From Single Species to Mesocosms: Responses of Freshwater Copepods and their Community to PCP

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"It isn't pollution that's harming the environment.
It's the impurities in our air and water that are doing it."

US Vice President Dan Quayle

Abstract

The results of single species toxicity tests were compared to tests at higher levels of biological organisation. Laboratory single species acute and chronic toxicity tests using three freshwater copepod species: *Calamoecia lucasi, Boeckella delicata*, and *Mesocyclops* cf. *leuckarti*, were compared to community-level responses in naturally derived laboratory microcosms and *in situ* mesocosms dosed with pentachlorophenol (PCP).

Acute 48 h lethality tests used two copepod life stages (nauplii and adults). Tests were conducted at 22°C with laboratory cultured animals of all species and at varying temperatures with seasonally collected *C. lucasi* adults. Chronic sublethal tests with <24 h nauplii measured time to metamorphosis. Copepod cultures were established using animals collected from Lake Rotomanuka, New Zealand.

A 12-day laboratory microcosm experiment was conducted during the summer of 1997 using naturally derived microcosms (1.6 L) established using water and planktonic organisms collected from Lake Rotomanuka. The microcosm experiment coincided with the summer mesocosm experiment so that community-level responses could be compared.

Four 20-day seasonal mesocosm experiments were undertaken between the winter of 1996 and autumn of 1997 to investigate seasonal variations in planktonic community responses to PCP. The mesocosms (860 L) were deployed in Lake Rotomanuka and established using water and planktonic organisms from the lake. Single applications of technical grade PCP dissolved in 95% ethanol were applied to the microcosms and mesocosms in a regression design experiment employing seven unreplicated nominal PCP concentrations (4, 10, 24, 36, 54, 81 and 121 $\mu g.L^{-1}$), with solvent and non-solvent controls.

Temperature, dissolved oxygen, pH, conductivity, chlorophyll *a*, zooplankton and phytoplankton abundance were monitored regularly during the microcosm and mesocosm experiments. PCP concentrations were measured over a 40-day period in the spring mesocosm experiment so that nominal concentrations could be confirmed and rates of decay calculated.

The effects of treatment and time on plankton species composition in the microcosms and mesocosms were analysed using redundancy analysis (RDA), with Monte Carlo permutation tests to identify significant treatment effects on each sample day. Daily EC50 estimates were calculated using total copepod % change in abundance so that effect concentrations could be compared to results from the laboratory copepod tests.

In the laboratory tests, *C. lucasi* was the most sensitive of the three copepods with 48 h LC50 values of 52 and 106 $\mu g.L^{-1}$ PCP for nauplii and adults respectively. *B. delicata*

nauplii and *M. leuckarti* adults were least sensitive (227 and 173 $\mu g.L^{-1}$ respectively). Summer collected *C. lucasi* adults at 22°C were least sensitive (106 $\mu g.L^{-1}$), while winter and spring collected adults at 12 and 17°C were most sensitive (70 and 71 $\mu g.L^{-1}$ respectively). PCP delayed metamorphosis in all species. *C. lucasi* were most sensitive (ChV 14.1 $\mu g.L^{-1}$) and *M. leuckarti* least sensitive (ChV 104.3 $\mu g.L^{-1}$).

Community-level responses to PCP were similar in the microcosms and summer mesocosms with treatment effects apparent at concentrations as low as 10 and 24 $\mu g.L^{-1}$ in both systems. There was a slight delay in response in the microcosms, with copepod EC50 values on days eight (85 $\mu g.L^{-1}$) and twelve (90 $\mu g.L^{-1}$) in the microcosms similar to those on days two (96 $\mu g.L^{-1}$) and four (89 $\mu g.L^{-1}$) in the mesocosms.

Following observation of a solvent effect, as reduced oxygen concentrations in the microcosms and mesocosms, a microcosm study was undertaken to investigate the effect of solvent concentration (8, 16, 32, 63, 94, and 125 $\mu L.L^{-1}$ ethanol) on pH and dissolved oxygen. Declines in oxygen and pH occurred in all treatments, although a recovery towards pretreatment levels was observed in the two lowest concentrations after eleven days.

Planktonic community sensitivity to PCP in the mesocosms varied seasonally. In the RDAs treatment effects were identified at concentrations of 24 $\mu g.L^{-1}$ in summer, 36 $\mu g.L^{-1}$ in spring and autumn, and 54 $\mu g.L^{-1}$ in winter. With the exception of *Cryptomonas*, which responded positively to PCP, all other taxa were most sensitive in winter and spring, with copepods showing the greatest sensitivity. The seasonal variation in mesocosm copepod 48 h EC50 values, which ranged from 47 to 90 $\mu g.L^{-1}$ in spring and summer respectively, corresponded with changes observed in the laboratory tests using seasonally collected *C. lucasi*. Measured PCP concentrations on day one in the spring mesocosms were similar to predicted nominal concentrations. The mean half-life of PCP was ~21 days.

In general, copepod LC50 values from laboratory tests were comparable to PCP concentrations causing acute responses in the microcosms and summer mesocosms. Copepod chronic values from sublethal tests were also similar to microcosm and mesocosm EC50 values and concentrations causing community-level treatment effects.

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Definitions of terms used

ACR — Acute to Chronic Ratio. The ratio of the acute toxicity of a toxicant (e.g. LC50) to its chronic toxicity (e.g. NOEC). For example:

$$ACR = \frac{LC50}{NOEC}$$

The ACR can be used to predict the probable chronic responses of similar species or toxicants for which chronic data is not available.

ChV — Chronic Value. The geometric mean of the NOEC and LOEC. The ChV represents a presumably safe concentration of a chemical.

EC50 — Median Effective Concentration. An effect other than death is the criterion. The concentration producing a specific response in 50% of the organisms tested.

LC50 — Median Lethal Concentration. The concentration that is lethal to 50% of the exposed organisms after continuous exposure for a fixed time (e.g. 48 h).

LOEC — Lowest Observable Effect Concentration. The lowest concentration of a toxicant that has a statistically significant adverse effect on the exposed population of test organisms when compared to the controls.

NEC — No Effect Concentration. The concentration of a toxicant producing no statistically significant effect.

NOEC — No Observable Effect Concentration. The highest concentration of a toxicant that has no statistically significant adverse effect on the exposed population of test organisms when compared with the controls.

CHAPTER ONE

General Introduction

Single species and multispecies toxicity tests

Single species aquatic toxicity tests conducted under standardised laboratory conditions constitute the basis for determination of most water quality criteria. They provide essential information on concentrations and durations of exposures to chemicals that result in changes in survival, reproduction, physiology, biochemistry and behaviour of individuals of a particular species (Cairns 1983). Furthermore, they are relatively easy to conduct, can be standardised and replicated, and are generally inexpensive.

Single species tests are undertaken because they are assumed to provide results that can be used to protect ecosystems. However, their ability to accurately predict the effects of toxicants on complex communities has been widely debated (Cairns 1983; Monk 1983; Kimball and Levin 1985; Cairns 1986a; Slooff et al. 1986; Chapman 1995; Ferson et al. 1996; Crane 1997).

The deficiencies of single species tests are summarised by Pontasch et al. (1989):

- Single species tests do not take into account interactions among species
- They utilize genetically homogeneous laboratory-stock test populations that lack the adaptive capability of heterogeneous natural populations
- They use species of unknown relative sensitivities
- They are often conducted under physical and chemical conditions that lack similarity to natural habitats
- They ultilize species that are usually not indigenous to the receiving system, thus complicating field validation of the test

In the past it was suggested that if the most sensitive species could be found then no-effect concentrations derived using this species would protect all organisms in the environment, although it is highly unlikely that such a species exists (Cairns 1986b; Cairns and Niederlehner 1987). However, as noted by Cairns et al. (1996), only rarely, if ever, has

there been a call for a complete shift away from single species tests but rather for the addition of data from tests that focus on higher levels of biological organisation.

Multispecies tests have a level of organisation higher than that of single species tests (Dickson et al. 1985), and include tests ranging from relatively simple systems containing two species in a predator-prey interaction to large artificial ponds.

Examples of uses for multispecies tests as listed by Dickson et al. (1985) include:

- Identification of species interactions or compensation that would be missed in single species tests
- Illustration of chemical-physical pathways
- Assessment of population, community, and ecosystem-level responses to toxicants
- Elucidation of particular mechanisms and interactions
- Identification of sensitive or critical species that should be used in single-species toxicity tests
- Testing of hypotheses

The most frequently cited justifications for conducting studies at higher levels of biological organisation are improved environmental realism and greater predictive ability (Clements and Kiffney 1994). Because they incorporate and evaluate more end-points that correspond more closely to those of natural systems it is suggested that their sensitivity, and hence ability to assess the potential effects of chemicals and effluents on ecosystems, should be enhanced relative to single species tests.

Community-level multispecies tests range in size and complexity from small laboratory microcosms to larger *in situ* mesocosms to artificial ponds and streams, and can be used to study the fate of chemicals, and their effects (both direct and indirect) on a variety of organisms at the individual, population and community-levels.

The term microcosm is usually applied to small laboratory systems (Taub 1997), although many outdoor systems are also commonly referred to as microcosms, thus they can range in size from one litre to thousands of litres. Development of laboratory microcosm protocols has concentrated primarily on generic systems derived from laboratory cultured organisms such as the Standardised Aquatic Microcosm, SAM (ASTM 1995), or natural

ecosystems such as Leffler microcosms (Leffler 1984). These systems are designed to simulate properties common to all ecosystems rather than replicating specific natural systems therefore ecological realism is reduced relative to larger microcosms and mesocosms. The greatest appeal of small microcosms lies in their ability to provide greater experimental control while at the same time still maintaining some characteristics of larger systems such as diversity, succession, and trophic dynamics (Niederlehner and Cairns 1994). They can also be easily replicated and more concentrations can be tested. Inter- and intra-laboratory comparisons of SAM have also demonstrated their ability to provide similar community-level responses (Taub et al. 1986; Conquest and Taub 1989; Taub et al. 1989).

Microcosms using naturally derived communities tend to be larger than generic systems and are designed to simulate effects on a specific community. There is some concern that microcosms using naturally derived communities, although more environmentally realistic, may cause test results to be quite variable. However, Cairns and Cherry (1998) suggest that if the number of organisms exposed is large enough then the range of sensitivities will be randomised and therefore repeatable, as illustrated by Sheehan et al. (1986) and Niederlehner and Cairns (1994) who found that distinctly different communities responded similarly to stress.

Mesocosms are defined by Odum (1984) as bounded and partially enclosed outdoor experimental units that closely simulate the natural environment. They provide simplifications or models of natural ecosystem behaviour and offer higher degrees of environmental realism than laboratory single species systems and small microcosms. Although they do not behave identically to the natural ecosystem, they allow greater degrees of experimental control as they can be manipulated and replicated, hence they provide an intermediate between entire ecosystems and laboratory toxicity tests.

Mesocosm studies are usually undertaken to negate a presumption of adverse ecological effects or unreasonable risks based upon laboratory derived information, and to define the intensity and duration of adverse effects of toxicants in aquatic systems (Rodgers 1994). Mesocosms have been used extensively for pesticide registration, and in 1988 became a requirement as the last step in the tiered registration or pre-registration process of potentially hazardous pesticides (Touart 1988). This decision was revoked by the USEPA in 1992 (Fisher 1992), apparently because it was perceived that mesocosm studies did not contribute substantially to existing laboratory test data in a timely and cost-effective manner (Boyle and Fairchild 1997; Landis et al. 1997).

As with single species testing, debates concerning the role of multispecies tests abound (Cairns 1988; Clements and Kiffney 1994; Crane 1995; Joern and Hoagland 1996; Landis 1996; Shaw and Kennedy 1996; Boyle and Fairchild 1997; Crane 1997). Opinions differ as to whether tests at higher levels of biological organisation can contribute significantly to data obtained from single species tests. Many argue that the added expense and time involved in undertaking mesocosm tests may outweigh their presumed advantages, i.e. increased environmental realism. Furthermore, it is argued that the inherent natural variability of complex communities reduces our ability to distinguish toxicant related changes from those occurring naturally, thus test sensitivity may be reduced rather than increased. In some cases the information gained from mesocosm studies may simply confirm the results of laboratory tests (Shaw and Kennedy 1996) or even suggest that single species tests are over-protective (Crane 1997).

Design of microcosm and mesocosm experiments

Microcosm and mesocosm studies have traditionally employed a replicated design relying on analysis of variance (ANOVA) and multiple range tests to determine the significance of treatment effects. Multiple range tests compare each treatment level with the non-dosed

controls and are useful in determining no-observable-effect-concentrations (NOEC) and lowest-observable-effect-concentrations (LOEC) for a toxicant. However, they are limited to the evaluation of effects only in those concentrations actually tested, and do not allow for inference of responses at untested concentrations (Liber et al. 1992). Thus the determination of endpoints, such as the NOEC and LOEC, in ANOVA experiments are entirely dependent on the concentrations chosen, and are a design artifact rather than a measurement of ecological risk. Furthermore, the ANOVA design restricts the concentration range, as it becomes logistically unfeasible to employ a large number of sufficiently replicated concentrations.

Regression design experiments such as that of Liber et al. (1992) are more suited to examining dose-response relationships as they employ greater numbers of concentrations, although they commonly have fewer or no replicates at any given treatment level. A regression design allows for the estimation of specific end points such as the no-effect concentration (NEC) and EC50, which are of interest from a regulatory point of view (Liber et al. 1992) in addition to facilitating comparisons with laboratory tests.

Therefore, the goals of a study will determine the experimental design. ANOVA designs are most appropriate when specific environmental concentrations of a chemical are of interest, whereas regression designs have the ability to define population responses over a wide range of chemical concentrations (Liber et al. 1992). In the current study regression designs were used for the microcosm and mesocosm experiments so that effective concentrations could be calculated with greater precision thus increasing the sensitivity of the tests and enhancing comparisons between them.

If sufficient resources are available, experiments could incorporate aspects of both ANOVA and regression designs in a 'hybrid' design, with either increased replication, or additional test concentrations around the predicted measurement endpoints so that more precise estimates can be obtained.

According to Liber et al. (1992), their study was the first to employ a regression design without replication of treatment concentrations. Replication is generally advocated so that an estimate of natural variability can be obtained, thus enabling differentiation between natural and treatment-related effects on the parameters under investigation. Increased replication at each treatment level should also increase the statistical power of the test. However, even with replication, the ability to detect statistically significant treatment effects may not be markedly improved, as often it is not possible to include a sufficient number of replicates to significantly reduce the influence of within treatment variability.

In the USEPA guidance document on aquatic mesocosm experiments (Touart 1988), it was recommended that a minimum of three replicates be used at each of four treatment levels (Touart 1994). However, Christman et al. (1994) suggest that six rather than three replicates would usually improve the ability to detect differences between treatments. The ability to replicate a sufficient number of treatments six times would be beyond the scope of most studies however. Similarly, in a regression design, increasing the number of treatment levels along the response gradient will increase the confidence in the fitted dose-response line (Touart 1994).

Multivariate analysis of community responses

Mesocosm studies are undertaken so that ecosystem-level responses may be examined, but few ecosystem-level analyses are performed on the data collected (Kennedy 1994). Instead, multispecies toxicity tests are predominantly analysed using conventional data analysis employed for single species tests (ANOVA and multiple comparison tests). These techniques are used to find statistically significant differences between treated and non-treated replicates by examining individual parameters in isolation, and are often incapable of detecting subtle treatment effects due to the inherent variability in the biological parameters measured. Therefore large treatment effects are usually required before

statistical differences can be detected (Kennedy 1994). Thus the common use of univariate data analyses may be partly to blame for the perception that multispecies tests are relatively insensitive and contribute little additional information to that obtained from laboratory tests.

Multivariate analyses are more appropriate for analysing complex community-level responses to toxicants in microcosms and mesocosms. A variety of multivariate methods are available and have the advantage of examining data sets in their entirety rather than isolating individual components. Landis et al. (1997) outline the attributes and deficiencies of some of the more commonly used multivariate methods such as principal components analysis (PCA), nonmetric multidimensional scaling (NMDS) and clustering. Each technique provides different insights into the patterns that exist within multispecies toxicity tests and is based on different assumptions and methodology.

Various studies have used principal components analysis (PCA) or its derivative, redundancy analysis (RDA), to identify treatment effects at the community level (see van Breukelen and Brock 1993; Lucassen and Leeuwangh 1994; Verdonschot and ter Braak 1994; van Wijngaarden et al. 1995; Shaw and Manning 1996; van den Brink et al. 1996; Sierszen and Lozano 1998). PCA and RDA, using the program CANOCO (ter Braak 1988), condense large data sets into relatively simple diagrams that plot the path of the various treatment groups over time and show the associated species. PCA gives an overview of the variation between the samples but does not specify the treatment contribution. Further analysis with RDA identifies the contribution of treatment with its introduction as an environmental variable, and in combination with Monte Carlo permutation tests, statistical significance of treatment-related effects can be determined.

Multivariate analyses in this study used RDA to visualise temporal and treatment-related changes in species composition.

Ecotoxicology in New Zealand

Aquatic toxicology is a relatively new discipline in New Zealand, and following implementation of the Resource Management Act, 1991 (RMA), its profile has increased as toxicity tests are increasingly used to determine compliance with RMA requirements (Hickey and Roper 1994). To date, studies have focussed largely on identifying native aquatic invertebrates and vertebrates that may have potential as laboratory test species so that standardised tests can be developed which are relevant to the New Zealand environment. Much of this work has focussed on native freshwater fish, limnetic cladocerans, and freshwater and estuarine benthic invertebrates.

Although there are localised areas of contamination in New Zealand which, in some instances, are known to be significant sources of pollutants to adjacent waterways, levels of contamination are generally below those found in many developed countries. These relatively low levels of chemical contamination present a challenge when attempting to quantify levels of environmental impact, necessitating further studies to identify suitable test species and development of sublethal tests to detect adverse effects (Hickey 1995).

Internationally recognised standard toxicity test protocols often use species that are not relevant in New Zealand as they do not occur here. Standardised tests using cladocerans, in particular *Daphnia magna*, are widely utilised because of the importance of cladocerans in freshwater ecosystems and their amenability to laboratory culture. In New Zealand *Daphnia* is uncommon, small *Bosmina* spp. are the most widespread of the cladoceran species, and cladocerans are often outnumbered numerically by calanoid copepods.

Thus selection of suitable species for use in laboratory bioassays should take into consideration the distinctive features of New Zealand zooplankton communities:

- Crustacean zooplankton show low species diversity, and predatory cladocerans and midges (e.g. *Chaoborus* spp.) are absent
- Large *Daphnia* are absent, with the exception of *D. carinata* which only rarely occurs in lakes
- Of the cladocerans, *Bosmina* spp. are the most widespread

- *Ceriodaphnia* spp. are also found throughout New Zealand, though numbers often fluctuate considerably with only brief periods of abundance
- Centropagid calanoid copepods belonging to the genera *Calamoecia* and *Boeckella* often dominate zooplankton communities numerically
- Large cyclopoid copepods belonging to the genus *Cyclops* are absent
- Smaller cyclopoid copepods are the main invertebrate predators, along with the water mite (*Piona exigua*)
- Many species have a ubiquitous distribution, implying they can tolerate a wide range of conditions

(Chapman et al. 1975; Chapman and Green 1987; Burns 1991).

