if over a slightly wider period. Daphnids were subsequently common through the remainder of the system at relatively consistent levels, again peaking in summer for pair 1, showing little seasonal variation for pairs 2 and 3, then tending towards a less pronounced late spring/early summer peak for pairs 4 and 5.

Although consistently present in the SP pond over winter under the original flow pattern (when it was fourth in series), daphnids were absent from this pond at this time during the equilibration period. Ponds 1N and 1S showed a corresponding dramatic change, becoming markedly more similar in terms of daphnid populations. Pond 1N developed a healthy daphnid population where previously there had been none, while abundance fell in 1S. The (originally linked) pairs 2 and 3 continued to contain similar daphnid numbers - and varied from each other to the same degree - as under the original flow pattern. For the three flow divisions, daphnid numbers remained similar between the SP outlets, and tended to remain higher on the northern side of the system irrespective of prevailing flow. Pairs 3, 4 and 5 displayed a mixture of alternating peaks and dominance on the high flow side, but were not obviously consistent in pattern.

Moinid cladocerans (Figure 4.20(a-g)) were more common in the earlier lagoons than *Daphnia*. They were found in higher numbers in both the SL and SPN ponds, and, while still restricted seasonally (to spring and summer), they were found over a longer period in these lagoons. *Moina* peaked and declined in late spring in SL, and in early summer in SP. Numbers were higher in the SP pond than in SL. Moinids developed at lower but more consistent numbers in pond pair 1, peaking in early and late spring and persisting year round. Unlike *Daphnia*, populations declined and became more sporadic in subsequent lagoon pairs, peaking in steadily lower numbers over late winter/spring, becoming absent in summer, and highly variable in general occurrence.

As with *Daphnia*, moinid numbers had been consistently present in the SP pond over the winter prior to the alteration of flow, but were absent over this period during the equilibrium period. Ponds 1N and 1S, however, did not show as dramatic a change in moinid abundance. During the three flow divisions, there was a tendency for moinid numbers to dominate on the low flow side of the SP pond. This reversed with pairs 1 and 2, where (after the start of the first flow division) higher *Moina* numbers tended to be found on the high flow side. Although this pattern continued into pair 3, moinid populations were highly variable by this point in the system, and ceased to provide meaningful or consistent results.

Again as with *Daphnia*, cyclopoid copepods (Figure 4.21(a-g)) were rare in the SL pond, where they were only found in limited numbers in summer. These numbers were higher in the SP pond, where cyclopoids were also found in low numbers in late spring. In both ponds, population peaks occurred in late summer. While cyclopoid numbers did not increase within subsequent lagoons, they became more common throughout the yearly cycle. Pond pairs 1, 2 and 3 displayed mid- to late spring peaks, while pairs 4 and 5 peaked in mid to late summer. In all ponds the cyclopoid populations appeared highly variable, with the exception of pair 2 where numbers were comparatively stable.

As with both *Daphnia* and *Moina*, cyclopoids had been present in the SP pond in the winter prior to the alteration of flow pattern in the 85wB, but were absent at the corresponding time during the equilibrium period. Cyclopoid numbers also underwent dramatic changes in 1N and 1S, which became markedly more similar under the new flow regime, while pairs 2 and 3 retained similar characteristics as before the change. Despite these trends, however, the highly variable and cyclic nature apparent in the cyclopoid populations defy identification of any underlying patterns related to the three divisions of flow.

Copepod nauplii (Figure 4.22(a-g)) represented a substantial portion of the crustacean zooplankton, and are responsible for much of the detail in the total crustacean plots (Figure 4.18(a-g)). In general,

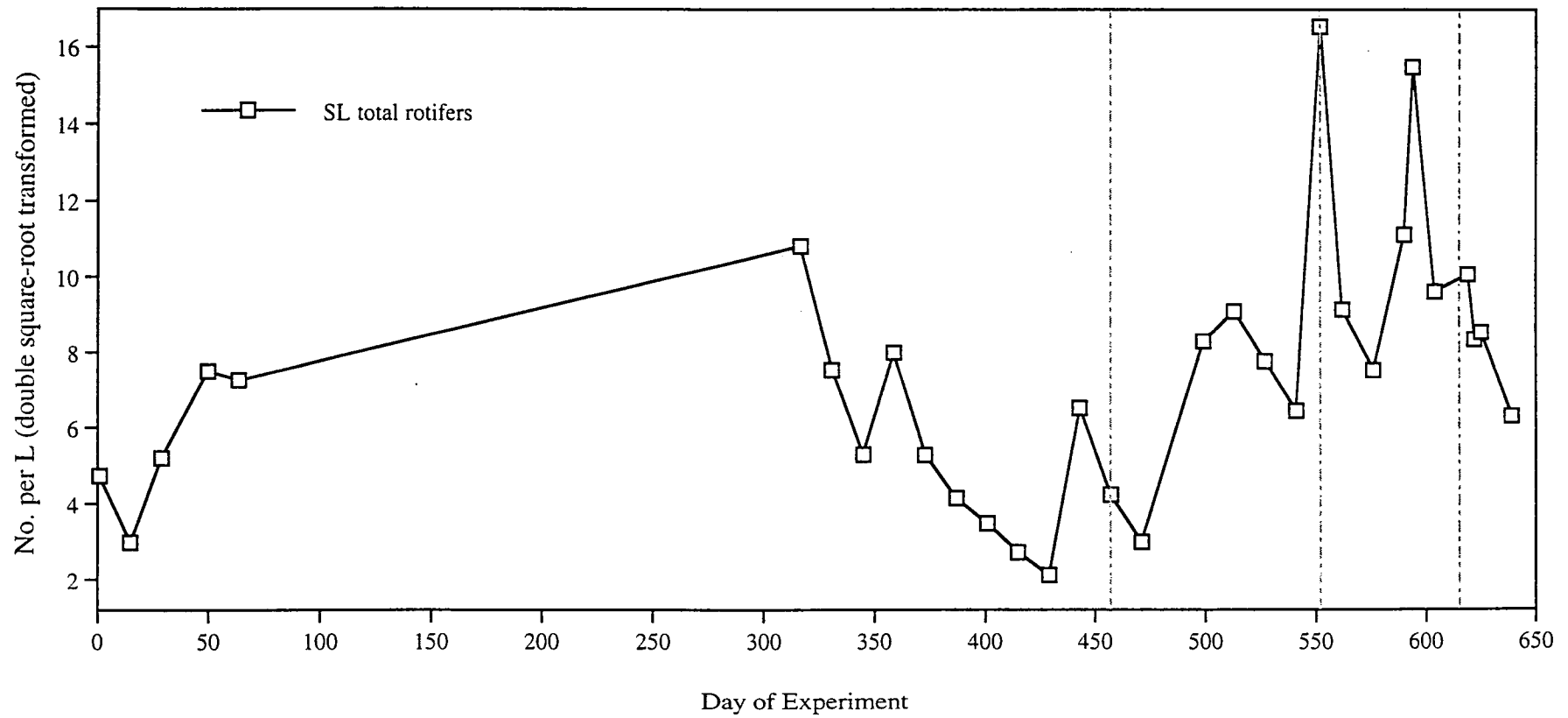
nauplii followed the trends set by the preceding crustacean groups. Numbers were minimal in the SL and SP ponds, with nauplii absent for much of the year before rising to a summer peak. In subsequent ponds, numbers peaked from late spring to summer, and were present throughout the year. Although winter troughs were present in pairs 1-5, this was relatively reduced in pair 2: the same pair in which cyclopoid numbers were markedly more stable. Seasonal variations aside, nauplii numbers were relatively similar across pond pairs 1-5, and were themselves far more stable than those for cyclopoids.

As with the other crustacean groups, nauplii were present in the SP pond in relatively consistent numbers over the winter period preceding alteration of flow, but were absent or reduced over the same period during the equilibrium period. In turn, nauplii responses to the different divisions in flow rate followed a curious pattern, as partially addressed in the description of total crustacean plots above.

In pair 1, the respective low flow pond exhibited higher nauplii numbers for most of the first and second flow divisions, but in both cases the high flow numbers became dominant towards the end of the flow period<sup>2</sup>. This pattern was consistent but reversed for pairs 2, 3 and 4, where the high flow side dominated initially before being exceeded by low flow numbers later within the division. Similar patterns can be seen for pair 5, but were becoming more variable by this point in the system. These patterns indicated a noticeably greater lag effect in this group than in the responses by the rotifers (and, to some degree, *Moina*), and, combined with the lack of discernible patterns in daphnids and cyclopoids, suggests that crustacean responses as a whole were much more complicated.

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<sup>2</sup> The third flow division was not conducted for long enough for this to occur again.



**Figure 4.13(a): Total rotifers (double square-root transformed) in the SL pond (85wB) over the main experimental period.**

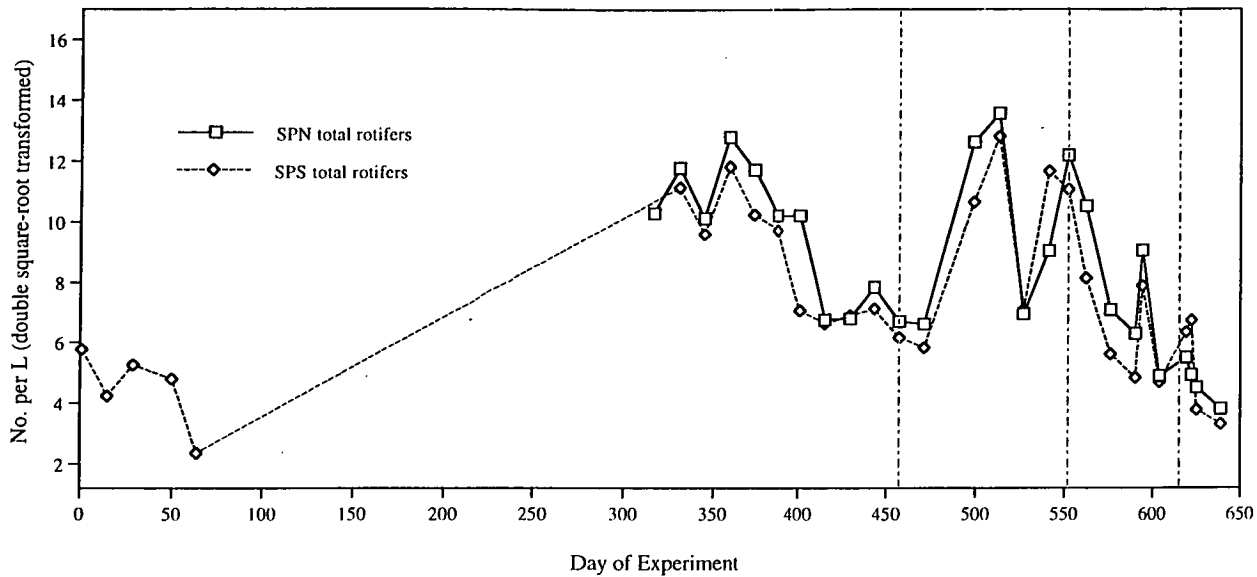


Figure 4.13(b): Total rotifers (double square-root transformed) in the SPN + SPS ponds (85wB) over the main experimental period.

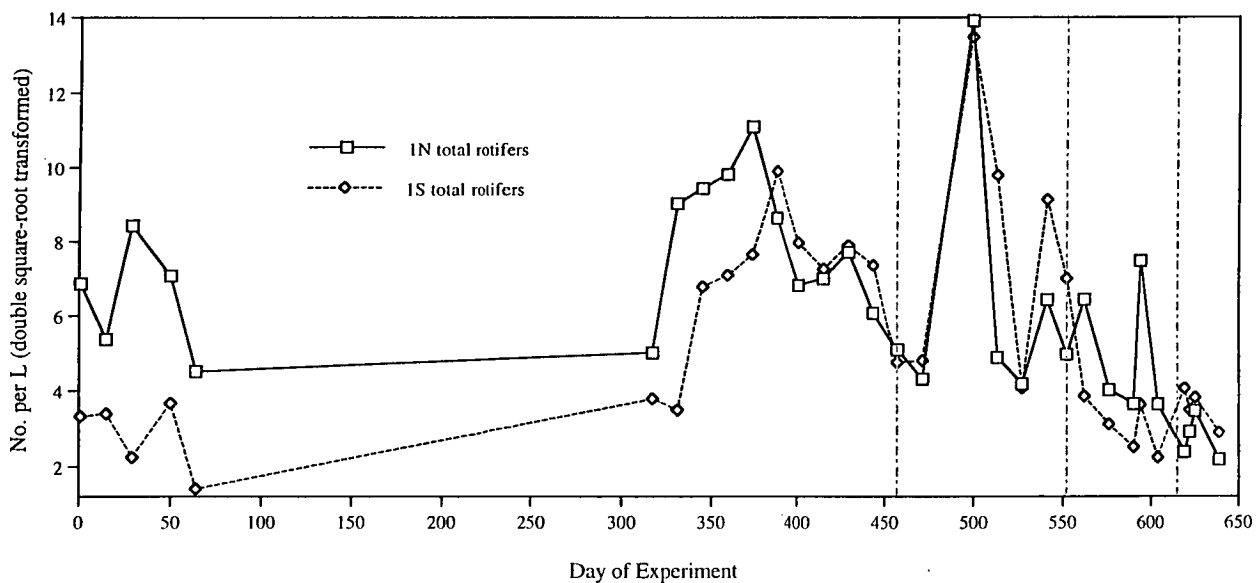


Figure 4.13(c): Total rotifers (double square-root transformed) in the 1N+1S ponds (85wB) over the main experimental period.

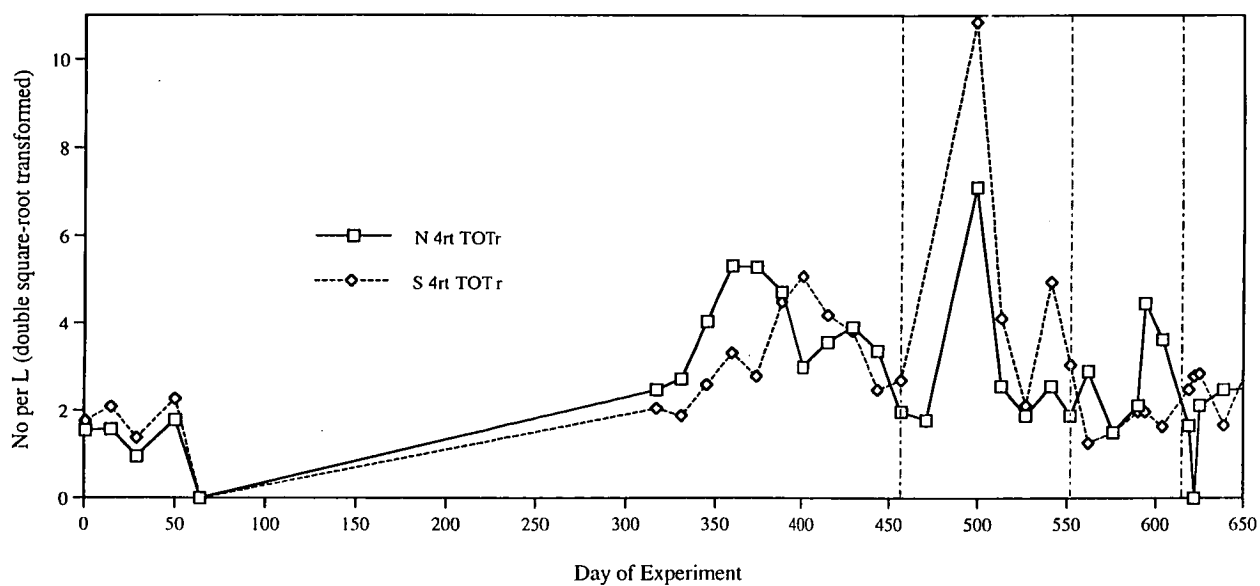


Figure 4.13(d): Total rotifers (double square-root transformed) in the 2N+2S ponds (85wB) over the main experimental period.



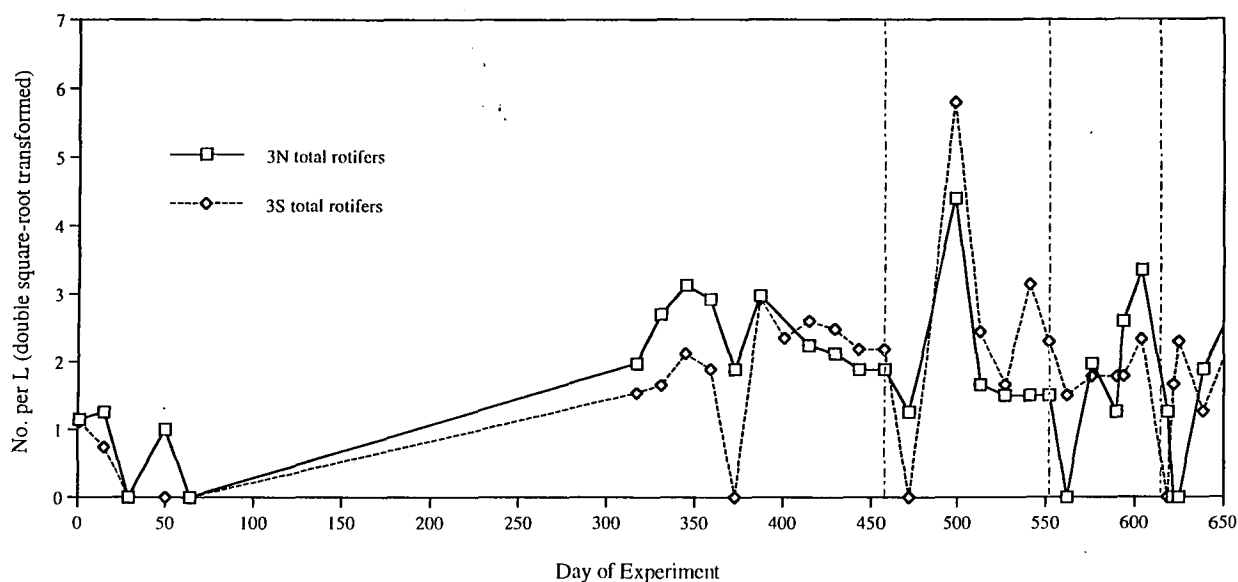


Figure 4.13(e): Total rotifers (double square-root transformed) in the 3N+3S ponds (85wB) over the main experimental period.

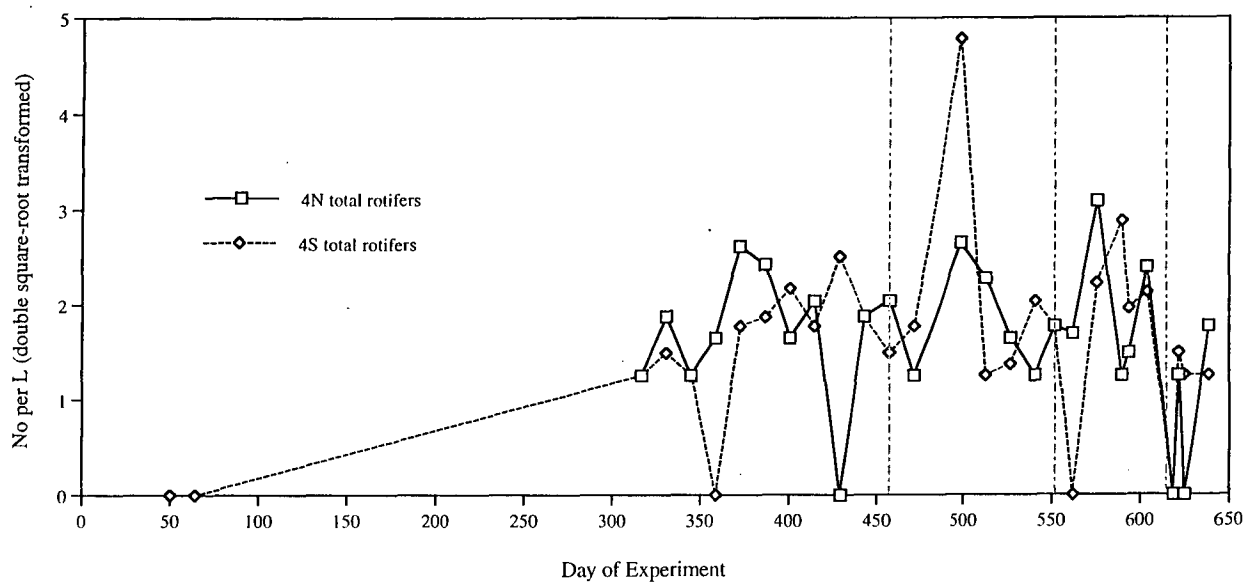


Figure 4.13(f): Total rotifers (double square-root transformed) in the 4N+4S ponds (85wB) over the main experimental period.

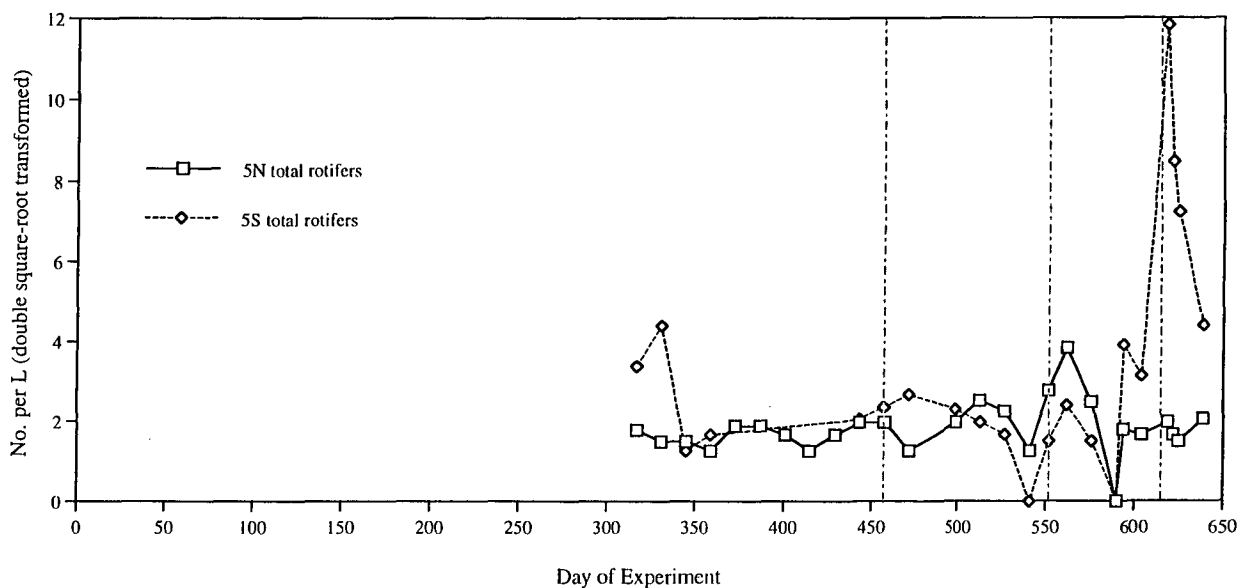
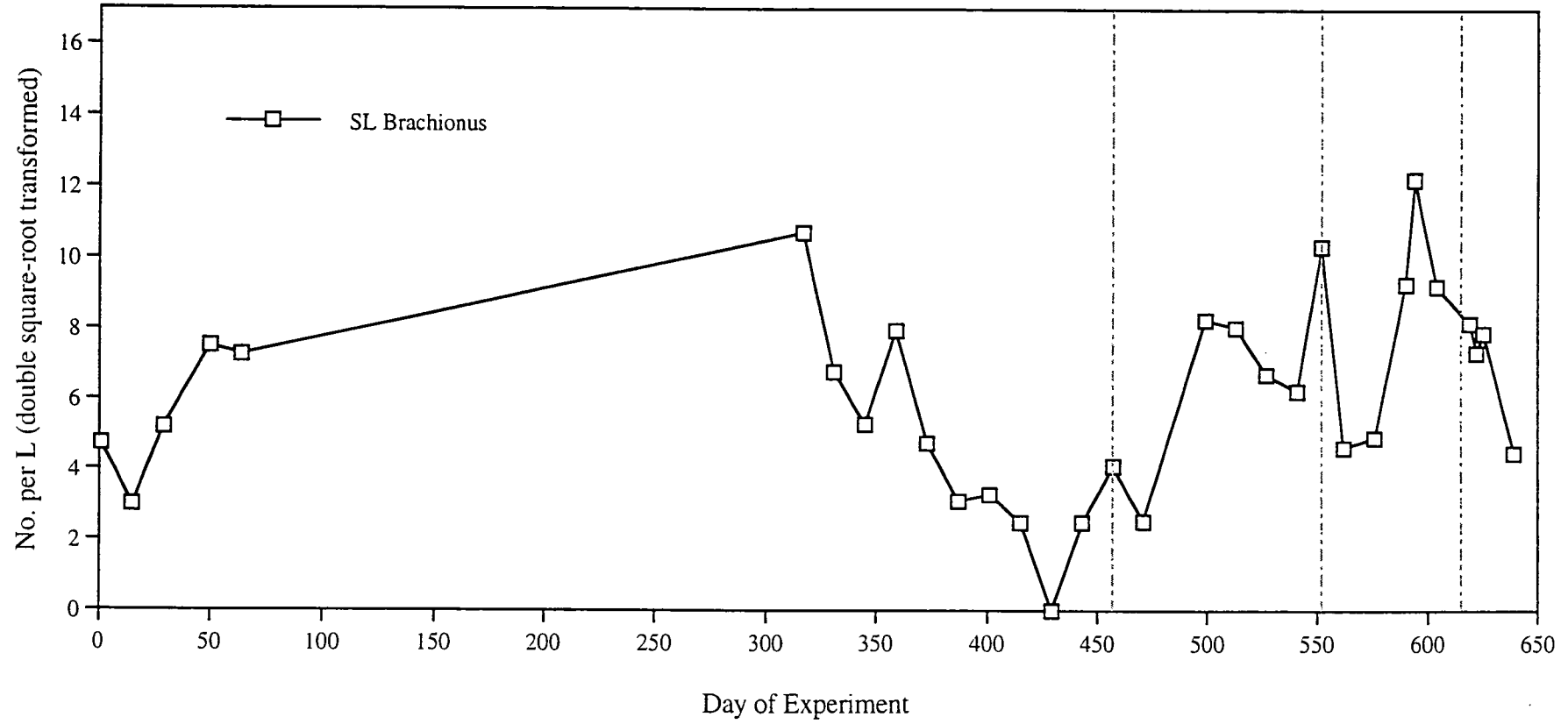
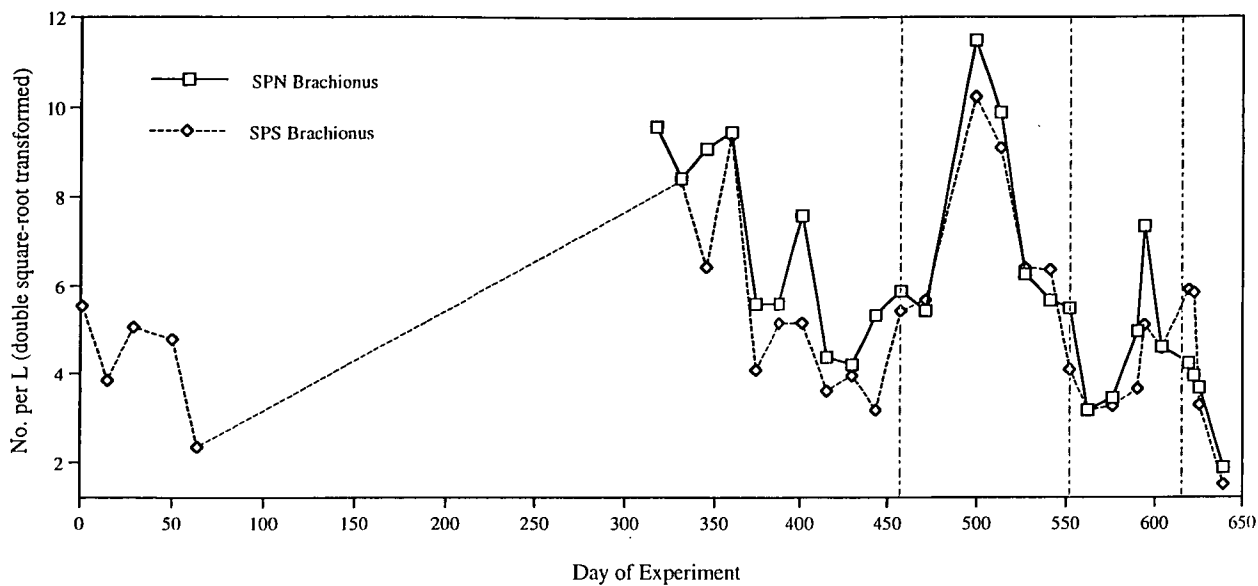


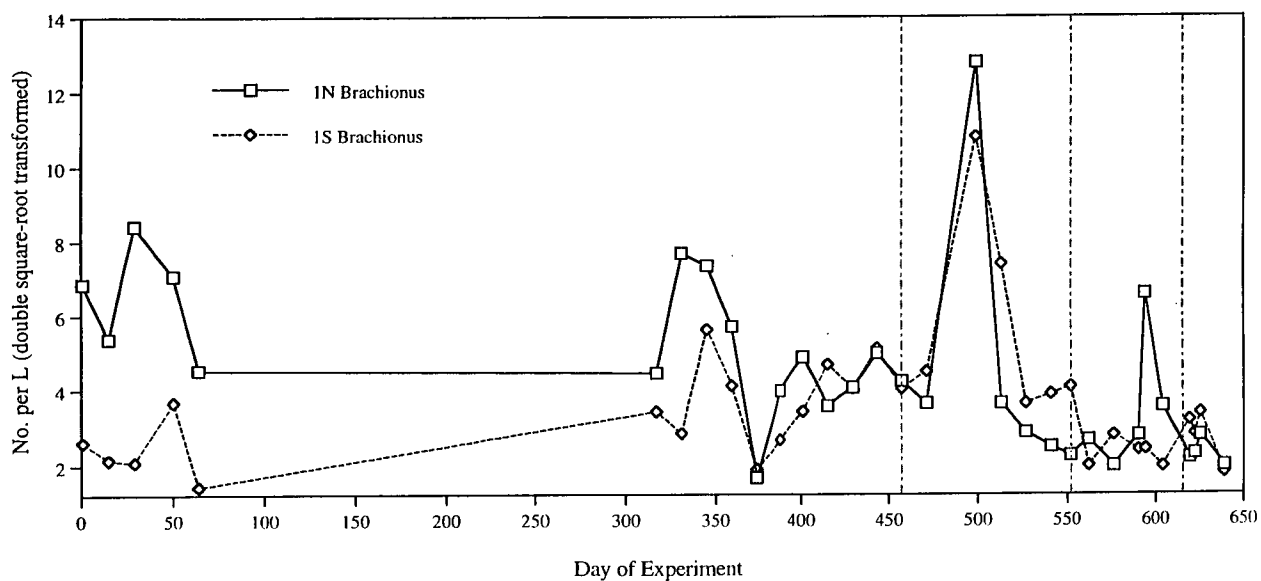
Figure 4.13(g): Total rotifers (double square-root transformed) in the 5N+5S ponds (85wB) over the main experimental period.



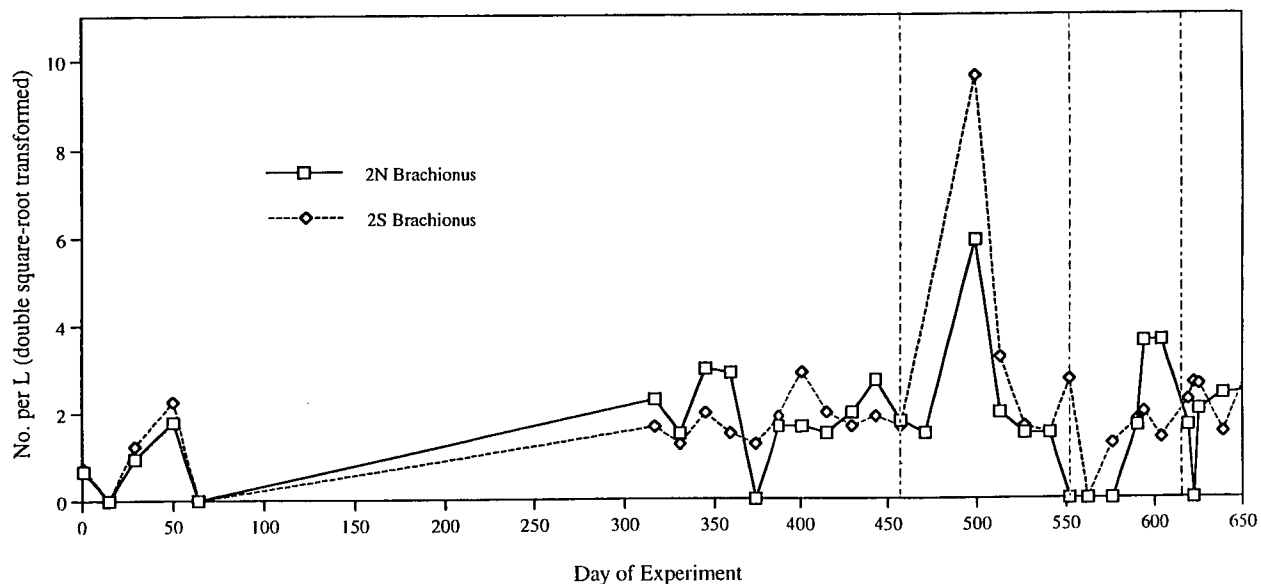
**Figure 4.14(a): Brachionus numbers (double square-root transformed) in the SL pond (85wB) over the main experimental period.**



**Figure 4.14(b): Brachionus numbers (double square-root transformed) in the SPN + SPS ponds (85wB) over the main experimental period.**



**Figure 4.14(c): Brachionus numbers (double square-root transformed) in the 1N+1S ponds (85wB) over the main experimental period.**



**Figure 4.14(d): Brachionus numbers (double square-root transformed) in the 2N+2S ponds (85wB) over the main experimental period.**

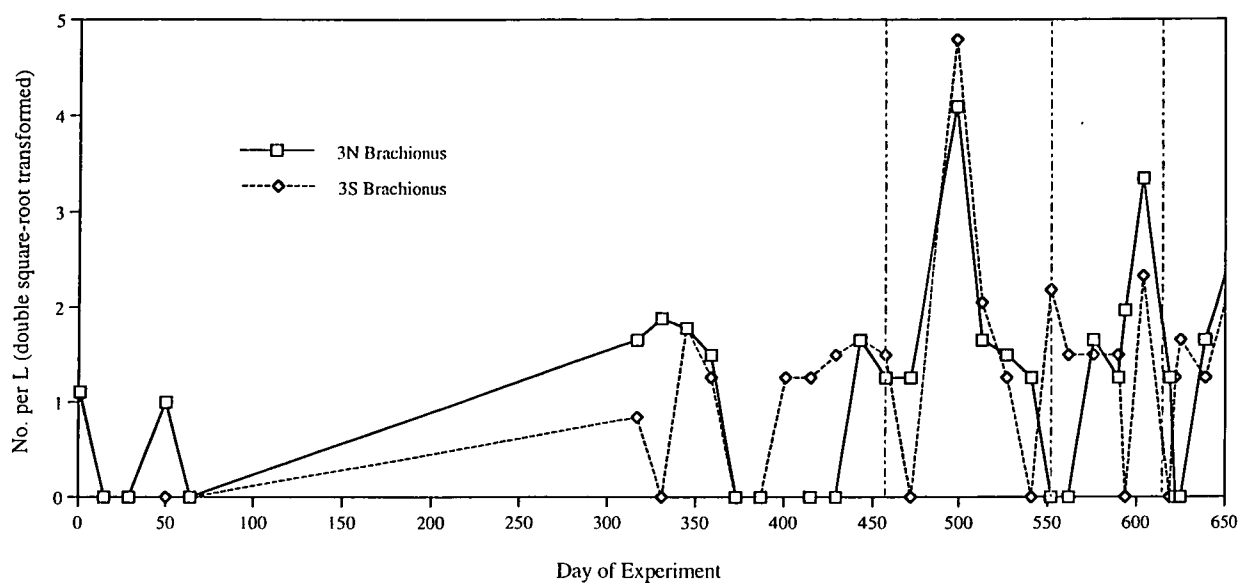


Figure 4.14(e): Brachionus numbers (double square-root transformed) in the 3N+3S ponds (85wB) over the main experimental period.

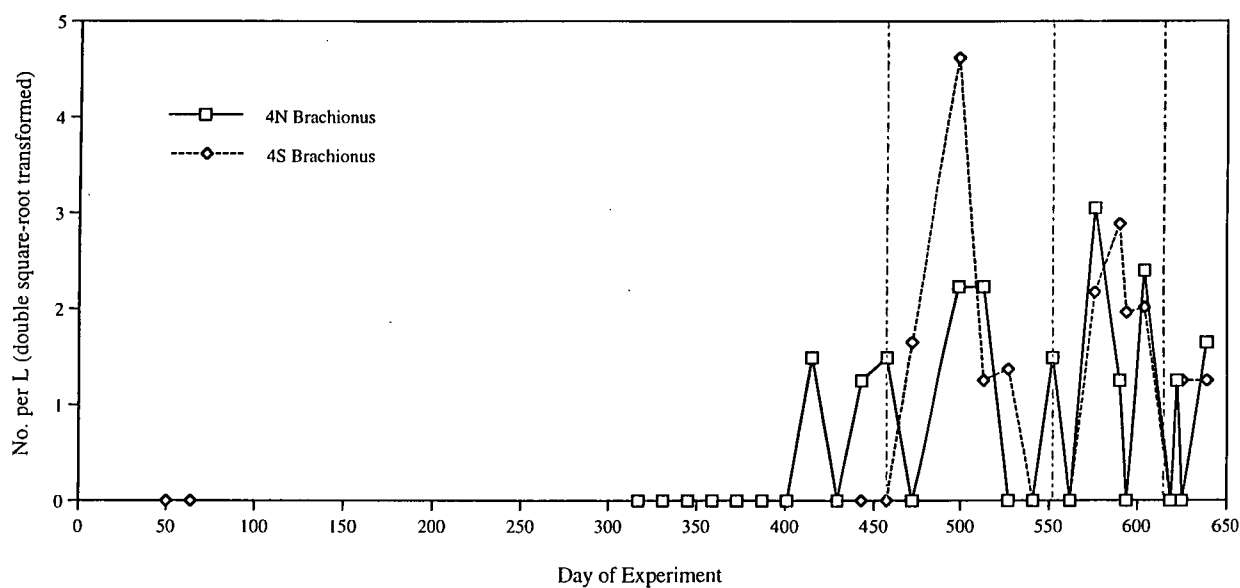


Figure 4.14(f): Brachionus numbers (double square-root transformed) in the 4N+4S ponds (85wB) over the main experimental period.

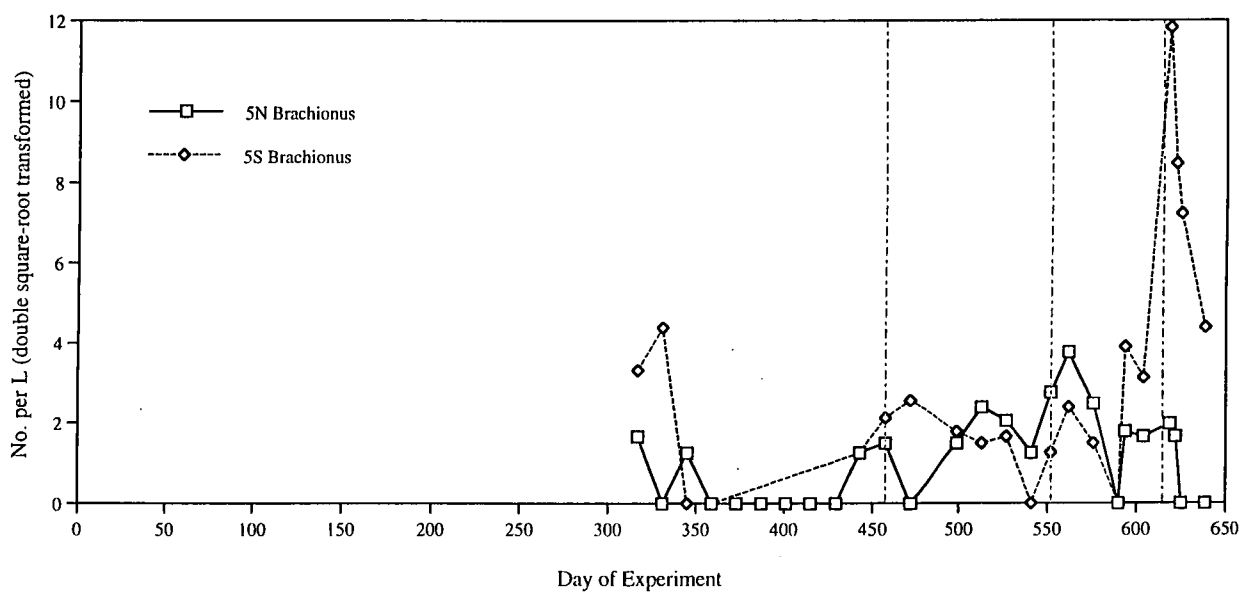
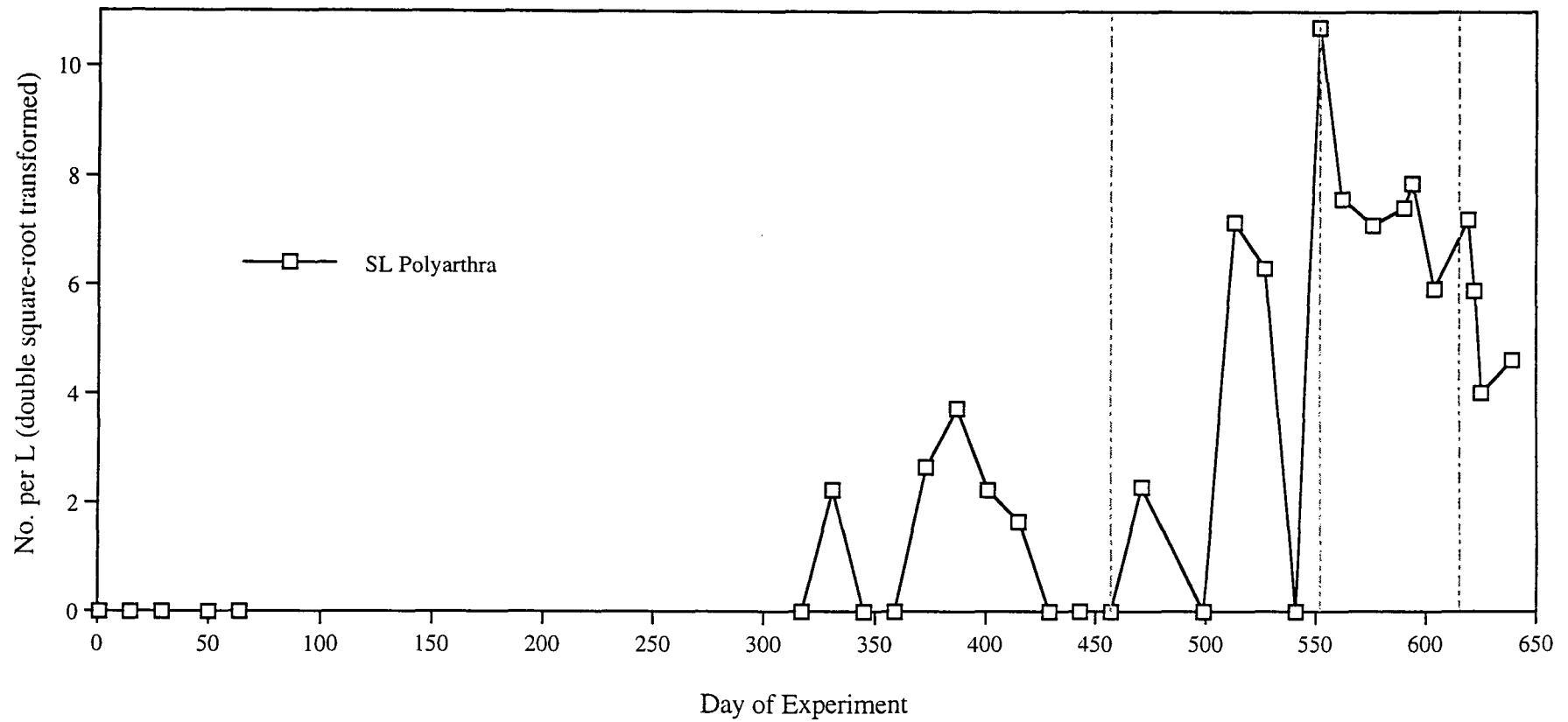
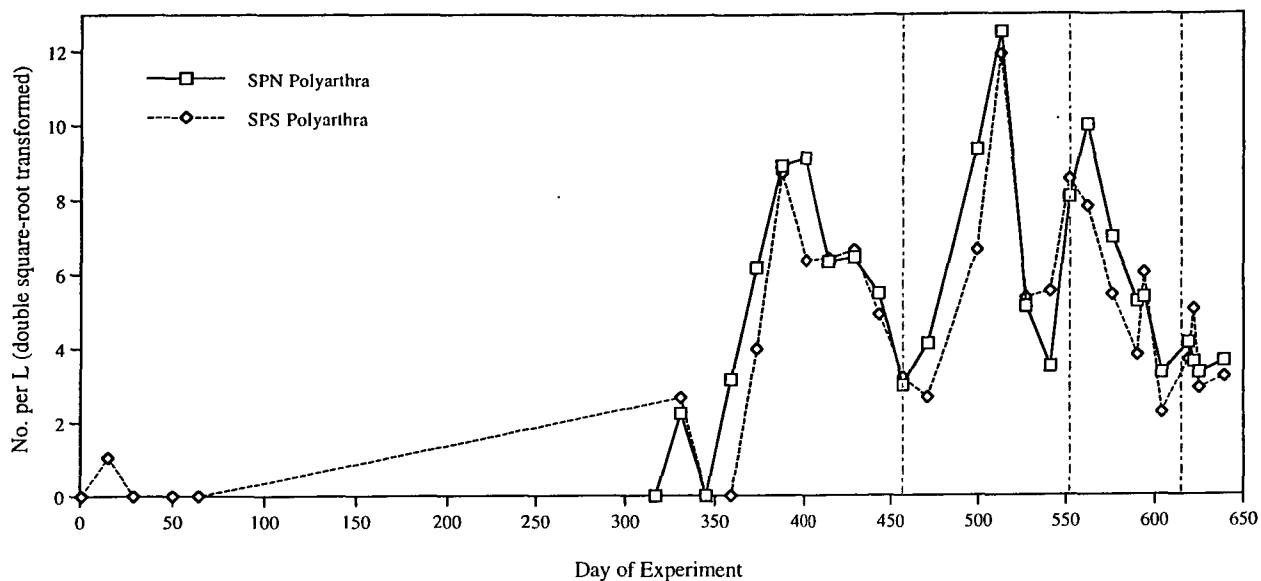


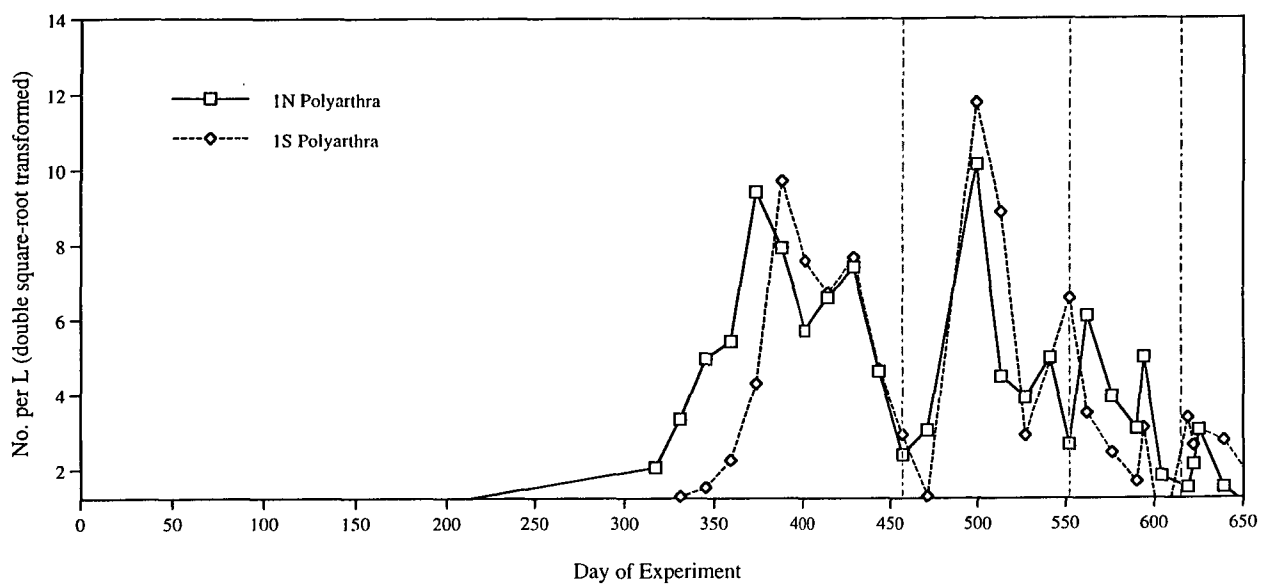
Figure 4.14(g): Brachionus numbers (double square-root transformed) in the 5N+5S ponds (85wB) over the main experimental period.



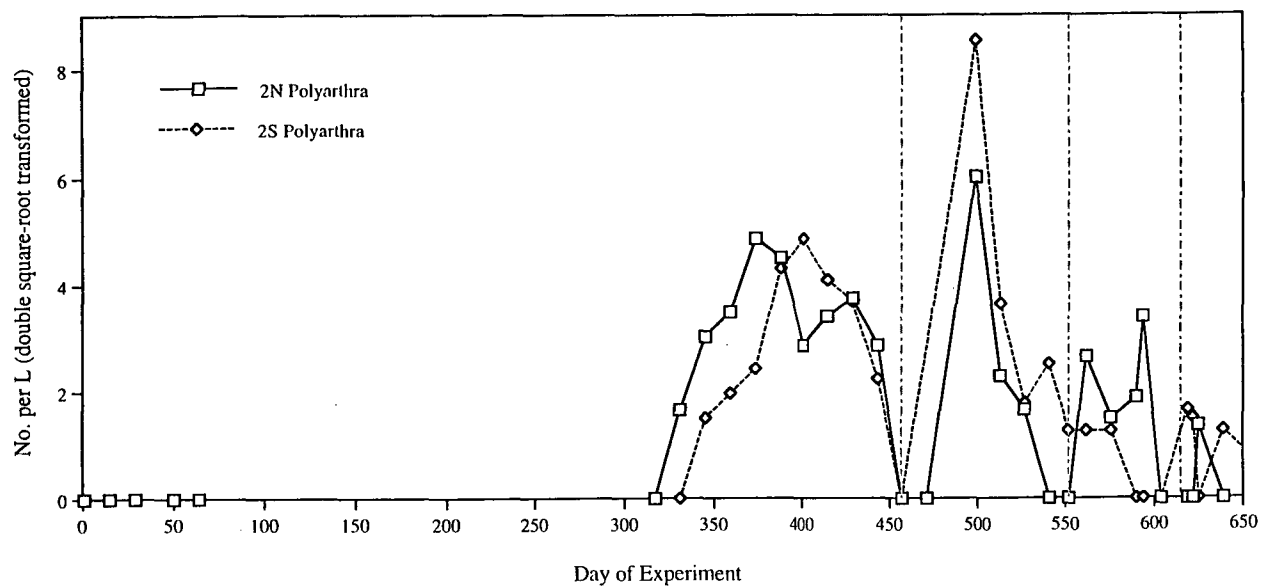
**Figure 4.15(a): Polyarthra numbers (double square-root transformed) in the SL pond (85wB) over the main experimental period.**



**Figure 4.15(b): Polyarthra numbers (double square-root transformed) in the SPN + SPS ponds (85wB) over the main experimental period.**



**Figure 4.15(c): Polyarthra numbers (double square-root transformed) in the 1N+1S ponds (85wB) over the main experimental period.**



**Figure 4.15(d): Polyarthra numbers (double square-root transformed) in the 2N+2S ponds (85wB) over the main experimental period.**

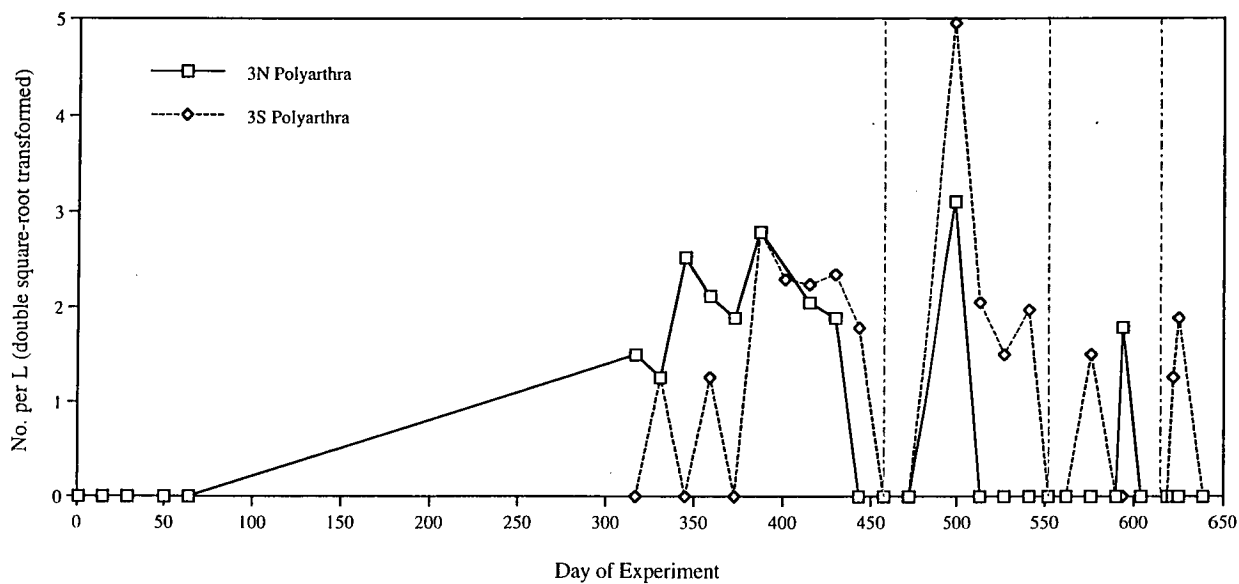


Figure 4.15(e): Polyarthra numbers (double square-root transformed) in the 3N+3S ponds (85wB) over the main experimental period.

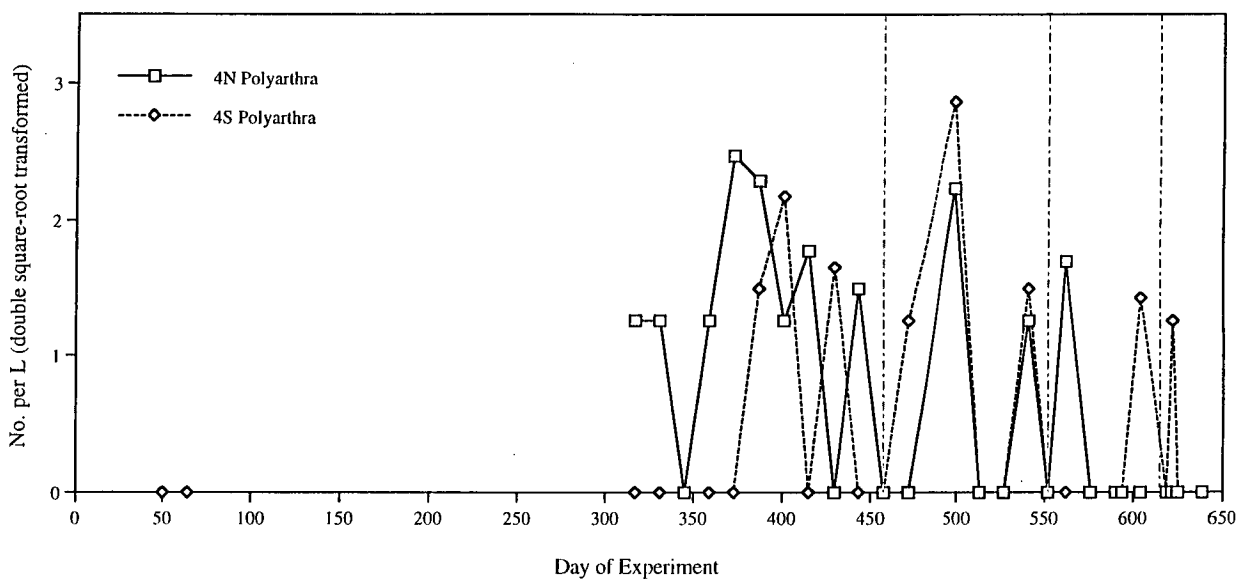


Figure 4.15(f): Polyarthra numbers (double square-root transformed) in the 4N+4S ponds (85wB) over the main experimental period.

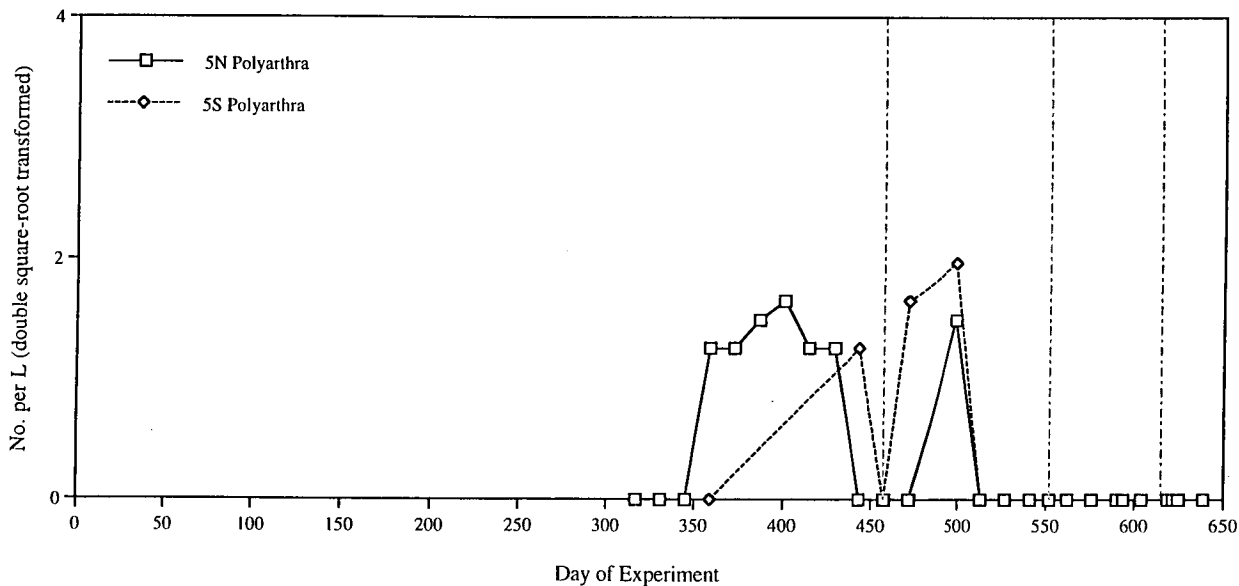


Figure 4.15(g): Polyarthra numbers (double square-root transformed) in the 5N+5S ponds (85wB) over the main experimental period.

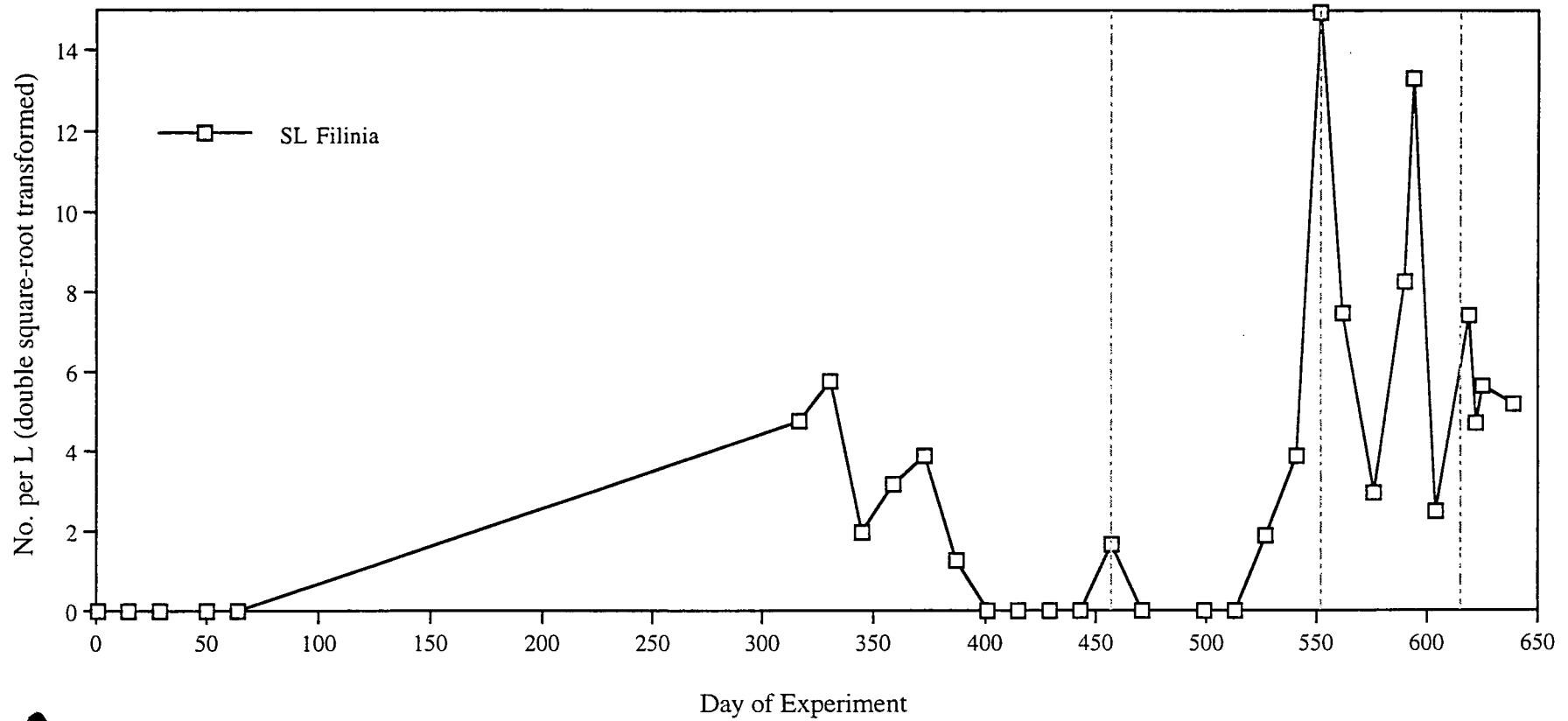


Figure 4.16(a): Filinia numbers (double square-root transformed) in the SL pond (85wB) over the main experimental period.



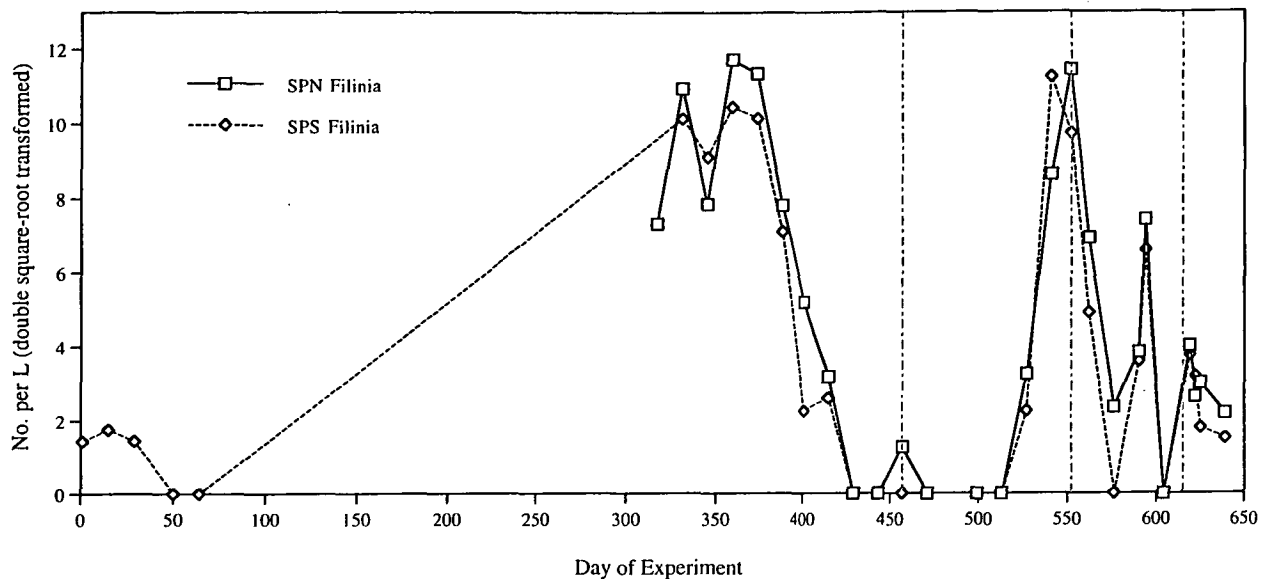


Figure 4.16(b): Filinia numbers (double square-root transformed) in the SPN + SPS ponds (85wB) over the main experimental period.

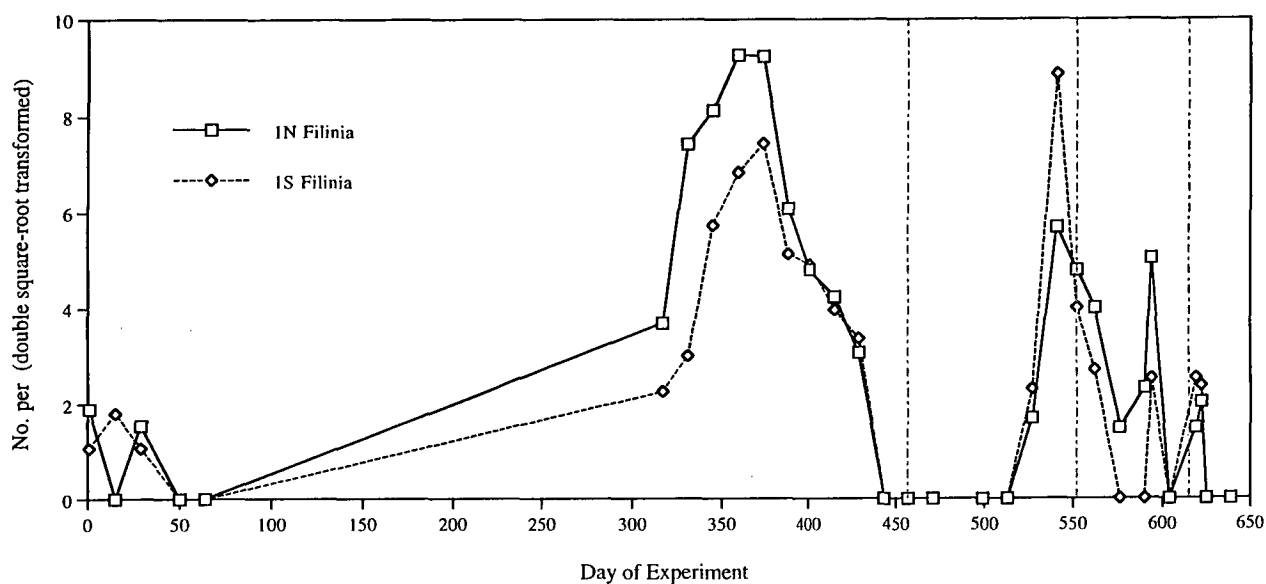


Figure 4.16(c): Filinia numbers (double square-root transformed) in the 1N+1S ponds (85wB) over the main experimental period.

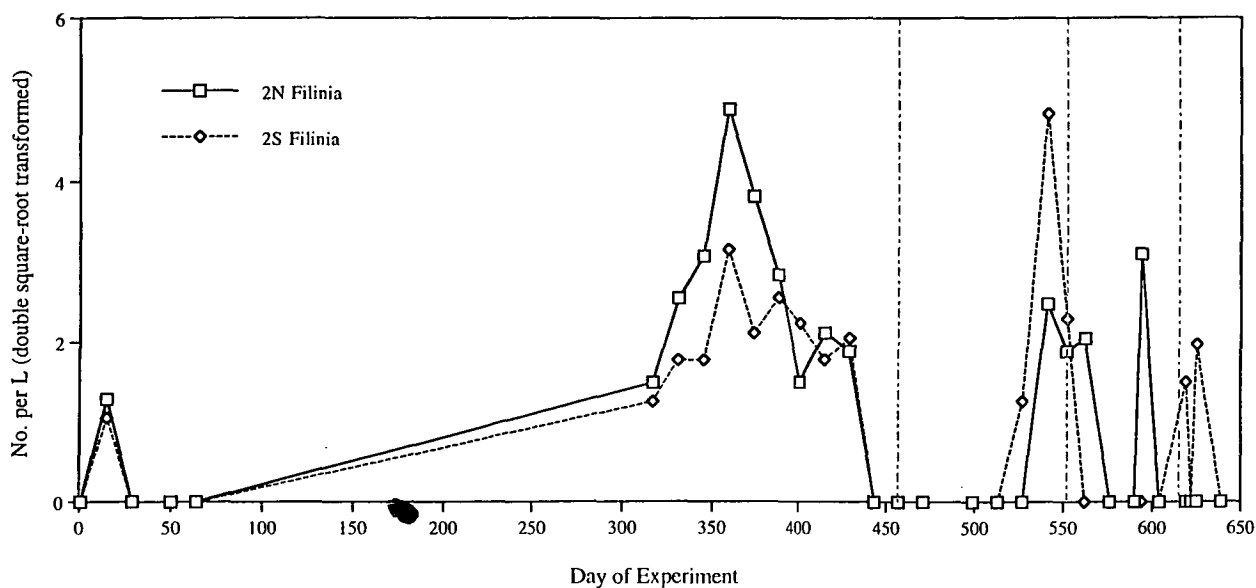


Figure 4.16(d): Filinia numbers (double square-root transformed) in the 2N+2S ponds (85wB) over the main experimental period.

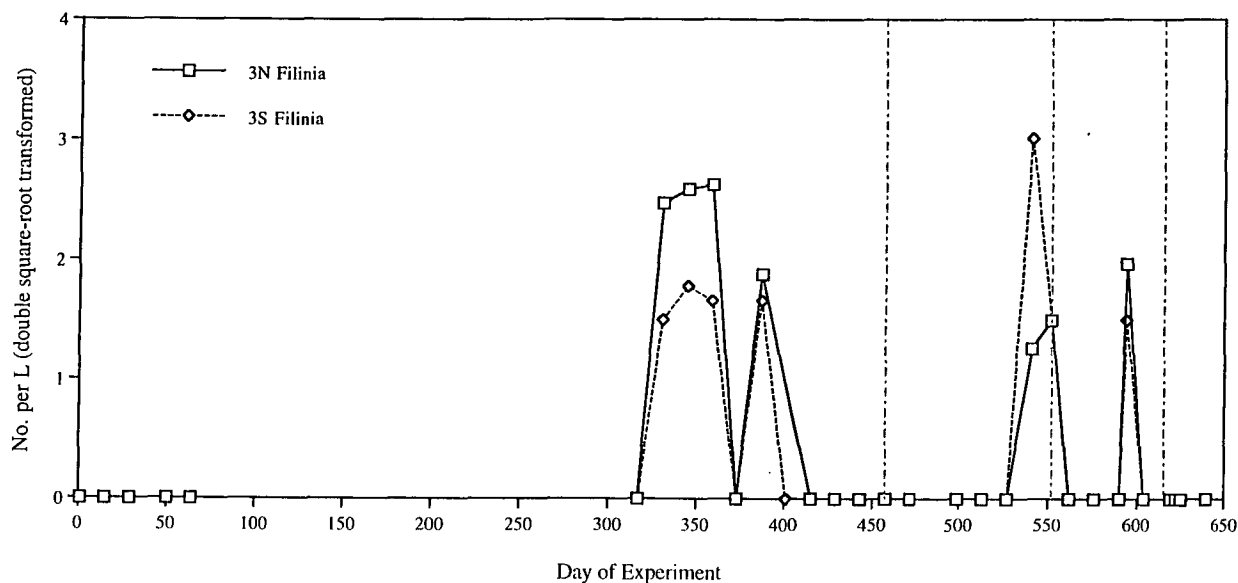


Figure 4.16(e): Filinia numbers (double square-root transformed) in the 3N+3S ponds (85wB) over the main experimental period.

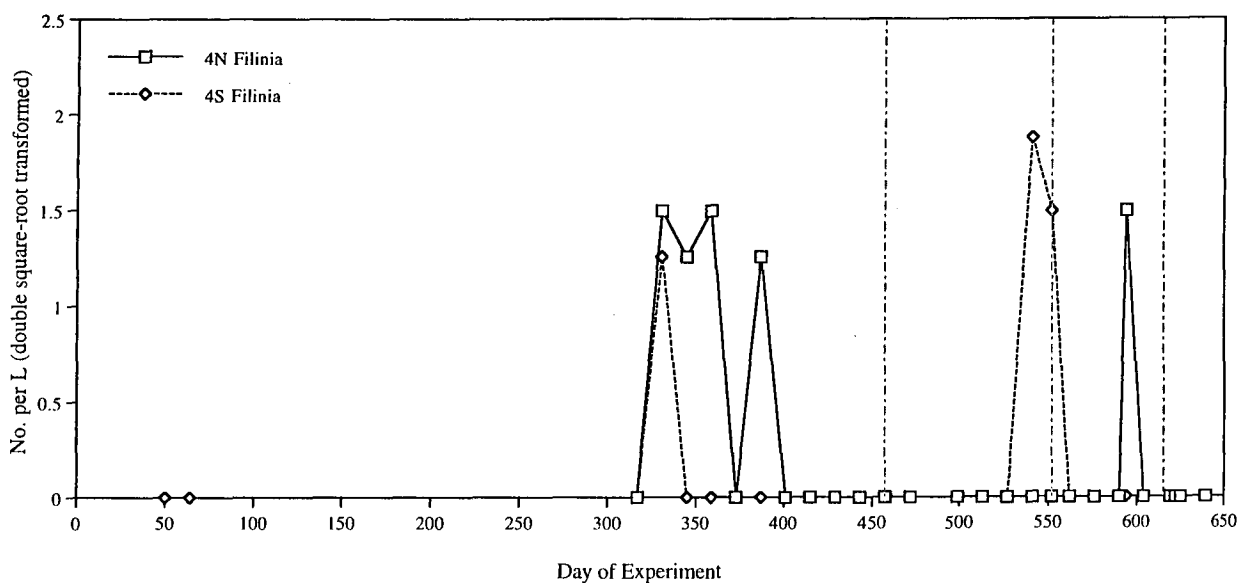


Figure 4.16(f): Filinia numbers (double square-root transformed) in the 4N+4S ponds (85wB) over the main experimental period.

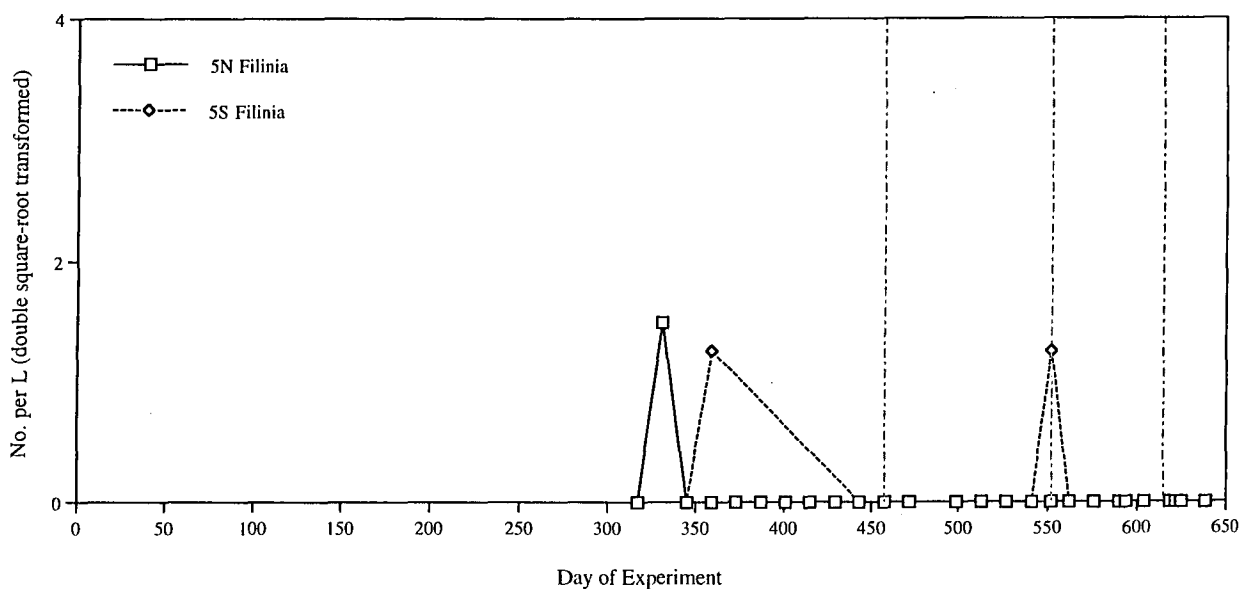
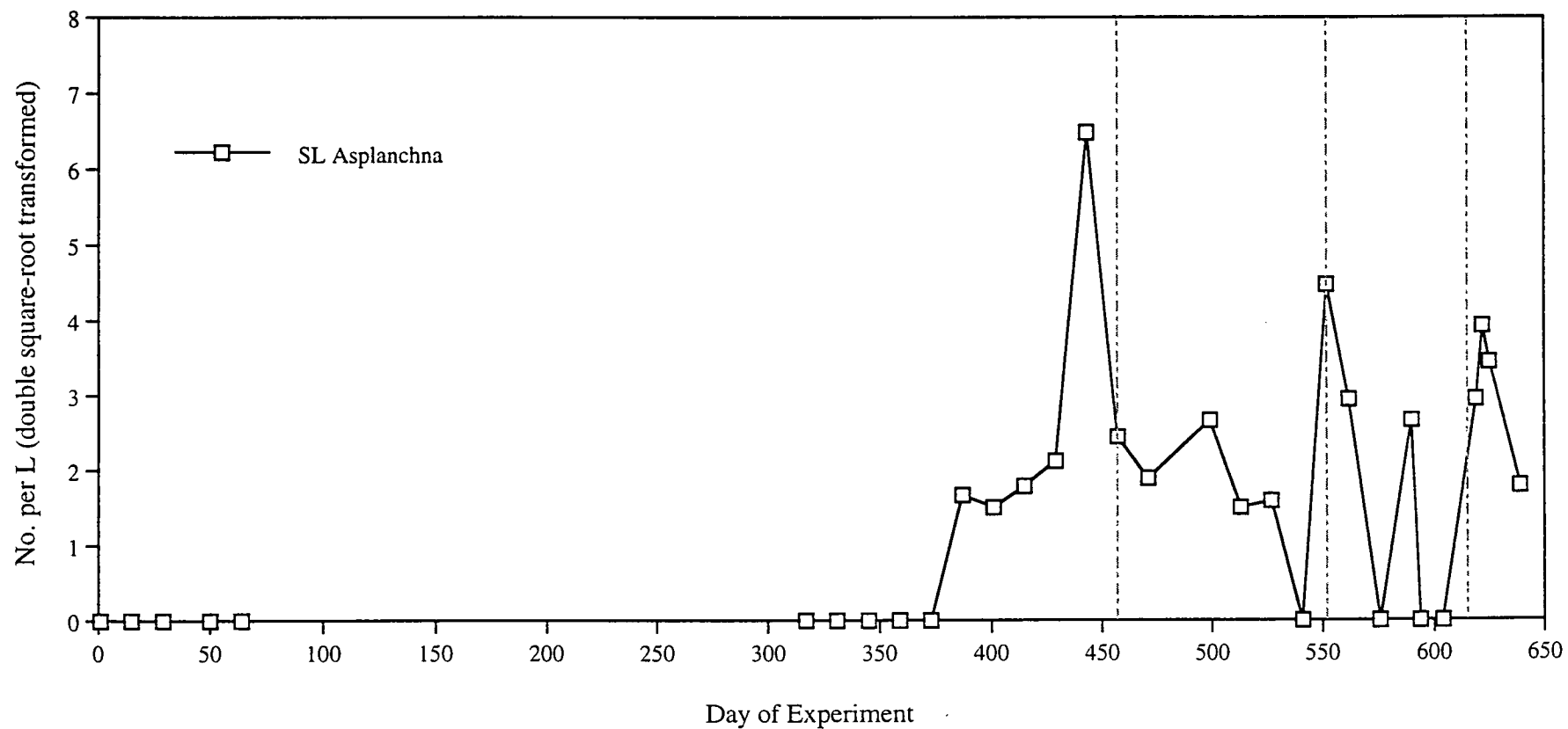


Figure 4.16(g): Filinia numbers (double square-root transformed) in the 5N+5S ponds (85wB) over the main experimental period.



**Figure 4.17(a): Asplanchna numbers (double square-root transformed) in the SL pond (85wB) over the main experimental period.**

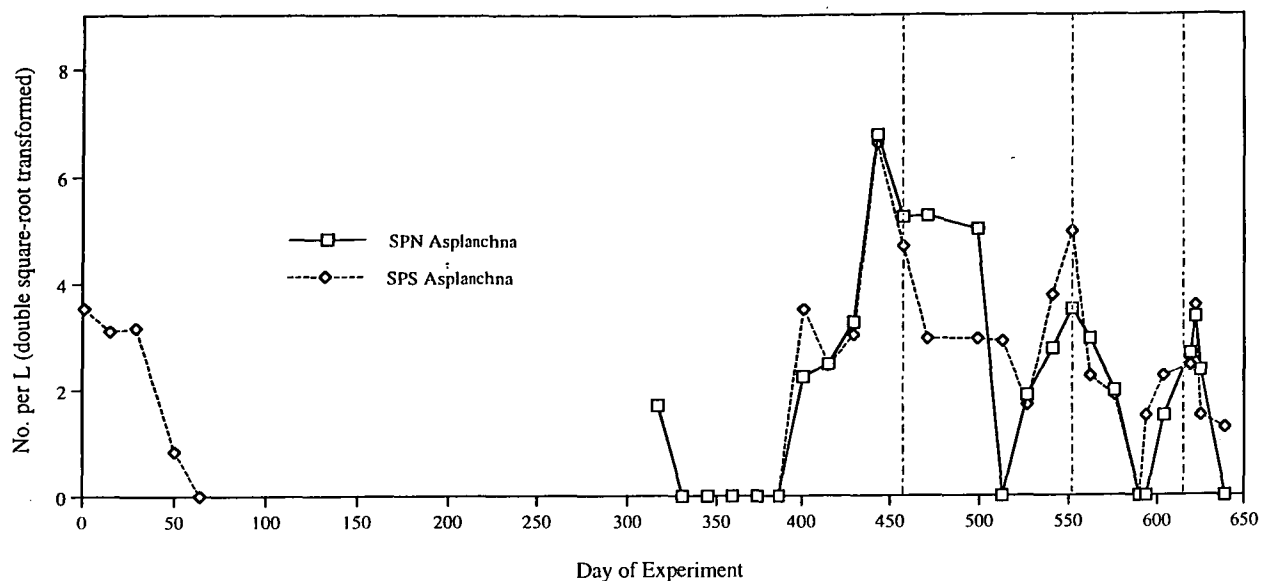


Figure 4.17(b): Asplanchna numbers (double square-root transformed) in the SPN + SPS ponds (85wB) over the main experimental period.

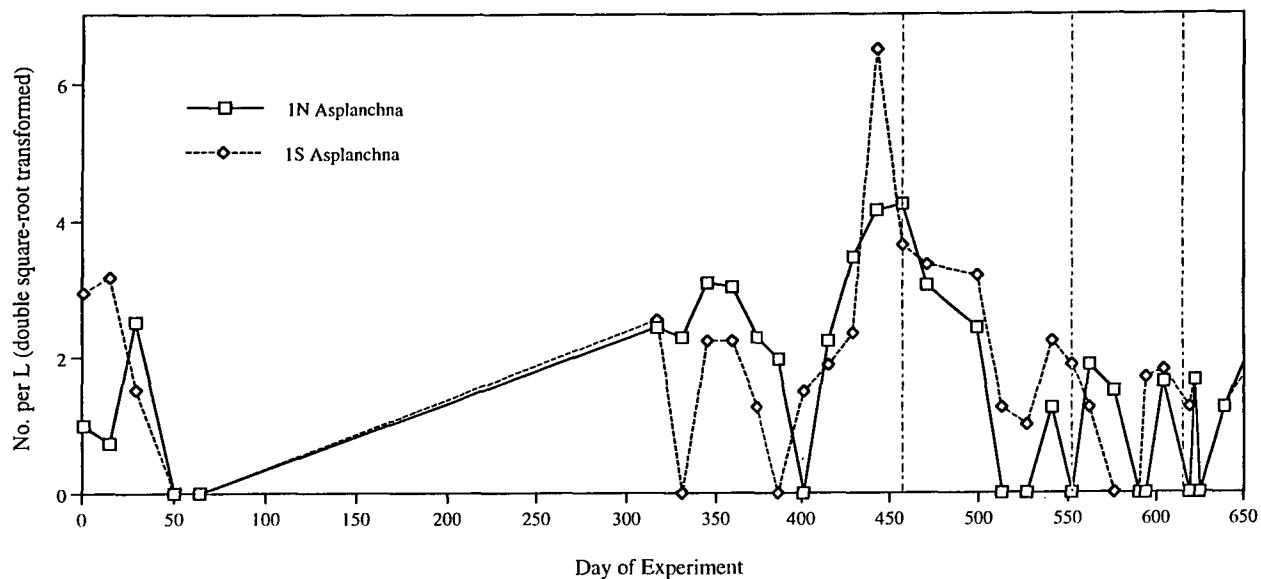


Figure 4.17(c): Asplanchna numbers (double square-root transformed) in the 1N+1S ponds (85wB) over the main experimental period.

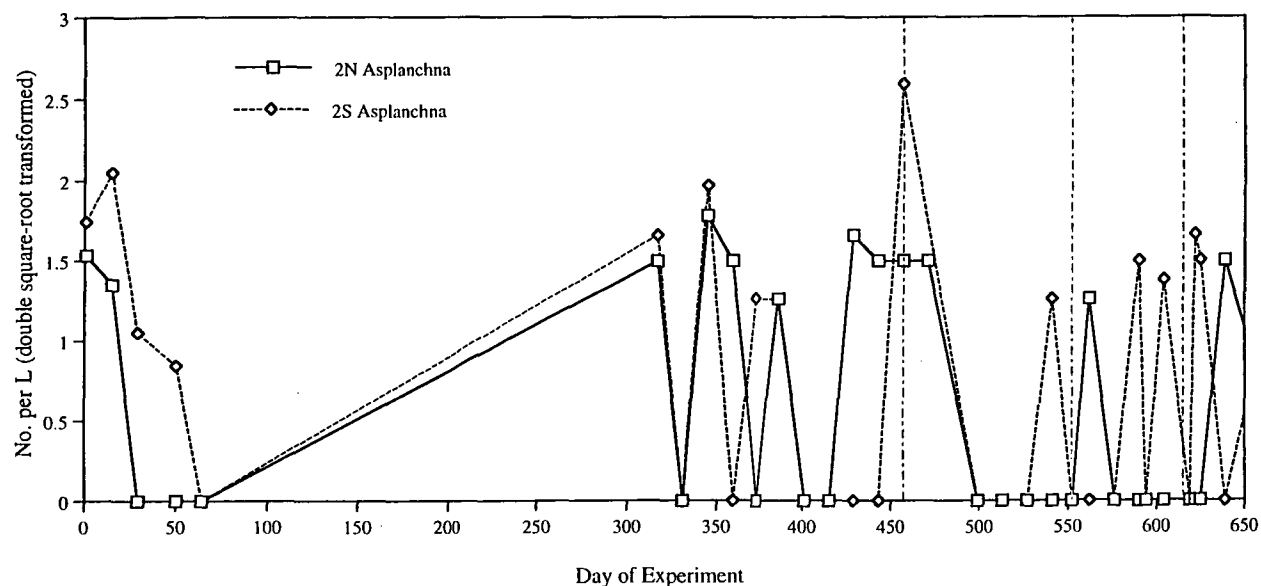


Figure 4.17(d): Asplanchna numbers (double square-root transformed) in the 2N+2S ponds (85wB) over the main experimental period.

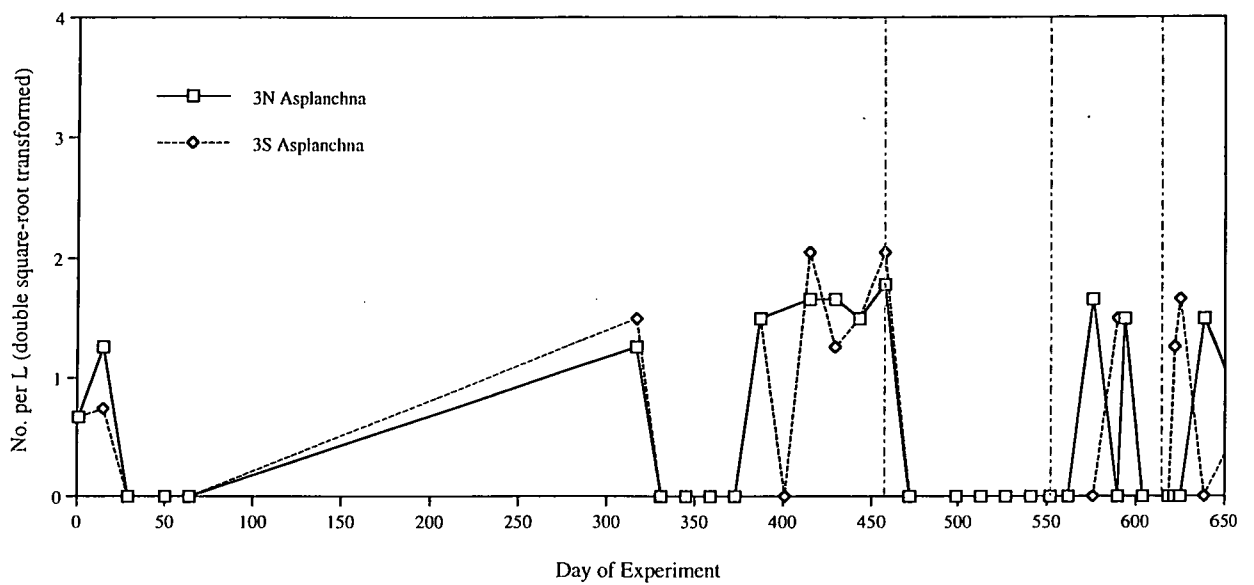


Figure 4.17(e): Asplanchna numbers (double square-root transformed) in the 3N+3S ponds (85wB) over the main experimental period.

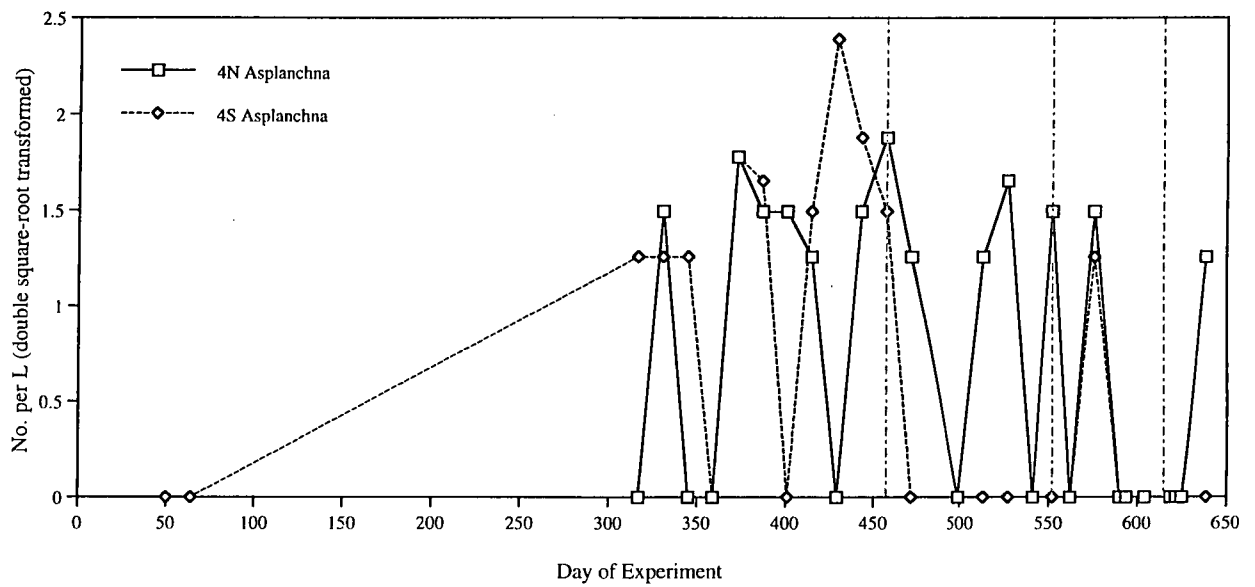


Figure 4.17(f): Asplanchna numbers (double square-root transformed) in the 4N+4S ponds (85wB) over the main experimental period.

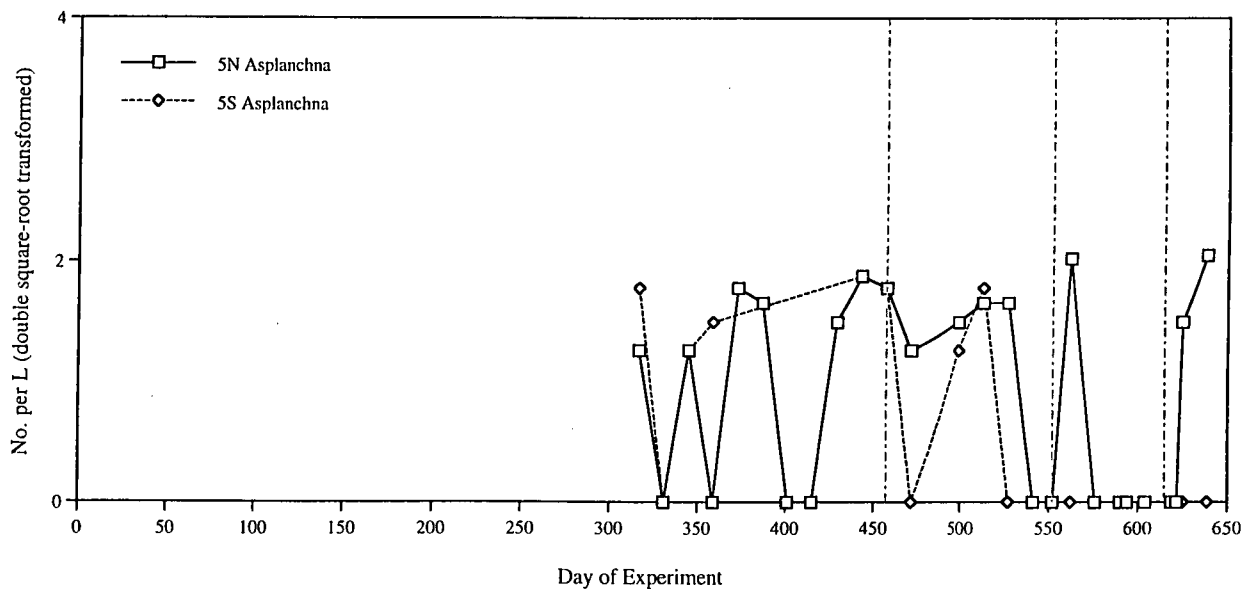
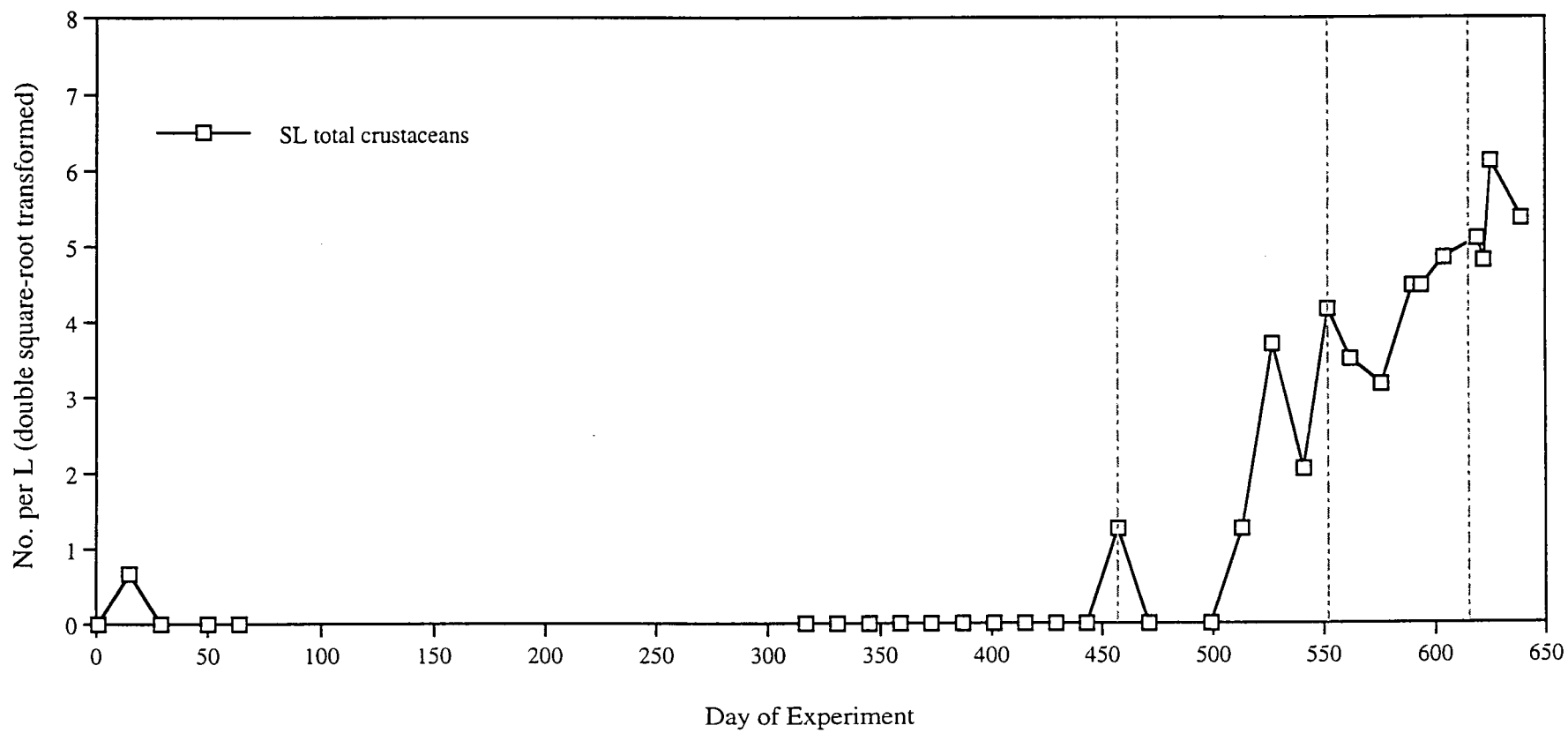


Figure 4.17(g): Asplanchna numbers (double square-root transformed) in the 5N+5S ponds (85wB) over the main experimental period.



**Figure 4.18(a): Total crustaceans (double square-root transformed) in the SL pond (85wB) over the main experimental period.**

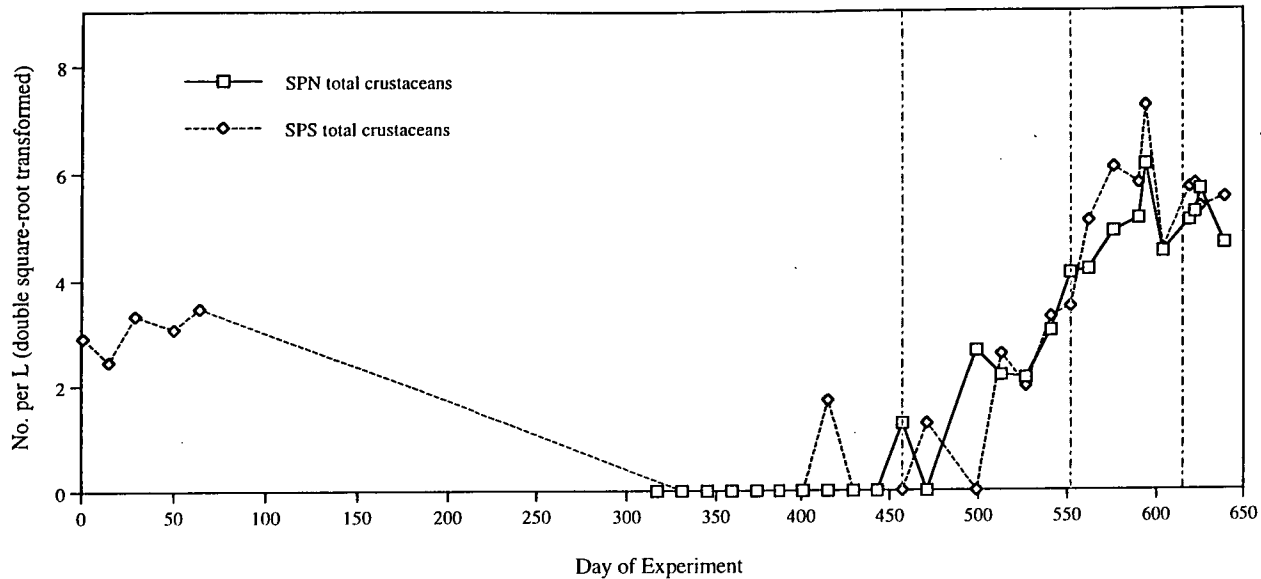


Figure 4.18(b): Total crustaceans (double square-root transformed) in the SPN + SPS ponds (85wB) over the main experimental period.

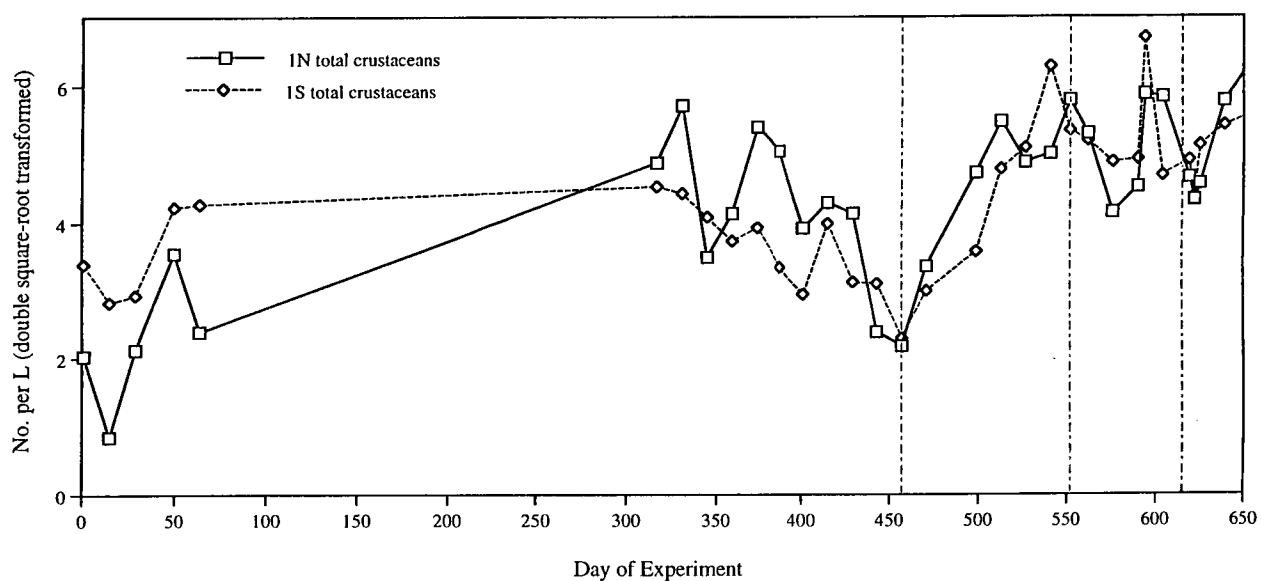


Figure 4.18(c): Total crustaceans (double square-root transformed) in the 1N+1S ponds (85wB) over the main experimental period.

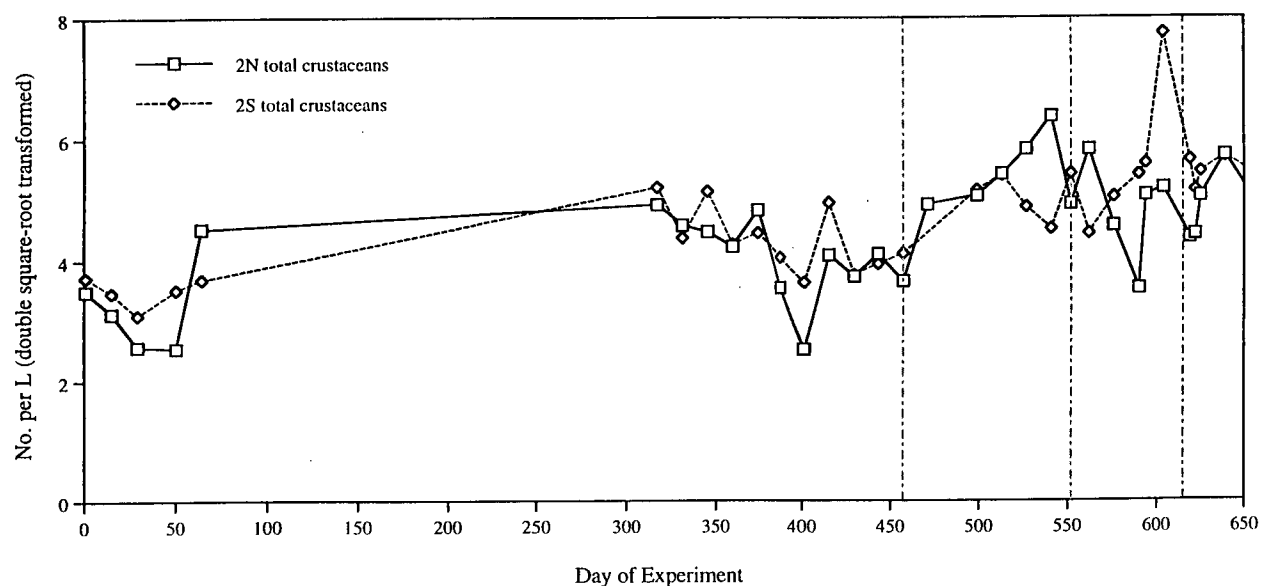


Figure 4.18(d): Total crustaceans (double square-root transformed) in the 2N+2S ponds (85wB) over the main experimental period.

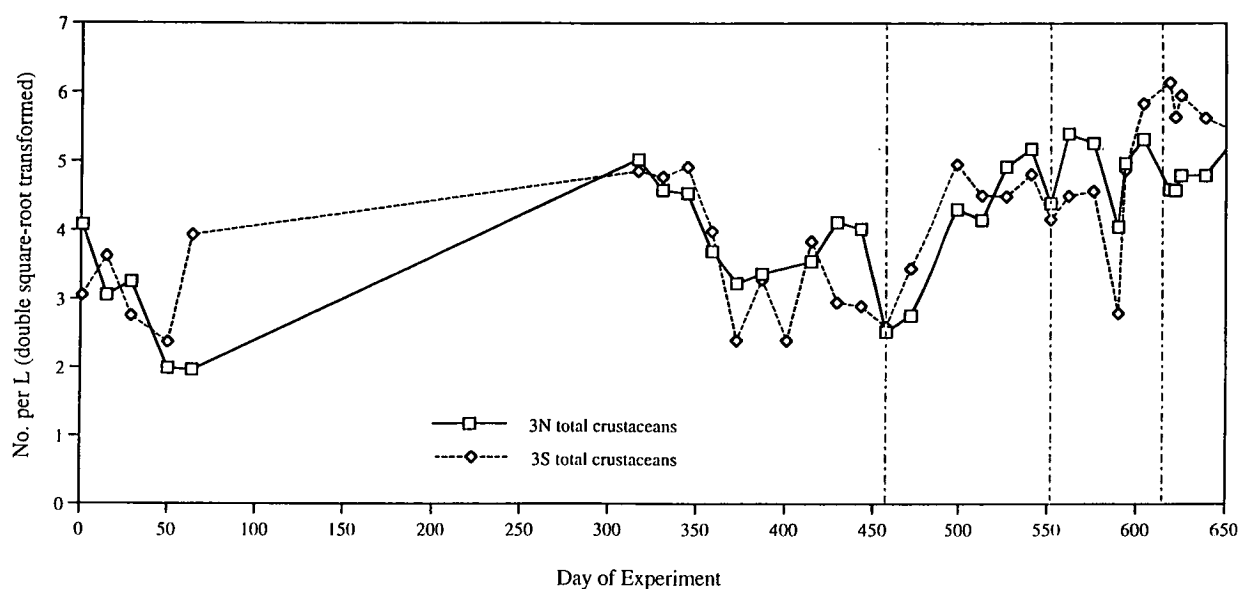


Figure 4.18(e): Total crustaceans (double square-root transformed) in the 3N+3S ponds (85wB) over the main experimental period.

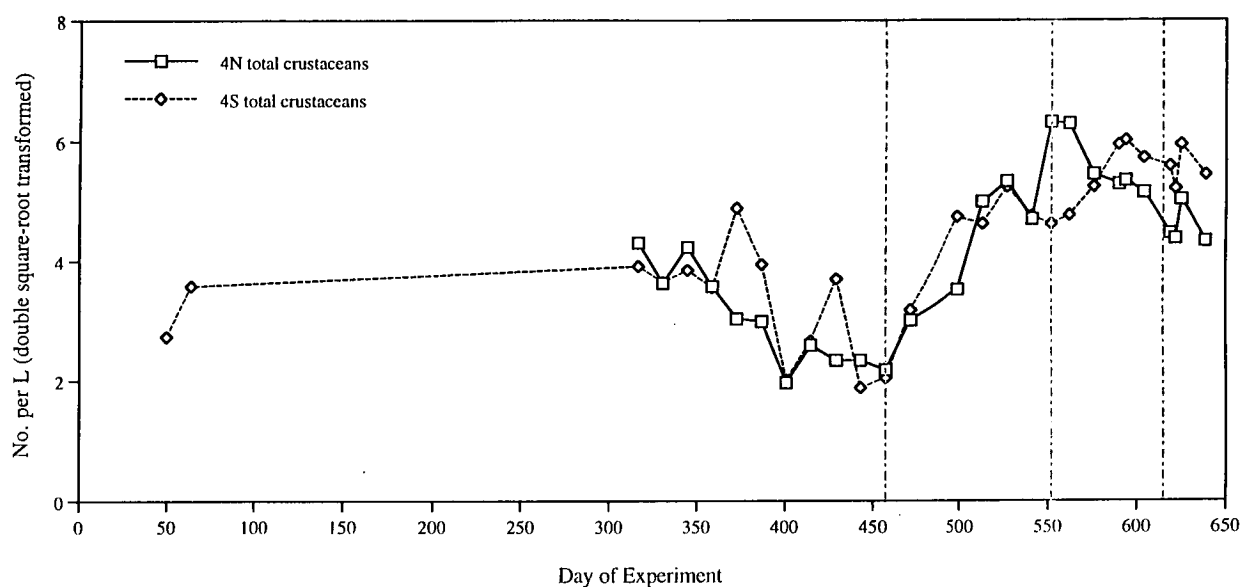


Figure 4.18(f): Total crustacean numbers (double square-root transformed) in the 4N+4S ponds (85wB) over the main experimental period.

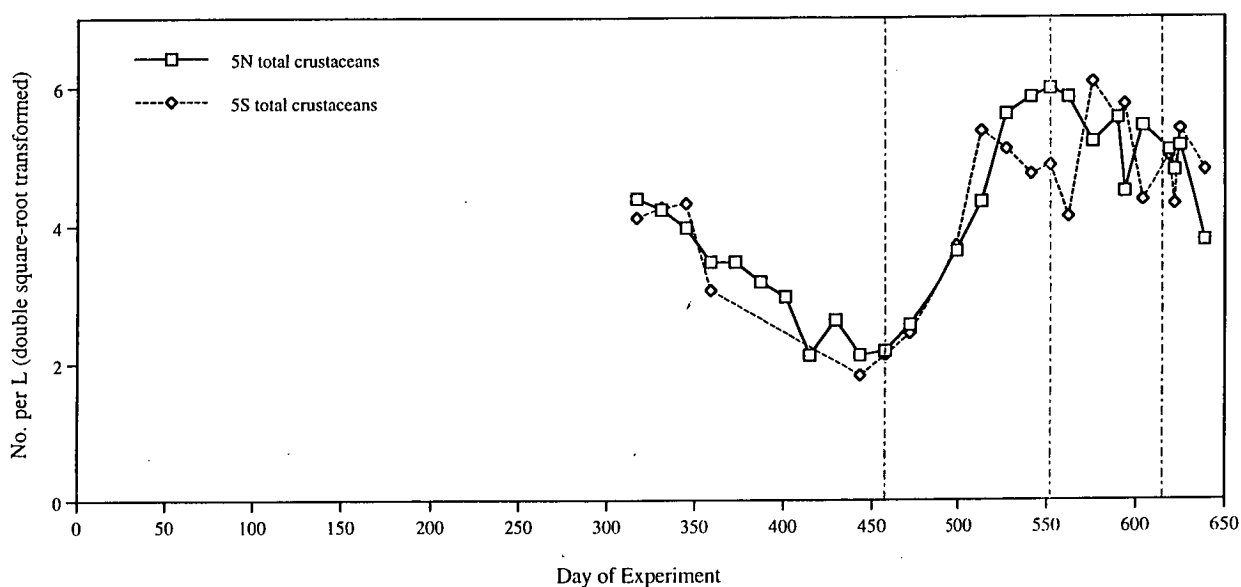
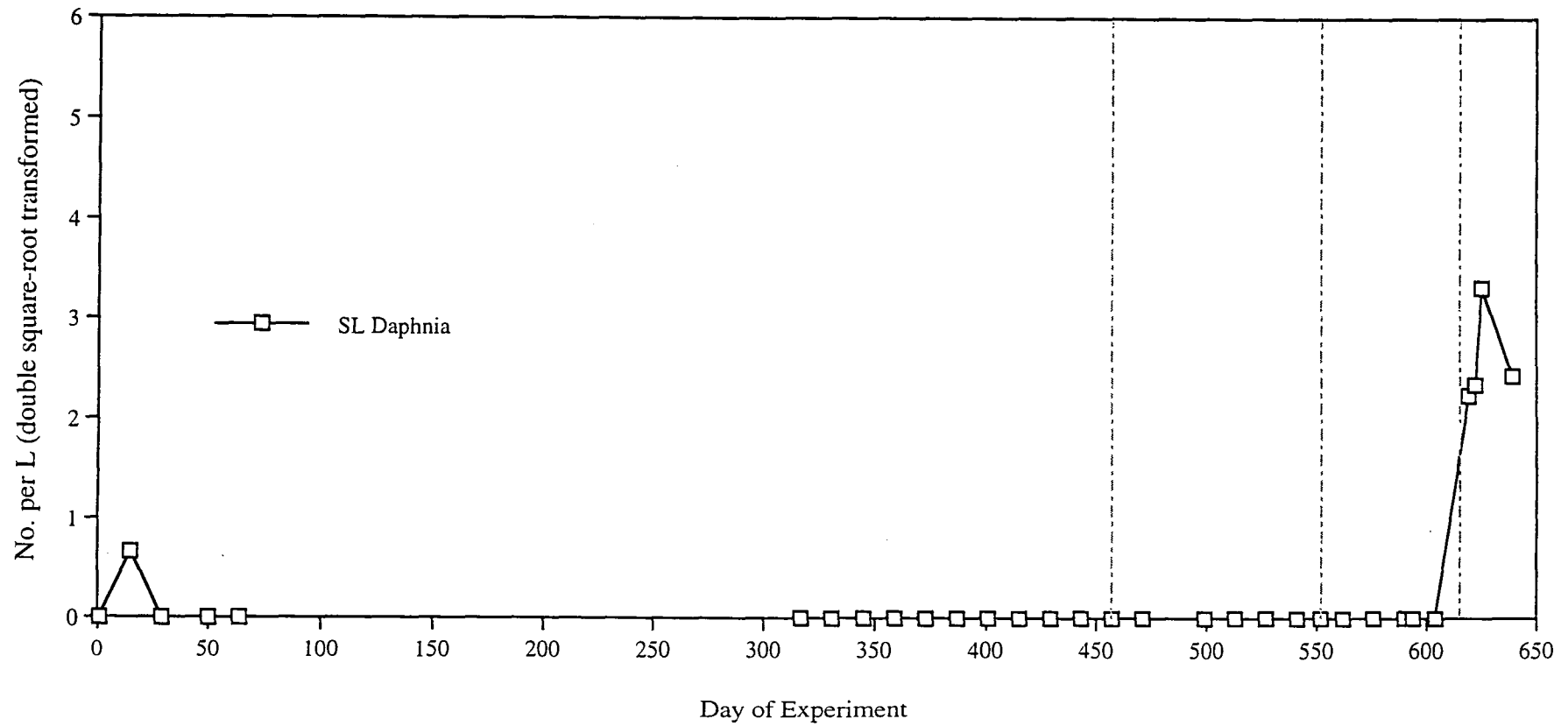
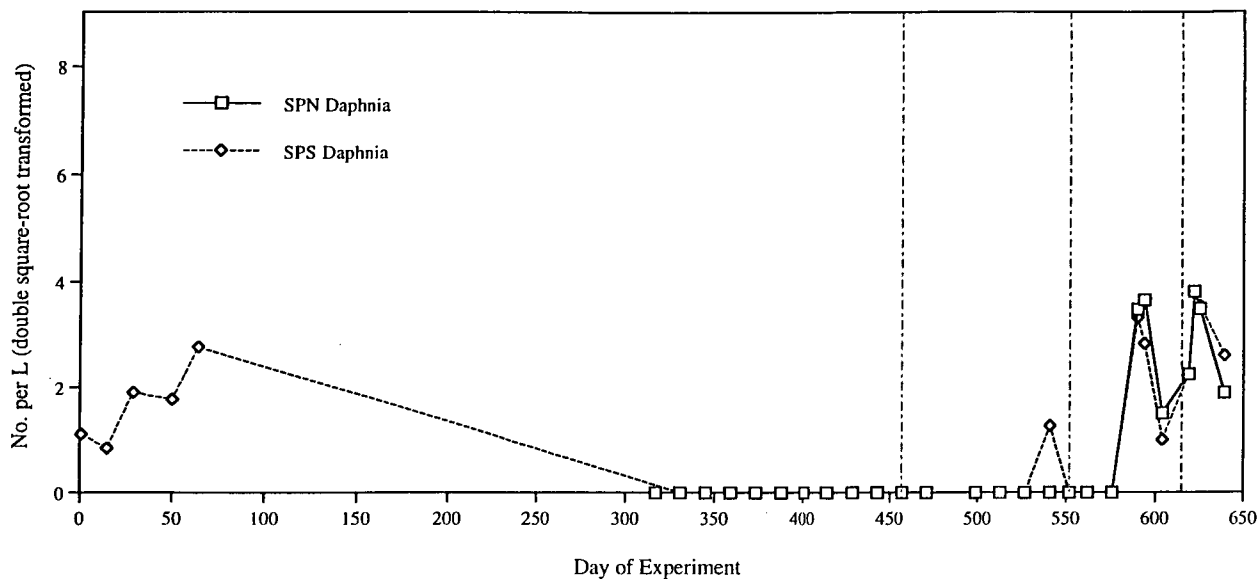


Figure 4.18(g): Total crustacean numbers (double square-root transformed) in the 5N+5S ponds (85wB) over the main experimental period.

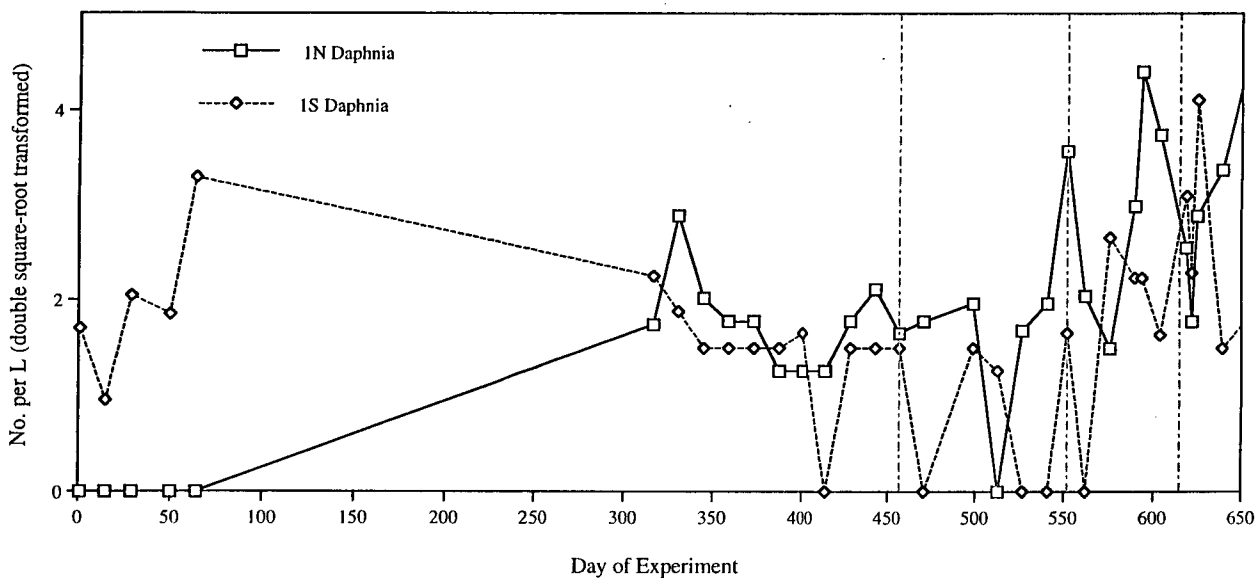




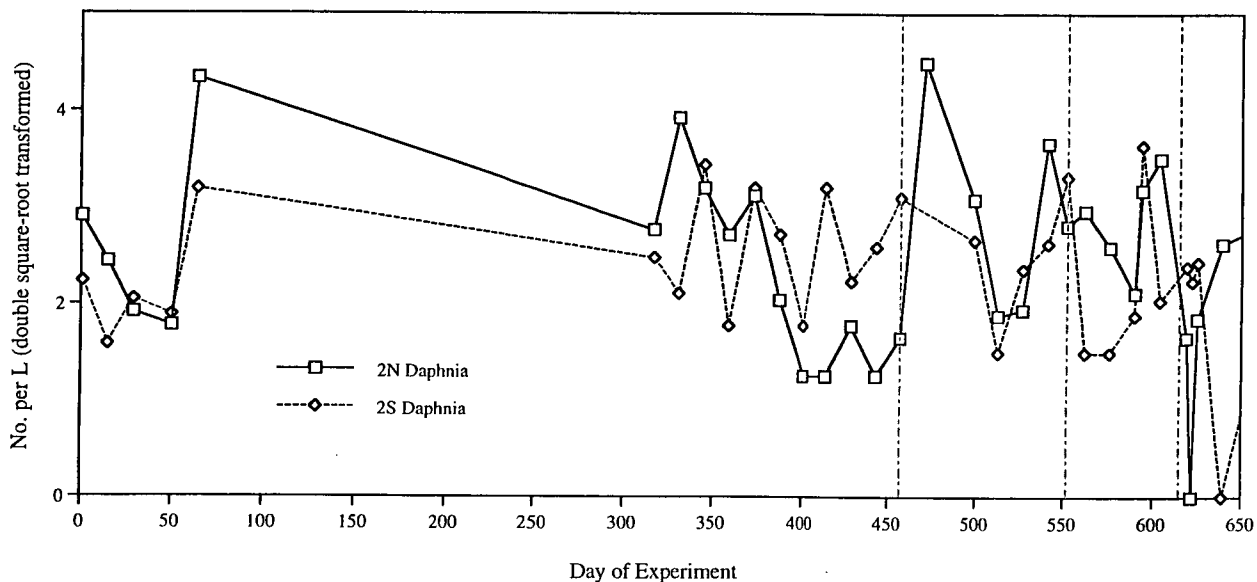
**Figure 4.19(a):** Daphnia numbers (double square-root transformed) in the SL pond (85wB) over the main experimental period.



**Figure 4.19(b): Daphnia numbers (double square-root transformed) in the SPN + SPS ponds (85wB) over the main experimental period.**



**Figure 4.19(c): Daphnia numbers (double square-root transformed) in the 1N+1S ponds (85wB) over the main experimental period.**



**Figure 4.19(d): Daphnia numbers (double square-root transformed) in the 2N+2S ponds (85wB) over the main experimental period.**

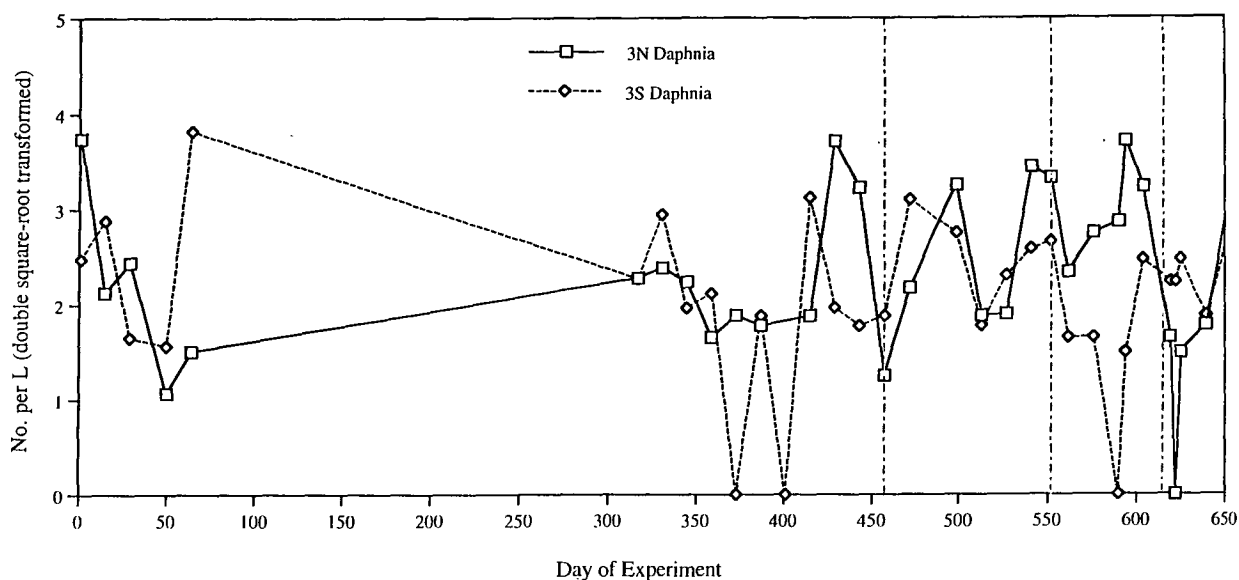


Figure 4.19(e): Daphnia numbers (double square-root transformed) in the 3N+3S ponds (85wB) over the main experimental period.

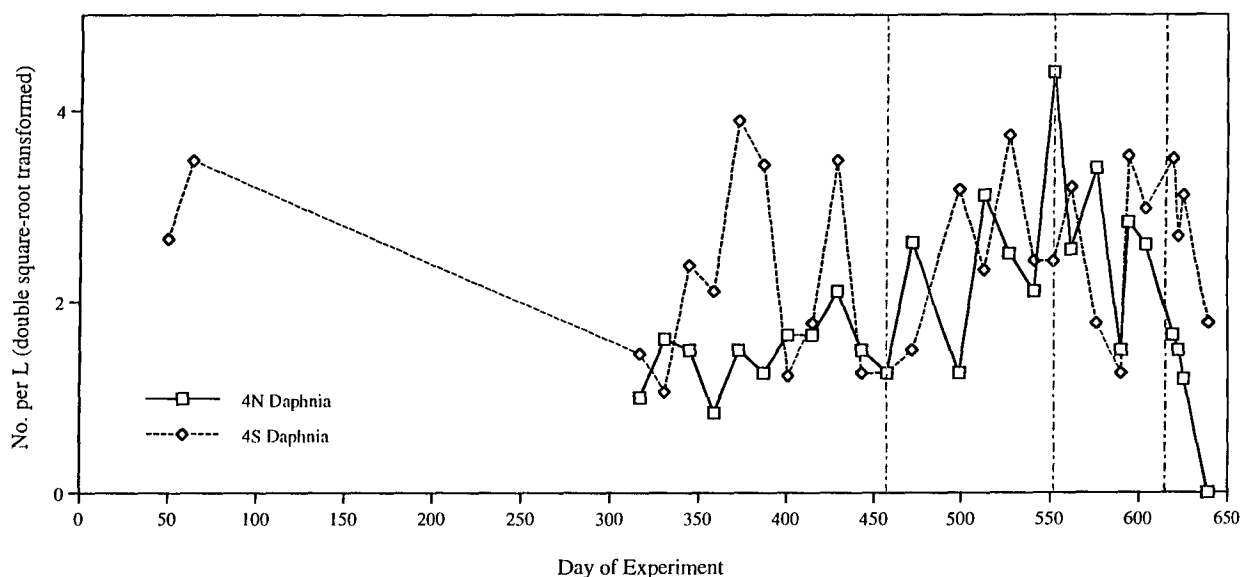


Figure 4.19(f): Daphnia numbers (double square-root transformed) in the 4N+4S ponds (85wB) over the main experimental period.

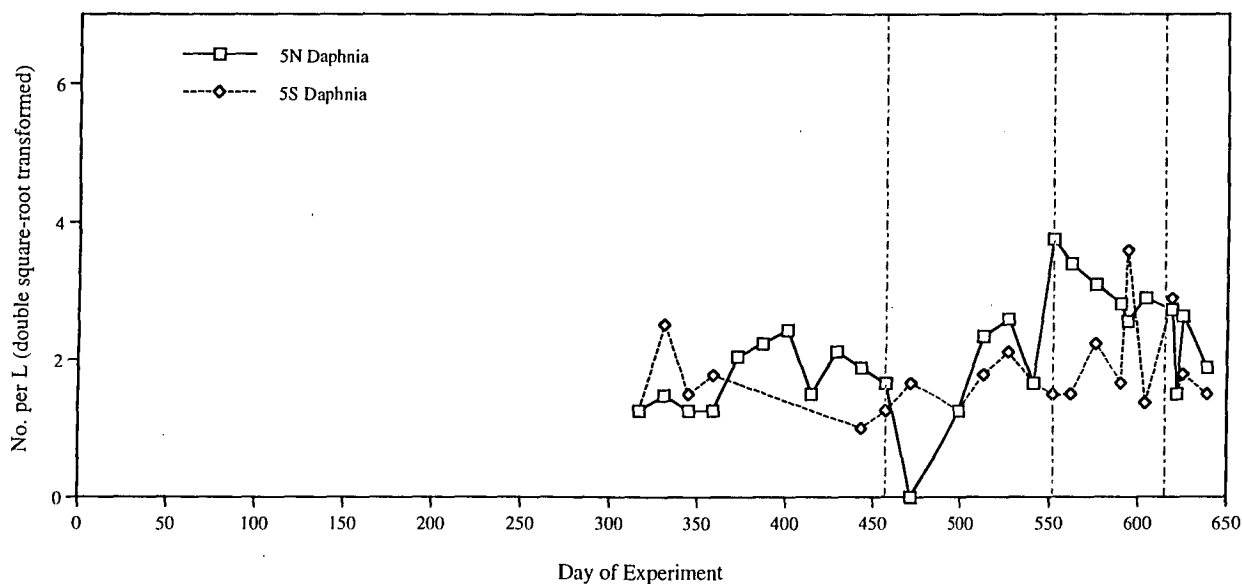
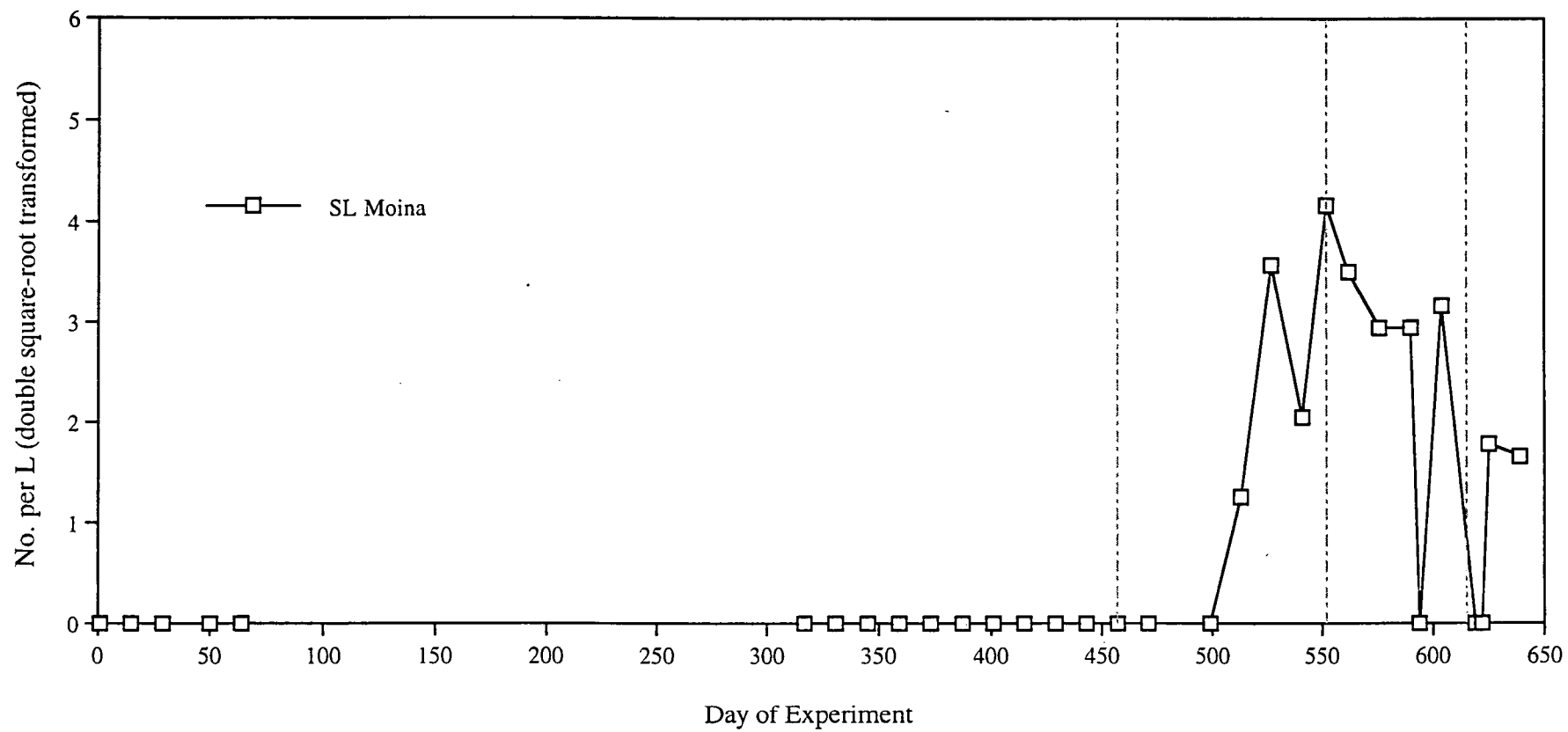


Figure 4.19(g): Daphnia numbers (double square-root transformed) in the 5N+5S ponds (85wB) over the main experimental period.



**Figure 4.20(a):** Moina numbers (double square-root transformed) in the SL pond (85wB) over the main experimental period.

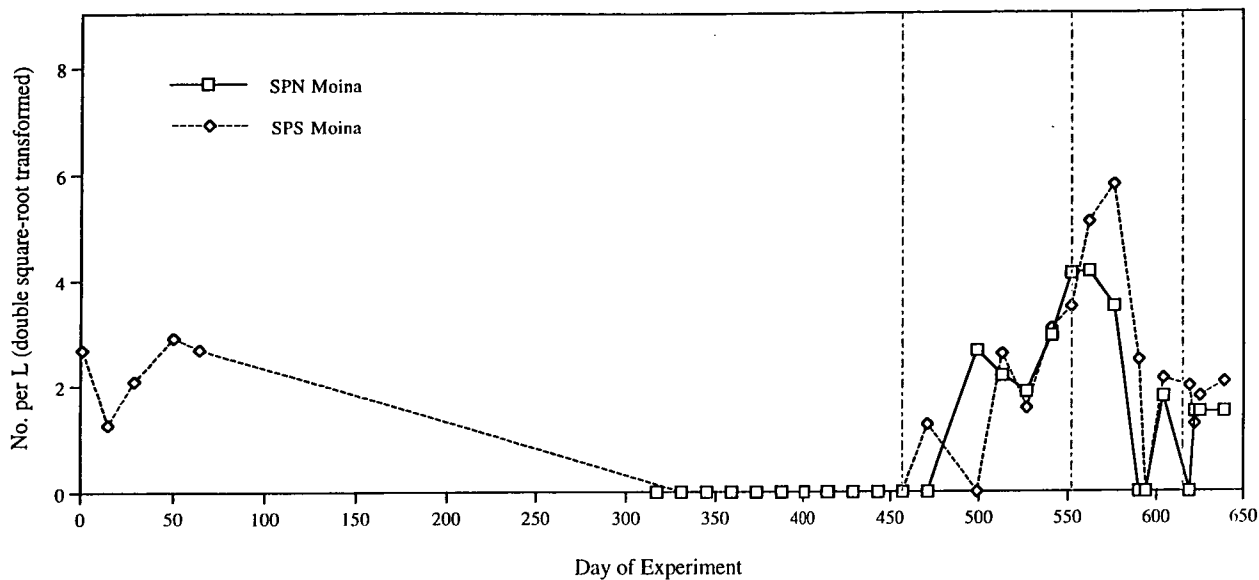


Figure 4.20(b): Moina numbers (double square-root transformed) in the SPN + SPS ponds (85wB) over the main experimental period.

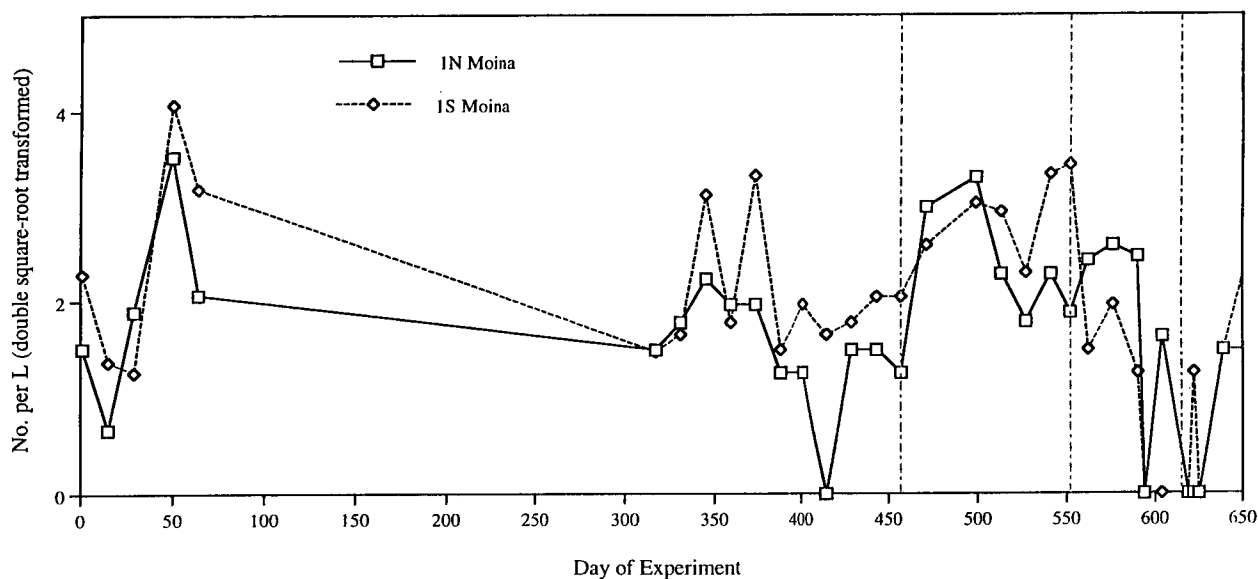


Figure 4.20(c): Moina numbers (double square-root transformed) in the 1N+1S ponds (85wB) over the main experimental period.

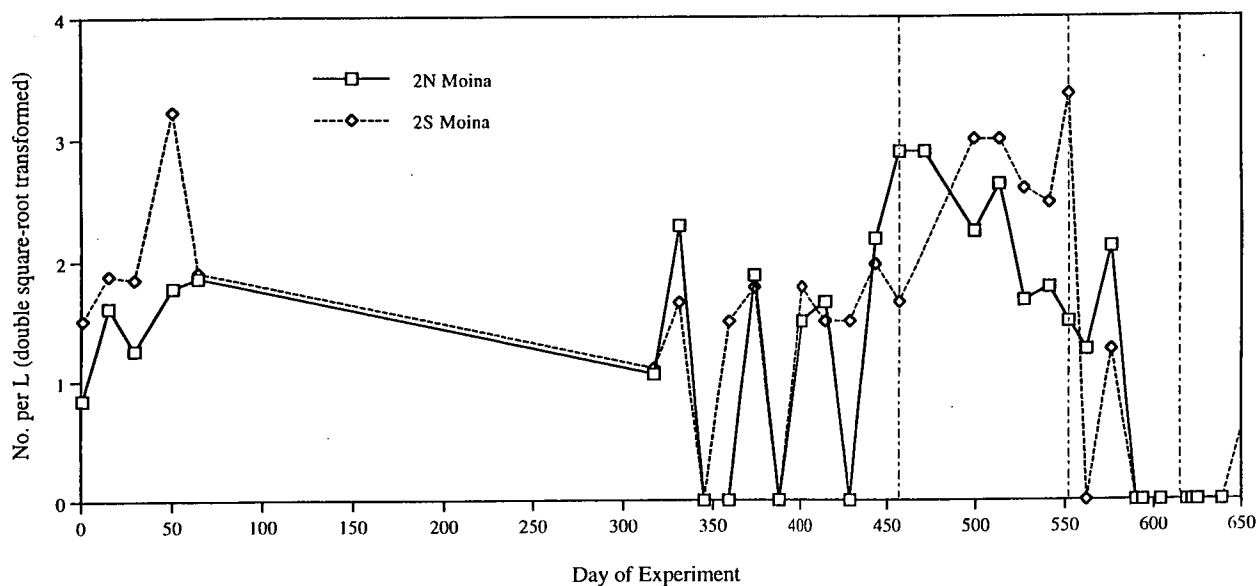


Figure 4.20(d): Moina numbers (double square-root transformed) in the 2N+2S ponds (85wB) over the main experimental period.

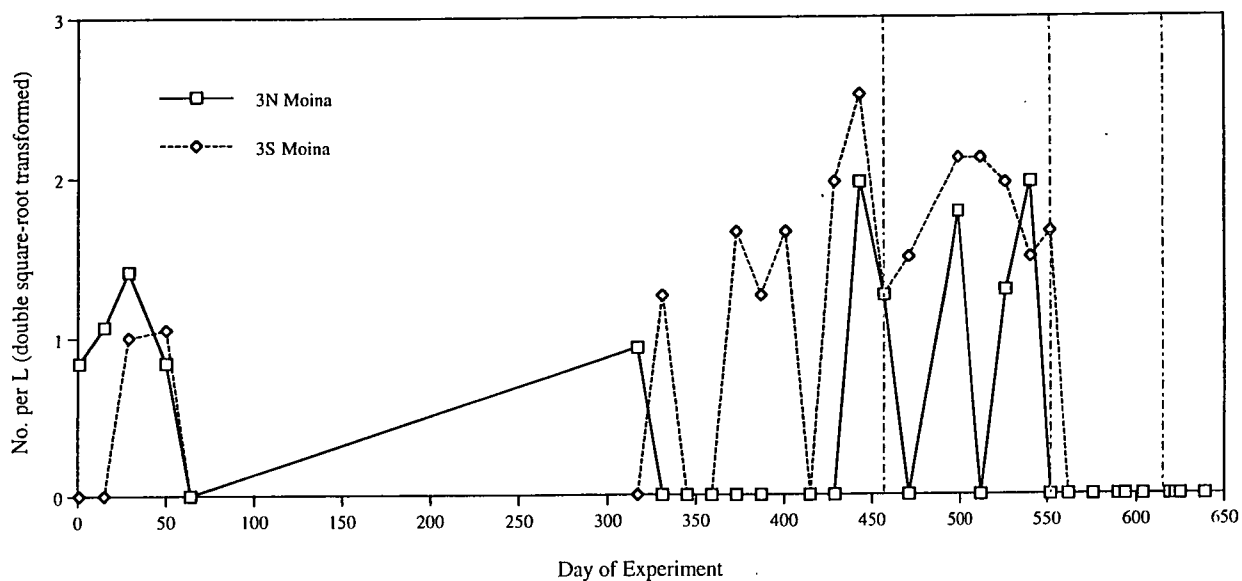


Figure 4.20(e): Moina numbers (double square-root transformed) in the 3N+3S ponds (85wB) over the main experimental period.

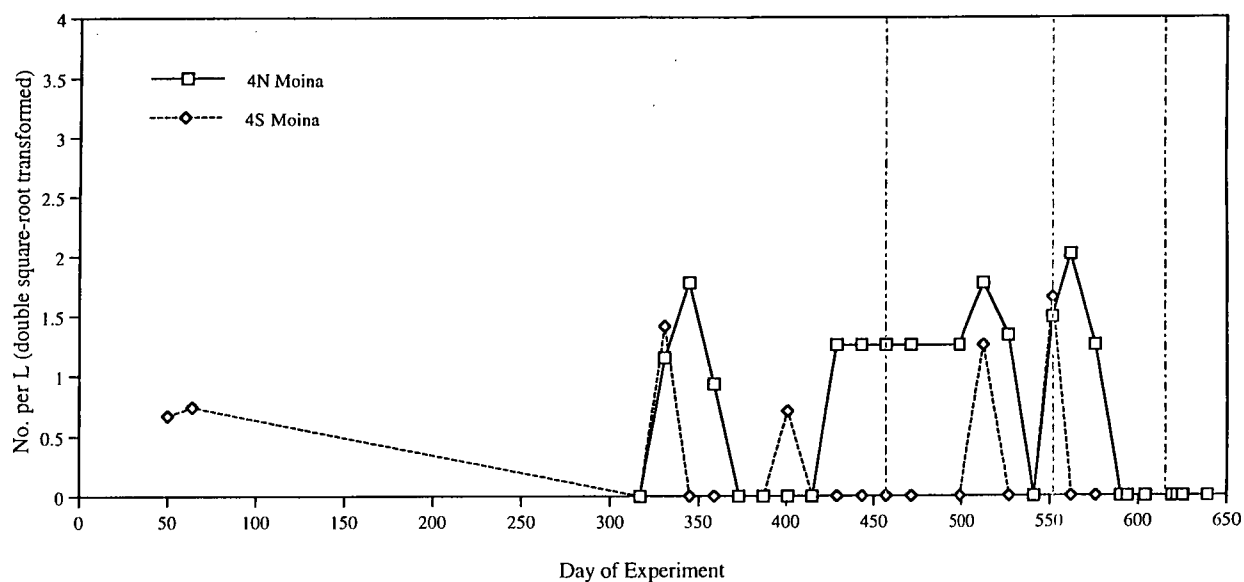


Figure 4.20(f): Moina numbers (double square-root transformed) in the 4N+4S ponds (85wB) over the main experimental period.

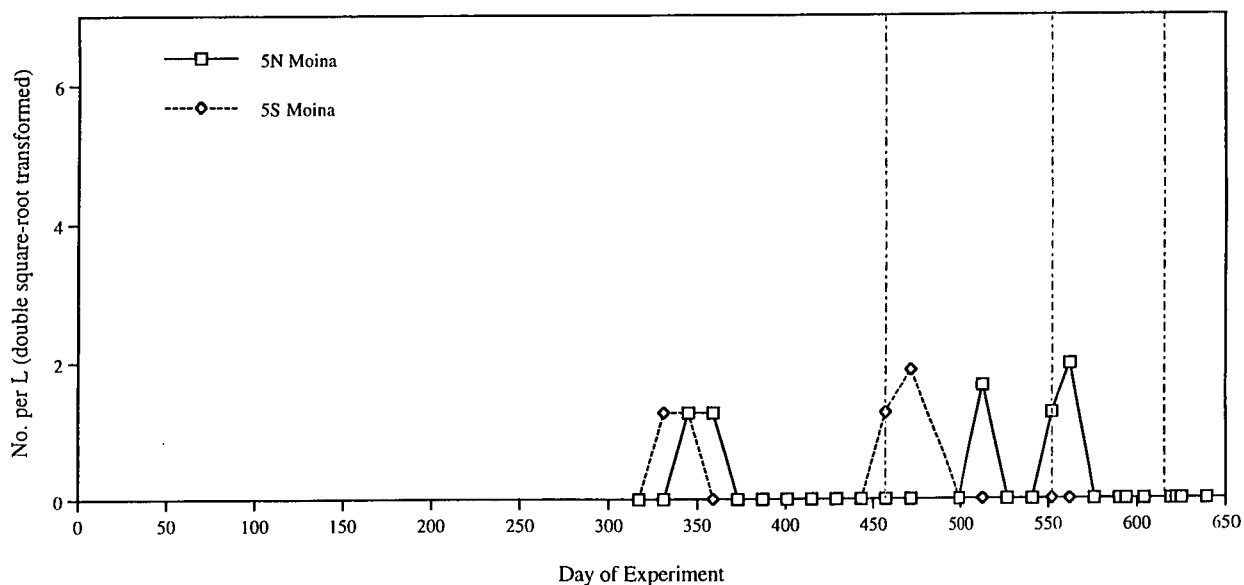
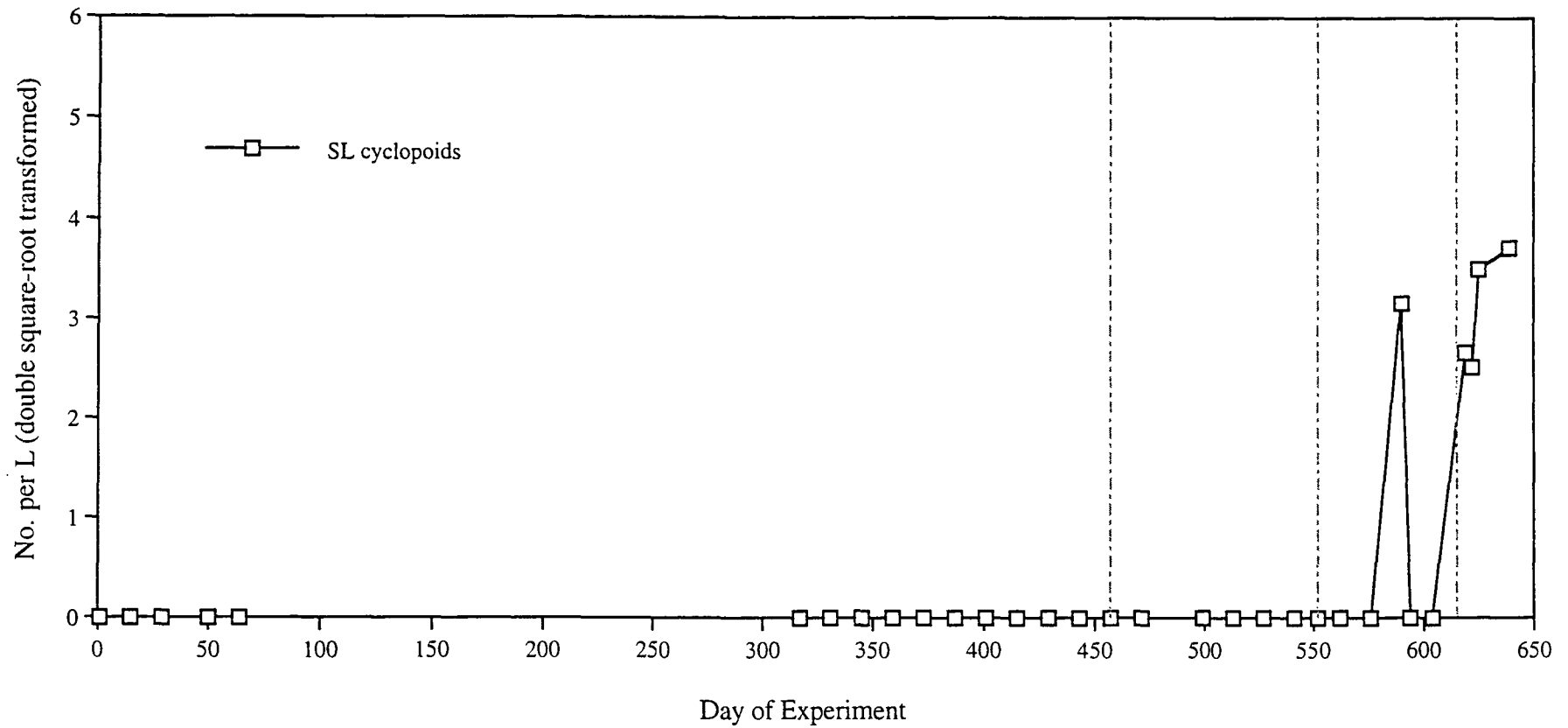
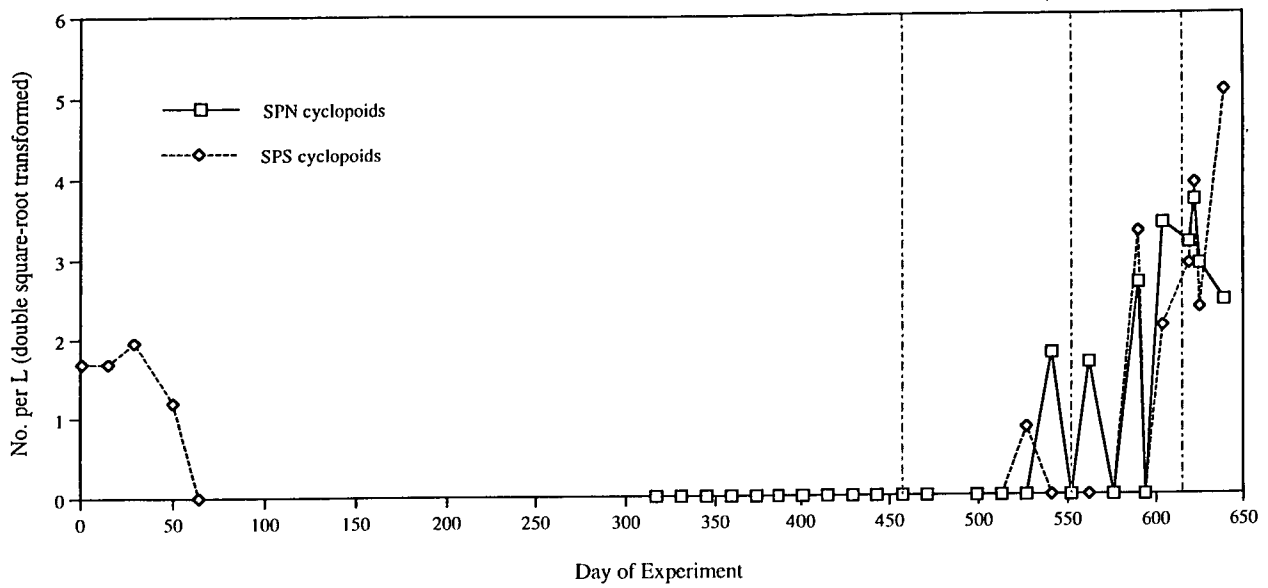


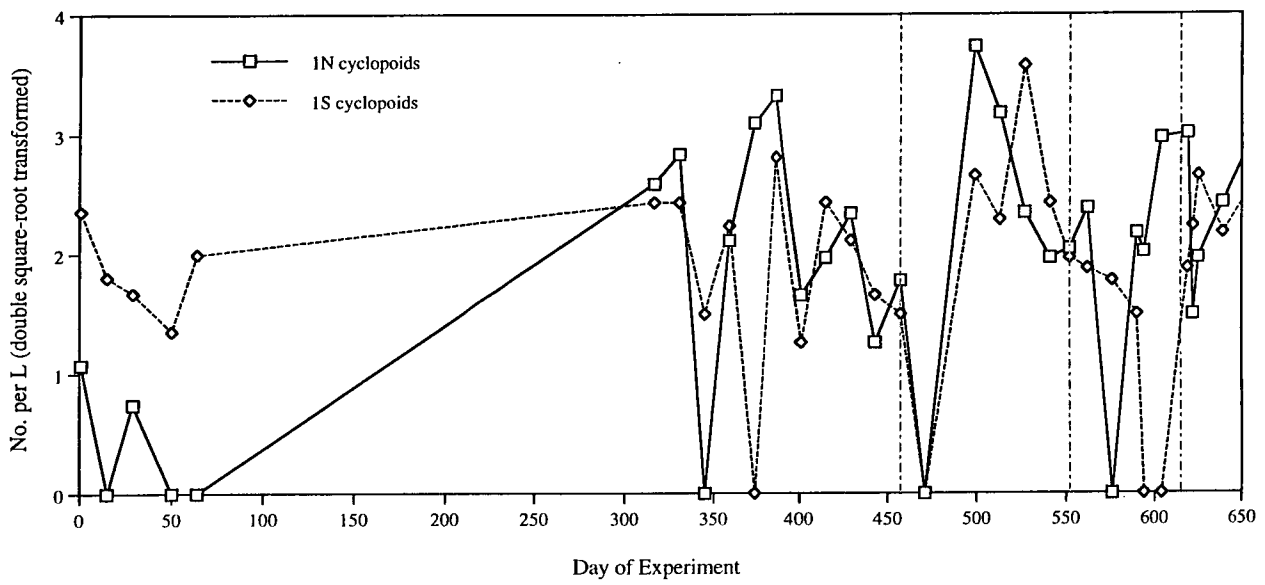
Figure 4.20(g): Moina numbers (double square-root transformed) in the 5N+5S ponds (85wB) over the main experimental period.



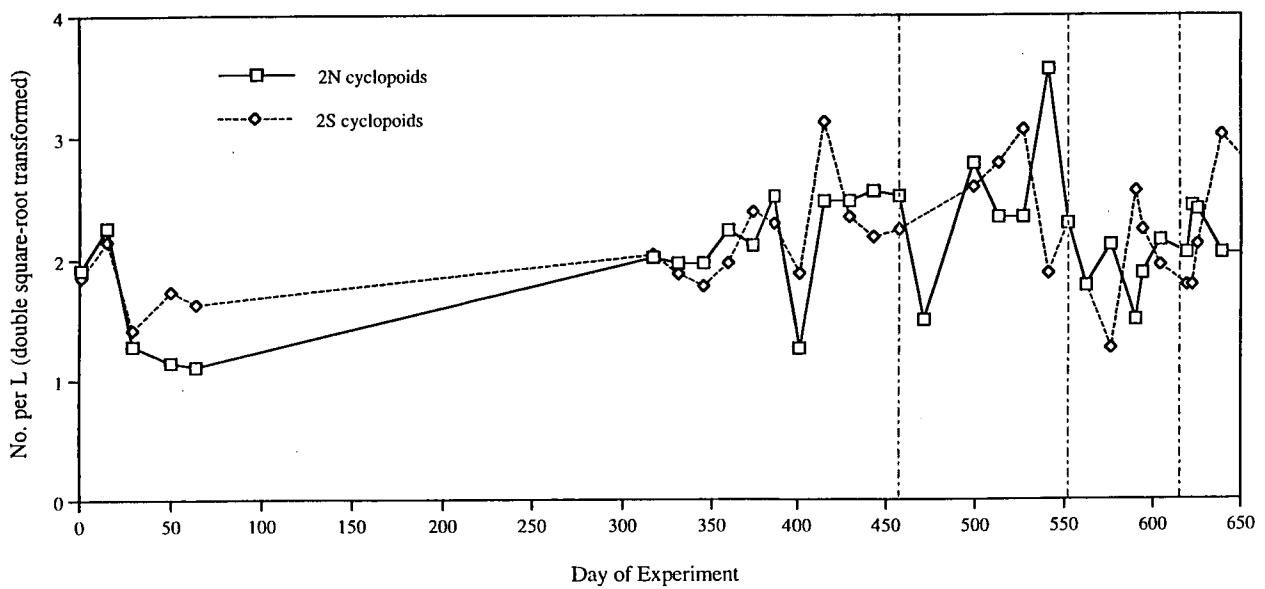
**Figure 4.21(a):** Cyclopid numbers (double square-root transformed) in the SL pond (85wB) over the main experimental period.



**Figure 4.21(b): Cyclopid numbers (double square-root transformed) in the SPN + SPS ponds (85wB) over the main experimental period.**



**Figure 4.21(c): Cyclopid numbers (double square-root transformed) in the 1N+1S ponds (85wB) over the main experimental period.**



**Figure 4.21(d): Cyclopid numbers (double square-root transformed) in the 2N+2S ponds (85wB) over the main experimental period.**



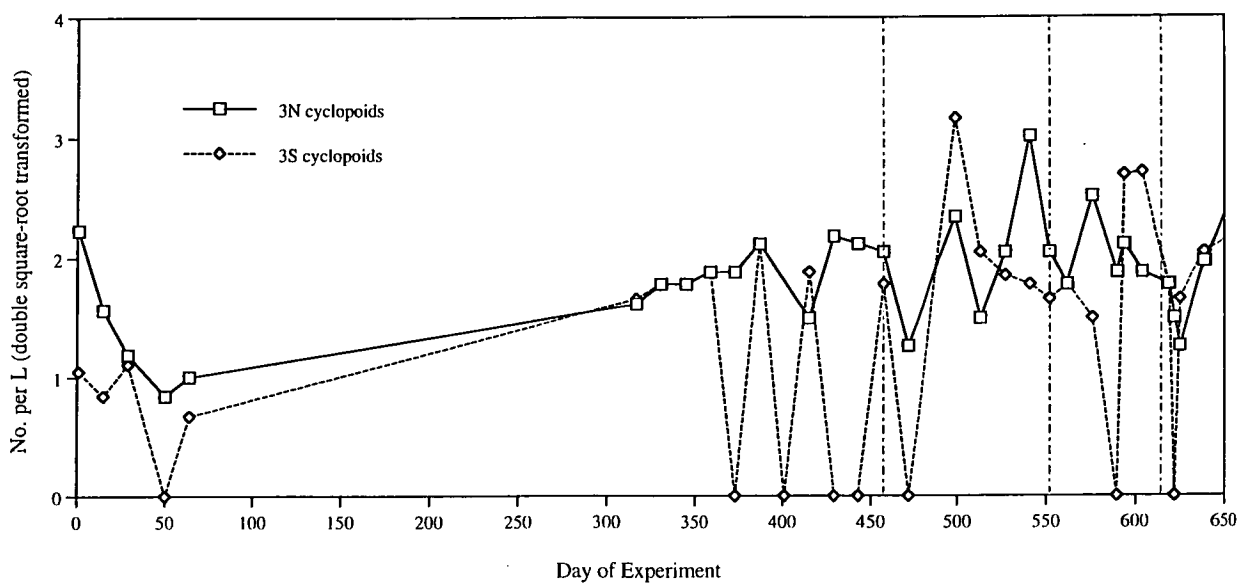


Figure 4.21(e): Cyclopoid numbers (double square-root transformed) in the 3N+3S ponds (85wB) over the main experimental period.

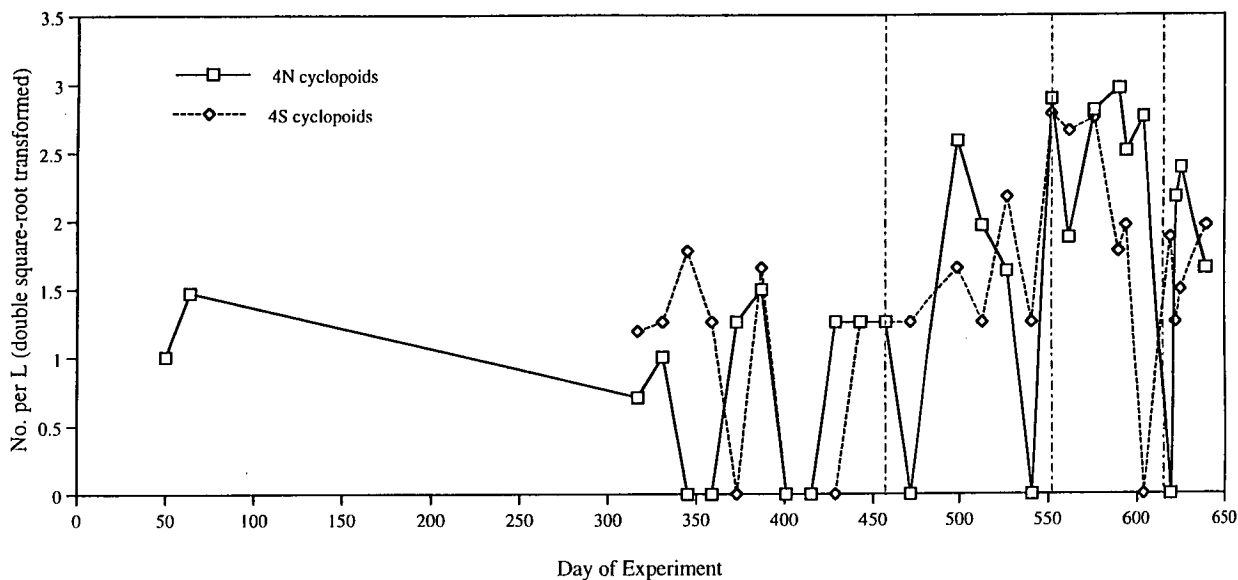


Figure 4.21(f): Cyclopoid numbers (double square-root transformed) in the 4N+4S ponds (85wB) over the main experimental period.

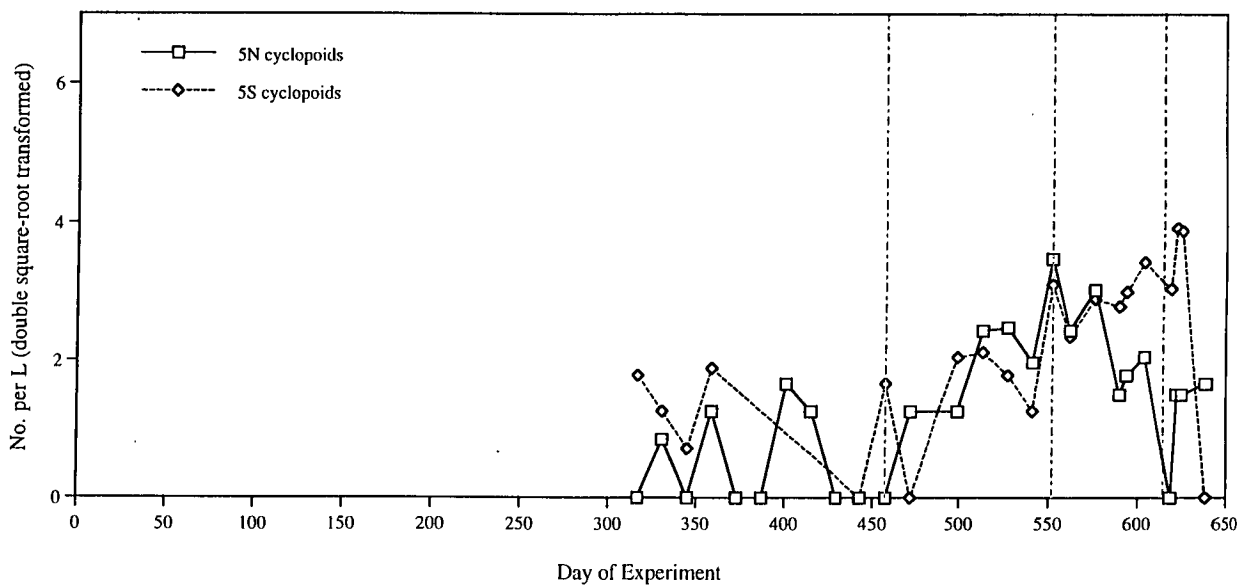
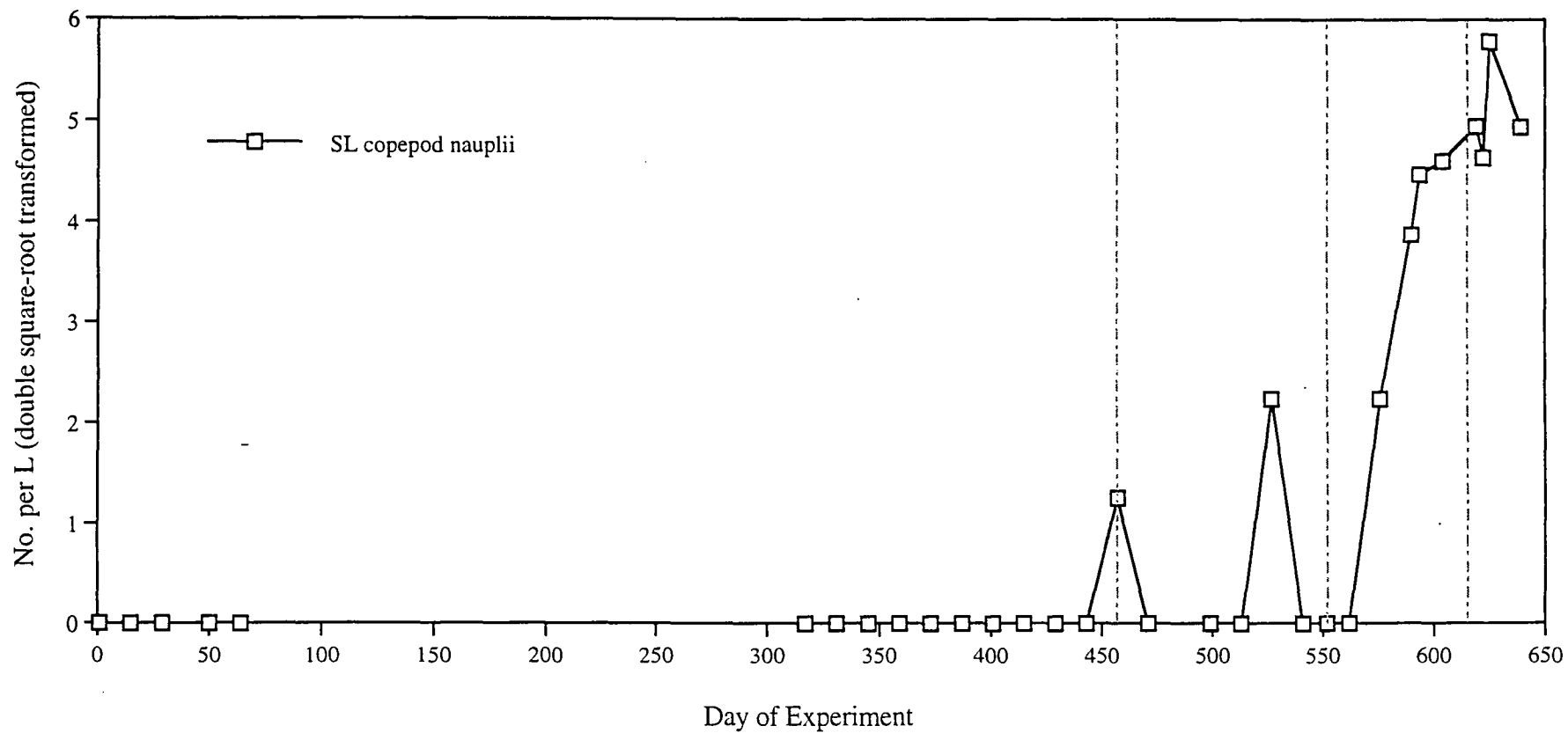
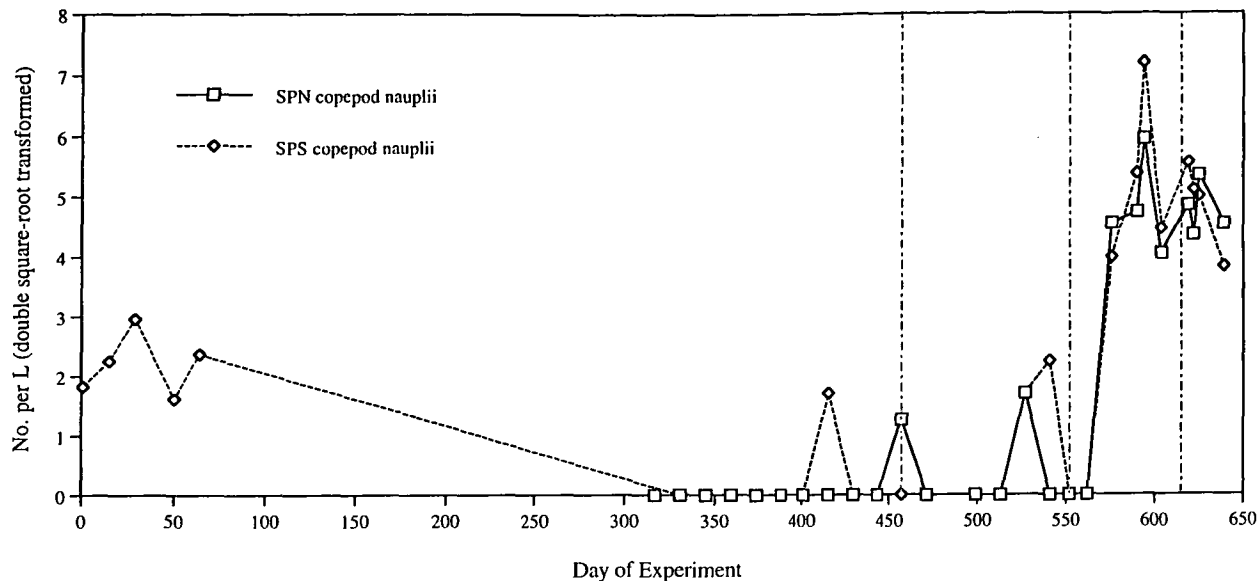


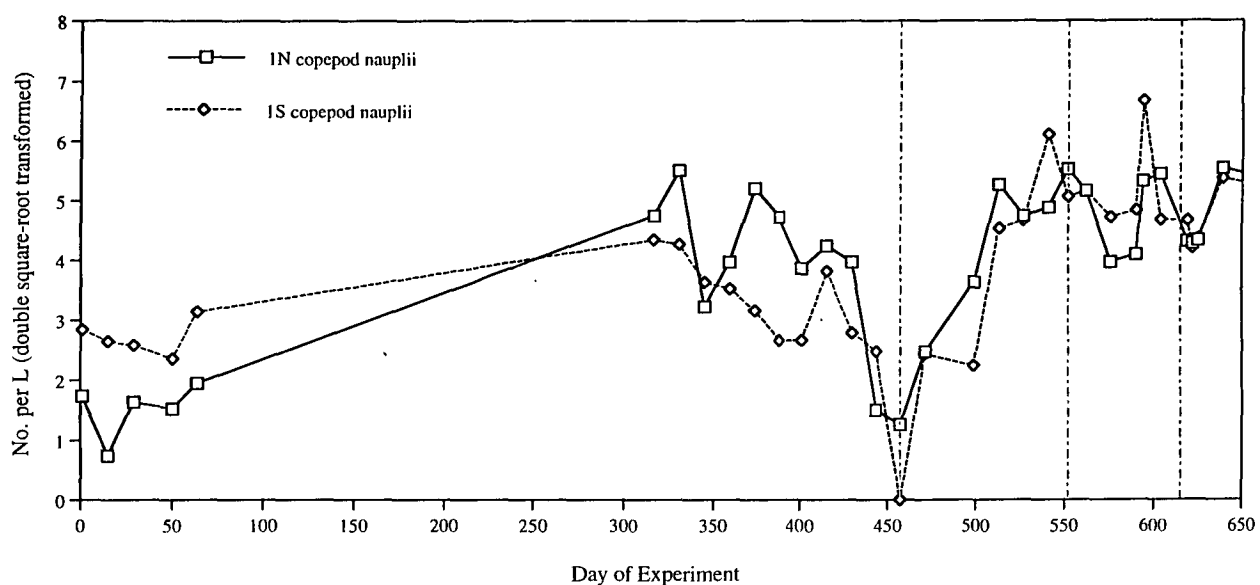
Figure 4.21(g): Cyclopoid numbers (double square-root transformed) in the 5N+5S ponds (85wB) over the main experimental period.