Three species of copepod were selected for use in laboratory toxicity tests in this study; *Calamoecia lucasi, Boeckella delicata* and *Mesocyclops* cf. *leuckarti.*

Freshwater copepods have been largely ignored in ecotoxicity testing, however their importance in freshwater ecosystems and numerical dominance in New Zealand lakes, suggests that they may have potential as toxicity test species. Southern Hemisphere calanoid copepods belong to the Centropagidae and are equivalents of the Northern Hemisphere diaptomids. The centropagids are well described taxonomically and occur in lakes throughout New Zealand. *C. lucasi* is found throughout the North Island and the north of the South Island, while species belonging to the genus *Boeckella* are widespread in both the South and North Islands (Burns 1991). Four *Boeckella* species also occur in the North Island, and of these *B. delicata* unusually co-occurs with *C. lucasi* in several lakes. Of the cyclopoid copepods found in New Zealand, *M. leuckarti* is common in northern North Island lakes (Green 1974).

Pentachlorophenol (PCP)

PCP was used in this study for a number of reasons:

1. Past use of PCP by the New Zealand timber industry means that many sites are likely to be contaminated to some extent, and may still be significant sources of contamination to streams, rivers and lakes.

2. PCP has been recommended as a reference toxicant by a number of workers (Davis and Hoos 1975; Adelman and Smith 1976; Lee 1980).
3. Previous toxicity assessments with New Zealand aquatic invertebrates (and vertebrates) have often included PCP as one of the toxicants, providing a (limited) database for comparisons of sensitivity between local species.
4. PCP has a well-described mode of action, is relatively non-selective, and is acutely toxic at low concentrations.

The environmental importance of PCP in New Zealand can be attributed primarily to its extensive use in the past by the timber industry, which used it as an antifungicide to treat sawn radiata pine. It is one of several contaminants identified in the New Zealand environment, and in some locations concentrations are high enough to cause concern with levels comparable to contaminated sites overseas.

To incorporate a greater degree of environmental relevance in this study it was decided to use a technical grade PCP formulation, as used by the New Zealand timber treatment industry.

Study objectives

Until recently freshwater ecotoxicity studies in New Zealand have been restricted to single species assessments of chemical toxicity. The feasibility of microcosms and *in situ* mesocosms for ecotoxicity studies in New Zealand has not been assessed and nothing is known about community-level toxicant effects on local planktonic organisms. On a wider scale, many comparative studies assessing the predictive abilities of tests at different levels of complexity have compared results from different studies rather than conducting a series of tests either sequentially or concurrently using organisms from the same source.

The overall objective of this thesis was to compare the predictive ability of toxicity tests at different levels of biological organisation, from laboratory single species acute and

sublethal tests using freshwater copepods to small naturally derived microcosms to *in situ* mesocosms. Copepods and microcosm species assemblages were collected from the lake in which the mesocosms were deployed.

Toxicity tests at each level of biological organisation are presented as self-contained chapters that have been written in a format suitable for submission to journals. Chapters two and three have already been submitted. The first and last chapters are written as a general introduction and discussion linking the experimental chapters together in relation to the overall objective. Because of this format there is some repetition between chapters, primarily in the methods sections.

Thus the objectives for each chapter are:

Chapter Two - Acute and chronic bioassays with freshwater copepods using PCP.

- To compare the sensitivity of three copepods to PCP with acute toxicity tests using nauplii and adults
- To determine the effect of season on sensitivity of adult *C. lucasi* to PCP
- To compare the sublethal toxicity of PCP to naupliar stages using metamorphosis as a test endpoint
- To evaluate the suitability of the three species for laboratory culture

Chapter Three - PCP decay in a New Zealand lake: a mesocosm study.

- To investigate the persistence of a technical formulation of PCP in the water column of a New Zealand lake using *in situ* mesocosms

Chapter Four - A comparative study of community-level responses in small laboratory microcosms and *in situ* mesocosms dosed with pentachlorophenol.

- To establish whether direct toxicant effects on species composition under controlled conditions in smaller and less costly microcosms would parallel those observed in larger and more environmentally realistic mesocosms

Chapter Five – Ethanol as a solvent carrier in microcosms and mesocosms: Effects on dissolved oxygen and pH in microcosms.

- To investigate the effect of different ethanol concentrations on the magnitude of pH and dissolved oxygen changes

Chapter Six - Seasonal variation in community-level responses of aquatic mesocosms dosed with pentachlorophenol

- To determine whether seasonality is important in modifying the response of a planktonic community to PCP

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CHAPTER TWO

Acute and chronic bioassays with freshwater copepods using pentachlorophenol

Abstract

The comparative sensitivity of three species of freshwater copepod (*Calamoecia lucasi* BRADY, *Boeckella delicata* PERCIVAL and *Mesocyclops* cf. *leuckarti* CLAUS) to pentachlorophenol (PCP) were assessed. Acute and chronic bioassays used two life stages (nauplii and adults). Acute 48 h lethality tests were conducted at 22°C with laboratory cultured animals of all species, and at varying temperatures with seasonally collected *C. lucasi* adults. Chronic sublethal tests with <24 h nauplii measured time to metamorphosis. *C. lucasi* was most sensitive, with 48 h LC50 values of 52 and 106 $\mu g.L^{-1}$ PCP for nauplii and adults respectively. *B. delicata* nauplii and *M. leuckarti* adults were least sensitive (227 and 173 $\mu g.L^{-1}$ respectively). Control survival was ≥ 95% in acute tests, with the exception of *C. lucasi* nauplii (60%). Summer collected *C. lucasi* adults at 22°C were least sensitive (106 $\mu g.L^{-1}$), while winter and spring collected adults at 12 and 17°C were most sensitive (70 and 71 $\mu g.L^{-1}$ respectively). PCP delayed metamorphosis in all species. *C. lucasi* were most sensitive (ChV 14.1 $\mu g.L^{-1}$) and *M. leuckarti* least sensitive (ChV 104.3 $\mu g.L^{-1}$). Control mortality was high in *C. lucasi* sublethal tests (65%), and of the three species, they were the most difficult to culture.

Keywords: freshwater copepods; metamorphosis; life-stage specific toxicity; seasonal sensitivity; PCP; New Zealand

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Introduction

Copepods are widespread in marine and freshwater ecosystems in a range of habitats and play an important role in the trophic dynamics of such systems. They have relatively short life cycles (3 to 4 weeks at ~20°C) and are easy to culture in the laboratory, with their small size requiring only small volumes of test and dilution water. In other words, they fulfil the requirements for the selection of species for routine use in toxicity tests. Various marine and estuarine copepods are frequently used as toxicity test species and LC50 values have been determined for a variety of toxicants.

Estuarine meiobenthic harpacticoid copepods have gained popularity as a test species since past problems associated with laboratory culturing and bioassays have largely been overcome (Chandler and Green 1996). Freshwater copepods have not been used widely in toxicity tests despite their importance in freshwater ecosystems and known sensitivity to pesticides (Fairchild et al. 1992; Webber et al. 1992; Fliedner and Klien 1996; Peither et al. 1996). Previous studies have used copepods from the Great Lakes (Latimer et al. 1975) and a groundwater copepod (Notenboom et al. 1992).

Compared with that of other countries, the crustacean zooplankton of New Zealand lakes show low species diversity, and centropagid calanoid copepods often dominate zooplankton communities numerically (Burns 1991). In New Zealand, Australia, and in southern and high altitude regions of South America, centropagid calanoid copepods belonging to the genera *Boeckella* and *Calamoecia* are equivalents of the diaptomids in Northern Hemisphere lakes. Centropagids also comprise a minor component of the freshwater calanoid fauna of Europe and North America (Bayly 1992). There are 21 Australasian species in the genus *Boeckella.* In New Zealand they are numerically dominant in South Island zooplankton communities (Burns 1991), although four *Boeckella* species also occur in the North Island. Of these four, *B. delicata* has a disjunct distribution occurring in northern New Zealand and New South Wales (Australia). *Calamoecia lucasi*

is widespread and abundant in North Island lakes but occurs in only isolated populations in the South Island. It is also found in large slow flowing rivers. In Australia *C. lucasi* is the most widely distributed of the 14 *Calamoecia* species, where it is found throughout with the exception of Tasmania (Bayly 1992).

Cyclopoid copepods have a worldwide distribution and are generally omnivorous with broad trophic niches (Hopp et al. 1997). Although they are widely distributed in New Zealand lakes and are present throughout the year, numbers fluctuate and they may be very abundant numerically for short periods. Along with the water mite *Piona exigua*, cyclopoid copepods are the main invertebrate predators in New Zealand lakes as predatory cladocerans and midge larvae (*Chaoborus* spp.) are absent (Burns 1991). *Mesocyclops* cf. *leuckarti* is common in northern North Island lakes (Green 1974).

Biological toxicity assessment in New Zealand has progressed considerably since 1986 (Hickey 1995), with development of freshwater toxicity tests based primarily on limnetic cladocerans (Hickey 1989; Willis et al. 1995) and benthic invertebrates (Hickey and Vickers 1992, 1994; Hickey and Martin 1995). Levels of environmental contamination in New Zealand are generally below those encounted at many overseas locations, and occur at sublethal concentrations, so tests need to be developed to detect adverse effects at low levels of contamination (Hickey 1995). This study was undertaken to provide information on the relative sensitivity of three New Zealand copepod species, *C. lucasi*, *B. delicata* and *M. leuckarti*, to PCP using acute and sublethal toxicity tests.

In addition to its use as a reference chemical (Lee 1980), large amounts of PCP were used by New Zealand timber treatment companies in the past, resulting in the contamination of numerous sites which may still be significant sources of contamination to streams, rivers and lakes (Shaw 1990).

The objectives of this study were a) to compare the sensitivity of three copepods to PCP with acute toxicity tests using nauplii and adults, b) to determine the effect of season on

sensitivity of adult *C. lucasi*, c) to compare the sublethal toxicity of PCP to naupliar stages using time to metamorphosis as a test endpoint, and d) to evaluate the suitability of the three species for laboratory culture.

Copepod Biology

Calanoid copepods are suspension feeding omnivores capable of selecting algae and small animals of particular dimensions (Vanderploeg 1994). Cyclopoid copepods are raptorial omnivores, seizing plant or animal food (Fryer 1957). Late ontogenetic stages of *M. leuckarti* (copepodites IV,V and adults) are carnivorous, and will cannibalise younger life stages (Gophen 1977). They also readily feed on dead organisms, and detritus from bottom sediments is sufficient for their survival (Papinska 1985). Nauplii and copepodite stages I to III are herbivorous.

Copepods reproduce sexually and females often produce multiple broods from one insemination. Nauplii hatch from fertilised eggs and are morphologically distinct from later developmental stages. There are five (cyclopoids) or six (harpacticoids and calanoids) naupliar stages prior to metamorphosis into copepodites, generally with one or two prefeeding naupliar instars (Vijverberg 1989). Following metamorphosis there are six copepodite stages. Development and somatic growth cease in the adult, the sixth copepodite instar.

In a study by Green (1976), *C. lucasi* nauplii took 19 to 6 days to develop at temperatures of 10 to 25°C and, in a North Island lake, average ecological longevities of adult *C. lucasi* throughout the year ranged between 1.1 to 76.9 days. Female *C. lucasi* have relatively small clutch sizes ranging between one to ten eggs (Chapman 1973; Green 1976), although clutches of up to 30 eggs have been found. *Boeckella* spp. are larger than *C. lucasi*, and clutch sizes vary with species. Jamieson and Burns (1988) reported clutch sizes as large as 48 eggs under high food levels, with up to eight clutches produced per female. Naupliar

development times of three *Boeckella* species varied between 5.4 and 21.5 days at 18 and 8.5°C (Jamieson 1986).

Reproductive parameters and adult longevity of *M. leuckarti* fed on a mixed diet of natural plankton and phytoplankton were measured by Hopp et al. (1997). Adults had a mean lifespan of 25 days (52 days maximum) at 18 to 20°C. Females produced up to eight clutches in their lifetime (mean of four clutches per female), with a maximum clutch size of 71 (mean clutch size 46). Naupliar and copepodite development times at 22°C were 15 to 16 days, and 10 to 15 days respectively, for males and females (Gophen 1976). In cool latitudes later stage copepodites are also known to overwinter in deeper water and may even hibernate in bottom sediments.

Methods

Cultures

Laboratory cultures of the three species were established from copepods collected from Lake Rotomanuka (37°55' S, 175°19' E) with a 100 μm mesh plankton net. On return to the laboratory adult copepods were isolated according to species and transferred into culture vessels (600 to 2000 mL borosilicate glass beakers). Species were identified using Chapman and Lewis (1976). Cultures were maintained in soft synthetic water (USEPA 1991), prepared using deionised water and reagent grade chemicals. Culture vessels were covered with petri dishes and maintained at 22 ±1°C in a temperature controlled water bath (Julabo F20 UC), under cool, white, fluorescent lighting (24 $\mu E.m^{-2}.s^{-1}$), with a photoperiod of L14:D10.

B. delicata and *C. lucasi* were fed 10^3 to 10^4 cells.mL^{-1} *Cryptomonas ozolini* (UTEX LB2194) and *Chlamydomonas reinhardtii* (UTEX 90), and 2 mL Banta's medium (Banta 1921) three times a week. *M. leuckarti* were fed <24 h washed brine shrimp nauplii (*Artemia salina*) daily. Algal species were cultured in Guillard's Woods Hole MBL

medium (Nichols 1973). Vitamins B_{12} (1 $\mu g.L^{-1}$), biotin (0.75 $\mu g.L^{-1}$), thiamine (75 $\mu g.L^{-1}$), and calcium pantothenate (5 $\mu g.L^{-1}$), were added to both copepod and algal media. Culture vessels were cleaned and the media changed weekly. When necessary individuals were transferred to new or larger culture vessels to prevent overcrowding.

Acute tests

The acute toxicity of PCP to the three copepods was investigated using 24 and 48 h static toxicity tests with <24 h nauplii and adult stages. Sufficient numbers of known age nauplii were obtained by isolating ovigerous females <24 h before beginning a test. Nauplii were isolated and transferred into test solutions using disposable pasteur pipettes in a minimum of culture medium to reduce dilution. Both males and females were used in adult tests. Depending on the availability of animals, two to five tests were conducted over time with two replicates of five to 20 animals employed for each of five concentrations of technical grade PCP (nominally 86%; Aldrich Chemical Co. Milwaukee, WI) and a solvent control. Because adult *M. leuckarti* are cannibalistic, only one adult *M. leuckarti* was placed in each of five replicates. Stock solutions (2 $g.L^{-1}$) were prepared by dissolving PCP in 95% ethanol. Test solutions were prepared by adding appropriate amounts of PCP stock solution to soft synthetic water (USEPA 1991). LC50 values were based on nominal PCP concentrations as test solutions were not analysed. pH was adjusted to 7.3 ± 0.2 and conductivity was 130 ± 10 $\mu S.cm^{-1}$. Tests with juveniles used 5 mL borosilicate glass beakers, containing 5 mL of test solution, covered with glass microscope slides. Adult tests used 50 mL borosilicate glass beakers, containing 50 mL of test solution, covered with parafilm (American National Can™). Test vessels were placed in a temperature controlled water bath (22 ±1°C) under cool, white, fluorescent lighting (24 $\mu E.m^{-2}.s^{-1}$) with a photoperiod of L14:D10. Animals were checked for mortality after 24 and 48 h by observation under a dissecting microscope. The criterion for mortality was a lack of

movement in combination with opaque colouring. Copepods were not fed during tests.

Seasonal variation in the toxicity of PCP to *C. lucasi* was investigated using adults collected from Lake Rotomanuka and tested at ambient water temperature. *C. lucasi* was chosen in preference to the other two copepods as it was present in the lake throughout the year in sufficient numbers, whereas numbers of the other two fluctuated. Collected *C. lucasi* were acclimated to laboratory conditions for 48 h before commencing acute toxicity tests. Test temperature was dependent on season. Tests were conducted at 12°C in winter (August 1996), 17°C in spring (October, November 1996), and 22°C in summer (February 1997) and autumn (March, April 1997). Three to four tests were conducted each season, with two replicates of 10 to 20 animals per concentration. All other test conditions were as described above.

Sublethal tests

The effect of PCP on time to metamorphosis was compared with tests using nauplii of the three copepods. Ovigerous females were isolated from the stock culture <24 h before beginning a test to ensure sufficient numbers of known age nauplii would be available.

<24 h nauplii were exposed to four PCP concentrations, a control and a solvent control in 5 mL borosilcate glass beakers containing approximately 5 mL of test solution, prepared as described previously. Depending on the availability of known aged nauplii there were one or two replicates per experiment, each containing five to 15 nauplii. Test conditions were as described for acute tests. Survival and the number metamorphosed were recorded every 48 h when the nauplii were transferred into new test vessels containing fresh toxicant solution. Test vessels were examined under a dissecting microscope and the nauplii individually transferred using disposable glass pasteur pipettes, copepodites were counted and discarded. This was essential in *M. leuckarti* tests to prevent cannabilism of remaining nauplii by copepodites.

The nauplii were fed every 48 h with *C. ozolini* (10^3 to 10^4 cells.mL^{-1}). Tests were terminated upon completion of metamorphosis in all treatments (8 to 10 days).

Statistical analysis

Acute LC50 values and their associated 95% confidence limits were calculated with probit analysis or Spearman-Karber using ToxCalc™ (Tidepool Scientific Software 1996). When required, data was smoothed and corrected for control mortality using Abbott's correction. The effect of season on PCP toxicity to *C. lucasi* adults was determined using ANOVA followed by Tukey multiple comparisons of the means.

Daily % metamorphosis for days five (*B. delicata*) and six (*C. lucasi* and *M. leuckarti*) were arcsin square root transformed and checked for normality (Shapiro-Wilk's test) and homoscedasticity (Bartlett's test) before being submitted for ANOVA and multiple comparison tests using ToxCalc™. Multiple comparison tests used to compare PCP treatments to solvent controls were either Dunnett's procedure or Bonferroni's t-test (one tailed, $p<0.05$). Naupliar mortality in controls and solvent controls for each species was tested with a two-way replicated ANOVA (treatment vs days). Differences in cumulative mortality (day 8) between species were compared using one-way ANOVA followed by Dunnett's procedure.

Results

Acute tests

Adult *C. lucasi* and *B. delicata* were 2.5 times more sensitive than adult *M. leuckarti* following a 24 h exposure to PCP, but only 1.3 to 1.5 times more so following a 48 h exposure (Table 1). *C. lucasi* and *M. leuckarti* nauplii were more sensitive than adults following a 48 h exposure, whereas *B. delicata* adults were almost twice as sensitive as the nauplii. Of the three species, *C. lucasi* nauplii and adults were the most sensitive, followed

by adult *B. delicata* and *M. leuckarti* nauplii. With the exception of *B. delicata* and *C. lucasi* nauplii, the 48 h LC50 values were similar.