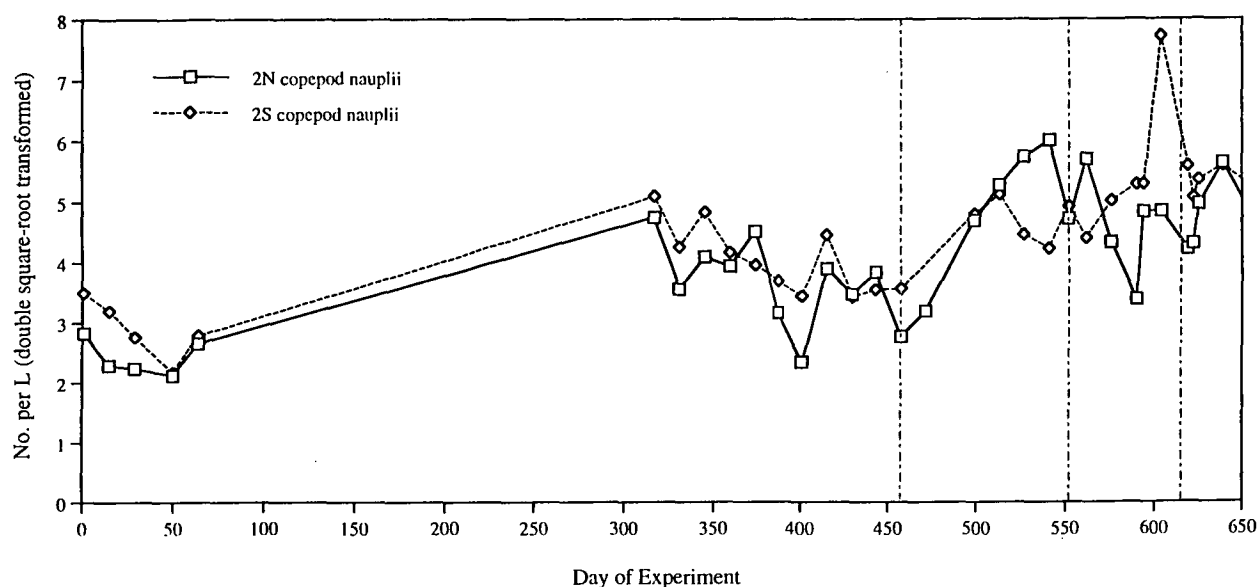
**Figure 4.22(a):** Copepod nauplii (double square-root transformed) in the SL pond (85wB) over the main experimental period.



**Figure 4.22(b):** Copepod nauplii (double square-root transformed) in the SPN + SPS ponds (85wB) over the main experimental period.



**Figure 4.22(c):** Copepod nauplii (double square-root transformed) in the 1N+1S ponds (85wB) over the main experimental period.



**Figure 4.22(d):** Copepod nauplii (double square-root transformed) in the 2N+2S ponds (85wB) over the main experimental period.

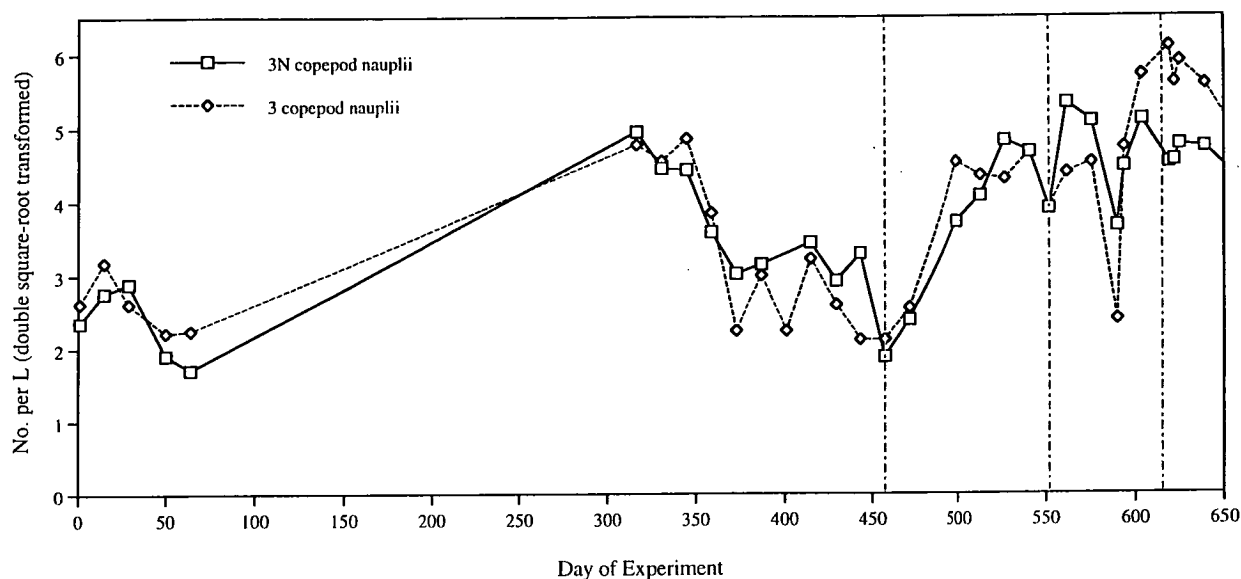


Figure 4.22(e): Copepod nauplii (double square-root transformed) in the 3N+3S ponds (85wB) over the main experimental period.

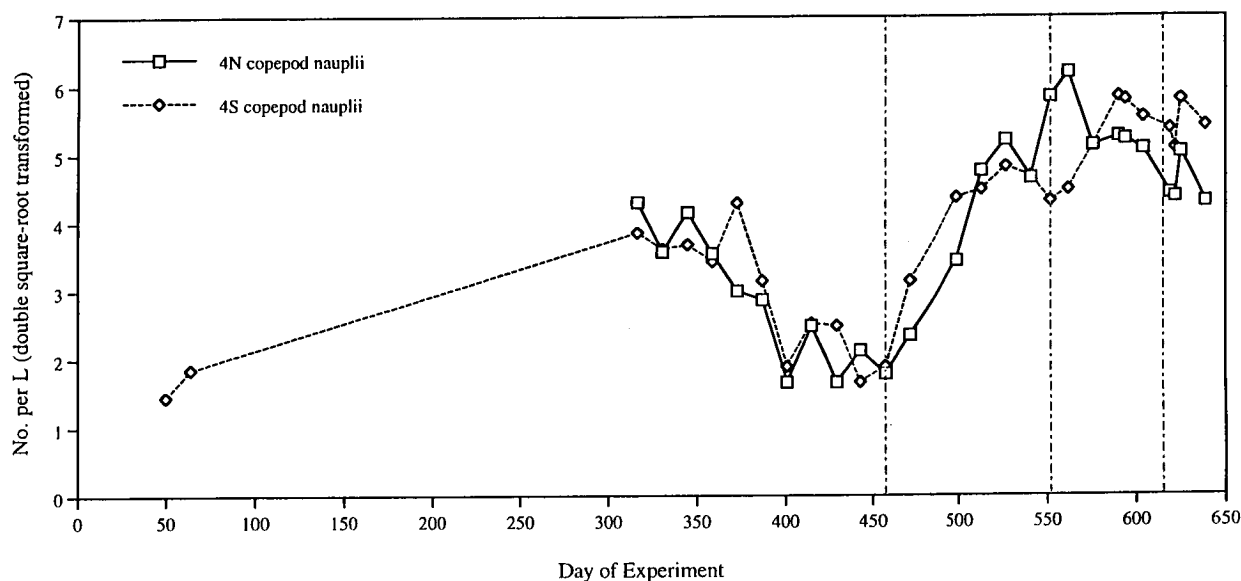


Figure 4.22(f): Copepod nauplii (double square-root transformed) in the 4N+4S ponds (85wB) over the main experimental period.

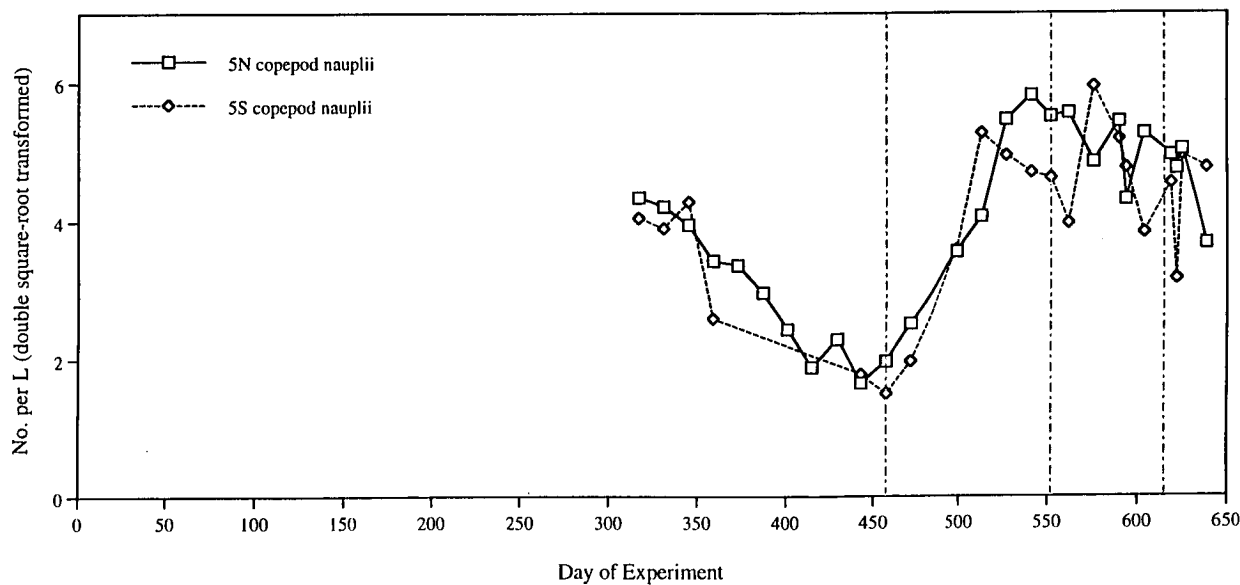


Figure 4.22(g): Copepod nauplii (double square-root transformed) in the 5N+5S ponds (85wB) over the main experimental period.

#### 4.5.3 Zooplankton: PRIMER analysis

While some general trends are evident in the preceding graphs and table, it was hoped that more definite patterns could be detected across the whole range of these species/groups by subjecting the data to multivariate analysis. Preliminary analysis of data using the SIMPER sub-routine of the PRIMER statistical package showed that the major species/groups within the plankton played the greatest role in similarity/dissimilarity between the lagoons, while rarer species only tended to add to the noise obscuring such patterns. As a result, analysis focussed on these major groups, representing four rotifer genera (*Brachionus*, *Polyarthra*, *Filinia*, and, despite the inconclusive graphs featured above, *Asplanchna*), and four crustacean genera/groups (daphnid cladocerans, moinid cladocerans, cyclopoid copepods, and total copepod nauplii). Analysis along these lines was also consistent with the objectives of the project, by focussing interest on the more common and potentially harvestable zooplankton fractions within the lagoons.

Ordination plots were generated from the data using the MDS program from PRIMER. All ordinations were generated from 20 random starts to ensure accurate plots were obtained and the Bray Curtis similarity files from which the plots originated were also examined for trends in the values they contained. Separate ordinations were produced for the four main rotifer genera and for the four main crustacean groups (representing both 'grass' and 'tree' levels of the zooplankton community) and for both together (to provide an overall view of total changes in that community).

The MDS ordination plots for the original flow pattern of the 85wB are shown in Figures 4.23-4.27, those for the equilibration period in Figures 4.28-4.37, and those for the three experimental divisions of flow in Figures 4.38-4.43, 4.44-4.49, and 4.50-4.53, respectively. In each Figure, the plot for the four main rotifer groups/genera is given at the top of the page, the plot for the four main crustaceans in the middle of the page, and the plot for all of these groups combined at the bottom.

Where large numbers of ponds group tightly, the plots are repeated at smaller size next to the original with outliers removed, to show the relationships between the remaining ponds in more detail. Stress values, which give an indication of how well an ordination represents the data, are given with the main plots. Stress values less than 0.1 are generally considered to be acceptable, those between 0.1 and 0.15 are acceptable but not ideal, those between 0.15 and 0.25 are poor, and those over 0.25 are unacceptable (Belbin 1994).

While Figures 4.23-4.53 allow direct comparison between rotifer, crustacean and combined zooplankton community patterns on any given day, the MDS plots for each zooplankton group are repeated alongside the corresponding section of text for that group. This provides easier reader access to corresponding graphs, as well as allowing direct comparison within each of the above groups over the course of a flow period. The MDS ordinations for rotifers during the original and equal flow patterns are presented in Figures 4.54(a-e) and 4.55(a-j), respectively, while those for the first, second and third flow divisions are presented in Figures 4.56(a-f), 4.57(a-f), and 4.58(a-d), respectively. Corresponding ordinations for crustaceans can be found in Figures 4.59-4.63, and those for both main groups combined in Figures 4.64-4.68.

Prevailing flow conditions are listed with each Figure, while, if required, the original and divided flow patterns are presented in Figures 3.2 & 3.4.

### *Expected patterns*

For the initial period, the major differences between lagoon pairs were expected to occur earlier, as the paired lagoons 1N and 1S were at this stage separated by the greatest distance (as ponds 3 and 5 in the original sequence, respectively). Not only were these ponds separated by an entire lagoon, but the intervening pond (lagoon 4, to become the SP pond) was itself separated from the SL pond (lagoon 1) by two other ponds. Under altered flow conditions, ponds SL and SP

would be linked, and so expected to show a greater degree of similarity, while the SP would also then precede 1N (original pond 3) in sequence, and be expected to change accordingly. In contrast, pond pairs 2S and 2N plus 3N and 3S followed each other directly in sequence under initial conditions (ponds 6 and 7 plus 8 and 9, respectively), and so could be expected to show greater similarity in the communities present. For the rotifers at least, differences could also be expected to be more pronounced between the earlier ponds, as it is here that the bulk of the rotifer assemblages occur. In addition to the changes above, the SP pond was initially outflowing in only one direction (so there are no SPN samples for the original flow period), and outlets still needed to be constructed so that later ponds could be added to the system.

Under equal flow conditions, it was expected that the patterns would change, with 1N + 1S and the SL + SPS (and SPN) lagoons becoming closer than under the original conditions. All paired lagoons were expected to show similar patterns, with these becoming established by the time of the first equal flow sample on 07.04.94, as the system had been refilling and equilibrating under this regime since the beginning of the year.

With unequal divisions in flow between the two sides, it was expected that the patterns and pairings established during the equal flow period would be altered and split, with the degree of difference becoming more pronounced as the new flow regime became established. With the reversal of the division in flow, it was predicted that any differences established during the preceding flow conditions would gradually be diminished and then reversed as the new flow regime became established. With the second reversal of the division in flow, it was again predicted that the prevailing patterns would diminish and reverse.

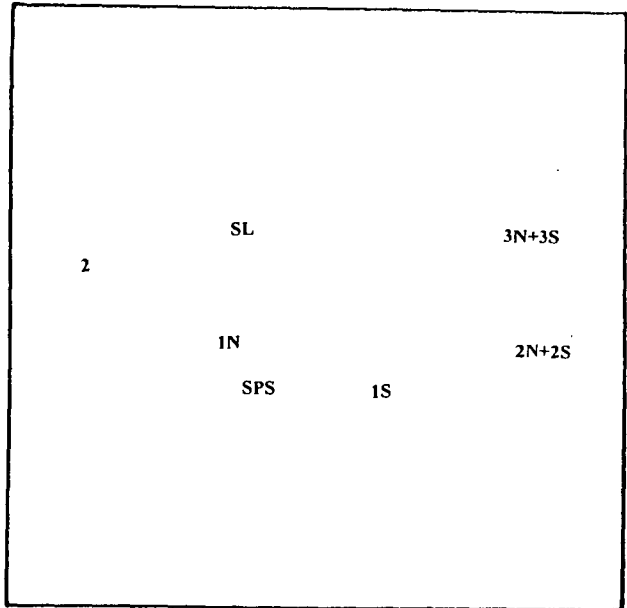
Figure 4.23

MDS ordinations for 26.05.93 (Day 1)  
Original flow conditions

Missing values:

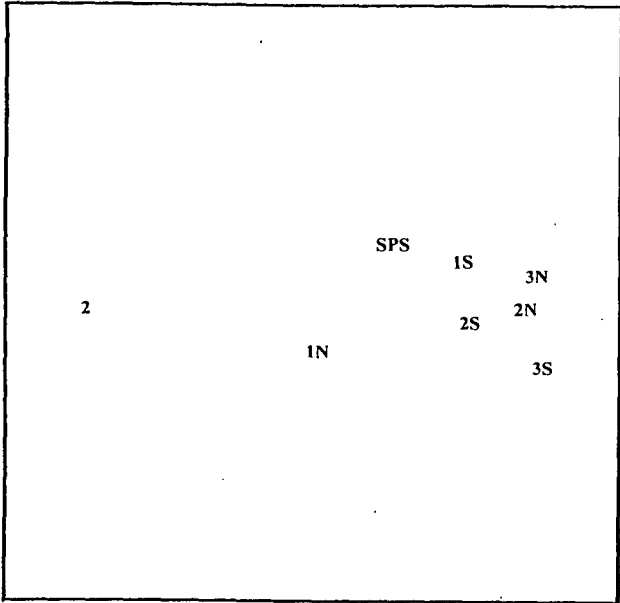
No SPN, 4N, 4S, 5N, 5S samples at this stage

No crustaceans in sample SL



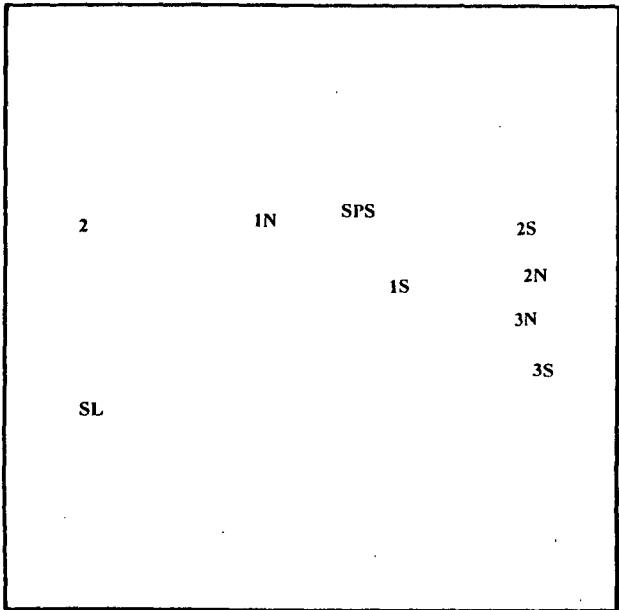
MAIN ROTIFERS

Stress = 0.01



MAIN CRUSTACEANS

Stress = 0.00



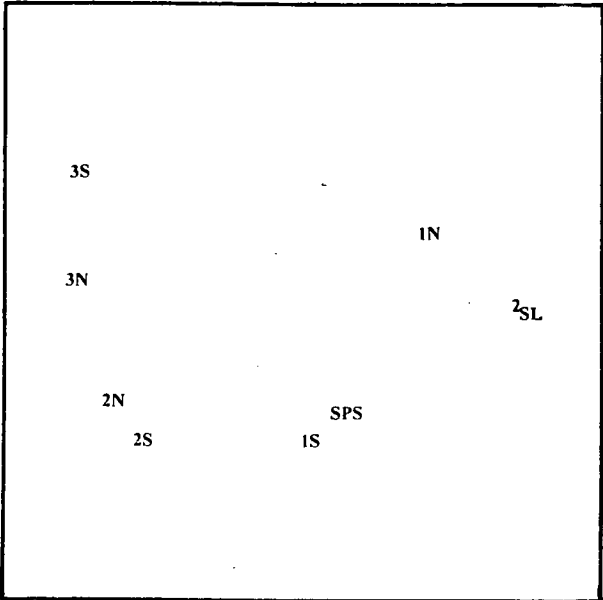
ALL MAIN TAXA

Stress = 0.01

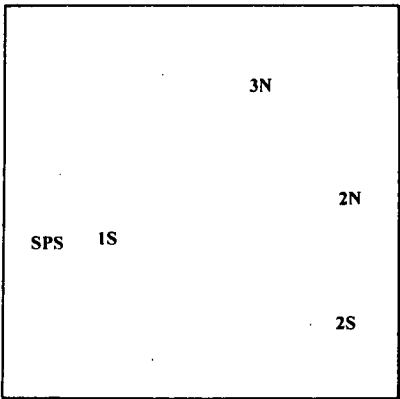


1B

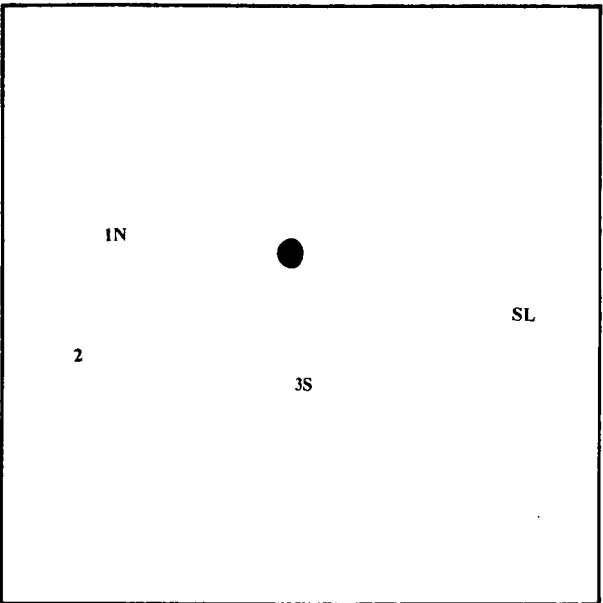
**Figure 4.24**  
**MDS ordinations for 09.06.93 (Day 15)**  
**Original flow conditions**  
*Missing values:*  
 No SPN, 4N, 4S, 5N, 5S samples at this stage



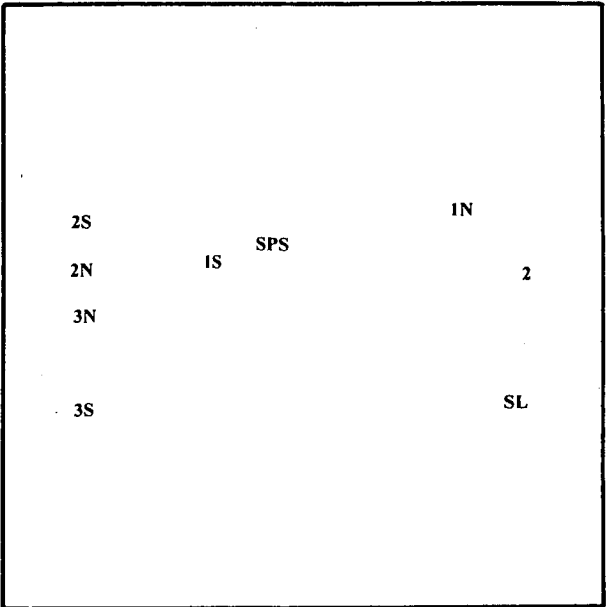
**MAIN ROTIFIERS** Stress = 0.00



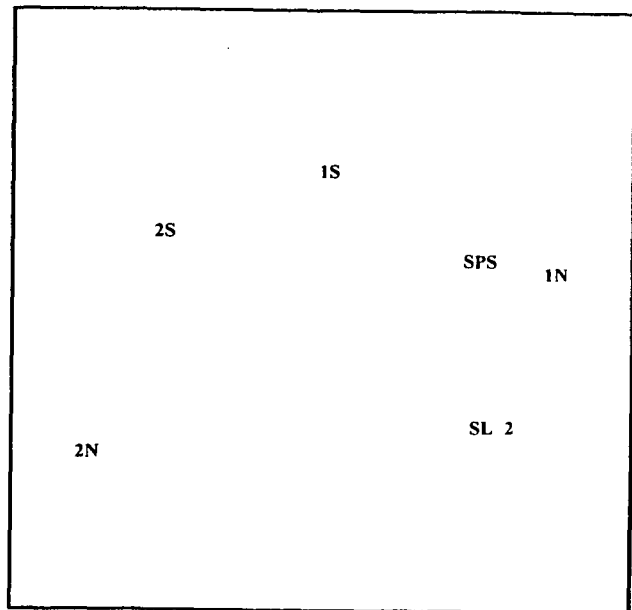
**MAIN CRUSTACEANS**  
 SL, 2, 1N, 3S removed



**MAIN CRUSTACEANS**  
 ● = SPS, 1S, 2N, 2S, 3N Stress = 0.01

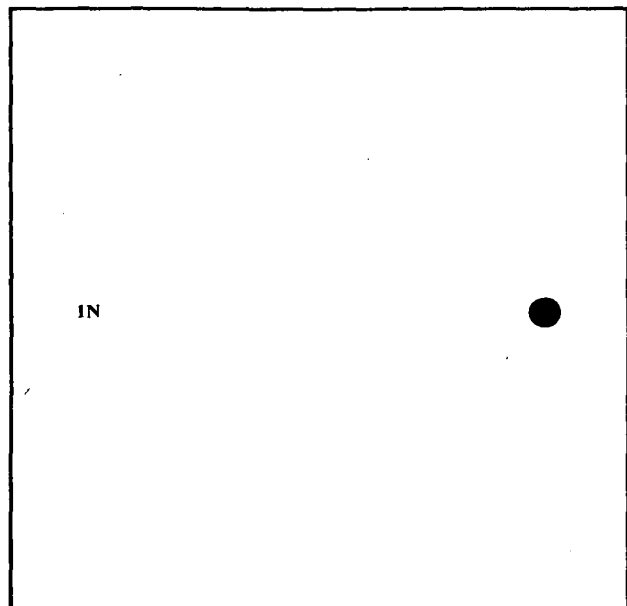


**ALL MAIN TAXA** Stress = 0.02



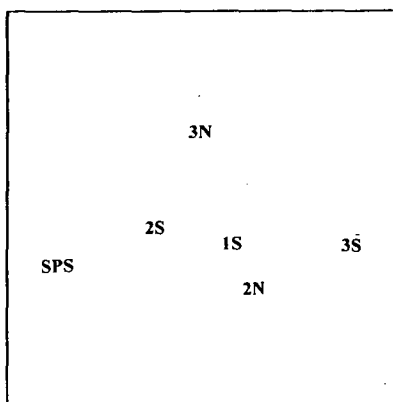
MAIN ROTIFERS

Stress = 0.00

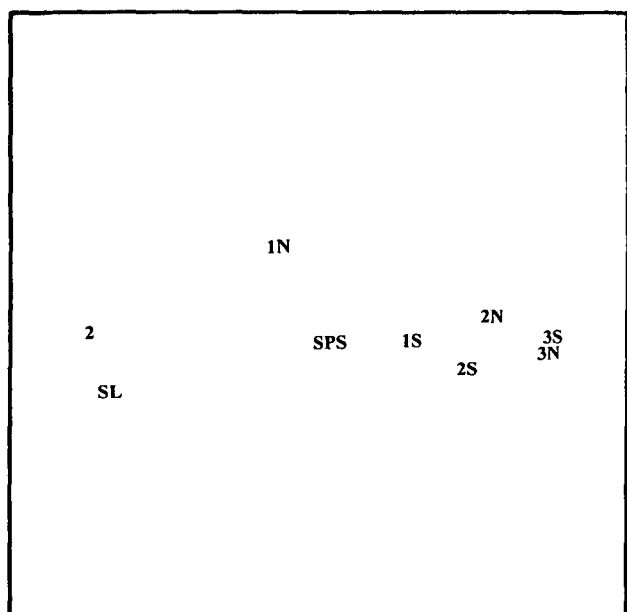


MAIN CRUSTACEANS  
● = SPS, 1S, pairs 2 + 3

Stress = 0.01



MAIN CRUSTACEANS  
1N removed



ALL MAIN TAXA

Stress = 0.01

Figure 4.25

MDS ordinations for 23.06.93 (Day 29)  
Original flow conditions

Missing values:

No SPN, 4N, 4S, 5N, 5S samples at this stage

No rotifers in samples 3N or 3S

No crustaceans in samples SL or (2)

**Figure 4.26**

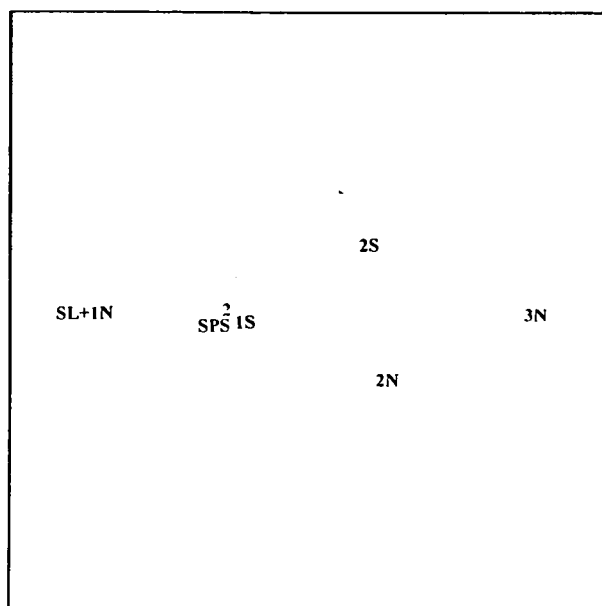
**MDS ordinations for 14.07.93 (Day 50)**  
**Original flow conditions**

*Missing values:*

No SPN, 4N, 5N, 5S samples at this stage

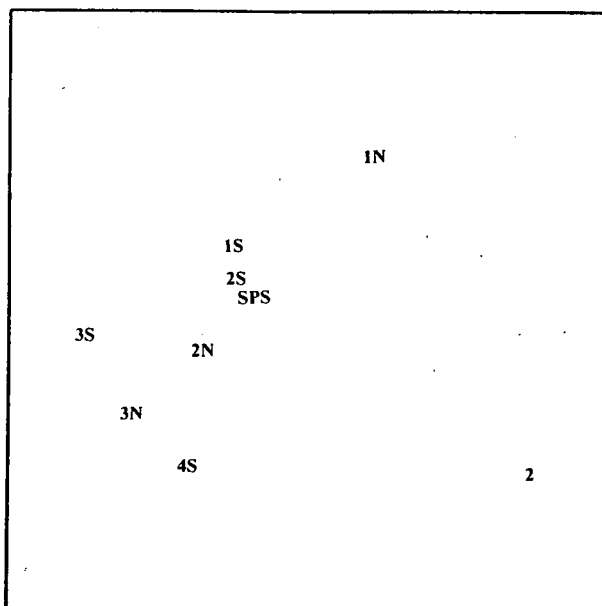
No rotifers in samples 3S or 4S

No crustaceans in sample SL



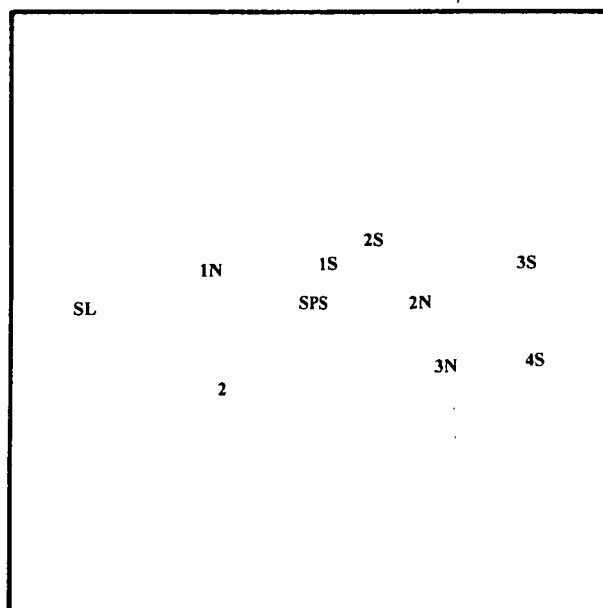
MAIN ROTIFERS

Stress = 0.01



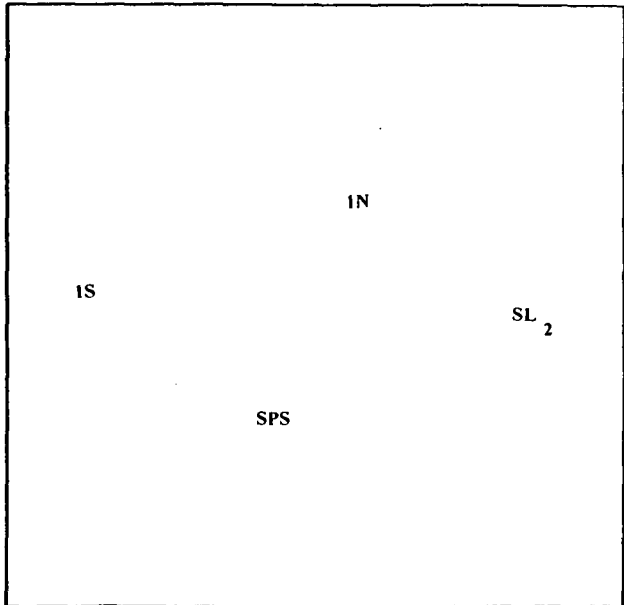
MAIN CRUSTACEANS

Stress = 0.05



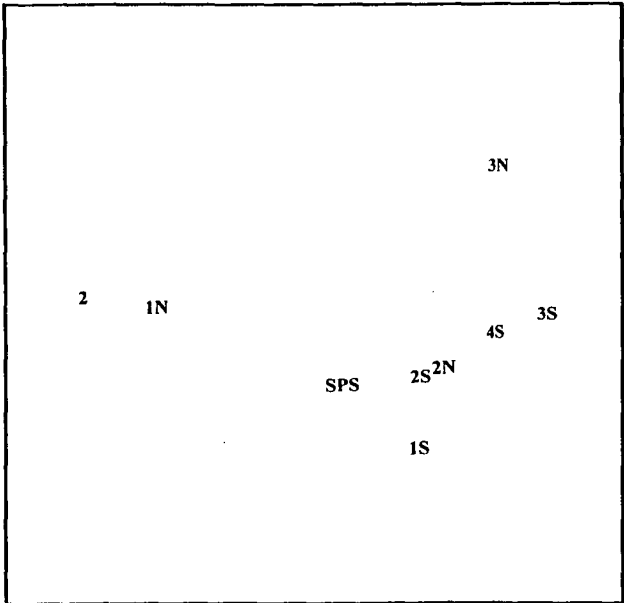
ALL MAIN TAXA

Stress = 0.04



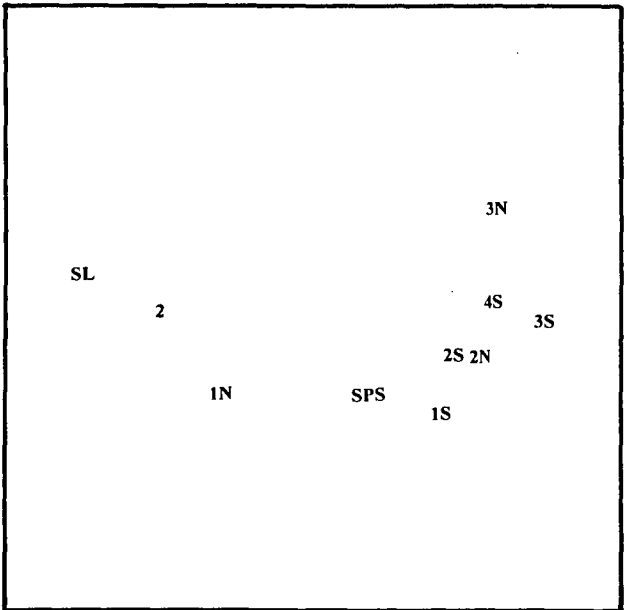
MAIN ROTIFERS

Stress = 0.00



MAIN CRUSTACEANS

Stress = 0.03



ALL MAIN TAXA

Stress = 0.01

**Figure 4.27**

**MDS ordinations for 28.07.93 (Day 64)**  
**Original flow conditions**

*Missing values:*

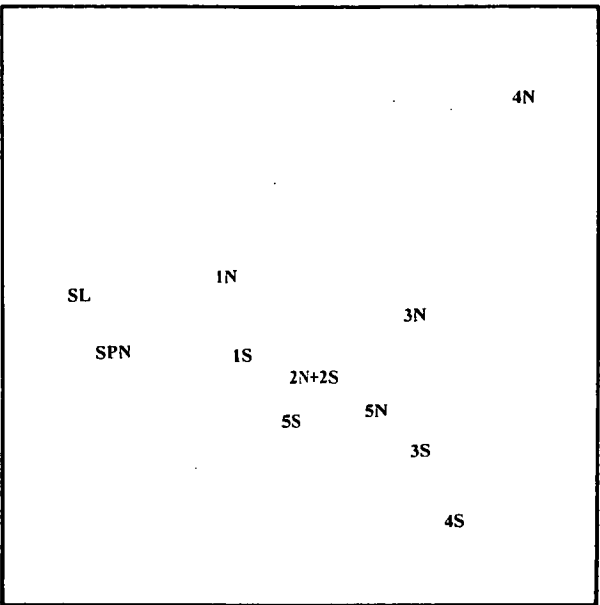
No SPN, 4N, 5N, 5S samples at this stage

No rotifers in samples 2N, 2S, 3N, 3S or 4S

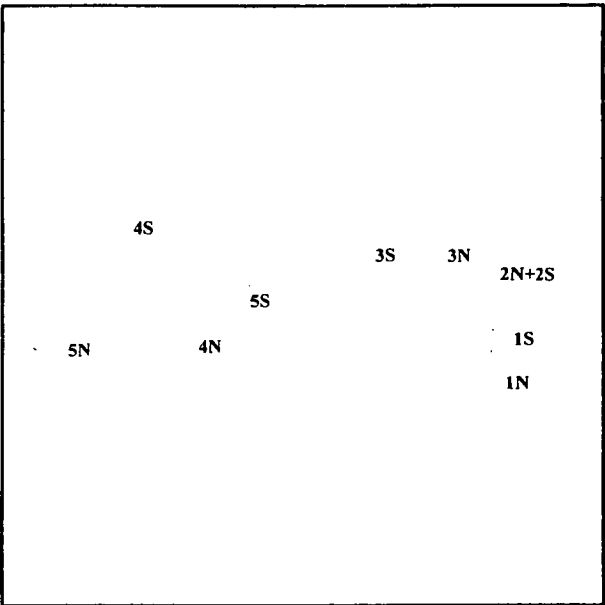
No crustaceans in sample SL

**Figure 4.28**  
**MDS ordinations for 07.04.94 (Day 317)**  
**Equal flow conditions (50%N: 50%S)**

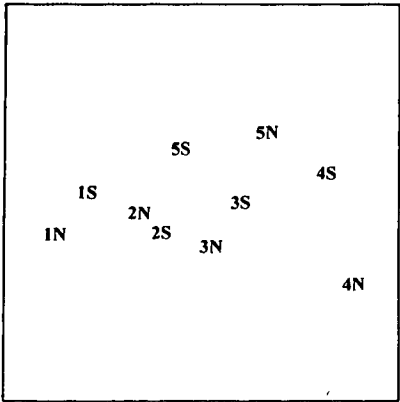
*Missing values:*  
 No SPS sample (preservation problem)  
 No crustaceans in samples SL or SPN



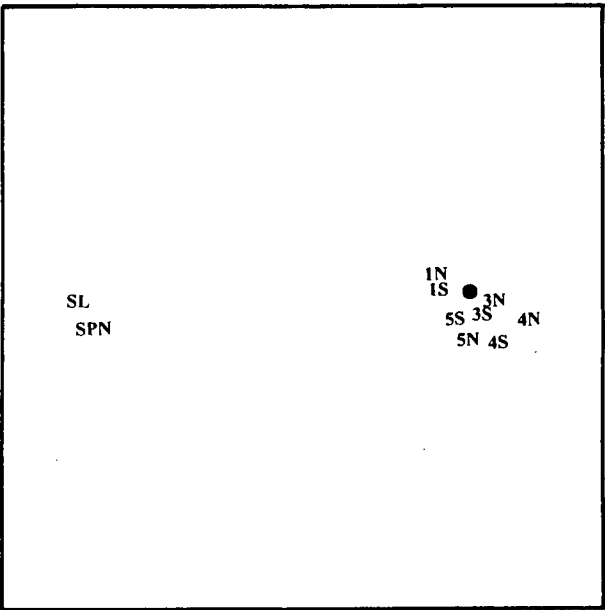
**MAIN ROTIFERS** Stress = 0.04



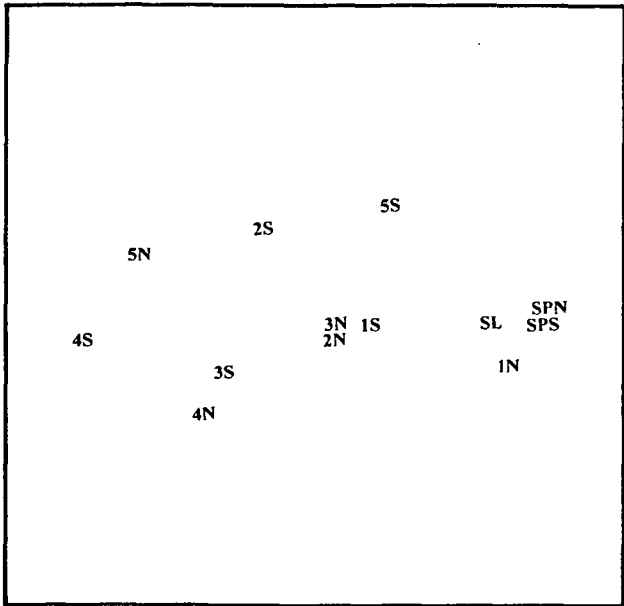
**MAIN CRUSTACEANS** Stress = 0.02



**ALL MAIN TAXA**  
 SL, SPN removed

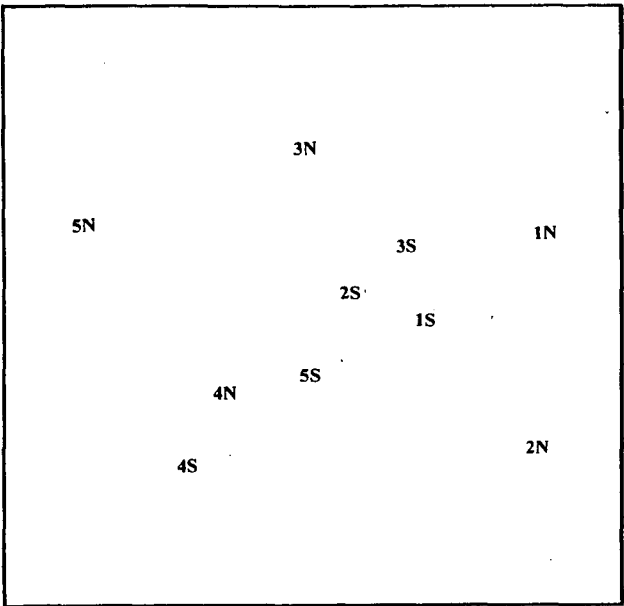


**ALL MAIN TAXA** ● = 2N+2S Stress = 0.01



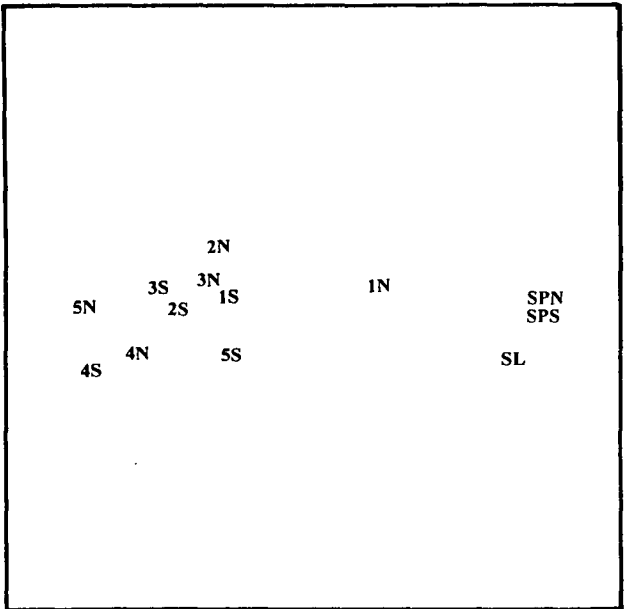
MAIN ROTIFERS

Stress = 0.05



MAIN CRUSTACEANS

Stress = 0.06



ALL MAIN TAXA

Stress = 0.04

Figure 4.29

MDS ordinations for 21.04.94 (Day 331)  
Equal flow conditions (50%N: 50%S)

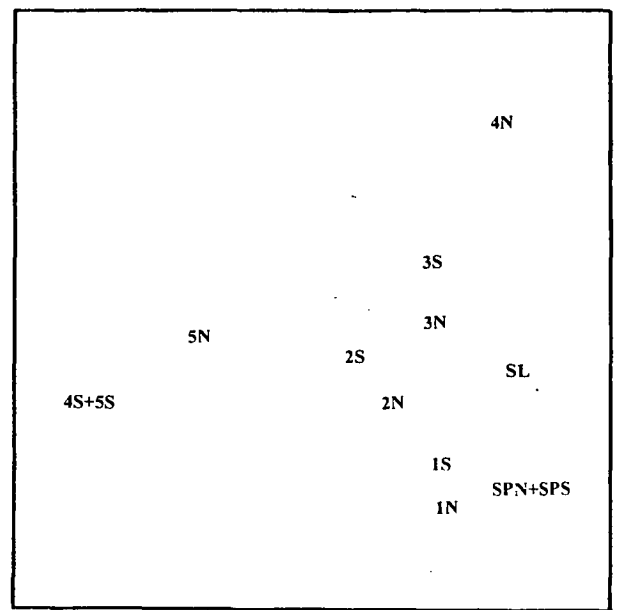
Missing values:  
No crustaceans in samples SL, SPN, or SPS

**Figure 4.30**

**MDS ordinations for 05.05.94 (Day 345)**  
**Equal flow conditions (50%N: 50%S)**

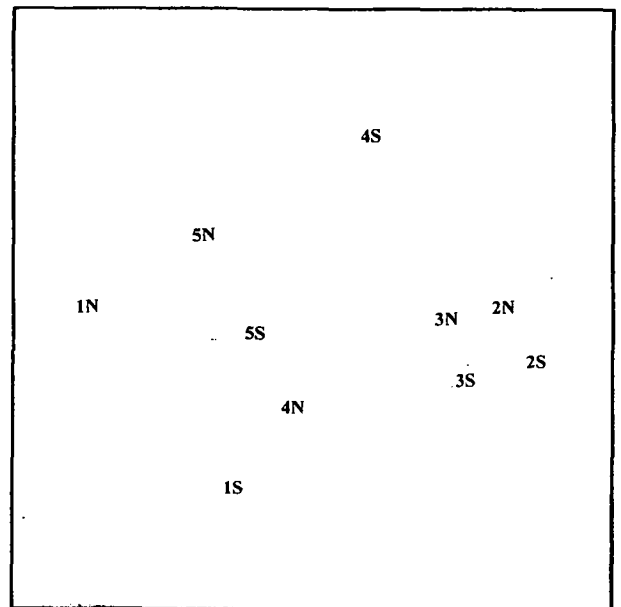
*Missing values:*

No crustaceans in samples SL, SPN, or SPS



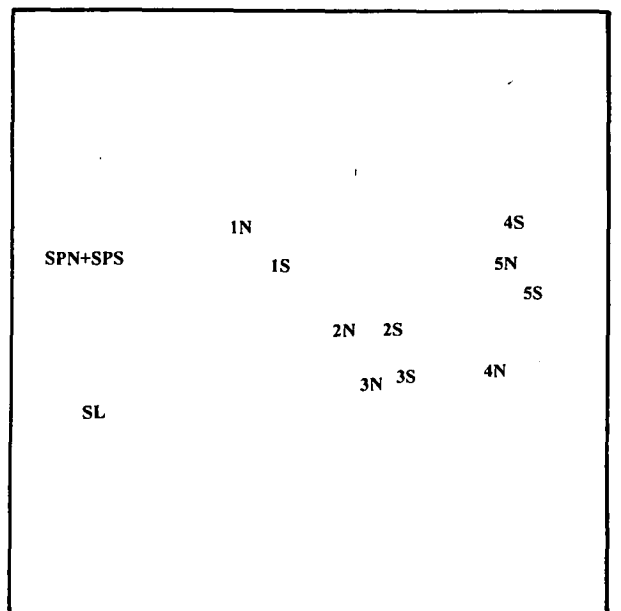
MAIN ROTIFERS

Stress = 0.04



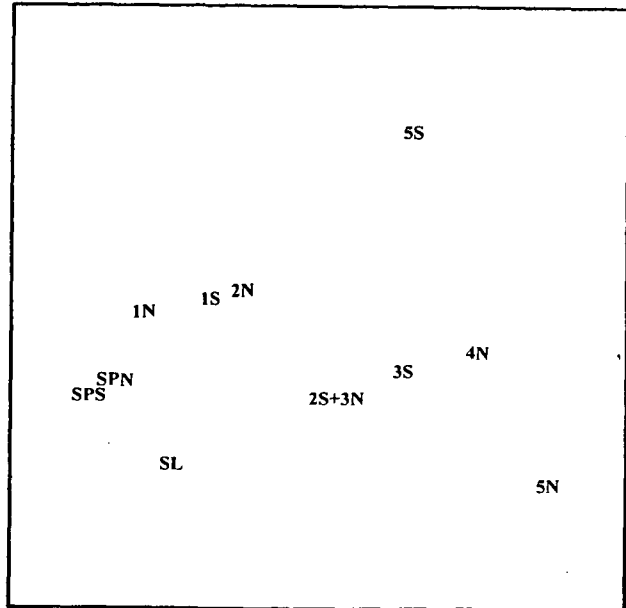
MAIN CRUSTACEANS

Stress = 0.07



ALL MAIN TAXA

Stress = 0.05



**Figure 4.31**

**MDS ordinations for 19.05.94 (Day 359)  
Equal flow conditions (50%N: 50%S)**

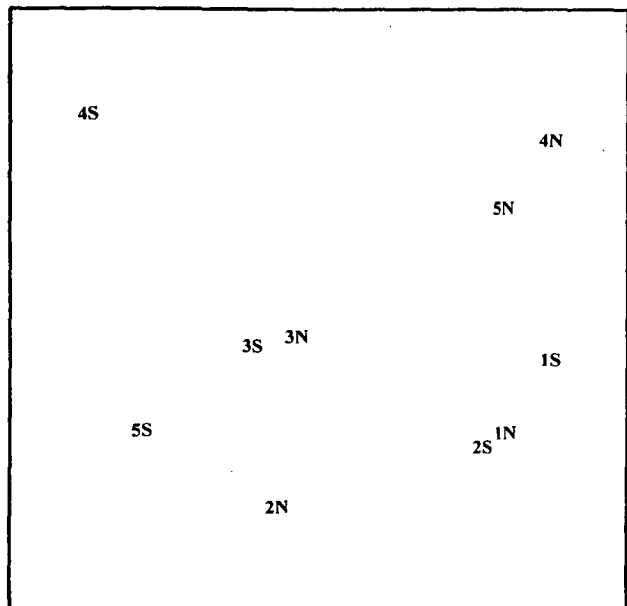
*Missing values:*

No rotifers in sample 4S

No crustaceans in samples SL, SPN, or SPS

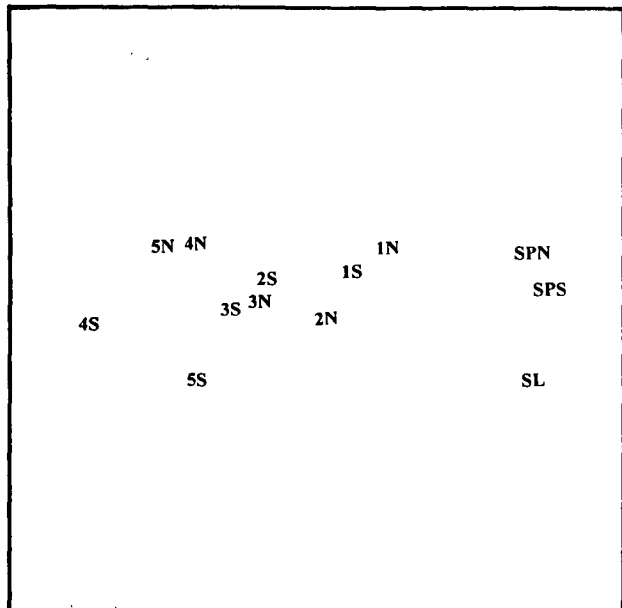
MAIN ROTIFERS

Stress = 0.04



MAIN CRUSTACEANS

Stress = 0.08



ALL MAIN TAXA

Stress = 0.03



**Figure 4.32**

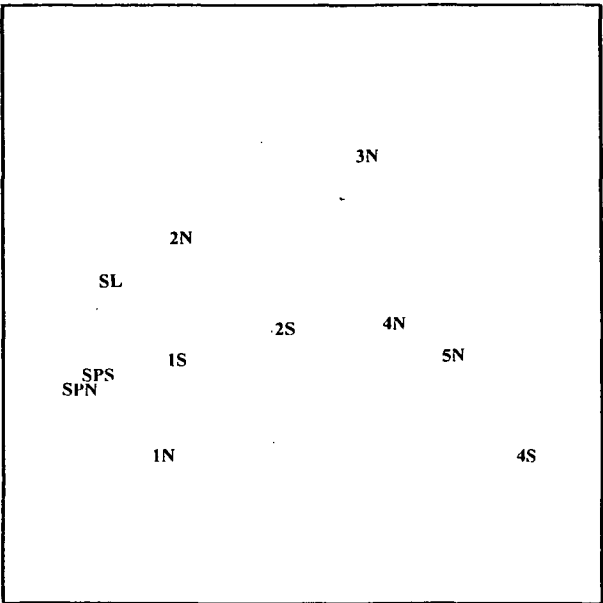
**MDS ordinations for 02.06.94 (Day 373)**  
**Equal flow conditions (50%N: 50%S)**

*Missing values:*

No 5S sample (pond cut out, MW works)

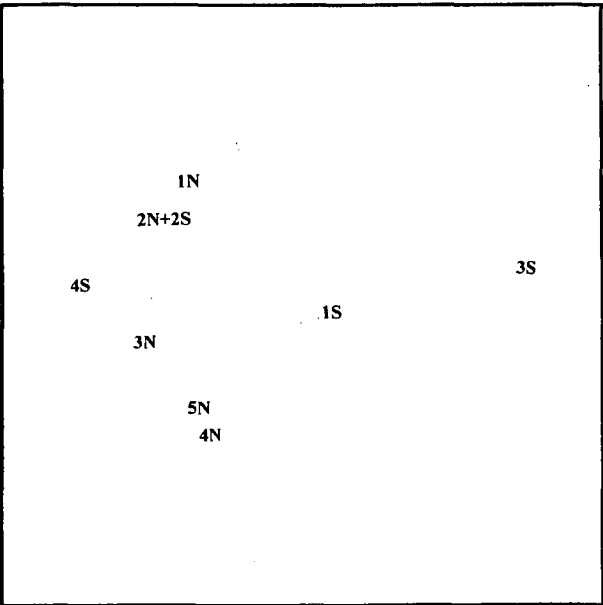
No rotifers in sample 3S

No crustaceans in samples SL, SPN, or SPS



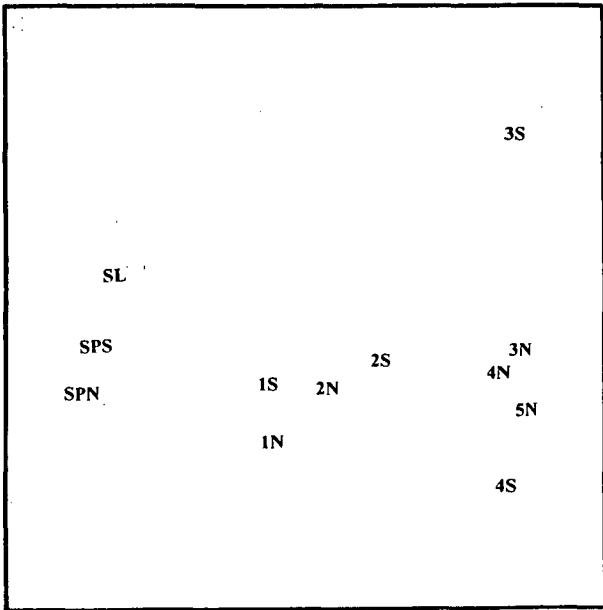
MAIN ROTIFERS

Stress = 0.05



MAIN CRUSTACEANS

Stress = 0.03



ALL MAIN TAXA

Stress = 0.05

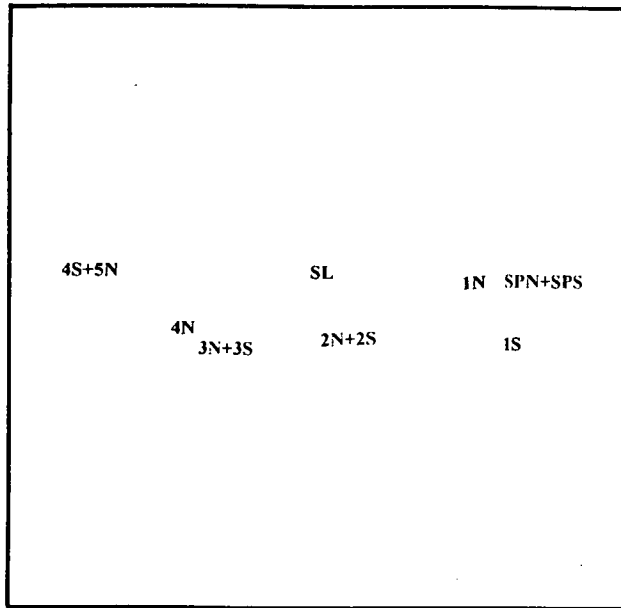
**Figure 4.33**

**MDS ordinations for 16.06.94 (Day 387)**  
**Equal flow conditions (50%N: 50%S)**

*Missing values:*

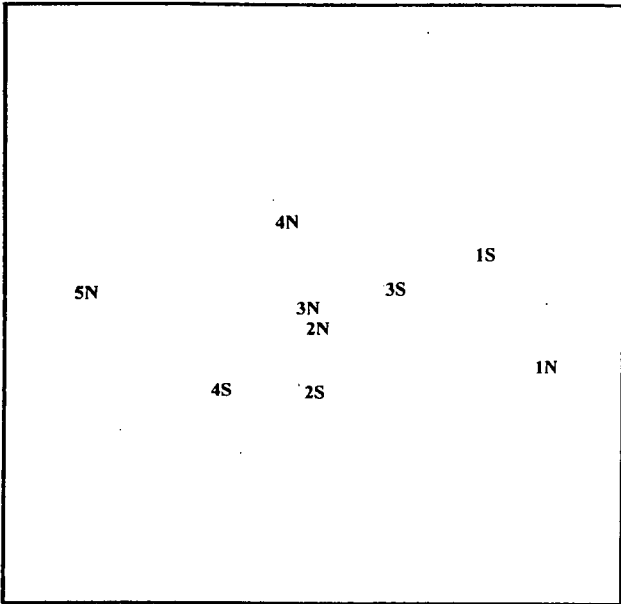
No 5S sample (pond cut out, MW works)

No crustaceans in samples SL, SPN, or SPS



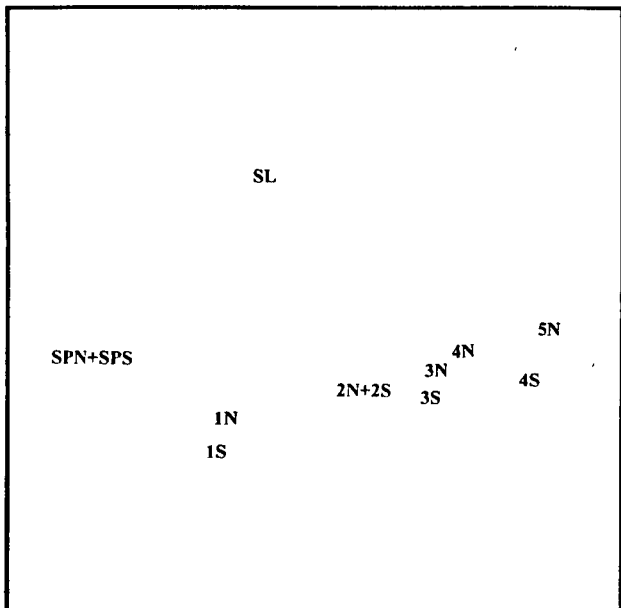
MAIN ROTIFERS

Stress = 0.01



MAIN CRUSTACEANS

Stress = 0.02



ALL MAIN TAXA

Stress = 0.03

**Figure 4.34**

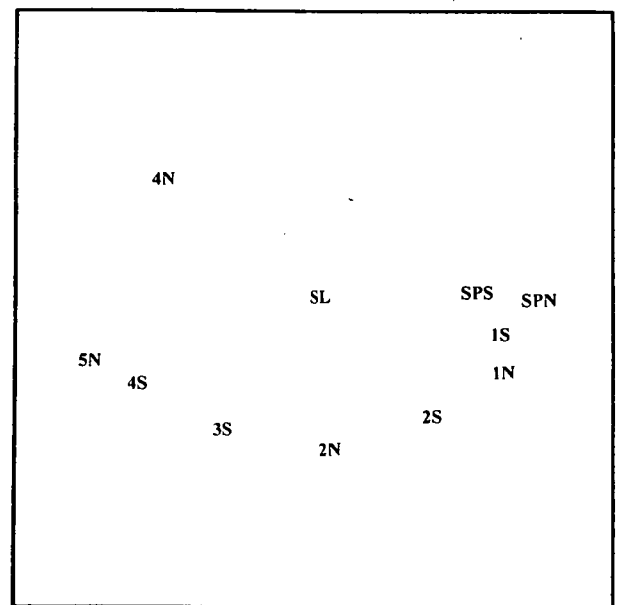
**MDS ordinations for 30.06.94 (Day 401)**  
**Equal flow conditions (50%N: 50%S)**

*Missing values:*

No 3N sample (preservation problem)

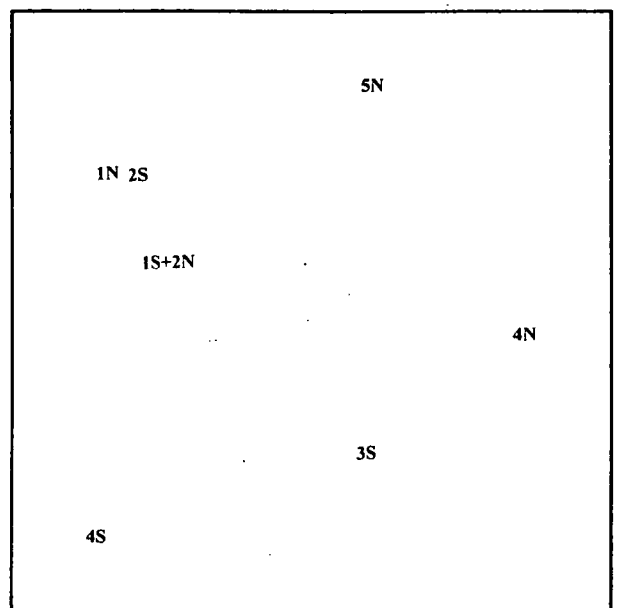
No 5S sample (pond cut out, MW works)

No crustaceans in samples SL, SPN, or SPS



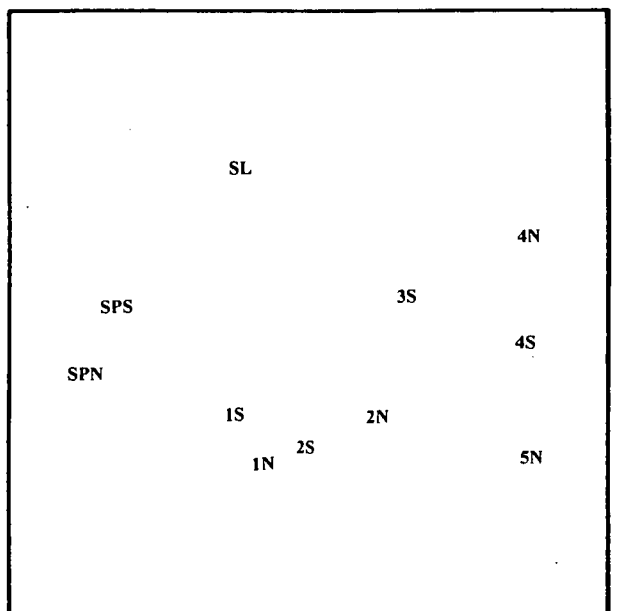
MAIN ROTIFERS

Stress = 0.03



MAIN CRUSTACEANS

Stress = 0.01

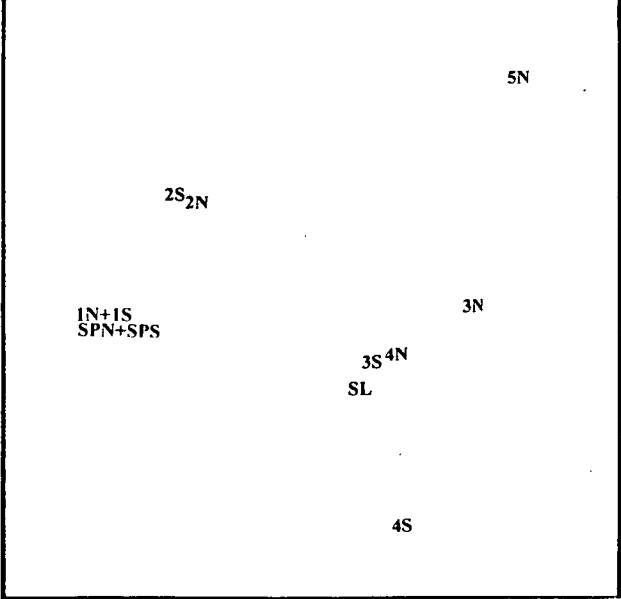


ALL MAIN TAXA

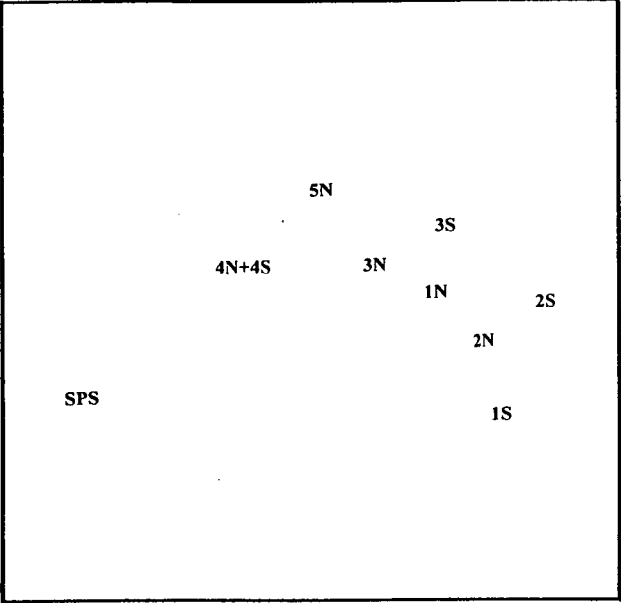
Stress = 0.08

**Figure 4.35**  
**MDS ordinations for 14.07.94 (Day 415)**  
**Equal flow conditions (50%N: 50%S)**

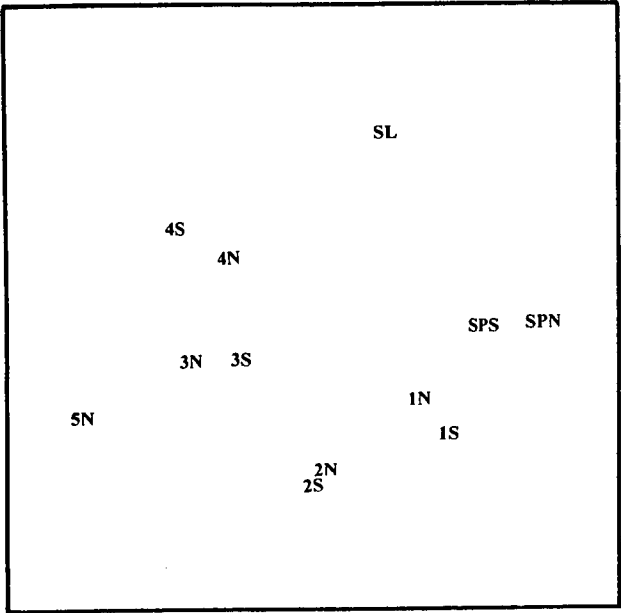
*Missing values:*  
No 5S sample (pond cut out, MW works)  
No crustaceans in samples SL or SPN



**MAIN ROTIFERS** Stress = 0.04



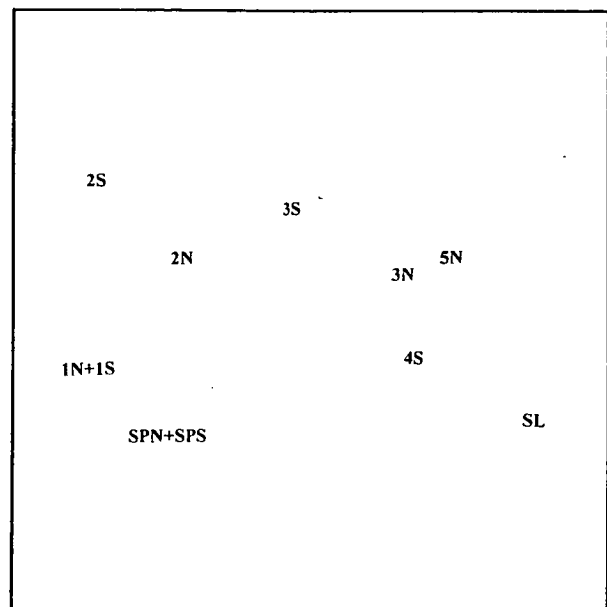
**MAIN CRUSTACEANS** Stress = 0.03



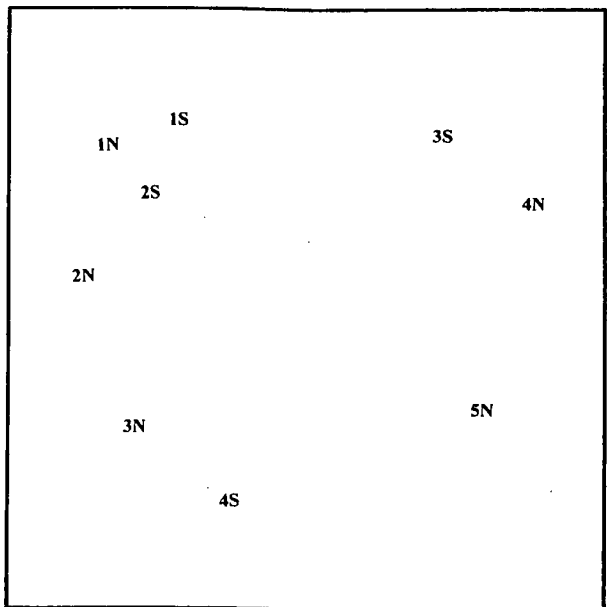
**ALL MAIN TAXA** Stress = 0.05

**Figure 4.36**  
**MDS ordinations for 28.07.94 (Day 429)**  
**Equal flow conditions (50%N: 50%S)**

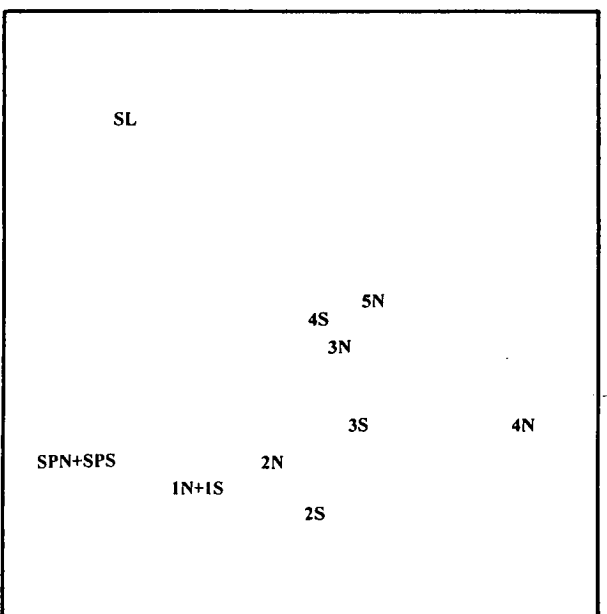
*Missing values:*  
No 5S sample (pond cut out, MW works)  
No rotifers in sample 4N  
No crustaceans in samples SL, SPN, or SPS



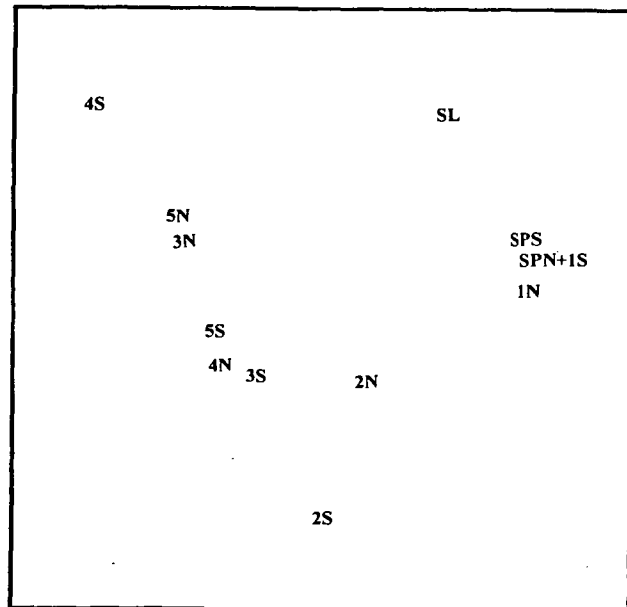
**MAIN ROTIFERS** **Stress = 0.02**



**MAIN CRUSTACEANS** **Stress = 0.04**



**ALL MAIN TAXA** **Stress = 0.05**



**Figure 4.37**

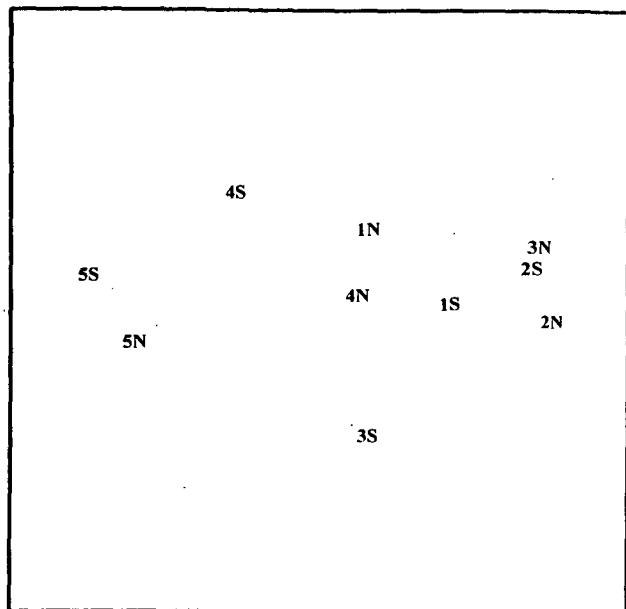
**MDS ordinations for 11.08.94 (Day 443)**  
**Equal flow conditions (50%N: 50%S)**

*Missing values:*

No crustaceans in samples SL, SPN, or SPS

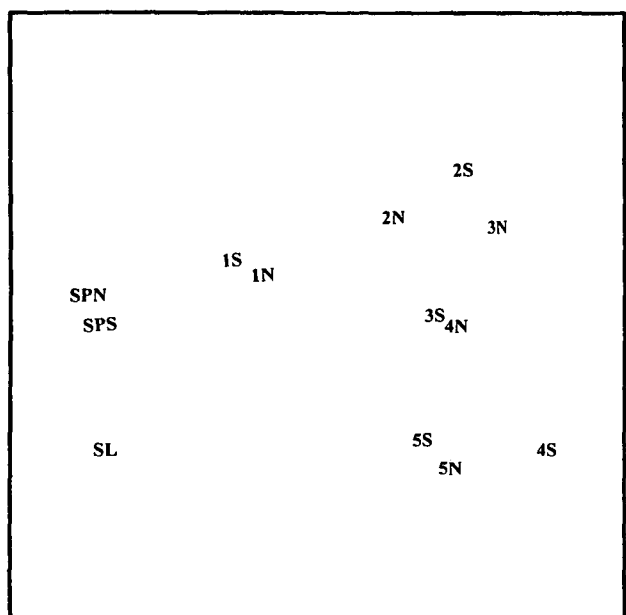
MAIN ROTIFERS

Stress = 0.04



MAIN CRUSTACEANS

Stress = 0.05



ALL MAIN TAXA

Stress = 0.06

**Figure 4.38**

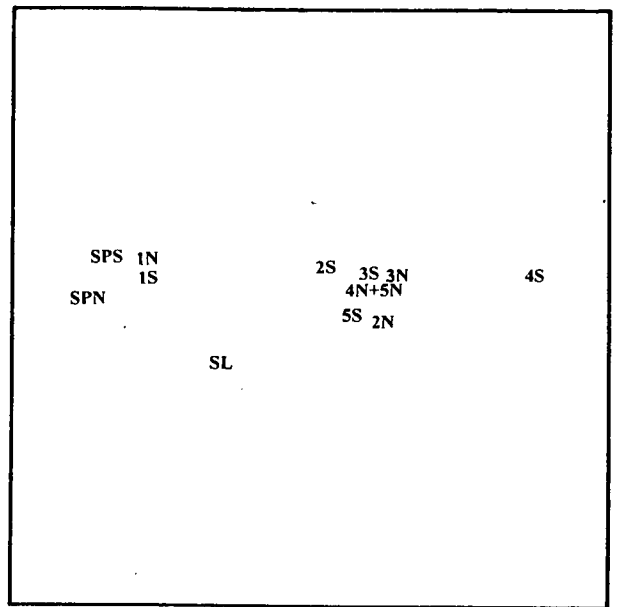
**MDS ordinations for 25.08.94 (Day 457)**

**First flow division (25%N: 75%S)**

**0 days since flow change**

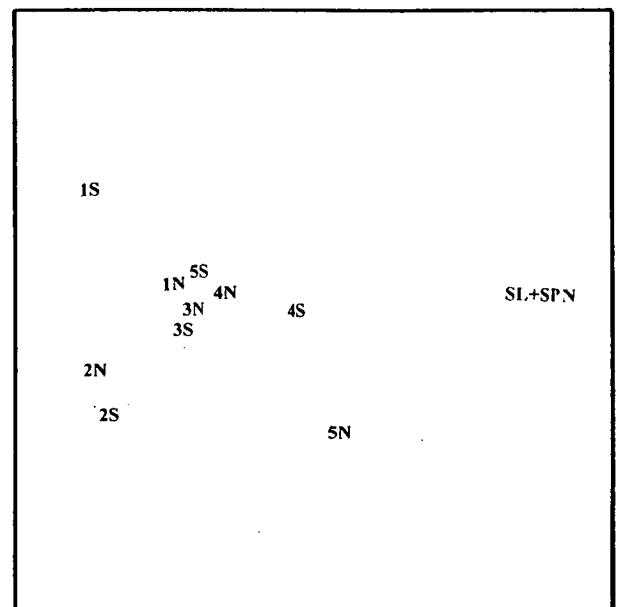
*Missing values:*

No crustaceans in sample SPS



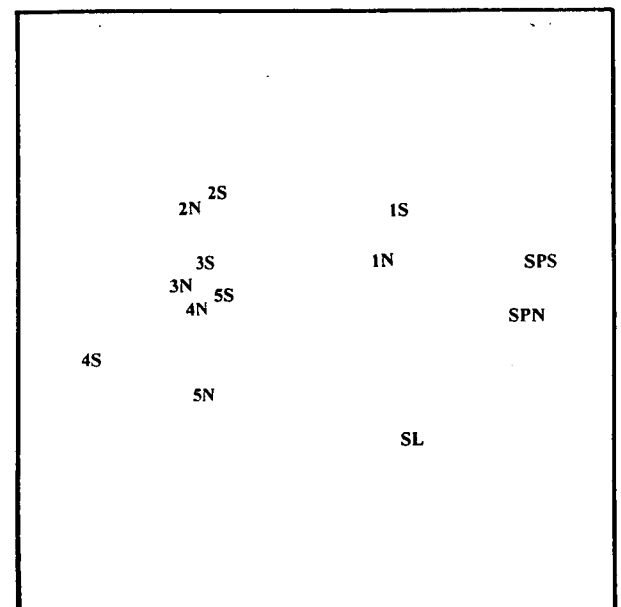
MAIN ROTIFERS

Stress = 0.01



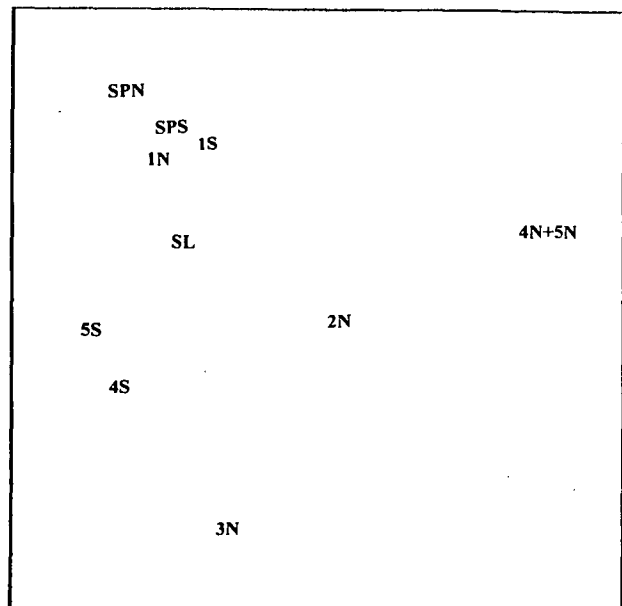
MAIN CRUSTACEANS

Stress = 0.02



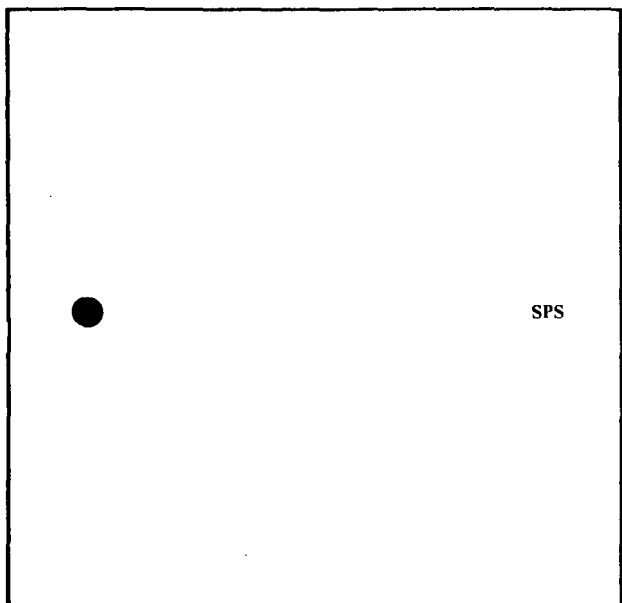
ALL MAIN TAXA

Stress = 0.04



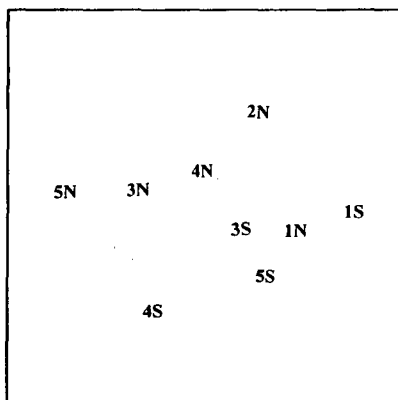
MAIN ROTIFERS

Stress = 0.02

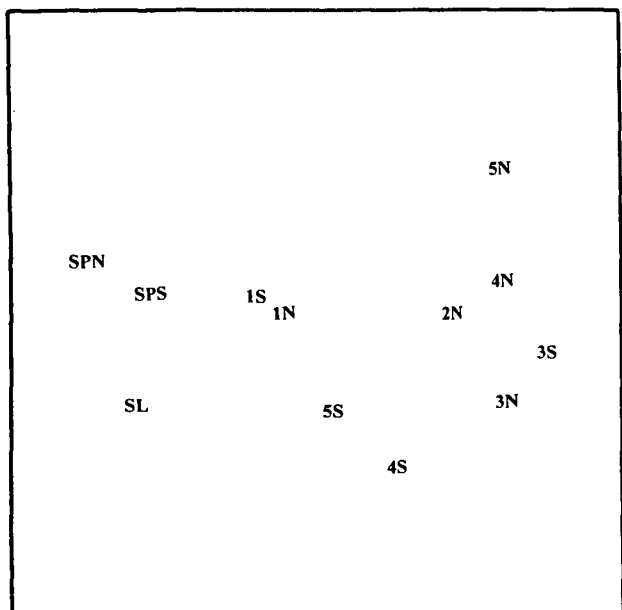


MAIN CRUSTACEANS  
● = 2N, pairs 1, 3-5

Stress = 0.00



MAIN CRUSTACEANS  
SPS removed



ALL MAIN TAXA

Stress = 0.07

Figure 4.39

MDS ordinations for 08.09.94 (Day 471)

First flow division (25%N: 75%S)

14 days since flow change

Missing values:

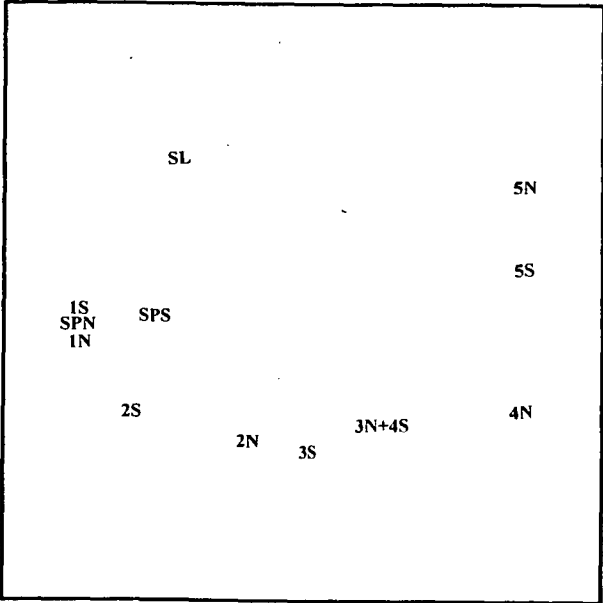
No 2S sample (preservation problem)

No rotifers in sample 3S

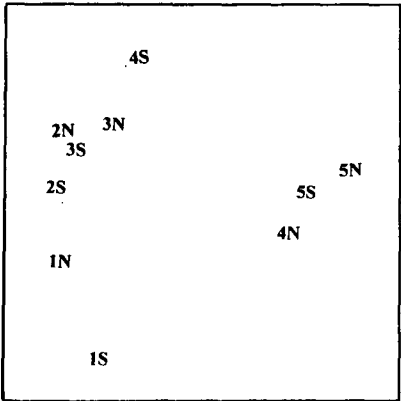
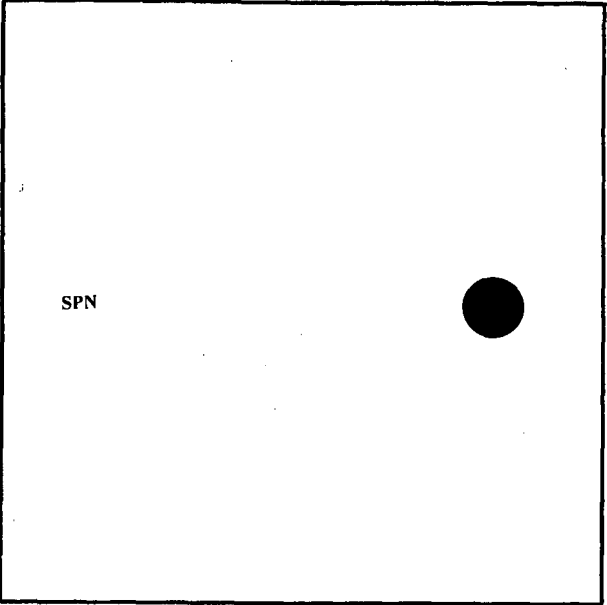
No crustaceans in samples SL or SPN



**Figure 4.40**  
**MDS ordinations for 06.10.94 (Day 499)**  
**First flow division (25%N: 75%S)**  
**42 days since flow change**  
*Missing values:*  
 No crustaceans in samples SL or SPS

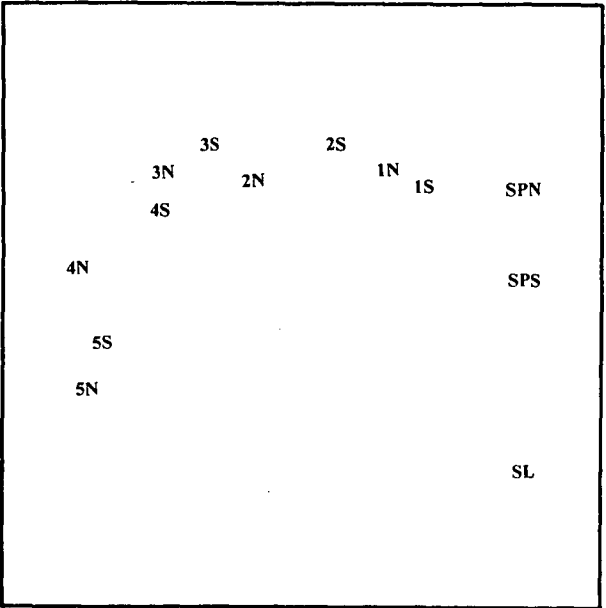


**MAIN ROTIFERS** Stress = 0.03



**MAIN CRUSTACEANS**  
 SPN removed

**MAIN CRUSTACEANS** Stress = 0.01  
 ● = all pairs 1-5

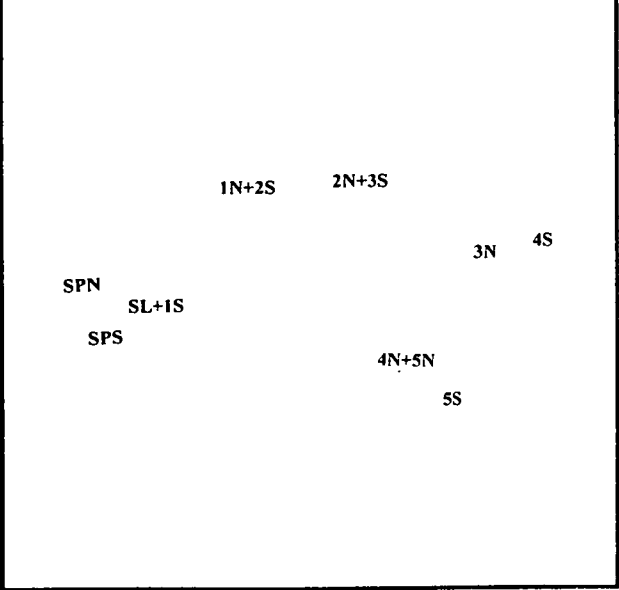


**ALL MAIN TAXA** Stress = 0.03

Figure 4.41

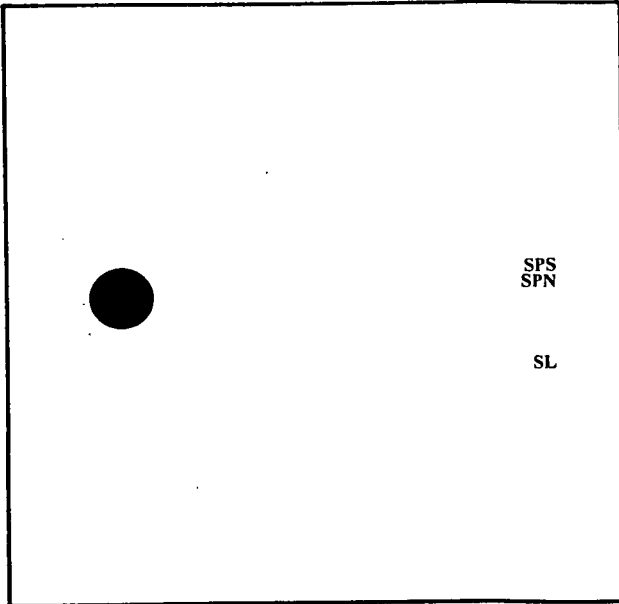
MDS ordinations for 20.10.94 (Day 513)  
First flow division (25%N: 75%S)  
56 days since flow change

No missing values



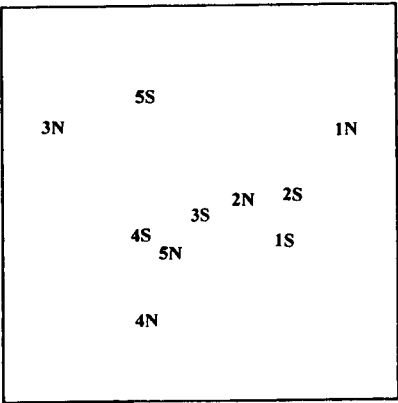
MAIN ROTIFERS

Stress = 0.01

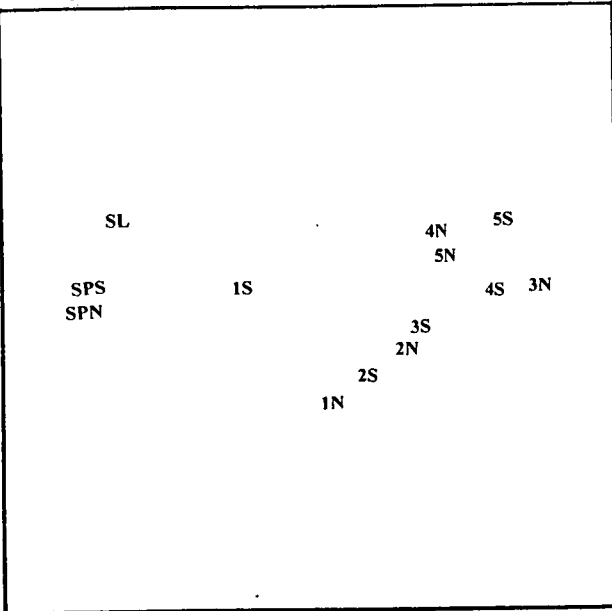


MAIN CRUSTACEANS  
● = all pairs 1-5

Stress = 0.01



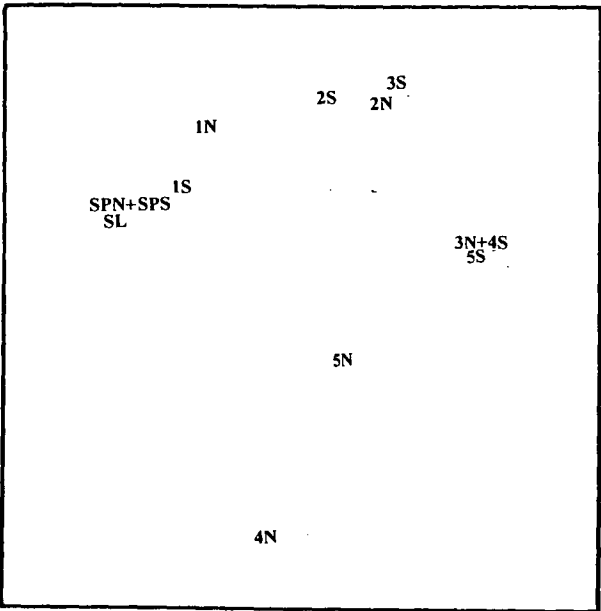
MAIN CRUSTACEANS  
SL, SPN, SPS removed



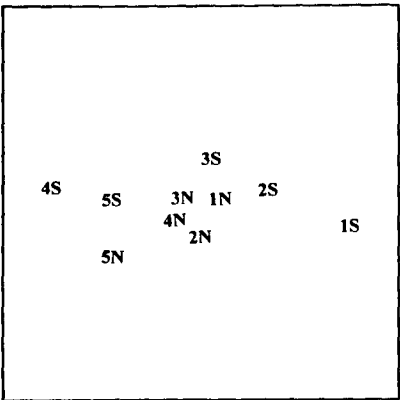
ALL MAIN TAXA

Stress = 0.02

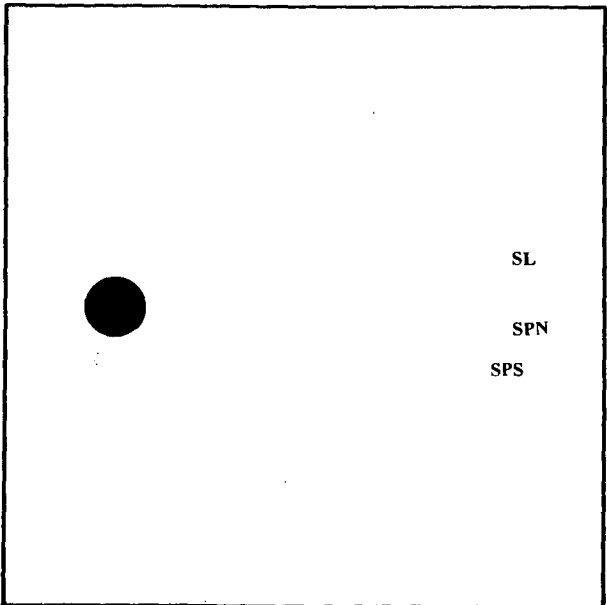
**Figure 4.42**  
**MDS ordinations for 03.11.94 (Day 527)**  
**First flow division (25%N: 75%S)**  
**70 days since flow change**  
*No missing values*



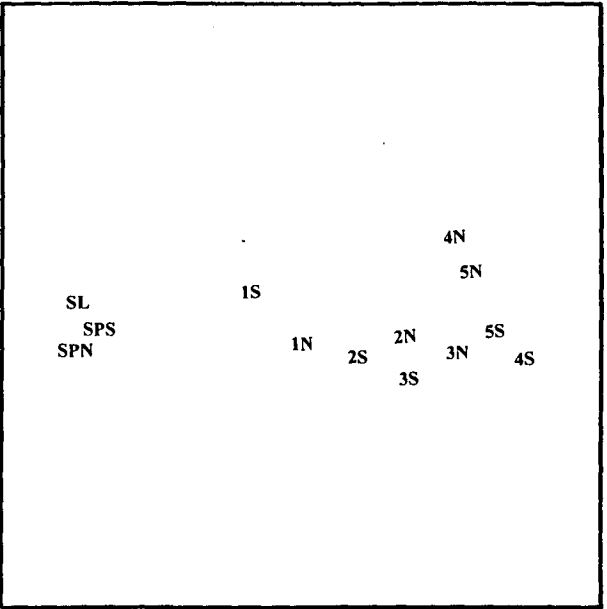
**MAIN ROTIFERS** Stress = 0.01



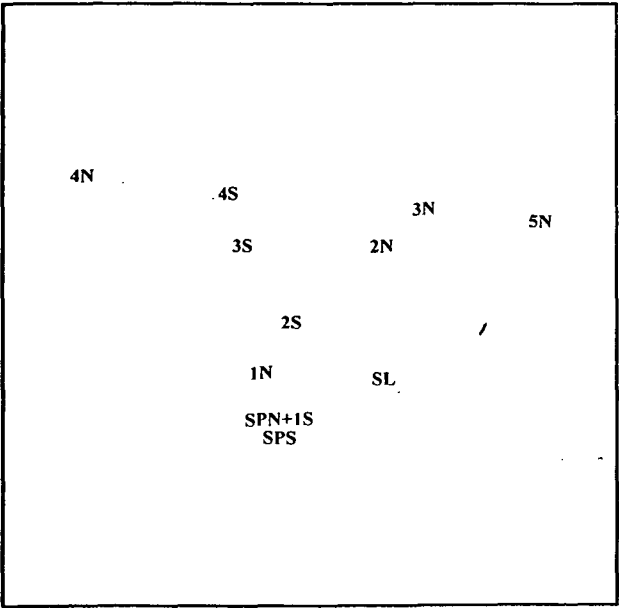
**MAIN CRUSTACEANS**  
**SL, SPN, SPS removed**



**MAIN CRUSTACEANS** Stress = 0.01  
 ● = all pairs 1-5

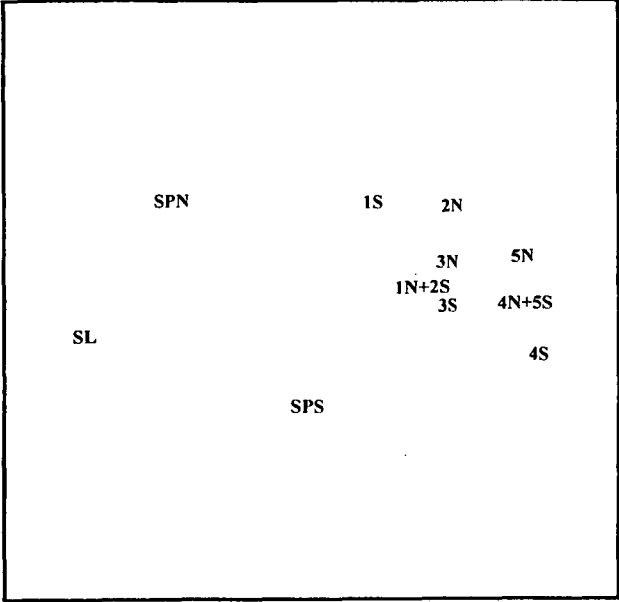


**ALL MAIN TAXA** Stress = 0.03



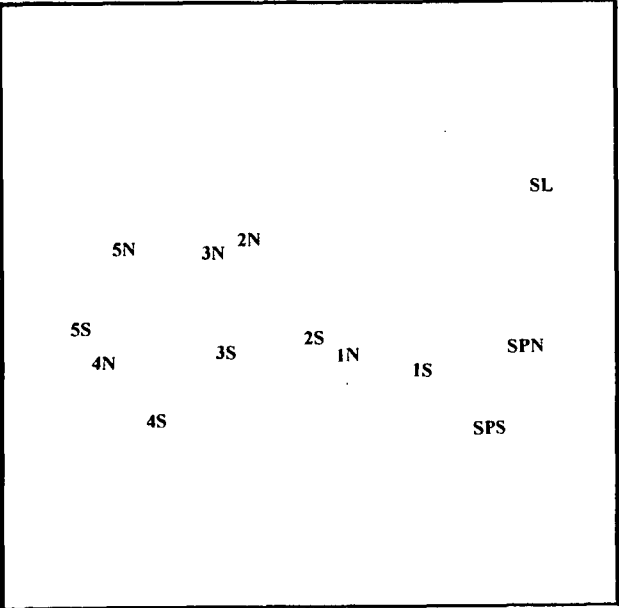
MAIN ROTIFERS

Stress = 0.04



MAIN CRUSTACEANS

Stress = 0.04



ALL MAIN TAXA

Stress = 0.04

**Figure 4.43**  
**MDS ordinations for 17.11.94 (Day 541)**  
**First flow division (25%N: 75%S)**  
**84 days since flow change**  
*Missing values:*  
No rotifers in sample 5S

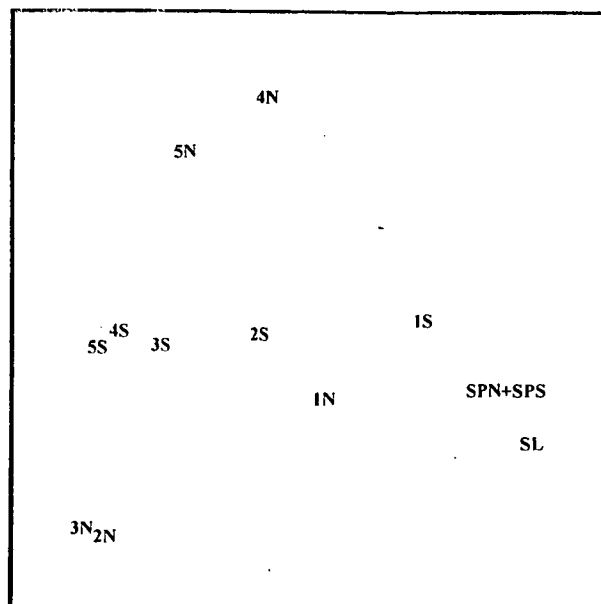
**Figure 4.44**

**MDS ordinations for 28.11.94 (Day 552)**

**Second flow division (75%N: 25%S)**

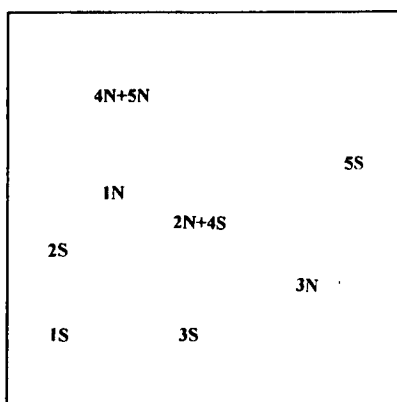
**0 days since flow change**

*No missing values*

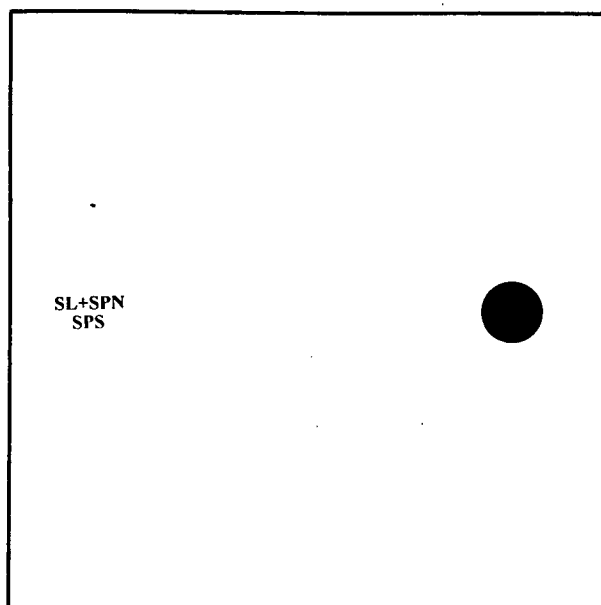


MAIN ROTIFIERS

Stress = 0.06



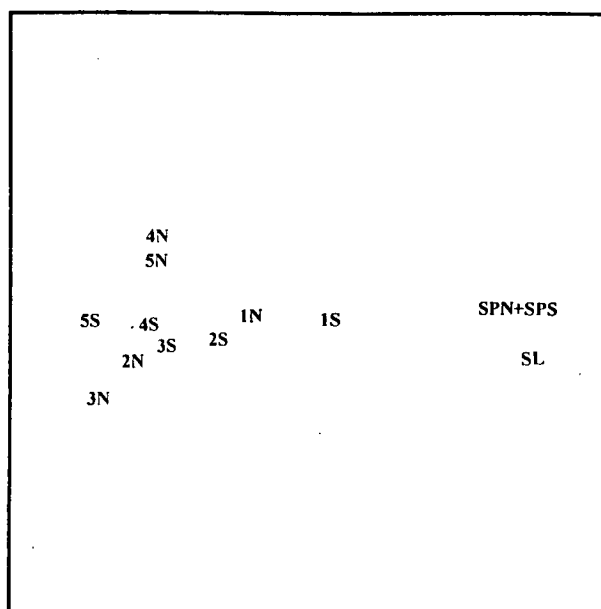
MAIN CRUSTACEANS  
SL, SPN, SPS removed



MAIN CRUSTACEANS

Stress = 0.01

● = all pairs 1-5



ALL MAIN TAXA

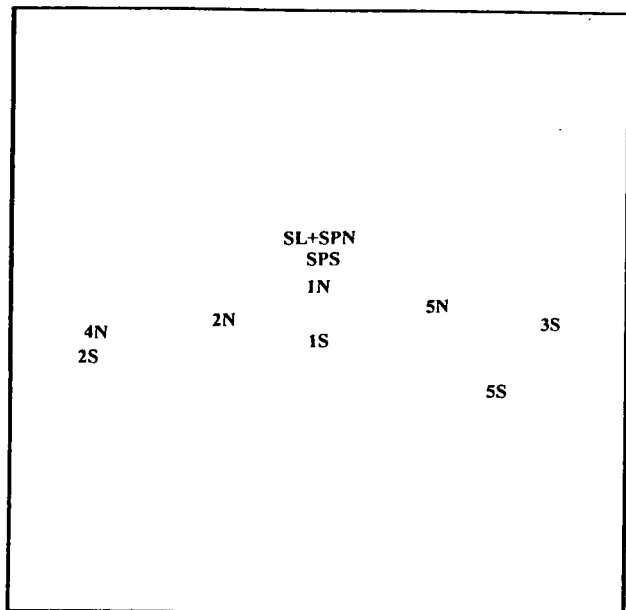
Stress = 0.03

Figure 4.45

MDS ordinations for 08.12.94 (Day 562)  
Second flow division (75%N: 25%S)  
10 days since flow change

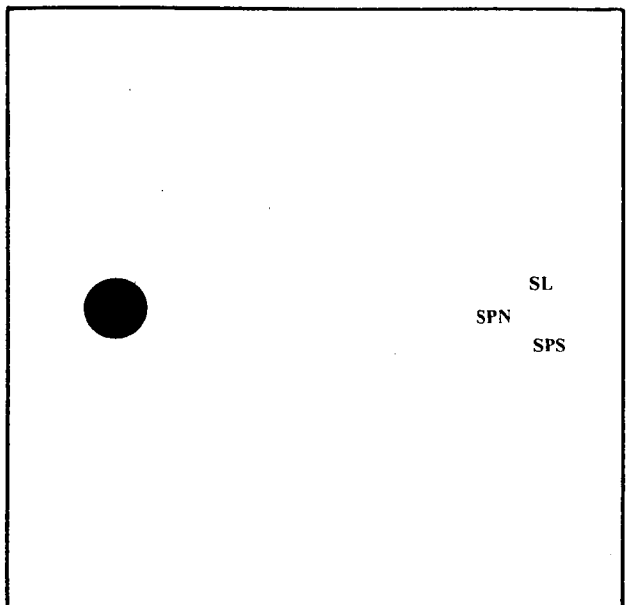
Missing values:

No rotifers in samples 3N or 4S



MAIN ROTIFERS

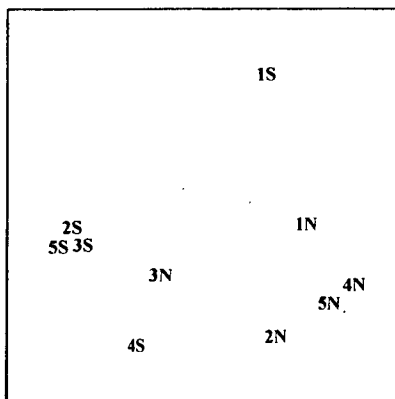
Stress = 0.01



MAIN CRUSTACEANS

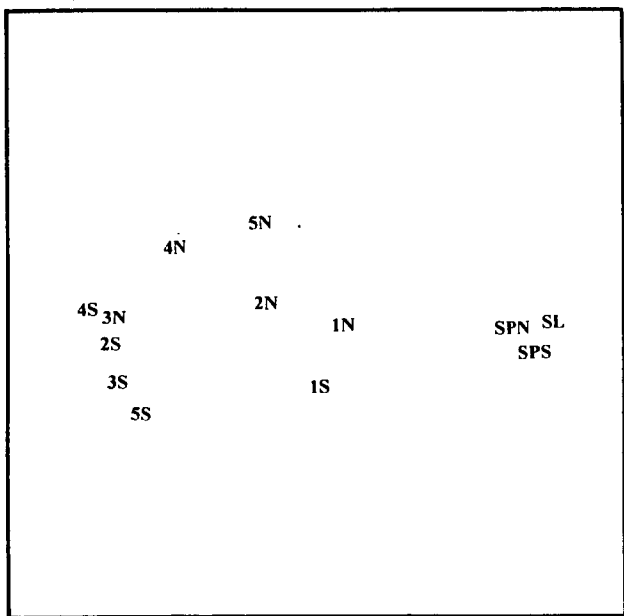
● = all pairs 1-5

Stress = 0.01



MAIN CRUSTACEANS

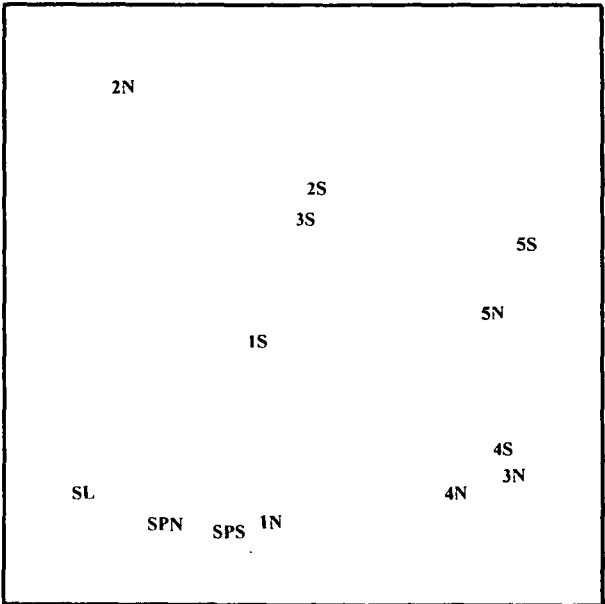
SL, SPN, SPS removed



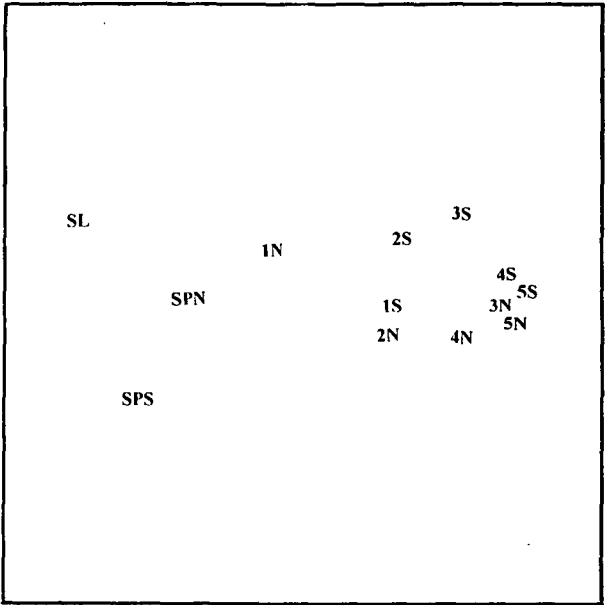
ALL MAIN TAXA

Stress = 0.05

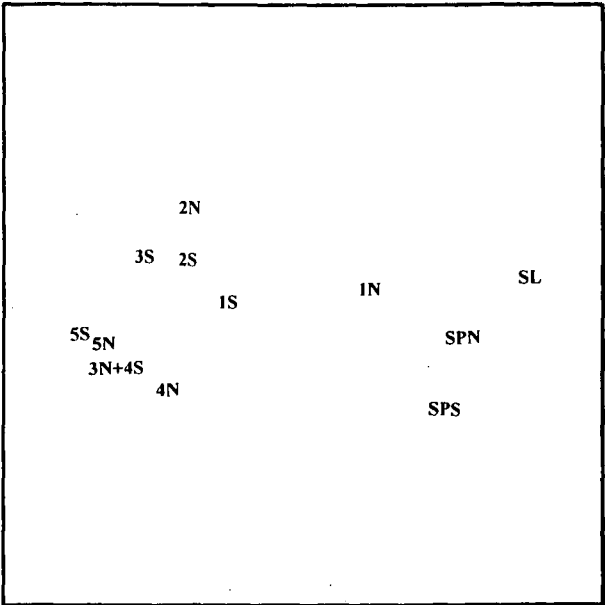
**Figure 4.46**  
**MDS ordinations for 22.12.94 (Day 576)**  
**Second flow division (75%N: 25%S)**  
**24 days since flow change**  
*No missing values*



**MAIN ROTIFERS** Stress = 0.06



**MAIN CRUSTACEANS** Stress = 0.03



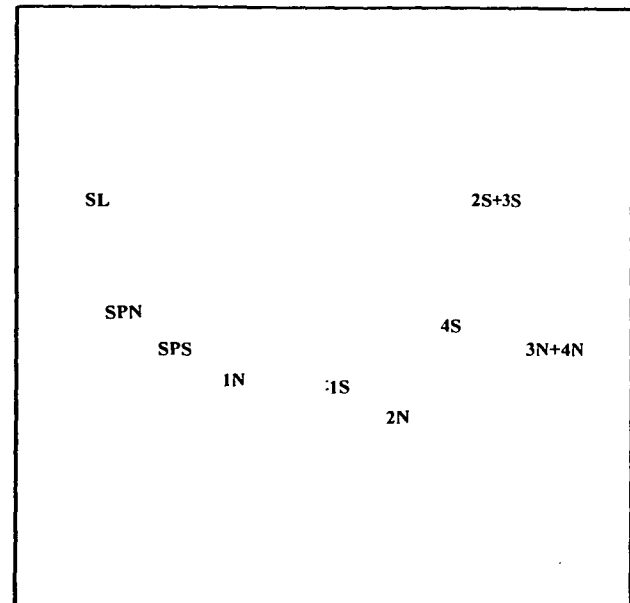
**ALL MAIN TAXA** Stress = 0.04

**Figure 4.47**

**MDS ordinations for 05.01.95 (Day 590)**  
**Second flow division (75%N: 25%S)**  
**38 days since flow change**

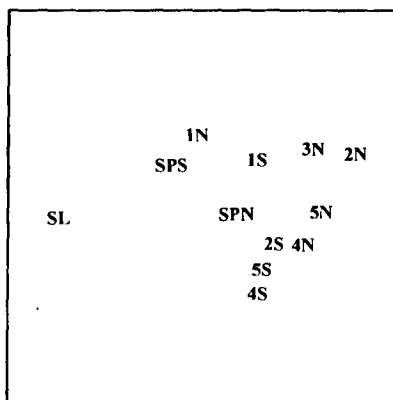
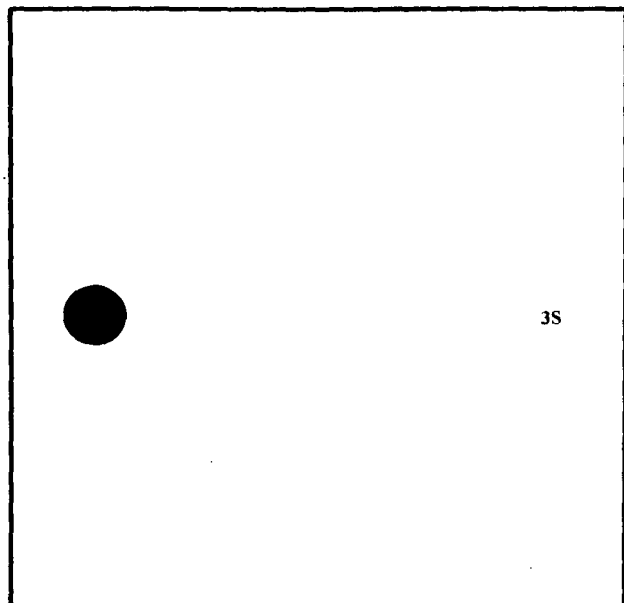
*Missing values:*

No rotifers in samples 5N or 5S



MAIN ROTIFERS

Stress = 0.04

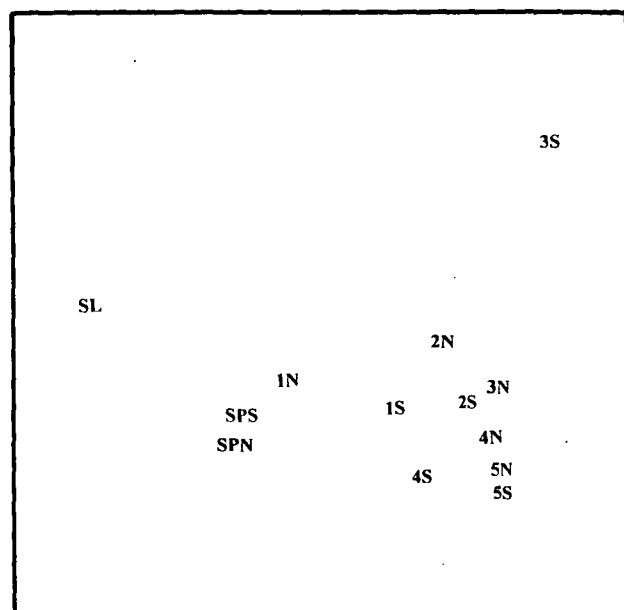


MAIN CRUSTACEANS

Stress = 0.01

● = all bar 3S

MAIN CRUSTACEANS  
3S removed

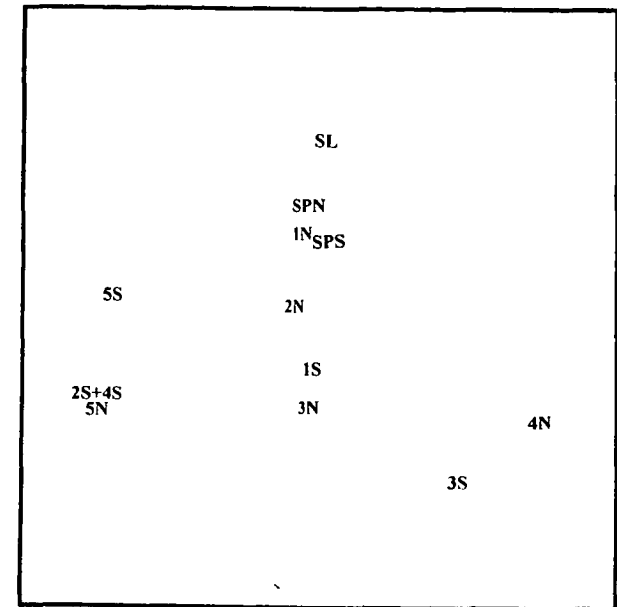


ALL MAIN TAXA

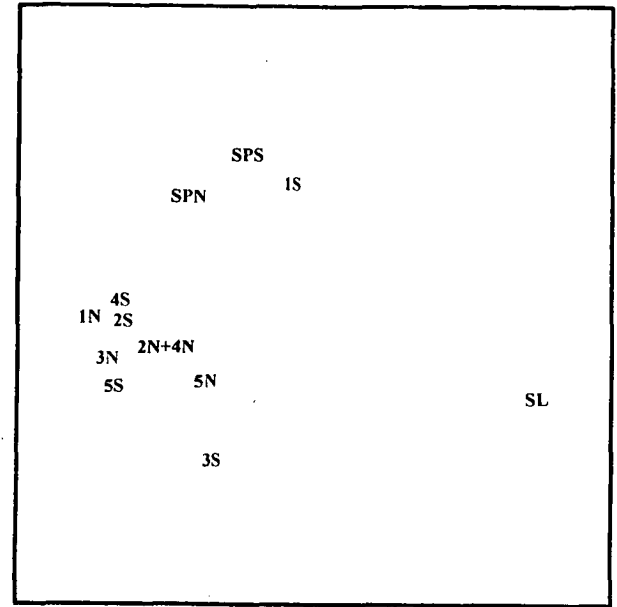
Stress = 0.07



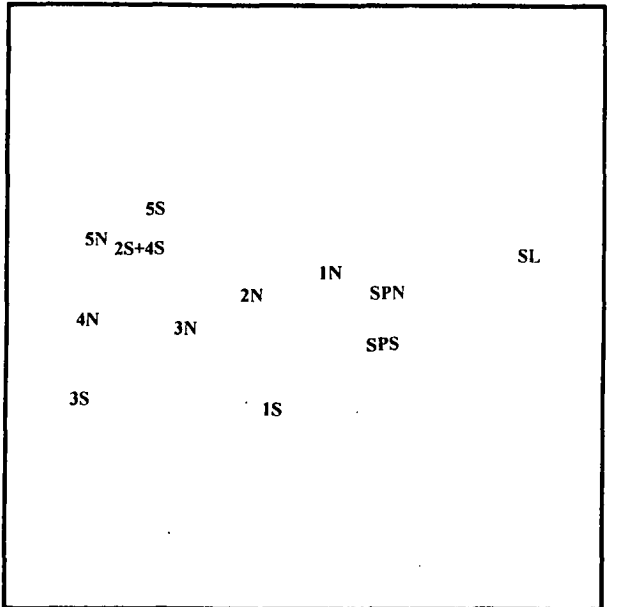
**Figure 4.48**  
**MDS ordinations for 09.01.95 (Day 594)**  
**Second flow division (75%N: 25%S)**  
**42 days since flow change**  
*No missing values*



MAIN ROTIFERS Stress = 0.02



MAIN CRUSTACEANS Stress = 0.05

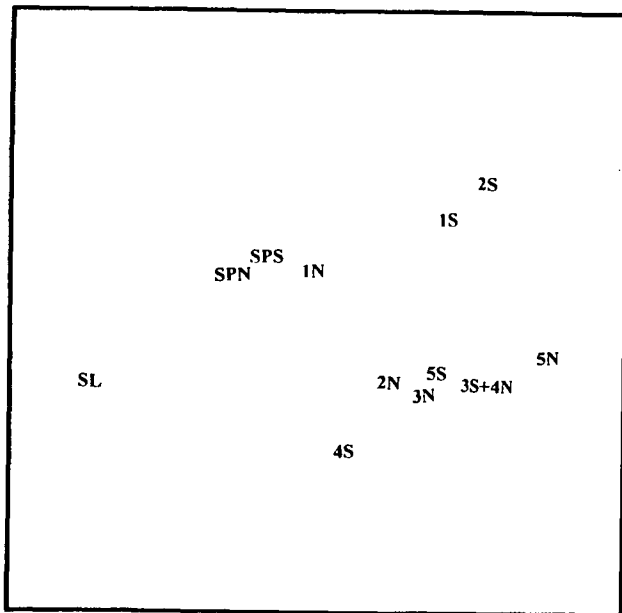


ALL MAIN TAXA Stress = 0.03

**Figure 4.49**

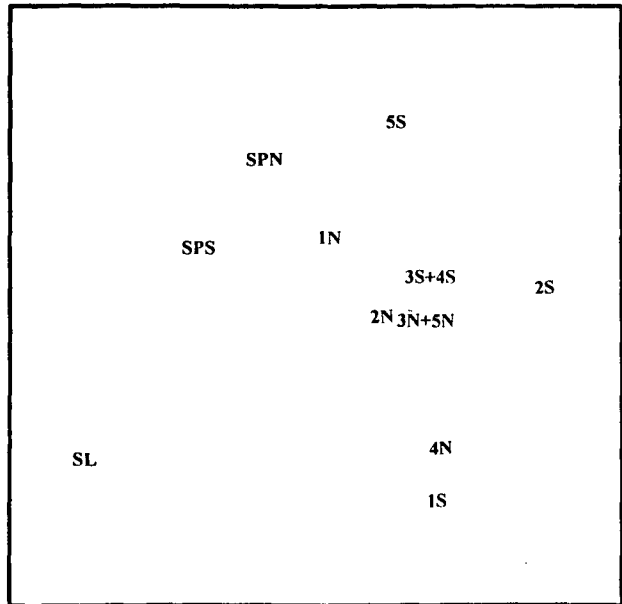
**MDS ordinations for 19.01.95 (Day 604)**  
**Second flow division (75%N: 25%S)**  
**52 days since flow change**

*No missing values*



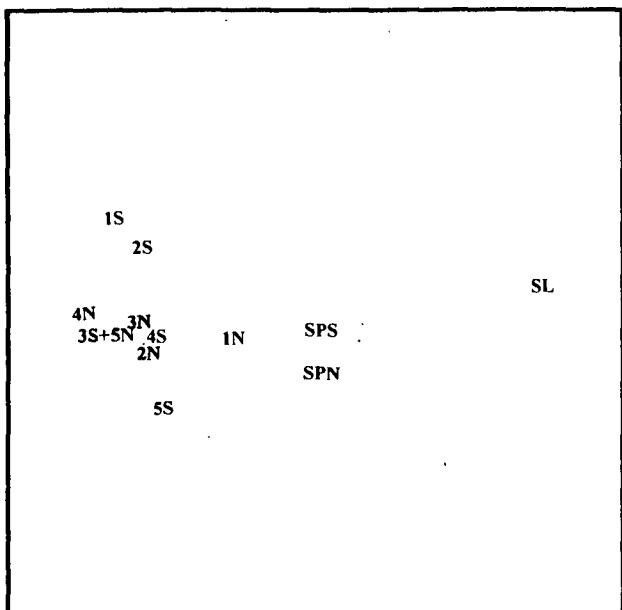
**MAIN ROTIFERS**

**Stress = 0.09**



**MAIN CRUSTACEANS**

**Stress = 0.08**



**ALL MAIN TAXA**

**Stress = 0.08**

**Figure 4.50**

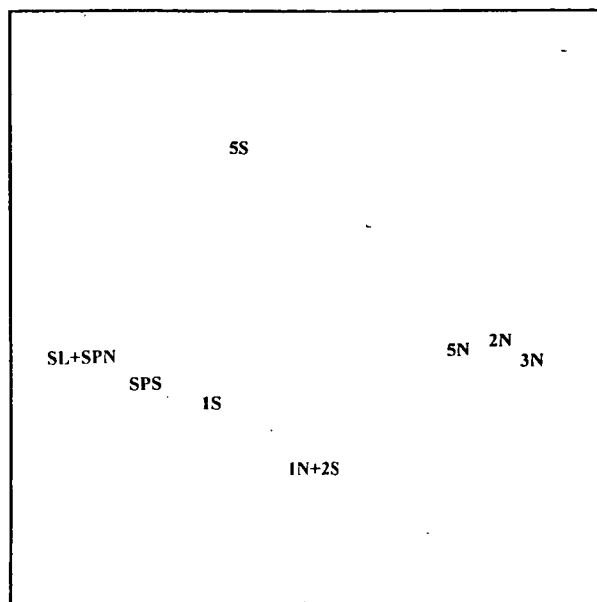
**MDS ordinations for 03.02.95 (Day 619)**

**Third flow division (25%N: 75%S)**

**4 days since flow change**

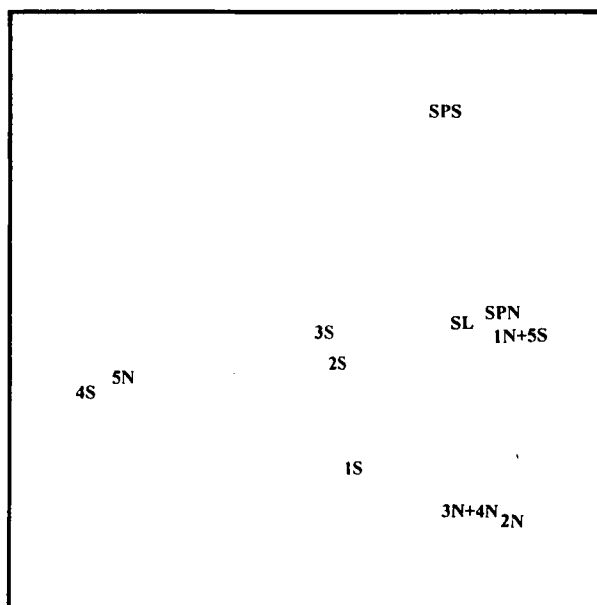
*Missing values:*

No rotifers in samples 3S, 4N, or 4S



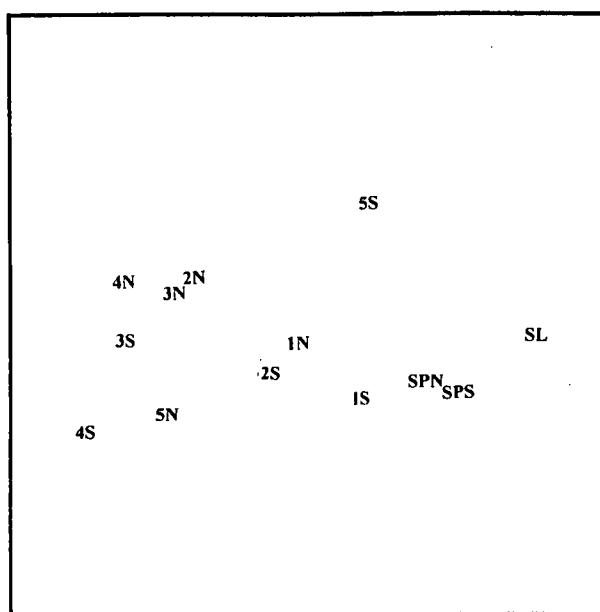
MAIN ROTIFERS

Stress = 0.01



MAIN CRUSTACEANS

Stress = 0.09



ALL MAIN TAXA

Stress = 0.05

**Figure 4.51**

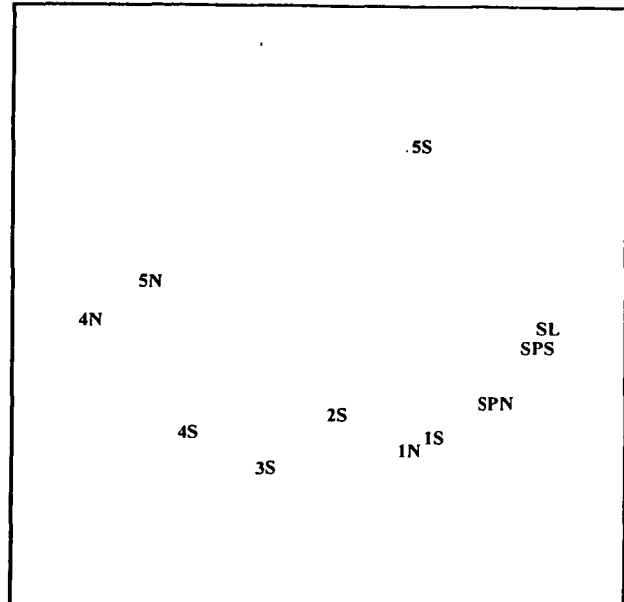
**MDS ordinations for 06.02.95 (Day 622)**

**Third flow division (25%N: 75%S)**

**7 days since flow change**

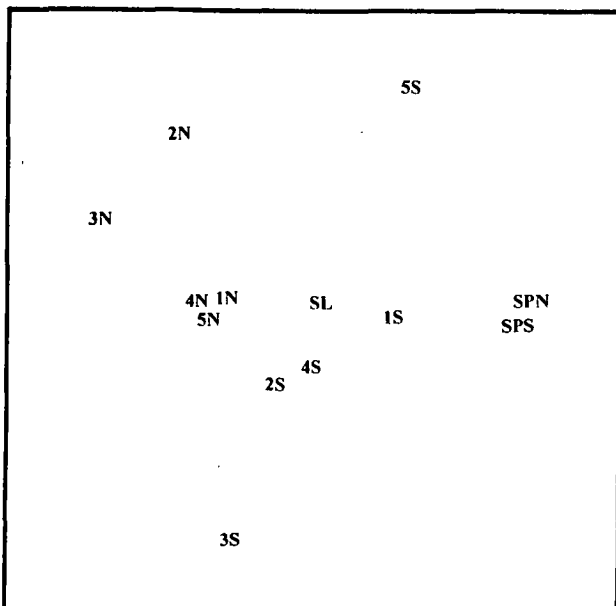
*Missing values:*

No rotifers in samples 2N or 3N



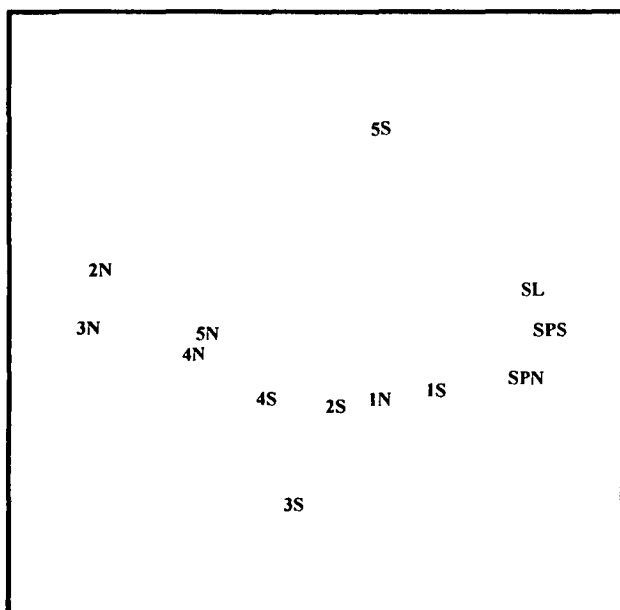
MAIN ROTIFERS

Stress = 0.02



MAIN CRUSTACEANS

Stress = 0.06



ALL MAIN TAXA

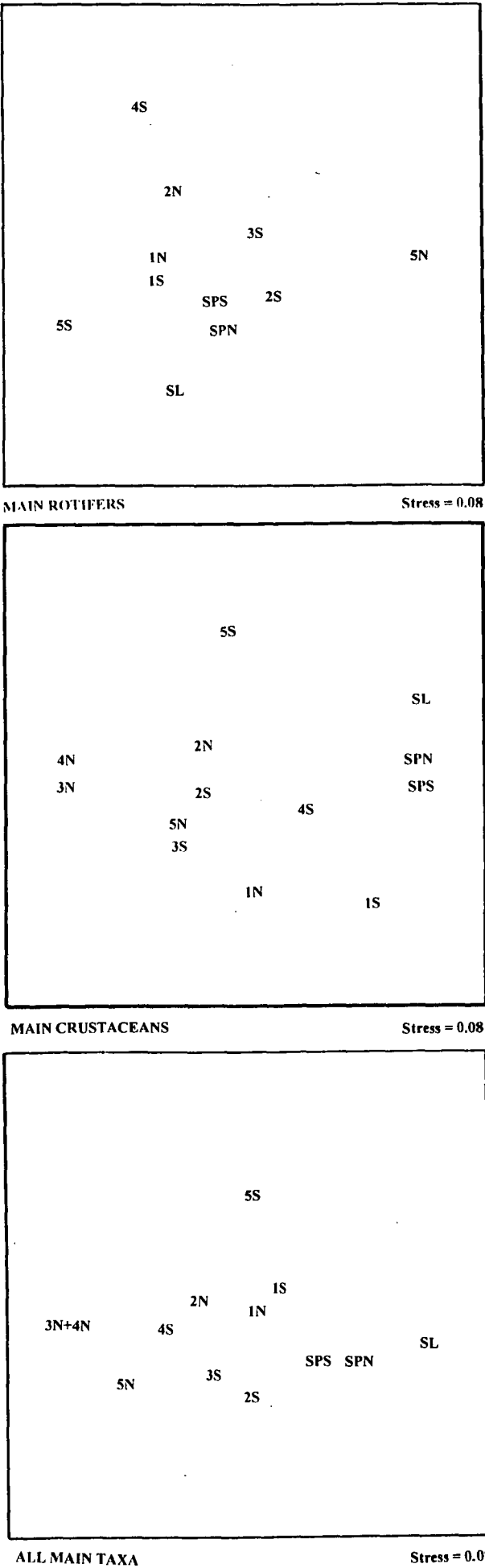
Stress = 0.04

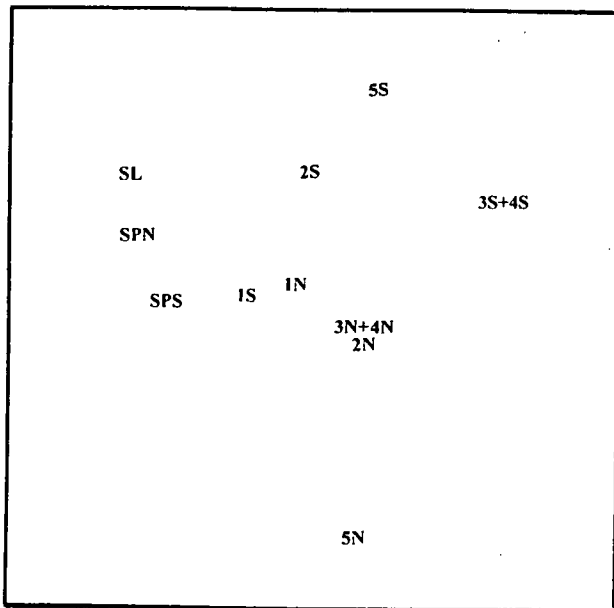
**Figure 4.52**

**MDS ordinations for 09.02.95 (Day 625)**  
**Third flow division (25%N: 75%S)**  
**10 days since flow change**

*Missing values:*

No rotifers in samples 3N or 4N





**Figure 4.53**

**MDS ordinations for 23.02.95 (Day 639)**

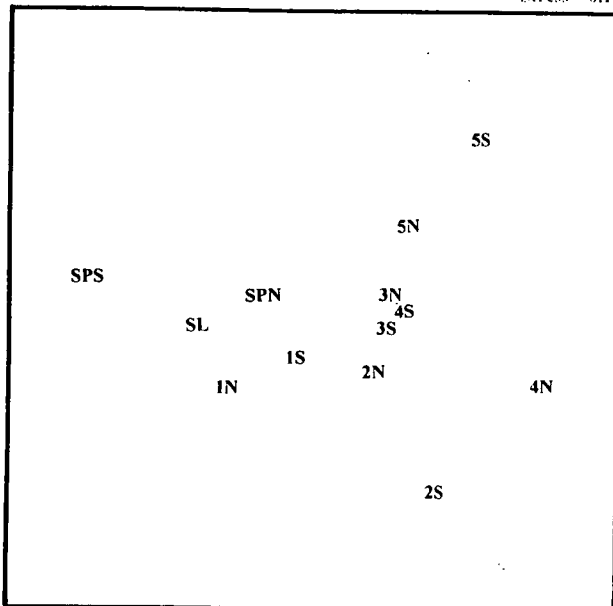
**Third flow division (25%N: 75%S)**

**24 days since flow change**

*No missing values*

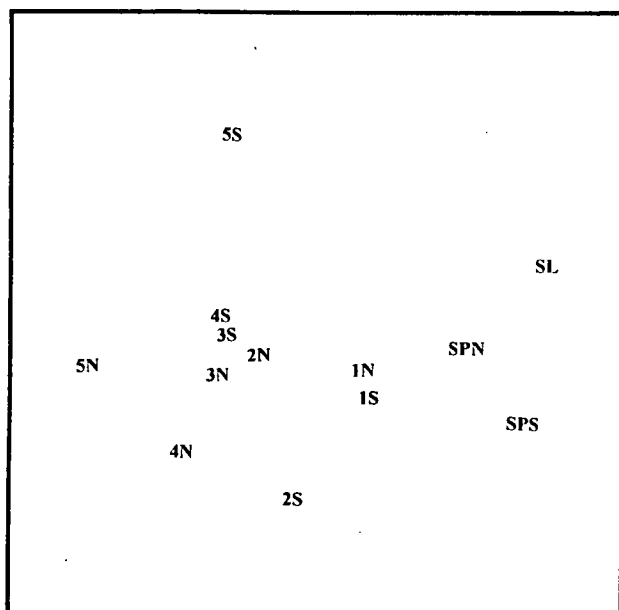
**MAIN ROTIFERS**

Stress = 0.10



**MAIN CRUSTACEANS**

Stress = 0.09



**ALL MAIN TAXA**

Stress = 0.11

#### 4.5.3.1 - Rotifer MDS ordinations

##### *Original flow pattern - single flow system (rotifers)*

Stress values for the rotifer plots over the original flow period ranged from 0.00 to 0.01 (average 0.005, standard error 0.003), which fall well within the ideal range for stress values as identified above. On Day 1 (26.05.93: Figure 4.23/4.54a), pond SL and the original pond 2<sup>3</sup> were very different to the later ponds (pairs 2 and 3) in terms of the rotifer community. As would be expected, 1N and 1S were separated by SPS in ordination space, while ponds 2N and 2S plus 3N and 3S grouped closely together.

It is important to note that the axes of MDS ordinations do not represent any particular values, but that it is the relative position of points within the plot that is important. Figure 4.23/4.54a shows the lagoons to follow a serpentine pattern in the ordination, when following the trajectory of the original flow sequence: SL (pond 1) to 2 (2) to 1N (3) to SPS (4) to 1S (5) to 2S+2N (6+7) to 3N+3S (8+9). The highest Bray Curtis percentage similarities between lagoons were 98.893 (3N+3S), 93.987 (2N+2S), 78.721 (1N+SPS), 77.324 (SPS+1S) and 67.950 (2N+3S). The similarity between the SL and SPS outlets was 62.018, while between 1N and 1S it was 57.181.

On Day 15 (09.06.93: Figure 4.24/4.54b), the ordination for rotifers showed a similar serpentine pattern, running from pond SL to 2 to 1N to SPS to 1S to 2S to 2N to 3N to 3S. [While the plot is reversed compared to Figure 4.23/4.54a, this is an artifact of the random starting point for the ordination. Again, it is the pattern, not the orientation of axes, that is important]. In contrast to the previous plot, pond 2 had swung more in line between SL and 1N, and SPS was closer to 1S than 1N. While pairs 2N+2S and 3N+3S remained close, they were slightly more open, and more readily reflected the actual order of these ponds in series (2S to 2N to 3N to 3S). The highest Bray Curtis similarities between lagoons were 85.449 (SL+2), 83.698 (2N+2S), 83.020 (SPS+1S), 79.085 (2+1N), and 74.100 (3N+3S). The similarity between the SL and

---

<sup>3</sup> Original pond 2, to be cut out of the system once the flow pattern was modified. This is not associated with pond pair 2 (2N and 2S), but occupied the same position in sequence as would later be occupied by the SP pond.

SPS outlets was 46.866, while between 1N+1S it was only 43.650.

On day 29 (23.06.93: Figure 4.25/4.54c), the same pattern was again evident, with slightly greater spacing between values in the ordination due to the absence of 3N and 3S (neither of which contained rotifers)<sup>4</sup>. SL and 2 remained close to each other and quite separate from the other lagoons, while 1N and SPS were also close, with the latter falling between 1N and 1S in the arc of the sites, followed by 2S and then 2N. The highest Bray Curtis similarities between lagoons were 87.569 (SL+2), 81.393 (1N+SPS), 69.705 (2+1N), 68.075 (SL+SPS), and 65.626 (1S+2S). The similarity between pairs 1N+1S was 54.474, while between 2N+2S, uncharacteristically out of the highest values, it was 58.671.

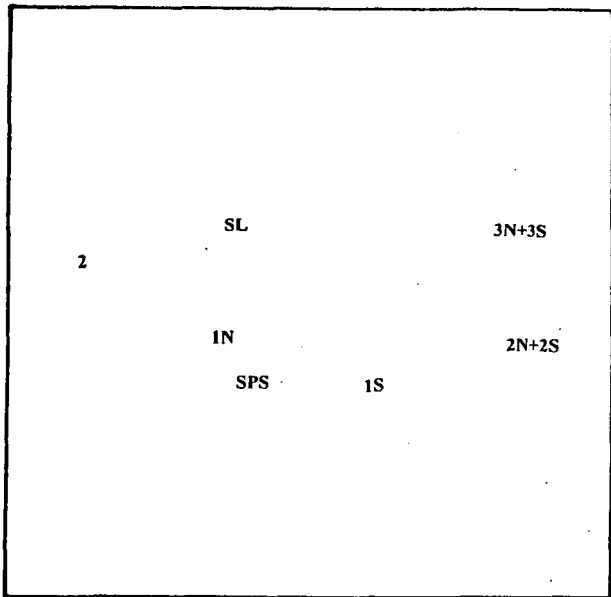
On Day 50 (14.07.93: Figure 4.26/4.54d), there was a slight variation to the general pattern, which was not as serpentine in nature, but still ran SL to 1N to SPS to 1S to 2S to 2N to 3N, with no rotifers in 3S. SL and 1N grouped very closely, with 2 falling out of the usual sequence and close to SPS and 1S. SPS continued to oscillate in grouping closer to 1N or 1S. The highest Bray Curtis similarities between lagoons were 96.967 (SL+1N), 92.000 (2+1S), 86.763 (2+SPS), 78.986 (SPS+1S), and 75.756 (2+1N). The similarity between the SL and SPS outlets was 72.834, while between 1N+1S it was 68.370, between 2N+2S it was 72.986, and between 3N+3S it was 0.000.

On the final sampling day of the original flow period (Day 64, 28.07.93: Figure 4.27/4.54e), there were no rotifers in lagoons 2N, 2S, 3N, or 3S, and so again the remaining ordination was less tight. SL and 2 remained close, with 1N, SPS and 1S well spaced out. Pond 1N was closer to the earlier ponds, while 1S fell on the opposite side of the ordination space. The highest Bray Curtis similarities between lagoons were 96.750 (SL+2), 76.737 (SL+1N), 75.216 (SP+1S), 73.686 (2+1N), and 68.346 (1N+SPS). The similarity between the SL and SPS outlets was 48.849, while between 1N+1S it was 47.667.

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<sup>4</sup> Without rigid values on the axes of an ordination plot, the absence of sites allows the remaining ones to spread out at greater distances within the ordination in order to maximise the reduction of stress values. As a result, the ordination plot, and pairings within that plot, appear to be less tight.



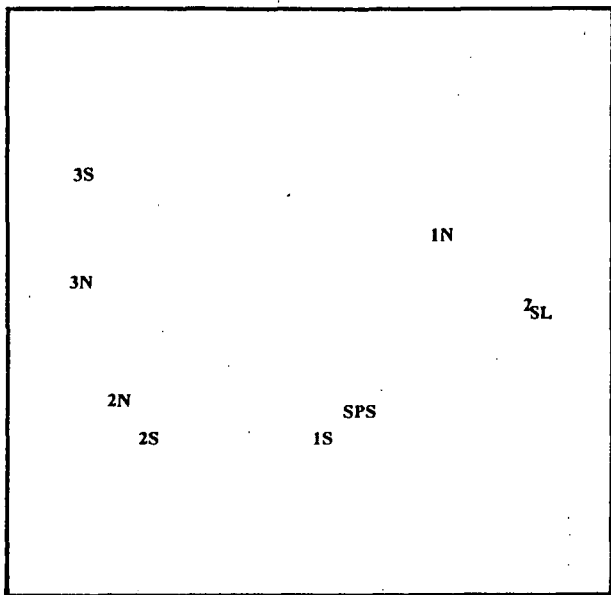


(a) Day 1: 26 May 1993

No SPN, 4N, 4S, 5N, 5S samples at this stage.

MAIN ROTIFERS

Stress = 0.01

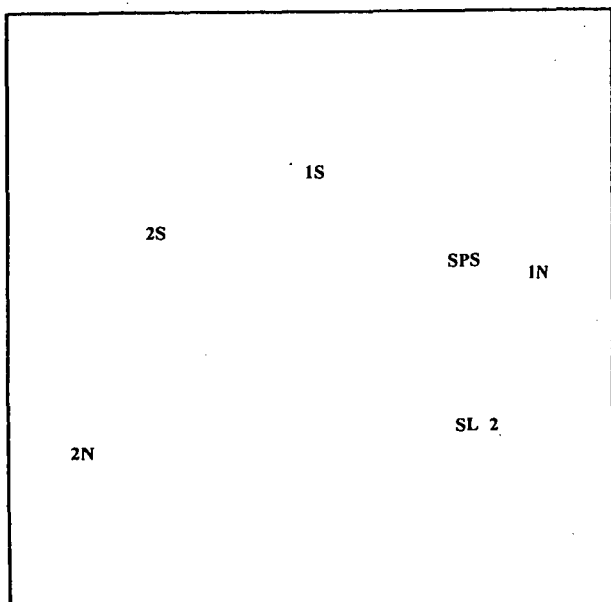


(b) Day 15: 9 June 1993

No SPN, 4N, 4S, 5N, 5S samples at this stage.

MAIN ROTIFERS

Stress = 0.00



(c) Day 29: 23 June 1993

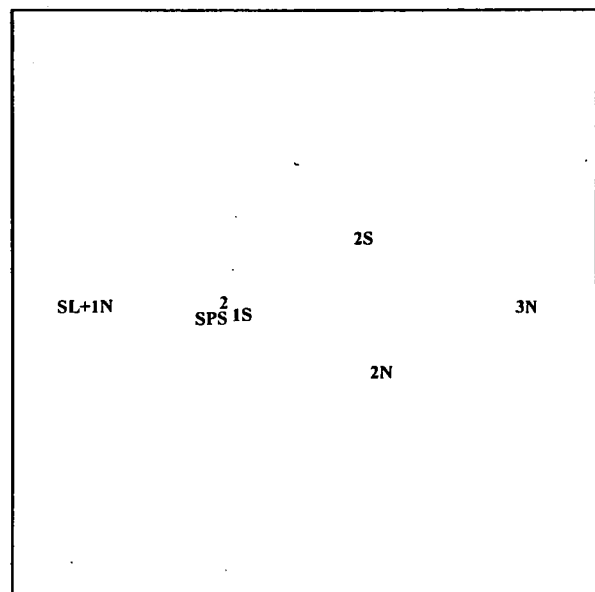
No SPN, 4N, 4S, 5N, 5S samples at this stage.

No rotifers in samples 3N or 3S.

MAIN ROTIFERS

Stress = 0.00

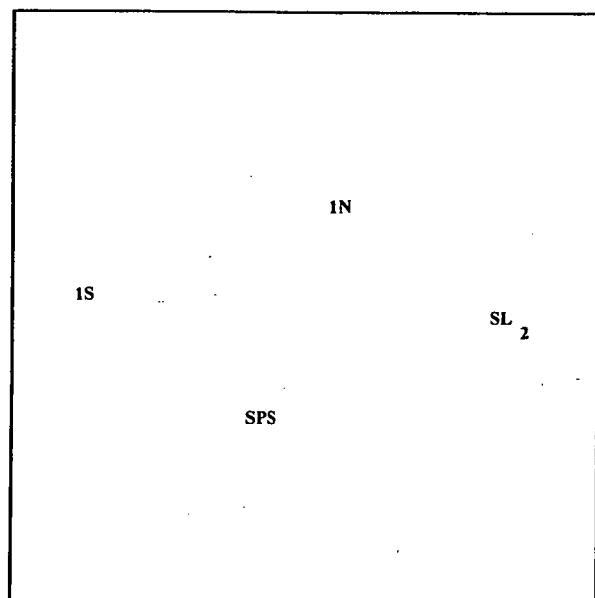
**(d) Day 50: 14 July 1993**  
 No SPN, 4N, 5N, 5S samples at this stage.  
 No rotifers in samples 3S or 4S.



MAIN ROTIFERS

Stress = 0.01

**(e) Day 64: 28 July 1993**  
 No SPN, 4N, 5N, 5S samples at this stage.  
 No rotifers in samples 2N, 2S, 3N, 3S, 4S.



MAIN ROTIFERS

Stress = 0.00

**Figure 4.54 (a-e)**

**Rotifer MDS ordination patterns for the original (pre-alteration) flow period.**

### *Equal flow pattern - 50%N, 50%S (rotifers)*

Stress values for the rotifer plots over the equal flow period ranged from 0.01 to 0.05 (average 0.036, standard error 0.004). These values again fall well within the acceptable range, although they are higher than those under the equal flow conditions. This is most likely due to the increased number of values within the ordination due to the inclusion of SPN and the later ponds, and the resultant increase in complexity of the plot.

On Day 317 (07.04.94: Figure 4.28/4.55a), the system maintained a distinct grading pattern, with the later ponds tending to fall away from the earlier ponds. No SPS sample was available on this day, but pairs 1N+1S, 2N+2S, and 5N+5S were all relatively close. Ponds 3N and 3S fell in the same area within the ordination, but were separated by 5N, while 4N and 4S were very separate. In contrast to the original flow regime, pond 2 was no longer sampled at this stage. While SPS was not included in this plot, 1N and 1S were very close compared to previously. The highest Bray Curtis similarities between lagoons were 89.515 (2N+2S), 84.207 (SL+SPN), 79.958 (3S+5N), 79.572 (3N+5N), and 78.138 (2N+1S). The similarity between 1N+1S was 77.892, between 3N+3S was 62.229, between 4N+4S was 0.000, and between 5N+5S was 72.805.

On Day 331 (21.04.94: Figure 4.29/4.55b), the same general gradation was present, with some differences in specifics of the pattern. SPN and SPS were grouping closely, but with SL falling between them and the later ponds. Pond 1N was still grouping close to SPS, but with that pond no longer falling between the 1N and 1S pair, as per the original flow pattern. Some noticeable breaks had occurred in previous pairings, with pairs 2N+2S, 3N+3S and 5N+5S no longer close and tending to pair with other ponds, while 4N and 4S were closer than previously. The highest Bray Curtis similarities between lagoons were 96.974 (SPN+SPS), 92.391 (2N+3N), 88.224 (3N+1S), 84.831 (SPS+1N), and 83.163 (SL+1N). The similarities between SL and the SPN and SPS outlets were 81.213 and 82.224, respectively, while between 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S they were 51.057, 69.473, 65.816, 74.371, and 40.626, respectively.

On Day 345 (05.05.94: Figure 4.30/4.55c), the pairings were far more distinct, and once again in a serpentine pattern running SL to SPN+SPS to 1N+1S to 2N+2S to 3N+3S to 4N to 5N to 4S+5S. This pattern now appeared to strongly reflect the new flow pattern (SL to SP to 1-5N and 1-5S), and was in definite contrast to the original one. The ordination pattern now graded from SPS to 1N to 1S to 2N to 2S instead of from 1N to SPS to 1S to 2S to 2N. The new pattern, however, was still breaking apart later in the system, with the newly incorporated lagoons. The highest Bray Curtis similarities were 100.000 (4S+5S), 87.874 (SPN+SPS), 78.160 (1N+1S), 77.589 (2N+2S), and 77.476 (2N+3N). The similarities between SL and the SPN and SPS outlets were 59.922 and 63.622, respectively, while between 3N+3S, 4N+4S, and 5N+5S they were 68.135, 0.000, and 66.667, respectively.

This distinct pattern continued on Day 359 (19.05.94: Figure 4.31/4.55d), with good pairings of SPS+SPN, 1N+1S, and 3N+3S, although 1N remained close to 2N and 3N was closer to 2S. Both 2N+2S and 5N+5S were well separated, while there were no rotifers at all in pond 4S. The highest Bray Curtis similarities were 94.683 (3N+2S), 89.749 (SPN+SPS), 81.645 (2N+1S), 80.148 (3N+3S) and 79.531 (4N+3S). The similarities between SL and the SPN and SPS outlets were 62.634 and 71.805, respectively, while between 1N+1S, 2N+2S, 4N+4S, and 5N+5S they were 79.433, 68.308, 0.000, and 0.000, respectively.

By Day 373 (02.06.94: Figure 4.32/4.55e), there were still good pairs early (SPN+SPS, 1N+1S, and 2N+2S), while 4N+4S were still separated and 3S contained no rotifers. No sample was available for the 5S lagoon on this day or for the next four sample days due to Melbourne Water drain works in the area. The highest Bray Curtis similarities were 88.107 (SPN+SPS), 83.300 (4N+5N), 80.495 (SPS+1S), 78.144 (1N+1S), and 74.676 (SPN+1N). The similarities between SL and the SPN and SPS outlets were 65.559 and 72.030, respectively, while between 2N+2S, 3N+3S, and 4N+4S they were 57.721, 0.000, and 58.970, respectively.

On Day 387 (16.06.94: Figure 4.33/4.55f), the arc of the ordination pattern was very flattened, with SL starting almost in the

middle. The 1N+1S pair were almost split by SPN+SPS, but pairs SPN+SPS, 2N+2S, and 3N+3S were otherwise good. Pair 4N+4S was again split by 5N. The highest Bray Curtis similarities were 100.000 (5N+4S), 98.136 (3N+3S), 96.861 (SPN+SPS), 96.471 (2N+2S), and 91.886 (4N+3S). The similarities between SL and the SPN and SPS outlets were 50.382 and 52.612, respectively, while between 1N+1S and 4N+4S they were 83.947 and 73.006, respectively.

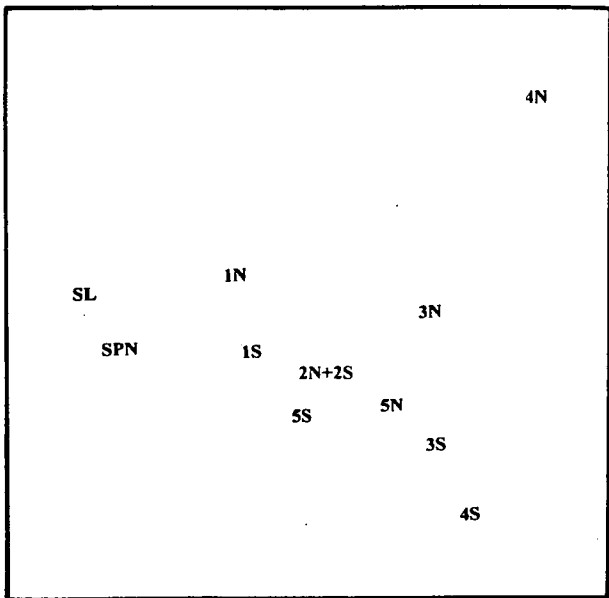
On Day 401 (30.06.94: Figure 4.34/4.55g), good pairs remained in place for SPN+SPS, 1N+1S, and 2N+2S, although the later pair was drifting slightly. Preservation problems meant that no 3N sample was counted, while 4S was still matching very close to 5N and was not near 4N. The highest Bray Curtis similarities were 86.353 (5N+4S), 84.991 (1N+1S), 83.917 (SPN+1S), 78.751 (1N+2S), and 78.567 (1N+SPS). The similarities between the SL and the SPN and SPS outlets were 45.032 and 57.819, respectively, while those between SPN+SPS, 2N+2S, and 4N+4S were 77.240, 75.136, and 51.004, respectively.

On Day 415 (14.07.94: Figure 4.35/4.55h), the general pattern was still there, but was slightly more varied, with the serpentine pattern of the ordination looping around on itself. Pairs SPN+SPS, 1N+1S, and 2N+2S remained very well matched, while 3N+3S were close but split by 4N. Ponds 4N and 4S were separated, but both were now removed from 5N. The highest Bray Curtis similarities were 95.370 (SPN+SPS), 94.539 (1N+1S), 93.708 (SPN+1S), 93.426 (1N+SPS), and 92.799 (SPN+1N). The similarities between SL and SPN and SPS were 53.155 and 56.413, respectively, while those between 2N+2S, 3N+3S, and 4N+4S were 89.963, 80.099, and 73.195, respectively.

On Day 429 (28.07.94: Figure 4.36/4.55i), SL broke from the general pattern, while the SPN+SPS and 1N+1S pairs remained good. Ponds 2N+2S and 3N+3S occurred in the same general area within the ordination, although 5N was much closer to 3N than the latter was to 3S. No rotifers were present in pond 4N. The highest Bray Curtis similarities were 97.471 (SPN+SPS), 95.216 (1N+1S), 87.557 (3N+5N), 87.355 (3N+4S), and 86.816 (2N+2S). The similarities between SL and SPN and SPS were 26.451 and 26.948, respectively, while those between

3N+3S and 4N+4S were 72.731 and 0.000, respectively.

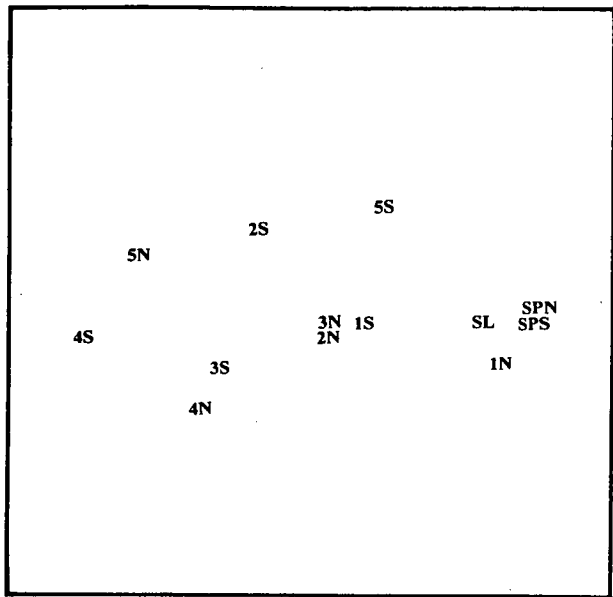
For the final sampling day of the equilibrium period, (Day 443, 11.08.94: Figure 4.37/4.55j), pairs SPN+SPS and 1N+1S remained close, but were slightly split by the closer pairing of SPN+1S. Ponds 2N+2S were wide of each other, while 3N and 3S were definitely split by both 4N and 5S. Neither of pond pairs 4 or 5 were pairing closely in the ordination, although the similarity between 5N+5S was high (as below). The highest Bray Curtis similarities were 96.185 (SPN+1S), 92.794 (4N+5S), 92.643 (SPS+1S), 92.586 (4N+3S), and 91.620 (1N+1S). The similarities between SL and the SPN and SPS outlets were 67.578 and 75.832, respectively, while those between SPN+SPS, 2N+2S, 3N+3S, 4N+4S and 5N+5S were 91.044, 73.498, 77.988, 48.800, and 83.307, respectively.



(a) Day 317: 7 April 1994  
No SPS sample (preservation problem).

MAIN ROTIFERS

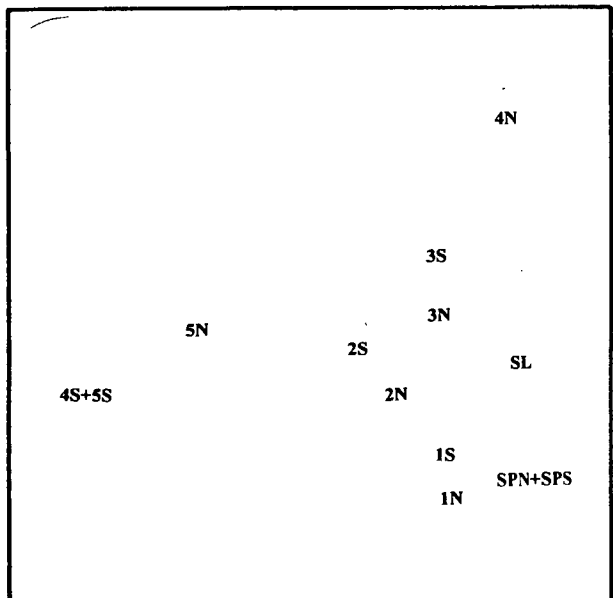
Stress = 0.04



(b) Day 331: 21 April 1994

MAIN ROTIFERS

Stress = 0.05

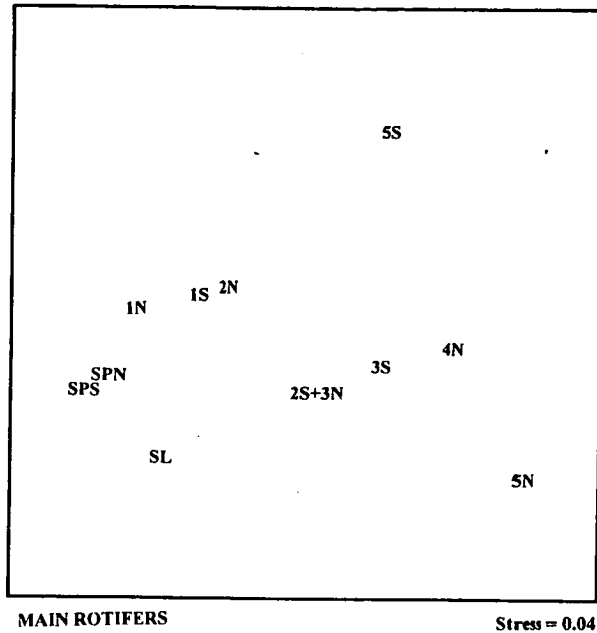


(c) Day 345: 5 May 1994

MAIN ROTIFERS

Stress = 0.04

(d) Day 359: 19 May 1994  
No rotifers in sample 4S.



(e) Day 373: 2 June 1994  
No 5S sample (pond cut out, MW works).  
No rotifers in sample 3S.

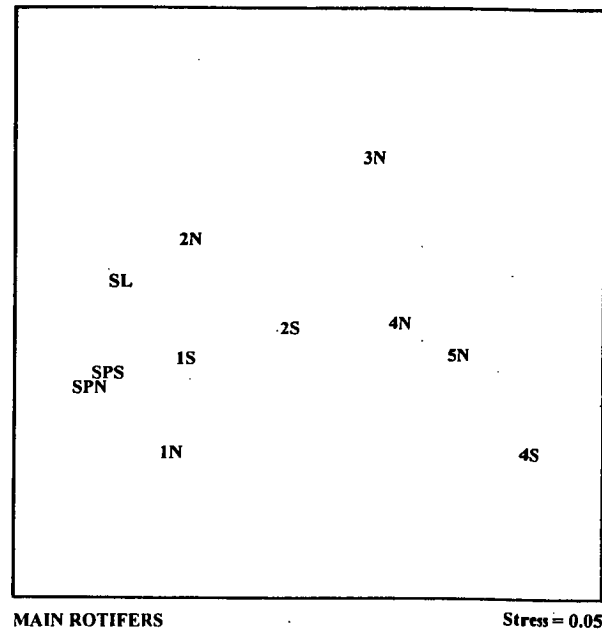
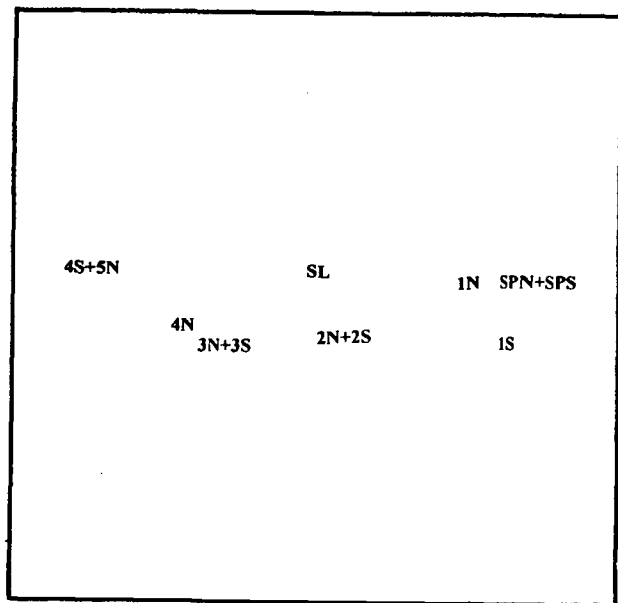


Figure 4.55 (a-e) *continued overleaf*

Rotifer MDS ordination patterns for the  
equal flow period (50%N: 50%S).



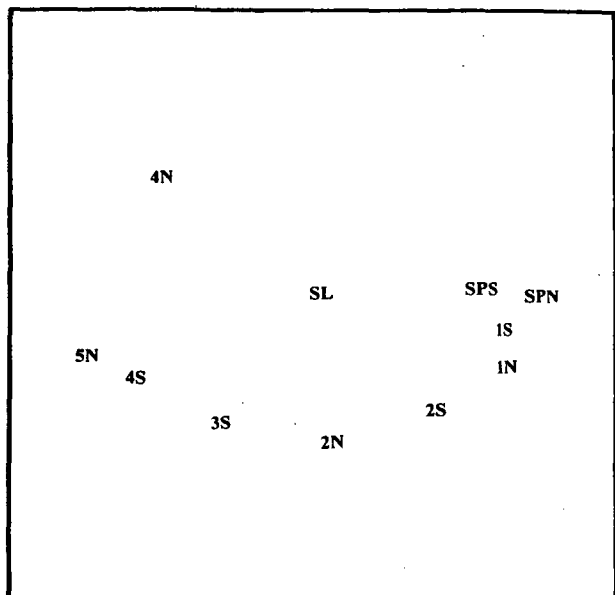


(f) Day 387: 16 June 1994

No 5S sample (pond cut out, MW works).

MAIN ROTIFERS

Stress = 0.01



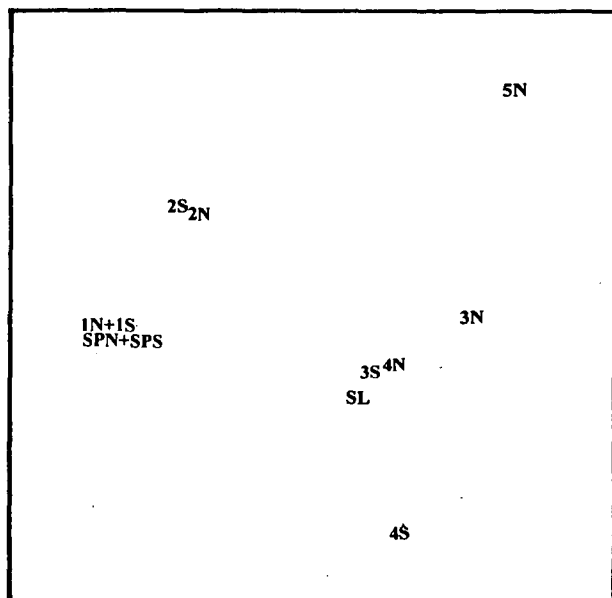
(g) Day 401: 30 June 1994

No 3N sample (preservation problem).