Table 1. Mean LC50 ($\mu g.L^{-1}$ PCP) values (± S.E.M) for cultured naupliar and adult stages tested at 22°C, plus mean control mortality (%).

species	stage	24 h LC50	control mortality (%)	48 h LC50	control mortality (%)
C. lucasi	adult[a]	137 (23)	4	106 (17)	5
	nauplii[b]	93 (61,123)	13	52 (42,63)	40
B. delicata	adult	141 (33)	2	126 (28)	2
	nauplii	246 (42)	0	227 (42)	3
M. leuckarti	adult	370 (101)	0	173 (19)	0
	nauplii	389 (42)	2	138 (9)	5

[a] *C. lucasi* adults collected in summer
[b] 95% CL from two combined tests

Adult *C. lucasi* collected during winter and spring, and tested at temperatures of 12 and 17°C respectively, were most sensitive to PCP (Table 2). Spring collected adults were more sensitive than those collected in autumn, perhaps because they were tested at 17°C rather than 22°C, however, autumn collected adults were more sensitive than those collected during the summer and also tested at 22°C. Summer collected adults were the least sensitive with 24 and 48 h LC50 values of 137 and 106 $\mu g.L^{-1}$ PCP respectively, 1.5 times those of the winter and spring values.

Survival of control adults and nauplii in all the acute tests was very good (≥95%) with the exception of *C. lucasi* naupliar tests where up to 40% mortality was recorded (Table 1). Adult *C. lucasi* control mortality (48 h) in the seasonal tests using collected animals varied between 5 and 16% (Table 2).

Table 2. Mean LC50 ($\mu g.L^{-1}$ PCP) values (± S.E.M) and control mortality (48 h) for seasonally collected and tested adult *C. lucasi*.

	winter	spring	summer	autumn
temperature (°C)	12	17	22	22
24 h LC50 (± S.E.M)	78 (7)[a]	76 (4)[b]	137 (23)[ab]	103 (8)
48 h LC50 (± S.E.M)	70 (8)	71 (7)	106 (17)	82 (1)
% control mortality	14	6	5	16

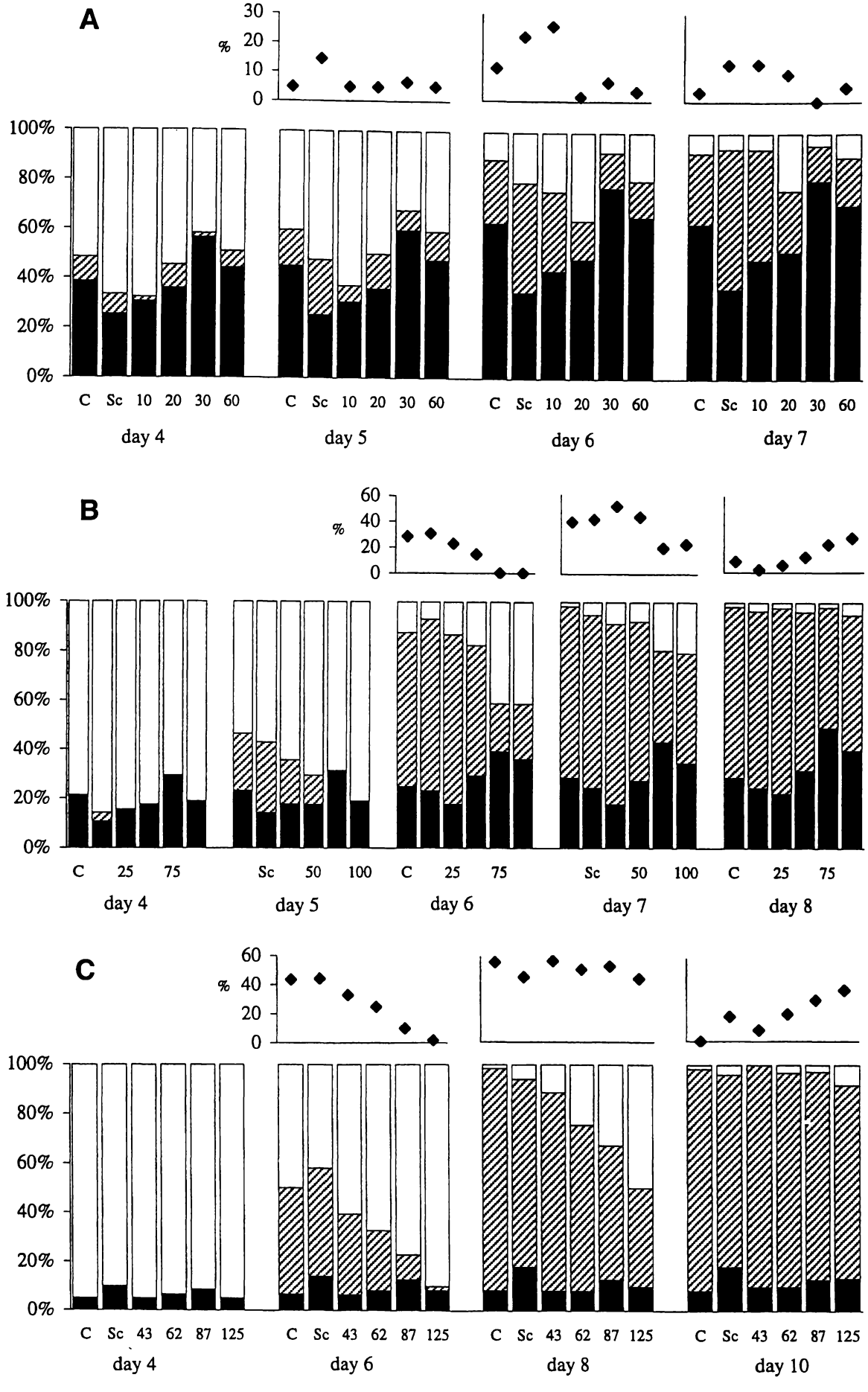
a, b significant difference at $p<0.05$

Sublethal tests

Time to metamorphosis was delayed at the higher PCP concentrations in all species (Fig. 1). The delay in metamorphosis was most apparent on days five (*B. delicata*) and six (*B. delicata*, *C. lucasi* and *M. leuckarti*). Cumulative metamorphosis by days eight (*B. delicata*) and ten (*M. leuckarti*) was similar in all PCP concentrations, as was *M. leuckarti* daily metamorphosis on day eight. On days eight (*B. delicata*) and ten (*M. leuckarti*), daily metamorphosis was increased in the higher concentrations, as metamorphosis was completed in the lower concentrations. This pattern was not observed in *C. lucasi* tests, metamorphosis was reduced in the higher concentrations throughout the tests. High *C. lucasi* naupliar mortality prior to metamorphosis reduced numbers undergoing metamorphosis, and this may have accounted, to some extent, for the different pattern observed.

Following page:

Figure 1. Column graphs show % cumulative metamorphosis, naupliar mortality, and naupliar survival in sublethal tests with *C. lucasi* (A), *B. delicata* (B) and *M. leuckarti* (C). Scatter graphs depicting % daily metamorphosis are above species cumulative data. Abscissa values represent PCP concentration in $\mu g.L^{-1}$. (C = control; Sc = solvent control). n = 45 to 70 depending on species and treatment.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metamorphosis
naupliar survival

Chronic toxicity values are presented in Table 3. *C. lucasi* nauplii were the most sensitive species tested with a ChV of 14.1 $\mu g.L^{-1}$ PCP, seven times lower than the ChV for *M. leuckarti* and four times lower than for *B. delicata*. 48 h LC50 values (Table 1) and ChV for *M. leuckarti* were very similar (ACR_{50} 1.6), whereas time to metamorphosis appears to be a more sensitive test endpoint for the calanoid copepods (ACR_{50} 4.5 and 5.2).

Table 3. Chronic toxicity values ($\mu g.L^{-1}$ PCP) for the three copepods (ACR_{50} acute chronic ratio using 48 h LC50 values from Table 1).

	Day	NOEC	LOEC	ChV	ACR_{50}
C. lucasi	6	10	20	14.1	5.2
B. delicata	5	50	75	61.2	4.5
M. leuckarti	6	87	125	104.3	1.6

Control mortality

In sublethal tests using <24 h old nauplii control mortality was lowest in *M. leuckarti* tests with a mean of 8% (± 4) by day eight (Fig. 2a). The highest control mortality was recorded in *C. lucasi* tests with a mean of 65% (±12). Control mortality in *B. delicata* tests was 28% (±13). *C. lucasi* mortality in the solvent controls (37 ±10%) was significantly lower ($p<0.05$) than in the controls, whereas it increased slightly in *M. leuckarti* (18 ±7%) and *B. delicata* (31 ±7%) tests (Fig. 2b).

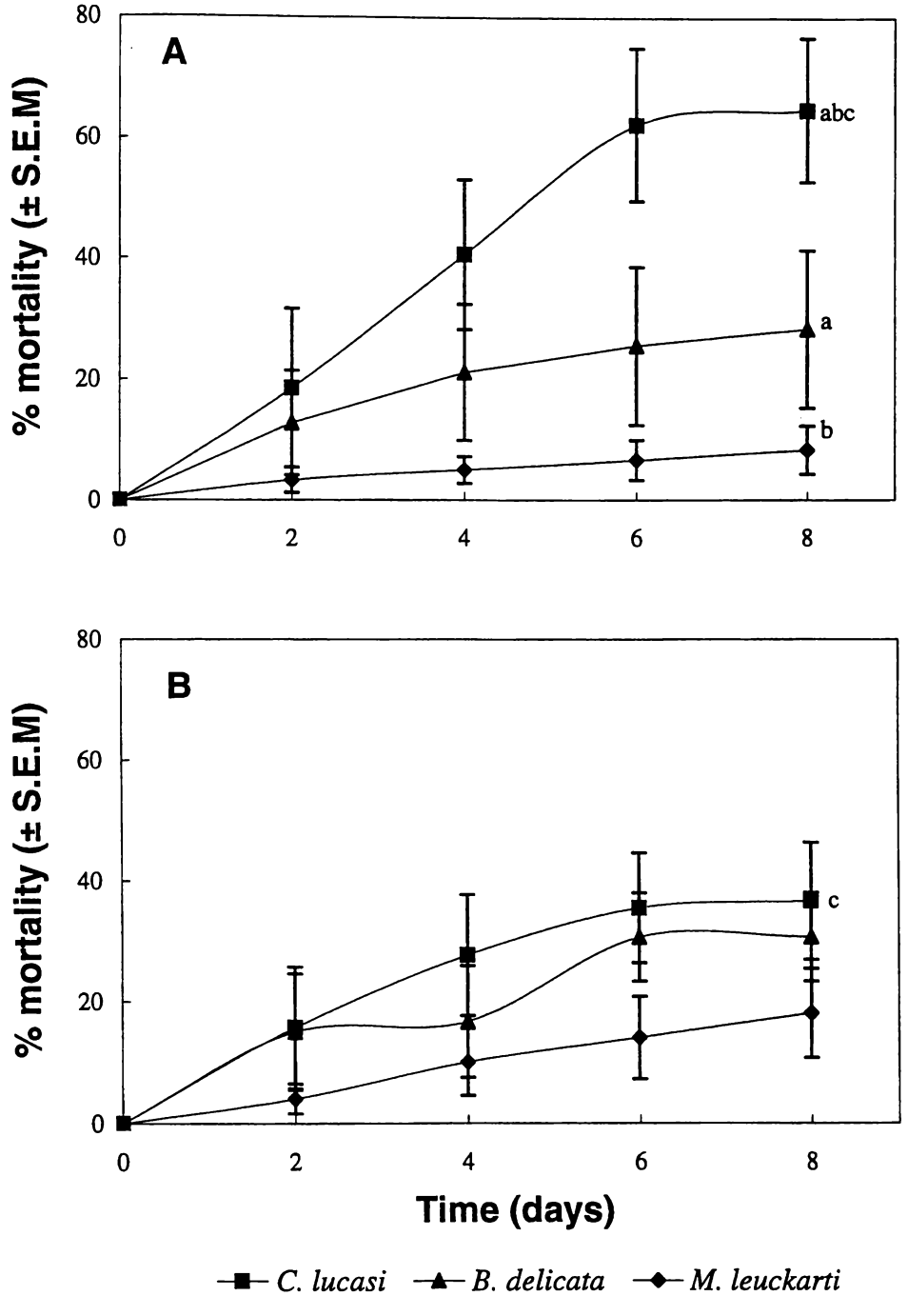

Figure 2. Naupliar mortality in controls (A) and solvent controls (B) in sublethal tests. Significant difference ($p<0.05$) between species (a,b); between treatments (c). n = 50 to 63 depending on species and treatment.

Discussion

The calanoid *C. lucasi* was the most sensitive of the three copepods in both acute and sublethal tests. LC50 values for adults of the three species were similar, while there was a 4-fold difference between LC50 values for *B. delicata* and *C. lucasi* nauplii (Table 1). Comparison of the 48 h LC50 values with other New Zealand freshwater invertebrates (Table 4), suggests that copepods are among the more sensitive of the species tested. Of the five cladocerans, *S. vetulus* and *C. dubia* show similar sensitivity, along with *D. magna*, which does not occur in New Zealand but is used as a reference species. Adults of the amphipod *Chaetocorophium lucasi* are also sensitive to PCP with a 96 h LC50 of 130 $\mu g.L^{-1}$ (Hickey and Martin 1995).

Table 4. Acute EC50 ($\mu g.L^{-1}$ PCP) estimates for New Zealand aquatic invertebrates.

species name		temp (°C)	life stage	test duration	EC50	source
Daphnia carinata	Cladoceran	20	<24 h	24 h	570	Hickey (1989)
Ceriodaphnia dubia	Cladoceran	20	<24 h	24 h	202	
Ceriodaphnia cf. *pulchella*	Cladoceran	20	<24 h	24 h	1790	
Simocephalus cf. *vetulus*	Cladoceran	22	<24 h	48 h	140	Willis et al. (1995)
*Daphnia magna**	Cladoceran	20	<24 h	48 h	187	Hickey and Vickers (1992)
Deleatidium spp.	Mayfly	15	larvae	48 h	287	
Chaetocorophium cf. *lucasi*	Amphipod	20	adult	96 h	130	Hickey and Martin (1995)
Sphaerium novaezelandiae	Bivalve	20	adult	96 h	1100	
Lumbricus variegatus	Oligochaete	20	adult	96 h	690	
Tanais standfordi	Shrimp	20	adult	96 h	1800	

**Daphnia magna* not native to New Zealand.

Similar sensitivity of copepod species has been noted before. Notenboom et al. (1992) exposed the adults of a groundwater copepod, *Parastenocaris germanica*, commonly found

in The Netherlands, to four toxicants under normoxic and hypoxic conditions with 48 h LC50 values for PCP of 80 and 36 $\mu g.L^{-1}$ respectively.

In order to standardise conditions and maximise replication, toxicity tests are typically conducted in a controlled laboratory environment, and do not take into account that environmental exposures are likely to occur under fluctuating conditions. Seasonal variations in toxicant sensitivity using field collected animals, rather than those cultured in the laboratory, have not been widely studied. Hedtke et al. (1986) found that the toxicity of PCP to eleven aquatic species tested in Mississippi River water varied seasonally, and Sosnowski et al. (1979) observed a wide range in sensitivity of field collected *Acartia tonsa* to copper. *A. tonsa* sensitivity was directly related to the interaction of changes in algal and zooplankton density, expressed in terms of food rations per animal. Furthermore, increased sensitivity was coupled with higher natural (control) mortality. The variation in PCP toxicity to adult *C. lucasi* in the present study may have been due to an inverse effect of temperature, or may have been a seasonal effect since the autumn collected adults were more sensitive than those collected in summer and tested at the same temperature. The effect of temperature on PCP toxicity to cladocerans has been investigated in several studies. Lewis and Horning (1991) observed no change in PCP toxicity to temperature acclimated *D. magna* and *D. pulex*, whereas Hedtke et al. (1986) found that PCP toxicity to seasonally collected *S. vetulus* increased with temperature.

It is likely that the increased toxicity of PCP to adult *C. lucasi* at the lower temperatures was due to seasonal variation in nutrition, as it is well known that food quality affects toxicity test results with laboratory cultured cladocerans (Winner et al. 1977; Cooney et al. 1992; Norberg-King and Schmidt 1993). It has been suggested that zooplankton communities in New Zealand lakes may be food limited (Chapman 1973; Green 1976), and Chapman (1973) concluded that seasonal changes observed in numbers of *C. lucasi* in two eutrophic North Island lakes could have been the result of fluctuating food levels. During

summer months the quantity and quality of food available for grazing may be improved relative to that during other months of the year, therefore the reduced sensitivity of copepods collected and tested in summer may have reflected their improved nutritional status. Lower control mortality in the summer tests would tend to support this hypothesis.

Early life stages of many species are generally more sensitive to toxicants than later developmental stages. In acute toxicity tests, with the exception of *B. delicata*, the copepod nauplii were more sensitive to PCP than the adults. The naupliar stages of several harpacticoid copepods have also shown increased sensitivity compared to adult and copepodite stages following acute exposures to toxicants (Verriopoulos and Moraitou-Apostolopoulou 1982; O'Brien et al. 1988; Green et al. 1996). Bushong et al. (1990) exposed nauplii of the marine copepod *Acartia tonsa* to tributyltin for six days and determined them to be significantly more sensitive than adults tested by U'ren (1983). The size difference alone may explain differences in susceptibility between the two life stages, and Green et al. (1996) suggested that differences in the ability of nauplii and later stages to metabolise toxicants might account for their increased susceptibility.

Chronic tests (>96 h) with marine and meiobenthic copepods have generally used test endpoints spanning full life cycle and reproductive conditions, in addition to lethality. Hutchinson and Williams (1989) investigated the chronic toxicity of industrial effluent discharges to adult females and nauplii of the benthic copepod *Tisbe battaglia*. They measured effects on female reproduction and survival over nine days, and naupliar survival over seven days, concluding that naupliar survival appeared to be a more sensitive indicator of effluent toxicity than adult reproduction. Furthermore, collection of survivorship data required the least effort. In the present study it was decided that a test endpoint such as time to metamorphosis might be more sensitive than naupliar survival. The change in body form would also be very easy to detect, and tests could be completed within eight to ten days.

Copepod nauplii are an important component of planktonic communities and the change in body form from nauplius to copepodite is a major developmental event. In contrast to the usual inverse relationship between metabolic rate and body size in invertebrates, naupliar mass-dependent metabolism remains constant during growth (Epp and Lewis 1980). The naupliar body form is energy efficient at small body sizes, but because of constant increases in metabolism with size, later stages have relatively greater energetic requirements. Metabolic stressors such as PCP could therefore be expected to delay an event such as metamorphosis because they reduce the amount of energy available for naupliar growth. In sublethal tests the calanoid nauplii were more sensitive to PCP than *M. leuckarti* (Table 3). The transition in body form occurs at a much higher weight (and therefore metabolic rate) in calanoid species than in cyclopoids (Epp and Lewis 1980), which may account for the enhanced sublethal toxicity of PCP to the calanoids.

Delays in the onset of reproduction and time to peak production have been noted in studies with estuarine calanoid and harpacticoid copepods (Daniels and Allan 1981; Verriopoulos and Moraitou-Apostolopoulou 1981; Allan and Daniels 1982; Moraitou-Apostolopoulou et al. 1983; Misitano and Schiewe 1990). Daniels and Allan (1981) observed a lengthening in developmental time to reproductive maturity in the estuarine copepod *Eurytemora affinis*, exposed to 5 and 10 $\mu g.L^{-1}$ dieldrin. Although time to metamorphosis was not recorded, it is likely that this would also have been delayed. The intrinsic rate of population increase (*r*) was also reduced at these concentrations due to a reduction in the number surviving to maturity and delayed onset of reproduction. This was more important in reducing *r* than the slightly impaired fecundity in those surviving to reproduce.