No 5S sample (pond cut out, MW works).

MAIN ROTIFERS

Stress = 0.03



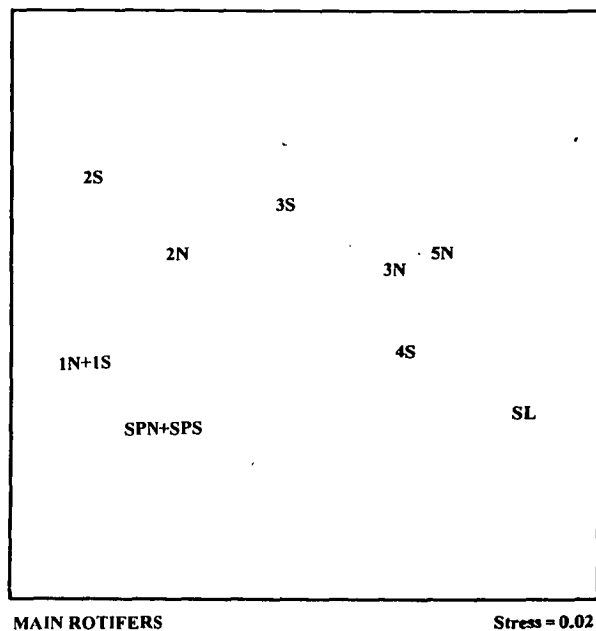
(h) Day 415: 14 July 1994

No 5S sample (pond cut out, MW works).

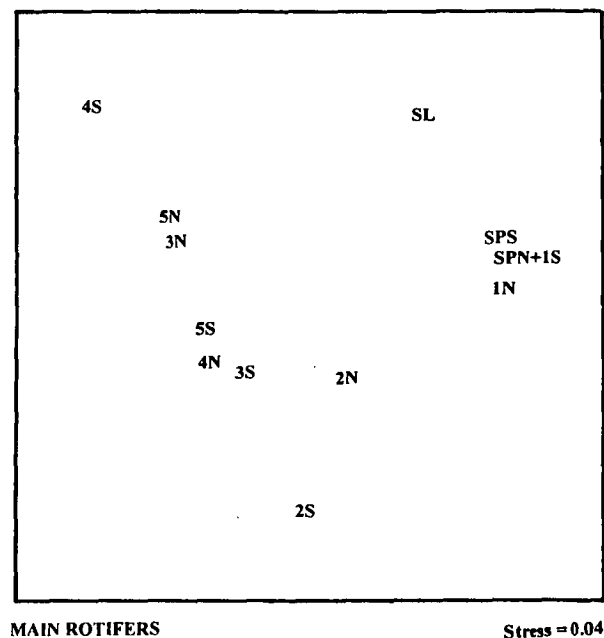
MAIN ROTIFERS

Stress = 0.04

**(i) Day 429: 28 July 1994**  
 No 5S sample (pond cut out, MW works).  
 No rotifers in sample 4N.



**(j) Day 443: 11 August 1994**



**Figure 4.55 (f-j) *cont. from previous page***

**Rotifer MDS ordination patterns for the equal flow period (50%N: 50%S).**

***First flow division - 25%N, 75%S (rotifers)***

Stress values for the rotifer plots over the first flow division ranged from 0.01 to 0.04 (average 0.02, standard error 0.006), again well within the acceptable range, and lower on average than for the equilibrium period.

The division of flow was changed on Day 457 (25.08.94: Figure 4.38/4.56a). At this stage, pond pairs SPN+SPS, 1N+1S, and 3N+3S were close, while 2N and 2S were separated by several other ponds in the ordination. Pond 4N paired with 5N, while 5S was near to these, and 4S was on its own. The highest Bray Curtis similarities were 98.466 (4N+5N), 97.614 (4N+3S), 96.229 (3N+5N), 96.081 (5N+3S), and 94.698 (3N+4N). The similarities between the SL and SPN and SPS outlets were 66.058 and 60.670, respectively, while those between the SPN+SPS, 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 91.341, 93.799, 83.799, 92.322, 61.398, and 91.357 respectively.

On Day 471 (08.09.94: Figure 4.39/4.56b), 14 days after the change of flow, the SPN+SPS and 1N+1S pairs remained intact, but with 1S tending slightly towards the SPS pair. Unfortunately there was no 2S sample due to a preservation problem, and no rotifers were present in pond 3S. Pond 4N paired with 5N and 4S with 5S, with these pairs in turn well separated. The highest Bray Curtis similarities were 100.000 (4N+5N), 88.024 (1N+SPS), 85.311 (SPS+1S), 84.609 (SPN+SPS), and 84.446 (1N+1S). The similarities between the SL and SPN and SPS outlets were 62.254 and 74.506, respectively, while those between the 3N+3S, 4N+4S, and 5N+5S pond pairs were all 0.000.

Unfortunately the preservation problems that claimed the SPS sample from 07.04.94, the 3N sample from 30.06.94, and the 2S sample from 08.09.94 also claimed the entire run of samples from Day 485 (22.09.94, 28 days after the flow change), with these all drying out in storage. However, on Day 499 (6.10.94: Figure 4.40/4.56c), 42 days after the division in flow, a marked change was evident in the ordination pattern for the early ponds. Ponds 1N and 1S were now split with the SPN pond between them, and 1S (high flow) falling on the SL side. The SPN/SPS pair were also split by this distribution. Pond 2S was also

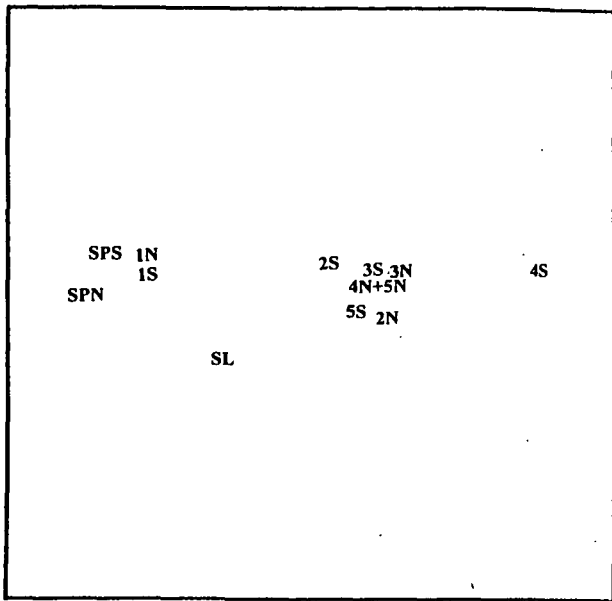
separating from the 2N pond, and falling marginally closer to 1N, while 3S was closer to 2N and 4S coupled with 3N. Pond 4N was well separated from 4S, but while the former fell in the direction of 5S, the 5N+5S pair were still closer to each other than to 4N. The highest Bray Curtis similarities were 94.803 (3N+4S), 91.467 (1N+1S), 90.744 (SPN+1N), 90.411 (SPN+1S), and 89.938 (2N+3S). The similarities between the SL and SPN and SPS outlets were 59.493 and 71.103, respectively, while those between the SPN+SPS, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 86.850, 79.309, 84.971, 74.804, and 89.531 respectively.

On Day 513 (20.10.94, 56 days since the flow change: Figure 4.41/4.56d), the pond pairs were very definitely split, with 1S now pairing with SL, and both intruding on the pairing of the SP outlets. Pond 2S was now pairing distinctly with 1N, 3S with 2N, and 4S with 3N, as had started to be seen with the preceding ordination plot. Ponds 5N and 5S were still close, but 4N was now pairing with the former pond. The highest Bray Curtis similarities were 96.143 (2N+3S), 92.715 (4N+5N), 92.332 (SL+1S), 91.768 (1N+2S), and 90.733 (SPN+SPS). The similarities between the SL and SPN and SPS outlets were 77.779 and 82.279, respectively, while those between the 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 63.246, 76.488, 57.606, 52.934, and 86.117, respectively.

On Day 527 (03.11.94, 70 days since the change: Figure 4.42/4.56e), SPN+SPS were pairing again, but 1S remained very close. A slight gap had also opened up between 1N and 2S, but 3S was still pairing closely to 2N, and 4S to 3N. Pond 5S was close to the latter pair, while 4N and 5N were both out on their own at the tail of the ordination. The highest Bray Curtis similarities were 95.792 (3N+4S), 95.183 (SPN+SPS), 94.936 (3N+5S), 94.584 (SL+SPS), and 93.267 (2N+3S). The similarities between the SL and SPN and SPS outlets were 90.014 and 94.584, respectively, while those between the 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 81.382, 80.355, 59.199, 0.000, and 61.806, respectively.

For the final sample of the first flow division (Day 541, 17.11.94, 84 days post-change: Figure 4.43/4.56f), the pattern appears to

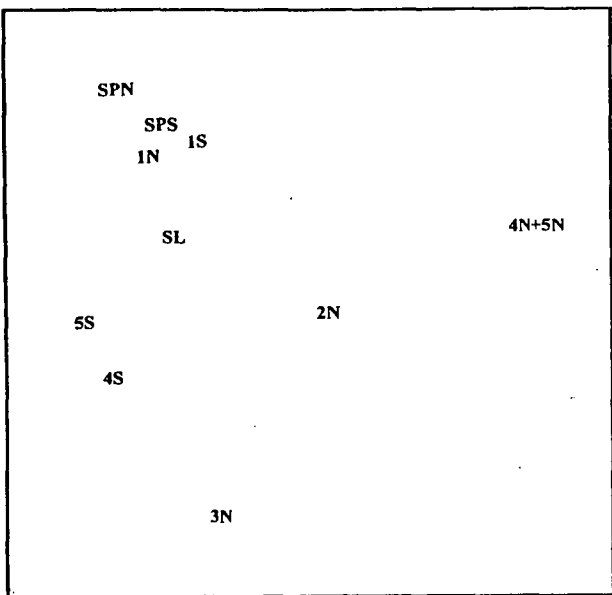
have developed beyond the preceding ordinations for the later ponds. Pond 1S paired with SPN and 2S fell close to 1N, leaving 2N to pair with 3N, and 3S with 4S. Ponds 3S and 4S occupied a similar location on the opposite side of the ordination to 2N and 3N. Ponds 4N and 5N again fell on the outer and away from each other, while no rotifers were found in 5S. The highest Bray Curtis similarities were 90.187 (SPN+1S), 86.623 (SPN+SPS), 85.062 (SPS+1S), 83.637 (1N+1S), and 82.365 (1N+2S). The similarities between the SL and SPN and SPS outlets were 62.276 and 54.655, respectively, while those between the 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 56.457, 33.547, 54.281 and 0.000 respectively.



(a) Day 457: 25 August 1994  
0 days since flow change.

MAIN ROTIFERS

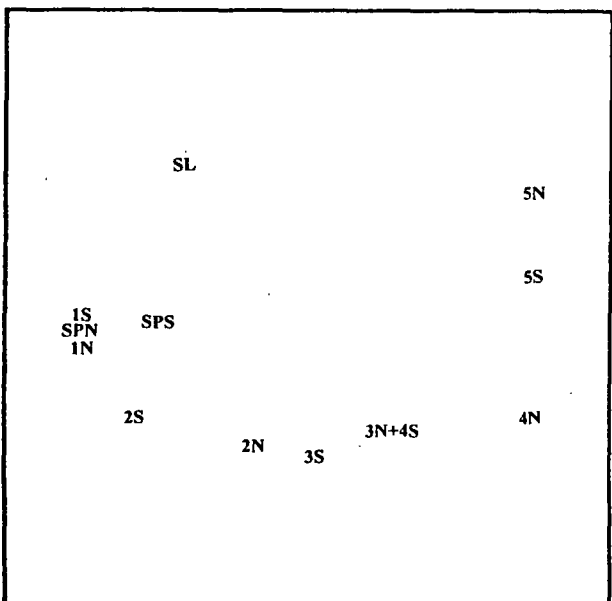
Stress = 0.01



(b) Day 471: 8 September 1994  
14 days since flow change.  
No 2S sample (preservation problem).  
No rotifers in sample 3S.

MAIN ROTIFERS

Stress = 0.02

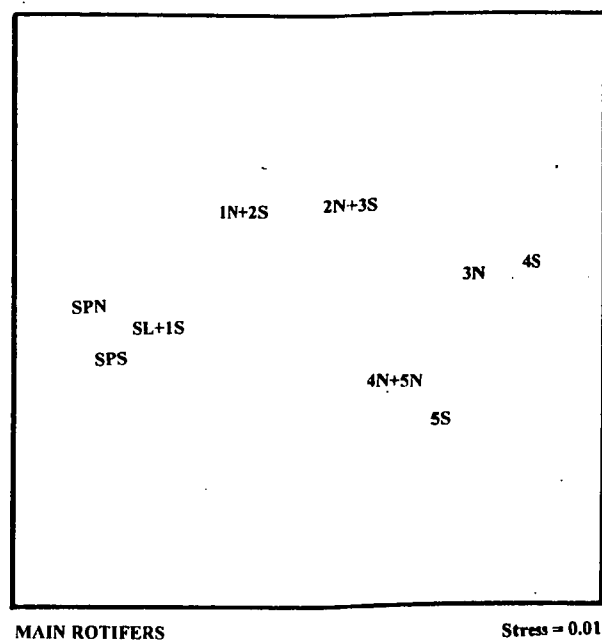


(c) Day 499: 6 October 1994  
42 days since flow change.

MAIN ROTIFERS

Stress = 0.03

(d) Day 513: 20 October 1994  
56 days since flow change.



(e) Day 527: 3 November 1994  
70 days since flow change.

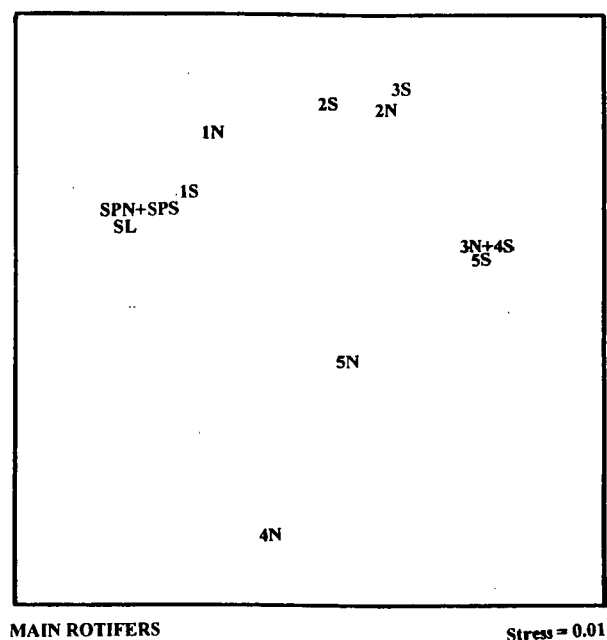
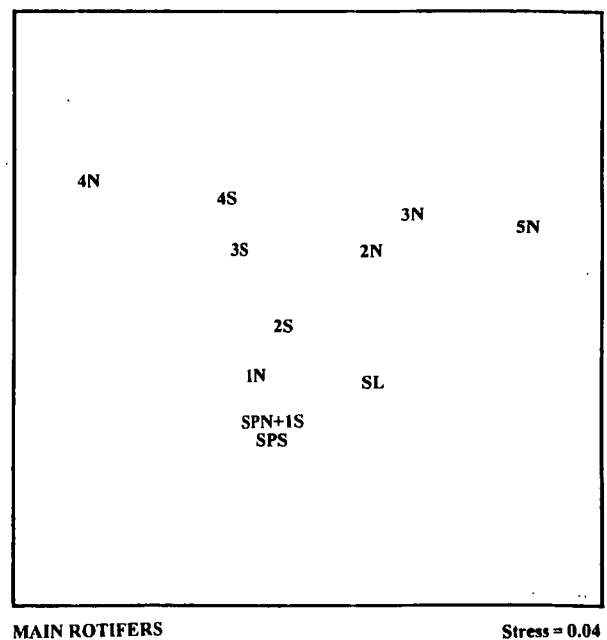


Figure 4.56 (a-f)

Rotifer MDS ordination patterns for the  
first flow division (25%N: 75%S).

(f) Day 541: 17 November 1994  
84 days since flow change.  
No rotifers in sample 5S.



### *Second flow division - 75%N, 25%S (rotifers)*

Stress values for the rotifer plots over the second flow division ranged from 0.01 to 0.09 (average 0.047, standard error 0.013). While these values again fell within the acceptable range, the average level and the standard error were much higher than for the previous flow division. Two stress values were at 0.06, while one (at 0.09) was close to the upper limit of the acceptable range. Notably, this high value occurred at the end of this flow division (Day 604), when the new flow regime had been established for the longest period.

The division of flow was reversed on Day 552 (28.11.94: Figure 4.44/4.57a). The ordination maintains trends from the previous flow division, although these are not as pronounced. SPN+SPS were closely paired, with 1S closer to these than 1N, and the latter closer to 2S. Ponds 3S, 4S and 5S followed in a tight group, with the remaining northern ponds (2N+3N and 4N +5N) pairing on their own in peripheral (and opposite) sides of the ordination. The highest Bray Curtis similarities were 91.357 (4S+5S), 90.966 (SPN+SPS), 89.757 (3S+4S), 88.596 (2N+3N), and 82.554 (SL+SPN). The similarities between the SL and the SPS outlet was 79.083, while those between the 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 67.946, 46.137, 57.862, 50.000, and 47.733 respectively.

By Day 562 (08.12.94, 10 days since the change: Figure 4.45/4.57b), SPN (on the new high flow side) was pairing with the SL pond, 1N was moving closer to SPS, and 2N was moving closer to 1S, although it was not closer than 1S was to 1N. No rotifers were recorded in 3N or 4S, while (the low flow) 2S paired with 4N and, on the other side of the ordination, 5N fell closer to the earlier ponds than either 3S or 5S. The highest Bray Curtis similarities were 90.380 (SL+SPN), 89.623 (1N+SPS), 88.042 (SPN+SPS), 87.938 (SL+SPS), 85.068 (4N+2S). The similarities between the 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 78.312, 34.835, 0.000, 0.000, and 58.457, respectively.

On Day 576 (22.12.94, 24 days from change: Figure 4.46/4.57c), SPN fell closer to SL than SPS fell to SL, while 1N paired with SPS. The rest of the distribution was quite scattered, with 4N and 1S falling



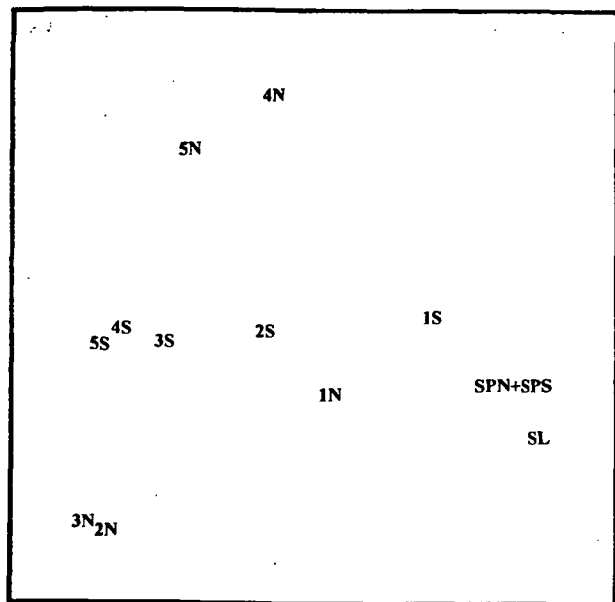
similar distances from the earlier ponds, and 3N falling between 4N and 4S to pair with the latter. Pond 5N fell on the earlier side of 5S, while 2S+3S grouped well away from the early ponds, and pond 2N was an outlier. The highest Bray Curtis similarities were 91.357 (2S+3S), 86.353 (3N+4S), 86.001 (4N+4S), 85.892 (SL+SPN), and 83.595 (SPN+SPS). The percentage similarity between the SL and SPS outlets was 68.042, while the similarities between the 1N+1S, 2N+2S, 3N+3S, and 5N+5S pond pairs were 62.371, 62.712, 47.468, and 75.333, respectively.

On Day 590 (05.01.95, 38 days from change: Figure 4.47/4.57d), SPN still fell closer to SL than its pair, while 1N remained equally close to SPS. Ponds 1S and 2N were as close as SPS and 1N, followed by 4S and the pair of 3N+4N. Ponds 2S+3S finished the ordination, and were furthest removed from the early ponds. No rotifers were present in either 5N or 5S. The highest Bray Curtis similarities were 100.000 (3N+4N), 95.483 (2S+3S), 87.986 (SPN+SPS), 87.355 (2N+1S), and 85.451 (1N+SPS). The similarities between the SL and SPN and SPS outlets were 67.254 and 56.934, respectively, while those between the 1N+1S, 2N+2S, 3N+3S, and 4N+4S pond pairs were 66.021, 48.610, 59.199, and 60.600, respectively.

On Day 594 (09.01.95, 42 days since reversal: Figure 4.48/4.57e), SPN remained closer to SL than SPS to SL. Pond 1N moved between the SPN and SPS ponds, well away from 1S. Pond 2N was also now closer to the earlier ponds, falling half way between SPS and 1S. Pond 3N was now the pond pairing closest to 1S, while 5N grouped with the paired earlier ponds 2S+4S. Pond 5S was on its own, while 4N and 3S were near each other, but not really paired. The highest Bray Curtis similarities were 100.000 (2S+4S), 94.936 (5N+2S), 94.936 (5N+4S), 90.592 (SPN+1N), and 86.757 (SPN+SPS). The similarities between the SL and SPN and SPS outlets were 75.089 and 67.223, respectively, while those between the 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 60.971, 32.638, 58.639, 0.000, and 62.697, respectively.

For the final sampling day of the second flow division (Day 604, 19.01.95, 52 days since the reversal: Figure 4.49/4.57f), the pattern remains similar to the previous ordinations. SPN fell on the SL side of

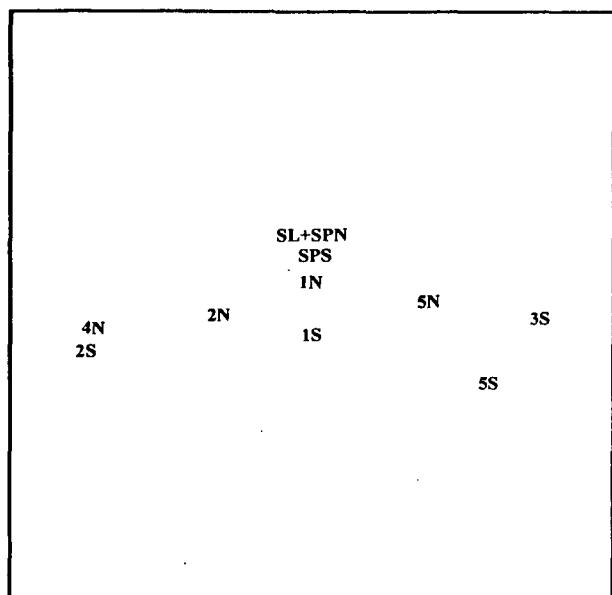
SPS, with 1N very close to SPS, and 4N pairing with 3S. Ponds 2N, 3N, and (unusually) 5S were grouping together, 1S paired with 2S, and 5N and 4S both fell on their own. The highest Bray Curtis similarities were 98.281 (4N+3S), 96.571 (3N+5S), 96.054 (2N+3N), 92.635 (2N+5S), 89.939 (SPN+SPS). The similarities between the SL and SPN and SPS outlets were 58.497 and 50.961, respectively, while those between the 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 66.543, 43.174, 82.008, 69.023, and 69.332 respectively. As noted previously, the stress value was high on this ordination (0.09), although still just within the acceptable range.



(a) Day 552: 28 November 1994  
0 days since flow change.

MAIN ROTIFERS

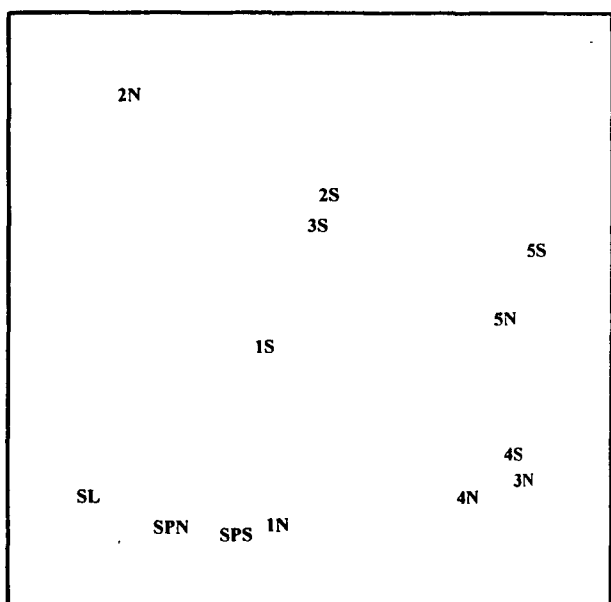
Stress = 0.06



(b) Day 562: 8 December 1994  
10 days since flow change.  
No rotifers in samples 3N or 4S

MAIN ROTIFERS

Stress = 0.01

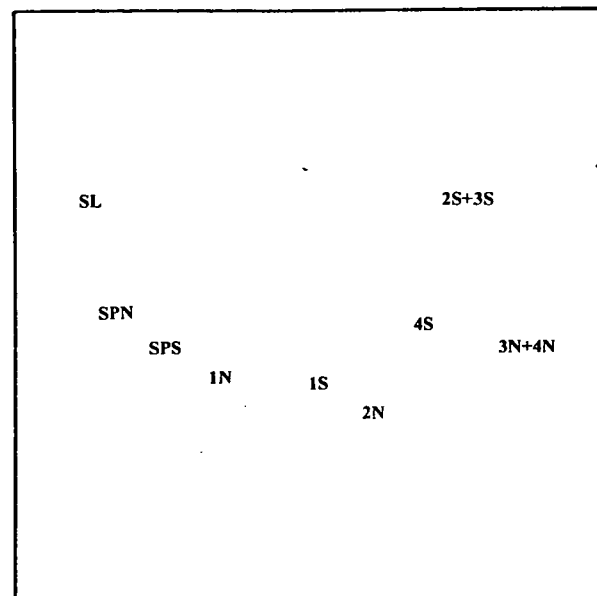


(c) Day 576: 22 December 1994  
24 days since flow change.

MAIN ROTIFERS

Stress = 0.06

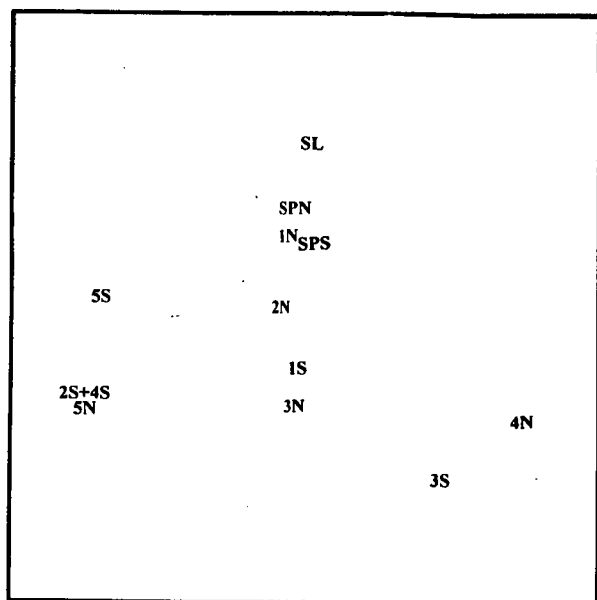
(d) Day 590: 5 January 1995  
38 days since flow change.  
No rotifers in samples 5N or 5S



MAIN ROTIFERS

Stress = 0.04

(e) Day 594: 9 January 1995  
42 days since flow change.



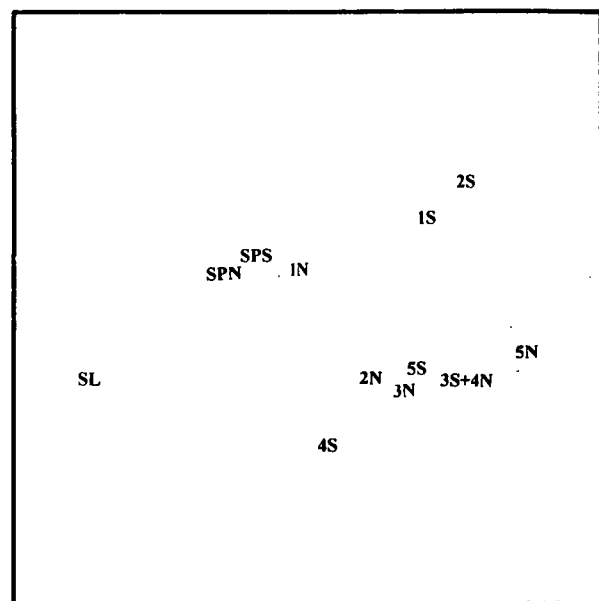
MAIN ROTIFERS

Stress = 0.02

Figure 4.57 (a-f)

Rotifer MDS ordination patterns for the  
second flow division (75%N: 25%S).

(f) Day 604: 19 January 1995  
52 days since flow change.



MAIN ROTIFERS

Stress = 0.09

### *Third flow division - 25%N, 75%S (rotifers)*

Stress values for the rotifer plots over the third flow division ranged from 0.01 to 0.10. Once again, the average (0.053) and standard error (0.026) of these stress values increased, and the highest values of 0.08 and 0.1 occurred after the longest periods under the new flow regime (Days 625 and 639, 10 and 24 days since the change, respectively). The 0.1 stress value for Day 639 fell on the threshold for acceptable values as listed previously.

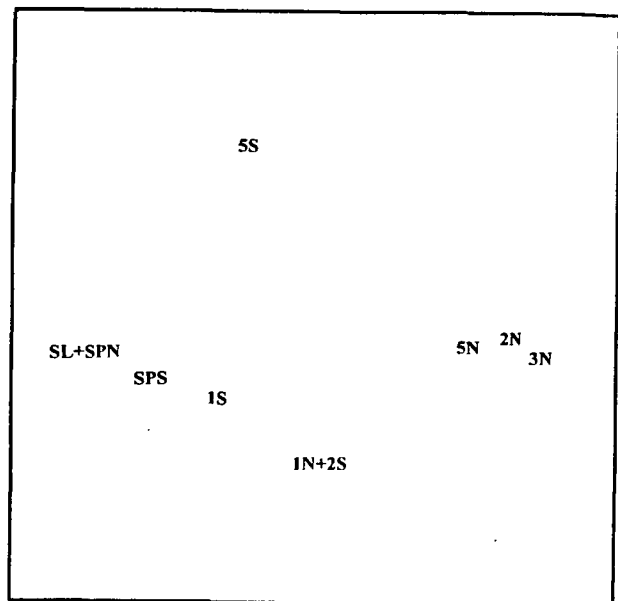
On Day 619 (03.02.95, 4 days after the change: Figure 4.50/4.58a), SPN remained paired with SL, SPS fell close to these ponds, and 1S was close to SPS. Pond 1N now paired with 2S. Ponds 2N, 3N and 5N were grouped next in the ordination, while 5S was out on its own and no rotifers were recorded in 3N, 4N, or 4S. The highest Bray Curtis similarities were 97.938 (1N+2S), 91.442 (SPN+SPS), 91.357 (2N+5N), 86.353 (2N+3N), and 81.687 (SPN+1S). The similarities between the SL and SPN and SPS outlets were 73.651 and 75.874, respectively, while those between the 1N+1S, 2N+2S, 3N+3S, and 5N+5S pond pairs were 66.600, 47.006, 0.000, and 28.553, respectively.

On Day 622 (06.02.95, 7 days post-reversal: Figure 4.51/4.58b), SPS (high-flow) was now pairing with SL, and 1S fell on the SPN side of its pair 1N. Ponds 2S, 3S, and 4S fell almost in a line closer to these earlier ponds, while 4N and 5N were much further away and 5S remained out on its own. No rotifers were found in 2N or 3N. The highest Bray Curtis similarities were 92.183 (1N+1S), 89.157 (SL+SPS), 86.966 (SPN+SPS), 86.353 (4N+5N), and 82.447 (SPN+1S). The percentage similarity between the SL and SPN outlets was 76.456, and those between the 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 0.000, 0.000, 66.667, and 32.735, respectively.

By Day 625 (09.02.95, 10 days post-reversal: Figure 4.52/4.58c), SPN surprisingly fell on the SL side of SPS, although 2S was close to these. Pond 3S was also close; 1S fell on the earlier side of the 1N+1S pair. Pond 5S had now moved markedly closer to the earlier ponds, while 5N was now out on its own. In general; all of the southern ponds (with the exception of 4S) fell earlier in the ordination pattern. No

rotifers were found in 3N or 4N (low flow side). The highest Bray Curtis similarities were 95.148 (1N+1S), 86.834 (SPN+SPS), 77.801 (SPS+1S), 75.688 (SPS+2S), and 74.384 (1N+SPS). The similarities between the SL and SPN and SPS outlets were 73.960 and 62.094, respectively, while those between the 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 42.879, 0.000, 0.000, and 0.000 respectively.

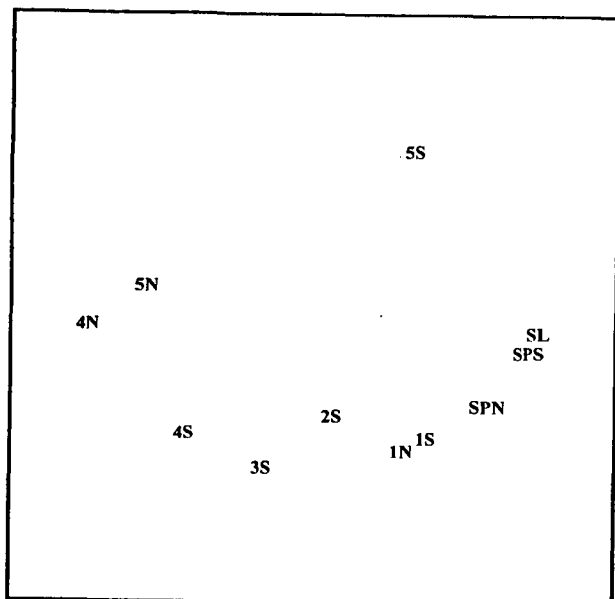
Finally, on Day 639 (23.02.95, 24 days post-reversal: Figure 4.53/4.58d), SPN was still on the SL side of SPS and half-way between the two. Ponds 1N+1S remained paired, but with the latter still closer to the initial ponds. All of the remaining southern ponds fell in the upper half of the ordination (with 3S+4S paired), while the northern ponds fell lower. Pond 2N grouped closely with 3N+4N, while 5N fell on its own at the tail of the ordination. The highest Bray Curtis similarities were 100.000 (3S+4S), 96.076 (3N+4N), 89.581 (2N+3N), 86.222 (1N+1S) and 85.715 (2N+4N). The similarities between the SL and SPN and SPS outlets were 64.841 and 63.498, respectively, while those between the SPN+SPS, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 81.839, 45.069, 57.057, 60.312, and 0.000, respectively.



(a) Day 619: 3 February 1995  
4 days since flow change.  
No rotifers in samples 3S, 4N or 4S.

MAIN ROTIFERS

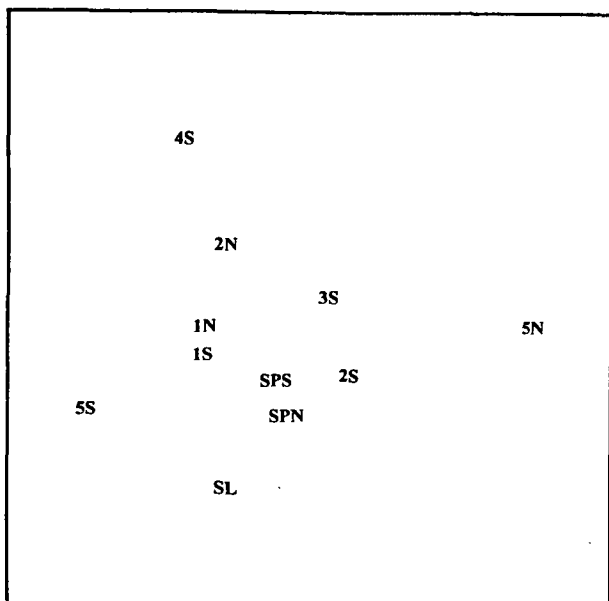
Stress = 0.01



(b) Day 622: 6 February 1995  
7 days since flow change.  
No rotifers in samples 2N or 3N.

MAIN ROTIFERS

Stress = 0.02

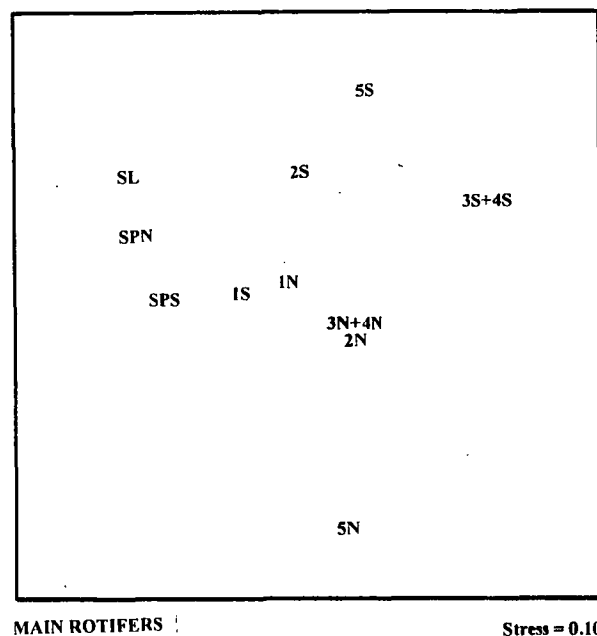


(c) Day 625: 9 February 1995  
10 days since flow change.  
No rotifers in samples 3N or 4N.

MAIN ROTIFERS

Stress = 0.08

**(d) Day 639: 23 February 1995  
24 days since flow change.**



**Figure 4.58 (a-d)**

**Rotifer MDS ordination patterns for the  
third flow division (25%N: 75%S).**



**Table 4.2: Bray-Curtis similarities between paired lagoons (rotifers).**

Bold, underlined values represent pairs among the five most similar lagoons for that day. Asterisk values represent pairs between which no Bray-Curtis value can be calculated, as rotifers were absent from both ponds. Bold zero values represent pond pairs for which rotifers were present in one pond but not the other.

FLOW	DAY	SPN+SPS	1N+1S	2N+2S	3N+3S	4N+4S	5N+5S
ORIGINAL	1	NA	57.181	<u><b>93.987</b></u>	<u><b>98.893</b></u>	NA	NA
ORIGINAL	15	NA	43.65	<u><b>83.698</b></u>	<u><b>74.1</b></u>	NA	NA
ORIGINAL	29	NA	54.474	58.671	*****	NA	NA
ORIGINAL	50	NA	68.37	72.986	<b>0</b>	NA	NA
ORIGINAL	64	NA	47.667	*****	*****	NA	NA
EQUAL	317	NA	77.892	<u><b>89.515</b></u>	62.229	0	72.805
EQUAL	331	<u><b>96.974</b></u>	51.057	69.473	65.816	74.371	40.626
EQUAL	345	<u><b>87.874</b></u>	<u><b>78.16</b></u>	<u><b>77.589</b></u>	68.135	0	66.667
EQUAL	359	<u><b>89.749</b></u>	79.433	68.308	<u><b>80.148</b></u>	<b>0</b>	0
EQUAL	373	<u><b>88.107</b></u>	<u><b>78.144</b></u>	57.721	<b>0</b>	58.97	NA
EQUAL	387	<u><b>96.861</b></u>	83.947	<u><b>96.471</b></u>	<u><b>98.136</b></u>	73.006	NA
EQUAL	401	77.24	<u><b>84.991</b></u>	75.136	NA	51.004	NA
EQUAL	415	<u><b>95.37</b></u>	<u><b>94.539</b></u>	89.963	80.099	73.195	NA
EQUAL	429	<u><b>97.471</b></u>	<u><b>95.216</b></u>	<u><b>86.816</b></u>	72.731	<b>0</b>	NA
EQUAL	443	91.044	<u><b>91.62</b></u>	73.498	77.988	48.8	83.307
1st DIV.	457 (0)	91.341	93.799	83.799	92.322	61.398	91.357
1st DIV.	471 (14)	<u><b>84.609</b></u>	<u><b>84.446</b></u>	NA	<b>0</b>	0	0
1st DIV.	499 (42)	86.85	<u><b>91.467</b></u>	79.309	84.971	74.804	89.531
1st DIV.	513 (56)	<u><b>90.733</b></u>	63.246	76.488	57.606	52.934	86.117
1st DIV.	527 (70)	<u><b>95.183</b></u>	81.382	80.355	59.199	0	61.806
1st DIV.	541 (84)	<u><b>86.623</b></u>	<u><b>83.637</b></u>	56.457	33.547	54.281	<b>0</b>
2nd DIV.	552 (0)	<u><b>90.966</b></u>	67.946	46.137	57.862	50	47.733
2nd DIV.	562 (10)	<u><b>88.042</b></u>	78.312	34.835	<b>0</b>	<b>0</b>	58.457
2nd DIV.	576 (24)	<u><b>83.595</b></u>	62.371	62.712	47.468	<u><b>86.001</b></u>	75.333
2nd DIV.	590 (38)	<u><b>87.986</b></u>	66.021	48.61	59.199	60.6	*****
2nd DIV.	594 (42)	<u><b>86.757</b></u>	60.971	32.638	58.639	0	62.697
2nd DIV.	604 (52)	<u><b>89.939</b></u>	66.543	43.174	82.008	69.023	69.332
3rd DIV.	619 (4)	<u><b>91.442</b></u>	66.6	47.006	<b>0</b>	****(+3S)	28.553
3rd DIV.	622 (7)	<u><b>86.966</b></u>	<u><b>92.183</b></u>	<b>0</b>	<b>0</b>	66.667	32.735
3rd DIV.	625 (10)	<u><b>86.834</b></u>	<u><b>95.148</b></u>	42.879	<b>0</b>	<b>0</b>	0
3rd DIV.	639 (24)	81.839	<u><b>86.222</b></u>	45.069	57.057	60.312	0

### *Summary of rotifer MDS ordination patterns and similarities*

The MDS ordinations based on the four main rotifer groups initially reflected the original flow pattern through the 85wB system. With the change in the pattern of flow, ponds broke from this initial pattern to pair well over the equilibration period, with these pairings reflecting the new pattern and the equal division of flow between the halves. These pairings were less consistent for the later ponds (pairs 4 and 5).

As the first flow division (25%N:75%S) established itself, later southern (high flow) ponds tended to move forwards in the ordination patterns to pair with or pass earlier northern ponds (these differences were not as pronounced for the SPN and SPS outlets, which reflected low and high flow sides of the same pond). With the second flow division (75%N:25%S), this pattern steadily reversed as the new flow regime became established. Later northern (high flow) ponds began to match or precede earlier southern ponds, although patterns became more confused as the change-over took effect, leaving lagoons to group into north and south clusters (with the former falling earlier). With the final flow division (25%N:75%S), similar reversals were seen in the pattern, with the southern ponds moving forwards in the ordination and becoming much more chaotic in comparison to the equilibrium period.

Similarity values between paired ponds based on rotifer data are presented in Table 4.2. Underlined values represent those ranking among the five highest (most similar) of all ponds for the system on each sampling day. As can be seen, greater numbers of corresponding pairs ranked among the highest similarities during the equilibrium period than during the following flow divisions, particularly beyond the SP outlets.

Pair 1N+1S (which were initially separated in the 85wB series) showed low similarity under the original flow pattern before establishing as consistently similar ponds by the end of the equilibrium period. Although this similarity decreased only slightly during the first flow division, it dropped markedly when flow was reversed for the

**Table 4.3: Highest Bray-Curtis similarities between lagoons (rotifers).**

Bold, underlined values represent those between corresponding lagoon pairs.

Red values represent combinations of later southern ponds with earlier northern ponds.

Blue values represent combinations of later northern ponds with earlier southern ponds.

FLOW	DAY	HIGHEST PAIRS				
ORIGINAL	1	<u>98.893 (3N+3S)</u>	<u>93.987 (2N+2S)</u>	78.721 (1N+SPS)	77.324 (SPS+1S)	67.950 (2N+3S)
ORIGINAL	15	85.449 (SL+02)	<u>83.698 (2N+2S)</u>	83.020 (SPS+1S)	79.085 (02+1N)	<u>74.100 (3N+3S)</u>
ORIGINAL	29	87.569 (SL+02)	81.393 (1N+SPS)	69.705 (02+1N)	68.075 (SL+SPS)	65.626 (1S+2S)
ORIGINAL	50	96.967 (SL+1N)	92.000 (02+1S)	86.763 (02+SPS)	78.986 (SPS+1S)	75.756 (02+1N)
ORIGINAL	64	96.750 (SL+02)	76.737 (SL+1N)	75.216 (SPS+1S)	73.686 (02+1N)	68.346 (1N+SPS)
EQUAL	317	<u>89.515 (2N+2S)</u>	84.207 (SL+SPN)	79.958 (3S+5N)	79.572 (3N+5N)	78.138 (2N+1S)
EQUAL	331	<u>96.974 (SPN+SPS)</u>	92.391 (2N+3N)	88.224 (3N+1S)	84.831 (SPS+1N)	83.163 (SL+1N)
EQUAL	345	100.00 (4S+5S)	<u>87.874 (SPN+SPS)</u>	<u>78.160 (1N+1S)</u>	<u>77.589 (2N+2S)</u>	77.476 (2N+3N)
EQUAL	359	94.683 (3N+2S)	<u>89.749 (SPN+SPS)</u>	81.645 (2N+1S)	<u>80.148 (3N+3S)</u>	79.531 (4N+3S)
EQUAL	373	<u>88.107 (SPN+SPS)</u>	83.300 (4N+5N)	80.495 (SPS+1S)	<u>78.144 (1N+1S)</u>	74.676 (SPN+1N)
EQUAL	387	100.00 (5N+4S)	<u>98.136 (3N+3S)</u>	<u>96.861 (SPN+SPS)</u>	<u>96.471 (2N+2S)</u>	91.886 (4N+3S)
EQUAL	401	86.353 (5N+4S)	<u>84.991 (1N+1S)</u>	83.917 (SPN+1S)	78.751 (1N+2S)	78.567 (1N+SPS)
EQUAL	415	<u>95.370 (SPN+SPS)</u>	<u>94.539 (1N+1S)</u>	93.708 (SPN+1S)	93.426 (1N+SPS)	92.799 (SPN+1N)
EQUAL	429	<u>97.471 (SPN+SPS)</u>	<u>95.216 (1N+1S)</u>	87.557 (3N+5N)	87.355 (3N+4S)	<u>86.816 (2N+2S)</u>
EQUAL	443	96.185 (SPN+1S)	92.794 (4N+5S)	92.643 (SPS+1S)	92.586 (4N+3S)	<u>91.620 (1N+1S)</u>
1st DIV.	457 (0)	98.466 (4N+5N)	97.614 (4N+3S)	96.229 (3N+5N)	96.081 (5N+3S)	94.698 (3N+4N)
1st DIV.	471 (14)	100.00 (4N+5N)	88.024 (1N+SPS)	85.311 (SPS+1S)	<u>84.609 (SPN+SPS)</u>	<u>84.446 (1N+1S)</u>
1st DIV.	499 (42)	94.803 (3N+4S)	<u>91.467 (1N+1S)</u>	90.744 (SPN+1N)	90.411 (SPN+1S)	89.938 (2N+3S)
1st DIV.	513 (56)	96.143 (2N+3S)	92.715 (4N+5N)	92.332 (SL+1S)	91.768 (1N+2S)	<u>90.733 (SPN+SPS)</u>
1st DIV.	527 (70)	95.792 (3N+4S)	<u>95.183 (SPN+SPS)</u>	94.936 (3N+5S)	94.584 (SL+SPS)	93.267 (2N+3S)
1st DIV.	541 (84)	90.187 (SPN+1S)	<u>86.623 (SPN+SPS)</u>	85.062 (SPS+1S)	<u>83.637 (1N+1S)</u>	82.365 (1N+2S)
2nd DIV.	552 (0)	91.357 (4S+5S)	<u>90.966 (SPN+SPS)</u>	89.757 (3S+4S)	88.596 (2N+3N)	82.554 (SL+SPN)
2nd DIV.	562 (10)	90.380 (SL+SPN)	89.623 (1N+SPS)	<u>88.042 (SPN+SPS)</u>	87.938 (SL+SPS)	85.068 (4N+2S)
2nd DIV.	576 (24)	91.357 (2S+3S)	86.353 (3N+4S)	<u>86.001 (4N+4S)</u>	85.892 (SL+SPN)	<u>83.595 (SPN+SPS)</u>
2nd DIV.	590 (38)	100.00 (3N+4N)	95.483 (2S+3S)	<u>87.986 (SPN+SPS)</u>	87.355 (2N+1S)	85.451 (1N+SPS)
2nd DIV.	594 (42)	100.00 (2S+4S)	94.936 (5N+2S)	94.936 (5N+4S)	90.592 (SPN+1N)	<u>86.757 (SPN+SPS)</u>
2nd DIV.	604 (52)	98.281 (4N+3S)	96.571 (3N+5S)	96.054 (2N+3N)	92.635 (2N+5S)	<u>89.939 (SPN+SPS)</u>
3rd DIV.	619 (4)	97.938 (1N+2S)	<u>91.442 (SPN+SPS)</u>	91.357 (2N+5N)	86.353 (2N+3N)	81.687 (SPN+1S)
3rd DIV.	622 (7)	<u>92.183 (1N+1S)</u>	89.157 (SL+SPS)	<u>86.966 (SPN+SPS)</u>	86.353 (4N+5N)	82.447 (SPN+1S)
3rd DIV.	625 (10)	<u>95.148 (1N+1S)</u>	<u>86.834 (SPN+SPS)</u>	77.801 (SPS+1S)	75.688 (SPS+2S)	74.384 (1N+SPS)
3rd DIV.	639 (24)	100.00 (3S+4S)	96.076 (3N+4N)	89.581 (2N+3N)	<u>86.222 (1N+1S)</u>	85.715 (2N+4N)

second, before returning to (possibly transient) high levels for the shorter period of the third. In contrast to pair 1, pairs 2 and 3 were initially more similar under the original flow pattern, and retained reasonable similarity into the equilibrium period. Similarity values for both of these pairs, however, were markedly lower during the three flow divisions. Pairs 4 and 5 were rarely among the highest similarity values, and varied markedly in similarity within and between different flow regimes.

It should be noted that Table 4.2 is likely to include underestimation of pond similarity in certain cases, as similarity values are (unavoidably) designed not to treat zero occurrences as comparable events between sites. In broader situations, this is desirable, as it helps avoid invalid conclusions (e.g. the absence of penguins from both the North Pole and the Simpson Desert does not mean that these locations or the processes within them are necessarily similar or comparable). In the terms of *this study*, however, there is some value to treating such absences as indicative (if not conclusive) of similarity due to patterns of nutrient loading and flow manipulation at WTC (e.g. Table 4.1).

This shortfall is largely accounted for by combining rotifer and crustacean data in the analysis (as at the end of this chapter) to remove the chances of zero-only data sets, but needs to be kept in mind when examining these groups separately. Consequently, pond pairs for which no Bray-Curtis values could be calculated have been treated in the following ways in Table 4.2: (i) for pairs in which *both* ponds were lacking rotifer fauna, similarity can effectively be considered 100% *in the terms of this project*, but, to distinguish this from properly calculated values, it is denoted only as a series of asterisks in the table; (ii) for pairs in which only one pond was lacking rotifer fauna, similarity has been declared as 0%, and is presented as a bold zero in the table to distinguish it from calculated zero values. As this method cannot account for comparisons of samples containing no rotifers and those containing only extremely low rotifer numbers (e.g. solitary species or specimens, ), rare cases may still underestimate 'similarity' as it would ideally be applied in this project. For further reference, ponds lacking rotifer or crustacean fauna are listed in Table 4.1.

Table 4.3 presents another way of looking at the similarities in rotifer distribution within the system, including a visual indication

of the shift in community types between the two halves. This table lists the highest similarities between all lagoons on each sampling day, with underlined values representing corresponding pond pairs, red values representing 'out of phase' pairings between later southern ponds and earlier northern ponds, and blue values representing 'out of phase' pairings between later northern ponds and earlier southern ponds (these combinations include pairings with the SL pond).

As can be seen, pairings between corresponding ponds dominated during the equilibration period, but became less common during the three divisions of flow. Late S/early N pond combinations tended to dominate during the first flow division (75%S), reversing to mainly late N/early S pairings during the second (75%N). Although more late S/early N combinations were present for the third division (75%S), no defined pattern had developed by the end of the period.

As before, it should be noted that Table 4.3 is subject to the omission of rotifer-free ponds from Bray Curtis calculations, and as such does not list the sites of "assumed" 100% similarity as denoted by asterisks in Table 4.2. For the same reason, it also omits "assumed" 100% similarities for 3N+4S on Day 562 (late S/early N, second flow division) and 4N+3S on Day 619 (late N/early S, third flow division).

#### 4.5.3.2 - Crustacean MDS ordinations

##### *Original flow pattern- single flow system (crustaceans).*

Flow characteristics and expected patterns remain as discussed in the preceding section on rotifers. Stress values for the crustacean plots over the original flow period ranged from 0.00 to 0.05 (average 0.02, standard error 0.01), which fall well within the ideal range for stress values.

On Day 1 (26.05.93: Figure 4.23/4.59a), no Crustacea occurred in the SL pond, and the original pond 2 separated quite distinctly from the remaining ponds. These ponds then generally followed a serpentine fashion across the remainder of the ordination space, running 1N to SPS to 1S to 2S to 2N to both 3N and 3S (opposite sides). As with the rotifers, this pattern followed the order of the lagoons under the original flow pattern, grading from earlier ponds to later ones. The highest Bray Curtis percentage similarities between lagoons were 90.839 (2N+3N), 88.176 (2N+2S), 86.495 (1S+2S), 84.004 (2N+3S), and 83.728 (SP+1S). The similarity between the SL and SPS outlets was 0.000, as Crustacea were absent at the former outlet but present at the latter one, while between the 1N+1S, and 3N+3S outlets the similarities were 63.945 and 76.787, respectively.

On Day 15 (09.06.93: Figure 4.24/4.59b), the ordination for Crustacea shows the early (SL, 2, and 1N) ponds and the last (3S) pond separating from a tight grouping of the intervening ponds. Within the tighter group the ponds followed the typical arc from early to later ponds, although this was not as prominent in the full ordination. The highest Bray Curtis similarities between lagoons were 94.300 (SPS+1S), 87.706 (2N+2S), 87.398 (2N+3N), 87.077 (3N+1S), and 86.783 (1S+2S). The similarity between the SL and SPS outlets was only 19.994, while between 1N+1S and 3N+3S it was 34.541 and 79.489, respectively.

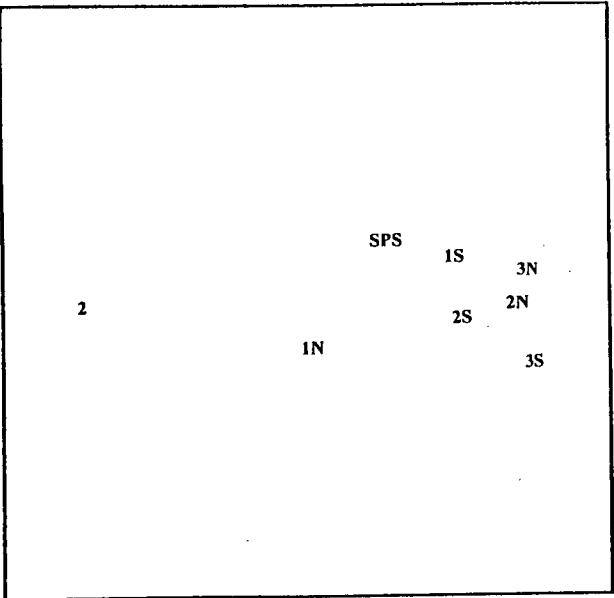
On day 29 (23.06.93: Figure 4.25/4.59c), there were no Crustacea in the first two ponds (SL+2), while the third pond (1N) separated very distinctly from the remaining ponds. The remaining ponds graded from earlier to later, although 1S fell slightly out of place



within this gradation. The highest Bray Curtis similarities between lagoons were 93.964 (2N+1S), 93.438 (1S+2S), 93.359 (SPS+2S), 92.602 (3N+2S), and 91.755 (2N+3S). The similarity between the SL and SPS outlets was 0.000, as Crustacea were again absent at the former outlet while present at the latter one. The similarities between pond pairs 1N+1S, 2N+2S, and 3N+3S were 61.418, 90.643, and 89.125, respectively.

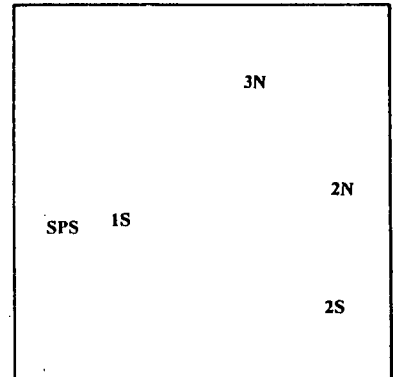
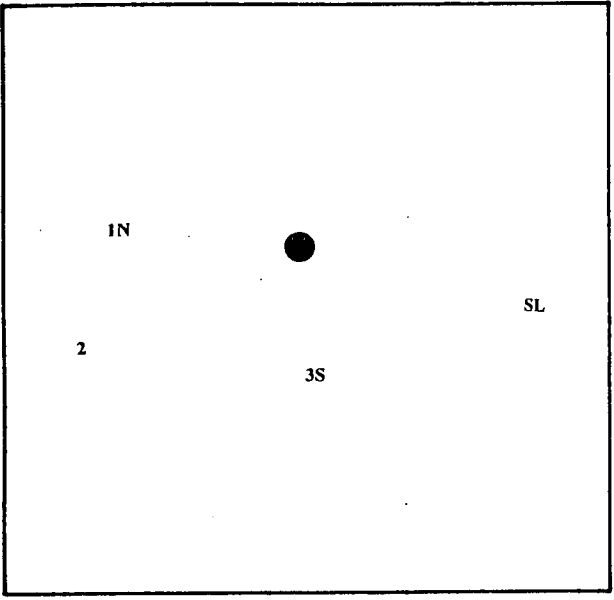
On Day 50 (14.07.93: Figure 4.26/4.59d), Crustacea were absent from the SL pond, and the original pond 2 separated quite distinctly from the remaining ponds in the ordination. The remaining ponds fell in a distinct gradation from earlier to later lagoons, with 1N itself quite separate from the rest, 1S+2S+SPS grouping closely together and followed by 2N, and 3S+3N+4S falling in a more widely spread group towards the end. The highest Bray Curtis similarities between lagoons were 92.186 (1S+2S), 90.675 (SPS+2S), 88.171 (SPS+2N), 87.475 (SP+1S), and 86.092 (2N+2S). Again, the similarity between the SL and SPS outlets was 0.000 due to the absence of Crustacea from the former pond, while similarities between the 1N+1S and 3N+3S ponds were 68.754 and 80.474, respectively.

For the final sampling day of the original flow period (Day 64, 28.07.93: Figure 4.27/4.59e), Crustacea remained absent from the SL pond, and both the second and third ponds (2+1N) grouped closely and very separate to the remaining ponds. The same gradation from earlier to later ponds was present, although pond 4S occurred earlier in the sequence than would have been expected. Ponds 2S+2N were closely paired. The highest Bray Curtis similarities between lagoons were 90.541 (2N+2S), 90.039 (1S+2S), 85.033 (2S+4S), 84.161 (2+1N), and 83.995 (3S+4S). SL and SPS were again completely dissimilar due to the absence of Crustacea from the SL pond, while similarities between pairs 1N+1S and 3N+3S were 51.287 and 70.996, respectively.



(a) Day 1: 26 May 1993  
No SPN, 4N, 4S, 5N, 5S samples at this stage.  
No crustaceans in sample SL.

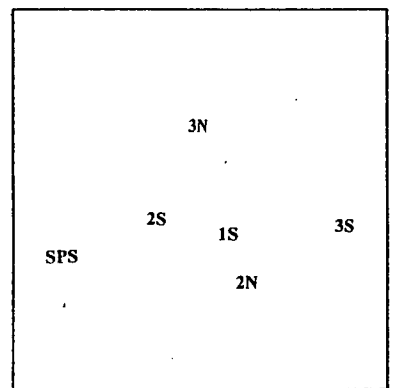
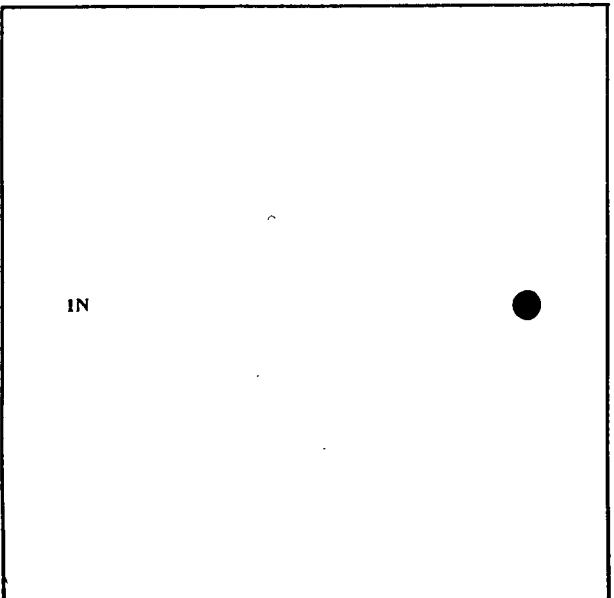
MAIN CRUSTACEANS Stress = 0.00



MAIN CRUSTACEANS  
SL, 2, 1N, 3S removed

(b) Day 15: 9 June 1993  
No SPN, 4N, 4S, 5N, 5S samples at this stage.

MAIN CRUSTACEANS Stress = 0.01  
● = SPS, 1S, 2N, 2S, 3N



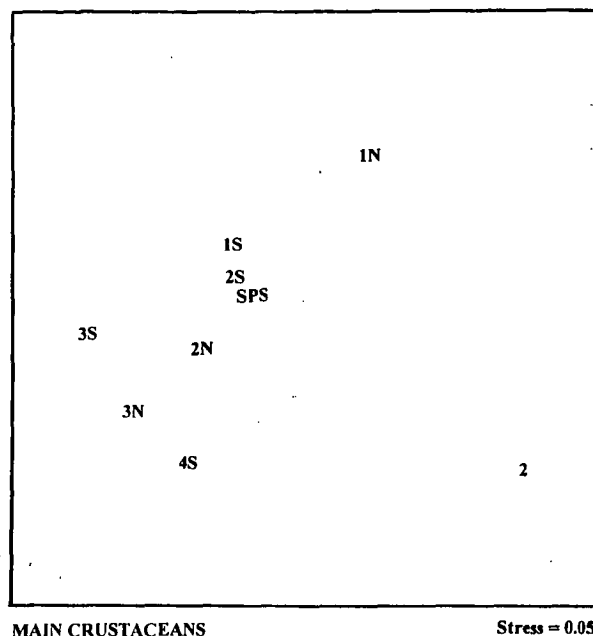
MAIN CRUSTACEANS  
1N removed

(c) Day 29: 23 June 1993  
No SPN, 4N, 4S, 5N, 5S samples at this stage.  
No crustaceans in samples SL or (2).

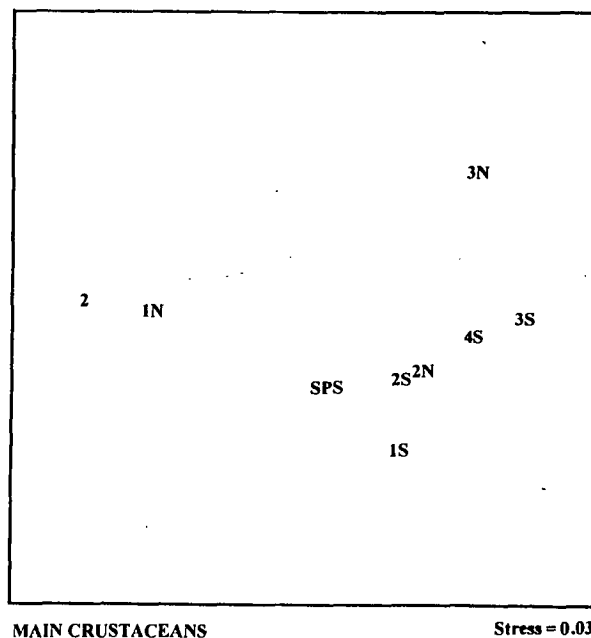
MAIN CRUSTACEANS Stress = 0.01  
● = SPS, 1S, pairs 2 + 3



**(d) Day 50: 14 July 1993**  
 No SPN, 4N, 5N, 5S samples at this stage.  
 No crustaceans in sample SL.



**(e) Day 64: 28 July 1993**  
 No SPN, 4N, 5N, 5S samples at this stage.  
 No crustaceans in sample SL.



**Figure 4.59 (a-e)**

**Crustacean MDS ordination patterns  
 for the original (pre-alteration) flow  
 period.**

### *Equal flow pattern - 50%N, 50%S (crustaceans)*

Under equal flow conditions, these patterns had changed to reflect the new order of flow through the system. Stress values for this period ranged from 0.01 to 0.08 (average 0.041, standard error 0.008), with the increase at least partly due to the increased number of values being included in the ordination. Again, all stress values fall within the acceptable range.

On Day 317 (07.04.94: Figure 4.28/4.60a), no Crustacea were present in the SL or SPN samples (the SPS sample had unfortunately deteriorated in storage). The remaining ponds had already established themselves in a distinct pattern grading from earlier to later according to the new flow pattern. Pairs 1N+1S and 2N+2S were quite distinct, while 3N+3S were also noticeably close to each other. Ponds 4N, 4S, 5N, and 5S all fell in the same part of ordination space, although pairing between these lagoons was not tight. With the exception of 5N, the northern ponds of each lagoon pair fell towards the earlier side of the gradation than their partners. The highest Bray Curtis similarities between lagoons were 96.684 (2N+2S), 95.370 (3N+2S), 94.835 (1N+1S), 93.983 (2N+3N), and 93.727 (3N+3S). While the absence of Crustacea from both the SL and SPN outlets gives a technical similarity of 0.000, it should be noted that this absence in the early ponds is exactly the sort of convergence in spatial pattern that was predicted for the system (as discussed on p. 4.40 & 4.56). Similarities not already given for paired lagoons were 88.891 and 83.635 for 4N+4S and 5N+5S, respectively.

On Day 331 (21.04.94: Figure 4.29/4.60b), Crustacea remained absent from the SL, SPN, and SPS ponds. For the remaining ponds both the pairings and the general gradation were less distinct, but were still present to some degree. All ponds 1-3 fell in one half of the ordination, while 4-5 all fell in the other. While not paired, ponds 1N plus 1S and 3N plus 3S fell in the same regions of ordination space with nothing between them, while 4N and 4S in particular were very close. Ponds 2N plus 2S and 5N plus 5S, however, were quite separate and divided by 1S and (to some degree) 4N, respectively. Again with the exception of 5S, the northern ponds of each pair tended to fall to the earlier side of the

gradation. The highest Bray Curtis similarities between lagoons were 95.973 (1S+2S), 92.391 (4N+4S), 92.024 (2S+3S), 91.921 (4N+5S), and 91.766 (3S+5S). Technically the similarities between the SL, SPN and SPS outlets remained zero, but again this agreed with the expected pattern of absence of Crustacea from the early ponds. The similarities between the remaining pairs of 1N+1S, 2N+2S, 3N+3S, and 5N+5S were 88.018, 85.103, 90.029, and 80.387, respectively.

On Day 345 (05.05.94: Figure 4.30/4.60c), the pattern remained similar, although the 1N and 1S lagoons now fell on the other side of the 4N/4S and 5N/5S ponds than 2N/2S and 3N/3S. Pond 2N paired with 2S and 3N with 3S, and both of these pairs were close to each other. Ponds 5N and 5S fell in the same region within the ordination, 1N and 1S were less close, and 4N and 4S were very separate. The tendency for either northern or southern sides to fall to the earlier side of the gradation was no longer as clear by this stage. The highest Bray Curtis similarities were 95.939 (3N+3S), 93.943 (2N+2S), 91.990 (2S+3S), 91.563 (2N+3N), and 91.317 (3N+2S). The absence of crustaceans from the SL, SPN and SPS lagoons remained in keeping with the expected trend, while the similarities between ponds 1N+1S, 4N+4S, and 5N+5S were 80.749, 67.734, and 91.006, respectively.

The pattern became more confused by Day 359 (19.05.94: Figure 4.31/4.60d), with only 3N+3S providing a distinct pairing. Ponds 1N and 1S fell close, but while 2N and 2S also fell with nothing between them, 1N and 2S paired closest of the four. Ponds 4N and 5N fell near to each other and on the opposite side of the ordination to their counterparts. The highest Bray Curtis similarities were 95.817 (1N+2S), 95.211 (3N+3S), 94.480 (1N+1S), 93.742 (4N+5N), and 93.664 (2N+3S). SL, SPN, and SPS continued to 'match' each other with Crustacea absent, while the similarities for the remaining pairs 2N+2S, 4N+4S, and 5N+5S were 83.965, 70.438, and 75.936, respectively.

By Day 373 (02.06.94: Figure 4.32/4.60e), the general gradation from earlier to later ponds had returned to the ordination, with the exception of pond 3S which was notably separate to the rest. Pairings between ponds, however, were not exceptionally good. Ponds 2N and 2S paired well, and while 1N and 1S were separate, they fell on the

same side of the bulk of the other ponds. By this stage the 5S pond was removed from circulation by Melbourne Water, but the 3N and 5N ponds fell directly between the 4N and 4S 'pair'. The highest Bray Curtis similarities were 95.595 (2N+2S), 90.704 (4N+5N), 86.848 (1N+2N), 84.685 (1N+2S), and 82.261 (2N+4S). Crustacea were again absent in SL, SPN, and SPS, while similarity values for pairs 1N+1S, 3N+3S, and 4N+4S were remarkably low at 66.113, 41.931, and 64.436, respectively.

On Day 387 (16.06.94: Figure 4.33/4.60f), the gradation from early to later ponds was distinct and pairings between lagoons, while still not close, were much improved. Ponds 1N+1S, 2N+2S, and 3N+3S all fell within the same areas within the ordination, and while values were closer between some of these pairs than within them, no values separated the corresponding ponds from each other. Ponds 4N and 4S were separated by a greater distance, however, with 2N and 3N beginning to intrude between them. The highest Bray Curtis similarities were 95.344, (2N+3N), 91.302 (2N+2S), 90.193 (3N+4N), 90.114 (1S+3S), and 90.110 (3N+3S). SL, SPN, and SPS remained similarly bereft of crustaceans, while the similarity values for pairs 1N+1S and 4N+4S had increased to 83.919 and 81.107, respectively.

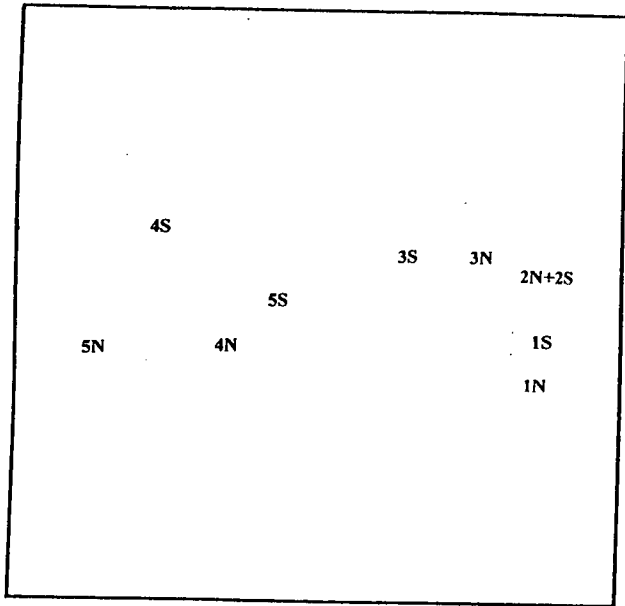
On Day 401 (30.06.94: Figure 4.34/4.60g), the early pairs 1N/1S and 2N/2S again fell within the same area of the ordination, and were quite separate to the later ponds. Pond 2S paired closer to 1N, however, and 2N closer to 1S. No 3N sample was available, and ponds 4N and 4S were well separated. The highest Bray Curtis similarities were 91.441 (2N+1S), 89.951 (1N+2S), 89.543 (1S+2S), 84.937 (1N+2N), and 83.411 (2N+2S). Again no Crustacea were recorded from ponds SL, SPN or SPS, while similarities between pairs 1N+1S and 4N+4S were 82.549 and 80.867, respectively.

Crustacea returned to the SPS outlet on Day 415 (14.07.94: Figure 4.35/4.60h), and this site was distinctly separate from the remaining sites in ordination space. The pattern of the remaining sites again maintained the gradation from early to later ponds, and 4N+4S now paired well. Ponds 2N+2S and 3N+3S again fell close to each other and were not separated by other ponds, while 1N and 1S were separated by 2N. The highest Bray Curtis similarities were 98.057

(4N+4S), 91.993 (2N+1S), 87.721 (3N+3S), 86.690 (1N+3N), and 84.999 (1N+2N). SL and SPN contained no Crustacea, while their presence in SPS meant that it had zero similarity to the preceding ponds. Similarity values between pairs 1N+1S and 2N+2S were 75.258 and 84.654, respectively.

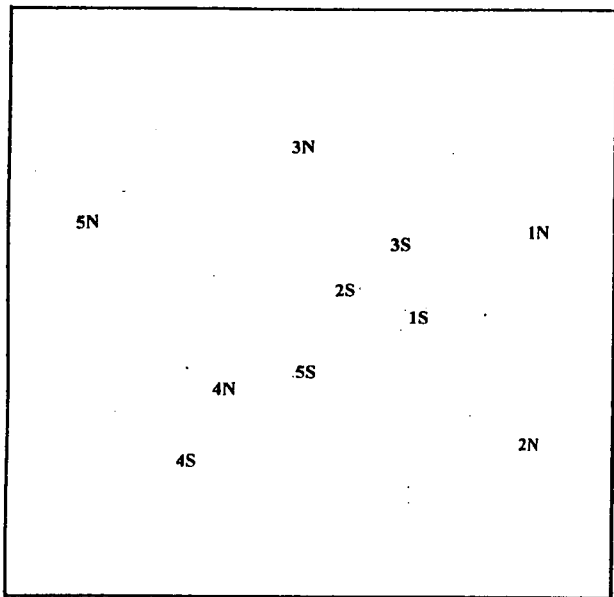
The ordination based on crustacean values for Day 429 (28.07.94: Figure 4.36/4.60i), maintained the gradation from earlier to later ponds, but with pairs becoming more separated further into the system. Ponds 1N+1S and 2N+2S fell close and the pairs were not separated by other values, although 2S fell closer to 1N than to 2N. Pairs 3 and 4 were very separate, with 3S+4N and 3N+4S falling much closer to each other than to their counterparts. The highest Bray Curtis similarities were 94.709 (1N+2S), 90.068 (3N+4S), 89.300 (1S+2S), 88.826 (1N+1S), and 87.663 (1N+2N). The early ponds SL, SPN and SPS once more reflected expected patterns by containing no crustaceans, while the similarity values between ponds 2N+2S, 3N+3S, and 4N+4S were 87.612, 59.447, and 61.575, respectively.

The gradation from early to later ponds was slightly more confused in the ordination for crustacean zooplankton on Day 443, 11.08.94: Figure 4.37/4.60j). Ponds 2N, 2S and 3N fell to one side of the 1N+1S pair; 4N, 4S, 5N and (the returned) 5S fell to the other side, and 3S was out on its own. Ponds 5N+5S paired well, and 2N+2S were separated by a similar distance, although 2S fell closer to 3N. Ponds 1N and 1S fell in the same area but were intruded upon by 4N, while this pond and 4S were slightly further apart. The highest Bray Curtis similarities were 95.481 (3N+2S), 89.030 (2N+2S), 88.799 (4N+1S), 88.175 (4N+1N), and 84.998 (2N+1S). SL, SPN, and SPS maintained the absence of crustaceans seen up until this point, while the similarity values between 1N+1S, 3N+3S, 4N+4S and 5N+5S were 81.857, 68.941, 81.008, and 84.102, respectively.



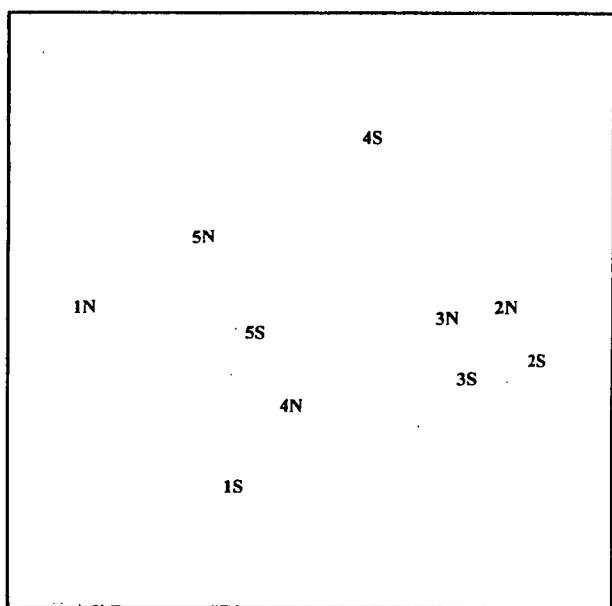
(a) Day 317: 7 April 1994

No SPS sample (preservation problem).  
No crustaceans in samples SL or SPN.



(b) Day 331: 21 April 1994

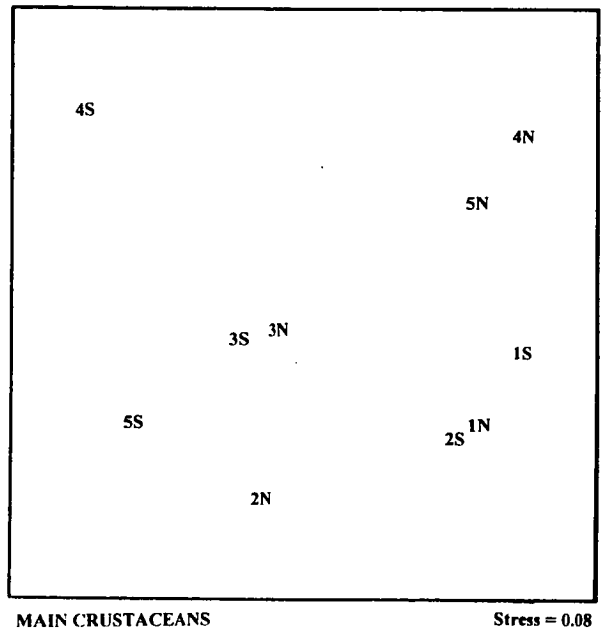
No crustaceans in samples SL, SPN, or SPS.



(c) Day 345: 5 May 1994

No crustaceans in samples SL, SPN or SPS.

(d) Day 359: 19 May 1994  
 No crustaceans in samples SL, SPN or SPS.



(e) Day 373: 2 June 1994  
 No 5S sample (pond cut out, MW works).  
 No crustaceans in samples SL, SPN or SPS.

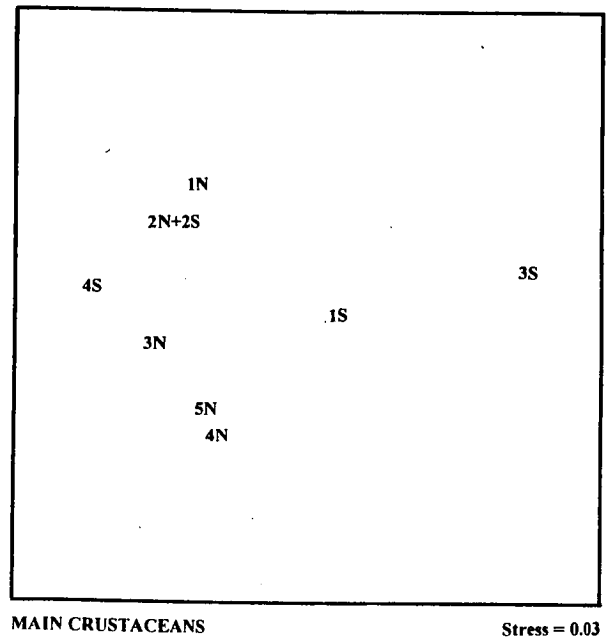
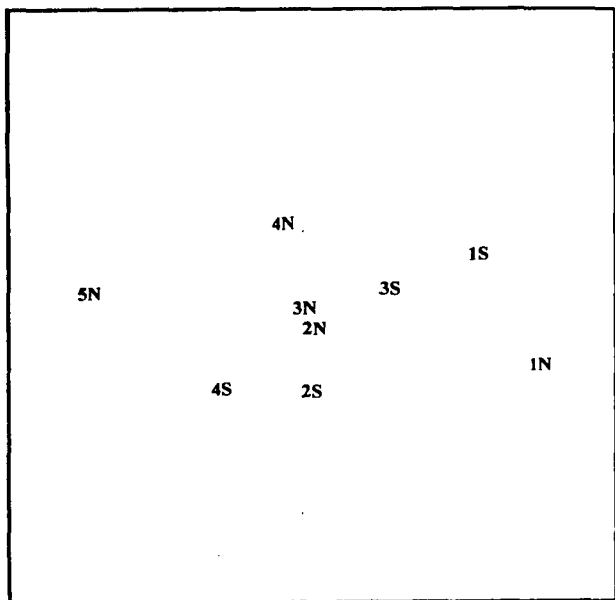


Figure 4.60 (a-e) continued overleaf

Crustacean MDS ordination patterns for  
 the equal flow period (50%N: 50%S).

C  
R  
U  
S  
T  
A  
C  
E  
A  
N



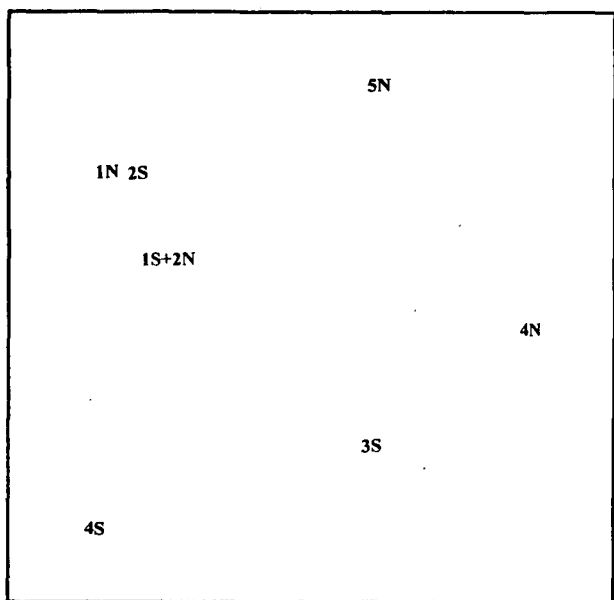
**(f) Day 387: 16 June 1994**

No 5S sample (pond cut out, MW works).

No crustaceans in samples SL, SPN or SPS.

MAIN CRUSTACEANS

Stress = 0.02



**(g) Day 401: 30 June 1994**

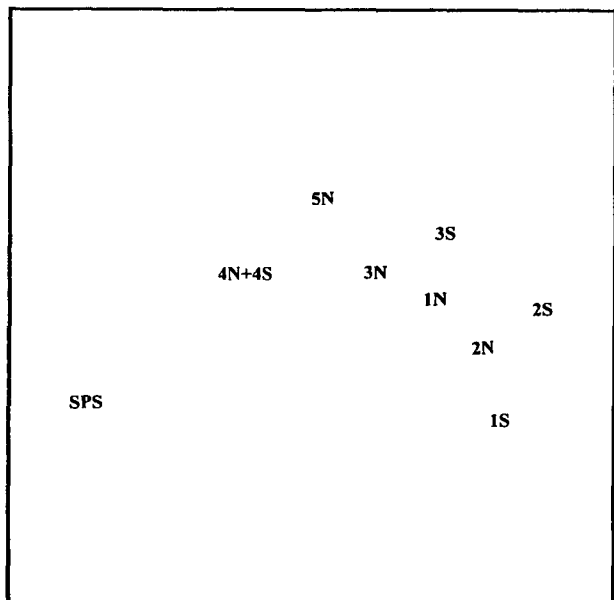
No 3N sample (preservation problem).

No 5S sample (pond cut out, MW works).

No crustaceans in samples SL, SPN or SPS.

MAIN CRUSTACEANS

Stress = 0.01



**(h) Day 415: 14 July 1994**

No 5S sample (pond cut out, MW works).

No crustaceans in samples SL or SPN.

MAIN CRUSTACEANS

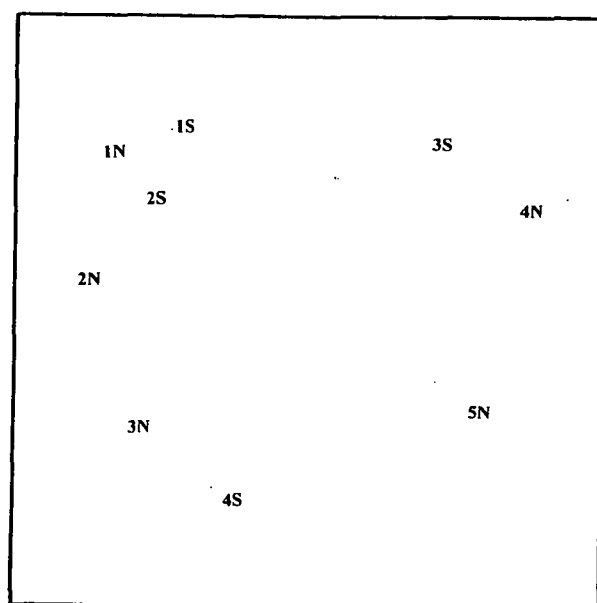
Stress = 0.03

C  
R  
U  
S  
T  
5



**(i) Day 429: 28 July 1994**

No 5S sample (pond cut out, MW works).  
No crustaceans in samples SL, SPN or SPS.

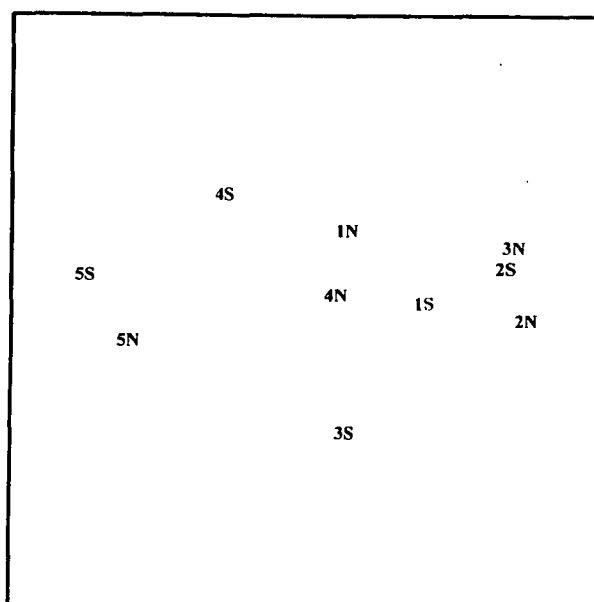


MAIN CRUSTACEANS

Stress = 0.04

**(j) Day 443: 11 August 1994**

No crustaceans in samples SL, SPN or SPS.