A potential confounding factor in toxicity tests with naupliar stages are high control mortality rates. It is known that harpacticoid naupliar mortality is generally high relative to later life stages (Green et al. 1995), although high levels of natural mortality would suggest that they might also be more sensitive to toxicants (Green et al. 1996).

Control mortality of 40% occurred in the 48 h tests using *C. lucasi* nauplii, and mortality was also very high in *C. lucasi* sublethal tests (Fig. 2). Although still quite high (37%), mortality was significantly lower in *C. lucasi* solvent controls. It is possible that the addition of a carbon source enhanced bacterial growth, providing an additional food source, as *C. lucasi* are known to feed on bacteria (Forsyth and James 1984). Bushong et al. (1990) considered that control survival during *A. tonsa* nauplii tests of 59 to 77% was acceptable given the life stage, and Green et al. (1996) observed control mortality was 5 to 6% higher than that of the adult or copepodite stages of *A. tenuiremis*. In contrast to *C. lucasi*, naupliar control survival in *B. delicata* and *M. leuckarti* 48 h acute tests was good (≥95%), however >20% control mortality occurred in *B. delicata* sublethal tests (Fig. 2). Overall mortality was low in *M. leuckarti* tests, with the highest mortality (18%) recorded in the solvent controls. Green et al. (1996) have suggested that 20% control mortality be set as an acceptable limit for harpacticoid copepod toxicity tests using naupliar stages, rather than the test acceptability criterion (≤10%) for standardised toxicity tests (USEPA 1991).

The variation in control mortality between species during the tests reflects, to some degree, the performance of each species in laboratory cultures. Of the three species, *M. leuckarti* were by far the easiest to culture, and thrived on a diet of *Artemia* nauplii, producing large numbers of offspring. Of the calanoids, *B. delicata* were more suitable for laboratory culture than were *C. lucasi*, as they are larger, more robust, and have greater fecundity. Both calanoid species were fed the same diet, although *B. delicata* fed preferentially on *C. ozolini*. *C. lucasi* clutch sizes varied, and at times it was quite difficult to obtain sufficient numbers of nauplii for tests. The success of *C. lucasi* cultures appeared to vary depending on when the animals used to start the cultures were collected, presumably because of seasonal variability in their nutritional status. Green (1976) also had difficulty culturing *C. lucasi*, and reported high naupliar mortality, especially at lower temperatures.

The selection of toxicity test species is based on, among other things, the ecological importance of the species. In New Zealand, copepods are often numerically dominant in freshwater ecosystems, and the results from this study suggest they may be very sensitive to toxicants, although this needs to be determined for a range of chemicals. Optimal culture conditions also need to be established. Early life stages are usually the most sensitive part of the life cycle, and copepod nauplii are, in general, no exception. The retention of different juvenile and adult body forms provides an easy to measure test endpoint in sublethal bioassays and also reduces the test duration. Whether time to metamorphosis is as sensitive as chronic endpoints based on reproduction has yet to be determined. The effects of a range of toxicants on time to metamorphosis also need to be investigated.

Despite the sensitivity of *C. lucasi* to PCP, poor naupliar control survival, in addition to small clutch sizes and variable offspring production in cultures, do not currently favour the use of this species in toxicity tests unless suitable culture conditions are established.

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CHAPTER THREE

Pentachlorophenol decay in a New Zealand lake: A mesocosm study

Abstract

Mesocosms (860 L) in Lake Rotomanuka, New Zealand, were treated with technical grade pentachlorophenol (PCP) to assess its persistence in the water column of a New Zealand lake. Nominal PCP concentrations of 4, 10, 24, 36, 54, 81, and 121 $\mu g.L^{-1}$ were applied in a regression design experiment as single applications that were mixed to ensure an even distribution of PCP with depth. 2,3,4,6-tetrachlorophenol (TeCP) was the main contaminant (10% w/w). By 40 days 74% of the PCP and 83% of the TeCP had disappeared from the water in the treated mesocosms. Mean half-lives for PCP and TeCP were 21 and 17 days respectively. Photolysis is suggested as the primary degradation process with PCP and TeCP disappearing from the water column in all treatments at a similar rate.

Keywords: pentachlorophenol; tetrachlorophenol; degradation; half-life; photolysis; mesocosm; New Zealand

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Introduction

New Zealand is reputed to have been among the heaviest per capita users of PCP in the world (Stevenson 1992). The timber industry used up to 200 tonnes annually for almost 40 years until mid-1988 (Shaw 1990), primarily as an antisapstain fungicide for sawn timber. PCP was deregistered for sale in New Zealand in December 1991. Up to 600 sites have been identified in New Zealand where PCP may have been used to treat timber and many of these are likely to be contaminated to some extent (National Task Group [NTG] 1992). The potential for pollution of the aquatic environment is likely to have been fairly high in the past (Shaw 1990) and it is possible that PCP leaching may still be contaminating waterways.

The largest known contaminated site in New Zealand, the Waipa Sawmill 5 km south of Rotorua, used up to 1000 tonnes of PCP between 1956 and 1988 (NTG 1992). In 1993, Gifford et al. (1993) estimated that approximately 150 kg of PCP was being transported annually via the Puarenga Stream into Lake Rotorua. Concentrations of PCP in water and sediment samples from Lake Rotorua and selected tributaries ranged from <0.01 to 3.62 $\mu g.L^{-1}$ and <1 to 418 $ng.g^{-1}$ DW respectively (Gifford et al. 1995), with highest sediment concentrations at the southern end of the lake near the Puarenga Stream outflow. PCP concentrations in sediments from remote lakes in New Zealand are similar to overseas background levels, ranging from 0.6 to 1.3 $ng.g^{-1}$ DW, indicating that PCP contamination in New Zealand is localised.

The New Zealand timber processing industry used technical grade PCP, typically as a 0.5 to 1 % w/w aqueous solution either in dip baths or spray booths for antisapstain treatment, or mixed in oil, usually at 5 % w/w, for longer term protection (Gifford et al. 1995). The use of technical grade formulations imported from a variety of sources means that sites have been contaminated with other chlorophenol compounds in addition to PCP.

PCP is subject to abiotic (photochemical) and biotic (microbial) degradation in sediment and water. Various laboratory studies have examined the effects of dissolved oxygen, light, temperature, pH, water hardness, and the presence of sediment on decay rates (Baker et al. 1980; Boyle et al. 1980; Liu et al. 1981; Brockway et al. 1984; Hwang et al. 1987). Experimental field studies employing ponds, mesocosms, and artifical streams report half-lives of PCP in the water column varying from one to 22 days (Lee et al. 1982; Crossland and Wolff 1985; Liber et al. 1997). Following the accidental release of wood treatment wastes into a Mississippi lake, Pierce and Victor (1978) found that PCP persisted in the water column for over six months.

It is known that many organic contaminants accumulate in lake sediments, usually at concentrations considerably higher than those found in the overlying water, but contrary to field observations, the results from enclosure studies suggest that neither PCP nor TeCP accumulate to any extent in the sediments of experimental systems.

This study was undertaken to investigate the persistence of a technical formulation of PCP at different concentrations in the water column of a small New Zealand lake using mesocosms. Mesocosms, *in situ* enclosures, isolate part of the natural ecosystem providing higher degrees of ecological realism in experimental systems. They have proven valuable in the validation of toxicological laboratory data and for extrapolating hazard to the ecosystem level (Liber 1994), and have been widely recommended as tools for the ecological risk assessment of pesticides (Graney et al. 1994; Hill et al. 1994; Shaw and Kennedy 1996).

Methods

Study site

Lake Rotomanuka (37°55'S, 175° 19'E) is a small lake (surface area 136,730 m^2) situated approximately fifteen kilometres south of Hamilton, New Zealand. The lake is surrounded by farmland and is protected from prevailing westerly winds making it an ideal site for mesocosm deployment. It is the deepest (maximum and mean depths are 8.7 and 5.0 m respectively; Lowe and Green 1987) and the clearest of the Waikato lakes with secchi depths ranging from 2.25 to 4.8 m (Green 1989). Lake Rotomanuka is mesotrophic (Etheredge 1983) and normally stratifies in summer with a thermocline at 3 to 4 m. The hypolimnion deoxygenates.

Experimental design

The mesocosm experiment was conducted during the spring of 1996, from October 9 to November 18. Ten mesocosms were anchored in a row at the eastern end of the lake in approximately 6 m of water, positioned 2 m apart in a north-south orientation to avoid shading by adjacent mesocosms at low sun angles. Each mesocosm was made from 100 µm clear polyethylene tubing sealed at the bottom end and attached to a float made from a galvanised steel tube (extending 240 mm above the water surface) with a polystyrene collar to provide buoyancy. Protective covers made from 4.5 mm clear acrylic (perspex) prevented dilution by rainwater and fouling by birds. The polyethylene bags were 3 m deep with an internal diameter of 0.6 m to give a total volume of approximately 860 L. Two days prior to adding PCP the bags were filled with lake water pumped from a depth of 2 m using a diaphragm pump.

The experiment employed a regression design (Liber et al. 1992) with seven unreplicated treatments and three controls. A technical formulation of PCP (nominally 86%, Aldrich Chemical Co. Milwaukee, WI) was used and contained TeCP as the major contaminant.

Nominal PCP concentrations of 4, 10, 24, 36, 54, 81, and 121 $\mu g.L^{-1}$ were applied to the mesocosms as single applications on day 0. Nominal TeCP concentrations were 0.7, 1.7, 3.9, 5.9, 8.8, 13.2, and 19.7 $\mu g.L^{-1}$ respectively. Appropriate amounts of the PCP formulation were dissolved in 100 mL of 95% ethanol followed by dilution in 15 L of lake water before being added to each mesocosm. To ensure an even distribution of PCP in the water column, each mesocosm was mixed using a secchi disk. Based on the results of single species laboratory tests and LC50 values reported in the literature, the concentrations were within the range where significant biological responses occur.

Temperature, dissolved oxygen, pH, and conductivity were measured on each sampling day between the hours of 10.00 and 13.00. Measurements were taken at three depths (surface, middle and bottom) and the daily mean for each treatment calculated. Conductivity remained stable at 160 $\mu S.cm^{-1}$ (S.D. 1.1), while the temperature increased from 16.4 to 19.6°C during the experiment. Mean (±1 S.D.), initial (day 0) and final (day 40) values for dissolved oxygen and pH are presented in Table 5. Light intensity was measured at 0.5 m depth intervals in the water column with a Quantum Light Meter equipped with a LI-COR 2π sensor.

Table 5. Dissolved oxygen and pH in the mesocosms.

nominal PCP conc.	Dissolved oxygen ($mg.L^{-1}$)		pH	
($\mu g.L^{-1}$)	mean (S.D.)	initial / final	mean (S.D.)	initial / final
*control	8.9 (1.8)	9.5 / 5.8	7.4 (0.1)	7.4 / 7.4
4	7.4 (2.5)	9.6 / 3.4	7.2 (0.2)	7.3 / 7.0
10	6.0 (3.5)	9.0 / 1.2	7.1 (0.4)	7.4 / 6.5
24	5.6 (3.9)	9.6 / 0.4	7.0 (0.4)	7.4 / 6.6
36	6.8 (2.7)	9.6 / 2.9	7.2 (0.3)	7.4 / 6.9
54	4.8 (3.9)	9.4 / 0.6	6.9 (0.5)	7.3 / 6.2
81	7.4 (1.7)	9.1 / 5.0	7.7 (1.3)	7.3 / 7.0
121	4.7 (3.5)	9.6 / 4.6	7.6 (1.3)	7.4 / 6.8

* mean of three controls

Two depth-integrated water column samples were collected from each mesocosm using a 3 m length of PVC pipe (80 mm I.D.) on days 1, 5, 8, 12, 16, 20, and 40. One litre subsamples were placed in brown glass bottles after being filtered through a 40 μm mesh plankton net to remove most of the zooplankton. The remaining water was returned to the mesocosms. On return to the lab the water samples were frozen at -20°C for later analysis.

Sample analysis

Each water sample (1 L) was acidified to pH 4 after the addition of 20 $g.L^{-1}$ Na_2SO_4 and 5 $mL.L^{-1}$ methanol. 2,4,6-tribromophenol (10 μL of c. 1 $mg.mL^{-1}$ in acetone) was added as the surrogate standard. The samples were extracted using SPEC•47•C18AR solid phase extraction discs (ANSYS). Samples were loaded onto the SPEC® discs using a Zymark® Autotrace™ SPE Workstation at a load rate of 30 $mL.min^{-1}$. They were then dried for 45 mins under nitrogen at pressure (~150 kPa) and placed in a desiccator overnight.

Chlorophenols were extracted from the SPEC® discs using a Hewlett Packard 7680T SFE module using supercritical CO_2 (32.8 MPa) at a flow rate of 1.6 $mL.min^{-1}$ to separate analytes from the sample matrix. The chamber was held static for 4 min (density 0.85 $g.mL^{-1}$; temperature 60°C) with a dynamic extraction time of 20 min. Trap (ODS) and nozzle temperatures were 30 and 45°C respectively. The trap was eluted with 1.5 mL *t*-Butyl methyl ether (MtBE) into GC vials for derivatisation and analysis. Dibromoanthracene (50 μL of c. 1 $mg.mL^{-1}$ in pyridine) was added as the injection standard and the extract was silylated using 50 μL BSTFA/TMCS (Alltech). Chlorophenols were quantified using a Hewlett Packard 5890 series II gas chromatograph equipped with a 50 m HP Ultra-2 column, and a 5971A mass selective detector operated in the selected ion mode (SIM). Column conditions were: solvent delay 10 min; initial and final temperatures 60° and 300°C; rate $10°C.min^{-1}$. The detection limit was 20 $ng.L^{-1}$. All

chlorophenols were quantified using calibration curves from pure compound standards and HP EnviroQuant Software.

Day 1 and day 40 samples were sent for independent analysis by a commercial laboratory. PCP was analysed using hexane extraction and GC-ECD analysis.

Apparent half-lives for PCP and TeCP in each treatment were calculated by linear regression of natural log-transformed concentrations vs time using the day five to 20 samples. The half-lives were calculated by dividing ln(0.5) by the slope of the regression [ln(0.5)/-*k*] (Giddings 1994). Initial, day one and day 40 PCP and TeCP concentrations were estimated from regression equations.

Extraction efficiency and background chlorophenol levels

Three replicate double distilled water samples (1 L) were spiked with 10 µL of a chlorophenol standard containing 4-chlorophenol (0.74 $mg.mL^{-1}$), 2,4-dichlorophenol (0.695 $mg.mL^{-1}$), 2,4,6-trichlorophenol (0.6325 $mg.mL^{-1}$), 2,4,6-tetrachlorophenol (0.5525 $mg.mL^{-1}$) and pentachlorophenol (0.5425 $mg.mL^{-1}$).

Three replicate double distilled water samples (1 L) with 2,4,6-tribromophenol (10 µL of 1.0945 $mg.L^{-1}$) added as the surrogate standard were extracted to determine background levels of chlorophenols. The highest background levels recovered were 0.45 $\mu g.L^{-1}$ and 0.20 $\mu g.L^{-1}$ PCP and TeCP respectively. These values were used to correct the PCP and TeCP concentrations calculated for the mesocosm samples. No other chlorophenol compounds were detected in the blank samples.

A water sample (1 L) taken from Lake Rotomanuka was also analysed. No chlorophenol compounds were detected in this sample.

Results

Chlorophenol extraction recovery

Recovery of the surrogate standard (tribromophenol) ranged from 83% to 116% with a mean of 102% (S.D. 0.09). Two samples were excluded due to low recovery of the surrogate (41 and 72%). Calculated PCP and TeCP concentrations were corrected for recovery of the surrogate standard.

Mean recoveries from the three replicate samples spiked with the chlorophenol standard were: Chlorophenol 13%; Dichlorophenol 14%; Trichlorophenol 90%; Tetrachlorophenol 109%; Pentachlorophenol 116%. Acidification to pH 4 prior to extraction in order to maximise PCP and TeCP recovery and the SPEC® disk drying step, contributed to the poor recovery rates for chloro- and dichlorophenol.

PCP and TeCP concentrations

PCP and TeCP disappeared from the water column at a similar rate (mean daily loss rates for TeCP and PCP were 0.03 and 0.025) in all treatments (Fig. 3). The calculated half-lives were similar for both PCP and TeCP being 21 and 17 days respectively (Tables 6 and 7). Initial PCP concentrations were extrapolated from the regression equations and generally were in agreement with predicted nominal values. Extrapolated PCP concentrations for days 1 and 40 compared well with samples analysed by the commercial laboratory. On average there was a 74% loss of PCP by day 40 and an 83% loss of TeCP.

The technical grade PCP formulation contained 10% TeCP as the major contaminant, which corresponds well with a ratio of 0.09 (S.D. 0.0042) TeCP:PCP from the mesocosm samples. The ratio of TeCP to PCP did not change in the mesocosms between days 5 to 20.

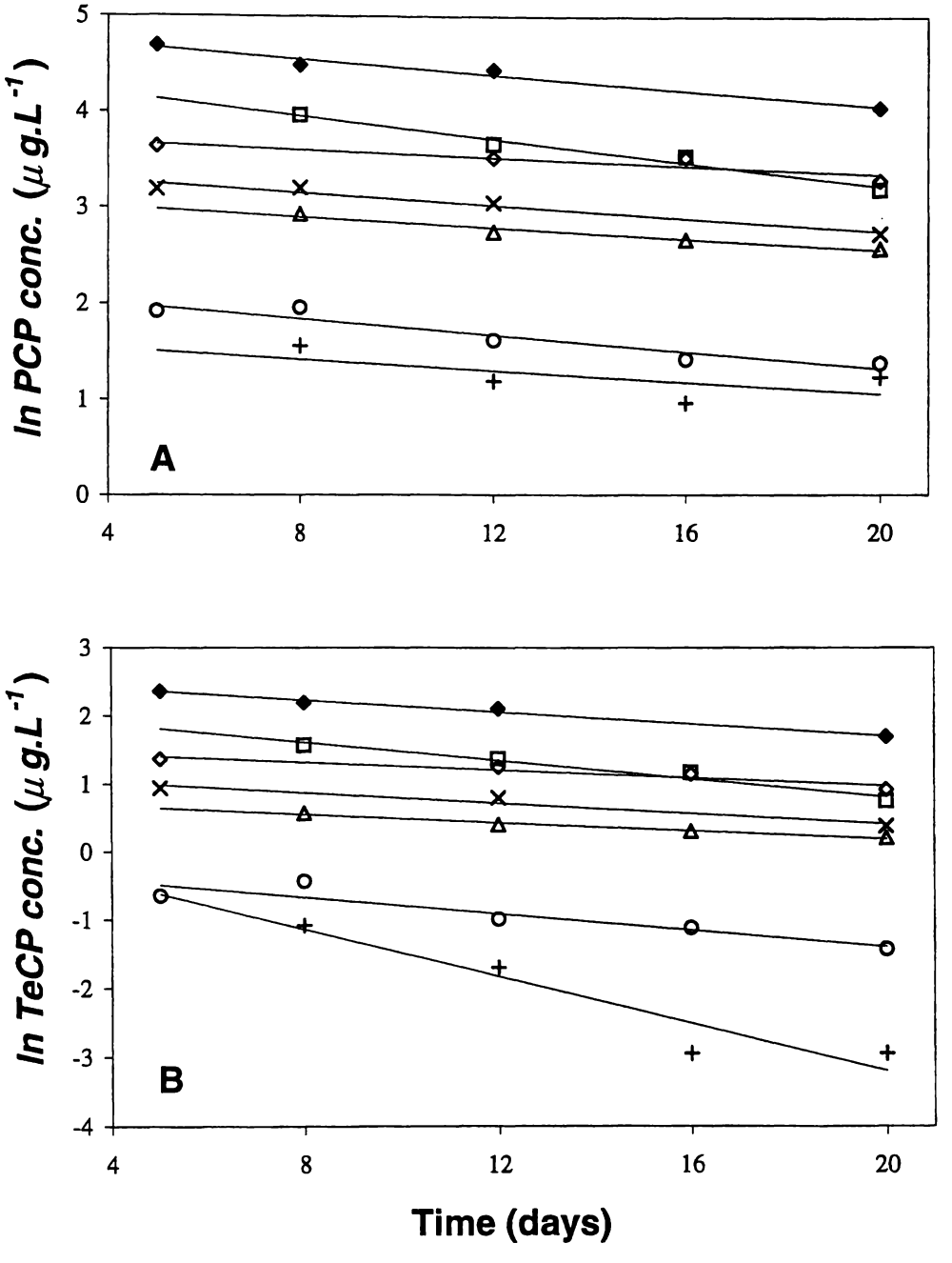

Figure 3. Loss of PCP (A) and TeCP (B) from PCP treated mesocosms (days 5 to 20). 4 (+), 10 (○), 24 (△), 36 (×), 54 (◇), 81 (□), and 121(◆) µg.L^{-1} PCP (nominal conc). Regression equations for each treatment are listed in Tables 6 and 7.

Table 6. Estimated half-lives (days) and PCP concentrations. (% loss calculated as the difference between estimated values for days 0 and 40. Values in brackets are for the samples analysed by a commercial laboratory).

nominal					extrapolated PCP conc. ($\mu g.L^{-1}$)		
PCP conc.	regression equation	r^2	t ½	% loss	day 0	day 1	day 40
4 $\mu g.L^{-1}$	y=-0.0296x + 1.6532	0.3943	23	68	5	5 (*2*)	1.6
10	y=-0.0424x + 2.1745	0.9042	16	78	9	8 (*10*)	2
24	y=-0.0281x + 3.1219	0.9572	25	70	23	22 (*24*)	7 (*8*)
36	y=-0.0326x + 3.4084	0.9489	21	73	30	29 (*27*)	8 (*11*)
54	y=-0.0207x + 3.7643	0.8005	33	56	43	42 (*40*)	19 (*22*)
81	y=-0.0607x + 4.4371	0.9726	11	92	85	80 (*52*)	7 (*5*)
121	y=-0.0401x + 4.8658	0.9669	17	80	130	125 (*140*)	26 (*27*)
Mean			21	74			
Std dev			7	11			

Table 7. Estimated half-lives (days) and TeCP concentrations (% loss calculated as the difference between estimated values for days 0 and 40).

nominal					extrapolated TeCP conc.($\mu g.L^{-1}$)		
PCP conc.	regression equation	r^2	t ½	% loss	day 0	day 1	day 40
4 $\mu g.L^{-1}$	y=-0.1714x + 0.2295	0.896	4	100	1.3	1.1	0.0
10	y=-0.0603x - 0.1891	0.8531	11	88	0.8	0.8	0.1
24	y=-0.0306x + 0.795	0.9826	23	68	2.2	2.2	0.7
36	y=-0.0383x + 1.1769	0.9468	18	78	5.9	5.6	1.3
54	y=-0.0287x + 1.5458	0.9083	24	68	4.7	4.6	1.5
81	y=-0.067x + 2.1475	0.964	10	93	8.6	8.0	0.6
121	y=-0.0437x + 2.5783	0.9846	16	83	13.2	12.6	2.3
Mean			*17	83			
Std dev			6	12			

*half-life from the 4 $\mu g.L^{-1}$ PCP treated mesocosm not included

Light conditions in the mesocosms

In full sunlight there was a 77 to 85% reduction in light intensity from 0 to 0.5 m depth in the mesocosms compared to the lake, and under overcast conditions a 24 to 41% reduction (Table 8). At depths greater than 1 m, light intensity in the mesocosms was similar to that of the lake at the same depth.

Table 8. Percentage reduction in light intensity in the mesocosms compared to the lake at the same depth.

	water column depth (m)				
conditions	surface	0.5	1.0	1.5	2.0
overcast	24	41	11	18	0
sunny	77	85	27	0	0

Discussion

The results from this study indicate that both PCP and TeCP persisted in the water column for a considerable time with approximately 20% remaining after 40 days. The mean half-lives for PCP and TeCP were 21 and 17 days respectively.

The persistence of PCP has been investigated in a number of field studies with reported half-lives ranging from 2 to 4.7 days in a 1 m deep pond (Crossland and Wolff 1985) to 22 days in 5.5 m deep marine mesocosms (Lee et al. 1982). The rapid disappearance of PCP from the water column, and evidence of shorter half-lives in shallow ponds following a subsurface application (Crossland and Wolff 1985), implicates photolysis as the principal mode of degradation. With the absorption of light energy PCP undergoes photochemical reduction to form lower chlorinated phenols, chlorinated dihydroxybenzenes, and non-aromatic fragments (Wong and Crosby 1978).

Under aerobic conditions in aquaria containing pond water, Boyle et al. (1980) found half-lives ranging from 13.9 to 18.6 days. Brockway et al. (1984) calculated half-lives of 10 to

15 days for PCP added to aquaria containing sediment, synthetic medium and a weekly inoculum of pond plankton. In a series of experiments, Liber et al. (1997) estimated times for 50% dissipation of TeCP and PCP from depth-integrated water samples were 0.4 to 1.1 days and 3.4 to 5.2 days respectively, depending on whether the formulation was applied in the morning or evening as a surface application. Half-lives were calculated from nominal values for the morning application and measured concentrations for the evening application. Liber et al. (1997) reported differences in times for 50% dissipation of TeCP and PCP at depths of 5 cm (8.8 hr), 50 cm (12 hr) and 100 cm (55 and 58 hr). The surface application undoubtedly enhanced the rate of photolytic degradation of PCP and TeCP, however, the contribution of photolysis could not be determined because of the confounding effects of mixing during the first two days. Between days two to seven, following the mixing period, rates of dissipation were the same at all depths. In the present study the PCP formulation was mixed throughout the water column, and this was reflected in the longer half-lives compared to those of Liber et al. (1997).

In the present study, photolysis was probably the primary mechanism of PCP degradation within the mesocosms, however, it is possible that the rate of decay was slowed due to their small surface area to volume ratio enhancing the effects of shading by the floats. A 77% reduction in light intensity was recorded in full sunlight at the mesocosm surface, although at depths greater than 1.5 m the light intensity was the same as that of the lake. Despite the reduced light intensity in the mesocosms, the initial pH values (Table 5) were conducive to higher rates of photolysis. At pH values recorded in this study PCP and TeCP were more than 99% ionised, with pKa values of 4.8 and 5.4 respectively (Crosby 1981; Fox and Joshi 1984). The dissociated phenoxide ions have a stronger absorbance than the nonionized compound (Hwang et al. 1987) and therefore undergo photolysis more rapidly.

PCP also undergoes aerobic microbial degradation in water, but it is generally accepted that this biodegradation will start only after a long incubation period if no adapted bacteria

are present (Crossland and Wolff 1985). Biodegradation of PCP in outdoor experimental streams was not significant until three weeks after the start of dosing (Pignatello et al. 1983), and after 40 days there was no microbial degradation of PCP added to stream-water in a study by Baker et al. (1980). Because the daily decay rates for PCP and TeCP did not increase with time in the present study it is unlikely that biodegradation of PCP and TeCP occurred.

The relative persistence of PCP in the water column in the present study could be attributed to the absence of sediment, in addition to the reduced light conditions already discussed. PCP may persist for considerable periods in lakes, especially in the sediments where degradation rates are generally much slower than in the overlying water column (WHO 1987). Knowlton and Huckins (1983) investigated the fate of radiolabeled PCP in littoral microcosms containing sediment and macrophytes. After 180 days 40 to 43% of the radioactivity was present in the sediments and 14% in macrophytes and filamentous algae. In limnocorrals containing sediment Liber et al. (1997) calculated that PCP sediment concentrations accounted for 55.9% of the total PCP in the system 43 days after being treated with a TeCP formulation. In agreement with other research, however, Liber et al. concluded that neither TeCP nor PCP accumulates to any significant extent in the sediments of experimental systems. Crossland and Wolff (1985) also reported that adsorption of PCP onto sediment was negligible, accounting for only 2% of the total PCP in outdoor ponds, and Pignatello et al. (1983) found that PCP adsorption by sediment and uptake by biota accounted for 5 to 15%. Consequently, it is unlikely that the absence of sediment in the present study contributed significantly to the longer half-lives in the water column. Further, PCP and TeCP were present in their water soluble ionic forms, so would not adsorb onto sediment in significant quantities. For chlorophenols to be adsorbed they have to be in their molecular form, not the ionic phenolate form (Liber et al. 1997).

It is also possible that some loss of PCP and TeCP may be accounted for by adsorption onto the polyethylene mesocosm walls and its associated periphyton. This was observed by Liber et al. (1997), although the amount of PCP and TeCP associated with the entire limnocorral liner was less than 0.05% of the total. Yunker (cited in Liber et al. 1997) similarly reported that in marine enclosures, PCP was not adsorbed onto the walls in significant amounts. In the current study PCP and TeCP loss due to adsorption onto suspended particulates or uptake by zooplankton and phytoplankton is unknown as the water samples were filtered through 40 μm mesh, thereby removing most of this material. At the higher PCP concentrations, chlorophyll *a* and plankton biomass decreased with time (Chapter 6) without changes in PCP and TeCP decay rates, so it is unlikely that uptake by the plankton contributed significantly to PCP and TeCP loss. Following a PCP spill in a Mississippi lake Pierce and Victor (1978) found that suspended particulates generally contained <10% of the PCP in the water column.

Reported differences in half-life may also be accounted for by variations in temperature, season and latitude. Some photochemical reactions require a temperature dependent activation step so that photodegradation rates increase exponentially with temperature. However, Hwang et al. (1987) observed no change in chlorophenol photolysis rates when the temperature was altered. In the present study there was a small temperature increase (~3°C) over the experimental period with no change in the rate of decay. Changes in photodegradation rates could also be expected with latitude and between seasons as a result of changes in irradiance. Hwang et al. (1987) suggested higher summer irradiance is an important determinant of photolytic degradation rates, although this may be offset to some extent by the extinction of light due to increased suspended particulates, especially in deeper waters. New Zealand experiences high UV levels, especially during spring and summer months because of seasonal ozone depletion and atmospheric clarity, which could be expected to increase PCP decay rates. The mesocosm design in this experiment

increased shading so may have counteracted any effects of higher UV levels. Therefore it is expected that under natural conditions, photolysis rates and subsequent decay would be higher, resulting in shorter half-lives.

The ratio of TeCP to PCP remained the same throughout this experiment (0.09) and was similar to that of the PCP formulation used. Although 2,3,4,6- and 2,3,5,6- TeCP isomers are the predominant lower chlorinated phenols produced when PCP in water is exposed to sunlight their contribution to the total of photolysis products is minor (Wong and Crosby 1978). 2,3,4,6-TeCP was the only isomer detected in quantifiable amounts in the present study, since this was the most abundant isomer in the PCP formulation. If any lower chlorophenols were produced from degradation of the TeCP they were not detected in quantifiable amounts, although this may be due, in part, to the lower recovery rates recorded for these compounds.

In summary, the half-lives for PCP and TeCP recorded in this study were at the upper end of those reported in the literature, and suggest photolysis as the primary loss process during the entire experimental period. Differences in half-lives reported from a range of published experimental field studies may, to some extent, be accounted for by differences in experimental design such as sample application, mesocosm size and design, and water depth. Although conditions in the mesocosms did not exactly match those of the lake, the results indicate that PCP may persist for considerable periods in the water column of New Zealand lakes. This may not be a problem for acute contamination, but may be of concern under periods of continuous leaching.

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CHAPTER FOUR

A comparative study of community-level responses in small laboratory microcosms and *in situ* mesocosms dosed with pentachlorophenol: Does size matter?

Abstract

Community-level responses of naturally derived laboratory microcosms (1.6 L) and *in situ* mesocosms (860 L) dosed with pentachlorophenol (PCP) were compared. The microcosms were established using water and plankton collected from Lake Rotomanuka, where the mesocosms were deployed. Single applications of technical grade PCP (nominally 86%) dissolved in 95% ethanol, were applied to the microcosms and mesocosms in regression design experiments employing seven unreplicated PCP concentrations (4, 10, 24, 36, 54, 81 and 121 $\mu g.L^{-1}$), a solvent control and non-solvent controls.

Temperature, dissolved oxygen, pH, conductivity, chlorophyll *a*, zooplankton and phytoplankton abundance were monitored on days 2, 4, 8, and 12 after PCP addition. The effects of treatment and time on plankton species composition were analysed using redundancy analysis (RDA), with Monte Carlo permutation tests to identify significant treatment effects on each sample day. Daily EC50 estimates were calculated using total copepod % change in abundance.

Changes in community structure unrelated to PCP were observed in the microcosms with marked declines in rotifer and phytoplankton abundance. Abiotic conditions and community responses to PCP were similar in the microcosms and mesocosms. In the RDA, treatment effects were apparent at concentrations as low as 10 and 24 $\mu g.L^{-1}$ PCP in both the mesocosms and microcosms. There was a slight delay in response in the microcosms, with copepod EC50 values on days eight (85 $\mu g.L^{-1}$) and twelve (90 $\mu g.L^{-1}$) in the microcosms similar to those on days two (96 $\mu g.L^{-1}$) and four (89 $\mu g.L^{-1}$) in the mesocosms. Possible modifications to improve the microcosm experimental design are discussed.

Keywords: microcosm; mesocosm; community-level response; multivariate statistics; copepods; PCP; New Zealand

Introduction

Microcosms and mesocosms are experimental systems that encompass higher levels of biological organisation and have higher degrees of environmental realism than conventional laboratory tests (Cairns 1988). Mesocosms are larger in size, accommodate greater ecological complexity, and experiments are generally conducted *in situ*, thus providing greater environmental realism than microcosms. The term 'microcosm' is usually applied to small laboratory systems (Taub 1997). Their smaller size and controlled laboratory surroundings decrease their similarity to natural systems but increase the ability to standardise test protocols, replicate treatments and undertake repeatable tests.

Microcosms may be either gnotobiotic (species defined) such as the Standardised Aquatic Microcosm (SAM) developed by Taub and colleagues (ASTM 1995), or naturally derived, using organisms collected from natural ecosystems. Gnotobiotic microcosms contain synthetic media and sediment, and organisms that are easily and reliably cultured. They are not intended to represent any particular ecosystem, but are generic systems that possess properties common to all ecosystems and may be used for predictive purposes (Giesy and Allred 1985). Derived microcosms are site specific, can be used to assess localized effects on a specific area, and are easier to initiate and maintain, as they do not require cultured organisms. Derived microcosms generally contain greater than 50 litres, although Leffler (1984) has developed a protocol utilising one litre microcosms containing synthetic media and organisms collected from a natural community.

It is uncertain how complex test systems need to be in order to give realistic assessments of toxicant effects on natural communities (Lampert et al. 1989). Sugiura (1992) suggests that, due to low species diversity, small microcosms have only limited use in predicting ecosystem level effects because they are not representative of the complexity that exists in natural systems. However, Landis et al. (1993) have shown that SAM do express complex

dynamics. Comparison of results from both Taub and Leffler microcosms with data from experimental ponds has demonstrated the sensitivity of small microcosms for the prediction of environmental effects (Harrass and Taub 1985; Stay et al. 1985; Larsen et al. 1986; Stay et al. 1989). Similar conclusions have been drawn from comparisons between larger microcosms (>60 L) containing natural planktonic communities and experimental ponds (Giddings and Franco 1985; Hook et al. 1986; Fliedner and Klein 1996). However, the larger volumes contained in many naturally derived experimental systems increase the associated costs, which may make them unrealisable in some instances.

This chapter presents the results of a short-term study comparing community-level responses of small (1.6 L) naturally derived microcosms and larger (860 L) *in situ* mesocosms dosed with PCP. The laboratory microcosms were established using water and organisms collected from the lake in which the mesocosms were deployed. The primary objective of this study was to establish whether direct toxicant effects on species composition under controlled conditions in smaller and less costly microcosms would parallel those observed in larger and more environmentally realistic mesocosms.

Methods

Study site

Lake Rotomanuka (37°55'S, 175° 19'E) is a small lake (surface area 136,730 m^2) situated approximately fifteen kilometres south of Hamilton, New Zealand. The lake is surrounded by farmland and is protected from prevailing westerly winds making it an ideal site for mesocosm deployment. It is the deepest (maximum and mean depths are 8.7 and 5.0 m respectively; Lowe and Green 1987) and the clearest of the Waikato lakes with secchi depths ranging from 2.3 to 4.8 m (Green 1989). Etheredge (1983) found that the lake had a mesotrophic or eutrophic dinoflagellate community dominated by *Peridinium* species. Lake Rotomanuka normally stratifies in summer with a thermocline at 3 to 4 m, and has a

deoxygenated hypolimnion.

In situ mesocosms

The mesocosm experiment was conducted during the summer of 1997, from January 20 to February 11. Data for only the first twelve days post-treatment are presented in this chapter. Ten mesocosms were anchored in a row at the eastern end of the lake in approximately 6 m of water, positioned 2 m apart in a north-south orientation to avoid shading by adjacent mesocosms at low sun angles (Fig. 4a). Each mesocosm was made from 100 μm clear polyethylene tubing sealed at the bottom end with wooden battens (Fig. 4b), and attached to a float made from a galvanised steel tube (extending 240 mm above the water surface) with a polystyrene collar to provide buoyancy (Fig. 5). Protective covers made from 4.5 mm clear acrylic (perspex) prevented dilution by rainwater and fouling by birds. The polyethylene bags were 3 m deep with an internal diameter of 0.6 m to give a total volume of approximately 860 L. Two days prior to adding PCP the bags were filled with lake water pumped from a depth of 2 m using a diaphragm pump.

The experiment employed a regression design (Liber et al. 1992) with seven unreplicated treatments, two controls and a solvent control. A technical formulation of PCP (nominally 86%, Aldrich Chemical Co. Milwaukee, WI) was used and contained TeCP as the major contaminant (Chapter 3). Nominal PCP concentrations of 4, 10, 24, 36, 54, 81, and 121 $\mu g.L^{-1}$ were applied to the mesocosms as single applications on day 0 respectively. Chemical analysis of water samples from a previous mesocosm experiment (Chapter 3) to determine initial PCP concentrations and rates of decay suggest that measured PCP concentrations closely reflect predicted nominal concentrations. Loss of PCP from the water column was also relatively slow with a calculated half-life of 21 days (Chapter 3). Thus nominal concentrations have been used throughout this study.