MAIN CRUSTACEANS

Stress = 0.05

**Figure 4.60 (f-j) *cont. from previous page***

**Crustacean MDS ordination patterns for  
the equal flow period (50%N: 50%S).**

***First flow division - 25%N, 75%S (crustaceans)***

Stress values for the crustacean plots over the period of the first flow division ranged from 0.00 to 0.04 (average 0.015, standard error 0.006), well within the acceptable range and markedly lower on average than for the equilibrium period.

With the change of flow on Day 457 (25.08.94: Figure 4.38/4.61a), the ordination maintained the same general patterns as seen during the equilibrium period. Crustacea were present in the SL and SPN samples, although still absent from SPS. The crustacean assemblage for these two lagoons was markedly different to the remaining ponds, though, with both ponds grouping together and distinct from the others. The gradation from early to later ponds could be seen across pairs 1-5, with pond 1S some way out on its own at the start. Good pairings could be seen between pairs 2N+2S and 3N+3S, and while 1S fell away from 1N, the latter remained its closest pond. Ponds 4N and 4S similarly fell in the same region of the ordination without any ponds between them, while 5N and 5S were separated both by distance and 4N. The highest Bray Curtis similarities were 100.000 (SL+SPN), 93.934 (4N+5S), 93.595 (3N+5S), 93.466 (1N+5S), and 92.579 (3N+4N). With no Crustacea, SPS had zero similarity to both SL and SPN, while similarities between pairs 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S were 77.351, 81.509, 91.654, 86.331, and 59.276, respectively.

On Day 471 (08.09.94, 14 days after the change of flow: Figure 4.39/4.61b), no Crustacea were found in the SL and SPN samples, although some were present at the SPS outlet. This sample remained very different to the remaining sites, which grouped tightly at the opposite side of the ordination. Within this group, the usual gradation could be seen (this time from right to left), although there was a greater tendency for the southern ponds to fall to one side of the ordination (lower right) and the northern ponds to the other (upper left). Ponds 1N+1S fell close, however, with 1S remaining the earliest of the ponds in the gradation. Ponds 3N+3S and 4N+4S were not paired but did fall in line with each other, with no ponds falling between them. Ponds 5N+5S were more separate, with the space between them intruded

upon by pairs 3 and 4. Without a 2S sample, 2N fell by itself at one side of the main group. The highest Bray Curtis similarities were 86.830 (3N+4N), 86.346 (1N+5S), 85.097 (4N+3S), 81.891 (1N+1S), and 80.930 (3S+5S). SL and SPN again matched each other in terms of the expected spatial succession by containing no Crustacea, while SPS matched neither as crustaceans were present at this outlet. Similarity values for the 3N+3S, 4N+4S, and 5N+5S pond pairs were 70.411, 63.305, and 42.434, respectively.

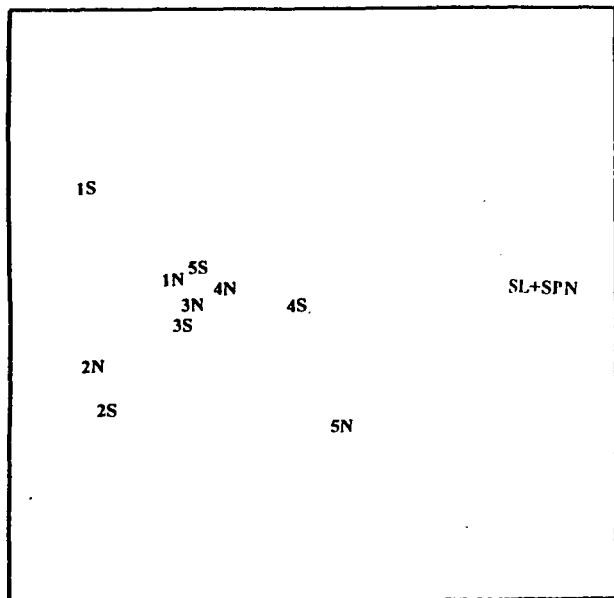
By Day 499 (6.10.94, 42 days after the flow change: Figure 4.40/4.61c), there were still no Crustacea in the SL pond, while they had reappeared in SPN and disappeared from SPS. SPN fell very separate to the remaining lagoons in ordination space. The gradation can be seen running from bottom to top on the left side of the ordination, and finishing running from ponds 4N to 5N on the right. Importantly, the southern ponds now all preceded their northern counterparts quite distinctly in the gradation. The pattern begins with 1S out on its own. No values fell between 1S and 1N, although the latter was as close to 2S as it was to its pair. Ponds 2S, 3S, and 5S all preceded their corresponding ponds by about the same distance, and 3S even preceded, and paired close to, 2N. While pairs 2, 3 and 5 were relatively close, 4S was very separate to 4N. The highest Bray Curtis similarities were 96.130 (2N+3S), 94.285 (2N+2S), 93.708 (5N+5S), 92.955 (2S+3S), and 91.431 (2N+3N). SL and SPS displayed a 'similar' absence of Crustacea, while both shared zero similarity with SPN on that count. Similarities between pairs 1N+1S, 3N+3S, and 4N+4S were 85.394, 89.530, and 71.594, respectively.

On Day 513 (20.10.94, 56 days since the flow change: Figure 4.41/4.61d), the trio of SL, SPN, and SPS all contained Crustacea, and these lagoons separated very distinctly from the remainder. SPN and SPS paired closely, with SPN on the SL side. Within the larger group of ponds, 1N fell towards the start of the gradation, and was well separated from 1S. Pond 2S preceded 2N, although the two remained relatively close. Pond 3S preceded and was very separate to 3N, and also preceded 4N and 5N. Pairs 4N and 5N were not particularly close, and 4S preceded its partner to fall closer to 5N. Pond 5S was on the outer, and fell closer to 3N than to the other lagoons. The highest Bray Curtis similarities were 94.526 (2N+2S), 94.216 (1S+2S), 93.745 (5N+4S), 92.594

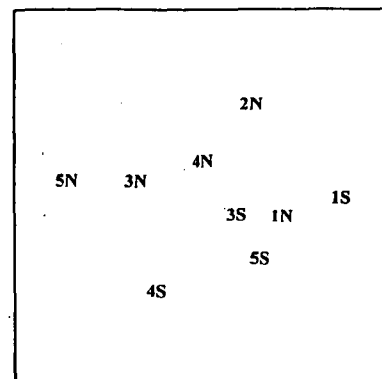
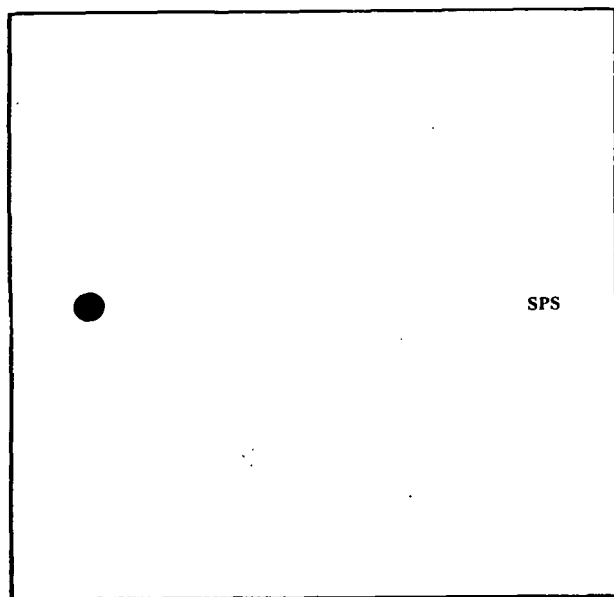
(2N+1S), and 91.964 (3S+4S). The similarities between the SL and SPN and SPS outlets were 73.205 and 65.364, respectively, while those between the SPN+SPS, 1N+1S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 91.357, 83.755, 82.843, 89.102, and 80.954, respectively.

On Day 527 (03.11.94, 70 days since the change: Figure 4.42/4.61e), SL, SPN, and SPS were again grouping very separately to the remaining ponds. SPN and SPS were paired, with the former on the SL side of the two. Within the larger group, pond 1S fell to the beginning of the gradation, followed by pond 2S. Pond 1N was separated from 1S by some distance, with 2S falling between them. Pond 1N was, in turn, intruding between 2N and 2S, which were not paired. Ponds 3S and 3N fell much closer to each other, although 3S fell earlier in the ordination (towards 1S and 2S), while 1N, 2N, 3N and 4N were grouping tightly together. Ponds 5S and 5N fell close to each other, and while neither was especially forwards of the other, 5S was slightly closer to the cluster of the earlier northern ponds. Pond 4S was the last of the ponds in the line of the gradation, and was separated from 4N by 5S, which fell half way between the two. The highest Bray Curtis similarities were 94.662 (1N+3N), 94.438 (3N+4N), 93.803 (1N+2N), 93.105 (2N+4N), and 92.506 (2N+3N). The similarities between the SL and SPN and SPS outlets were 76.144 and 65.863, respectively, while those between the SPN+SPS, 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 85.059, 83.482, 86.163, 91.226, 83.624, and 91.275, respectively.

For the final sample of the first flow division (Day 541, 17.11.94, 84 days post-change: Figure 4.43/4.61f), the SL, SPN, and SPS lagoons were again separate from the remaining ponds, but were not particularly close to each other. Pond 1S was again the outermost of the remaining ponds, and was the closest of this group to SPN. Pond 2S paired with 1N and 5S with 4N, while the former pair split 3N and 3S. The northern ponds again tended towards one side of the grouping (away from the SL and SP ponds), and 4S was again on its own. The highest Bray Curtis similarities were 96.786 (4N+5S), 92.831 (2S+3S), 92.809 (1N+2S), 91.842 (2N+3N), and 91.496 (1N+3S). The similarities between the SL and SPN and SPS outlets were 60.455 and 47.461, respectively, while those between the SPN+SPS, 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 52.110, 79.553, 80.180, 89.143, 89.436 and 89.292 respectively.

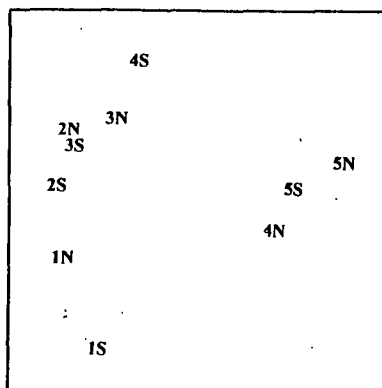
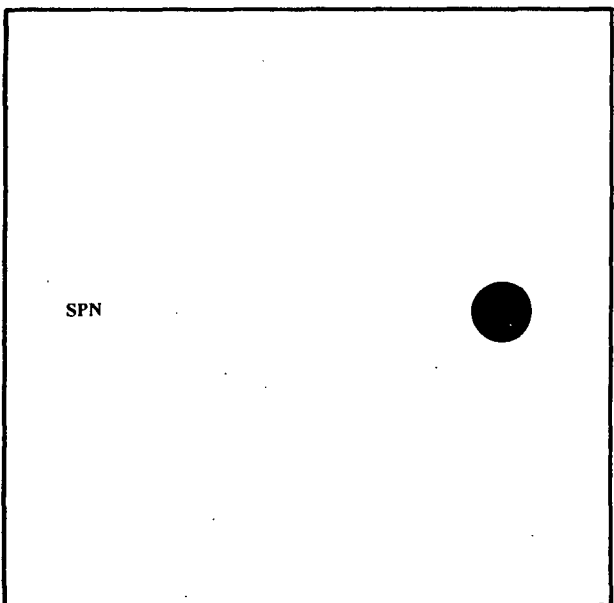


(a) Day 457: 25 August 1994  
0 days since flow change.  
No crustaceans in sample SPS.



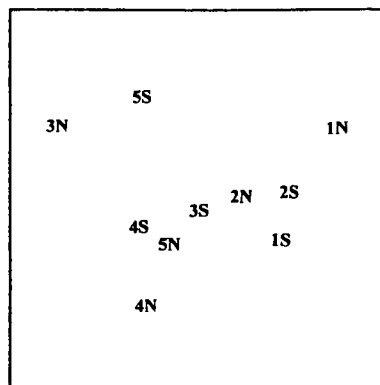
MAIN CRUSTACEANS  
SPS removed

(b) Day 471: 8 September 1994  
14 days since flow change.  
No 2S sample (preservation problem).  
No crustaceans in samples SL or SPN.



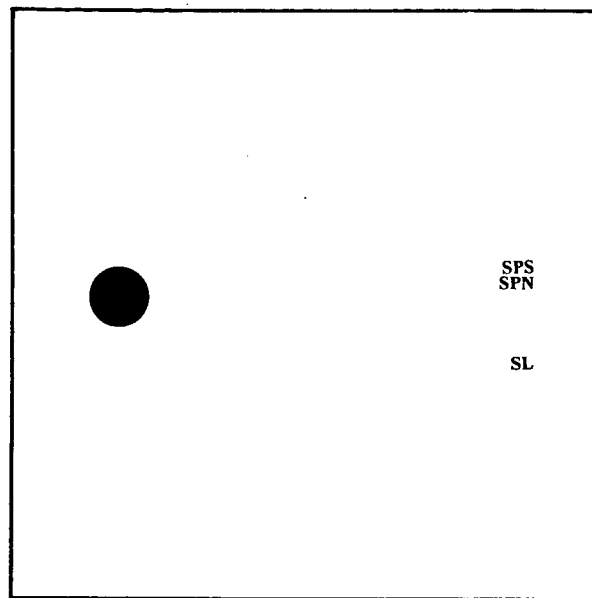
MAIN CRUSTACEANS  
SPN removed

(c) Day 499: 6 October 1994  
42 days since flow change.  
No crustaceans in samples SL or SPS.



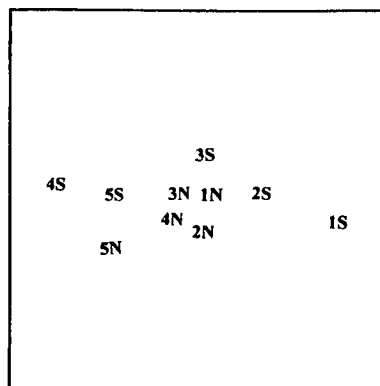
MAIN CRUSTACEANS  
SL, SPN, SPS removed

(d) Day 513: 20 October 1994  
56 days since flow change.



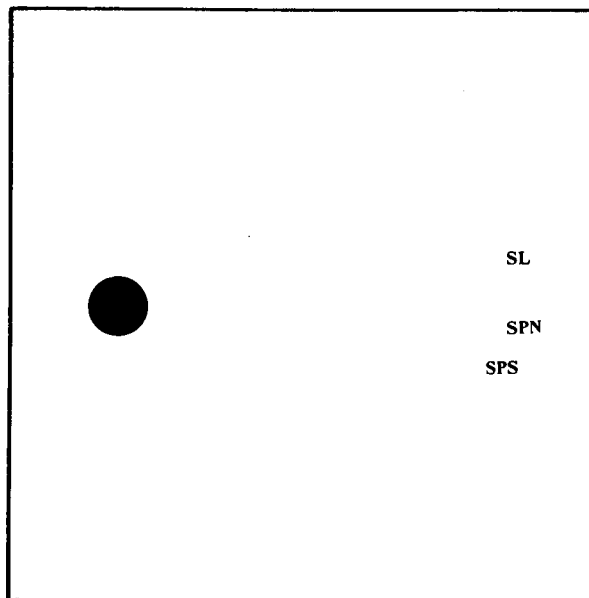
MAIN CRUSTACEANS  
● = all pairs 1-5

Stress = 0.01



MAIN CRUSTACEANS  
SL, SPN, SPS removed

(e) Day 527: 3 November 1994  
70 days since flow change.



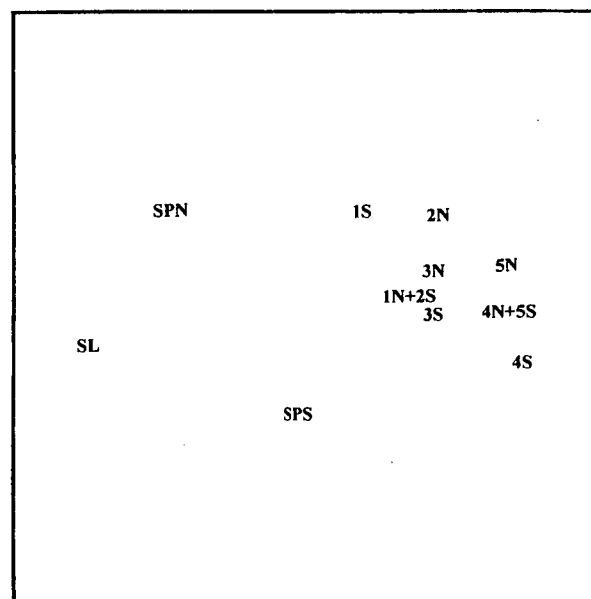
MAIN CRUSTACEANS  
● = all pairs 1-5

Stress = 0.01

Figure 4.61 (a-f)

Crustacean MDS ordination patterns for  
the first flow division (25%N: 75%S).

(f) Day 541: 17 November 1994  
84 days since flow change.



MAIN CRUSTACEANS

Stress = 0.04

### *Second flow division - 75%N, 25%S (crustaceans)*

Stress values for the crustacean plots over the second flow division ranged from 0.01 to 0.08 (average 0.032, standard error 0.013). These values again fell within the acceptable range, and the average level and the standard error were not greatly different than those for the previous flow division. As with the rotifers, however, the higher stress values occurred towards the end of this flow division period, possibly indicating a greater degree of variation between the ponds once the new regime had established itself.

For the day of flow reversal, (Day 552, 28.11.94: Figure 4.44/4.62a), the ordination maintained trends from the previous flow division. SL paired with SPN, and SPS in turn grouped close to these. These three outlets were very separate to those of the remaining ponds, which grouped tightly on the other side of the ordination. Within this group the southern ponds 1S, 2S and 3S all fell on the earlier side of the gradation, with 1S to the fore. All three of these ponds preceded 1N, 2N and 3N, which fell in a line behind them, while 4S paired with 2N. Ponds 4N and 5N also paired, while 5S was out on its own. The highest Bray Curtis similarities were 99.468 (SL+SPN), 93.315 (4N+5N), 93.211 (2N+4S), 91.723 (2N+3S), and 91.663 (1N+5N). The similarity between the SL and SPS outlets was 91.149, while those between the SPN+SPS, 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 91.677, 84.056, 89.795, 85.771, 85.450, and 79.445, respectively.

By Day 562 (08.12.94, 10 days post flow reversal: Figure 4.45/4.62b), the SL, SPN and SPS ponds were still very separate to the remainder. The SPN pond, however, was beginning to fall out of its position directly between the SPS and SL. Pond 1S was very separate to the remaining ponds, while 2S, 3S and 5S grouped closely together. The southern ponds again appeared separate to their northern counterparts, with the former group tending to fall across the upper left of the ordination and the latter in the lower right. The highest Bray Curtis similarities were 98.969 (2S+3S), 93.652 (2S+5S), 93.489 (4N+5N), 92.696 (3S+5S), and 92.353 (2N+5N). The percentage similarities between the SL and the SPN and SPS outlets were 75.129 and 81.417, respectively,

while the similarities between the SPN+SPS, 1N+1S, 2N+2S, 3N+3S, 4N+4S and 5N+5S pond pairs were 76.267, 83.020, 79.162, 90.537, 77.599, and 73.747, respectively.

On Day 576 (22.12.94, 24 days from change: Figure 4.46/4.62c), SL, SPN and SPS were not as separate from the other lagoons as they had been previously. SPN was still falling less directly between SL and SPS than previously, and pond 1N was beginning to break from the other group and was moving closer to SL and the SPN pond. The southern ponds were still falling to one side of the larger group than the remaining northern ponds (upper versus lower sides). While this group still graded from earlier ponds to later ponds (left to right), 2N was beginning to precede 1S and 2S, 4N was drawing level with 3S, and 5N was slightly ahead of 5S. The highest Bray Curtis similarities were 95.710 (2N+1S), 94.922 (3N+5N), 93.475 (4S+5S), 93.474 (3N+4S), and 91.830 (3N+5S). The percentage similarities between the SL, SPN and SPS outlets were 78.356 and 69.298, respectively, while the similarities between the SPN+SPS, 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 83.963, 77.514, 82.673, 85.123, 86.748, and 90.570, respectively.

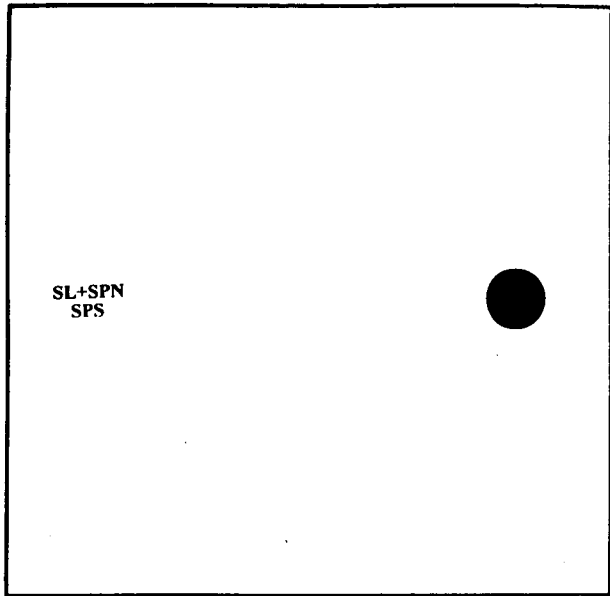
On Day 590 (05.01.95, 38 days from change: Figure 4.47/4.62d), the 3S pond separated very distinctly from the other lagoons. While much closer than 3S, SL fell at the extreme edge of the gradation from earlier to later ponds, while 1N was pairing with SPS next on the fringe of the group. SPS was now much closer than SPN to SL. Ponds 2N and 3N were moving up towards the earlier ponds, while 5N and 4N were moving ahead of pond 2S. Ponds 2S, 5S and 4S were falling together in a group towards the end of the gradation. The highest Bray Curtis similarities were 97.170 (2S+5S), 93.737 (4S+5S), 93.548 (4N+2S), 93.175 (4N+5S), and 91.925 (2S+4S). The similarities between the SL and SPN and SPS outlets were 62.723 and 77.872, respectively, while those between SPN+SPS, 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S were 84.509, 84.231, 80.882, 44.228, 89.224, and 86.065, respectively.

On Day 594 (09.01.95, 42 days since reversal: Figure 4.48/4.62e), the SL pond was separating some distance from the remaining ponds. Lagoon 1S was now moving into the outermost



position from the remaining group, closely followed by SPS and then SPN. Pond 1N was then falling close to 4S and 2S, with the other northern ponds falling in the centre of the larger group. The highest Bray Curtis similarities were 96.073 (2S+4S), 95.841 (2N+4N), 95.482 (1N+2S), 95.333 (3N+2S), and 94.724 (4N+2S). The similarities between the SL and SPN and SPS outlets were 63.636 and 61.789, respectively, while those between the SPN+SPS, 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 89.426, 73.254, 94.005, 84.097, 91.755, and 86.501, respectively.

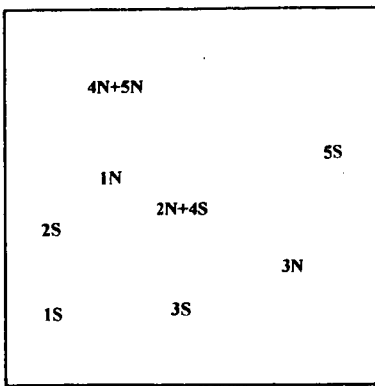
For the final sampling day of the second flow division (Day 604, 19.01.95, 52 days post-reversal: Figure 4.49/4.62f), the SL pond remained quite separate from the other lagoons. SPS fell the closest to it, followed by SPN and 1N. Pond 5S fell not far from these lagoons on its own. Pond 3S paired with 4S, while 2N fell very close to paired lagoons 3N+5N. Pond 2S fell on its own, and separated from 2N by these paired ponds. Ponds 4N and 1S fell close together, also isolated from other ponds and at the tail end of the ordination. The highest Bray Curtis similarities were 96.743 (3N+5N), 96.691 (3S+4S), 96.123 (2N+3N), 95.003 (5N+4S), and 94.521 (2N+5N). The similarities between the SL and SPN and SPS outlets were 62.894 and 75.208, respectively, while those between the SPN+SPS, 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 87.568, 62.793, 79.529, 89.596, 81.007, and 76.932, respectively. As noted previously, the stress value was highest on this ordination (0.08), although still within the acceptable range.



MAIN CRUSTACEANS

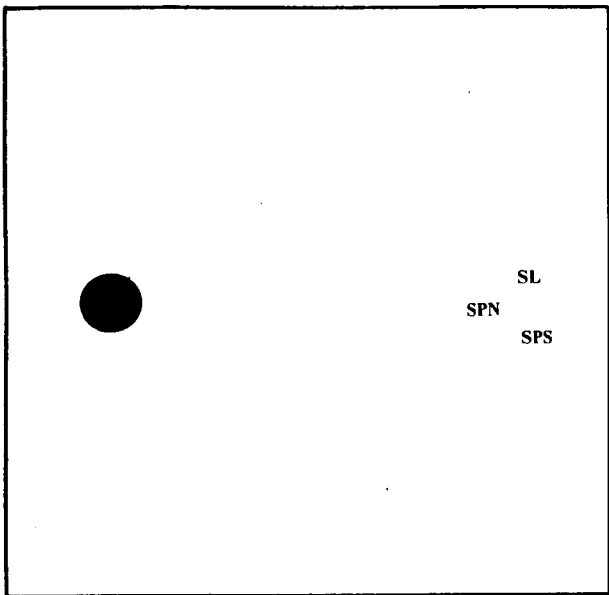
● = all pairs 1-5

Stress = 0.01



MAIN CRUSTACEANS  
SL, SPN, SPS removed

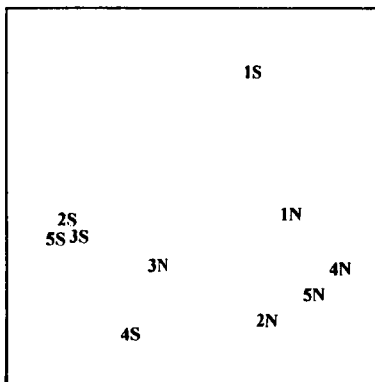
(a) Day 552: 28 November 1994  
0 days since flow change.



MAIN CRUSTACEANS

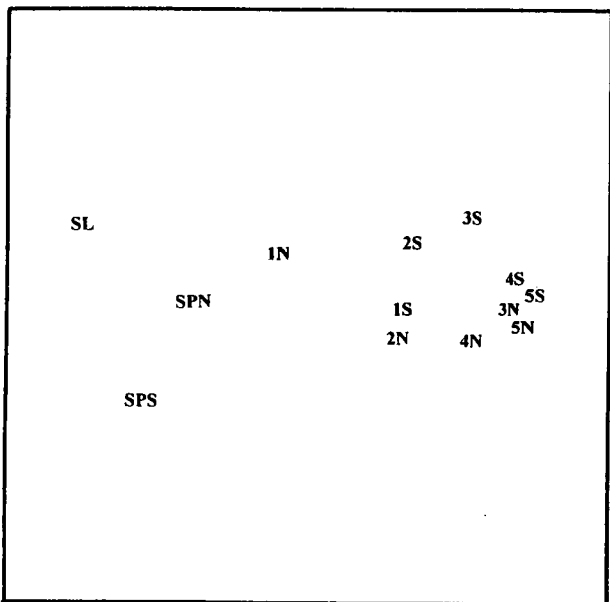
● = all pairs 1-5

Stress = 0.01



MAIN CRUSTACEANS  
SL, SPN, SPS removed

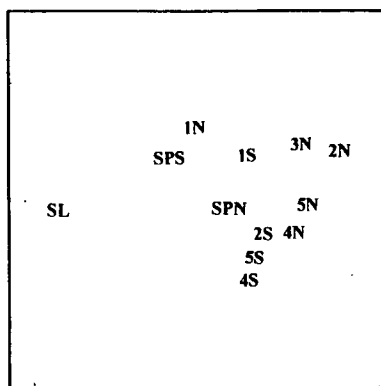
(b) Day 562: 8 December 1994  
10 days since flow change.



MAIN CRUSTACEANS

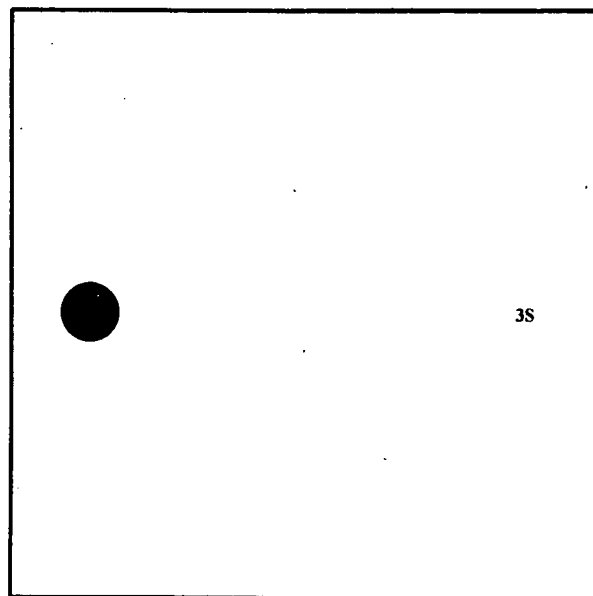
Stress = 0.03

(c) Day 576: 22 December 1994  
24 days since flow change.



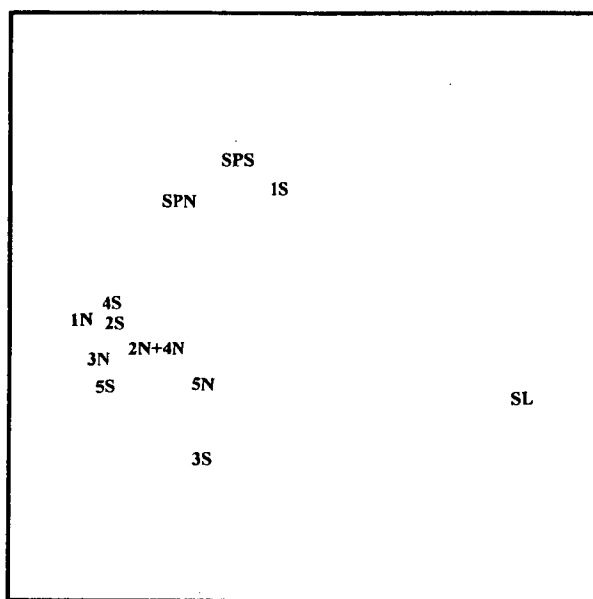
MAIN CRUSTACEANS  
3S removed

(d) Day 590: 5 January 1995  
38 days since flow change.



MAIN CRUSTACEANS  
● = all bar 3S

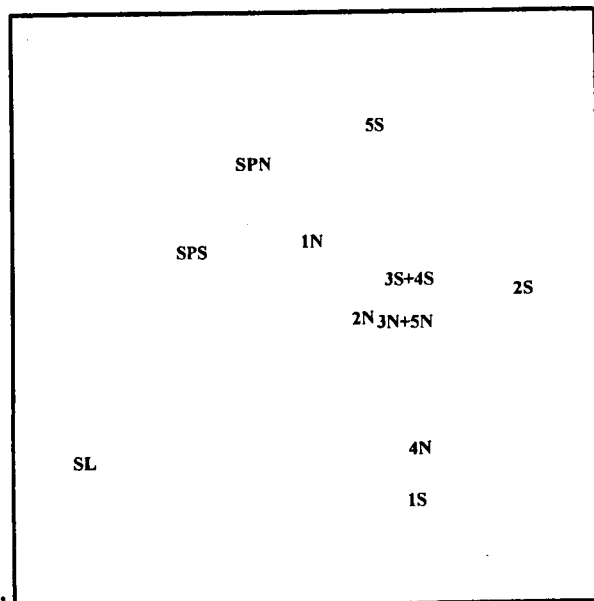
Stress = 0.01



(e) Day 594: 9 January 1995  
42 days since flow change.

MAIN CRUSTACEANS

Stress = 0.05



(f) Day 604: 19 January 1995  
52 days since flow change.

MAIN CRUSTACEANS

Stress = 0.08

Figure 4.62 (a-f)

Crustacean MDS ordination patterns for  
the second flow division (75%N: 25%S).

### *Third flow division - 25%N, 75%S (crustaceans)*

Stress values for the crustacean plots over the third flow division ranged from 0.06 to 0.09 (average 0.08, standard error 0.008). These values remain within, but are beginning to approach the threshold of, the acceptable range. The stress values begin high (0.09, Day 619, 4 days after change), in keeping with the high stress values at the end of the previous flow division. They then fall (0.06, Day 622, 7 days after change), before rising again (0.08, Day 625, 10 days after change) and peaking once more once the new regime is established (0.09, Day 639, 24 days after change).

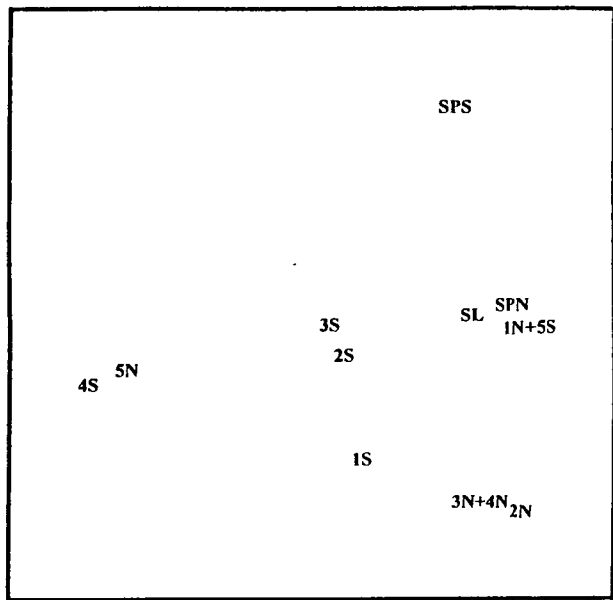
On Day 619 (03.02.95, 4 days post-reversal: Figure 4.50/4.63a), a distinct group was formed by SL, SPN, and the paired ponds 1N+5S. Away from these, pair 3N+4N grouped closely with 2N, 2S paired with 3S, and 4S paired with 5N. Ponds 1S and SPS fell on their own. The highest Bray Curtis similarities were 98.744 (3N+4N), 97.640 (2N+4N), 97.167 (1N+5S), 96.965 (SL+SPN), and 96.655 (2S+3S). The similarity between the SL and SPS outlets was 87.534, while those between the SPN+SPS, 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 87.126, 89.612, 86.610, 88.091, 72.242, and 80.077, respectively.

On Day 622 (06.02.95, 7 days post-reversal: Figure 4.51/4.63b), SPN paired with SPS, while SL fell in the middle of the ordination with 1S nearby. Ponds 2S+4S were paired and near SL and 1S. Ponds 1N, 4N and 5N grouped closely to the opposite side of SL than 1S, while the remaining ponds fell on the outer. The southern ponds tended to fall to the lower right half of this ordination plot, with most of the northern ponds to upper left. The highest Bray Curtis similarities were 95.876 (1N+4N), 95.835 (4N+5N), 95.553 (SPN+SPS), 95.378 (2S+4S), and 95.106 (1N+5N). The similarities between the SL, SPN and SPS outlets was 80.663 and 80.741, respectively, while those between the 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 85.286, 76.928, 65.564, 83.434, and 75.423, respectively.

By Day 625 (09.02.95, 10 days post-reversal: Figure 4.52/4.63c), SL was again separate from the other ponds, while SPN and SPS were paired (with SPN closer to SL). Ponds 2N and 2S were close to pairing

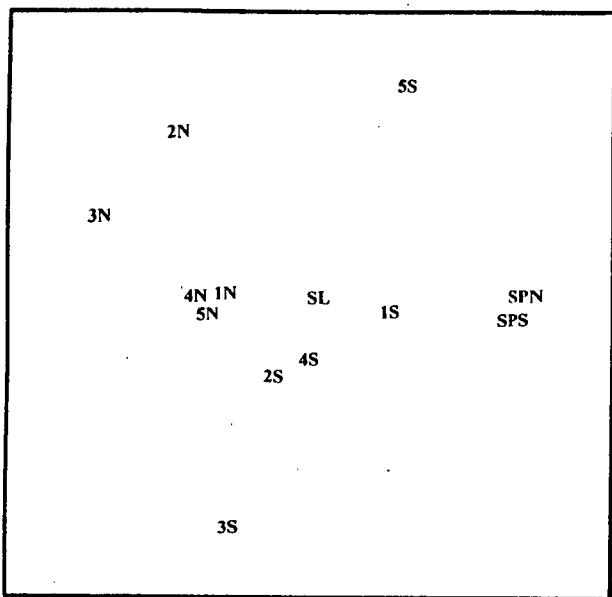
(with 2S also close to 5N and 3S), while 1N and 1S were in the same part of the ordination but not quite paired. Ponds 3N and 3S were separated and pairing with 4N and 5N, respectively, while 4S and 5S were falling on their own. The gradation pattern was still quite disrupted, with the southern ponds all tending towards the earlier (in this case, right) side of the ordination. The highest Bray Curtis similarities were 95.176 (SPN+SPS), 94.765 (2S+3S), 94.722 (3N+4N), 94.588 (SL+SPN), and 94.075 (5N+2S). The similarity between the SL and SPS outlets was 91.955, while those between the 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 90.474, 93.387, 85.756, 81.124, and 83.245, respectively.

Finally, on Day 639 (23.02.95, 24 days post-reversal: Figure 4.53/4.63d), SPS was on its own, with SL and SPN close together and near the main group of values. Ponds 1N+1S were also close, as were 3N and 3S, although these were separated by 4S. Ponds 2N and 5N occurred close to, but on opposite sides of this group. Ponds 4N, 2S and 5S were on the outer from the group, but were nearest to their counterparts in the centre. The highest Bray Curtis similarities were 96.397 (3S+4S), 96.007 (2N+3S), 94.280 (3N+3S), 94.265 (3N+4S), and 92.856 (SPN+1S). The similarities between the SL and SPN and SPS outlets were 89.562 and 88.529, respectively, while those between the SPN+SPS, 1N+1S, 2N+2S, 4N+4S, and 5N+5S pond pairs were 80.763, 90.139, 80.763, 78.802, and 76.777, respectively.



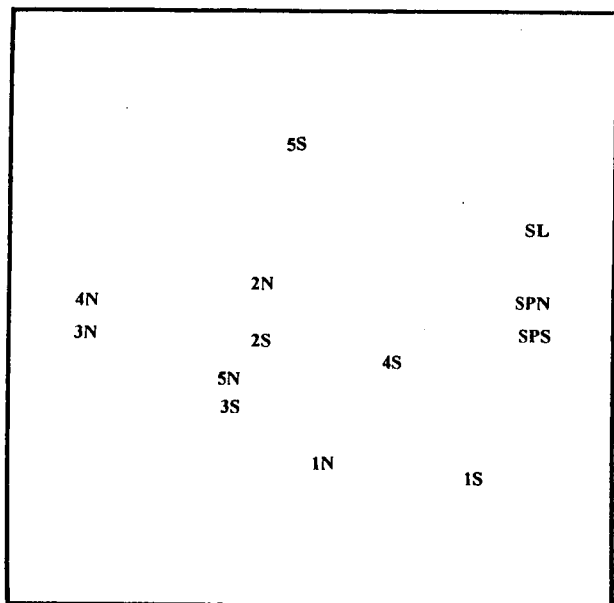
(a) Day 619: 3 February 1995  
4 days since flow change.

MAIN CRUSTACEANS Stress = 0.09



(b) Day 622: 6 February 1995  
7 days since flow change.

MAIN CRUSTACEANS Stress = 0.06



(c) Day 625: 9 February 1995  
10 days since flow change.

MAIN CRUSTACEANS Stress = 0.08

(d) Day 639: 23 February 1995  
24 days since flow change.

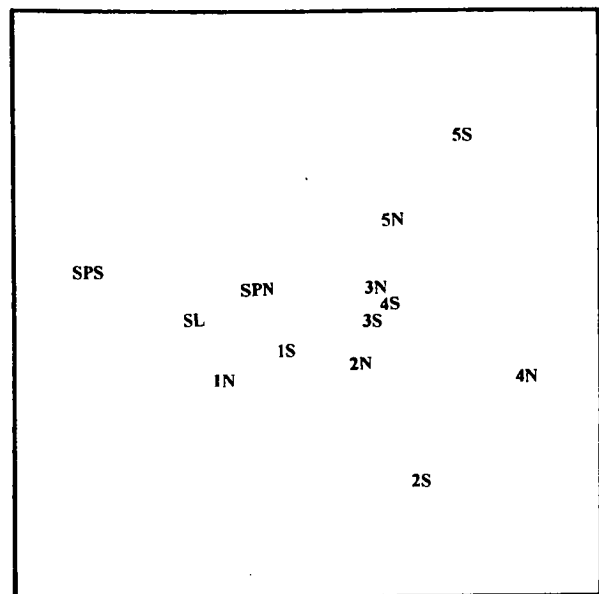


Figure 4.63 (a-d)

Crustacean MDS ordination patterns for  
the third flow division (25%N: 75%S).

**Table 4.4: Bray-Curtis similarities between paired lagoons (crustaceans).**

Bold, underlined values represent pairs among the five most similar lagoons for that day. Asterisk values represent pairs between which no Bray-Curtis value can be calculated, as crustaceans were absent from both ponds. Bold zero values represent pond pairs for which crustaceans were present in one pond but not the other.

FLOW	DAY	SPN+SPS	1N+1S	2N+2S	3N+3S	4N+4S	5N+5S
ORIGINAL	1	NA	63.945	<b><u>88.176</u></b>	76.787	NA	NA
ORIGINAL	15	NA	34.541	<b><u>87.706</u></b>	79.489	NA	NA
ORIGINAL	29	NA	61.418	90.643	89.125	NA	NA
ORIGINAL	50	NA	68.754	<b><u>86.092</u></b>	80.474	NA	NA
ORIGINAL	64	NA	51.287	<b><u>90.541</u></b>	70.996	NA	NA
EQUAL	317	NA	<b><u>94.835</u></b>	<b><u>96.684</u></b>	<b><u>93.727</u></b>	88.891	83.635
EQUAL	331	*****	88.018	85.103	90.029	<b><u>92.391</u></b>	80.387
EQUAL	345	*****	80.749	<b><u>93.943</u></b>	<b><u>95.939</u></b>	67.734	91.006
EQUAL	359	*****	<b><u>94.48</u></b>	83.965	<b><u>95.211</u></b>	70.438	75.936
EQUAL	373	*****	66.113	<b><u>95.595</u></b>	41.931	64.436	NA
EQUAL	387	*****	83.919	<b><u>91.302</u></b>	<b><u>90.11</u></b>	81.107	NA
EQUAL	401	*****	82.549	<b><u>83.411</u></b>	NA	80.867	NA
EQUAL	415	<b>0</b>	75.258	84.654	<b><u>87.721</u></b>	<b><u>98.057</u></b>	NA
EQUAL	429	*****	<b><u>88.826</u></b>	87.612	59.447	61.575	NA
EQUAL	443	*****	81.857	<b><u>89.03</u></b>	68.941	81.008	84.102
1st DIV.	457 (0)	<b>0</b>	77.351	81.509	91.654	86.331	59.276
1st DIV.	471 (14)	<b>0</b>	<b><u>81.891</u></b>	NA	70.411	63.305	42.434
1st DIV.	499 (42)	<b>0</b>	85.394	<b><u>94.285</u></b>	89.53	71.594	<b><u>93.708</u></b>
1st DIV.	513 (56)	91.357	83.755	<b><u>94.526</u></b>	82.843	89.102	80.954
1st DIV.	527 (70)	85.059	83.482	86.163	91.226	83.624	91.275
1st DIV.	541 (84)	52.11	79.553	80.18	89.143	89.436	89.292
2nd DIV.	552 (0)	91.677	84.056	89.795	85.771	85.45	79.445
2nd DIV.	562 (10)	76.267	83.02	79.162	90.537	77.599	73.747
2nd DIV.	576 (24)	83.963	77.514	82.673	85.123	86.748	90.57
2nd DIV.	590 (38)	84.509	84.231	80.882	44.228	89.224	86.065
2nd DIV.	594 (42)	89.426	73.254	94.005	84.097	91.755	86.501
2nd DIV.	604 (52)	87.568	62.793	79.529	89.596	81.007	76.932
3rd DIV.	619 (4)	87.126	89.612	86.61	88.091	72.242	80.077
3rd DIV.	622 (7)	<b><u>95.553</u></b>	85.286	76.928	65.564	83.434	75.423
3rd DIV.	625 (10)	<b><u>95.176</u></b>	90.474	93.387	85.756	81.124	83.245
3rd DIV.	639 (24)	80.763	90.139	80.763	<b><u>94.28</u></b>	78.802	76.777



### *Summary of crustacean ordination patterns and similarities*

Whether due to a less well defined response by the plankton, the forced lower degree of taxonomic resolution for the group, or more open ordinations due to crustacean absences over the equilibrium period, the MDS plots for the Crustacea do not exhibit as distinct or consistent a pattern as those for the rotifers, although some similar trends and characteristics can be seen. Ordinations from the original flow pattern reflect the initial order of ponds, with this pattern then changing markedly with the alteration of flow for the equilibration period. While the ordinations over this period generally reflect the new flow pattern, pairings are not as distinct or consistent, and Crustacea are frequently lacking from the earliest ponds. More distinct patterns are seen with the first flow division (25%N:75%S), where the southern ponds begin to precede their northern counterparts in a more consistent fashion. With the second flow division (75%N:25%S), the northern ponds tend to move forwards, although there is some variation in this pattern later in the flow period. With the third and final (and shortest) flow division (25%N:75%S), some of the southern ponds again move forwards and divide into one half of the ordination spaces, while other pairs may be moving closer together in the process of swapping over.

Similarity values between paired ponds based on crustacean data are presented in Table 4.4. Underlined values represent those ranking among the five highest (most similar) of all ponds for the system on each sampling day. As with the rotifers (end of Section 4.5.3.1), Bray-Curtis calculations have omitted ponds with no crustacean fauna (Table 4.1). Missing values have therefore been substituted according to the method outlined before, with asterisk values effectively representing 100% similarity. [Bold zero values also represent substituted estimates, and in these cases may strongly underestimate pond similarity, given the consistent absence of crustaceans from the SPN/SPS ~~lagoons~~<sup>outlets</sup> in the early portion of the table, and the continued high similarity between these ~~lagoons~~<sup>outlets</sup> in the later part of the table].

**Table 4.5: Highest Bray-Curtis similarities between lagoons (crustaceans).**

Bold, underlined values represent those between corresponding lagoon pairs.

Red values represent combinations of later southern ponds with earlier northern ponds.

Blue values represent combinations of later northern ponds with earlier southern ponds.

FLOW	DAY	HIGHEST PAIRS				
ORIGINAL	1	90.839 (2N+3N)	<b><u>88.176 (2N+2S)</u></b>	86.495 (1S+2S)	84.004 (2N+3S)	83.728 (SPS+1S)
ORIGINAL	15	94.300 (SPS+1S)	<b><u>87.706 (2N+2S)</u></b>	87.398 (2N+3N)	87.077 (3N+1S)	86.783 (1S+2S)
ORIGINAL	29	93.964 (2N+1S)	93.438 (1S+2S)	93.359 (SPS+2S)	92.602 (3N+2S)	91.755 (2N+3S)
ORIGINAL	50	92.186 (1S+2S)	90.675 (SPS+2S)	88.171 (SPS+2N)	87.475 (SPS+1S)	<b><u>86.092 (2N+2S)</u></b>
ORIGINAL	64	<b><u>90.541 (2N+2S)</u></b>	90.039 (1S+2S)	85.033 (2S+4S)	84.161 (02+1N)	83.995 (3S+4S)
EQUAL	317	<b><u>96.684 (2N+2S)</u></b>	95.370 (3N+2S)	<b><u>94.835 (1N+1S)</u></b>	93.983 (2N+3N)	<b><u>93.727 (3N+3S)</u></b>
EQUAL	331	95.973 (1S+2S)	<b><u>92.391 (4N+4S)</u></b>	92.024 (2S+3S)	91.921 (4N+5S)	91.766 (3S+5S)
EQUAL	345	<b><u>95.939 (3N+3S)</u></b>	<b><u>93.943 (2N+2S)</u></b>	91.990 (2S+3S)	91.563 (2N+3N)	91.317 (3N+2S)
EQUAL	359	95.817 (1N+2S)	<b><u>95.211 (3N+3S)</u></b>	<b><u>94.480 (1N+1S)</u></b>	93.742 (4N+5N)	93.664 (2N+3S)
EQUAL	373	<b><u>95.595 (2N+2S)</u></b>	90.704 (4N+5N)	86.848 (1N+2N)	84.685 (1N+2S)	82.261 (2N+4S)
EQUAL	387	95.344 (2N+3N)	<b><u>91.302 (2N+2S)</u></b>	90.193 (3N+4N)	90.114 (1S+3S)	<b><u>90.110 (3N+3S)</u></b>
EQUAL	401	91.441 (2N+1S)	89.951 (1N+2S)	89.543 (1S+2S)	84.937 (1N+2N)	<b><u>83.411 (2N+2S)</u></b>
EQUAL	415	<b><u>98.057 (4N+4S)</u></b>	91.993 (2N+1S)	<b><u>87.721 (3N+3S)</u></b>	86.690 (1N+3N)	84.999 (1N+2N)
EQUAL	429	94.709 (1N+2S)	90.068 (3N+4S)	89.300 (1S+2S)	<b><u>88.826 (1N+1S)</u></b>	87.663 (1N+2N)
EQUAL	443	95.481 (3N+2S)	<b><u>89.030 (2N+2S)</u></b>	88.799 (4N+1S)	88.175 (4N+1N)	84.998 (2N+1S)
1st DIV.	457 (0)	100.00 (SL+SPN)	93.934 (4N+5S)	93.595 (3N+5S)	93.466 (1N+5S)	92.579 (3N+4N)
1st DIV.	471 (14)	86.830 (3N+4N)	86.346 (1N+5S)	85.097 (4N+3S)	<b><u>81.891 (1N+1S)</u></b>	80.930 (3S+5S)
1st DIV.	499 (42)	96.130 (2N+3S)	<b><u>94.285 (2N+2S)</u></b>	<b><u>93.708 (5N+5S)</u></b>	92.955 (2S+3S)	91.431 (2N+3N)
1st DIV.	513 (56)	<b><u>94.526 (2N+2S)</u></b>	94.216 (1S+2S)	93.745 (5N+4S)	92.594 (2N+1S)	91.964 (3S+4S)
1st DIV.	527 (70)	94.662 (1N+3N)	94.438 (3N+4N)	93.803 (1N+2N)	93.105 (2N+4N)	92.506 (2N+3N)
1st DIV.	541 (84)	96.786 (4N+5S)	92.831 (2S+3S)	92.809 (1N+2S)	91.842 (2N+3N)	91.496 (1N+3S)
2nd DIV.	552 (0)	99.468 (SL+SPN)	93.315 (4N+5N)	93.211 (2N+4S)	91.723 (2N+3S)	91.663 (1N+5N)
2nd DIV.	562 (10)	98.969 (2S+3S)	93.652 (2S+5S)	93.489 (4N+5N)	92.696 (3S+5S)	92.353 (2N+5N)
2nd DIV.	576 (24)	95.710 (2N+1S)	94.922 (3N+5N)	93.475 (4S+5S)	93.474 (3N+4S)	91.830 (3N+5S)
2nd DIV.	590 (38)	97.170 (2S+5S)	93.737 (4S+5S)	93.548 (4N+2S)	93.175 (4N+5S)	91.925 (2S+4S)
2nd DIV.	594 (42)	96.073 (2S+4S)	95.841 (2N+4N)	95.482 (1N+2S)	95.333 (3N+2S)	94.724 (4N+2S)
2nd DIV.	604 (52)	96.743 (3N+5N)	96.691 (3S+4S)	96.123 (2N+3N)	95.003 (5N+4S)	94.521 (2N+5N)
3rd DIV.	619 (4)	98.744 (3N+4N)	97.640 (2N+4N)	97.167 (1N+5S)	96.965 (SL+SPN)	96.655 (2S+3S)
3rd DIV.	622 (7)	95.876 (1N+4N)	95.835 (4N+5N)	<b><u>95.553 (SPN+SPS)</u></b>	95.378 (2S+4S)	95.106 (1N+5N)
3rd DIV.	625 (10)	<b><u>95.176 (SPN+SPS)</u></b>	94.765 (2S+3S)	94.722 (3N+4N)	94.588 (SL+SPN)	94.075 (5N+2S)
3rd DIV.	639 (24)	96.397 (3S+4S)	96.007 (2N+3S)	<b><u>94.280 (3N+3S)</u></b>	94.265 (3N+4S)	92.856 (SPN+1S)

Table 4.4 shows that corresponding ponds were more consistently among the highest similarities for the 85wB system during the equilibrium period than during the three flow divisions. The SPN and SPS outlets showed a high degree of similarity throughout the project, particularly during the equilibrium period when Crustacea were completely absent. Similarity for these outlets was effectively lower over the different flow divisions, but passed through a transient stage of high similarity early in the third division. As with the rotifers, 1N+1S were relatively dissimilar under the original flow regime, but similarity increased during the equilibration period and remained high (but less frequently among the highest) for the three flow divisions. Ponds 2N+2S were consistently among the highest similarity values throughout the original flow and equilibration periods, but ceased to be among them after the middle of the first flow division, while relative similarity for 3N+3S increased during the equilibration period and decreased after it. The later ponds were rarely among the highest similarity values, but varied both within and between different flow periods.

Table 4.5 lists the highest crustacean similarities between all lagoons on each sampling day, with underlined values representing corresponding pond pairs, red values representing 'out of phase' pairings between later southern ponds and earlier northern ponds, and blue values representing 'out of phase' pairings between later northern ponds and earlier southern ponds (these combinations include pairings with the SL pond).

As can be seen, pairings between corresponding ponds were most prevalent (if not quite dominant) during the equilibrium period. [These pairings would become more dominant with the inclusion of 100% similarity values for the SPN/SPS pond pair as per Table 4.4]. Pairings between corresponding ponds became markedly less prevalent during the three divisions in flow, and were completely absent during the second division. Mixed pairings between earlier/later north/south ponds followed a less distinct pattern than for the rotifers (Table 4.3), but finished with late S/early N pond combinations at the end of the first division (75%S). These persisted into the second division (75%N) before belatedly developing into late N/early S combinations, which carried over into the third (75%S). At the end of this period, pairings were predominantly late S/early N.

#### 4.5.3.3 - Combined rotifer and crustacean MDS ordinations

##### *Original flow pattern - single flow system (all main groups)*

The four main rotifer and four main crustacean groups were also analysed together to give an indication not only of what was happening in the separate rotifer and crustacean assemblages, but also to see what patterns were occurring across the whole zooplankton community. It was intended that the resulting plots would not just be a combination of the previous patterns, but may both clarify them and balance out the absences that occurred among sites for the individual groups. Patterns were expected to show greater distinction and discrimination between similar crustacean assemblages occurring with differing rotifer communities, and *vice versa*, and would also help compensate for the effect of missing taxa in measuring similarity.

Stress values for the total plots over the original flow period ranged from 0.01 to 0.04 (average 0.018, standard error 0.0065), which again fall well within the ideal range for stress values previously identified. On Day 1 (26.05.93: Figure 4.23/4.64a), both the SL pond and the original pond 2 fell on their own, very separate to each other and the other lagoons. Ponds 1N, SPS, and 1S fell closer together, followed by a linear grouping of 2S, 2N, 3N and 3S. As before, the original flow pattern of the lagoons was reflected in a circular/serpentine pattern, running SL, 2, 1N, SPS, 1S, 2S, 2N, 3N and 3S. The highest Bray Curtis percentage similarities between lagoons were 89.397 (2N+2S), 86.510 (2N+3N), 80.910 (3N+3S), 80.601 (2N+3S), and 80.463 (SPS+1S). The similarity between the SL and SPS outlets was only 41.942, while between 1N+1S it was only 60.236.

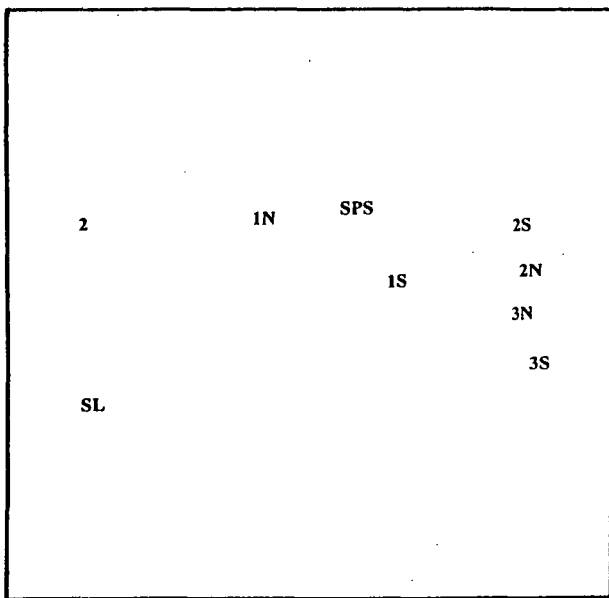
On Day 15 (09.06.93: Figure 4.24/4.64b), an almost identical pattern was seen (albeit reversed left to right in the ordination orientation). Pond 1N was closer to pond 2 and more separate from SPS/1S, while a slightly greater distance separated 3S from 3N. The highest Bray Curtis similarities between lagoons were 87.883 (SPS+1S), 86.714 (2N+2S), 82.988 (2N+3N), 79.673 (3N+2S), and 78.833 (3N+3S). The similarity between the SL and SPS outlets was only 37.602, while

between 1N+1S it was only 40.175.

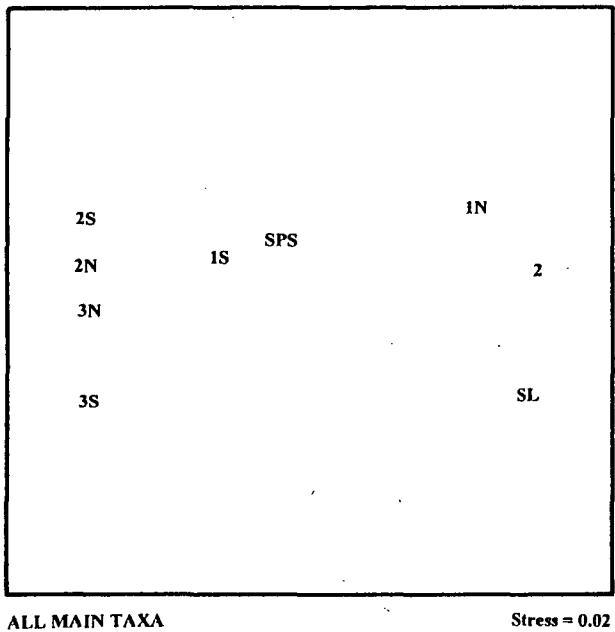
On day 29 (23.06.93: Figure 4.25/4.64c), the same pattern remained intact, with only minor variations. Pond 2 was now closer to SL (both absent from the crustacean plot), while 1N had moved back towards SPS. Pond 1S still fell between, and was close to, SPS and 2S, while 3N+3S (absent from the rotifer plot) were now pairing closely. The highest Bray Curtis similarities between lagoons were 89.125 (3N+3S), 87.569 (SL+2), 85.560 (2N+3S), 84.910 (2N+2S), and 84.878 (1S+2S). The similarity between the SL and SPS outlets was 42.581, while between 1N+1S it was 57.307.

On Day 50 (14.07.93: Figure 4.26/4.64d), the serpentine pattern was more sharply cornered, but still following the same trend. Pond 1N was still falling away from the linear group of SPS/1S/2S, although it was closer to this than to either SL or 2. The inclusion of 4S showed it to impose between the 3N and 3S ponds, although this could also be interpreted as another turn in the serpentine pattern. The highest Bray Curtis similarities between lagoons were 85.382 (1S+2S), 84.486 (SPS+1S), 83.874 (SPS+2S), 83.007 (2N+2S), and 79.394 (2N+3N). The similarity between the SL and SPS outlets was 46.406, while between 1N+1S and 3N+3S it was 68.591 and 72.796, respectively.

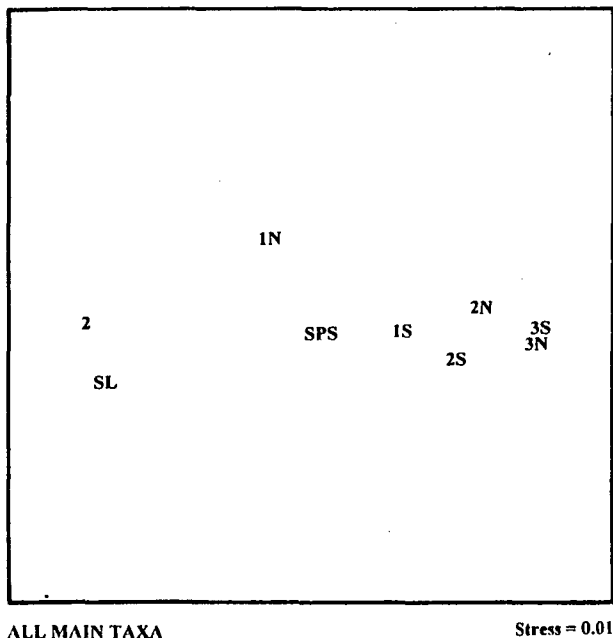
On the final sampling day of the original flow period (Day 64, 28.07.93: Figure 4.27/4.64e), a similar pattern was maintained, although more arced than serpentine. Pond 1N was again drawing away towards SL and 2, while 2S+2N were grouping closely and 4S was again interposing between 3N and 3S. The only major change was that pond 3N, rather than 3S, was now the pond falling away from the remaining ponds at the end of the ordination. The highest Bray Curtis similarities between lagoons were 90.541 (2N+2S), 85.033 (2S+4S), 84.388 (1S+2S), 83.995 (3S+4S), and 81.966 (2N+4S). The similarity between the SL and SPS outlets was only 26.934, while between 1N+1S and 3N+3S it was 50.290 and 70.996, respectively.



No SPN, 4N, 4S, 5N, 5S samples at this stage.



No SPN, 4N, 4S, 5N, 5S samples at this stage.

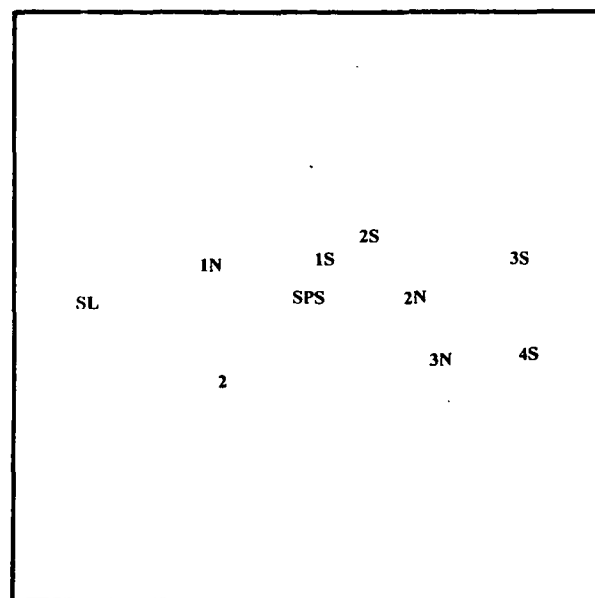


No SPN, 4N, 4S, 5N, 5S samples at this stage.

ALL (D)

**(d) Day 50: 14 July 1993**

No SPN, 4N, 5N, 5S samples at this stage.

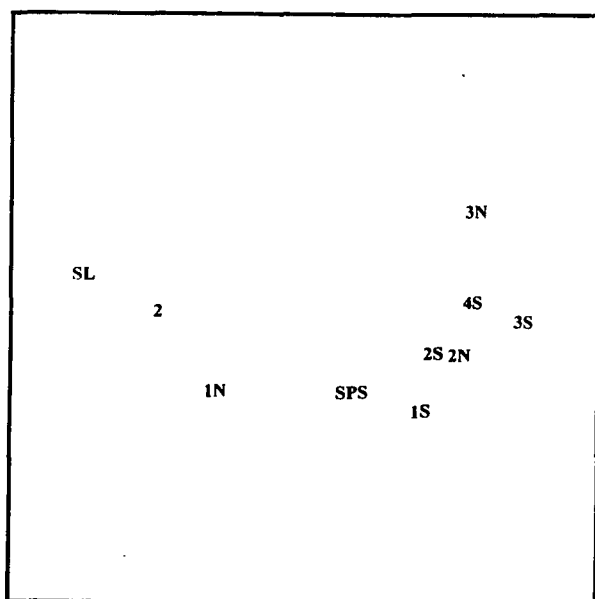


ALL MAIN TAXA

Stress = 0.04

**(e) Day 64: 28 July 1993**

No SPN, 4N, 5N, 5S samples at this stage.



ALL MAIN TAXA

Stress = 0.01

**Figure 4.64 (a-e)**

**All main zooplankton genera/group  
MDS ordination patterns for the  
original (pre-alteration) flow period.**

*Equal flow pattern - 50%N, 50%S (all main groups)*

Stress values for the total group plots over the equal flow period ranged from 0.01 to 0.08 (average 0.045, standard error 0.006). These values still fall well within the acceptable range, and, as with both the rotifer and crustacean plots, the increase in these values would largely represent the increased number of available sites and their effect on the complexity of the ordination plots.

On Day 317 (07.04.94: Figure 4.28/4.65a), the SL and SPN ponds fell well away from the remaining lagoons (no SPS sample was available). Pairings 1-5 were good, reflecting the new flow pattern and the equal flow across the two halves. Within these pairs, the largest gap appeared between 4N+4S (with 4N falling away from the main group), while pair 5 (particularly 5S) broke with the general gradation from earlier to later ponds across the ordination. The highest Bray Curtis similarities between lagoons were 94.418 (2N+2S), 86.409 (1N+1S), 86.401 (2N+1S), 86.089 (3N+2S), and 85.302 (3N+3S). The similarity between SL and SPN was 84.207, while similarities between the remaining pairs 4N+4S and 5N+5S were 74.009 and 79.445, respectively.

On Day 331 (21.04.94: Figure 4.29/4.65b), the general gradation from earlier to later ponds remained, although the pairings were not as good. SPN+SPS paired closely, and with SL fell well away from the other lagoons. Pond 1N broke from 1S and the rest of following lagoons to fall midway between these and the earlier ponds. Ponds 2N and 2S were separated by 3N and 3S, and although the latter ponds remained close they paired closer to 1S and 2S respectively. Ponds 4N and 4S now paired closely, and fell between 5N and 5S, with 5S again falling further forwards in the gradation than would be expected. The highest Bray Curtis similarities between lagoons were 96.974 (SPN+SPS), 86.705 (4N+4S), 85.764 (3N+1S), 83.865 (1S+2S), and 83.111 (2S+3S). The similarities between SL and the SPN and SPS outlets were 81.213 and 82.224, respectively, while between 1N+1S, 2N+2S, 3N+3S, and 5N+5S they were 67.878, 80.606, 82.666, and 67.557, respectively.

On Day 345 (05.05.94: Figure 4.30/4.65c), the pairings were far more distinct, and clearly refined from both the rotifer and crustacean



plots. The ordination fell into the typical serpentine gradation from earlier to later ponds, and pairs SPN+SPS, 1N+1S, 2N+2S, 3N+3S, and 5N+5S fell into obvious and well matched pairs. Only ponds 4N and 4S failed to match each other, and were instead separated by pair 5. The highest Bray Curtis similarities were 87.874 (SPN+SPS), 86.032 (2N+2S), 85.897 (5N+5S), 85.378 (3N+3S), and 84.506 (2N+3N). The similarities between SL and the SPN and SPS outlets were 59.922 and 63.622, respectively, while between 1N+1S and 4N+4S they were 78.959 and 58.155, respectively. Notably, ponds 4S and 5S, which were previously identical in rotifer assemblages (100.000), were now put into context for the overall rotifer/crustacean community (78.797).

The grading pattern and pairings remained good for Day 359 (19.05.94: Figure 4.31/4.65d), with SPN+SPS, 1N+1S, and 3N+3S all remaining clearly matched. Ponds 2N and 2S were relatively close but were split by 3N, while 4N and 5N fell together and separate from their corresponding numbers. The highest Bray Curtis similarities were 89.749 (SPN+SPS), 89.899 (3N+2S), 89.031 (3N+3S), 86.742 (4N+5N), and 84.355 (1N+1S). The similarities between SL and the SPN and SPS outlets were 62.634 and 71.805, respectively, while between 2N+2S, 4N+4S, and 5N+5S they were 75.908, 57.392, and 58.490, respectively.

For Day 373 (02.06.94: Figure 4.32/4.65e), the gradation and early pairings were good (SPN+SPS, 1N+1S, 2N+2S), although 3N, 4N and 5N were grouping tightly to the exclusion of 3S and 4S. Pond 3S fell well away from all other ponds (5S was absent from this and the following four sample dates due to MW works). The highest Bray Curtis similarities were 88.107 (SPN+SPS), 87.563 (4N+5N), 80.179 (2N+2S), 73.592 (1N+1S), and 73.203 (3N+4N). The similarities between SL and the SPN and SPS outlets were 65.559 and 72.030, respectively, while between 3N+3S and 4N+4S they were only 35.647 and 62.784, respectively.

On Day 387 (16.06.94: Figure 4.33/4.65f), the gradation from early to later ponds was as consistent as ever, while pairings between lagoons were also strong. Pairs SPN+SPS, 1N+1S, 2N+2S, and 3N+3S were all very distinct. Ponds 4N and 4S were relatively close to each other, although tending more towards 3N and 5N, respectively. The

highest Bray Curtis similarities were 96.861 (SPN+SPS), 94.157 (2N+2S), 93.656 (3N+3S), 90.120 (3N+4N), and 86.578 (4N+3S). The similarities between SL and the SPN and SPS outlets were 50.382 and 52.612, respectively, while between 1N+1S and 4N+4S they were 83.938 and 78.097, respectively.

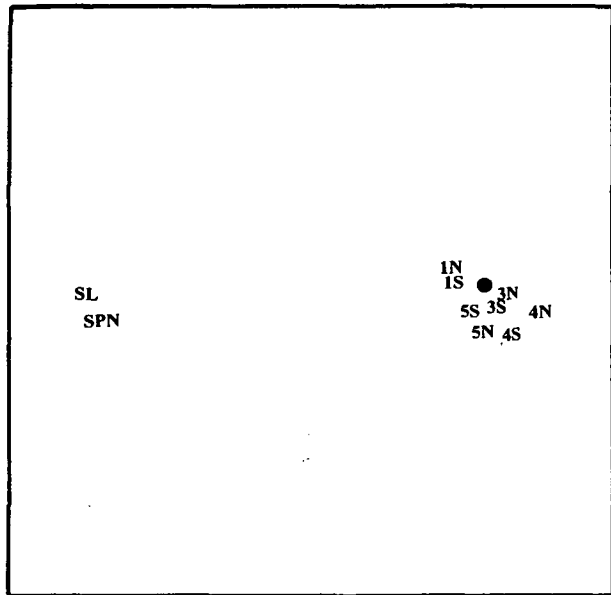
On Day 401 (30.06.94: Figure 4.34/4.65g), the gradation remained in place, again appearing in a serpentine fashion. Pairings SP, 1, and 2 remained good, even though 1N and 2S appeared to fall closer to each other than to their partners (1N+1S remained slightly closer in terms of similarity percentages, however). Sample 3N was not available for comparison to 3S, while, although not paired, 4N and 4S fell in the same region of the ordination. The highest Bray Curtis similarities were 84.205 (1N+1S), 83.229 (1N+2S), 79.169 (2N+2S), 79.136 (1S+2S), and 77.240 (SPN+SPS). The similarities between the SL and the SPN and SPS outlets were 45.032 and 57.819, respectively, while the similarity between 4N+4S was 68.650.

On Day 415 (14.07.94: Figure 4.35/4.65h), gradation followed a more circular than serpentine fashion, but still ran from earlier to later ponds in a clear pattern. Pairings SPN+SPS, 1N+1S, 2N+2S, 3N+3S, and 4N+4S were all excellent, while 5N was still missing its partner due to the MW works. Not surprisingly, the highest Bray Curtis similarities all correspond to the above pairs, with 90.469 (SPN+SPS), 88.521 (1N+1S), 86.820 (2N+2S), 86.328 (4N+4S), and 84.821 (3N+3S). The similarities between SL and SPN and SPS were 53.155 and 52.180, respectively.

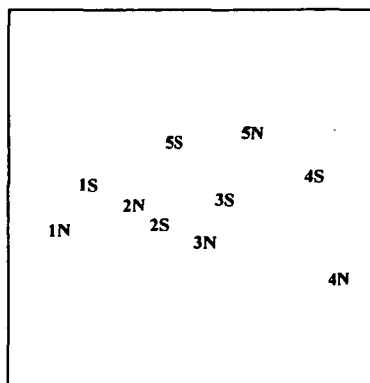
On Day 429 (28.07.94: Figure 4.36/4.65i), the gradation remained semi-circular, with SL out on its own, SPN+SPS and 1N+1S pairing well, 2N+2S close to each other, and 3N and 3S slightly apart with 3N close to 4S and 5N. Pair 4 was separated, with 4N an outlier at the tail of the ordination pattern. The highest Bray Curtis similarities were 97.471 (SPN+SPS), 93.083 (1N+1S), 89.197 (3N+4S), 87.221 (2N+2S), and 77.727 (5N+4S). The similarities between SL and SPN and SPS were 26.451 and 26.948, respectively, while those between 3N+3S and 4N+4S were 64.230 and 46.291, respectively.

For the final sampling day of the equilibrium period, (Day

443, 11.08.94: Figure 4.37/4.65j), the gradation remained in a semi-circular fashion, and pairings remained very good. SPN+SPS and 1N+1S remained clearly paired, while the renewed availability of 5S showed it to pair closely to 5N. Ponds 2N and 2S fell quite close, with 3N nearby (but not intruding between or before them in the gradation). Ponds 3N+3S and 4N+4S had no other ponds falling between them, but 3S and 4N paired with each other much more closely than with their corresponding partners. The highest Bray Curtis similarities were 91.044 (SPN+SPS), 88.508 (1N+1S), 83.979 (4N+3S), 83.670 (5N+5S), and 83.471 (2N+2S). The similarities between SL and the SPN and SPS outlets were 67.578 and 75.832, respectively, while those between 3N+3S and 4N+4S were 71.855 and 68.989, respectively.

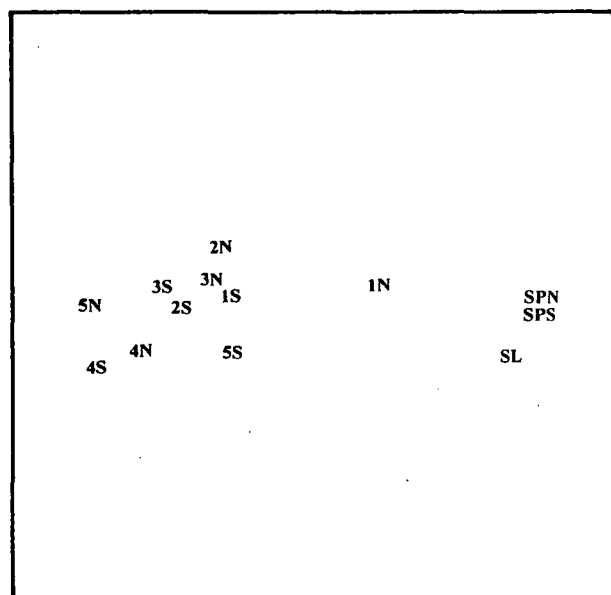


ALL MAIN TAXA      ● = 2N+2S      Stress = 0.01



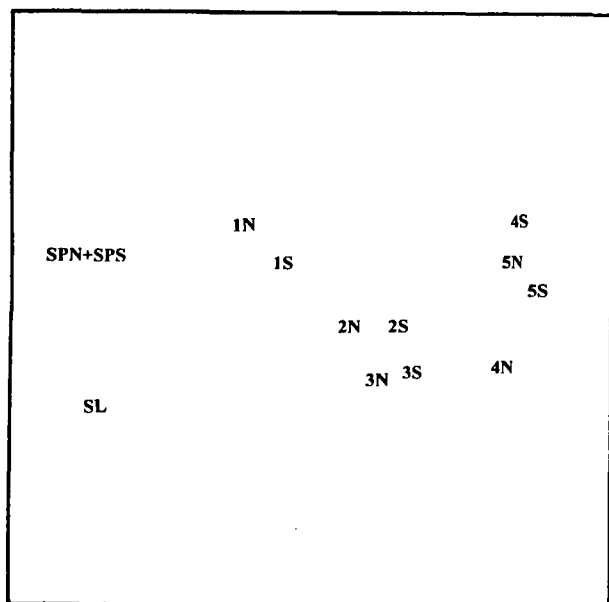
ALL MAIN TAXA  
SL, SPN removed

(a) Day 317: 7 April 1994  
No SPS sample (preservation problem).



ALL MAIN TAXA      Stress = 0.04

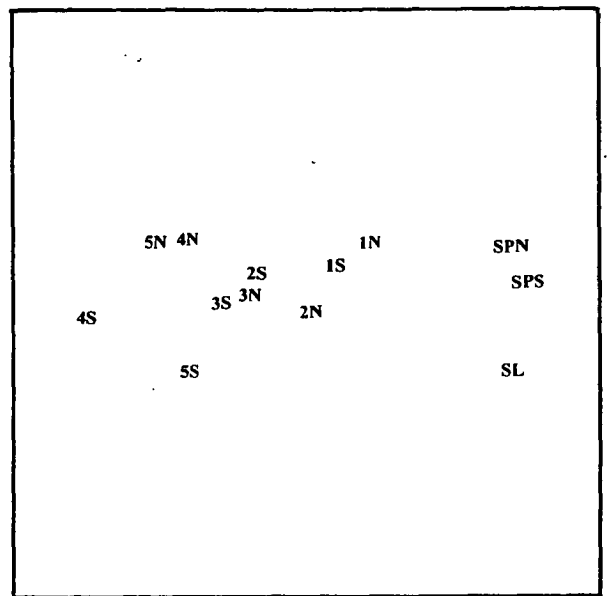
(b) Day 331: 21 April 1994



ALL MAIN TAXA      Stress = 0.05

(c) Day 345: 5 May 1994

(d) Day 359: 19 May 1994

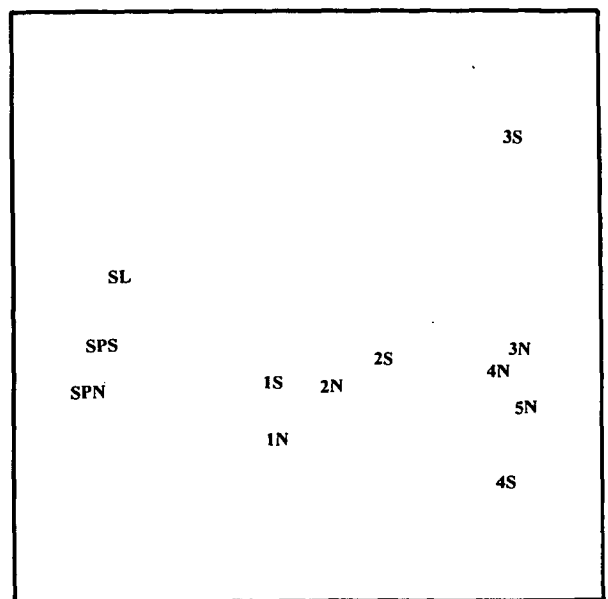


ALL MAIN TAXA

Stress = 0.03

(e) Day 373: 2 June 1994

No 5S sample (pond cut out, MW works).

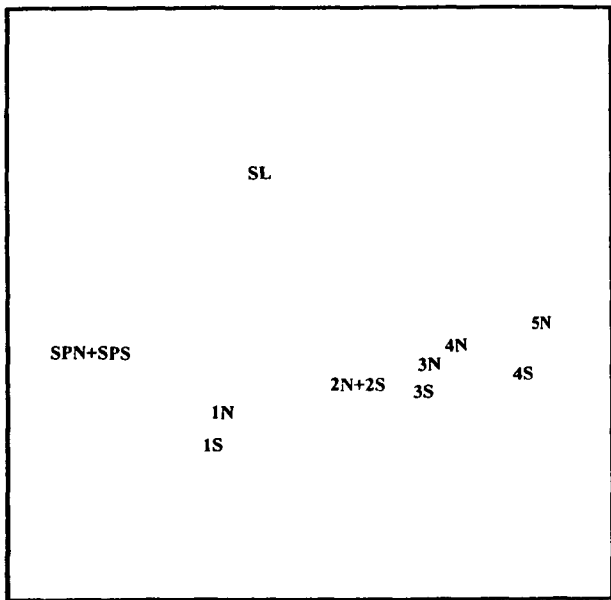


ALL MAIN TAXA

Stress = 0.05

Figure 4.65 (a-e) *continued overleaf*

All main zooplankton genera/group  
MDS ordination patterns for the equal  
flow period (50%N: 50%S).

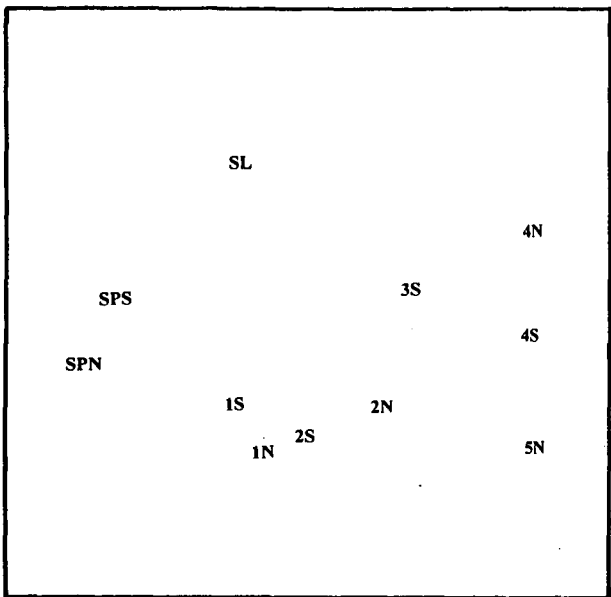


(f) Day 387: 16 June 1994

No 5S sample (pond cut out, MW works).

ALL MAIN TAXA

Stress = 0.03



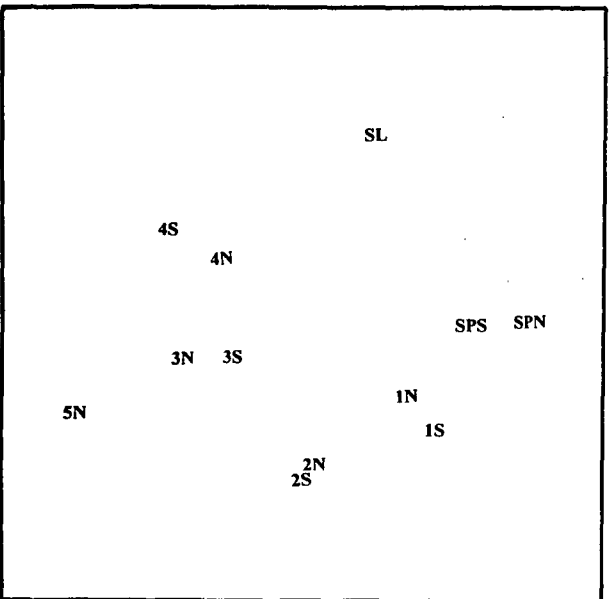
(g) Day 401: 30 June 1994

No 3N sample (preservation problem).

No 5S sample (pond cut out, MW works).

ALL MAIN TAXA

Stress = 0.08



(h) Day 415: 14 July 1994

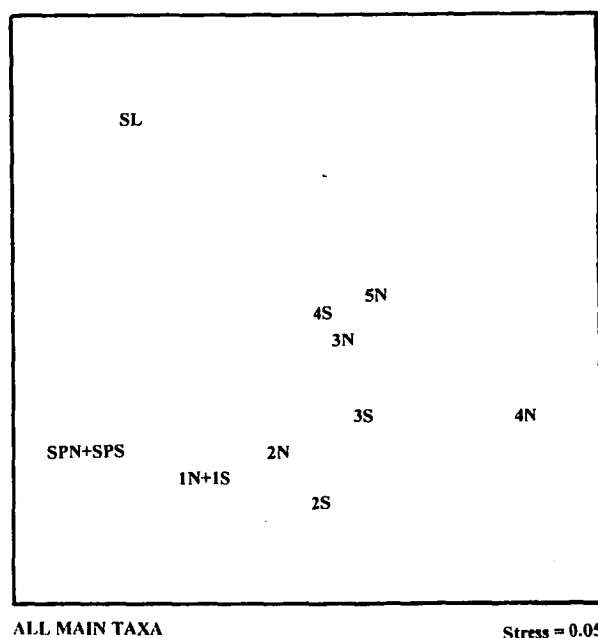
No 5S sample (pond cut out, MW works).

ALL MAIN TAXA

Stress = 0.05

A  
L  
L  
(S)

(i) Day 429: 28 July 1994  
No 5S sample (pond cut out, MW works).



(j) Day 443: 11 August 1994

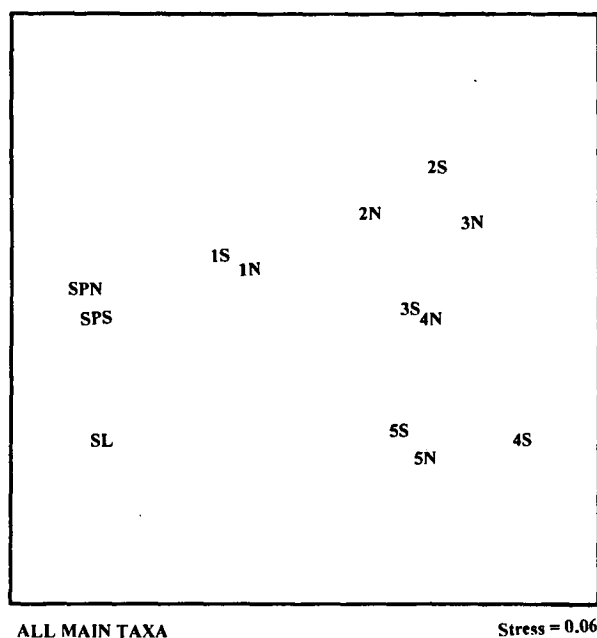


Figure 4.65 (f-j) *cont. from previous page*

All main zooplankton genera/group  
MDS ordination patterns for the equal  
flow period (50%N: 50%S).

*First flow division - 25%N, 75%S (all main groups)*

Stress values for the total plots over the first flow division ranged from 0.02 to 0.07 (average 0.038, standard error 0.008), again well within the acceptable range, and not greatly different to the average of the equilibrium period.

On Day 457 (25.08.94: Figure 4.38/4.66a), the day of flow change, the ordination pattern remained similar to those for the equilibrium period, with a clear circular gradation from earlier to later ponds and good pairings for SPN+SPS, 1N+1S, 2N+2S, and 3N+3S. Pairings remained variable for the later lagoons, however, with 4N and 5S falling together and 4S and 5N out on their own. It should be noted that the 1N+1S pair fell on its own half way between the earlier ponds and the main group of the later ponds, as was seen throughout the equilibration period. The highest Bray Curtis similarities were 93.317 (3N+4N), 91.873 (3N+3S), 91.562 (4N+3S), 91.422 (3N+5S), and 92.416 (4N+5S). The similarities between the SL and SPN and SPS outlets were 69.339 and 57.309, respectively, while those between the SPN+SPS, 1N+1S, 2N+2S, 4N+4S, and 5N+5S pond pairs were 87.497, 88.230, 82.126, 78.134, and 73.249, respectively.