Appropriate amounts of the PCP formulation were dissolved in 100 mL of 95% ethanol,

Figure 4a - The mesocosms deployed in Lake Rotomanuka

Figure 4b - The polyethylene tubing sealed with wooden battens

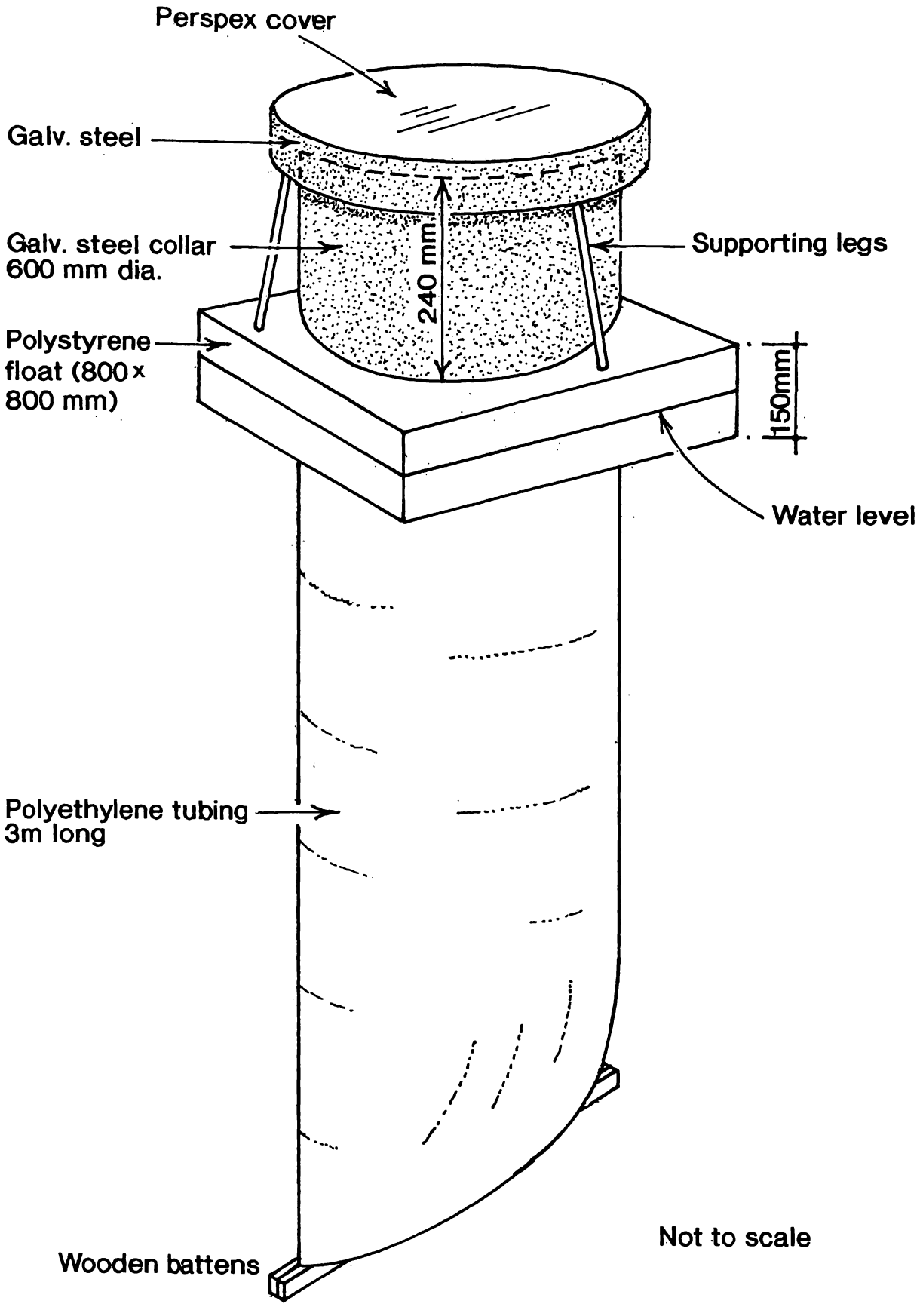


Figure 5. Mesocosm design.

followed by dilution in 15 L of lake water before being added to each mesocosm.

To ensure an even distribution of PCP in the water column, each mesocosm was mixed using a secchi disk. Based on the results of single species laboratory tests and LC50 values reported in the literature, the concentrations were within the range where significant biological responses occur. The mesocosms were sampled from a boat on days 0, 2, 4, 8 and 12, between the hours of 10.00 and 13.00. Temperature and dissolved oxygen were measured at three depths (surface, middle and bottom) and the daily mean for each treatment calculated. Conductivity was measured at the surface. Two depth-integrated water column samples were collected from each mesocosm using a 3 m length of PVC pipe (80 mm I.D.) and combined. Samples for pH, chlorophyll *a* and phytoplankton were taken from the combined sample, before filtering the water through a 40 µm mesh plankton net to collect the zooplankton for later enumeration. Zooplankton and phytoplantkon samples were preserved with 10% formalin and Lugol's iodine, respectively. The remaining water was returned to its respective mesocosm.

Laboratory microcosms

A 12 day laboratory microcosm experiment was conducted using water and planktonic organisms from Lake Rotomanuka on 3 February 1997 (day -1). Water was collected in 20 L buckets from a depth of two metres using a diaphragm pump. On return to the laboratory, the lake water was combined and mixed using a secchi disk before adding 1600 mL to each microcosm (2 L glass jars). Five additional samples were taken to determine the homogeneity of the zooplankton samples (Appendix I), and initial species abundance for day 0, which are presented as the mean of the five samples. A single sample was taken from the composite water to determine phytoplankton abundance on day 0.

Temperature, dissolved oxygen, conductivity and pH of the composite water were 23.7°C, 97.1 %, 162 $\mu S.cm^{-1}$, and 7.5 respectively.

The experimental design was similar to that employed for the *in situ* mesocosms, with one control and a solvent control. Appropriate amounts of PCP stock solution (2 $g.L^{-1}$) dissolved in 95% ethanol, were added as a single application on day 0 to give nominal PCP concentrations of 4, 10, 24, 36, 54, 81, and 121 $\mu g.L^{-1}$. Additional ethanol was added so that concentrations were the same as those in the mesocosms. The microcosms were covered with plastic petri dishes to reduce evaporation. Surface illumination of 17.5 $\mu E.m^{-2}.s^{-1}$ was provided by randomly positioning the microcosms under a bank of fluorescent lights (Sylvania GRO-LUX F18W/GRO) in a temperature controlled laboratory (22 ± 2°C), with a photoperiod of 14L:10D. The microcosms were stirred gently every day with a glass rod to resuspend non-motile phytoplankton. There were five replicates per concentration. A replicate microcosm from each concentration was destructively sampled on days 1, 2, 4, 8 and 12. Dissolved oxygen and conductivity were measured in the undisturbed water column prior to taking samples for pH, chlorophyll *a* (50 mL) and phytoplankton. The remaining water was filtered through a 40 µm mesh plankton net to collect the zooplankton, which was preserved with 10% formalin for later enumeration.

Sample analysis

Zooplankton samples were resuspended with distilled water and made up to known volumes in a measuring cylinder. Appropriate dilutions were chosen to give counts of 100 to 200 individuals of the dominant taxa per subsample. Counts of ~100 individuals have been calculated to reduce subsampling coefficients to ±10% (Cryer 1983 and papers therein). After thorough mixing, a 5 mL subsample was transferred to a gridded perspex counting tray mounted on the movable stage of a model MZ12 Leica stereomicroscope. One subsample was counted per sample. Cryer (1983) has shown that subsamples drawn from a sample were random. Copepods were separated according to family and life stage

(nauplii, copepodites, adults). Rotifers were identified to species level according to Shiel (1995).

Phytoplankton samples were counted using the sedimentation technique (Utermöhl 1958). Each sample was thoroughly mixed and a 50 mL aliquot sedimented for 24 h (Etheredge 1987). Samples were counted using an Olympus model IMT-2 inverted microscope. Larger taxa were counted during a scan of the entire basal plate at low magnification (x 60). Smaller, more numerous taxa were counted at higher magnifications (x 300; x 600) along diameter belt transects. To achieve 20% counting accuracy, an acceptable level for phytoplankton analyses (Utermöhl 1958, Hobro and Willén 1977), counting continued until each major species had been recorded at least 100 times (Etheredge 1987). The randomness of phytoplankton distribution on the basal plate of the sedimentation chamber, and in subsamples, was tested using Fisher's Index of Dispersion (Fisher 1948) (Appendix II). Texts consulted for species identification were Prescott (1978), Pridmore and Hewitt (1982), Croasdale and Flint (1986) and Etheredge (1983, 1987).

Chlorophyll a

As soon as possible after collection, water samples were filtered onto Whatman GF/C glass fibre filters (25 mm diameter), and stored at -20°C for no longer than one month. The filters were homogenised, and Chl. *a* extracted for 1 to 2 h at 4°C, using 10 mL of 90% acetone. The homogenate was centrifuged, and fluorescence of the supernatant measured before and after acidification with 2M HCl, using an Aminco SPF-500 spectrofluorometer set to 430 nm (excitation) and 665 nm (emission) (Strickland and Parsons 1968). Machine readings were calibrated using a chlorophyll *a* standard (Sigma Chemical Co. St. Louis, MO). Chl. *a* concentration was calculated as:

$$\mu g/L = \left((unacidified - acidified) \times 0.4575 - 1.6353\right) \times \left(\frac{extract\ volume\ (mL)}{volume\ filtered\ (mL)}\right)$$

Multivariate analysis of treatment effects

Effects at the community level were analysed by redundancy analysis (RDA) using CANOCO version 3.1 (ter Braak 1988). RDA is based on a linear response model in which species abundance either increases or decreases monotonically in relation to environmental variables (ter Braak 1987). Initial analysis using detrended correspondence analysis (DCA) confirmed linearity of the data as the gradient lengths were less than three standard deviation units (see van Wijngaarden et al. 1995). In contrast to principal components analysis (PCA), which takes into account all variance of a data set, RDA is constrained to the fraction of the total variance that is explained by the explanatory variables. In RDA, "PCP concentration" and "time," plus their interaction, were combined as explanatory variables, thereby focussing on the variance attributed to treatment or time. There was an obvious solvent effect on dissolved oxygen concentrations between the controls and non-solvent control, so solvent (presence or absence) was used as a covariable to correct for differences between treatments which may be attributed to its addition. To test for a treatment effect on species composition, data for each sampling day were tested separately for statistical significance using unrestricted Monte Carlo permutation tests. The Monte Carlo permutation test is non-parametric and randomly permutes the sampling data repeatedly in order to calculate a test statistic (van Wijngaarden et al. 1995).

The ordination results are presented as correlation biplots of species, sites and environmental variables using species-centred RDA in which each species is implicitly weighted by the variance of its abundance values. Species with high variance, often the abundant ones, therefore dominate the RDA solution, whereas species with low variance, often the rare ones, have only minor influence (ter Braak 1987). Euclidean scaling was used so that resulting ordination plots were optimal for interpreting distances between sites (ter Braak 1988). Species abundance was $\ln(1x + 1)$ transformed, and each treatment at each sampling time was considered to be a site.

EC50 calculation

Calculation of EC50 values used the method of Liber et al. (1992). The abundance of each species and total Crustacea, copepods, rotifers and phytoplankton were plotted vs. time to determine whether dose-response relationships were apparent. Total copepod abundance (predominantly *C. lucasi*) was used to estimate EC50 values by calculating the percent change in abundance relative to day 0 for each treatment, and plotting them as probit values vs. log PCP concentration. Daily EC50 values were estimated from the graphs by eye. EC50 values were not estimated for other taxonomic groups, as dose-response relationships were not as obvious or abundance was higher in PCP treatments than in controls. In the microcosms, total rotifer and phytoplankton abundance declined in all treatments including the controls.

Results

Physicochemical parameters

Temperature, conductivity and pH were similar in the microcosms and mesocosms (Table 9). Light intensity at the surface was considerably lower in the microcosms than mesocosms, but there was a marked reduction in light intensity with depth in the mesocosms.

Table 9. Mean (± 1 S.D.) physicochemical parameters.

	temperature (°C)	conductivity ($\mu S.cm^{-1}$)	pH	*light ($\mu E.m^{-2}.s^{-1}$)
microcosms	22.7 (0.9)	171 (12)	7.7 (0.5)	17.5
mesocosms	22.6 (0.8)	159 (3)	7.5 (0.3)	160 - 2.9

* microcosm value = surface illumination; mesocosm values = range from surface to 3 m on a sunny day.

Dissolved oxygen concentrations were highest in the non-solvent controls, in both the microcosms and mesocosms (Fig. 6). A solvent effect was apparent, with decreased

oxygen in the solvent controls. Oxygen concentrations were similar in the solvent controls and lower PCP treatments. The lowest oxygen concentrations were in the 121 $\mu g.L^{-1}$ PCP treatments. Oxygen concentrations initially increased in the microcosms, before decreasing to similar levels as those in the mesocosms.

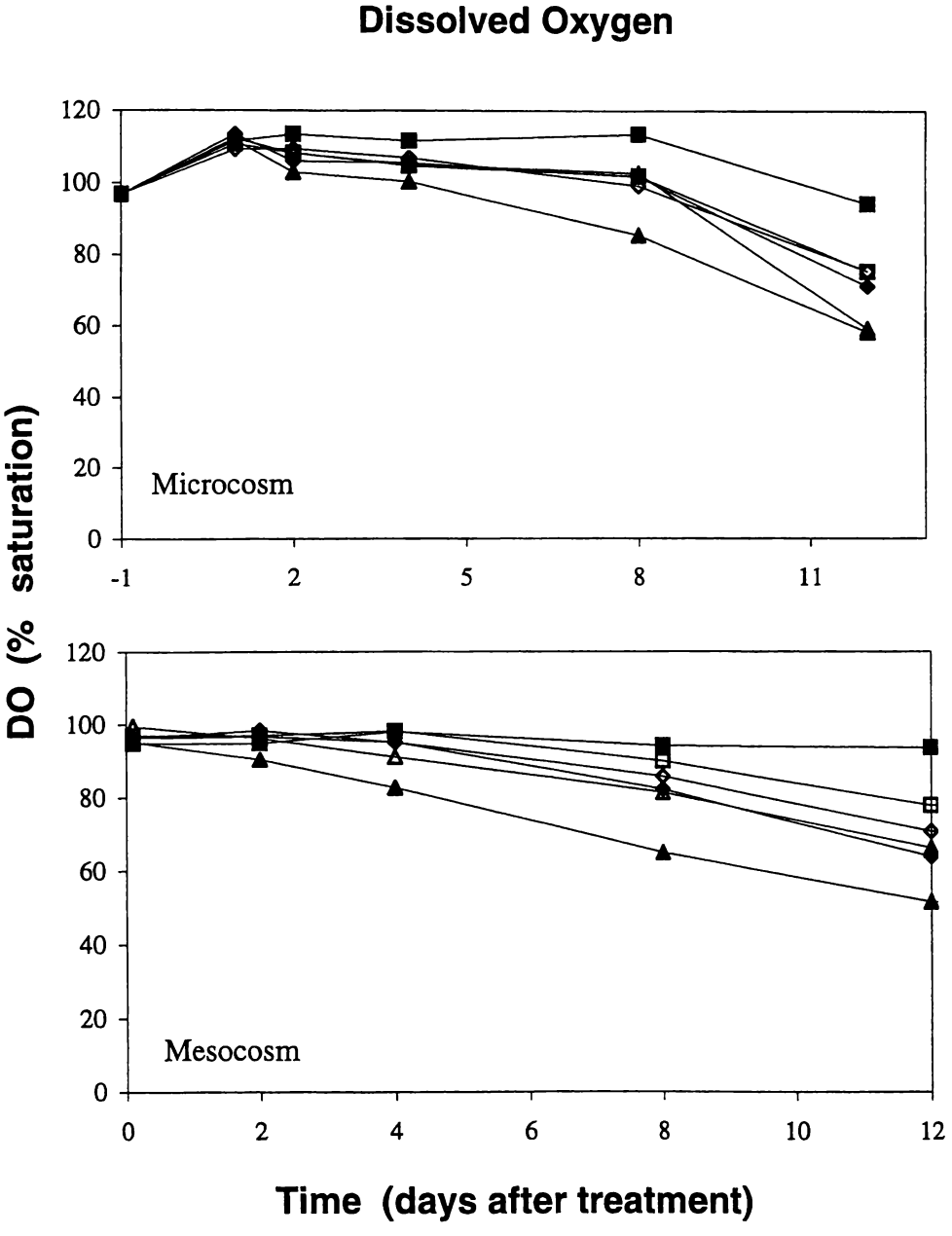


Figure 6. Dissolved oxygen concentrations (% saturation) in microcosms and mesocosms. (control (■), solvent control (□), 10 (◇), 36 (◆), 54 (△)and 121(▲) $\mu g.L^{-1}$ PCP)

Chlorophyll a

Chlorophyll *a* initially increased in all microcosms before decreasing (Fig. 7). The largest declines occurred in the higher PCP treatments. Chlorophyll *a* decreased with time in all mesocosm treatments including the controls, although concentrations initially increased in the 10 $\mu g.L^{-1}$ PCP treatment before decreasing.

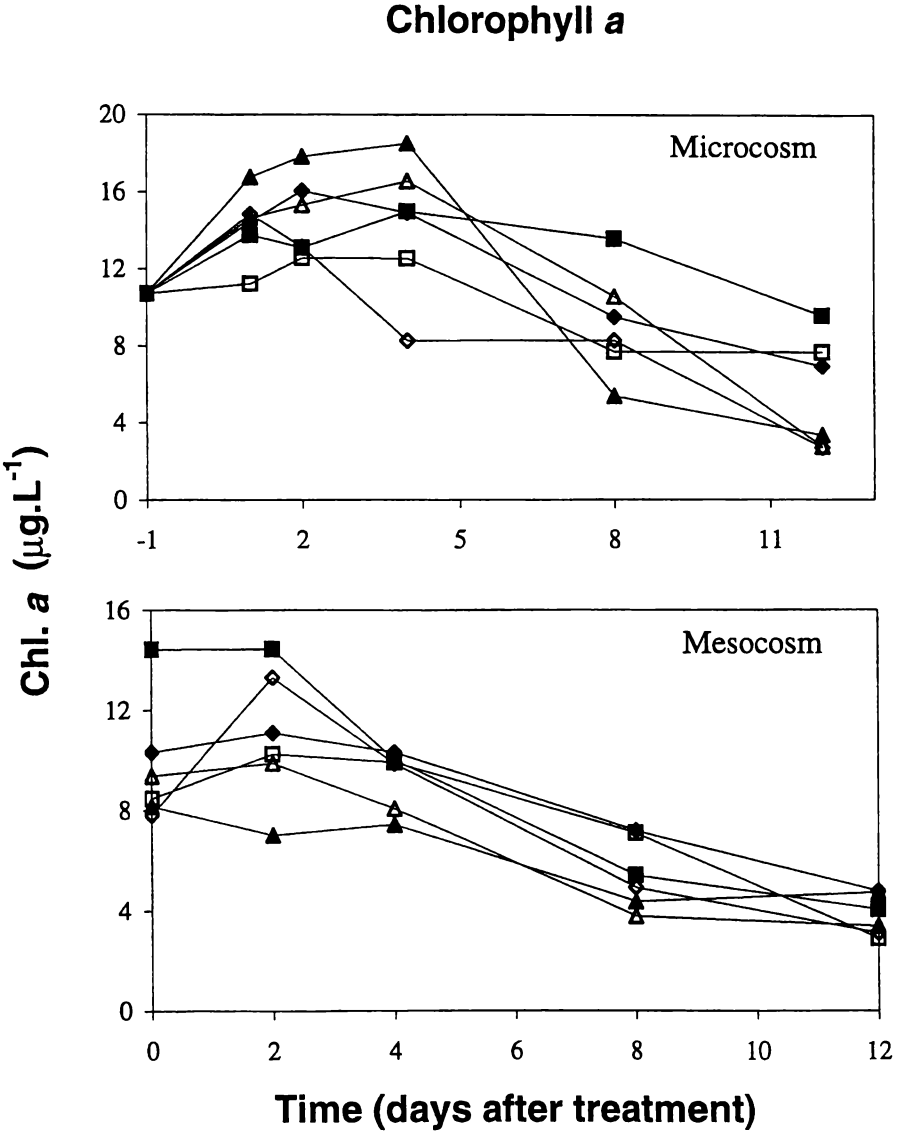


Figure 7. Chlorophyll *a* concentrations ($\mu g.L^{-1}$) in microcosms and mesocosms. (control (■), solvent control (□), 10 (◇), 36 (◆), 54 (△)and 121(▲) $\mu g.L^{-1}$ PCP)

Total phytoplankton

Phytoplankton abundance remained similar, or increased in all mesocosms, with no obvious dose-response relationship (Fig. 8). Abundance declined with time in all microcosm treatments except the control.

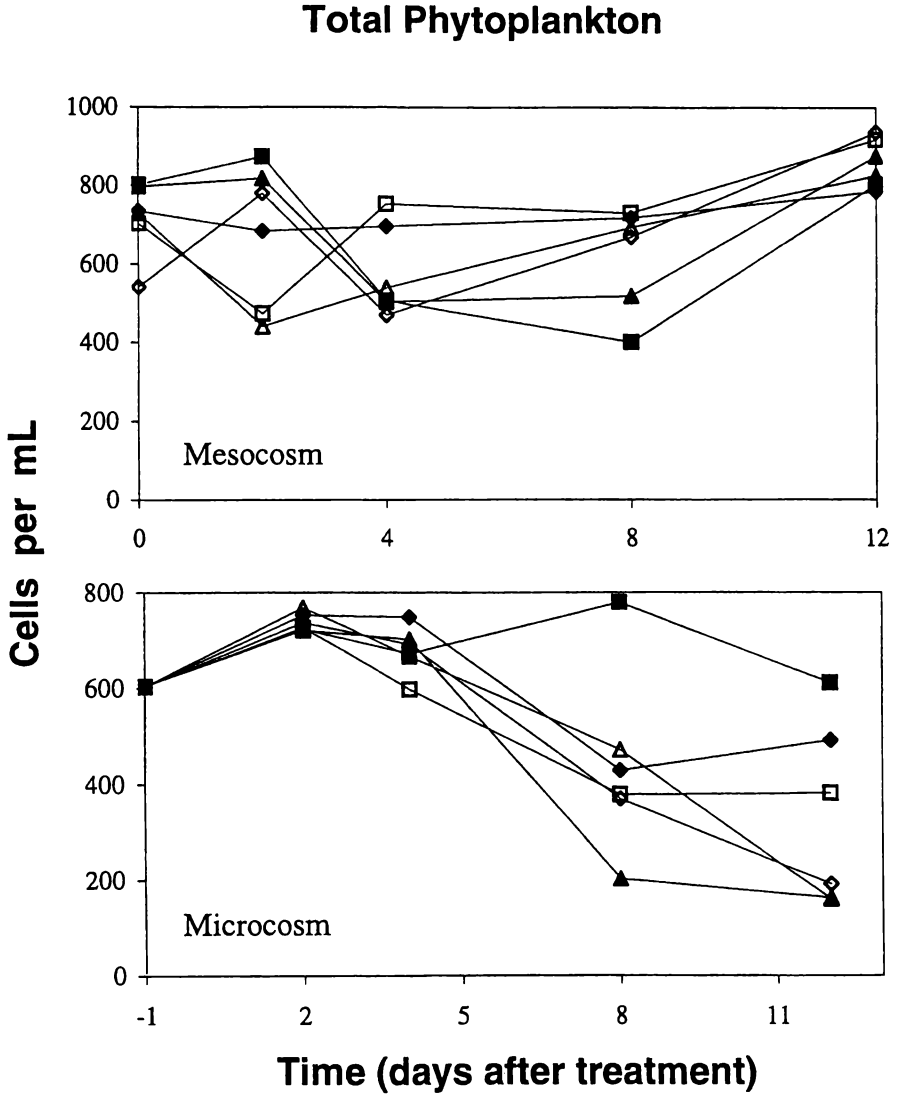


Figure 8. Total phytoplankton in microcosms and mesocosms.
(control (■), solvent control (□), 10 (◇), 36 (◆), 54 (△)and 121(▲) $\mu g.L^{-1}$ PCP)

Zooplankton

Total numbers of Crustacea increased, or remained stable, in all microcosms. In the mesocosms, numbers remained relatively stable, although there was an initial decrease in the highest mesocosm treatment and numbers doubled in the 10 $\mu g.L^{-1}$ PCP mesocosm (Fig. 9). The cladocerans, predominantly *Bosmina longirostris* and to a lesser extent *Ceriodaphnia pulchella*, increased in all microcosms and mesocosms (Fig 9). The greatest increases were in the highest treatment in the microcosms, and in the 121 and 10 $\mu g.L^{-1}$ mesocosm treatments. Rotifers decreased in all microcosms including the controls (Fig 9). In the mesocosms, rotifers decreased in the higher treatments and the control, but increased in the solvent control and lower treatments. An acute response to PCP was apparent in the highest concentration in both systems with rotifer numbers declining two days after treatment.

Copepod numbers remained similar in controls and lower PCP treatments, but decreased in the highest PCP treatment in both microcosms and mesocosms (Fig. 9). Changes in abundance of the different copepod life-stages were similar in the microcosms and mesocosms, with a pronounced treatment effect at the highest concentration (Fig. 10). Nauplii were most sensitive with marked decreases occurring within two to four days after PCP addition. Nauplii decreased in all microcosms and mesocosms, as did copepodites in the microcosms. Adult copepods increased in abundance in all but the highest microcosm and mesocosm treatments, as did copepodites in the mescosms.

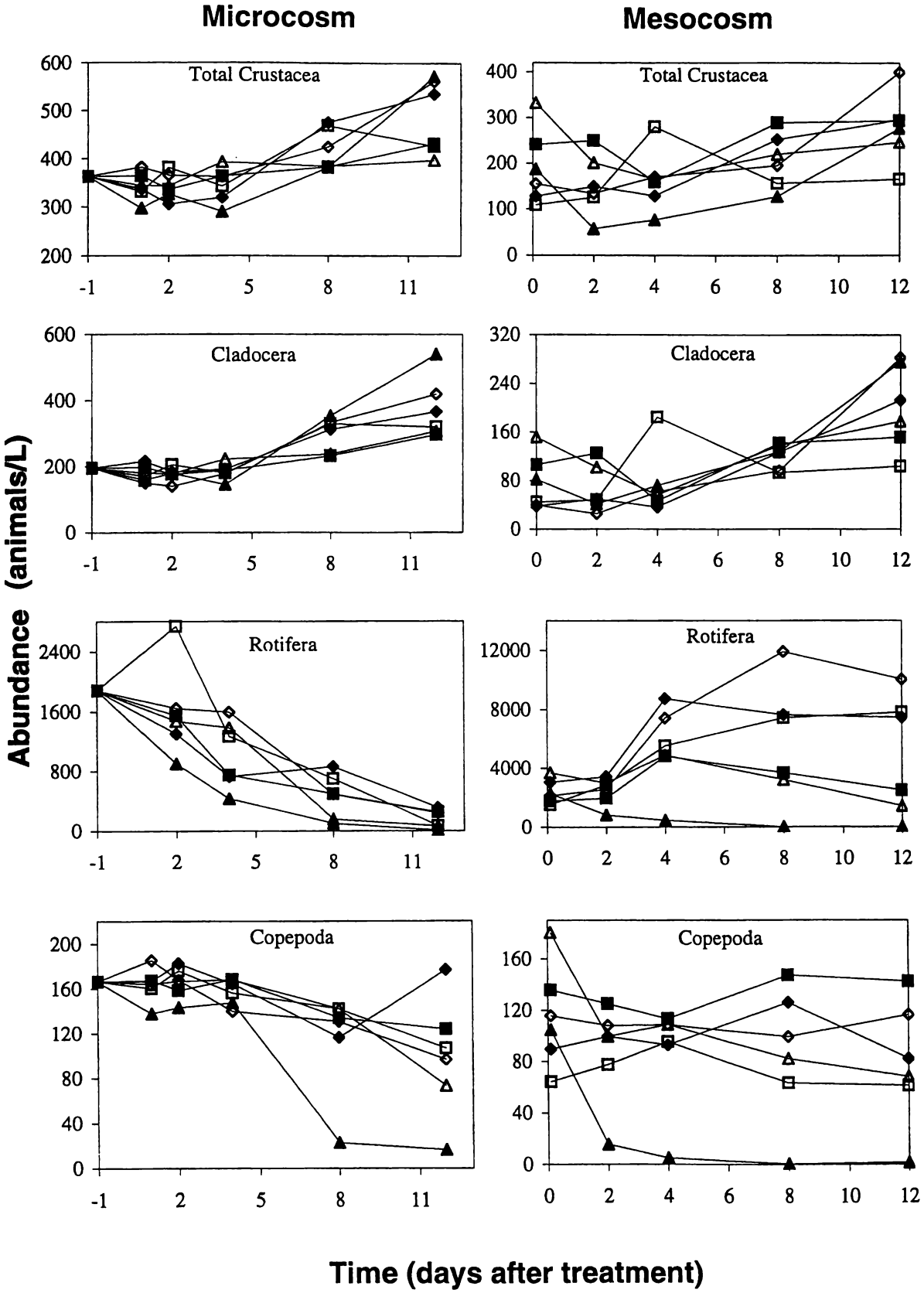


Figure 9. Changes in abundance of total Crustacea, Cladocera, Rotifera and Copepoda. (control (■), solvent control (□), 10 (◇), 36 (◆), 54 (△)and 121(▲) $\mu g.L^{-1}$ PCP)

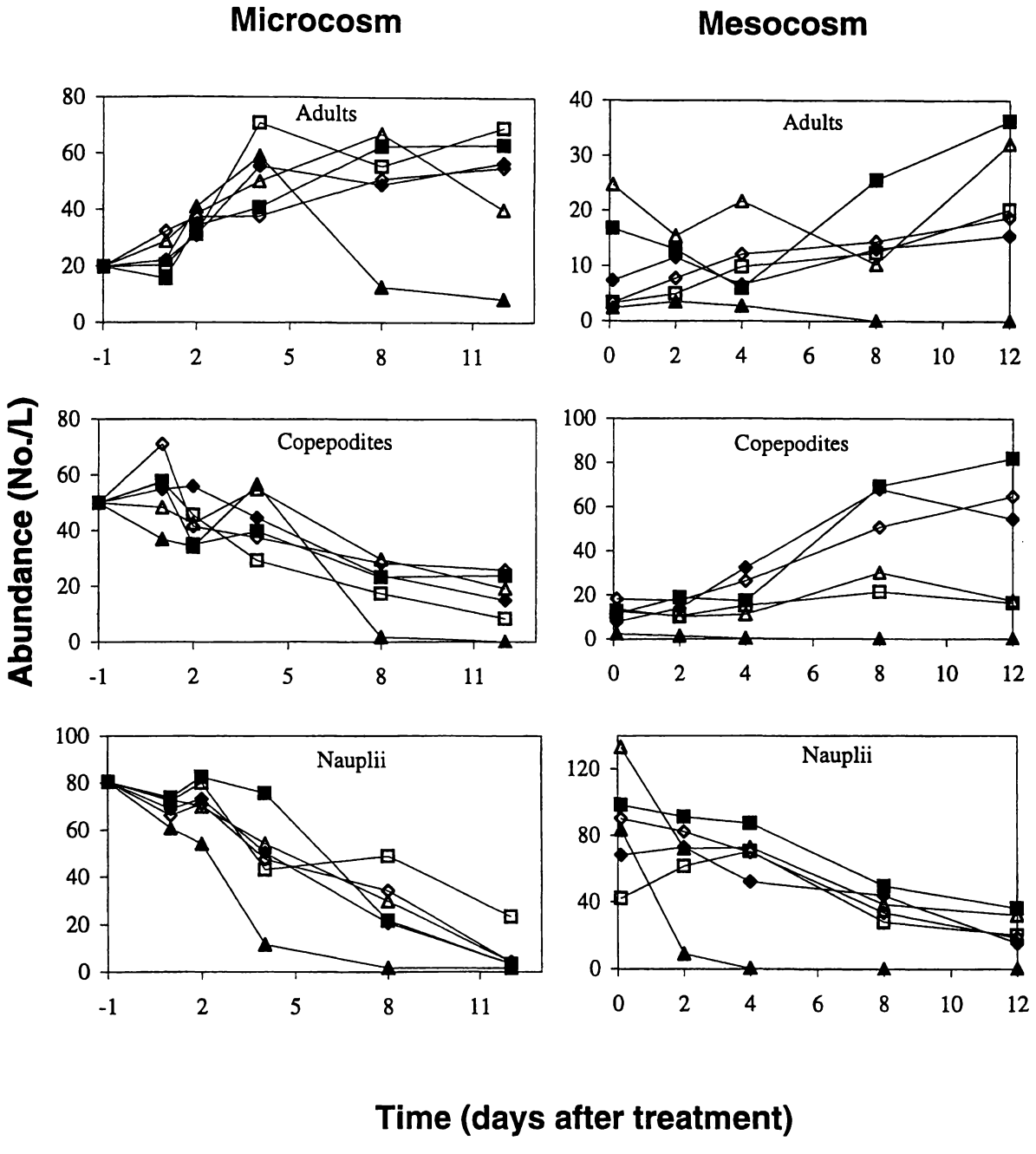


Figure 10. Changes in abundance of copepod life-stages (adults, copepodites, nauplii). (control (■), solvent control (□), 10 (◇), 36 (◆), 54 (△)and 121(▲) $\mu g.L^{-1}$ PCP)

EC50 estimates

EC50 values were estimated using total copepod % change in abundance relative to day 0 for those sample days on which relative abundance spanned 50% (Fig. 11). EC50 values for the microcosms on days eight and twelve were similar to those on days two and four in the mesocosms (Table 10), indicating a delay in response in the microcosms.

Table 10. Estimated EC50 values ($\mu g.L^{-1}$ PCP) for total copepod % change in abundance.

	day 2	day 4	day 8	day 12
microcosm	-	-	85	90[a]
mesocosm	96	89	55	52

[a] EC50 estimated as the highest concentration corresponding to 50% effect (see Fig. 11)

Multivariate analyses

The RDA biplots (Figs. 12 and 13) summarise treatment and temporal effects on the planktonic communities in the mesocosms and microcosms. The goodness-of-fit of the axes constrained to the explanatory variables is indicated by the eigenvalues. The higher the eigenvalue, the more variation is explained by the axis (van Wijngaarden et al. 1995). The eigenvalues for the first and second axes are presented in Table 11.

Table 11. Eigenvalues for the first and second RDA axes.

	axis 1	axis 2	% of total variance
microcosms	57	4.5	61.5
mesocosms	40	17	57

Examination of the ordination plots suggests that 'time' is the environmental variable associated with axis one, whereas 'treatment' is associated with axis two. Hence, the high first axis value in the microcosms and corresponding low second axis value suggests that 'time' was the overriding process driving shifts in species composition.

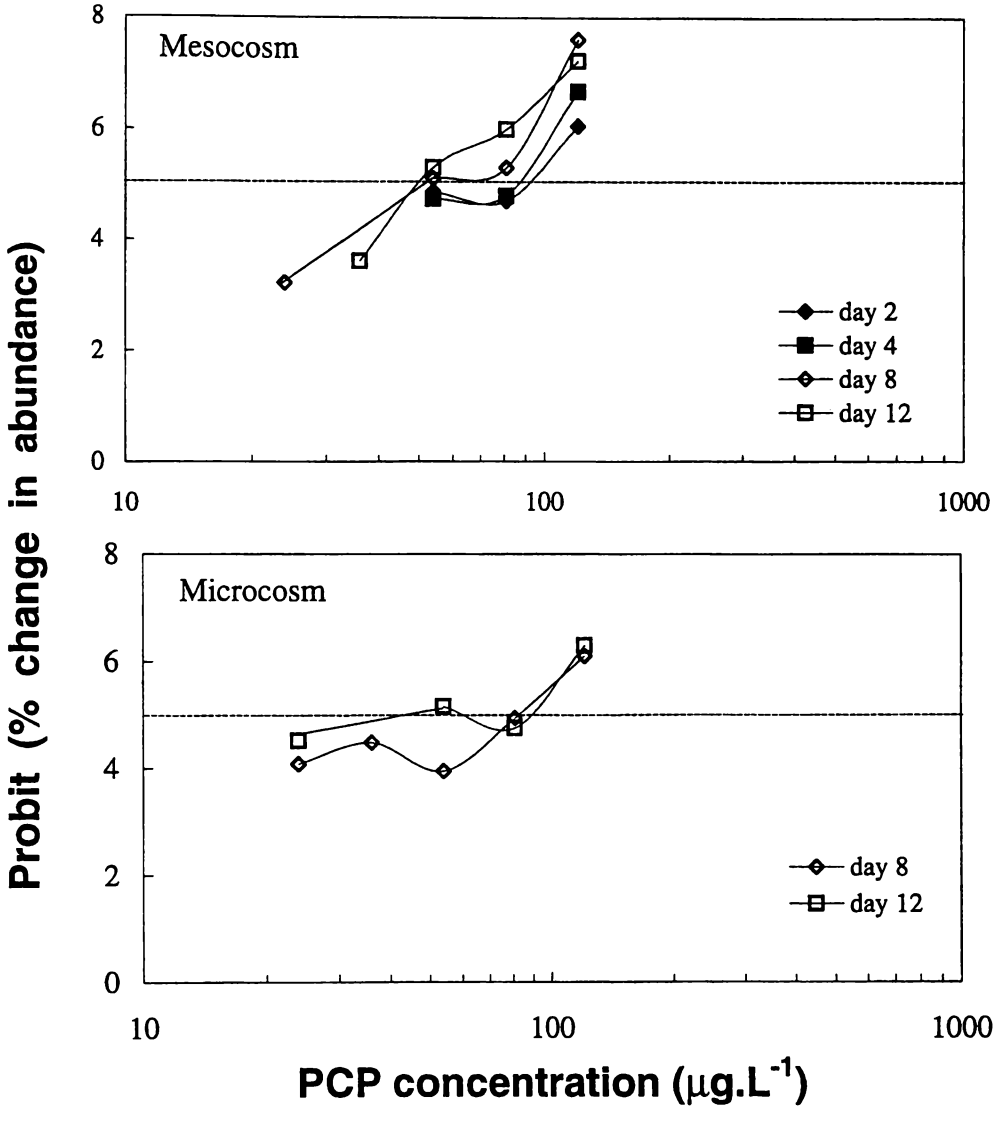


Figure 11. Probit plots for estimation of EC50 values for total copepod % change in abundance (compared to day 0) in microcosms and mesocosms on days when values spanned 50%.