On Day 471 (08.09.94: Figure 4.39/4.66b), 14 days after the change of flow, the SPN+SPS and 1N+1S pairs remained intact, but with SPS falling on the SL side and 1S falling on the SP side of their respective pairs. No 2S sample was available, 3N+3S remained paired, while 4S and 5S were separated from their corresponding northern lagoons and appeared to fall towards the earlier side of the gradation. The highest Bray Curtis similarities were 83.438 (1N+1S), 80.714 (SPN+SPS), 78.360 (4N+3S), 74.642 (SPS+1S), and 73.872 (4S+5S). The similarities between the SL and SPN and SPS outlets were 62.254 and 69.627, respectively, while those between the 3N+3S, 4N+4S, and 5N+5S pond pairs were 64.187, 47.099, and 26.701, respectively.

By Day 499 (6.10.94: Figure 4.40/4.66c), 42 days after the division in flow, the subtle changes present in the previous ordination were becoming slightly more pronounced. The SP pair were more separate than before, with SPS (high flow) moving further towards the SL pond. Ponds 1N+1S remained paired, with 1S on the SP side of the gradation. Ponds 2N+2S had split, with 2S moving closer to pair 1, while 3S (although still close to its pair) was moving closer to 2N and 4S was moving closer to 3N. Ponds 5N+5S were paired, although 5S

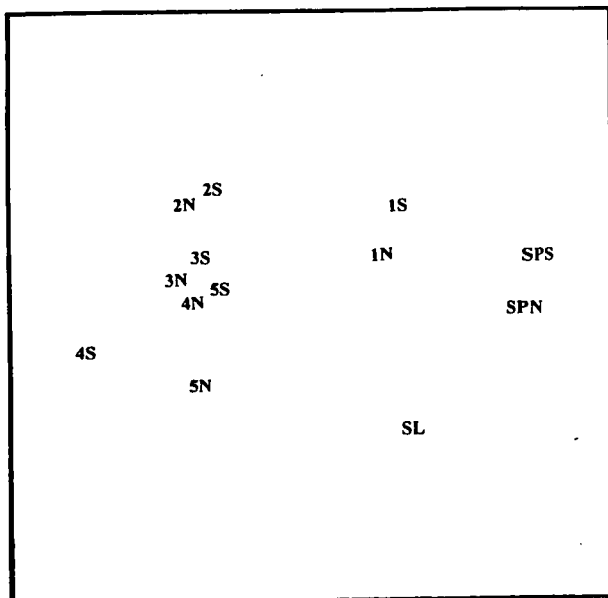


also fell earlier in the circular gradation than its partner. The highest Bray Curtis similarities were 93.277 (2N+3S), 91.942 (5N+5S), 90.205 (3N+4S), 89.636 (1N+1S), and 87.628 (3N+3S). The similarities between the SL and SPN and SPS outlets were 55.474 and 71.103, respectively, while those between the SPN+SPS, 2N+2S, and 4N+4S pond pairs were 82.062, 86.229, and 72.886 respectively.

On Day 513 (20.10.94, 56 days since the flow change: Figure 4.41/4.66d), SPS still fell on the SL side of its pair, while 1S was now well out on its own and falling half way between the earlier ponds and 1N. 'Out of sync' pairings had developed between 1N+2S, 2N+3S, and 4S+3N, with relative flow levels between the sides now appearing to impose themselves over pond order. Ponds 4N+5N paired towards the tail of the gradation, with 5S falling last. The highest Bray Curtis similarities were 93.024 (2N+3S), 90.791 (SPN+SPS), 89.536 (1N+2S), 88.890 (2N+2S), and 88.515 (4N+5N). The similarities between the SL and SPN and SPS outlets were 77.409 and 80.814, respectively, while those between the 1N+1S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 72.665, 76.667, 82.421, and 82.355, respectively.

On Day 527 (03.11.94, 70 days since the change: Figure 4.42/4.66e), SPS remained between SL and SPN in a tight group. Pond 1S still fell away from 1N and the later ponds, 2S fell between 1N and 2N, and 3S fell level with 2N. Ponds 4S and 5S followed closely behind and 3N in the gradation, while 5N and 4N fell at its tail. The highest Bray Curtis similarities were 93.232 (SPN+SPS), 90.709 (3N+5S), 90.129 (4S+5S), 89.382 (2N+3S), and 87.816 (SL+SPS). The similarity between the SL and SPN was 86.941, while those between the 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 82.506, 84.739, 85.723, 73.271, and 84.891, respectively.

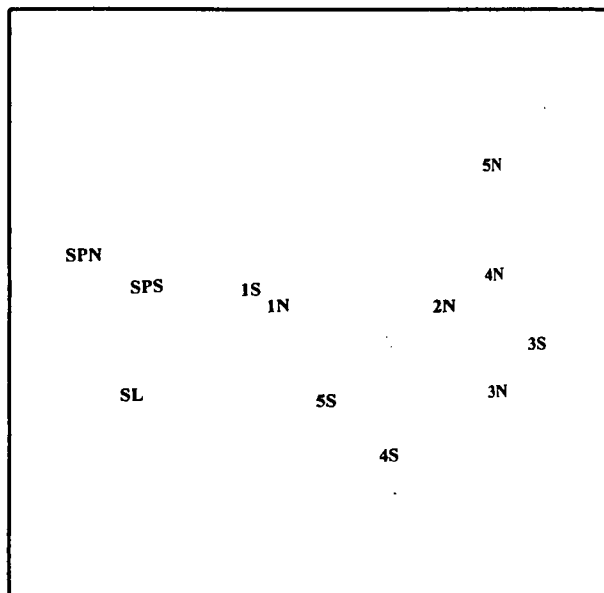
For the final sample of the first flow division (Day 541, 17.11.94, 84 days post-change: Figure 4.43/4.66f), SPS had fallen back to the non-SL side of SPN, although 1S remained half-way between 1N and the SP ponds, 2S paired with 1N, 5S paired with 4N, and 3S and 4S fell forwards of their northern partners in the gradation. The highest Bray Curtis similarities were 89.584 (4N+5S), 89.164 (2N+3N), 87.343 (1N+2S), 83.158 (5N+5S), and 81.997 (1N+1S). The similarities between the SL and SPN and SPS outlets were 61.946 and 53.294, respectively, while those between the SPN+SPS, 2N+2S, 3N+3S, and 4N+4S pond pairs were 79.977, 71.894, 75.747, and 81.199, respectively.



(a) Day 457: 25 August 1994  
0 days since flow change.

ALL MAIN TAXA

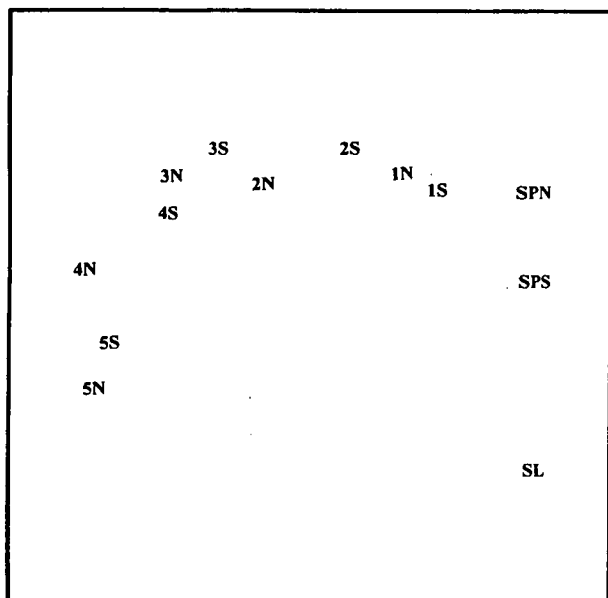
Stress = 0.04



(b) Day 471: 8 September 1994  
14 days since flow change.  
No 2S sample (preservation problem).

ALL MAIN TAXA

Stress = 0.07

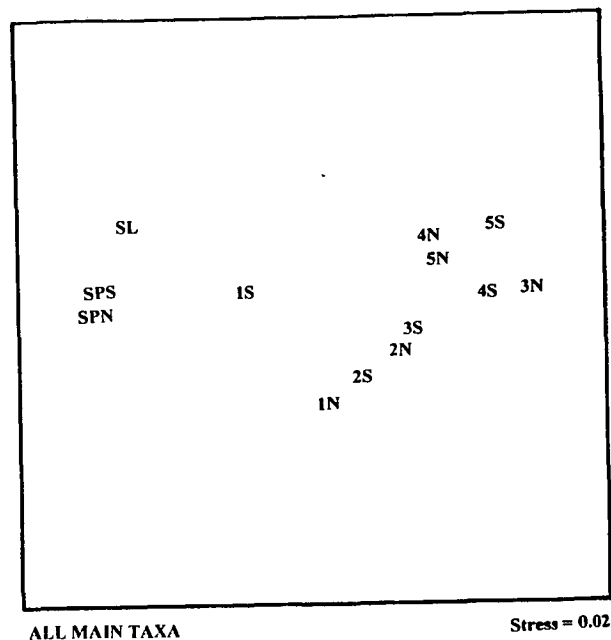


(c) Day 499: 6 October 1994  
42 days since flow change.

ALL MAIN TAXA

Stress = 0.03

(d) Day 513: 20 October 1994  
56 days since flow change.



(e) Day 527: 3 November 1994  
70 days since flow change.

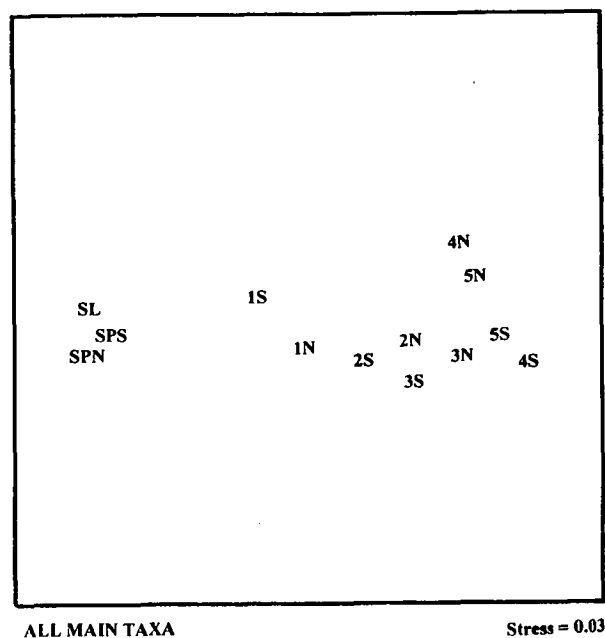
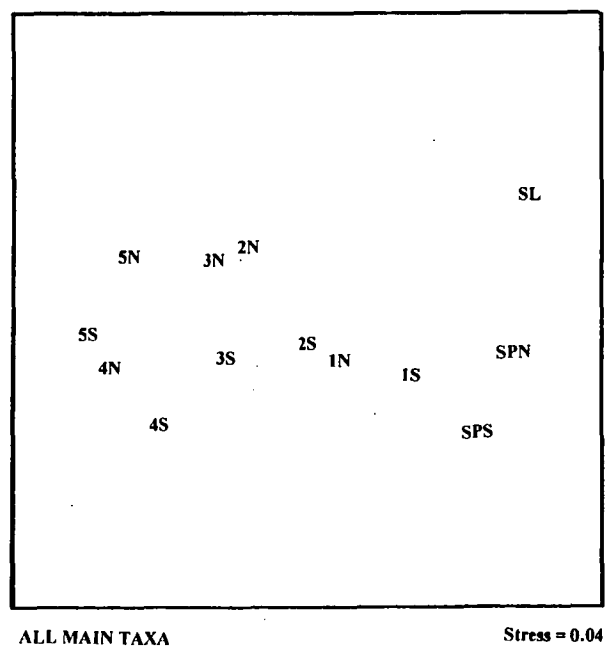


Figure 4.66 (a-f)

All main zooplankton genera/group  
MDS ordination patterns for the first  
flow division (25%N: 75%S).

(f) Day 541: 17 November 1994  
84 days since flow change.



*Second flow division - 75%N, 25%S (all main groups)*

Stress values for the total plots over the second flow division ranged from 0.03 to 0.08 (average 0.05, standard error 0.009). These values remain well within the acceptable range, and only marginally higher than for the equilibrium period and the first flow division. The highest stress value (0.08) did, however, occur at the end of this period (Day 604), when the 75%N:25%S flow regime had been established for the longest period.

With the reversal of flow on Day 552 (28.11.94: Figure 4.44/4.67a), the ordination pattern remained much as it had been for the previous flow division. SPN+SPS paired close to SL, while 1S fell towards the earlier ponds and away from 1N. Pond 2S fell nearest to 1N, and 3S, 4S and 5S fell close to 2N, leaving the remaining northern ponds to fall at the periphery of the main group of ponds. The highest Bray Curtis similarities were 91.051 (SPN+SPS), 90.804 (3S+4S), 87.571 (2N+4S), 86.395 (4N+5N), and 86.250 (4S+5S). The similarities between the SL and the SPN and SPS outlets were 84.368 and 80.307, respectively, while those between the 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 75.830, 79.121, 79.837, 78.781, and 73.576, respectively.

By Day 562 (08.12.94, 10 days since the change: Figure 4.45/4.67b), SPN was moving closer to the SL pond, while 1N was beginning to edge out in front of 1S in the gradation from earlier to later ponds (1N and 1S were effectively pairing again). Pond 2N was now well in front of 2S, while 4N and 5N, still on the outer of the main group, were also preceding their corresponding southern ponds. Pond 3N remained grouped with the bulk of the southern ponds towards the end of the ordination. The highest Bray Curtis similarities were 90.476 (3N+4S), 89.575 (4S+5S), 87.792 (SL+SPN), 86.800 (SL+SPS), and 85.570 (SPN+SPS). The similarities between the 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 80.479, 67.118, 83.323, 72.259, and 69.488, respectively.

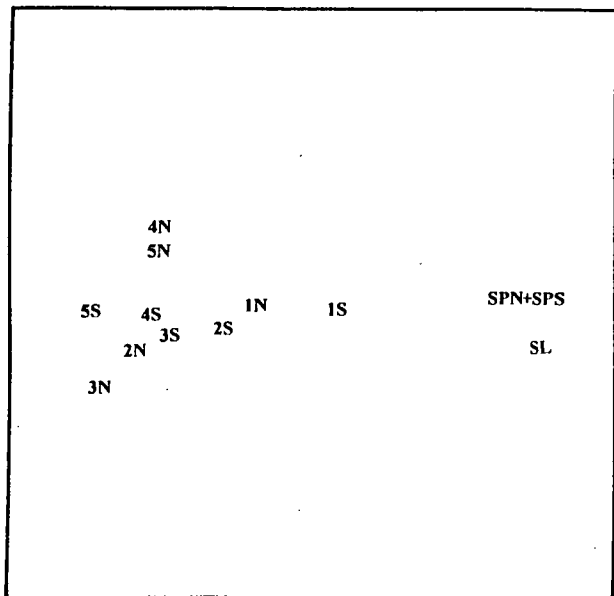
On Day 576 (22.12.94, 24 days from change: Figure 4.46/4.67c), SPN was noticeably closer to SL than SPS was to SL. Ponds 1N and 1S

were no longer paired, with 1N now well out on its own and closer to the the earlier ponds, while 1S had fallen back into the main group. Ponds 2N, 2S, and 3S were all close, while 3N+4S and 5N+5S were paired, and 4N fell on the earlier side of the gradation than (the nearby) 4S. The highest Bray Curtis similarities were 91.683 (3N+4S), 88.245 (5N+5S), 88.211 (2S+3S), 87.791 (3N+4N), and 87.391 (5N+4S). The similarities between the SL and SPN and SPS outlets were 83.568 and 68.506, respectively, while those between the SPN+SPS, 1N+1S, 2N+2S, 3N+3S, and 4N+4S pond pairs were 83.747, 71.098, 79.362, 75.372, and 86.551, respectively.

On Day 590 (05.01.95, 38 days from change: Figure 4.47/4.67d), SPN and SPS were again pairing, now well away from SL and with SPS on the SL side of the two. Pond 1N had moved close to this pair and the SPS pond in particular, while 1S remained back towards the main group of later ponds. Pond 2N preceded 2S, which was paired with 3N. Pond 4N was the next closest to these lagoons, with 4S out on its own and 5N+5S paired. Pond 3S was very separate to all other lagoons. The highest Bray Curtis similarities were 87.890 (1N+SPS), 87.140 (4N+5S), 86.237 (SPN+SPS), 86.065 (5N+5S), and 85.978 (4N+2S). The similarities between the SL and SPN and SPS outlets were 65.743 and 65.044, respectively, while those between the 1N+1S, 2N+2S, 3N+3S, and 4N+4S were 77.639, 71.534, 48.455, and 84.001, respectively.

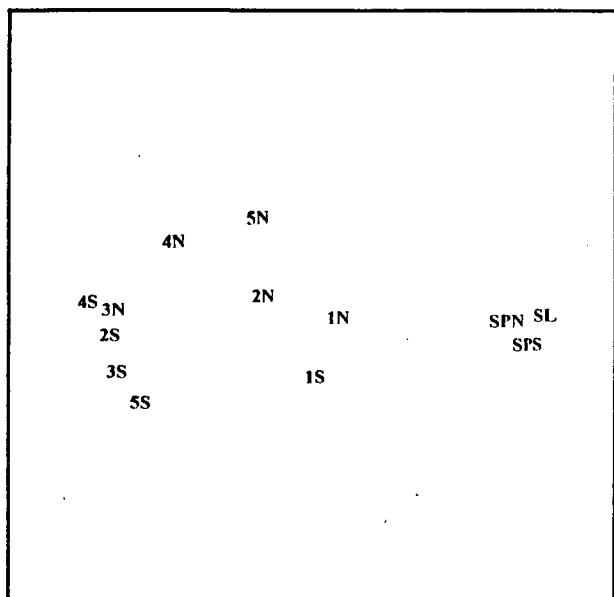
On Day 594 (09.01.95, 42 days since reversal: Figure 4.48/4.67e), SPN and SPS fell close together, with SPN the closer of the two to the distant SL. Pond 1N fell closer to SPN than either pond fell to SPS. Ponds 2N and 1S were moving out of the main group of ponds, with 2N now closer to the earlier ponds than 1S. Pond 3N was also moving out of the group of the later ponds, followed by 4N and 3S, while 2S+4S fell with the final ponds 5N and 5S. The highest Bray Curtis similarities were 96.647 (2S+4S), 88.548 (2S+5S), 88.538 (5N+2S), 88.196 (SPN+1N), and 87.996 (4S+5S). The similarities between the SL and SPN and SPS outlets were 72.706 and 66.050, respectively, while those between the SPN+SPS, 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 87.646, 66.368, 71.645, 75.271, 79.209, and 81.237, respectively.

For the final sampling day of the second flow division (Day 604, 19.01.95, 52 days post-reversal: Figure 4.49/4.67f), the SL pond was out on its own, while SPN and SPS were paired. Pond 1N was not as close to these ponds as in the previous ordination, but still fell half way between them and the group of the later ponds. The pattern was more confused within this group, with 4S grouping with 2N and 3N, and 3S pairing with 5N. Ponds 1S, 2S, and 5S fell on the periphery. The highest Bray Curtis similarities were 96.106 (2N+3N), 91.234 (5N+3S), 91.179 (3S+4S), 90.750 (3N+5N), and 89.222 (5N+4S). The similarities between the SL and SPN and SPS outlets were 60.283 and 60.553, respectively, while those between the SPN+SPS, 1N+1S, 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 88.693, 64.099, 71.424, 87.991, 78.181, and 75.394, respectively.



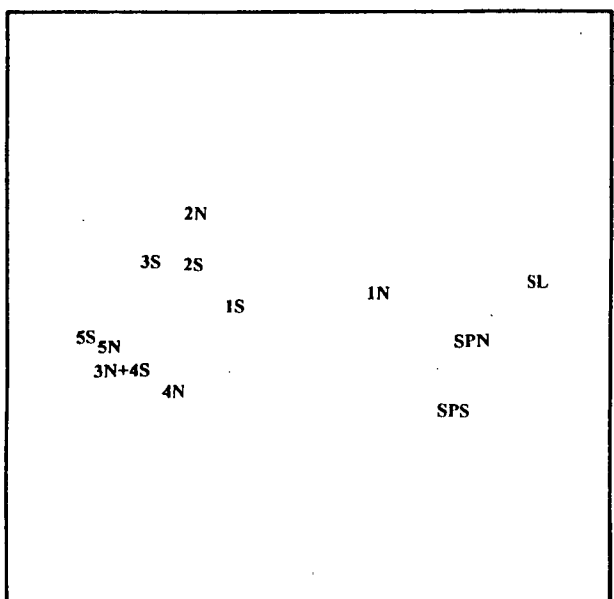
(a) Day 552: 28 November 1994  
0 days since flow change.

ALL MAIN TAXA Stress = 0.03



(b) Day 562: 8 December 1994  
10 days since flow change.

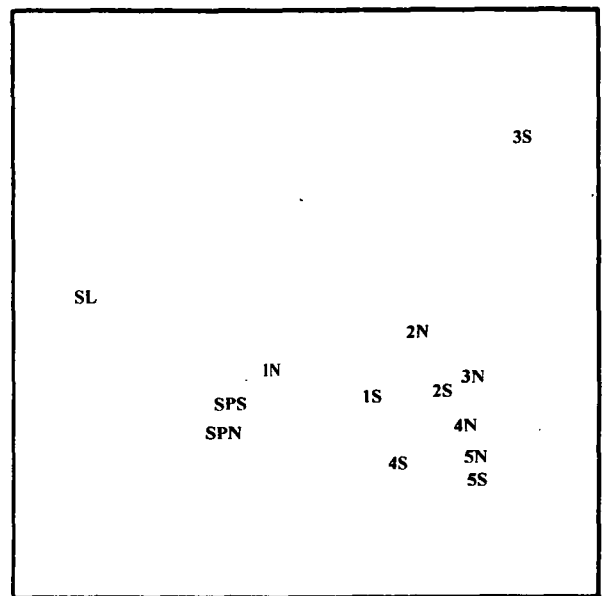
ALL MAIN TAXA Stress = 0.05



(c) Day 576: 22 December 1994  
24 days since flow change.

ALL MAIN TAXA Stress = 0.04

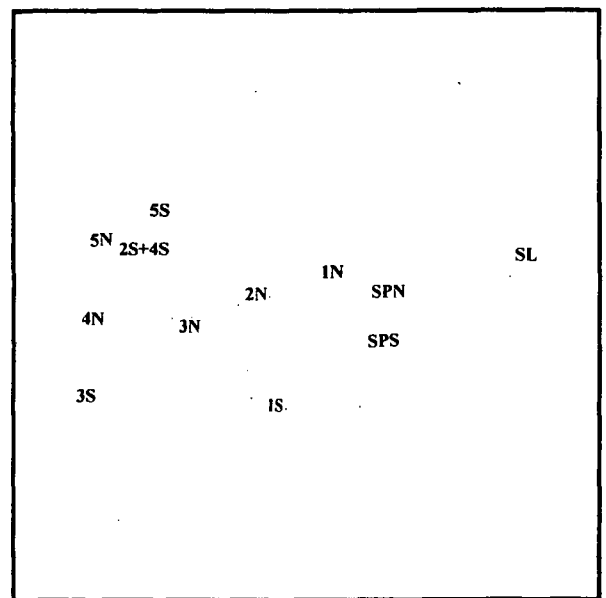
(d) Day 590: 5 January 1995  
38 days since flow change.



ALL MAIN TAXA

Stress = 0.07

(e) Day 594: 9 January 1995  
42 days since flow change.



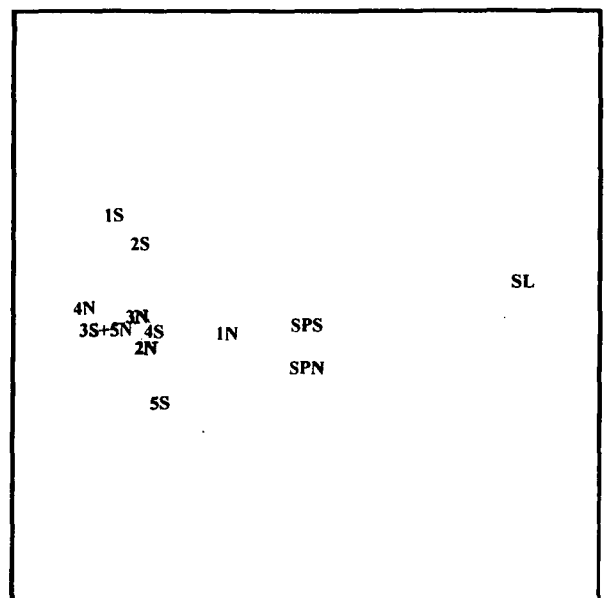
ALL MAIN TAXA

Stress = 0.03

Figure 4.67 (a-f)

All main zooplankton genera/group  
MDS ordination patterns for the second  
flow division (75%N: 25%S).

(f) Day 604: 19 January 1995  
52 days since flow change.



ALL MAIN TAXA

Stress = 0.08



### *Third flow division - 25%N, 75%S (all main groups)*

Stress values for the total plots over the third flow division ranged from 0.04 to 0.11, with an average of 0.07 and a standard error of 0.018. This average is noticeably higher than for the previous flow divisions, and for the first time a stress value exceeded the desirable threshold (0.11: still acceptable, but not ideal). Notably, this high stress value occurred at the end of the main experiment, when this regime had been established for the longest period, while the second highest stress level (0.08) occurred on the sampling date immediately before. Similar rises in stress values were previously noted for the corresponding rotifer and crustacean ordinations.

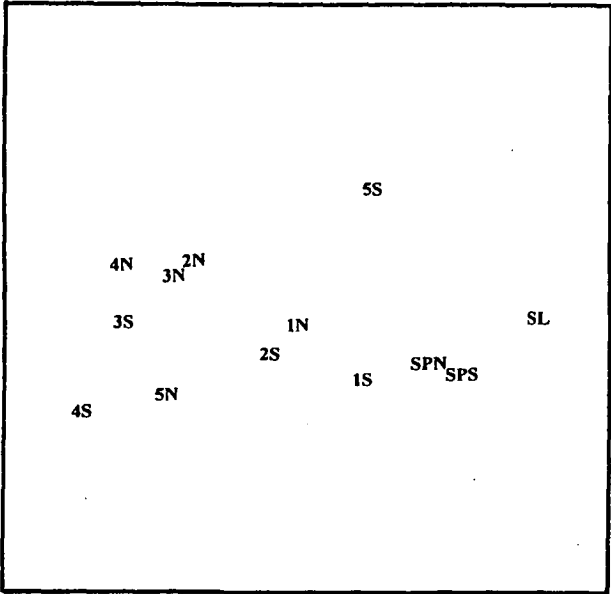
On Day 619 (03.02.95: Figure 4.50/4.68a), after four days under the new flow regime, the (high flow) SPS pond was becoming the closer of the SP pair to the SL pond. Following the pattern from bottom right to top left of this ordination, 1S was clearly moving away from the remaining ponds and closer to the SP pair, while 2S paired with 1N. Ponds 5N and 5S were well separated, but fell on the same general line towards the earlier ponds, with 5S the slightly closer of the two. Pond 4S followed 5N, while 3S was close to the remaining group of 2N, 3N and 4N. The highest Bray Curtis similarities were 94.825 (2N+3N), 91.512 (3N+4N), 90.492 (1N+2S), 89.599 (SPN+SPS), and 88.420 (2N+4N). The similarities between the SL and SPN and SPS outlets were 81.353 and 79.976, respectively, while those between the 1N+1S, 2N+2S, 3N+3S, 4N+4S and 5N+5S pond pairs were 79.427, 75.319, 82.357, 72.242, and 57.830, respectively.

On Day 622 (06.02.95, 7 days post-reversal: Figure 4.51/4.68b), the SPS pond was now closer to SL than it was to SPN. The 1S pond was still falling towards SPN, with 2S falling towards 1N. Ponds 4S and 3S were next, with 5S well off to the far side of the ordination. Ponds 4N+5N, followed by 2N and 3N, finished the gradation of early to later and high flow to low flow ponds. The highest Bray Curtis similarities were 94.282 (4N+5N), 90.985 (SPN+SPS), 90.701 (2N+3N), 88.737 (1N+1S), and 86.012 (SL+SPS). The percentage similarity between the SL and SPN outlets was 78.106, and those between the 2N+2S, 3N+3S,

4N+4S, and 5N+5S pond pairs were 56.343, 51.562, 80.400, and 59.091, respectively.

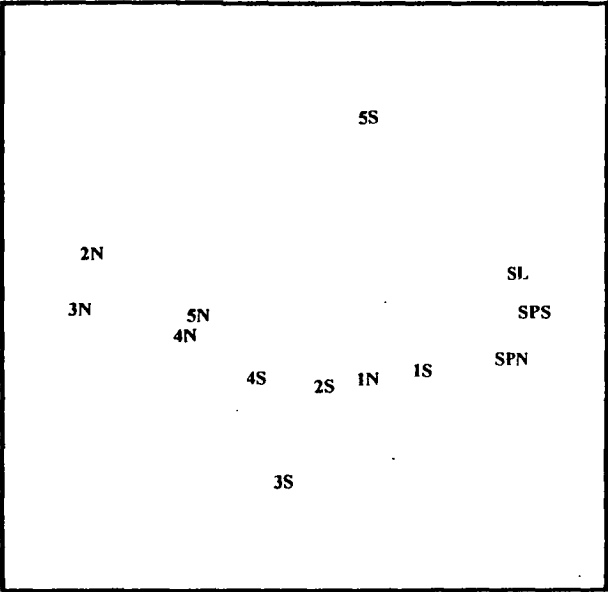
By Day 625 (09.02.95, 10 days post-reversal: Figure 4.52/4.68c), SPN had returned to fall between SL and its pair, while 1N+1S were pairing with 1N the slightly closer of the two to the SP pair. Ponds 2S and 3S, however, were still forwards in the ordination, and 4S fell close to 2N. Ponds 5N and 5S remained on the same line but opposite sides of the main group, while 3N+4N were paired at the end of the pattern. The highest Bray Curtis similarities were 94.722 (3N+4N), 92.229 (1N+1S), 91.364 (SPN+SPS), 84.011 (4N+5N), and 83.531 (1N+2N). The similarities between the SL and SPN and SPS outlets were 83.308 and 76.141, respectively, while those between the 2N+2S, 3N+3S, 4N+4S, and 5N+5S pond pairs were 76.703, 66.178, 76.093, and 57.773, respectively.

Finally, on Day 639 (23.02.95, 24 days post-reversal: Figure 4.53/4.68d), SPN was still the closer of the two SP ponds to SL, while 1N+1S remained paired. Ponds 2N and 2S were separate but on a similar line in the ordination, while 3N+3S were close with the latter pairing with 4S. Pond 5S was still an outlier but falling back in the ordination, while 5N fell at the tail end of the group. The highest Bray Curtis similarities were 96.831 (3S+4S), 90.111 (2N+3N), 88.925 (1N+1S), 88.234 (3N+4N), 86.955 (3N+3S). The similarities between the SL and SPN and SPS outlets were 77.038 and 76.714, respectively, while those between the SPN+SPS, 2N+2S, 4N+4S, and 5N+5S pond pairs were 81.181, 71.489, 74.805, and 51.985, respectively.



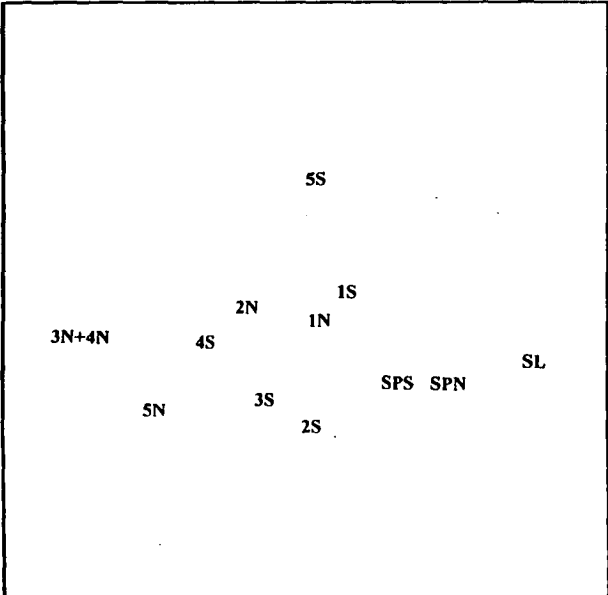
(a) Day 619: 3 February 1995  
4 days since flow change.

ALL MAIN TAXA                      Stress = 0.05



(b) Day 622: 6 February 1995  
7 days since flow change.

ALL MAIN TAXA                      Stress = 0.04



(c) Day 625: 9 February 1995  
10 days since flow change.

ALL MAIN TAXA                      Stress = 0.08

(d) Day 639: 23 February 1995  
24 days since flow change.

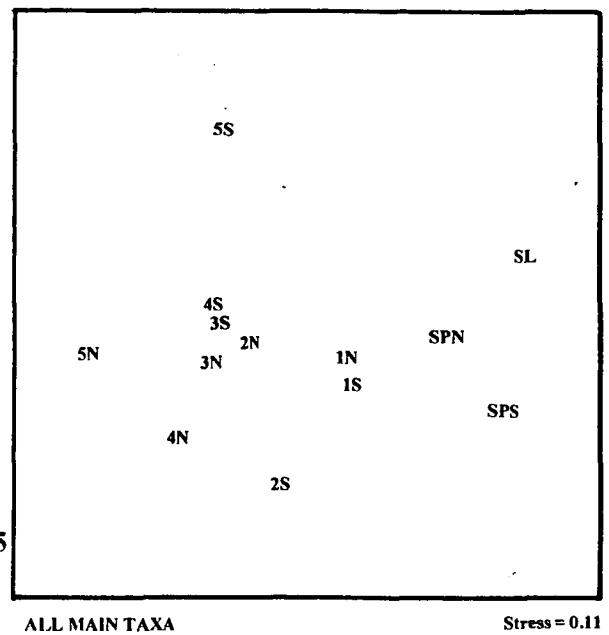


Figure 4.68 (a-d)

All main zooplankton genera/group  
MDS ordination patterns for the third  
flow division (25%N: 75%S).

**Table 4.6: Bray-Curtis similarities between paired lagoons (all main groups).**  
**Bold, underlined values represent pairs among the five most similar lagoons for that day.**

FLOW	DAY	SPN+SPS	1N+1S	2N+2S	3N+3S	4N+4S	5N+5S
ORIGINAL	1	NA	60.236	<u>89.397</u>	<u>80.91</u>	NA	NA
ORIGINAL	15	NA	40.175	<u>86.714</u>	<u>78.833</u>	NA	NA
ORIGINAL	29	NA	57.307	<u>84.91</u>	<u>89.125</u>	NA	NA
ORIGINAL	50	NA	68.591	<u>83.007</u>	72.796	NA	NA
ORIGINAL	64	NA	50.29	<u>90.541</u>	70.996	NA	NA
EQUAL	317	NA	<u>86.409</u>	<u>94.418</u>	<u>85.302</u>	74.009	79.445
EQUAL	331	<u>96.974</u>	67.878	80.606	82.666	<u>86.705</u>	67.557
EQUAL	345	<u>87.874</u>	78.959	<u>86.032</u>	<u>85.378</u>	58.155	<u>85.897</u>
EQUAL	359	<u>89.749</u>	<u>84.355</u>	75.908	<u>89.031</u>	57.392	58.49
EQUAL	373	<u>88.107</u>	<u>73.592</u>	<u>80.179</u>	35.647	62.784	NA
EQUAL	387	<u>96.861</u>	83.938	<u>94.157</u>	<u>93.656</u>	78.097	NA
EQUAL	401	<u>77.24</u>	<u>84.205</u>	<u>79.169</u>	NA	68.65	NA
EQUAL	415	<u>90.469</u>	<u>88.521</u>	<u>86.82</u>	<u>84.821</u>	<u>86.328</u>	NA
EQUAL	429	<u>97.471</u>	<u>93.083</u>	<u>87.221</u>	64.23	46.291	NA
EQUAL	443	<u>91.044</u>	<u>88.508</u>	<u>83.471</u>	71.855	68.989	<u>83.67</u>
1st DIV.	457 (0)	87.497	88.23	82.126	<u>91.873</u>	78.134	73.249
1st DIV.	471 (14)	<u>80.714</u>	<u>83.438</u>	NA	64.187	47.099	26.701
1st DIV.	499 (42)	82.062	<u>89.636</u>	86.229	<u>87.628</u>	72.886	<u>91.942</u>
1st DIV.	513 (56)	<u>90.791</u>	72.665	<u>88.89</u>	76.667	82.421	82.335
1st DIV.	527 (70)	<u>93.232</u>	82.506	84.739	85.723	73.271	84.891
1st DIV.	541 (84)	79.977	<u>81.997</u>	71.894	75.747	81.199	<u>83.158</u>
2nd DIV.	552 (0)	<u>91.051</u>	75.83	79.121	79.837	78.781	73.576
2nd DIV.	562 (10)	<u>85.57</u>	80.479	67.118	83.323	72.259	69.488
2nd DIV.	576 (24)	83.747	71.098	79.362	75.372	86.551	<u>88.245</u>
2nd DIV.	590 (38)	<u>86.237</u>	77.639	71.534	48.455	84.001	<u>86.065</u>
2nd DIV.	594 (42)	87.646	66.368	71.645	75.271	79.209	81.237
2nd DIV.	604 (52)	88.693	64.099	71.424	87.991	78.181	75.394
3rd DIV.	619 (4)	<u>89.599</u>	79.427	75.319	82.357	72.242	57.83
3rd DIV.	622 (7)	<u>90.985</u>	<u>88.737</u>	56.343	51.562	80.4	59.091
3rd DIV.	625 (10)	<u>91.364</u>	<u>92.229</u>	76.703	66.178	76.093	57.773
3rd DIV.	639 (24)	81.181	<u>88.925</u>	71.489	<u>86.955</u>	74.805	51.985

*Summary of combined rotifer and crustacean ordination patterns/similarities*

The ordinations for the overall rotifer/crustacean community provide a much more defined, complete and consistent pattern for the 85wB. For the original flow period, this pattern is clearly in keeping with the serpentine gradation from early to later ponds as previously seen. This changes over the equilibration period, and while there is some variation over this period, it generally shows very good pairings of lagoons as predicted, reflecting both the new flow pattern and the equal flow between sides. For the first flow division (25%N:75%S), there is a clear shift in the pattern, with the southern (high flow) ponds moving forwards to match or precede earlier northern ponds. This pattern is clearly reversed as the second flow division (75%N:25%S) becomes established, while the third and shortest flow division (25%N:75%S) begins to show a good reversal before the pattern becomes more confused.

Similarity values between paired ponds based on combined rotifer and crustacean data are presented in Table 4.6. Underlined values represent those ranking among the five highest (most similar) of all ponds for the system on each sampling day. As the combined data contain no sites in which all taxa are absent, the shortfalls encountered in analysing the individual groups have been bypassed and a better overall view of community changes is presented.

As can be seen in the table, corresponding ponds (pairs SP to 3) were very commonly among the highest similarity values during the equilibrium period, and became markedly less common over the course of the divisions in flow. Pair 1N+1S showed a distinct increase in similarity between the original and equilibrium flow periods, while 2N+2S and 3N+3S were relatively high in both periods.

Table 4.7 lists the highest combined rotifer/crustacean community similarities between all lagoons on each sampling day, with underlined values representing corresponding pond pairs, red values representing 'out of phase' pairings between later southern ponds and earlier northern ponds, and blue values representing 'out of

Table 4.7: Highest Bray-Curtis similarities between lagoons (all main groups).

Blue values represent combinations of later southern ponds with earlier southern ponds.  
Red values represent combinations of later southern ponds with earlier northern ponds.  
Bold, underlined values represent those between corresponding lagoon pairs.

FLOW	DAY	HIGHEST PAIRS
ORIGINAL	1	<u>88.397 (2N+2S)</u> 86.510 (2N+3N)
ORIGINAL	15	<u>87.883 (SPS+1S)</u> <u>86.714 (2N+2S)</u> 82.988 (2N+3N)
ORIGINAL	29	<u>89.125 (3N+3S)</u> 87.569 (SL+02)
ORIGINAL	50	85.382 (1S+2S) 84.486 (SPS+1S)
ORIGINAL	64	<u>90.541 (2N+2S)</u> 85.033 (2S+4S)
EQUAL	317	<u>94.418 (2N+2S)</u> <u>86.409 (1N+1S)</u> <u>86.705 (4N+4S)</u> <u>86.032 (2N+2S)</u> <u>89.749 (SPN+SPS)</u> 88.107 (SPN+SPS) 96.861 (SPN+SPS) <u>84.205 (1N+1S)</u> <u>90.469 (SPN+SPS)</u> <u>97.471 (SPN+SPS)</u> <u>91.044 (SPN+SPS)</u>
EQUAL	373	88.107 (SPN+SPS) 87.563 (4N+5N)
EQUAL	387	<u>94.157 (2N+2S)</u> <u>93.656 (3N+3S)</u> <u>79.169 (2N+2S)</u> <u>86.820 (2N+2S)</u> 89.197 (3N+4S) 83.979 (4N+3S)
EQUAL	401	<u>83.229 (1N+2S)</u> <u>88.521 (1N+1S)</u> <u>93.083 (1N+1S)</u> <u>88.508 (1N+1S)</u>
EQUAL	415	<u>90.469 (SPN+SPS)</u> <u>88.521 (1N+1S)</u> <u>93.083 (1N+1S)</u> <u>88.508 (1N+1S)</u>
EQUAL	429	<u>97.471 (SPN+SPS)</u> <u>91.044 (SPN+SPS)</u>
EQUAL	443	<u>91.044 (SPN+SPS)</u>
1st DIV.	457 (0)	93.317 (3N+4N)
1st DIV.	471 (14)	<u>83.438 (1N+1S)</u> <u>80.714 (SPN+SPS)</u> <u>91.873 (3N+3S)</u> 91.562 (4N+3S) 78.360 (4N+3S) 90.205 (3N+4S) 89.536 (1N+2S) 90.129 (4S+5S) 87.343 (1N+2S)
1st DIV.	499 (42)	<u>93.277 (2N+3S)</u> <u>91.942 (5N+5S)</u> <u>90.791 (SPN+SPS)</u> <u>90.709 (3N+5S)</u> 89.164 (2N+3N)
1st DIV.	513 (56)	<u>93.024 (2N+3S)</u> <u>90.791 (SPN+SPS)</u> <u>90.709 (3N+5S)</u>
1st DIV.	527 (70)	<u>93.232 (SPN+SPS)</u> <u>89.584 (4N+5S)</u>
1st DIV.	541 (84)	<u>89.584 (4N+5S)</u>
2nd DIV.	552 (0)	<u>91.051 (SPN+SPS)</u> 90.804 (3S+4S) 89.575 (4S+5S) <u>88.245 (5N+5S)</u> <u>87.140 (4N+5S)</u> 88.548 (2S+5S) 91.234 (5N+3S)
2nd DIV.	562 (10)	<u>90.476 (3N+4S)</u> <u>91.683 (3N+4S)</u> <u>87.890 (1N+SPS)</u> 96.647 (2S+4S) 96.106 (2N+3N)
2nd DIV.	576 (24)	<u>91.683 (3N+4S)</u> <u>87.890 (1N+SPS)</u> 96.647 (2S+4S) 96.106 (2N+3N)
2nd DIV.	590 (38)	<u>87.890 (1N+SPS)</u> 96.647 (2S+4S) 96.106 (2N+3N)
2nd DIV.	594 (42)	96.647 (2S+4S) 96.106 (2N+3N)
2nd DIV.	604 (52)	96.106 (2N+3N)
3rd DIV.	619 (4)	94.825 (2N+3N) 91.512 (3N+4N) <u>90.985 (SPN+SPS)</u> <u>92.229 (1N+1S)</u> 90.111 (2N+3N)
3rd DIV.	622 (7)	94.282 (4N+5N) <u>90.985 (SPN+SPS)</u> <u>92.229 (1N+1S)</u> 90.111 (2N+3N)
3rd DIV.	625 (10)	94.722 (3N+4N) <u>92.229 (1N+1S)</u> 90.111 (2N+3N)
3rd DIV.	639 (24)	96.831 (3S+4S) 90.111 (2N+3N)
80.463 (SPS+1S)		<u>80.601 (2N+3S)</u> 79.673 (3N+2S) <u>84.910 (2N+2S)</u> <u>83.007 (2N+2S)</u> 83.995 (3S+4S)
84.878 (1S+2S)		<u>84.910 (2N+2S)</u> <u>83.007 (2N+2S)</u> 83.995 (3S+4S)
79.394 (2N+3N)		<u>83.007 (2N+2S)</u> 83.995 (3S+4S)
81.966 (2N+4S)		83.995 (3S+4S)
85.302 (3N+3S)		86.089 (3N+2S) 83.865 (1S+2S) <u>85.378 (3N+3S)</u> 86.742 (4N+5N) <u>73.592 (1N+1S)</u> 90.120 (3N+4N) 79.136 (1S+2S) <u>86.328 (4N+4S)</u> <u>87.221 (2N+2S)</u> <u>83.670 (5N+5S)</u>
83.111 (2S+3S)		83.865 (1S+2S) <u>85.378 (3N+3S)</u> 86.742 (4N+5N) <u>73.592 (1N+1S)</u> 90.120 (3N+4N) 79.136 (1S+2S) <u>86.328 (4N+4S)</u> <u>87.221 (2N+2S)</u> <u>83.670 (5N+5S)</u>
84.506 (2N+3N)		<u>85.378 (3N+3S)</u> 86.742 (4N+5N) <u>73.592 (1N+1S)</u> 90.120 (3N+4N) 79.136 (1S+2S) <u>86.328 (4N+4S)</u> <u>87.221 (2N+2S)</u> <u>83.670 (5N+5S)</u>
84.355 (1N+1S)		<u>84.355 (1N+1S)</u> 90.120 (3N+4N) 79.136 (1S+2S) <u>86.328 (4N+4S)</u> <u>87.221 (2N+2S)</u> <u>83.670 (5N+5S)</u>
73.203 (3N+4N)		90.120 (3N+4N) 79.136 (1S+2S) <u>86.328 (4N+4S)</u> <u>87.221 (2N+2S)</u> <u>83.670 (5N+5S)</u>
86.578 (4N+3S)		90.120 (3N+4N) 79.136 (1S+2S) <u>86.328 (4N+4S)</u> <u>87.221 (2N+2S)</u> <u>83.670 (5N+5S)</u>
<u>77.240 (SPN+SPS)</u>		<u>77.240 (SPN+SPS)</u> 84.821 (3N+3S) 77.727 (5N+4S) 83.471 (2N+2S)
92.416 (4N+5S)		91.422 (3N+5S) 74.642 (SPS+1S) <u>89.636 (1N+1S)</u> <u>88.890 (2N+2S)</u> <u>89.382 (2N+3S)</u> <u>83.158 (5N+5S)</u>
73.872 (4S+5S)		74.642 (SPS+1S) <u>89.636 (1N+1S)</u> <u>88.890 (2N+2S)</u> <u>89.382 (2N+3S)</u> <u>83.158 (5N+5S)</u>
87.628 (3N+3S)		<u>89.636 (1N+1S)</u> <u>88.890 (2N+2S)</u> <u>89.382 (2N+3S)</u> <u>83.158 (5N+5S)</u>
88.515 (4N+5N)		<u>88.890 (2N+2S)</u> <u>89.382 (2N+3S)</u> <u>83.158 (5N+5S)</u>
87.816 (SL+SPS)		<u>89.382 (2N+3S)</u> <u>83.158 (5N+5S)</u>
81.997 (1N+1S)		<u>83.158 (5N+5S)</u>
86.250 (4S+5S)		86.395 (4N+5N) 87.792 (SL+SPN) 88.211 (2S+3S) <u>86.237 (SPN+SPS)</u> <u>88.538 (5N+2S)</u> 91.179 (3S+4S)
86.570 (SPN+SPS)		86.395 (4N+5N) 87.792 (SL+SPN) 88.211 (2S+3S) <u>86.237 (SPN+SPS)</u> <u>88.538 (5N+2S)</u> 91.179 (3S+4S)
87.391 (5N+4S)		87.791 (3N+4N) <u>86.065 (5N+5S)</u> 88.196 (SPN+1N) 90.750 (3N+5N)
85.978 (4N+2S)		87.791 (3N+4N) <u>86.065 (5N+5S)</u> 88.196 (SPN+1N) 90.750 (3N+5N)
87.996 (4S+5S)		87.996 (4S+5S) 89.222 (5N+4S)
88.420 (2N+4N)		88.420 (2N+4N) <u>86.012 (SL+SPS)</u> 83.531 (1N+2N) <u>86.955 (3N+3S)</u>
88.737 (1N+1S)		<u>88.737 (1N+1S)</u> 84.011 (4N+5N) 88.234 (3N+4N)
91.364 (SPN+SPS)		<u>91.364 (SPN+SPS)</u> <u>88.925 (1N+1S)</u>
90.492 (1N+2S)		<u>90.492 (1N+2S)</u>
90.701 (2N+3N)		90.701 (2N+3N)

phase' pairings between later northern ponds and earlier southern ponds (these combinations include pairings with the SL pond).

Once again, corresponding pond pairs strongly dominated the highest similarity values over the equilibrium period, and, although initially persisting into the first, became markedly less dominant during the three divisions in flow (particularly during the second). Mixed pairings between late S/early N ponds predominated through the first flow division (75%S), reversing to late N/early S combinations during the second. No pattern established during the shorter third flow division, although increased corresponding pond pairings may have reflected a transient stage in the reversal of community types within some lagoons.



## 5. DISCUSSION

### 5.1 Manipulated patterns at WTC

Large scale ecological experiments allow the investigation and manipulation of functional components within the context of a complete ecosystem. Such experiments bypass the limitations of artificial, single-factor laboratory studies, less complex enclosure environments, and inference from field monitoring alone, but are in turn prone to problems of natural variation, pseudoreplication, statistical invalidity, and limitations in the time and resources available to conduct them adequately (Stewart-Oaten *et al.* 1986, Hairston 1989, Eberhard & Thomas 1991). Large scale experimental design needs to remain simple in order to counterbalance environmental complexity, and the experimental questions posed and results gathered may often be less precise than in investigations conducted under ideal (controlled) conditions. In some cases, replication in large scale manipulations is not possible, but results may remain distinct and convincing (Hairston 1989, outlining Schindler 1974 as an example). In short, the assumptions and short-falls of ecological experiments need to be recognised, as with any scientific study.

As outlined in Sections 3.2-3.4, the design of this experimental manipulation tried to take such factors into account within the limitations of the available system (85wB) at the Werribee Treatment Complex (WTC). The difficulties involved in conducting an experiment at such a scale were offset by the rarity of such manipulations (Section 2.6), the established need for them in regard to sewage treatment and nutrient recycling through faunal harvest (Kawai *et al.* 1987), and the practical, large scale requirements of industry. Mindful of the attendant assumptions and potential short-falls of the project design (as discussed further in Section 6.3), it is suggested that definite patterns can be identified in the data recorded for the manipulations of the 85wB system.

Distinct changes in the physical and biological conditions of

the 85wB lagoons occurred following the alteration of flow from its original pattern to the divided system. Marked changes were recorded both for the equilibrium flow period, and for the subsequent unequal divisions in flow. Patterns in zooplankton populations reflected both the position of lagoons in the system and the flow rate/nutrient loading passing through the system.

During the equilibrium period, the magnitude of change depended on the original position of individual ponds prior to and following establishment of the new flow pattern. Corresponding pond pairs on the northern and southern sides began to match each other in chemical and biological parameters, with ponds that were initially separated in sequence (and so initially very different) showing the greatest degree of relative change. This was the case for ponds 1N+1S, which were separated by an entire lagoon (SP) under original flow conditions. In contrast, ponds 2S, 2N, 3N and 3S were consecutive lagoons in the original sequence. The SL and SP ponds also became markedly more similar, as they changed from being separated by another two ponds (the original 'pond 2' and 1N) to being directly linked.

Good overall matches developed between corresponding north and south ponds for measured nutrient values under equal flow conditions. Ammonia, nitrate and phosphorus values matched well between pond pairs, even between those which were initially quite different. Nitrite values also paired well, although initial differences were not as great. While little difference or change was recorded for pH, minor differences in conductivity and salinity in the later ponds became even less apparent during the equal flow period.

Biologically, paired north and south lagoons reflected the same pattern. Chlorophyll-a levels shifted from initial differences between paired ponds to match more closely. Rotifer and crustacean numbers and types also changed markedly, from differences reflecting the original flow pattern to generally good matches between corresponding ponds under the new flow pattern. Some differences persisted within these pairings, however, with less strong matches between the later ponds for rotifers (pairs 4 & 5), where rotifer numbers

were much lower and their relative contribution to the zooplankton assemblage was less. Differences may also have arisen as the corresponding ponds in pairs 4 and 5 were less well matched in terms of area, capacity and retention time, and, as nutrient loading was greatly reduced by this stage in the system, the nutrient-mediated pattern may have been becoming less defined.

While generally reflecting the altered flow pattern, pairings for crustaceans were not as distinct as those for rotifers. This was potentially due to a mixture of the exclusion/underestimation of absence and low abundance information from the calculation of similarities (as outlined at the end of Sections 4.5.3.1 & 4.5.3.2), the tendency of the crustaceans to persist at more stable levels throughout the remaining lagoons in which they occurred (with sub-target flow providing less pronounced differences between different pond pairs), and the lower taxonomic resolution for crustacean identifications in this study.

These discrepancies disappeared when both rotifers and crustaceans were considered in combination, as a means of assessing trends in the overall zooplankton community. Trends were very consistent, and shifted from reflecting the original flow pattern to strong pairings for the new pattern under equal division of flow. For rotifers, crustaceans and both combined, corresponding ponds were frequently among the highest similarity pairings during the equilibration period.

Distinct changes also followed the subsequent experimental manipulations, with flow rate divided unequally between the halves of the system. While these changes were not to the same degree as the larger shifts observed between the original and equilibrium flow patterns (both because flow rates were low and the pond sequence remained the same), they still followed definite and identifiable trends.

Ammonia again displayed direct responses to the three flow divisions, with markedly higher levels consistently recorded throughout the system on the high flow side. Nitrate, nitrite and (initially to a lesser degree) phosphorus all broke with the matching patterns they had developed between paired ponds under equal flow.

Subsequent patterns for these nutrients were far more erratic than those for ammonia. As suggested in Sections 4.4.2 & 4.4.3, this variation may have been due to the effects of compounding chemical or biological interactions on these nutrients, in contrast to the higher, swamping concentrations of influent ammonia. Whatever the exact mechanism, levels of these nutrients became less well matched between corresponding pond pairs following the changes in division of flow. As during the equilibrium period, pH showed little change, while conductivity and salinity displayed increased variation between pairs (although timing suggests this may not have been directly related to the changes in flow).

Chlorophyll-a levels showed the same break in similarity between corresponding pond pairs over the unequal divisions in flow. Ponds displayed increased variation and differences in magnitude, particularly in later ponds and at later times. Although chlorophyll-a levels reached an extremely high peak of  $2.731\text{mg.l}^{-1}$ , this corresponded to a previous recorded level of  $2.8\text{mg.l}^{-1}$  at WTC (Hussainy 1979) and did not equal levels recorded in high rate algal ponds (higher than  $5\text{mg.l}^{-1}$ ; Canovas *et al.* 1995) nor those achieved under artificial conditions ( $17.5\text{mg.l}^{-1}$ ; Wrigley & Toerien 1990, reporting earlier work).

As with non-ammonia nutrients, chlorophyll patterns and cycles may have been complicated by an interplay of strong bottom-up and top-down effects (Blomqvist 1997, Scharf 1997), as well as by the effects of unequal flow division compounding to some degree across the experimental period and between the three unequal flow regimes. [As alluded to in Chapter 3 and at the beginning of this discussion, the necessary constraints of large scale ecological experiments leave them particularly prone to the effects of pseudoreplication (Hairston 1989), and the time-frame and limitations imposed by the availability of the 85wB lagoons means that this remains a concern. It should be noted, however, that this experiment was designed to examine not only the effects of flow/nutrient loading, but also of change and reversal in that flow/loading in a continuously operating system].

As with the other parameters, the zooplankton showed a definite change with the unequal divisions in flow. For this group, however, top-down influences were effectively absent (as with

ammonia), and so the resulting patterns were less complex and more consistent.

For rotifers, high-flow ponds shifted forwards in the ordination patterns as a consequence of the new flow regimes, and corresponding ponds (particularly beyond the SP outlets) fell markedly in similarity relative to the rest of the system. These trends were reversible, as predicted, but again with increasing variability towards the end of the experimental period.

Crustacean populations also reflected the changes in flow division, and similarities between corresponding ponds also fell markedly relative to the rest of the system. Crustacean patterns did tend to show high-flow ponds moving away from their low-flow counterparts, but also exhibited a north/south component, with ponds from the same side tending to group together rather than intermixing along the gradient. The Crustacea were more consistent in pattern than they had been during the equilibrium period, although some variation occurred in later ponds/times and for the last flow division it is possible that the populations were still changing in response to the altered flow regime when the experiment came to a close.

With both rotifer and crustacean data combined, these patterns and trends became more sharply defined, and displayed clear community shifts with all three flow divisions and reversals. There was a sharp decline in similarities between corresponding ponds relative to the rest of the system. The highest similarities reflected the new flow regimes, with later high-flow ponds clearly pairing with earlier low-flow ponds. Again, this pattern starts to become more confused at the end of the final (shortest) flow division, either due to accumulating effects across the whole experimental period or because the system was still responding to the flow reversal.

In short, large scale manipulations between halves of a lagoon system that - in location, orientation, shape, capacity and influent quality - were as similar and comparable as possible, produced remarkably similar trends in chemical and biological conditions between corresponding ponds under equal flow and less similar conditions under unequal flow. For ammonia concentrations and zooplankton communities, the dissimilarities produced under unequal

flow developed in consistent and predictable patterns, in keeping with the expected patterns as described in Section 4.5.3.

With all other conditions remaining the same, these differences can be attributed to either the physical effects of flow itself or the nutrient loading that particular flow rates represent. Flow rate alone, however, does not adequately explain the gradation in zooplankton that is seen across a system (where flow rate remains the same from beginning to end) nor the changes in lagoon character that accompanied alterations of flow pattern (e.g. the shift of the SP pond from fourth to second in sequence). While actual flow rate may help explain the persistence of rotifers further through a system under high flow conditions (due to physical washout), it does not fully explain the surge in crustacean numbers that occurs in earlier ponds under lower flow conditions.

Prior observations at WTC (Section 3.3) have indicated that nutrient loading is as important as flow rate, and that zooplankton populations are most strongly correlated with ammonia concentrations in the lagoons (David Cartwright, unpublished data). Within the current project, ammonia displayed a strong and direct response to changes in the division of flow, and graded from high to low levels along the same gradient of earlier-to-later ponds that characterised the pattern in zooplankton distribution.

Representative overlays of scaled ammonia concentrations on overall community patterns are presented in Figures 5.1-5.3, highlighting the zooplankton composition and ammonia profiles established per pond by the mid-to-late equilibration period and the end of each flow division. There is a strong coupling of patterns, with those ponds grouped on the basis of similar planktonic communities also exhibiting comparable ammonia characteristics. These are in keeping both with the earlier-to-later gradation mentioned above and, in general, with the variations in pattern and pond pairings produced by the divisions in flow. While the pattern of zooplankton distribution through the 85wB may still have been changing and developing at the end of the (shorter) third flow division, it was still clearly correlated with the prevailing ammonia conditions at that time (Figure 5.3).

While zooplankton distribution and ammonia concentration would appear to be strongly linked, this does not necessarily prove a

causal relationship between the two. Ammonia may play a direct role in determining zooplankton community structure, or it may simply reflect the overall nutrient loading on the system and the role that that plays, or both may be affected by the same base processes. As can be seen in Plate 3.4, the physical appearance of water in the 85wB system grades markedly from the earlier to later ponds. Matters are further complicated by sampling limitations, and the assumption that physico-chemical conditions at the time of collection reflect those that produced the populations extant at the same time. Although influent nutrient concentration at WTC remains relatively consistent (Constable 1988), flow rate within the course of this study was highly variable and would have directly influenced overall nutrient loading. Different fractions of the zooplankton community would respond to such variations in conditions with varying lag times, and, alongside chance variation and reduced definition due to sub-target flow levels, these may account for some of the noise and occasional variation seen in the general patterns recorded at WTC and in the 85wB system.

While changes in biological communities according to pollution have been well recognised before (indeed, they form the basis of WSP treatment systems), WTC is unusual for the distinct, prolonged and predictable patterns it exhibits. As reviewed in Section 2.6, the number of comparable studies in the literature is limited, and these have usually focussed on zooplankton dynamics and environmental interactions from a different perspective to that reported here; e.g. between discrete sites rather than covering an elongated nutrient gradient such as across the 85wB lagoons.

Although not investigated or manipulated on the same scale, however, previous references to loading-related zooplankton distributions have been made in the literature (as discussed in detail in the following Section). These references further support the apparent link between the spatial succession of zooplankton in the 85wB and the (flow-mediated) nutrient loadings of the lagoons. Previous recognition of such patterns in eutrophic waters also supports the incredible overall stability achieved in manipulations of the 85wB, in which population structures may well have been buffered against change and chance by the characteristic low diversity/high biomass status of polluted waters.

SL

SPS

<sup>1</sup>N  
1S

<sup>3</sup>N<sup>4</sup>N  
2S3S

4S

SL

4S

4N

<sup>3</sup>N3S

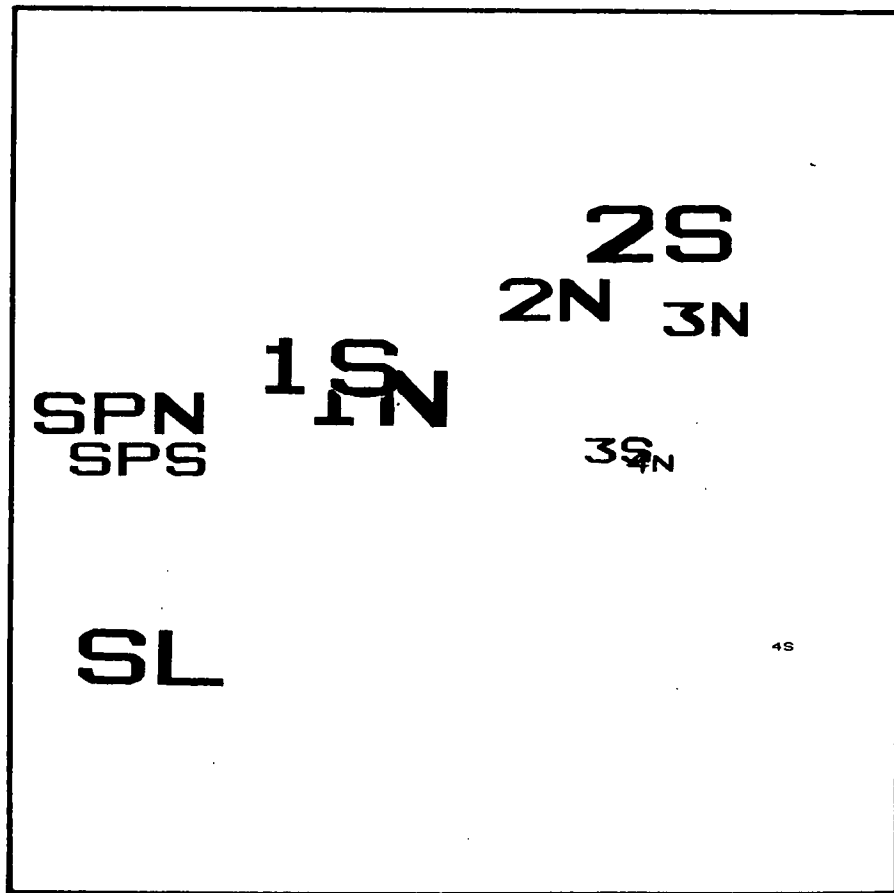
SPS<sup>2</sup>N

5N

<sup>1</sup>N  
1S

2S





**Figure 5.1: Combined rotifer and crustacean zooplankton community patterns as established under equal flow, overlaid with scaled environmental (ammonia) data.**

Larger symbols denote higher ammonia concentrations.

*N.B. Ponds 5N and 5S are mostly absent as water samples were not consistently collected for these lagoons.*

**Top left:** as per Figures 4.33/4.65(f), representing Day 387 (16 June 1994). Note that SPS and 2S directly overlap and obscure their corresponding northern pairs (SPN & 2N), which are of similar magnitude.

**Bottom left:** as per Figures 4.35/4.65(h), representing Day 415 (14 July 1994). SPS and 2S partially obscure their northern counterparts, which are again of similar magnitude. Chemical data was collected for pond 5N on this day, and so this lagoon is included in the plot.

**Top right:** as per Figures 4.37/4.65(j), representing Day 442 (11 August 1994).

4N

SL  
SPS  
SPN

1S  
1N  
2S

2N  
3N  
4S

3S

SL

3N  
2N

4N

3S

2S  
1N  
1S

SPN

4S

SPS

**Figure 5.2: Combined rotifer and crustacean zooplankton community patterns as established under the first flow division (25%N:75%S), overlaid with scaled environmental (ammonia) data.**

Larger symbols denote higher ammonia concentrations.

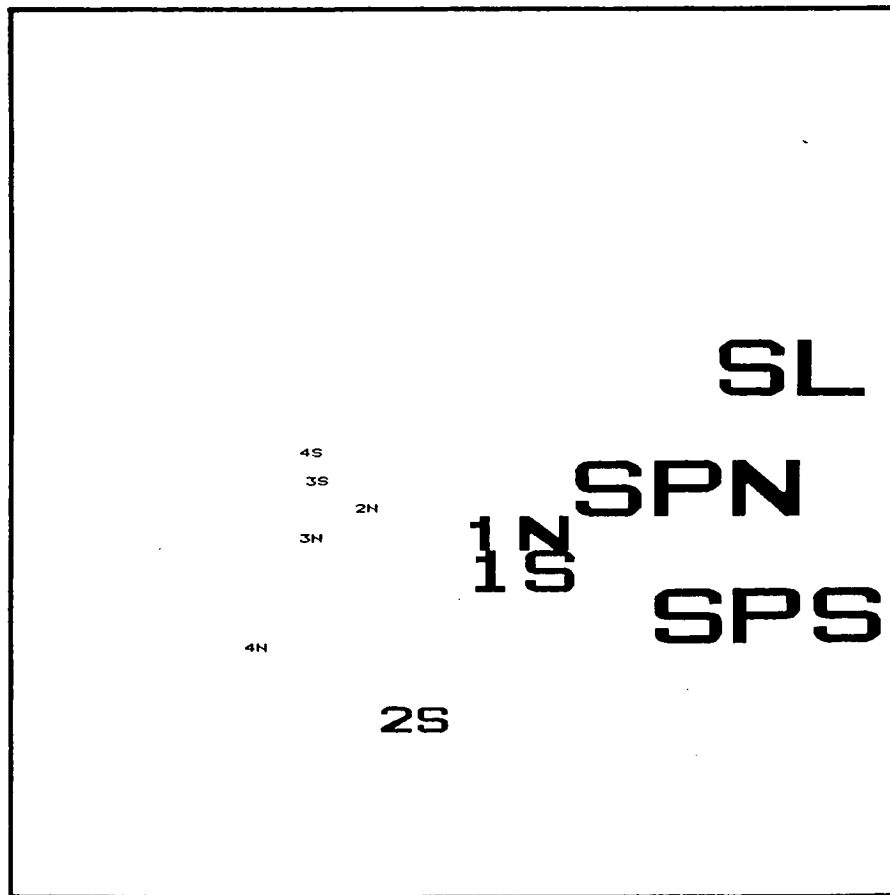
*N.B. Ponds 5N and 5S are absent as water samples were not collected for these lagoons.*

**Top left:** as per Figures 4.42/4.66(e), representing Day 527 (3 November 1994), 70 days following the change in flow.

**Bottom left:** as per Figures 4.43/4.66(f), representing Day 541 (17 November 1994), 84 days following the change in flow.

1S  
2S  
4N  
3N  
1N  
2N  
SPN  
SPS  
1S  
SL

1S  
2S  
4N  
3N  
2N  
1N  
SPS  
SPN  
SL



**Figure 5.3: Combined rotifer and crustacean zooplankton community patterns as established under the second and third flow divisions (75%N:25%S and 25%N:75%S), overlaid with scaled environmental (ammonia) data.**

Larger symbols denote higher ammonia concentrations.

*N.B. Ponds 5N and 5S are absent as water samples were not collected for these lagoons.*

**Top left:** as per Figures 4.48/4.67(e), representing Day 594 (9 January 1995), 42 days following the change in flow.

**Bottom left:** as per Figures 4.49/4.67(f), representing Day 604 (19 January 1995), 52 days following the change in flow.

**Top left:** as per Figures 4.53/4.68(d), representing Day 639 (23 February 1995), 24 days following the change in flow.

## 5.2 Nutrient-mediated successional patterns

Temporal succession in zooplankton populations has been widely reported, and is usually related to seasonal variation (Orcutt & Pace 1984, Sommer *et al.* 1986, Kawai *et al.* 1987, Sanders *et al.* 1989, Griggs 1993, Canovas *et al.* 1995, Mendes *et al.* 1995, Mezrioui & Oudra 1998, Pinto-Coelho 1991 & 1998). High nutrient availability appears to allow large zooplankton populations to persist throughout the year at WTC, although plankton levels appear to remain polarized between summer peaks and winter troughs (David Cartwright, unpublished data)<sup>1</sup>. Other patterns are overlaid on such seasonal cycles, however, and large temporal variation has been recorded in communities of bacteria, phytoplankton and zooplankton in waste stabilization ponds (WSPs) at low latitudes, where seasonal variation is slight (Uhlmann 1980, Pinto-Coelho 1998).

Mitchell (1980) has, in turn, noted that succession in waste stabilization lagoons “generally favours species less tolerant of high organic concentrations”. Phytoplankton biomass is directly influenced by nutrient loading (Moss *et al.* 1991), and the development of algae in sewage lagoons is recognised as the second step in sewage self-purification, following bacterial establishment (Mezrioui & Oudra 1998). In water bodies at opposite ends of the trophic spectrum, the dominant phytoplankton taxa are generally different (Watson *et al.* 1997). Zooplankton community type has also been linked with levels of eutrophy within a water body (Pejler 1983), while bacterial, phytoplankton and zooplankton biomass are all significantly enhanced by nutrient enrichment (Cottingham *et al.* 1997 & 1998). Within the zooplankton (and as recorded at WTC), the abundance of rotifers, cyclopoids, and cladocerans tends to increase with such loading, while calanoid abundance may fall to zero (Hussainy 1979, Maier 1996a; Adrian 1997, Pinto-Coelho 1998).

In smaller one or two pond waste stabilization systems, where treatment depends on extended retention times within each

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<sup>1</sup> High nutrient levels may help algal communities remain active throughout the year, despite other conditions being less than ideal. Seasonal variations still exist, however, with characteristic successions of dominance occurring over the yearly cycle (Mitchell 1980).

pond, loading-mediated succession may therefore present itself temporally, as nutrient concentrations begin to change within a lagoon. Such cycles of nutrient input and treatment may explain some non-seasonal temporal zooplankton successions (themselves overlaid on strong seasonal patterns) within such smaller systems as those investigated by Mitchell (1980) and Griggs (1993).

Such a temporal pattern in WSPs can be seen as analogous to that recorded in high rate oxidation ponds (HROPs). HROPs consist of single ponds in which treatment occurs via bacterial/algal interactions over time, while the development of zooplankton within the system is discouraged (due to the potential impact on phytoplankton dominance and stability: Sections 2.4.1& 5.3). Within the phytoplankton of HROPs, however, distinct temporal successions in species dominance occur according to the prevailing loading conditions (Borowitzka 1998). Inverse seasonal patterns between ciliates/rotifers and crustaceans may occur when these groups do develop in HROPs, but these patterns also include a strong component directly relating to influent nutrient concentration (Canovas *et al.* 1995). In the system studied by Canovas *et al.* (1995), concentrations varied seasonally due to different concentrations of organic input from agricultural operations, and shorter and longer retention times favoured the development of smaller and larger zooplankton species, respectively.

Non-seasonal temporal succession in zooplankton communities has been described in detail by Patil *et al* (1975), in regard to a large single pond system with high retention time. This lagoon displayed a distinct and "orderly process" of ecological succession in phytoplankton and zooplankton, culminating in a relatively stable community once water conditions stabilised. Bacterial and ciliate numbers were initially high, declining steadily over a period of weeks. *Brachionus* rotifers appeared during the third week after sewage influx to the pond, and peaked during the fourth.

Patil *et al* (1975) indicated that the predominance of certain plankton species was dependent on the loading conditions of the pond, with species assemblages depending on the degree of pretreatment and

mixing. They also stated that the reoccurrence of particular algal species during the purification process could be attributed to fluctuations in the nutrient loading entering the pond, and that taxa present under low nutrient concentrations were easily distinguishable from those in highly polluted waters. They concluded that food availability and associated biochemical changes influence plankton population dynamics, resulting in a marked succession of organisms during the course of sewage stabilization.

Hydraulic loading and the type of influent have elsewhere been identified as critical for the growth of the predominant planktonic species within particular lagoons (Uhlmann 1980). Nutrient availability is known to play an important role in the dynamics of planktonic communities, with phytoplankton dynamics related to effluent quality and zooplankton dynamics related to both phytoplankton abundance and effluent quality (Mitchell 1980). The growth of rotifer communities can also be reduced or prevented by implementing short retention times within a pond (Schluter & Groeneweg 1981). Increased eutrophication has led to direct changes in the structure and seasonal succession of zooplankton communities in polluted lake environments, and daphnid biomass in particular was strongly correlated with levels of total phosphorus and inorganic nitrogen (Pinto-Coelho 1998).

The distinct pattern of zooplankton distributions at WTC can be seen as a spatial expression of this type of nutrient-mediated succession. Because the layout of WTC permits extensive series of interconnected lagoons, treatment times can be spread across whole lagoon systems. Retention times within single lagoons are therefore much shorter than within smaller treatment systems (as per the observations of Schluter & Groeneweg (1981) listed above), and may be measured in days rather than weeks or months. Because of this, under constant flow, nutrient loading is more likely to vary between ponds than within them, and will vary markedly between ponds at each end of the system. With such a pattern at WTC, the extent of species appears to shift spatially across a system as nutrient loading varies, both at the inlet to the system and between subsequent ponds within that system.



Within each pond, the plankton populations respond to the prevailing conditions; conditions which theoretically correspond to nutrient concentrations at various stages of treatment within a single treatment pond of high retention time, and which host their own particular (temporal) planktonic communities.

Such spatial successions in zooplankton have been noted in the literature, ranging from passing observations to more detailed investigations. Mitchell (1980) reported a difference in a small, two pond system, noting that “fundamental biological differences” existed between the ponds, and that algae became more prominent in the second lagoon.

Such two pond systems do not offer the opportunity to examine or identify a full multiple-pond planktonic gradation in the form that is present at WTC. However, in another small, two pond system, Griggs (1993) not only recorded significant seasonal patterns in zooplankton abundance, but also noted biotic differences between the ponds that are in keeping with the patterns described here. Ciliates peaked at higher numbers in the first lagoon, while rotifer numbers were orders of magnitude higher in the second pond than in the first. Calanoid and cyclopoid copepods were absent and rare, respectively, from the first lagoon, and rare and relatively common, respectively, in the second. Daphnid cladocerans were also far more numerous (and seasonally persistent) in the second lagoon than the first (peaking at  $1750.l^{-1}$  in contrast to  $10.l^{-1}$ , respectively), while moinid cladocerans were more common in the earlier lagoon (peaking at  $3300.l^{-1}$ ), and generally far lower and more variable in number in the second (peaking at between  $100-200.l^{-1}$ ).

Bick & Scholtyseck (1960) reported species predominance in relation to loading in a three-pond sewage treatment system, including ciliates, rotifers, copepods, cladocerans and various phytoplankton in their results. Pudo (1978) described another three-pond system that can be seen to combine both temporal and spatial nutrient-mediated succession within its ponds. Within the first pond, bacteria and ciliates were initially very numerous, but decreased markedly as algal growth peaked. At the same time, rotifer numbers steadily increased towards

their own maximum. This can be compared to the type of temporal succession described by Patil *et al.* (1975), with the community structure matching the change in nutrient concentrations with time.

In other respects, however, the system described by Pudo (1978) can be seen to display characteristics of a spatial succession in keeping with that observed for WTC. The predominant organisms in the first pond did not extend into the second, and such pond characteristics as colour differed markedly between the two when algae dominated pond 1. Crustacean forms were relatively limited, and were restricted to the third pond. Notably, with the isolation of pond 1 and the inlet of sewage directly into pond 2, bacteria, ciliates and flagellates numbers rapidly increased in the latter lagoon. Pudo (1978) concluded that the development and succession of planktonic communities was largely dependent on the method of feeding sewage into the lagoons, combined with biochemical changes within each pond and seasonal influences.

Uhlmann (1980) also recorded a spatial succession - again in more compressed form - across connected waste stabilization ponds, and recommended it as a means of improving effluent quality by promoting zooplankton growth and clear-water phase grazing: in short an early recommendation for applying biomanipulation-style methods (Section 2.5). Uhlmann suggested that the arrangement of ponds in series would disconnect some feedback relationships within the food web but may increase stability. He indicated that this may particularly be the case for the final pond, where a 'persistent clearwater-state' could develop due to mass growths of *Daphnia* and other zooplankton in the absence of fish. Uhlmann noted that higher trophic levels, notably predatory zooplankton and fish, develop in such systems providing loading is not "too high", while microbial activity is in turn reduced to levels reflecting acceptable treatment.

Instead of designing and constructing large single ponds for wastewater treatment, Uhlmann (1980) promoted subdividing the total available area to provide a three-pond system. By using separate ponds, feedback loops in the foodweb were cut (as above, & Section 2.4.1), concentrating bacterial activity in the first pond, algal activity in the

second, and zooplankton in the third. [Mitchell (1980) also suggested connecting various ponds in series, but with a final pond promoting macrophyte growth to facilitate phytoplankton removal. Pudo (1978) notes that linking several ponds in series presents the simplest technical solution to treating sewage, while Canovas *et al.* (1995) highlight the harvesting potential of sequential ponds in which the later ones promote the growth of crustacean biomass].

In effect, Uhlmann (1980) was recommending a system like WTC, although, in keeping with Pudo (1978), with fewer ponds. He noted that single (non-series) pond systems are highly variable in terms of removal efficiencies, possibly because their “biological processes [are] nearly permanently... in a transient stage” (Uhlmann 1980). This is an accurate description of the continual, non-seasonal temporal cycling of plankton communities in such systems as those described by Patil *et al.* (1975).

Kawai *et al.* (1987) conducted further investigations in a small three pond system similar to that of Uhlmann. The system was again divided into anaerobic, algal and zooplankton ponds, with a view to promoting cladoceran growth in the final pond for potential harvest. The pilot system they trialled was very efficient in treatment quality and very productive for cladocerans; these tended to develop several days after the introduction of influent from the earlier ponds, while the authors report that operation of the system was at times “temporarily damaged” by the development of *Brachionus* rotifers.

Interestingly, the three pond systems of Uhlmann (1980) and Kawai *et al.* (1987) concentrate rotifer and crustacean zooplankton (including *Brachionus*, *Filinia*, *Daphnia*, and *Moina*) into the one pond, which is comparable to the overlap of these genera in the second lagoon of the two pond system studied by Griggs (1993). It should be noted that the greater extension in pond number (and consequent decrease in water retention time per pond) at WTC has permitted different zooplankton communities to establish across a range of ponds, with differences detectable even within specific zooplankton groups (e.g. moinid cladocerans tending to occur and peak in earlier lagoons than daphnids).

The beginnings of such a progression within zooplankton were observed by Wrigley & Toerien (1990) in a small four pond system where zooplankton biomass was low in the first three ponds and high in the fourth. Primary productivity was lowest in ponds 1 and 2, rotifers were present in ponds 2 and 3, copepods in ponds 2, 3, and 4, and cladocerans were restricted to and dominant in pond 4. Wrigley & Toerien (1990) related this distribution to high pH and ammonia levels in the first three lagoons.

### **5.3 Mechanics of nutrient-mediated successional patterns**

The basis for nutrient-mediated successional changes would appear to be an interplay of tolerance and competition between planktonic taxa. Whether loading is changing over time within a single pond system or spatially over lagoons in series, succession is closely related to the functional ecology of the lagoons, and the biological treatment that is occurring in each stage. An overview of the intricacies of such phytoplankton and zooplankton interactions and their interdependence on environmental conditions was given in Chapter 2, but will now be discussed specifically in relation to the nutrient-mediated successional patterns that have been identified here.

At early stages, bacteria predominate and reduce solids and dissolved organic matter, frequently under anaerobic conditions (Patil *et al.* 1975, Uhlmann 1980). Protozoa follow closely behind the bacteria and may play an important role in the stabilization of organic matter, with phagotrophic ciliates (Mitchell 1980) and flagellates (Sanders *et al.* 1989) grazing on the bacteria and regulating their numbers. Ciliate size and abundance have both been related to nutrient loading (Marchessault & Mazumder 1997).

As organic materials are broken down and carbon dioxide and mineral salts are produced (Mezrioui & Oudra 1998), conditions become more suitable for phytoplankton and associated forms, which begin to dominate. These forms are sensitive to several toxic substances

found within the early stages of sewage treatment, however, including heavy metals, pesticides and phenols, and -- at high concentrations -- compounds such as nitrate, ammonia and hydrogen sulphide (Mezrioui & Oudra 1998). High ammonia or sulphide concentrations may directly inhibit photosynthetic activity.

The occurrence of phytoplankton is therefore inhibited (as per Fanuko 1984) until such substances settle out or, as with ammonia, decrease in concentration across a system. Different algal species display differential sensitivity to toxic substances, and can be arranged in a successional sequence grading from high tolerance to low (Mezrioui & Oudra 1998). Algae in high rate oxidation ponds have also been observed to follow a specific pattern of species dominance and tolerance in accord with high, medium and low loadings of both organic carbon and ammonia (Borowitzka 1998). Overall phytoplankton biomass generally increases in response to increased nutrient levels, while species dominance will change and diversity will fall (Morgan 1985, Carpenter *et al.* 1995 & 1996, Blomqvist 1996 & 1997, Corkum 1996, Cottingham *et al.* 1998).

Once established, phytoplankton produce sufficient photosynthetic oxygen to maintain aerobic conditions (Uhlmann 1980), in turn suppressing anaerobic bacteria. Algae may also produce antibacterial substances and provide strong competition for available phosphates (Mitchell 1980, Sanders *et al.* 1989, DELM/DPIF 1996, Borowitzka 1998). Bacterial numbers may also be reduced by predation and flocculation.

Rotifer and crustacean zooplankton may initially be excluded by high ammonia concentrations (Uhlmann 1980, Wrigley & Toerien 1990), and oxygen availability may also be a major limiting factor (Kring & O'Brien 1976, Heisey & Porter 1977). Provided BOD levels fall below  $5\text{g.m}^{-3}\text{.d}^{-1}$ , "mass growths of filter-feeding zooplankton" will occur, including both rotifers and crustaceans (Uhlmann 1980, Section 2.3.1). Directly matching zooplankton and chlorophyll levels, however, is not an easy task (Moloney & Gibbons 1996), although the latter does provide an acceptable measure of food quantity and quality (Vanni 1986).

The rotifers appear to be more tolerant of highly eutrophic conditions than the Crustacea, and so will occur earlier in either a temporal or spatial nutrient-mediated succession. Small-particle feeding specialists are generally at a great advantage over macrofilterers in more highly eutrophic waters, as available food sources are limited to micro-algae and bacteria (Pejler 1983, Orcutt & Pace 1984, Mezrioui & Oudra 1998). Both rotifer and ciliate abundances may be related to the availability of particulate matter (Mendes *et al.* 1995). Pudo (1978) also noted that rotifers developed in particularly large numbers following peaks in algal numbers, when dissolved oxygen levels were high (Section 2.3.1) but not as high as those preferred by crustaceans (Uhlmann 1980). Rotifers may have a significant impact on bacterial and smaller-sized algal populations through grazing and may play a secondary role in the removal of organic matter (Mitchell 1980, Uhlmann 1980, Sanders *et al.* 1989).

Rotifer development is reduced under anoxic conditions, which may be maintained in early lagoons with short retention times (Schluter & Groeneweg 1981, Kawai *et al.* 1987). Freshwater zooplankton communities tend to be dominated by either large *or* small bodied species (Vanni 1986), however, and once the larger zooplankton begin to appear they rapidly outcompete the rotifers and other filter feeders (Uhlmann 1980, Gilbert 1985 & 1989, May & Jones 1989). Distinct inverse relationships develop between rotifer and crustacean taxa (Gilbert 1988, Lampert & Rothhaupt 1991, Fussmann 1996).

Cyclopoids outcompete and heavily predate upon rotifers (Williamson 1983, Plaßmann *et al.* 1997, Conde-Porcuna & Declerck 1998) and are in turn outcompeted by cladocerans (Vanni 1986, Maier 1996a). Although some cyclopoids may predate on cladocerans (Conde-Porcuna & Declerck 1998), the latter outcompete and reduce survivorship in naupliar stages which form a major cyclopoid food source (Vanni 1986). Daphnids are more effective filter feeders than smaller zooplankton (Plaßmann *et al.* 1997), and cladoceran grazing reduces algal and ciliate food resources available to rotifers, and may also impose direct predatory or mechanical damage on rotifers

themselves (Fussmann 1996). Rotifer numbers also significantly decline above pH 9 (Schluter & Groeneweg 1981), while cladocerans are able to tolerate pH ranges up to 10.5 (Vijverberg *et al.* 1996). Rotifers therefore develop in the period or ponds where oxygen levels are rising, but where photosynthetically-elevated pH levels and competition pressures are not yet too high (Mitchell 1980, Schluter & Groeneweg 1981).

Curiously, such pH and oxygen changes (along with temperature) may also act as two key factors driving seasonal successional patterns (Griggs 1993), and, in small pond systems, ciliates may dominate under winter conditions when rotifer and crustacean species are not present. Similar inverse correlations may also occur between rotifer and crustacean abundances on a seasonal basis (Orcutt & Pace 1984), while, as mentioned in the preceding section, trophic-driven changes in such factors may alter seasonal succession patterns (Pinto-Coelho 1998).

Although once considered detrimental to pond function, crustacean zooplankton (both cladocerans and copepods) can help reduce effluent BOD and suspended solids, as well as removing nutrients, phytoplankton and bacteria, and so are useful in pond management (Mitchell 1980, Uhlmann 1980, Dinges 1982, Mezrioui & Oudra 1998). Once rotifer and subsequent crustacean communities have developed, viral and bacterial numbers are low (Mitchell 1980), despite the release of nutrients via zooplankton grazing that would otherwise promote bacterial growth (Hargrave & Green 1968, Hygum *et al.* 1997, MacKay & Elser 1998).

Bacteria are reduced to less than 1% of their numbers at the start of the system and/or treatment period (Uhlmann 1980). Bacteria form a food source of varying importance to different species and size classes of rotifers (Ooms-Wilms 1997), while bacterial grazing by copepods is nil and has previously only been believed to be significant for cladocerans in the absence of algae (Hussainy 1979, Sanders *et al.* 1989). Such systems are not particularly well understood (Cottingham *et al.* 1997), however, as some cladocerans may predominantly live on bacteria (Ooms-Wilms 1997) and at high nutrient loading may act as the major regulating factor on bacterial abundance and production (Pace &

Cole 1996).

Cladocera are also known to take flagellates, and both copepods and cladocerans have a significant impact on ciliate numbers (Sanders *et al.* 1989, Burns & Schallenberg 1996, Fussmann 1996). Daphnids may reduce ciliate numbers by orders of magnitude, and dominance shifts towards larger grazers may be a more important regulating factor for ciliate communities than prevailing nutrient conditions *per se* (Marchessault & Mazumder 1997). In other systems, however, grazer control of ciliates may be minimal (Havens & Beaver 1997).

Phytoplankton levels may themselves be minimal due to grazing (Uhlmann 1980, Kawai *et al.* 1987). Rotifers, copepods and cladocerans consume different sizes and types of food, which greatly influences phytoplankton community structure and distribution (Green & Shiel 1992, Blomqvist 1997, Borowitzka 1998, Mezrioui & Oudra 1998, Pinto-Coelho 1998), although both large and small zooplankton fractions may display effective control of total phytoplankton biomass (Cottingham *et al.* 1997, Scharf 1997). Large diel variations in dissolved oxygen levels in early and facultative lagoons give way to smaller variation in later lagoons, reflecting zooplankton control of algal populations and photosynthesis (Mitchell 1980).

While initially absent due to other prevailing conditions (particularly oxygen and food availability, as above), daphnid cladocerans have high phosphorus and nitrogen requirements (Baudouin & Ravera 1972, Hussainy 1979, Sterner & Hessen 1994, Vrede *et al.* 1999) and, once present, may therefore become more directly linked to these concentrations than food availability *per se* (Gulati & Demott 1997, Van Donk & Lurling 1997, DeMott *et al.* 1998, Pinto-Coelho 1998). Daphnid numbers may therefore fall alongside such parameters towards the tail-end of a system.

Should there be higher level predation pressures, such as from fish, the numbers of larger zooplankton may also be greatly reduced, if not entirely removed (White 1975, Marchessault & Mazumder 1997, Pinto-Coelho 1998). Relieved of competition, rotifers and smaller crustaceans (including other Cladocera and copepods) will



continue to dominate (Benndorf 1990), and, while microbial levels will still be reduced to a minimum, phytoplankton levels will remain high (White 1975, Uhlmann 1980, Breen 1983).

#### **5.4 Stability of nutrient-mediated successional patterns**

The observations made at WTC during this study strongly support and enhance the previous records of temporal and spatial nutrient-related successional stages in zooplankton communities. It is suggested that such strong patterns as those listed above are responsible for the overall stability and consistency achieved within and between the plankton communities of the two sides of the 85wB system in this study.

Previous researchers, such as Vanni (1986), have commented on the notorious variability of plankton populations on a small scale and over a short term, even within the same site or season. Within WTC and other waste-water systems, however, it is possible that such variation is greatly reduced by the highly eutrophic status, scale, and specific characteristics of the lagoons themselves. Shallow water bodies are more resistant to change in high eutrophic status, due to (i) their higher surface area to volume ratios, which allow for greater relative impact from internal and external nutrient loading, and (ii) "inherent biological buffer mechanisms" in their plankton dominated systems (Moss *et al.* 1991, Griggs 1993).

Systems such as those at WTC may therefore be buffered against change by the decreased diversity but enhanced biomass that they contain, and the set conditions/responses that produce them. [Decreases in such stability - as nutrient levels fell across the system - may have combined with differences in pond size and shape to explain the increased variation observed between the later pond pairs in the 85wB (pairs 4 and 5)].

In recording the distinct temporal changes that occurred in their one and three pond systems, respectively, both Patil *et al.* (1975)

and Pudo (1978) commented on variability within and between wastewater lagoon systems, while Dinges (1982) stated that different systems are unique entities driven by their own particular influent and environmental regimes. Patil *et al.* (1975) also noted that waste stabilization ponds were unlikely to display universal behaviour in regard to exact plankton community composition, as this would vary according to region, light intensity, waste characteristics and overall environmental conditions. However, they did conclude that the (nutrient-related) ecological succession pattern that they had outlined was likely to be integral to waste stabilization ponds, and would be expressed by at least a few of, and possibly many, members of the planktonic community.

Despite large global and local variation in effluent quality and climatic conditions, WSP algal communities have been found to be composed of a restricted set of genera that is even less diverse than in natural eutrophic waters (Mezrioui & Oudra 1998). Similarly, the persistent occurrence and dominance of such staple zooplankton genera as *Brachionus*, *Filinia*, *Polyarthra*, *Asplanchna*, *Daphnia*, and *Moina* (Pudo 1978, Mitchell 1980, Uhlmann 1980, Vanni 1986, Griggs 1993, Mendes *et al.* 1995, and this study) suggests that WSP plankton community composition may be more consistent and cosmopolitan than Patil *et al.* (1975) thought.

In regards to phytoplankton, Mitchell (1980) noted that local and regional floras do not develop and that inoculations are largely ineffective in altering species composition. Zooplankton biomass and composition in other large systems also show some degree of stability in the face of experimental manipulation (Dawidowicz 1990). Mesocosms subjected to constant nutrient loading rates have ultimately developed similarities in character despite being subjected to different non-nutrient treatments (Cottingham *et al.* 1997), rapid whole-lake nutrient enrichments have produced comparable ecological changes to those of slower, longer term eutrophication (Cottingham *et al.* 1998), and consistent algal community responses have been recorded in systems irrespective of their developmental starting point (Blomqvist 1997).

Despite their doubts over the extent of universal character among WSP successional communities, Patil *et al.* (1975) still declared them to represent a distinct and “orderly process” that would be comparable between regions. In contrast, Pudo (1978) concluded that such communities were highly variable in community composition and size, both within individual ponds and between periods of time. This conclusion is not surprising, as the order and stability present in Pudo’s system was masked by both spatial and temporal successional changes as described in Section 5.2. The spatial (‘between pond’) changes are only apparent when viewed in the context of other systems, while the variability within ponds (particularly the first pond on isolation from the system, and the second on receiving direct sewage influent) reflect the “transient stages” of nutrient-mediated community shifts also observed by Patil *et al.* (1975) and identified by Uhlmann (1980) for high retention times.

In promoting the spatial expression of these successional changes by connecting lagoons in series and (effectively) reducing retention times<sup>2</sup>, Uhlmann (1980) indicated that the treatment efficiency and overall stability of a lagoon system and its planktonic communities would be greatly enhanced. Individual ponds would no longer remain in permanently transient biological stages, and would exhibit “high adjustment stability” in the face of potentially disruptive perturbations. Wrigley & Toerien (1990) found their series of four small sewage lagoons to represent a “robust and stable system”, and noted that the self-regulation potential of sewage lagoons was high.

At WTC, pond performance has been efficient and consistent in spite of highly variable weather conditions and physical (non-nutrient) influent characteristics, even over “critical seasonal changing periods” (Caldwell Connell Engineers, 1975). WTC systems have also recovered to normal operating efficiency within a week of disruptive events (*ibid.*)<sup>3</sup>. In Tasmania, even under-performing and inadequately

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<sup>2</sup> Either by dividing the total area of a single pond into several cells connected in series and maintaining the same flow rate, or by connecting lagoons covering a larger or smaller total area and increasing or decreasing the flow rate accordingly. Smaller ponds also provide the advantage of reducing the hydraulic short circuiting of pond retention times due to stratification (Uhlmann 1980).

<sup>3</sup> Including overloading events, which may have produced temporary nutrient-related successional shifts and corresponding changes in treatment ability and consistency.

designed/managed<sup>4</sup> lagoon systems show some degree of stability, with seasonal drop-offs restricted to only some performance measures (although recovery during winter periods may take months: DELM/DPIF 1996). Due to the relatively consistent chemical nature of these ecosystems and the biological responses they produce, WSPs can therefore present as very predictable and stable systems, surprisingly well buffered against change and chance variation.

It should be stressed that such 'stability' does *not* automatically preclude the "boom-and-bust" cycling that often occurs within zooplankton populations. Complex processes are rarely controlled by single factors (Clymo 1995), and cycles may be complicated by the interplay of both bottom-up and top-down forces (Scharf 1997). It is therefore suggested that nutrient-mediated succession (and the stability provided by maintaining nutrient levels) is expressed by community *type*, falling between both longer term seasonal succession patterns and shorter term population cycles. As such, cyclic change still occurs between and within each nutrient-matched community, and both seasonal and population level changes are strongly in evidence in the data presented in this thesis.<sup>5</sup>

Such mixtures of seasonal, trophic-related and shorter-term zooplankton community and population variation have recently been recorded in a eutrophic lake by Pinto-Coelho (1998), and it would appear that these are controlled by environmental, chemical and biotic factors, respectively (Fussmann 1996). In a unique large-scale study investigating the synchronous and identical nutrient enrichment of multiple neighbouring lakes, Cottingham *et al.* (1998) found that consistent planktonic responses were produced in community level characteristics such as biomass and productivity, but similarity declined dramatically in moving from broader taxonomic groupings to comparisons of specific species<sup>6</sup>. The same authors suggest that the high level of abundance expressed across such broader groupings

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<sup>4</sup> Frequently overloaded.

<sup>5</sup> These cycles may have been responsible for some of the variation in established patterns between the 85wB lagoons.

<sup>6</sup> Such a result agrees with the general findings and conclusions of this study, and indicates that the lower level of taxonomic resolution for the crustacean zooplankton is of lesser importance than the other factors suggested in Section 5.1 as explaining the responses of this group over the equilibration period.

provides a strong community level ecological buffer against all but the most disruptive events, whereas individual species within a given grouping may cycle according to finer scale variations and environmental factors (Cottingham *et al.* 1998).

The inter-dependent and cyclic nature of phytoplankton and zooplankton blooms (Hussainy 1979, Uhlmann 1980, Section 2.4.1) means that individual population levels are rarely constant. However, some populations may maintain steady conditions for months, potentially representing equilibrium points that are re-attainable after disturbance (Uhlmann 1980), such as when the balance between different taxa is disrupted. Following the suggestions of Uhlmann (1980), such cyclic patterns may be partially reduced by separating those taxa in a flow-through system, and promoting stability by removing certain feedback loops. While nutrient-mediated successions do not remove or replace seasonal or smaller scale cycles, the excess nutrient levels present in highly eutrophic systems may still allow species to persist or occur to some degree outside their normal seasonal patterns (Maier 1996a)<sup>7</sup>.

The phytoplankton and zooplankton present in such systems are obviously capable of surviving and thriving in extremely rich nutrient concentrations. The resulting reduction of diversity with high production and increased abundance is the case for WSPs (Mitchell 1980), HROPs (Canovas *et al.* 1995) and eutrophic waters generally (Cottingham *et al.* 1998), and is typical of highly polluted environments. As such, disrupting the standard WSP successional pattern is unlikely to be easy, but shifting it in either direction through the lagoon system is possible.

Unlike the systems of Patil *et al.* (1975) and Pudo (1978), large, continuous, and high-flow systems such as those at WTC generally avoid nutrient-related successional changes within a pond. This is achieved by maintaining relatively constant inflow levels<sup>8</sup> and

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<sup>7</sup> For example, *Daphnia* was prevalent in the 85wB system over summer, despite being listed as a cold water form by Mitchell (1980), with cyclopoids becoming seasonally abundant in its absence.

<sup>8</sup> Some variation in these levels does occur, although the general pattern is stable at WTC due to the large numbers of ponds per system and the ability of the pattern to shift across them. In the 85wB over the experimental period (where inflow levels were highly variable, and low flow rate meant the pattern was condensed towards the start of the system) there was still some variation within

lower retention times within each cell of the system. In analysing influent characteristics at WTC, Constable (1988) found that BOD and suspended solid levels varied significantly on a daily, weekly, monthly and long-term basis (which is not surprising given the seasonal and daily changes in phytoplankton alone). In contrast, nutrient levels -- including organic nitrogen and ammonia -- remained relatively consistent.

Within this study, the zooplankton community patterns associated with such nutrients have also proven to be remarkably stable. It is clear that the ecology of the WTC lagoons is highly dependent on the continued input of nutrients from external sources, and is strongly influenced by variations in loading as controlled by flow. Such inputs and alterations of flow therefore provide a direct and potentially profound method of manipulating nutrient-mediated successional changes and altering the established communities within lagoons. The potential value of these types of manipulations to plankton harvesting are discussed in the following chapter.

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particular ponds. In some cases, the spatial succession was drawn forwards to the point of rotifers peaking in the first lagoon.

## 6. GENERAL DISCUSSION

### 6.1 Zooplankton harvesting

The aim of this work was to attempt to manipulate the zooplankton populations of the 85wB lagoon system in a predictable manner, as a preliminary step towards the management and potential harvest of material from the lagoons. The manipulations have proved successful, demonstrating that community structure and distribution can be shifted (and potentially extended) within a lagoon system according to changes in the prevailing flow rate. However, the potential for harvesting this material from the lagoons still needs to be addressed.

Preliminary harvesting experiments were conducted within the 85wB system in the course of this project, but are not reported in detail here. [These experiments were designed to investigate the effect, if any, of intensive harvesting using the purpose built vessel 'Baleen' (Plate 3.2) on the zooplankton within harvest lagoons, and these data are still being analysed: Appendix 3].

Zooplankton harvesting at WTC is not only possible, but is definitely feasible. The number of large (cladoceran) zooplankton required for efficient Baleen harvesting is  $50.l^{-1}$ , while the relative costs of harvesting copepod nauplii are dramatically reduced when density increases beyond  $400-500.l^{-1}$  (Zootech Pty. Ltd. data). Passive harvesting techniques, such as through outfall screens (Plate 3.2), carry minimal initial costs and almost zero operational costs, and so are cost effective at even lower zooplankton densities.

These levels are frequently matched and exceeded by zooplankton populations in WSPs. In the third pond of his system, Uhlmann (1980) reported *Daphnia* concentrations of more than  $1000.l^{-1}$  -- some 20 times the harvestable level -- and commented that "in a properly managed 'zooplankton pond' excess zooplankton biomass can easily be harvested by a fine-meshed net in the outflow [as per Zootech's outfall screens] and used for fish farming (in particular, for fry) in ponds operated in parallel."

In the course of this project at WTC, zooplankton were present in harvestable quantities on a frequent basis, particularly over the warmer months (Figures 4.13-4.22 and Appendices 2 & 3)<sup>1</sup>. Within the spatial succession, specific zooplankton groups did not necessarily show high abundance in all ponds in which they occurred, but peaked (and cycled) at particular points within their distributions.

In the data presented in this project, *Daphnia* and *Moina* were recorded at densities of up to 407.5.l<sup>-1</sup> and 1125.l<sup>-1</sup> (pond 2N at low flow on 8 August 1994 and SPS at low flow on 22 December 1994, respectively). Adult copepods were recorded at up to 650.l<sup>-1</sup> (SPS at high flow on 23 February 1995), while copepod nauplii were recorded at up to 3546.l<sup>-1</sup> (pond 2S at low flow on 19 January 1995). Total rotifer numbers reached densities of up to 74,925.l<sup>-1</sup> (28 November 1994 in the SL pond), and were frequently in the thousands early in the system.

It should be noted that the low but highly variable flow rate into the system often shifted zooplankton communities and population peaks earlier in the system, and so rotifer peaks such as the one listed above often occurred in the SL pond. Despite this, the general pattern is still reflected in the above (if concentrated towards the start of the system), with the rotifer peak occurring in the earliest pond, moinid and copepod peaks occurring in the next pond, and daphnid/nauplii peaks occurring later in the system.

The numerical dominance, by orders of magnitude, of rotifers over crustaceans in their respective peaks is in keeping with general patterns exhibited in the literature, although such patterns have usually been observed in a seasonal context (Orcutt & Pace 1984). Such dominance indicates that despite their smaller size, rotifer biomass may at times be comparable to that of the crustaceans.

The most productive single date/pond during this project was recorded during the harvesting experiment (referred to above). On 13 March 1996, pond 1S contained 3350.l<sup>-1</sup> *Daphnia*, 975.l<sup>-1</sup> *Moina*, 1350.l<sup>-1</sup> adult copepods, 1850.l<sup>-1</sup> total nauplii, and rotifer numbers were extremely high<sup>2</sup> at 110,150.l<sup>-1</sup> (rotifer numbers may well have been

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<sup>1</sup> Summer/early autumn periods were also the preferred harvesting times for algae (Caldwell Connell Engineers 1975).

<sup>2</sup> Although extremely high, freshwater rotifers cultures in piggery waste have been recorded at



higher in earlier ponds not incorporated in this experiment). Such levels are markedly higher than those reported for non-sewage eutrophic waters (Orcutt & Pace 1984). In total, 117,675 organisms per litre were recorded in this pond with all significantly above harvestable levels (Cladocera alone were 86.5 times the harvestable level). The flow rate into the system at this stage was consistently above the target level (Day 1023, Figure 4.2).

Harvesting has been carried out at WTC with highly successful results. Outfall screens have harvested as much as 2 litres<sup>3</sup> (2++ kg wet weight) of zooplankton material a minute through larger screen apertures, and as much as 1 litre a minute through smaller apertures, which are subject to faster blockage (Zootech Pty Ltd data). In just under 15 hours, Baleen harvesting removed 794.5kg (wet weight) of primarily daphnid zooplankton from pond 2S in the 85wB system (Appendix 3). This equated to an average rate of 53.86kg.h<sup>-1</sup>, even though the daphnids were frequently well below desirable levels over that time. Baleen harvesting at peak population levels has been even more productive (Zootech Pty Ltd data).

It is worth noting that trials of an algal harvesting process at WTC have previously produced promising results, both in terms of the harvesting process and the quality of the material harvested (Caldwell Connell Engineers 1975). As with the current interest in zooplankton, the harvested algae were proposed as a means of reclaiming waste nutrient resources that could provide a value-added return as a high-protein feed source (in this case for cattle, sheep, pigs and poultry: Caldwell Connell Engineers 1975). In addition, algal harvesting presents a far greater avenue for enhancing nutrient removal from wastewater than the harvest of zooplankton alone (Mitchell 1980). The viability of algal harvesting was directly related to consistency in favourable algal growth conditions (Caldwell Connell Engineers 1975), which, alongside seasonal considerations, may be greatly influenced by the potential stability that can be induced in systems via the manipulation of nutrient related successional patterns (Section 5.4).

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200-580 animals per ml, or 200,000-580,000/l (Groeneweg & Schluter 1981), while high-density laboratory cultures of marine rotifers have been recorded at 10,000-30,000 animals per ml, or 10,000,000-30,000,000/l (Yoshimura *et al.* 1996).

<sup>3</sup> This figure reflects the volume of material harvested, *not* the volume of water filtered.

In addition to the aquacultural value of the harvested material (Section 1.0), its removal from these lagoon systems may be beneficial to wastewater treatment and nutrient removal strategies as a whole. Both algae and zooplankton represent large pools of nitrogen and phosphorus within the lagoons. By dry weight, *Daphnia* species alone contain 1-2% phosphorus and 6.6-9.25% nitrogen (Baudouin & Ravera 1972, Hussainy 1979, Mitchell 1980, Sterner & Hessen 1994, Vrede *et al.* 1999). As with fatty acid (carbon) composition (Sekino *et al.* 1997), these levels are relatively consistent between species, and represent some of the highest nutrient contents for zooplankton (Pinto-Coelho 1998). Annual net productivity for *D. carinata* in Australian sewage lagoons has been calculated at 345g dry wt per m<sup>2</sup>, which is among the highest recorded for planktonic crustacean populations (Mitchell 1980). In South America, daphnid productivity in sewage has been calculated as 35mg.l<sup>-1</sup> dry weight, representing 700kg per day for a city of 100, 000 people (Kawai *et al.* 1987).

As well as collecting nutrients, zooplankton continually re-release large quantities of these compounds through grazing activities and biological processes (Hargrave & Green 1968, Wright & Shapiro 1984, Hygum *et al.* 1997, MacKay & Elser 1998, Vrede *et al.* 1999). Calculations by Mitchell (1980) indicated that more nutrient is recycled into the water column through daphnid biomass than is contained in that biomass, and, as such, daphnid zooplankton represents a relatively low nutrient store of phosphorus and nitrogenous material compared to that retained within a lagoon annually. Mitchell concluded that the harvesting of such material would therefore not be useful for the overall removal of nutrients from sewage lagoons. However, his calculations were based on maintaining *status quo* conditions, and productivity of the zooplankton crop was calculated as *net* annual production.

Unharvested zooplankton populations not only continue to recycle nutrients, but also re-release the nutrients bound in their tissues once they die. Frequent harvesting of zooplankton material would bypass this additional loss, effectively enhancing nutrient removal by

exploiting productivity in a manner more closely reflecting gross rates than net ones. As Mitchell (1980) indicated, such harvesting would need to be conducted every few days to fully exploit population turnover times. With such methods as Baleen harvesting, this is no longer as expensive or logistically difficult a proposal as he believed. Indeed, passive harvesting through such methods as outfall screens can effectively be conducted both continuously and with minimal operational effort.

Zooplankton responses to combined active and passive harvesting pressures may in turn allow exploitation of the intrinsic rate of increase of zooplankton populations by stimulating greater incorporation of nutrient into biological material. Harvesting may also be timed to exploit diel cycles such as vertical migration, not only to harvest higher crustacean densities but to collect larger proportions of animals with markedly higher carbon and nitrogen content (which may exhibit a greater tendency to migrate due to better body condition: Hays *et al.* 1998).

The harvesting of other zooplankton groups may also be valuable with regard to nutrient removal, although these groups have received relatively little attention in the past. Orcutt & Pace (1984) point out that rotifers may actually play the dominant role in annual secondary production in some circumstances, and that their biomass may equal that of crustaceans. With their shorter generation times, higher production rates, and higher intrinsic rates of increase, rotifers can play a major role in the energy flow and nutrient recycling of particular lagoons, and so their turnover may be more valuable in terms of nutrient removal via harvesting. In a similar way, the harvesting of algae has also proven to be beneficial to such systems (Caldwell Connell Engineers 1975, Mitchell 1980).

In addition to nutrient removal, zooplankton harvesting may provide an efficient means of removing accumulated heavy metals such as copper, cadmium, zinc, nickel, lead, chromium and iron (Hussainy 1979), and this would still provide a treatment benefit even if nutrient removal should prove minimal. Such removal does not necessarily preclude the use of harvested material as fish feed,

however. Hussainy (1979) notes that the levels of these metals in zooplankton at WTC are comparable to figures for freshwater zooplankton found elsewhere, while substantial removal (85% +) of heavy metals tends to occur in the anaerobic and early lagoons of a system, where bacterial and viral levels are also at their highest (Section 5.3). It would therefore be possible to harvest for treatment purposes early in a lagoon system, and for aquacultural and (potentially) further treatment purposes later in a system.

## **6.2 Combining harvesting with the nutrient-mediated successional pattern**

Recognition of the nutrient-mediated successional pattern at WTC has potentially important benefits for harvesting zooplankton material. It identifies a specific and major mechanism of community change falling between those of seasonal successional patterns and cyclic variations in populations, and can potentially be manipulated to introduce stability to the system and maintain or prolong the presence of certain dominant community types.

With further investigation, control of nutrient loading and retention times (via variation of flow rate or the introduction of multiple inlet points between or bypassing set lagoons) may allow the shifting or elongation of chosen community types and distributions across specific lagoon systems. Larger numbers of ponds may be manipulated to host species of particular harvest value, or peaks in the abundance of target communities may be shifted to ponds providing more suitable access or harvesting characteristics.

As discussed in Section 5.5, however, while consistent nutrient loading may increase the stability of zooplankton community types within the system, populations of those zooplankton may frequently cycle below harvestable levels. It is therefore possible that harvesting may itself be used as a tool in conjunction with nutrient loading to minimise population peaks and troughs. In short, it may be possible to gear harvest regimes to rates which simulate or delay

population crashes, breaking or minimising the 'boom-or-bust' cycle to maintain zooplankton growth at a constant and rapid replacement rate.

Such short term variations in planktonic populations are usually the result of biotic interactions (Fussmann 1996). Mitchell (1980) noted that ciliate grazing may play an important role in preventing self-limitation in bacteria, and that the bacterial community of early ponds continues assimilating organic material at a high rate as a result. Moderate fish predation may similarly promote population stability among larger zooplankton by helping avoid mass starvation and anoxia-driven mortality due to overpopulation (Benndorf 1990). No fish are found in the lagoons of WTC (Hussainy 1979), and while birds, water beetles and predatory zooplankton may have some impact on zooplankton communities, their influence is obviously not enough to keep populations in check.

It is therefore possible that harvesting may act as a 'predatory' influence on the zooplankton communities in these lagoons, and could be used to minimise cyclic extremes in lagoons/community types of particular harvesting interest, or for specific harvesting periods. Algal biomass may at times be limited by losses within a system rather than by restrictions on growth (Knowlton & Jones 1996). Dawidowicz (1990) states that the biomass of zooplankton required to control phytoplankton density is not unreasonably high, and it may be possible, pending investigation, that harvesting effort for zooplankton need not be as intensive or sustained as would first be assumed. This may particularly be the case with the larger (crustacean) zooplankton, which have lower reproduction and growth rates than the rotifers. Low elimination rates are sufficient to maintain numbers and biomass of net algae, which in turn have longer generation times than nano-phytoplankton (Dawidowicz 1990). Harvesting efforts would need to be more dramatic for rotifer communities, however, due to their shorter generation times, higher production rates and higher intrinsic rates of increase (Orcutt & Pace 1984).

If harvesting efforts *can* be used to influence zooplankton dynamics, difficulties will lie in determining and adjusting harvesting regimes to suitable levels. Insufficient rates will not markedly affect the

usual population cycles, while excessive rates will suppress zooplankton populations by triggering and sustaining population crashes in the same way as high levels of predation (Sections 2.4.6 & 2.5). Large zooplankton populations can exert this level of control on phytoplankton, leading to the 'clear water phase' (Uhlmann 1980, Dawidowicz 1990) which can either result in their own population collapse or they can remain stable for some time (months or longer: Uhlmann 1980). Dense stocks of planktivorous fish can substantially increase the stability of phytoplankton blooms (Uhlmann 1980), and this effect may be reproducible through harvesting pressure.

Cultures of the marine copepod *Acartia tsuensis* have been found to withstand harvesting rates of up to 30% of their populations per day (Ohno *et al.* 1990). Harvest efficiency increases dramatically within the first 10%, plateaus between 10% and 30%, and has a negative impact on populations beyond that. Moderate harvesting pressure is beneficial for population stability by lowering density-dependent mortality and other inhibitory factors, including cannibalism between copepodid and naupliar life stages, reduced growth rates and fecundity, and increased developmental time prior to reproductive activity.

Under natural conditions, however, such biological manipulations may be fraught with complexities (Section 2.4.6). Excessive removal of larger zooplankton may result in the dominance of smaller species, which may in turn lead to an increase in net algae as grazing pressure is shifted to nanoplankton alone (Dawidowicz 1990). Changes may then occur in nutrient cycling within the lagoon, and phytoplankton-bound materials might be liberated directly and indirectly into the water column in greater quantities (Hargrave & Geen 1968, Mitchell 1980, Dawidowicz 1990). While influencing population dynamics and community structure, the constant removal of zooplankton material can, however, be carried out at rates that do not disrupt the ultimate and inherent stability of such systems (Cottingham *et al.* 1997).

As populations of phyto- and zooplankton increase in size and turn over at a greater rate in spring/summer (Mitchell 1980, Geller 1986, Griggs 1993), harvesting pressures would need to be adjusted on a

seasonal basis. While lagoon systems operate all year, loading patterns may also require some seasonal adjustment due to changing responses in the biota (Caldwell Connell Engineers 1975). In all, it is unlikely that any harvesting regime could precisely match the intrinsic rate of increase in a zooplankton population for long, but it may be able to help prolong cycles and maintain the rapid growth phase for a greater period. As this would minimise population peaks as well as troughs, it would serve to optimize plankton harvest over time rather maximize it at a particular time.

While requiring far more research, the potential of using nutrient-mediated successional patterns (as manipulated in this project) to stabilise zooplankton community type, while developing harvesting regimes to stabilise population numbers, poses an attractive possibility for optimizing zooplankton yield from wastewater lagoons.

### **6.3 Future work**

As foreshadowed in earlier chapters, the work presented in this thesis is only a preliminary investigation into the manipulation of zooplankton populations at WTC. While this project has demonstrated that successful manipulations can be implemented in a predictable manner and at a large scale at the Complex, that scale has in turn limited the ability of this project to characterize or test those manipulations in greater detail. Consequently, many avenues of investigation remain open, and several important questions remain unanswered.

While future work should concentrate on characterizing and manipulating the nutrient-mediated succession in more detail and investigating the potential interplay and regulating effects of harvesting intensity and frequency, the experimental protocol should also be refined. This type of manipulation should not only be repeated in time and over longer periods, but preferably also across a greater number of comparable and truly separate systems. The gap between the

comprehensive study of ecological responses permitted by full-scale manipulations and the greater experimental control and replication afforded by mesocosm, laboratory and enclosure scale experiments has long needed bridging (Mitchell 1980, Uhlmann 1980, Morgan 1985, Vanni 1986, Kawai *et al.* 1987, Bloesch *et al.* 1988, Hairston 1989, Benndorf 1990, Carpenter & Kitchell 1992, Clymo 1995, Cottingham *et al.* 1997).

The 85wC system at WTC provides a lattice-like arrangement of similar sized lagoons which could potentially be altered to provide several side by side, near-identical series of lagoons. (The construction of a large scale experimental system along these lines has also been discussed, and would prove a valuable addition to experimental investigations at WTC). Such systems would help reduce the risk of pseudo-replication, as was forced on this project by necessities and limitations in design.

Future experiments should also be run at higher and more consistent loadings/flow rates than were possible during this study, both to maximize differences between pond communities and to stabilise the patterns and conditions producing them. Greater flow rates will help determine the degree to which plankton types, communities and peaks can be shifted and extended through a system. Other methods of manipulating nutrient levels and successions should also be tested, potentially including the isolation of ponds (and documentation of changes over time) and the provision of multiple inlet points to a system, whereby different influent strengths to specific ponds can be mixed or altered by design.

The interplay and overlap of seasonal and nutrient-mediated successional effects requires further attention, as do the flow-on effects between one trial and the next. The influence of environmental factors (such as on large scale evaporation from lagoons) needs to be characterized, particularly where flow rate is adversely affected. Broader investigation of the effects of nutrients would also be of value, both in covering a greater range of compounds and determining lag times between nutrient changes and cycles within the planktonic web (*a la* Davidson & Cunningham 1996).



While this project has specifically investigated the most common rotifer and crustacean groups in these lagoons (i.e. those of greatest harvesting potential), other taxa and the overall biological pattern deserve further attention. The incorporation of bacteria, protozoa and algae, amongst other groups, can only produce a stronger and more defined successional pattern. Ultimately, these factors should be combined in a model of zooplankton manipulation, tailored to the unusual environments of such lagoons and the unusual community characteristics they display.

## 6.4 Summary

In conclusion, this project has shown that:

- A distinct pattern, or spatial succession, can be seen in the zooplankton communities of the 85wB lagoon system at the Werribee Treatment Complex, overlying long-term seasonal variation and short-term population cycles.
- The pattern appears to be related to the nutrient loading of the lagoons (as expressed through flow rate) and is in keeping with patterns previously identified at the Complex, as well as with similar but less defined loading-related patterns in the literature.
- This pattern can be successfully manipulated in a predictable and consistent manner, and these manipulations can be successfully conducted at a large scale.
- The use of this pattern to stabilise community types *between* lagoons (bottom-up nutrient control) may one day be coupled with specially devised harvesting regimes to stabilise population numbers *within* lagoons (top-down 'predatory' regulation), ultimately providing an optimized level of zooplankton product across a system.

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# **APPENDICES**

## **Appendix 1:**

Raw physical, chemical and chlorophyll data from the main experimental period, as presented in the main text.

## **Appendix 2:**

Raw zooplankton data from the main experimental period, as presented in the main text.

## **Appendix 3:**

Sample harvest data, as referred to in the General Discussion (Section 6.1).

N.B. further chemical & biological data and experimental work collected/conducted during the course of this project is not presented in this thesis.

<b>Appendix 1:</b>						
<b>Flow rate (FR) data, ML/d</b>						
Flow rates were calculated from cumulative wheel readings at the start of the 85wB system (Plate3.1) and elapsed time since the last reading (averaged to flow per day for the preceding period).						
<b>DATE</b>	<b>DAY</b>	<b>Av. ML per day</b>		<b>DATE</b>	<b>DAY</b>	<b>Av. ML per day</b>
04.02.94	255	7.618380356		29.11.94	553	7.24
10.02.94	261	9.186951741		30.11.94	554	9.47
17.02.94	268	11.76238294		01.12.94	555	9.61
24.02.94	275	11.69899259		02.12.94	556	8.2
03.03.94	282	10.9689048		05.12.94	559	8.792318504
10.03.94	289	10.20586268		08.12.94	562	8.185083135
17.03.94	296	11.77469388		16.12.94	570	7.954315157
24.03.94	303	10.27799087		22.12.94	576	7.830743802
31.03.94	310	10.52154506		29.12.94	583	8.032534055
07.04.94	317	9.127407407		05.01.95	590	8.407030663
15.04.94	325	12.12816754		09.01.95	594	10.35615424
21.04.94	331	9.721666667		12.01.95	597	4.788477543
28.04.94	338	9.569077599		19.01.95	604	6.810816902
05.05.94	345	12.31533199		27.01.95	612	7.581539804
12.05.94	352	8.937718777		03.02.95	619	9.138836301
19.05.94	359	6.161568325		06.02.95	622	10.30998904
26.05.94	366	6.257694026		09.02.95	625	10.73526325
02.06.94	373	4.63518937		16.02.95	632	10.68563724
09.06.94	380	7.437322976		23.02.95	639	7.927932968
16.06.94	387	7.614222748				
23.06.94	394	9.205556097		03.04.95 NUT	678	9.421411201
30.06.94	401	8.282118299		04.04.95 NUT	679	9.702173379
08.07.94	409	9.329749702		05.04.95 NUT	680	11.8
14.07.94	415	7.824074629		06.04.95 NUT	681	10.70822494
22.07.94	423	5.349439837		07.04.95 NUT	682	12.32278746
28.07.94	429	8.066539373		08.04.95 NUT	683	11.86823529
05.08.94	437	6.859010699		09.04.95 NUT	684	11.69940984
11.08.94	443	6.871902239		10.04.95 NUT	685	10.29146853
18.08.94	450	6.178715483		11.04.95 NUT	686	13.47882353
25.08.94	457	6.151950995		12.04.95 NUT	687	9.198062284
01.09.94	464	8.480242421				
08.09.94	471	10.54183928		04.12.95 DEG	923	11.08217591
16.09.94	479	9.927780287				
22.09.94	485	10.32666899		13.03.96 HAR	1023	12.98957362
29.09.94	492	10.25257843		14.03.96 HAR	1024	15.6913879
06.10.94	499	10.12244597		15.03.96 HAR	1025	14.53951557
13.10.94	506	10.15890176		18.03.96 HAR	1028	15.9233003
20.10.94	513	9.055368321		19.03.96 HAR	1029	15.24826118
27.10.94	520	8.146876157		20.03.96 HAR	1030	15.06675881
03.11.94	527	7.46497594		21.03.96 HAR	1031	14.56744186
10.11.94	534	10.30276588		22.03.96 HAR	1032	15.17676349
17.11.94	541	8.058171618		25.03.96 HAR	1035	14.92128199
24.11.94	548	8.342758597		26.03.96 HAR	1036	13.71927273
28.11.94	552	6.99375		27.03.96 HAR	1037	14.0680678

**Appendix 1:**  
**Ammonia (NH3) data, mg/l**

Date	Day of Expt	SL(1)	SPN(4)	1N(3)	2N(7)	3N(8)	4N(N1)	5N(N2)	SPS(4)	1S(5)	2S(6)	3S(9)	4S(10)	5S(11)
18.11.93	177	15.2	NS	27	8.8	1	0.8		17	6	3.6	4.8	5.6	
02.12.93	191	23	21	14	6	2.8	0.6	0.6	17.5	15.5	7.8	3.6	5.8	
06.01.94	226	NA	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
27.01.94	247	NA	NA	NA	NA	NA	NS		NA	NA	NA	NA	NA	
10.02.94	261	7	20	9.666	3.4	2.4	0	0.08	16	9.666	1.6	1.4	0	
24.02.94	275	16	15.5	10.5	6.4	1.4	0.8	0.8	11	10	4.4	1.2	0	1
10.03.94	289	26	36	18	9.5	2	1		35	25	6	2	1	2
24.03.94	303	39	39	23.5	9	5	5			25	16	4	0.75	
07.04.94	317	28	18	15	7	7	4.6		33	21	13	5.2	3	
21.04.94	331	34	15	24	15.5	10.4	6		38	28	14.5	10.6	6.4	
05.05.94	345	38	27	23	20	12	7.5		23.5	25	20.5	10	7.4	
19.05.94	359	28	25	27	21.25	15.5	13		30	28	25.75	18.5	10.667	
02.06.94	373	29	24	23	21	14.167	12		26	25	25.25	16	11.667	
16.06.94	387	43.75	39.17	37.5	21	15.5	11.5		38.33	31.67	27	22	10.5	
30.06.94	401	44	45	42	31	25	17		48	41	42	29	19	
14.07.94	415	30	34	28	23	16	17	12	31	27	24	19	16	
28.07.94	429	41.25	33.75	42	27.915	30	22		34.375	24.375	20	28.335	24	
11.08.94	443	34.17	25.83	25	24.17	22	19.5		22.5	31.67	29.17	21	15.5	
25.08.94	457	39	30	24	24	27	21		27	28	24	25	14	
08.09.94	471	40	35.415	30	25	20	16		34	39	42	23	20	
22.09.94	485	52	40	43	20	21	13		39	36	32	22	12	
06.10.94	499	43	52	41	27	15	11		48	44	34	25	19	
20.10.94	513	35	27.5	27.5	20	13	8.5		23.75	33.75	29.17	22.5	12.5	
03.11.94	527	20.625	30	24.375	12.5	8.5	6.25		28.75	30	22.5	8.125	13.5	
17.11.94	541	33.33	32.5	18.33	14.5	13	5		33.33	26.67	23.5	13.5	9.5	
01.12.94	555													
08.12.94	562	40	25.83	31.67	18.5	8.5	9		36.67	24.5	13.5	6.5	3.5	
22.12.94	576	38.33	30	29.17	20	11.5	10		29.17	16.5	5	3.5	4	
05.01.95	590	35	32.5	22	13	9.5	6.5		25.5	10.5	4.5	3.5	7.5	
09.01.95	594	34.167	30.83	25	17.5	11	7.5		29.17	12.5	5.5	4	7	
19.01.95	604	20	22.5	23.5	14.5	8.5	6		27.5	11	4.5	5	4.5	
03.02.95	619	27.085	26	12	7.5	4	2.5		26	8.5	2.5	1	1.5	
09.02.95	625	25.833	25	13.5	6	3.5	1.6		24	14.5	4.5	1	0.6	
23.02.95	639	35	38.54	17.5	6	2.5	1		34.375	25	11	2.5	1	

**Appendix 1:**  
**Nitrate (NO3) data, mg/l**

Date	Day of Expt.	SL(1)	SPN(4)	1N(3)	2N(7)	3N(8)	4N(N1)	5N(N2)	SPS(4)	1S(5)	2S(6)	3S(9)	4S(10)	5S(11)
18.11.93	177	0.4	NS	0.3	0.6	0.2	0.2	0.2	0.5	0.4	0.2	0.3	0.4	
02.12.93	191	0.8	0.7	0.5	0.6	0.2	0.7	0.4	0.7	0.4	0.3	0.2	0.4	
06.01.94	226	1.1	1.2	0.5	1.3	0.3	0.6	1.2	1.1	1.9	0.5	0.4	0.4	0.8
27.01.94	247	1.1	1.2	1.2	1.2	0.5			1	1	0.5	0.4	0.4	
10.02.94	261	0.7	0.6	0.4	0.5	0.4	0.3	0.8	0.5	0.6	0.4	0.4	0.2	
24.02.94	275	1.2	0.9	0.5	0.7	0.4	0.3	0.3	0.8	0.4	0.4	0.3	0.3	0.5
10.03.94	289	1.1	1	0.3	0.6	0.4	0.4		0.8	0.7	0.3	0.4	0.3	0.3
24.03.94	303	1.2	0.9	0.5	0.4	0.5	0.4			0.5	0.4	0.4	0.2	
07.04.94	317	1.4	0.9	0.6	0.8	0.5	0.8		1	0.8	0.9	0.5	0.3	
21.04.94	331	1.2	0.8	0.6	0.6	0.4	0.4		1	0.5	0.4	0.6	0.5	
05.05.94	345	1.5	1	0.7	0.7	0.7	0.9		1	0.7	0.6	0.7	0.5	
19.05.94	359	1	1	1	2	1.5	1.6		1.1	1	1	1.4	1.1	
02.06.94	373	0.9	1.2	0.8	1.1	0.9	1		1.1	0.9	0.7	1	0.7	
16.06.94	387	1.3	1.2	1.2	1.7	1.5	1.5		1.1	1.4	1.1	1.6	0.9	
30.06.94	401	1	1	0.8	0.8	0.8	0.9		1	0.9	0.7	0.7	0.7	
14.07.94	415	1.5	1.2	0.9	1.1	0.8	1.1	0.7	1.2	1	0.6	0.8	0.9	
28.07.94	429	1.5	1.2	1.1	1.2	1.2	1.4		1.4	1.2	0.9	1.5	1.2	
11.08.94	443	1.3	1.9	1.7	1.5	1.2	1.3		1.7	2	1	1.3	1.6	
25.08.94	457	1.1	1.8	2.2	1.5	1.6	1.8		1.9	2.2	0.9	1.5	1.7	
08.09.94	471	1.2	1.2	1.3	1	0.8	1.1		1.2	1.3	0.7	1	1	
22.09.94	485	1.2	1.6	1.4	1.3	1.3	1.5		1.7	1.3	1.1	1.3	1	
06.10.94	499	1.4	1.7	1.5	2	1.5	2.2		1.8	1.8	1.3	1.8	1.2	
20.10.94	513	1.3	1.7	1.6	3.8	2.1	2		1.7	2	2.2	2.4	3	
03.11.94	527	1.4	1.7	1.9	2.5	2.1	1.8		1.6	2.1	2	2.9	1.9	
17.11.94	541	1.2	1.7	1.4	1.1	1	1.2		1.5	1.9	1.6	1.1	1.5	
01.12.94	555													
08.12.94	562	1.1	0.8	0.8	1.5	1.5	0.7		0.9	3	1.9	2.6	1.5	
22.12.94	576	1.1	0.8	0.6	0.6	0.6	0.4		0.7	0.6	0.9	0.9	0.5	
05.01.95	590	1.4	0.9	0.7	0.8	0.6	0.5		0.6	0.6	0.5	0.5	0.5	
09.01.95	594	0.9	0.8	0.7	0.6	0.6	0.7		0.9	1	0.6	0.5	0.5	
19.01.95	604	0.7	0.9	0.6	0.7	0.5	0.7		0.6	0.7	0.6	0.4	0.5	
03.02.95	619	0.8	0.6	0.8	1.5	0.8	0.8		0.5	0.7	0.5	0.5	0.6	
09.02.95	625	0.6	0.6	0.8	0.8	0.6	0.7		0.6	0.7	0.7	0.5	0.4	
23.02.95	639	0.6	0.6	0.5	0.4	0.3	0.3		0.5	0.7	0.4	0.4	0.3	

**Appendix 1:**  
**Nitrite (NO2) data, mg/l**

	Day of expt.	SL(1)	SPN(4)	1N(3)	2N(7)	3N(8)	4N(N1)	5N(N2)	SPS(4)	1S(5)	2S(6)	3S(9)	4S(10)	5S(11)
18.11.93	177	NA	NS	NA	NA	NA	NA		NA	NA	NA	NA	NA	
02.12.93	191	NA	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
06.01.94	226	NA	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
27.01.94	247	NA	NA	NA	NA	NA	NS		NA	NA	NA	NA	NA	
10.02.94	261	0.001	0.089	0.093	0.117	0.034	0.011	0.001	0.052	0.197	0.086	0.014	0.003	
24.02.94	275	0.003	0.027	0.046	0.177	0.025	0.036	0.018	0.061	0.051	0.07	0.017	0.006	0.053
10.03.94	289	0.003	0.005	0.034	0.166	0.08	0.059		0.004	0.118	0.054	0.063	0.01	0.04
24.03.94	303	0.004	0.006	0.05	0.1	0.077	0.099			0.052	0.065	0.072	0.05	
07.04.94	317	0.013	0.007	0.101	0.275	0.126	0.212		0.015	0.183	0.277	0.147	0.057	
21.04.94	331	0.003	0.002	0.025	0.18	0.122	0.112		0.002	0.031	0.098	0.161	0.138	
05.05.94	345	0	0.004	0.028	0.176	0.153	0.256		0.011	0.068	0.102	0.167	0.105	
19.05.94	359	0.022	0.095	0.178	0.665	0.485	0.4875		0.113	0.236	0.29	0.4275	0.288	
02.06.94	373	0.02	0.188	0.1205	0.307	0.224	0.28		0.182	0.1825	0.142	0.293	0.155	
16.06.94	387	0.015	0.135	0.195	0.488	0.467	0.442		0.116	0.302	0.267	0.532	0.235	
30.06.94	401	0.005	0.052	0.089	0.17	0.175	0.204		0.051	0.116	0.115	0.165	0.178	
14.07.94	415	0.017	0.016	0.05	0.294	0.184	0.272	0.147	0.017	0.087	0.097	0.199	0.213	
28.07.94	429	0.007	0.048	0.098	0.186	0.312	0.32		0.052	0.12	0.114	0.3904	0.214	
11.08.94	443	0.027	0.152	0.257	0.275	0.247	0.232		0.231	0.316	0.156	0.265	0.304	
25.08.94	457	0.034	0.26	0.4825	0.3885	0.4275	0.4371		0.255	0.545	0.19	0.4396	0.38	
08.09.94	471	0.01	0.073	0.162	0.141	0.175	0.264		0.049	0.124	0.099	0.178	0.249	
22.09.94	485	0.035	0.269	0.256	0.305	0.34	0.347		0.283	0.216	0.256	0.3546	0.255	
06.10.94	499	0.021	0.203	0.166	0.4179	0.3271	0.5638		0.195	0.227	0.18	0.4429	0.247	
20.10.94	513	0.121	0.34	0.315	1.32	0.695	0.615		0.24	0.33	0.66	0.8	1.14	
03.11.94	527	0.2	0.32	0.505	0.775	0.78	0.615		0.295	0.565	0.63	1.05	0.695	
17.11.94	541	0.091	0.288	0.375	0.285	0.275	0.367		0.33	0.542	0.512	0.36	0.435	
01.12.94	555													
08.12.94	562	0.043	0.057	0.098	0.47	0.489	0.115		0.062	1.095	0.715	1.02	0.47	
22.12.94	576	0.012	0.043	0.064	0.087	0.094	0.024		0.021	0.08	0.213	0.255	0.057	
05.01.95	590	0.091	0.101	0.09	0.196	0.122	0.118		0.063	0.136	0.072	0.15	0.13	
09.01.95	594	0.094	0.133	0.101	0.145	0.117	0.195		0.16	0.29	0.126	0.094	0.109	
19.01.95	604	0.082	0.233	0.099	0.147	0.083	0.196		0.163	0.192	0.146	0.072	0.093	
03.02.95	619	0.075	0.124	0.269	0.5188	0.243	0.221		0.087	0.156	0.116	0.091	0.086	
09.02.95	625	0.045	0.077	0.202	0.188	0.119	0.141		0.086	0.152	0.17	0.066	0.035	
23.02.95	639	0.034	0.075	0.089	0.06	0.041	0.056		0.037	0.135	0.077	0.075	0.049	

**Appendix 1:**  
**Phosphate (PO4) data, mg/l**

Date	Day of expt.	SL(1)	SPN(4)	1N(3)	2N(7)	3N(8)	4N(N1)	5N(N2)	SPS(4)	1S(5)	2S(6)	3S(9)	4S(10)	5S(11)
18.11.93	177	11.2	NS	12.8	14	15.6	14.8		16.4	10.6	16	19	40	
02.12.93	191	13.2	15.6	13.6	13.6	17.4	NA		16.8	20.2	19.4	18	17.8	
06.01.94	226	NA	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	
27.01.94	247	NA	NA	NA	NA	NA	NS		NA	NA	NA	NA	NA	
10.02.94	261	16.2	16.4	13.2	14	7.8	10.8	17.2	16	16.8	18.2	14.2	11	
24.02.94	275	14	17.2	18	15.6	30.4	15.2	14.4	16.4	15.8	14	18.4	13.8	18.8
10.03.94	289	11.5	15	13	13.5	8	13.5		15	17	10	12	10	13
24.03.94	303	13	13	18.5	13	14	14			20	18	13	7.5	
07.04.94	317	17	14.4	22.2	16.4	15.2	17.6		20.2	20.2	17.8	16.6	15.6	
21.04.94	331	16.4	14.6	18.8	16.4	11.8	15.2		16	20.2	15.8	16	17	
05.05.94	345	22.8	23.2	29.2	26	23.4	23.4		23.4	26.2	28	25	22.6	
19.05.94	359	25.4	26.6	29.4	24.9	27.6	25.4		24.667	27.6	27.1	25.6	25.2	
02.06.94	373	26.2	28.6	30.8	24.4	23.2	20.8		28.8	29.8	28.4	25	23.8	
16.06.94	387	31.5	45.5	33.5	42.5	31.5	34.5		34	33	33	31	35.5	
30.06.94	401	13	18.5	17	22	10	11		17.5	18	23	11	16	
14.07.94	415	22.6	22.8	26.8	26.6	24.4	11.8	23.1	23.4	24.8	27.8	26.2	12.4	
28.07.94	429	23	18	9	27.5	14	11		23	16.5	27	20.5	25	
11.08.94	443	23	23.5	24.5	23.5	30.5	27		19	24.5	41	29	27.5	
25.08.94	457	11	6	10	16	8	12		14	14	18	10	17	
08.09.94	471	10	14.5	9	38.5	8	6		12	11	11	18	10	
22.09.94	485	27	16	19	24	28	27		13	23.5	14	20.5	21	
06.10.94	499	11	9	10	12	9	7		13	10	10	18	14	
20.10.94	513	17	79.5	31.5	27.5	26	30.5		27	33	44	26	24	
03.11.94	527	20.75	19	22.75	24.25	25	22		23	22	22.5	45.75	24	
17.11.94	541	17	13	20.5	21.5	28	22.5		17.5	19.5	22	21	22	
01.12.94	555													
08.12.94	562	18.5	20	30	21	17.5	25		25	23.5	19	20.5	25	
22.12.94	576	20.5	22.5	22.5	23.5	27	27		24.5	19	20.5	22.5	27.5	
05.01.95	590	23	22.5	30	15.5	15.5	16.5		22	39.5	27.5	36	25	
09.01.95	594	21	21	82.5	13	11	13.5		21	13	12	14	17.5	
19.01.95	604	17.5	21	20	21	14.5	23.5		20.5	19.5	14	19.5	21	
03.02.95	619	9	13.5	15.5	7	12	15		13	10	16.5	16.5	14	
09.02.95	625	25	16.5	17.5	17.5	18	20		17.5	14.5	17	15.5	23.8	
23.02.95	639	12.5	15	16.5	20.5	17	5.5		12.5	12	16	6	15	

**Appendix 1:**  
**pH data**

<b>SAMPLED</b>	<b>Day of Expt.</b>	<b>SL(1)</b>	<b>SPN(4)</b>	<b>1N(3)</b>	<b>2N(7)</b>	<b>3N(8)</b>	<b>4N(N1)</b>	<b>5N(N2)</b>	<b>SPS(4)</b>	<b>1S(5)</b>	<b>2S(6)</b>	<b>3S(9)</b>	<b>4S(10)</b>	<b>5S(11)</b>
<b>10.03.94</b>	<b>289</b>	7.82	8.92	9.18		9.41			8.8	9.05	9.23	9.27		9.19
<b>24.03.94</b>	<b>303</b>	8.87	8.96	8.6	7.44		9.29			8.87	9.13	9.32	9.1	
<b>16.06.94</b>	<b>387</b>	8.41	8.52	8.7	8.86	8.95	9		8.52	8.82	8.88	9.02	9.03	
<b>30.06.94</b>	<b>401</b>	8.04	8.13	8.85	8.93	9.02	9.08		8.15	8.86	8.66	8.98	8.84	
<b>14.07.94</b>	<b>415</b>	8.42	8.35	8.6	8.75	8.6	8.58	8.7	8.48	8.49	8.52	8.57	8.64	
<b>28.07.94</b>	<b>429</b>	7.72	7.55	8.61	6.79	8.89	8.84		7.73	7.02	7.13	6.99	7.18	
<b>11.08.94</b>	<b>443</b>	7.8	8.03	7.95	8.99	8.93	8.97		8.07	7.88	spilt	9.05	9.01	
<b>25.08.94</b>	<b>457</b>	8.13	7.84	7.95	8.62	8.71	8.97		7.86	8.32	8.53	8.83	8.61	
<b>08.09.94</b>	<b>471</b>	7.62	6.97	7.73	7.27	8.97	9.32		7.7	8.03	8.76	7.17	9.04	
<b>22.09.94</b>	<b>485</b>	8.44	8.51	8.8	9.07	9.09	9.12		8.46	8.13	9.05	8.64	9.56	
<b>06.10.94</b>	<b>499</b>	7.8	8.18	8.5	8.49	8.71	8.68		7.85	8.21	8.49	8.73	8.64	
<b>20.10.94</b>	<b>513</b>	8.26	8.55	8.51	8.68	8.78	8.64		8.42	8.5	8.58	8.7	8.9	
<b>03.11.94</b>	<b>527</b>	8.11	8.2	8.6	8.59	8.71	8.66		8.3	8.39	8.62	8.69	8.58	
<b>17.11.94</b>	<b>541</b>	7.42	8.07	8.95	8.72	8.94	9.02		8.42	8.82	8.99	9.05	9.03	
<b>01.12.94</b>	<b>555</b>													
<b>08.12.94</b>	<b>562</b>	8.27	8.92	8.87	9	9.22	8.86		8.7	8.91	8.84	8.71	9.2	
<b>22.12.94</b>	<b>576</b>	8.79	8.92	spilt	8.89	8.9	8.94		9.03	8.97	8.87	8.9	8.79	
<b>05.01.95</b>	<b>590</b>	8.33	8.42	8.44	8.62	8.76	8.77		8.53	8.67	8.76	8.77	8.75	
<b>09.01.95</b>	<b>594</b>	8.29	8.46	8.6	8.76	8.7	8.72		8.44	8.73	8.76	8.78	8.76	
<b>19.01.95</b>	<b>604</b>	8.82	8.81	8.75	8.73	8.95	8.87		8.75	8.82	8.97	8.97	8.95	
<b>03.02.95</b>	<b>619</b>	6.91	7.11	7.36	8.92	8.71	8.81		7.7	8.59	8.77	8.81	8.55	
<b>09.02.95</b>	<b>625</b>	8.51	8.41	8.56	8.65	8.75	8.74		8.51	8.56	8.77	8.75	8.9	
<b>23.02.95</b>	<b>639</b>	7.37	7.22	8.82	8.21	8.5	8.89		8.43	8.88	7.87	8.68	8.91	

Appendix 1:

Conductivity data (uS/cm)

Readings depended on the availability of probes at the time of analysis. Some readings in mV depending on probe (omited from Figure 4.9).

DATE	Day of Expt	SL(1)	SPN(4)	1N(3)	2N(7)	3N(8)	4N(N1)	5N(N2)	SPS(4)	1S(5)	2S(6)	3S(9)	4S(10)	5S(11)
10.03.94	289	1233	1663	1153	NR	1431	NR		1556	1682	941	1301	NR	1683
24.03.94	303	1852	1770	1447	1316	NR	1473		NR	1431	1656	1609	1666	
21.04.94	331	1785	1123	1605	1622	1359	1018		1837	1814	1437	1577	1704	
05.05.94	345	1838	1755	1829	1894	1882	1815		1617	1857	1885	1840	1790	
19.05.94	359	1771	1618	1933	1846	1889	1904		1926	1885	1890	1889	1939	
02.06.94	373	1976	1977	1995	1925	1888	1903		1969	1969	1950	1939	1959	
16.06.94	387	2060	1973	1966	1820	1920	1817		1907	1800	1829	1958	1930	
30.06.94	401	1780	1977	1822	1722	1875	1853		1929	1862	1940	1874	1912	
14.07.94	415	(71mV)	(67mV)	(79mV)	(85mV)	(79mV)	(77mV)	(82mV)	(75mV)	(73mV)	(73mV)	(76mV)	(79mV)	
28.07.94	429	1941	2270	1774	1855	1912	1894		1921	1423	1643	1988	1934	
11.08.94	443	2010	1940	1894	1823	1764	1778		1843	1951	split	1537	1823	
25.08.94	457	1791	1767	1796	1603	1827	1829		1920	1842	1468	1538	1568	
08.09.94	471	1777	1913	1771	1886	1671	1787		1593	1927	1890	1654	1851	
22.09.94	485	1955	1896	1953	1408	1768	1665		1814	1791	1807	1628	1273	
06.10.94	499	1724	1890	1941	1832	1772	1837		1891	1816	1823	1701	1666	
20.10.94	513	(-63mV)	(-79mV)	(-77mV)	(-87mV)	(-92mV)	(-84mV)		(-72mV)	(-76mV)	(-81mV)	(-88mV)	(-99mV)	
03.11.94	527	(-54mV)	(-64mV)	(-83mV)	(-82mV)	(-88mV)	(-86mV)		(-66mV)	(-71mV)	(-83mV)	(-87mV)	(-80mV)	
17.11.94	541	1928	2040	1868	1980	1726	1912		1982	1931	1881	1581	1860	
01.12.94	555													
08.12.94	562	2250	1432	1977	2030	2030	2070		2120	2000	1984	1984	1932	
22.12.94	576	2030	2030	split	1980	2070	2010		1732	1803	1909	1958	1828	
05.01.95	590	(-66mV)	(-71mV)	(-71mV)	(-81mV)	(-90mV)	(-90mV)		(-78mV)	(-84mV)	(-89mV)	(-91mV)	(-88mV)	
09.01.95	594	(-65mV)	(-73mV)	(-81mV)	(-89mV)	(-86mV)	(-87mV)		(-72mV)	(-87mV)	(-89mV)	(-90mV)	(-89mV)	
19.01.95	604	(-94mV)	(-92mV)	(-89mV)	(-88mV)	(-101mV)	(-97mV)		(-89mV)	(-93mV)	(-102mV)	(-102mV)	(-101mV)	
03.02.95	619	1809	1846	2060	2030	2120	2210		1890	1936	2250	2230	2350	
09.02.95	625	(-75mV)	(-70mV)	(-79mV)	(-84mV)	(-89mV)	(-87mV)		(-75mV)	(-79mV)	(-90mV)	(-89mV)	(-97mV)	
23.02.95	639	2160	2100	2080	2170	2310	2060		2060	2050	2140	2230	2350	



## Appendix 1:

### Salinity data

Reading frequency depended on probe availability at the time of analysis.

SAMPLED	Day of expt.	SL(1)	SPN(4)	1N(3)	2N(7)	3N(8)	4N(N1)	5N(N2)	SPS(4)	1S(5)	2S(6)	3S(9)	4S(10)	5S(11)
10.03.94	289	0.6	0.9	0.6		0.7			0.8	0.9	0.4	0.7		0.9
24.03.94	303	1	1	0.7	0.7		0.8			0.7	0.9	0.8	0.9	
21.04.94	331	1	0.6	0.9	0.9	0.7	0.5		1	1	0.7	0.8	0.9	
05.05.94	345	1	1	1	1	1	1		0.9	1	1	1	1	
19.05.94	359	1	0.9	1.1	1	1	1.1		1.1	1	1.1	1	1.1	
02.06.94	373	1.1	1.1	1.1	1.1	1	1.1		1.1	1.1	1.1	1.1	1.1	
16.06.94	387	1.1	1.1	1.1	1	1.1	1		1	1	1	1.1	1.1	
30.06.94	401	1	1.1	1	0.9	1	1		1	1	1.1	1	1	
28.07.94	429	1.1	1.2	1	1	1	1		1	0.7	0.9	1.1	1.1	
11.08.94	443	1.1	1.1	1	1	1	1		1	1.1	spilt	0.8	1	
25.08.94	457	1	1	1	0.8	1	1		1	1	0.8	0.8	0.8	
08.09.94	471	1	1	1	1	0.9	1		0.8	1	1	0.9	1	
22.09.94	485	1.1	1	1.1	0.7	0.9	0.9		1	1	1	0.9	0.7	
06.10.94	499	0.9	1	1.1	1	1	1		1	1	1	0.9	0.9	
17.11.94	541	1.1	1.1	1	1.1	0.9	1.1		1.1	1.1	1	0.8	1	
01.12.94	555													
08.12.94	562	1.3	0.7	1.1	1.1	1.1	1.1		1.2	1.1	1.1	1.1	1.1	
22.12.94	576	1.1	1.1	spilt	1.1	1.1	1.1		0.9	1	1.1	1.1	1	
03.02.95	619	1	1	1.1	1.1	1.2	1.2		1	1	1.2	1.2	1.3	
23.02.95	639	1.2	1.2	1.1	1.2	1.3	1.1		1.1	1.1	1.2	1.2	1.3	

### Chlorophyll (chl a) data, ug/l

[illegible]

<b>Appendix 2:</b>									
<b>Zooplankton, Day 1 (26 May 1993)</b>									
Numbers per litre									
	[1] = SL	[2]	[3] = 1N	[4] = SP(S)	[5] = 1S	[6] = 2S	[7] = 2N	[8] = 3N	[9] = 3S
<i>Brachionus</i>	496.3	24990	2199.5	940.8	46.5	0.3	0.2	1.5	1.3
<i>Polyarthra</i>	0	0	0	0	0	0	0	0	0
<i>Filinia</i>	0	0	13	4.2	1.3	0	0	0	0
<i>Asplanchna</i>	0	0	1	155.5	74.7	9.2	5.5	0.2	0.2
<b>TOTAL rotifers</b>	496.3	24990	2213.5	1100.5	122.5	9.5	5.7	1.7	1.5
<b>Ostracods</b>	0	0	1.3	0	0	0	0	23.5	0.5
<b>Chydoridae</b>	0	0	0	0	0	0	0	0.8	0
<i>Daphnia</i>	0	0	0	1.5	8.3	25	71.5	196	37.8
<i>Moina</i>	0	1.5	5.2	51.3	27	5.2	0.5	0.5	0
<b>Cyclopoids</b>	0	0	1.3	8	30.7	11.8	13.5	24.8	1.2
<b>Calanoids</b>	0	0	0	0	0	0	0	0	0
<b>TOTAL adult copep.</b>	0	0	1.3	8	30.7	11.8	13.5	24.8	1.2
<b>TOTAL adult crust.</b>	0	1.5	7.8	60.8	66	42	85.5	245.6	39.5
<b>cyclopoid nauplii</b>									
<b>calanoid nauplii</b>									
<b>TOTAL nauplii</b>	0	0	9	11	65	149.7	63.5	30.7	47.2
<b>TOTAL crust.</b>	0	1.5	16.8	71.8	131	191.7	149	276.3	86.7
<b>TOTAL organisms</b>	496.3	24991.5	2230.3	1172.3	253.5	201.2	154.7	278	88.2

<b>Appendix 2:</b>									
<b>Zooplankton, Day 15 (9 June 1993)</b>									
Numbers per litre									
	[1] = SL	[2]	[3] = 1N	[4] = SP(S)	[5] = 1S	[6] = 2S	[7] = 2N	[8] = 3N	[9] = 3S
<i>Brachionus</i>	78.8	254.5	829.5	217.3	21.2	0	0	0	0
<i>Polyarthra</i>	0	0	0	1.2	0	0	0	0	0
<i>Filinia</i>	0	0	0	9.3	10.5	1.2	2.7	0	0
<i>Asplanchna</i>	0	0	0.3	92.7	101	17.5	3.3	2.5	0.3
<b>TOTAL rotifers</b>	78.8	254.5	829.8	320.5	132.7	18.7	6	2.5	0.3
<b>Ostracods</b>	0	0	0	0	0	0	0	0.3	0.5
<b>Chydoridae</b>	0	0	0	0	0	0	0	0.2	0
<i>Daphnia</i>	0.2	0	0	0.5	0.8	6.3	35.5	20.5	68.7
<i>Moina</i>	0	0	0.2	2.5	3.5	12.5	6.8	1.3	0
<b>Cyclopoids</b>	0	0	0	8	10.5	21.3	26	6	0.5
<b>Calanoids</b>	0	0	0	0	0	0	0	0	0
<b>TOTAL adult copep.</b>	0	0	0	8	10.5	21.3	26	6	0.5
<b>TOTAL adult crust.</b>	0.2	0	0.2	11	14.8	40.1	68.3	28.3	69.7
<b>cyclopoid nauplii</b>									
<b>calanoid nauplii</b>									
<b>TOTAL nauplii</b>	0	0.3	0.3	25.2	48.2	103.7	27.2	58.2	101.8
<b>TOTAL crust.</b>	0.2	0.3	0.5	36.2	63	143.8	95.5	86.5	171.5
<b>TOTAL organisms</b>	79	254.8	830.3	356.7	195.7	162.5	101.5	89	171.8

<b>Appendix 2:</b>									
<b>Zooplankton, Day 29 (23 June 1993)</b>									
Numbers per litre									
	[1] = SL	[2]	[3] = 1N	[4] = SP(S)	[5] = 1S	[6] = 2S	[7] = 2N	[8] = 3N	[9] = 3S
<i>Brachionus</i>	726.8	1975	5000.3	652.5	18.7	2.3	0.8	0	0
<i>Polyarthra</i>	0	0	0	0	0	0	0	0	0
<i>Filinia</i>	0	0	5.7	4.3	1.3	0	0	0	0
<i>Asplanchna</i>	0	0	39.5	100	5.3	1.2	0	0	0
TOTAL rotifers	726.8	1975	5045.5	756.8	25.3	3.5	0.8	0	0
Ostracods	0	0	0	0	0	0.3	0	0.5	0.5
Chydoridae	0	0	0	0	0.8	0	0	0	0
<i>Daphnia</i>	0	0	0	13	17.5	17.7	13.5	35.5	7.5
<i>Moina</i>	0	0	12.7	18.7	2.5	11.7	2.5	4	1
Cyclopoids	0	0	0.3	14.5	7.7	4	2.7	2	1.5
Calanoids	0	0	0	0	0	0	0	0	0
TOTAL adult copep.	0	0	0.3	14.5	7.7	4	2.7	2	1.5
TOTAL adult crust.	0	0	13	46.2	28.5	33.7	18.7	42	10.5
cyclopoid nauplii									
calanoid nauplii									
TOTAL nauplii	0	0	7	76.8	44.2	57.5	24.8	69.5	46.7
TOTAL crust.	0	0	20	123	72.7	91.2	43.5	111.5	57.2
TOTAL organisms	726.8	1975	5065.5	879.8	98	94.7	44.3	111.5	57.2

<b>Appendix 2:</b>										
<b>Zooplankton, Day 50 (14 July 1993)</b>										
Numbers per litre										
	[1] = SL	[2]	[3] = 1N	[4] = SP(S)	[5] = 1S	[6] = 2S	[7] = 2N	[8] = 3N	[9] = 3S	[10] = 4S
Brachionus	3175.2	344.3	2491	522.5	181.3	25.8	10	1	0	0
Polyarthra	0	0	0	0	0	0	0	0	0	0
Filinia	0	0	0	0	0	0	0	0	0	0
Asplanchna	0	0	0	0.5	0	0.5	0	0	0	0
TOTAL rotifers	3175.2	344.3	2491	523	181.3	26.3	10	1	0	0
Ostracod	0	0	0	0	0	0	0	0	0	0
Chydoridae	0	0	0	0	0	0	0	0	0	0
Daphnia	0	0	0	9.7	11.7	12.8	10	1.3	6	50
Moina	0	11.3	153.7	71.5	273.5	108.2	10	0.5	1.2	0.2
Cyclopoids	0	0.2	0	2	3.3	9	1.7	0.5	0	1
Calanoids	0	0	0	0	0	0	0	0	0	0
TOTAL adult copep.	0	0.2	0	2	3.3	9	1.7	0.5	0	1
TOTAL adult crust.	0	11.5	153.7	83.2	288.5	130	21.7	2.3	7.2	51.2
cyclopoid nauplii										
calanoid nauplii										
TOTAL nauplii	0	0	5.3	6.8	30.7	22	20	13.2	24	4.3
TOTAL crust.	0	11.5	159	90	319.2	152	41.7	15.5	31.2	55.5
TOTAL organisms	3175.2	355.8	2650	613	500.5	178.3	51.7	16.5	31.2	55.5

<b>Appendix 2:</b>										
<b>Zooplankton, Day 64 (28 July 1993)</b>										
Numbers per litre										
	[1] = SL	[2]	[3] = 1N	[4] = SP(S)	[5] = 1S	[6] = 2S	[7] = 2N	[8] = 3N	[9] = 3S	[10] = 4S
Brachionus	2777.5	3602.5	417.2	30.3	4	0	0	0	0	0
Polyarthra	0	0	0	0	0	0	0	0	0	0
Filinia	0	0	0	0	0	0	0	0	0	0
Asplanchna	0	0	0	0	0	0	0	0	0	0
TOTAL rotifers	2777.5	3602.5	417.2	30.3	4	0	0	0	0	0
Ostracod	0	0	0	0	0	0	0	0	0	0.2
Chydoridae	0	0	0	0	0.5	0	0	0	0	0
Daphnia	0	0	0	57.8	118	103.8	354	5.2	212.7	146.8
Moina	0	11.5	18	52.3	102.5	13.2	12	0	0	0.3
Cyclopoids	0	0	0	0	15.8	7	1.5	1	0.2	4.7
Calanoids	0	0	0	3.5	0	0	0	0	0	0
TOTAL adult copep.	0	0	0	3.5	15.8	7	1.5	1	0.2	4.7
TOTAL adult crust.	0	11.5	18	113.6	236.8	124	367.5	6.2	212.9	152
cyclopoid nauplii										
calanoid nauplii										
TOTAL nauplii	0	1.3	14.3	31.5	97.2	60	49.5	8.5	25.3	11.5
TOTAL crust.	0	12.8	32.3	145.1	334	184	417	14.7	238.2	163.5
TOTAL organisms	2777.5	3615.3	449.5	175.4	338	184	417	14.7	238.2	163.5

**Zooplankton, Day 317 (7 April 1994)**

Numbers per litre	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100

	SL	SPN	1N	2N	3N	4N	5N	SPS	1S	2S	3S	4S	5S
Brachionus	13142.85	8433.85	392.5	27.5	7.5	7.5	0	7.5	137.78	7.5	0.5	0	120
Polyarthra	0	0	17.5	0	5	2.5	0	0	0	0	0	0	0
Filinia	506.74	2833.51	187.5	5	0	0	0	0	26.44	2.5	0	0	0
Asplanchna	0	8.33	35	5	2.5	0	2.5	41.75	7.5	5	2.5	10	
TOTAL rotifers	13649.59	11275.69	632.5	37.5	15	2.5	10	205.97	17.5	5.5	2.5	130	
Ostracod	0	0	0	0.25	0.5	0	0	0.25	2.5	1.5	0	5	
Chydoridae	0	0	0	0	0	0	0	0	0	0	0	0	
Daphnia	0	0	9.25	59	27	1	2.5	25.75	38	26.75	4.5	2.5	
Moina	0	0	5	1.25	0.75	0	0	4.75	1.5	0	0	0	
Cyclopoids	0	0	45	16.5	6.75	2	0	35	17.25	7.5	0.25	10	
Calanoids	0	0	0	0	0.5	0.25	12.5	0	0	0	10	0	
TOTAL adult copep.	0	0	45	16.5	7.25	2.25	12.5	35	17.25	7.5	10.25	10	
TOTAL adult crust.	0	0	59.25	77	35.5	3.25	15	65.75	59.25	35.75	14.75	17.5	
cyclopoid nauplii													
calanoid nauplii													
TOTAL nauplii	0	0	510	507.5	600	342.5	355	357.5	672.5	517.5	220	267.5	
TOTAL crust.	0	0	569.25	584.5	635.5	345.75	370	423.25	731.75	553.25	234.75	285	
TOTAL organisms	13649.59	11275.69	1201.75	622	650.5	348.25	380	629.22	749.25	558.75	237.25	415	
Collection time	10	10.09	10.14	10.45	10.5	11.28	11.36	10.21	10.3	10.38	11.02	11.1	11.18
No. Brach ovig	3568.74	813.38	50	2.5	5		0	9.74	0	0		22.5	
% Brach ovig	27.15347	9.644231	12.73885	9.090909	66.66667		0	7.069241	0	0		18.75	
No. Polyarth ovig			0		0	0							
% Polyarth ovig			0		0	0							
No. Filinia ovig	204.85	553.37	57.5	0				11.13	2.5				
% Filinia ovig	40.42507	19.52949	30.66667	0				42.09531	100				
No. cylop. ovig			0	0	0.25			2.5	0	0	0	0	
% cylop. ovig			0	0	3.703704	0		7.142857	0	0	0	0	
No. calan. ovig					0	0	0.5					0	
% calan. ovig					0	0	4					0	

## NOTES

**Clear sky, stiff SW wind.**

**Outlet SPN: Asplanchna caught engulfing a Brachionus**

Outlet SPS: preservation problem with sample - decayed in storage.

**Pond 1N: water beetles in sample**



**Zooplankton, Day 331 (21 April 1994)**

[illegible]

**Zooplankton, Day 345 (5 May 1994)**

[illegible]

**Zooplankton, Day 359 (19 May 1994)**

[illegible][illegible]



**Zooplankton, Day 387 (16 June 1994)**

[illegible]

## NOTES

Overcast, light NW wind.

Ponds 2N + 2S: both v. bitty/lot of debris.

No data from pond 5S due to MW works.

**Zooplankton, Day 401 (30 June 1994)**

[illegible]

**Zooplankton, Day 415 (14 July 1994)**

Numbers per litre	
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100	100

[illegible]

**Zooplankton, Day 429 (28 July 1994)**

Numbers per litre
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	SL	SPN	1N	2N	3N	4N	5N	SPS	1S	2S	3S	4S	5S
Brachionus	0	307.5	270	15	0	0	0	240	265	7.5	5	0	0
Polyarthra	0	1710	3030	197.5	12.5	0	2.5	1932.5	3460	185	30	7.5	0
Filinia	0	0	90	12.5	0	0	0	0	130	17.5	0	0	0
Asplanchna	20	112.5	145	7.5	7.5	0	5	82.5	30	0	2.5	32.5	0
TOTAL rotifers	20	2130	3535	232.5	20	0	7.5	2255	3885	210	37.5	40	0
Ostracod	0	0	0	0	0	0	0	0	0	0	0	0	0
Chydoridae	0	0	0	0	0	0	0	0	0	0	0	0	0
Daphnia	0	0	10	10	190	20	20	0	5	25	15	147.5	0
Moina	0	0	5	0	0	2.5	0	0	10	5	15	0	0
Cyclopoids	0	0	30	37.5	22.5	0	0	0	20	30	0	2.5	0
Calanoids	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL adult copep.	0	0	30	37.5	22.5	0	0	0	20	30	0	2.5	0
TOTAL adult crust.	0	0	45	47.5	212.5	22.5	20	0	35	60	30	150	0
cyclopoid nauplii	0	0	250	145	72.5	7.5	27.5	0	60	137.5	45	37.5	0
calanoid nauplii	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL nauplii	0	0	250	145	72.5	7.5	27.5	0	60	137.5	45	37.5	0
TOTAL crust.	0	0	295	192.5	285	30	47.5	0	95	197.5	75	187.5	0
TOTAL organisms	20	2130	3830	425	305	30	55	2255	3980	407.5	112.5	227.5	0
Collection time (9.45am-11.30am)													
No. Brach ovig		45	40	2.5				42.5	30	0	0		
% Brach ovig		14.63415	14.81481	16.66667				17.70833	11.32075	0	0		
No. Polyarth ovig		360	680	37.5	0		0	297.5	750	12.5	0	0	
% Polyarth ovig		21.05263	22.44224	18.98734	0		0	15.39457	21.6763	6.756757	0	0	
No. Filinia ovig			0	0					0	0			
% Filinia ovig			0	0					0	0			
No. cylop. ovig			0	2.5	2.5				0	0		0	
% cylop. ovig			0	6.66667	11.11111				0	0		0	
No. calan. ovig													
% calan. ovig													
NOTES													
Overcast, light W wind.													
GENERALLY - all samples with v.v.v. little algae (no green sludge)													
POND SL - preservation problem? (lots of organic debris, no obvious formalin smell).													
PONDS SPN+1S - all 'giant' ans. = grow bigger but fewer in winter?													
PONDS 3N, 4N, + 4S - lot of organic debris													
POND 4N - lot of debris, & sample liquid level v. low = preservation problem?													
Some of sample 5N lost/spilt in laboratory.													
No pond 5S sample due to MW works.													



**Zooplankton, Day 443 (11 August 1994)**

[illegible][illegible]



**Zooplankton, Day 471 (8 September 1994)**

[illegible][illegible]

Numbers per litre
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[illegible]

**Zooplankton, Day 513 (20 October 1994)**

SL	SPN	1N	2N	3N	4N	5N	SPS	1S	2S	3S	4S	5S
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[illegible]

Clear sky, strong SW-S wind. Cover put back on N outlet of pond 2N.  
Pond 5N: sample contained a lot of algal clumps.

**Zooplankton, Day 527 (3 November 1994)**

[illegible]

**Zooplankton, Day 541 (17 November 1994)**

[illegible][illegible]

**Zooplankton, Day 552 (28 November 1994)**

Numbers per litre
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	SL	SPN	1N	2N	3N	4N	5N	SPS	1S	2S	3S	4S	5S
Brachionus	11400	900	25	0	0	5	57.5	275	275	55	22.5	5	2.5
Polyarthra	13175	4200	50	0	0	0	0	5275	1862.5	2.5	0	0	0
Filinia	49950	17000	537.5	12.5	5	0	0	8900	262.5	27.5	5	5	2.5
Asplanchna	400	150	0	0	0	5	0	600	12.5	0	0	0	0
TOTAL rotifers	74925	22250	612.5	12.5	5	10	57.5	15050	2412.5	85	27.5	10	5
Ostracod	0	0	0	0	0	0	0	0	0	0	0	0	0
Chydoridae	0	0	0	0	0	0	5	0	0	0	0	0	0
Daphnia	0	0	162.5	62.5	122.5	375	197.5	0	7.5	120	50	35	5
Moina	300	287.5	12.5	5	0	5	2.5	147.5	140	127.5	7.5	7.5	0
Cyclopoids	0	0	17.5	27.5	17.5	60	145	0	15	27.5	7.5	70	92.5
Calanoids	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL adult copep.	0	0	17.5	27.5	17.5	60	145	0	15	27.5	7.5	70	92.5
TOTAL adult crust.	300	287.5	192.5	95	140	440	350	147.5	162.5	275	65	112.5	97.5
cyclopoid nauplii	0	0	937.5	492.5	230	1160	920	0	662.5	585	232.5	347.5	455
calanoid nauplii	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL nauplii	0	0	937.5	492.5	230	1160	920	0	662.5	585	232.5	347.5	455
TOTAL crust.	300	287.5	1130	587.5	370	1600	1270	147.5	825	860	297.5	460	552.5
TOTAL organisms	75225	22537.5	1742.5	600	375	1610	1327.5	15197.5	3237.5	945	325	470	557.5

## Collection time

[illegible]

## NOTES

Flow changed to 75%:25% north to south.



**Zooplankton, Day 562 (8 December 1994)**

Numbers per litre
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[illegible]

**Zooplankton, Day 576 (22 December 1994)**

[illegible]

Appendix 2:												
Zooplankton, Day 590 (5 January 1995)												
Numbers per litre												
SL	SPN	1N	2N	3N	4N	5N	SPS	1S	2S	3S	4S	5S
Brachionus	7400	600	60	7.5	2.5	2.5	0	175	32.5	10	5	70
Polyarthra	3050	750	90	12.5	0	0	0	206.25	7.5	0	0	0
Filinia	4675	212.5	30	0	0	0	0	162.5	0	0	0	0
Asplanchna	50	0	0	0	0	0	0	0	0	5	0	0
TOTAL rotifers	15175	1562.5	180	20	2.5	2.5	0	543.75	40	15	10	70
Ostracod	0	0	0	0	2.5	2.5	0	0	2.5	17.5	25	132.5
Chydoridae	0	0	0	0	5	2.5	0	18.75	7.5	5	2.5	0
Daphnia	0	143.75	80	20	67.5	62.5	118.75	25	12.5	0	2.5	0
Moina	75	0	37.5	0	0	0	37.5	2.5	0	0	0	0
Cyclopoids	100	50	22.5	5	12.5	10	118.75	5	42.5	0	77.5	60
Calanoids	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL adult copep.	100	50	22.5	5	12.5	10	118.75	5	42.5	0	77.5	60
TOTAL adult crust.	175	193.75	140	25	87.5	22.5	293.75	42.5	77.5	27.5	95	202.5
cyclopoid nauplii	225	500	282.5	130	180	765	831.25	552.5	775	32.5	1160	722.5
calanoid nauplii	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL nauplii	225	500	282.5	130	180	765	831.25	552.5	775	32.5	1160	722.5
TOTAL crust.	400	693.75	422.5	155	267.5	787.5	1125	595	852.5	60	1255	925
TOTAL organisms	15575	2256.25	602.5	175	270	790	1668.75	635	867.5	70	1325	925
Collection time (9.15am-11.35am)	700	206.25	20	2.5	0	0	62.5	5	0	0	7.5	
No. Brach ovig												
% Brach ovig	9.4594595	34.375	33.3333333	33.3333333	0	0	35.714286	15.384615	0	0	0	10.714286
No. Polyarth ovig	0	0	0	0			25	0				
% Polyarth ovig	0	1.6666667	0	0			12.121212	0				
No. Filinia ovig	1450	50	5				18.75					
% Filinia ovig	31.016043	23.529412	16.666667				11.538462					
No. cylop. ovig	0	0	0	0	2.5	2.5	18.75	0	5		5	2.5
% cylop. ovig	0	37.5	0	0	25	50	15.789474	0	11.764706		6.4516129	4.1666667
No. calan. ovig												
% calan. ovig												
NOTES												
100% cloud cover, fine rain, no wind												

**Zooplankton, Day 594 (9 January 1995)**

**Zooplankton, Day 604 (19 January 1995)**

**Zooplankton, Day 619 (3 February 1995)**

Numbers per litre
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[illegible]

**Zooplankton, Day 622 (6 February 1995)**

**Zooplankton, Day 625 (9 February 1995)**

[illegible]



**Zooplankton, Day 639 (23 February 1995)**

Numbers per litre	
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[illegible]

## NOTES

Cloud cover 35%, very slight southerly wind. 1S north outlet board fixed again.

Flow almost going over the top of the divider at SPN outlet. South side now overflowing the final lagoon boards.

POND 5S - lots of b/down material = pond returning to normal after low flow, drying out, & new flow rotif. bloom? (lots of 'speckled growths'/mats)

## Summary of Baleen harvest data

**Note: these data represent the results of a harvesting trial conducted in the 85wB specifically for this project,**

and do not represent peak harvesting levels for the Baleen at high zooplankton densities or targetting other zooplankton size classes.

The following information provides a summary of some of the harvesting trials conducted at WTC during the course of this project (as per Section 6.1). Physical, chemical and biological data collected as part of harvesting and other supplementary experiments are not presented in this thesis.			
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Ponds used: 1N-3N, 1S-3S (2S = harvest pond)

Dates run: 13-15.03.96 (pre-harvest), 18-22.03.96 (harvest), 25-27.03.96 (post-harvest)

Harvesting took place between 8.30am & 12.00pm in the harvest week.

Baleen screen aperture = 500  $\mu\text{m}$ . Horizontal area of screen = 0.62  $\text{m}^2$ , average boat speed = 0.9017  $\text{m/s}$ .

A theoretical estimate of 0.5591 cubic metres passed over the Baleen screen per second during harvest. This figure is an overestimate, however, as it does not account for the effects of water resistance to the screen (as per Bottrell *et al.* 1976).

Harvest subsamples were taken half way through and at the end of each harvest session to determine the mix of animals harvested using that screen size.				
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DATE	Length of harvest	Plankton harvested	Prevailing daphnid nos./L (2S)
18.03.96	2 hrs 55 min	190kg	30
19.03.96	3 hrs 20 min	207kg	57.5
20.03.96	3 hrs 30 min	184.5kg	5
21.03.96	3 hrs 25 min	113kg	32.5
22.03.96	1 hr 35 min	100kg	12.5

18.03.96	subsample 1	Daphnia (96.17%), Moina (0.64%), cyclopoids (1.92%), nauplii (0.32%), Brachionus (0.64%), Asplanchna (0.32%)	
18.03.96	subsample 2	Daphnia (86.89%), Moina (1.64%), cyclopoids (11.48%)	
19.03.96	subsample 1	Daphnia (99.42%), cyclopoids (0.58%)	
19.03.96	subsample 2	Daphnia (100%)	
20.03.96	subsample 1	Daphnia (99.47%), Moina (0.53%)	
20.03.96	subsample 2	Daphnia (88.30%), cyclopoids (10.64%), Moina (1.07%)	
21.03.96	subsample 1	Daphnia (95.77%), cyclopoids (3.52%), nauplii (0.70%)	
21.03.96	subsample 2	Daphnia (89.66%), Moina (1.72%), cyclopoids (8.62%)	
22.03.96	subsample 1	Daphnia (96.09%), Moina (1.12%), cyclopoids (2.79%)	
22.03.96	subsample 2	no second sample	

<b>Appendix 3:</b>				
<b>Example basket harvesting data (Zootech Pty. Ltd.)</b>				
<b>Pond 5, 115E system</b>				
18.08.93 - Daphnia 70/l, Copepod nauplii 193/l, rotifers 0/l, copepods 0/l (D. Cartwright, unpubl. data)				
27.08.93 - pond reincorporated into 115E system, daily FR reduced from approx. 60 ML/d to 35 ML/d.				
<b>Date</b>	<b>No. of baskets</b>	<b>Screen aperture</b>	<b>Harvest time</b>	<b>Zoopl harvested</b>
30.08.98	1	250um	30 min	30 litres
31.08.98	8	250-315um	(rapid blockage, no harvest*)	
01.09.98	7	250-315um	2 hr 30 min	36 litres
03.09.98	4	250-315um	3 hr 30 min	78 litres
09.09.98	1	530um	20 hr 30 min	85 litres
10.09.98	1	530um	30 min	60 litres
13.09.98	1	530um	6 hr	25 litres
14.09.98	1	530um	20 hr 15 min	45 litres **
20.09.98	1	530um	15 hr 40 min	40 litres
NB - the large harvest on 30/8 may be the result of a 'flushing effect' caused by the change in hydrology rather than a sudden zooplankton bloom of significance.				
Following this event, there were several wild fluctuations observed in harvest rates around the time of wet weather flows, with occasional high rates experienced for short periods (e.g. 10/9).				
* Heavy rain contributes approx. 10 ML/d, lifting the FR to about 45 ML/d				
** Record wet weather flow event for the farm, rising above emergency levels. 250 ML/d released through 115E.				