The Monte Carlo permutation test for Day 0 of the mesocosm experiment resulted in a *p*-value of 0.16 indicating pre-treatment community composition was similar in all mesocosms (Table 12). Subsequent days had *p*-values of 0.01 indicating significant treatment effects. In the microcosms, day four was the only sample day with a significant *p*-value (0.05). A Monte Carlo permutation test for the entire microcosm data set from day -1 to day 12, resulted in a *p*-value of 0.01, indicating significant effects of both treatment and time.

Table 12. *p*-values for Monte Carlo permutation tests on each sample day.

	day 0	day 2	day 4	day 8	day 12
microcosms	-	0.10	0.05	0.06	0.14
mesocosms	0.16	0.01	0.01	0.01	0.01

community composition in day 0 microcosms was determined from composite water samples.

In the RDA biplots, samples with similar species composition lie close together while samples with dissimilar species composition lie far apart. The origin (centre) of a species ordination diagram represents the mean abundance of the individual species in all samples. Species closely associated with a site and present in higher than average abundance occur on the same side of the origin, while sites on the opposite side of the origin from a species point contain less than average abundance of that species.

The pre-treatment mesocosms (Fig. 12) are clustered together in the upper left quadrant suggesting a relatively homogeneous species composition as confirmed by the permutation test. Shifts in the controls and lower PCP treated microcosms and mesocosms from left to right indicate time vectors in this direction. Separation of the high and low PCP treatments between the upper and lower right quadrants indicates a treatment effect.

The microcosm controls and solvent controls were closely associated for the experimental duration indicating similar community composition (Fig. 13), whereas the mesocosm solvent controls diverged slightly from the controls along with the lower PCP treatments.

The replicate mesocosm controls remained closely associated. On day four there was greater divergence among the mesocosms than the microcosms indicating a slightly delayed treatment response in the microcosms.

Initially, species composition differed slightly between the microcosms and mesocosms but thereafter followed similar trends. Rotifers were abundant initially, as were calanoid copepod nauplii, and are positioned on the left in the ordination plots. All rotifers declined with time in the microcosms, while *Hexarthra mira* and *Anuraeopsis fissa* increased with time in the mesocosm controls and lower treatments. Later copepod stages, in particular calanoid adults, increased in abundance over time in both systems and are more closely associated with the controls and lower treatments on day 12 indicating a dose-response relationship. Cyclopoid copepods only increased with time in the microcosms.

The cladocerans, *Bosmina longirostris* and *Ceriodaphnia pulchella* were the only zooplankton species to show a positive response to PCP. *Ceriodaphnia pulchella* abundance increased with time in the mesocosms regardless of treatment level, and numbers of *B. longirostris* increased in both the mesocosms and microcosms.

Of the phytoplankton, *Peridinium* spp. and *Closterium acutum* were most abundant initially, whereas *Staurastrum leptocladum* and *muticum,* and *Staurodesmus glaber* increased in abundance in the lower concentrations of the mesocosm treatments and controls. *Cryptomonas* spp. responded positively to PCP in the mesocosms and increased in abundance in the highest treatment. *Botryococcus braunii* remained stable or increased in abundance in the microcosms.

Changes in abundance of zooplankton and phytoplankton taxa are shown in Appendices III and IV.

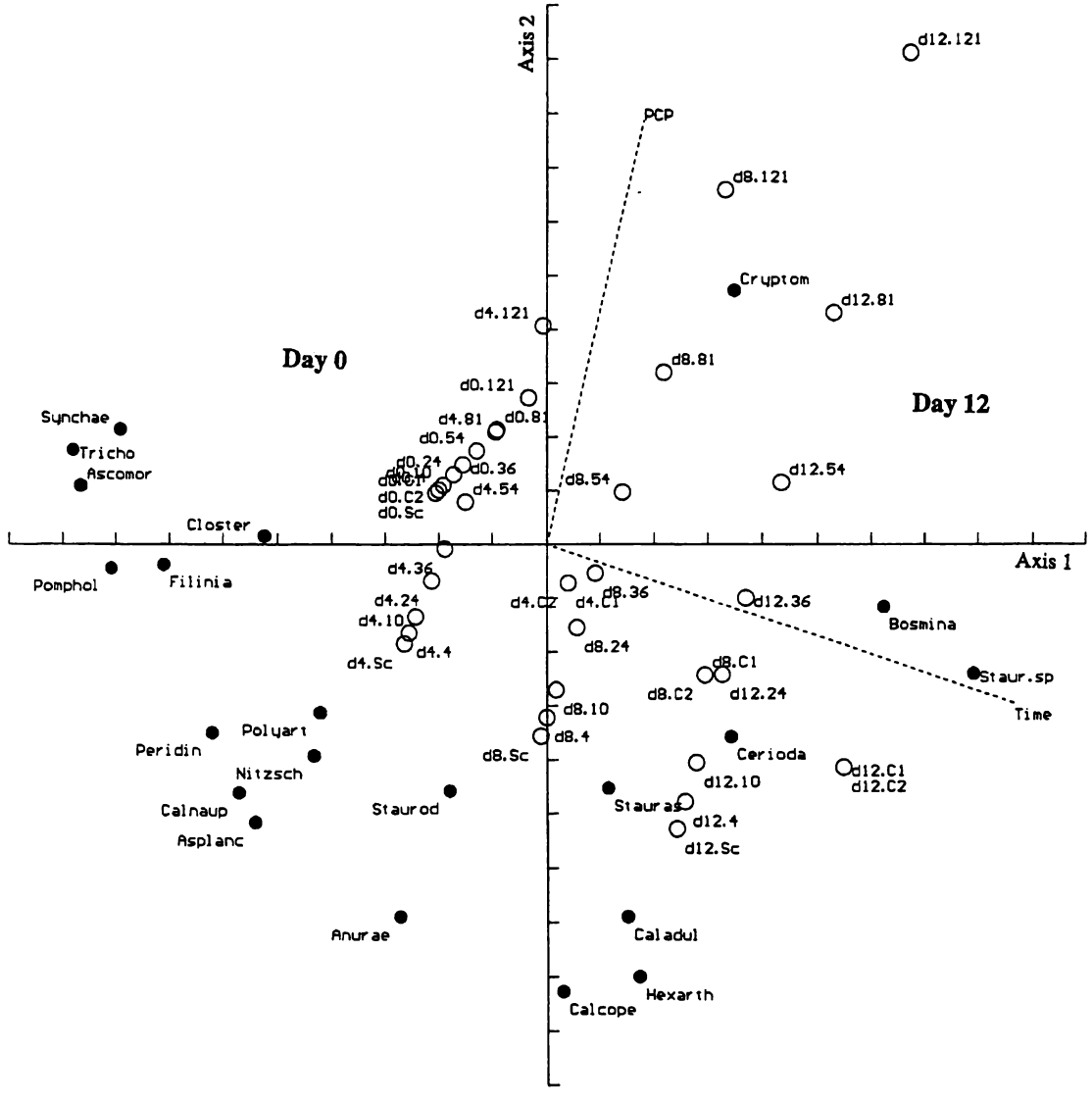
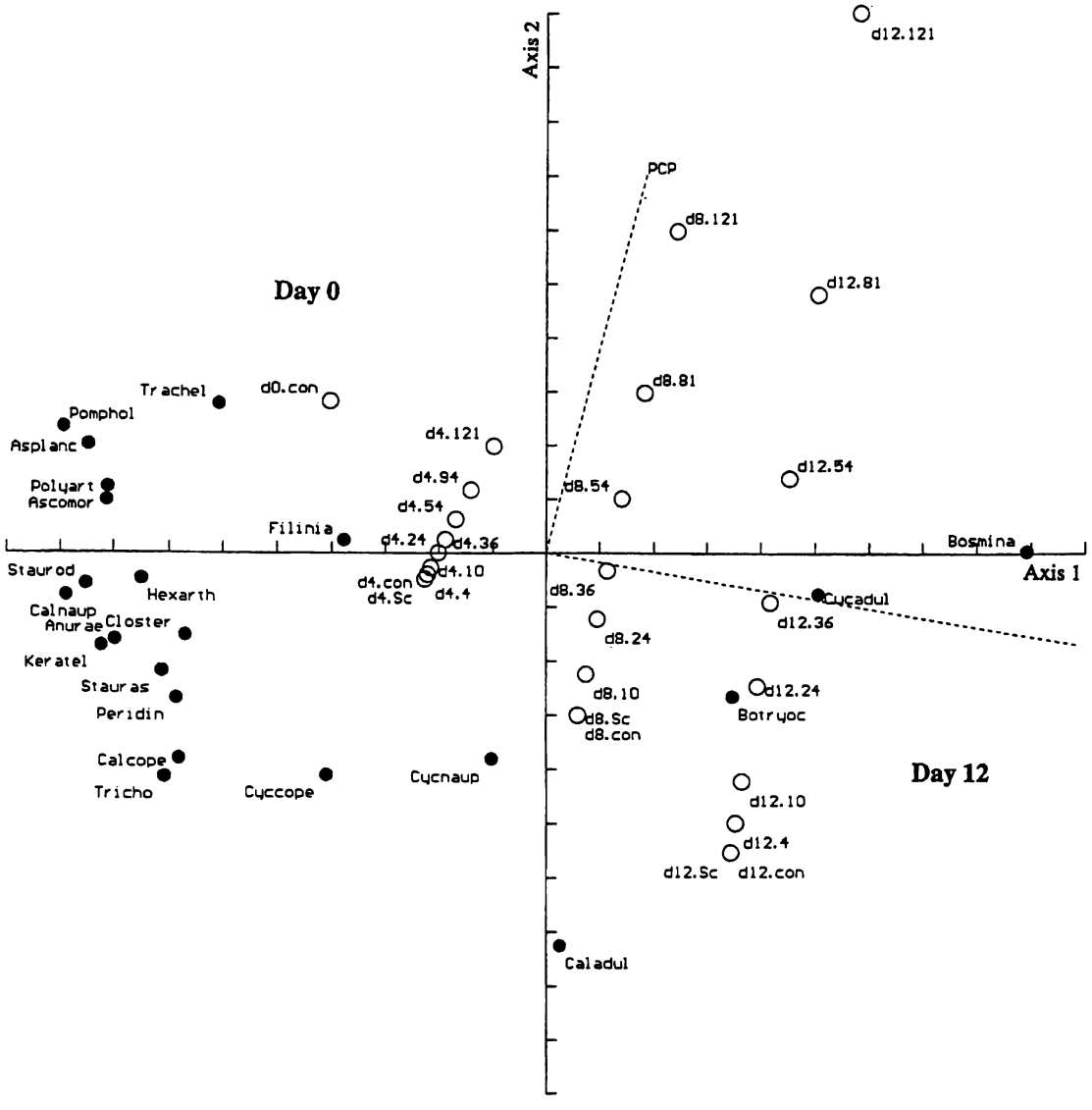


Figure 12. Mesocosm RDA biplot indicating effects of a single PCP application on planktonic community structure. Species are shown as closed circles and sites as open circles. Site codes indicate sampling day followed by PCP concentration ($\mu g.L^{-1}$). Site points for day two are not included in the diagram but lie intermediate between day 0 and four. Only those species well explained by the ordination are shown (see Figure 13 for species legend). Species in the centre of the diagram have been omitted.

RDA plots including site points for day two are shown in Appendix VIII.



Legend

Crustacea	Rotifers	Phytoplankton
Calnaup = *Calamoecia* nauplii	Synchae = *Synchaeta oblonga*	Cryptom = *Cryptomonas erosa* *Cryptomonas ovata*
Calcope = *Calamoecia* copepodite	Tricho = *Trichocerca similis*	Closter = *Closterium acutum*
Caladul = *Calamoecia* adult	Ascomor = *Ascomorpha ovalis*	Staurod = *Staurodesmus glaber* var. *limnophilus*
Bosmina = *Bosmina longirostris*	Pomphol = *Pompholyx complanata*	Nitzsch = *Nitzschia acicularis*
Cerioda = *Ceriodaphnia pulchella*	Filinia = *Filinia longiseta*, *Filinia novaezealandiae*	Stauras = *Staurastrum leptocladum* var. *insigne*
Cycnaup = Cyclopoid nauplii	Polyart = *Polyarthra dolichoptera*	Staur.sp = *Staurastrum muticum*
Cyccope = Cyclopoid copepodite	Asplan = *Asplanchna priodonta*	Peridin = *Peridinium* spp.
Cycadul = Cyclopoid adult	Anurae = *Anuraeopsis fissa*	Botryoc = *Botryococcus braunii*
	Hexarth = *Hexarthra mira*	Trachel = *Trachelomonas volvocina*

Figure 13. Microcosm RDA biplot. See Figure 12 for explanation of data and symbols.

Discussion

Despite changes in community structure in the microcosms unrelated to PCP treatment, overall sensitivity to the toxicant was similar in the microcosms and mesocosms, with multivariate analyses suggesting treatment effects at concentrations as low as 10 to 24 $\mu g.L^{-1}$ PCP. With the exception of surface illumination, which was considerably lower in the laboratory, abiotic conditions were similar in both experimental systems.

Habitat size has been suggested as a possible factor influencing the structure of food webs (Schoener 1989; Cohen and Newman 1991), with larger habitats predicted to support more complex food webs (Spencer and Warren 1996). In the present study, the general decline in rotifer abundance in the microcosms probably reflected the small size of the microcosms and hence their ability to support a complex community. Intuitively, larger microcosms better approximate natural systems, so they should successfully support more trophic levels and greater population densities over a longer period of time (Stephenson et al. 1984). Spencer and Warren (1996) tested this prediction using laboratory microcosms of different sizes, and not unexpectedly, larger beakers containing 305 mL supported communities with significantly more species and longer food chain lengths than smaller beakers containing 128 mL.

However, low species diversity may not necessarily be detrimental to the detection of community level effects. Although SAM contain a relatively simple community consisting of approximately 15 species (ASTM 1995), their potential to simulate effects in natural communities has been shown. Harrass and Taub (1985) compared SAM and field responses to copper and suggested that SAM can serve to demonstrate ecological effects likely to be shown by some natural communities. Larsen et al. (1986) also found that SAM provided reasonable estimates of atrazine concentrations producing direct effects in outdoor ponds.

It has been suggested that results obtained from multispecies tests may show no greater

sensitivity, predictive power, or greater interpretability, than single species tests (Kooijman 1985; Emans et al. 1993; Okkerman et al. 1993; Crane 1995). Such criticism may be attributed, in part, to the often inappropriate use of univariate statistical procedures for the analysis of multispecies data. Examination of individual and isolated biological parameters was shown to provide a limited and somewhat distorted view of the dynamic responses of SAM to turbine fuel by Landis et al. (1993). The most evident individual biological responses were those which displayed the most pronounced impacts, whereas multivariate analysis revealed a complex pattern of multiple divergences and convergences between treatment groups. The inability of univariate statistical techniques to identify an impact as significant until major reductions (~ 50%) in population size have occurred was also reported by Liber et al. (1992).

In the current study, multivariate analysis in combination with a dose range utilising seven treatments revealed community-level sensitivity at lower concentrations than could be clearly identifed on examination of individual variables and indicated similar sensitivity in both systems. Although individual responses in the microcosms were blurred by the confounding effect of enclosure, they still provided valuable information concerning the timing of responses that were lost in the multivariate analyses. In general, copepod abundance declines at the highest treatment level were delayed in the microcosms.

Modification of aspects of the microcosm experimental design, as described below, may have alleviated some of the problems associated with their smaller volume.

Microcosm experimental design

Enclosure effects arise because of the increased influence of edge effects that reduce water circulation and increase phytoplankton settling rates. Subsequent adherence of phytoplankton to the bottom reduces primary production and available food for filter feeding zooplankton (Harte et al. 1980). In an attempt to reduce the effect of settling in the

present study the microcosms were stirred daily to resuspend settled phytoplankton. Other microcosm studies have employed continuous mixing by air bubbling (Perez et al. 1977; Harte et al. 1980; Levy et al. 1985), although this means effects of treatment on oxygen concentrations can not be studied. Levy et al. (1985) investigated the effect of agitation on community composition in 58 L naturally derived microcosms with stirring blades or air bubbling out of capillary tubing. No agitation or the use of non-mechanical agitation with air bubbles was most successful for maintaining species composition similar to that of the parent community. Harte et al. (1980) suggest that mixing and the elimination of unwanted wall growth can also be accomplished by pouring the contents from a used microcosm into a clean one. In SAM, algae and zooplankton are reinoculated on a weekly basis, and twice weekly the sides are scraped and the microcosms stirred (Taub and Crow 1980). Leffler microcosms are also reinoculated on a weekly basis. Reinoculation permits demonstration of ecosystem recovery and simulates immigration or refugia in natural communities (Harrass and Taub 1985), therefore extending the life of the system. It also allows temporary toxic conditions to be distinguished from chronic toxicity (Taub 1997).

Pretreatment acclimation periods of up to three months in larger microcosm studies allow communities to develop and stabilize prior to treatment, and in many cases interconnection of microcosms in the pretreatment period allows mixing to ensure all microcosms have a similar community composition. Leffler microcosms also undergo a substantial acclimation period. In a study by Stay et al. (1989) using Leffler microcosms, stock cultures of organisms developed from a natural community were acclimated to experimental conditions in aquaria (37 L) for not less than two months. 50 mL of the stock was then added to 950 mL of synthetic media in one litre beakers, and the microcosms were left for a further six weeks to stabilise before addition of the toxicant.

Pretreatment periods in the present study of one and two days were considered sufficient to allow the organisms to acclimate to the conditions. With the exception of several rotifer

species, initial community composition was similar among the microcosms, but subsequent declines in rotifer abundance offset any initial dissimilarity. Pretreatment mesocosm community composition was also similar, as indicated by their close association in the ordination and the permutation test, however, when compared to the microcosms initial variability in abundance at the species level was increased between mesocosms. For example, in the mesocosms, initial numbers of total Crustacea and copepod nauplii ranged between 100 to 300 per L, and 40 to 130 per L respectively. The spatial distribution of organisms in the mesocosms may also have contributed to sample variability, although Stephenson et al. (1984) found no consistent pattern (ie. edge effect) in zooplankton distribution in enclosures considerably larger than those used in this study. The effects of enclosure on zooplankton distribution and sampling design are discussed in greater detail in Chapter six.

With the exception of Leffler microcosms (Leffler 1984), naturally derived microcosms usually contain considerably greater volumes (>50 L) than were used in the present study, so could be expected to support planktonic communities for longer periods of time in addition to maintaining greater complexity. However, reductions in species diversity in larger microcosms do occur. For example, Fliedner and Klein (1996) observed a reduction in species diversity during the three month conditioning phase in naturally derived 300 L microcosms. Despite this, comparable effects were observed between the microcosms and complex outdoor systems after treatment with lindane. Effects on species diversity in the smaller Leffler microcosms are unknown, as population dynamics are not investigated using this protocol and emphasis is placed on functional rather than structural responses.

Incorporation of various components from previously described microcosm protocols, such as the acclimation of stock cultures prior to establishing the microcosms, may have enhanced the current experimental design by reducing the magnitude of rotifer declines. Moreover, reinoculation from a stock culture may have allowed extension of the

experiment duration so that longer-term effects could be investigated.

Solvent effects

Test guidelines (e.g. OECD, HMSO) suggest that the concentration of organic solvents should not exceed either 0.1 or 0.5 $mL.L^{-1}$ of test solution. The amount of ethanol added to the systems in this study (0.125 $mL.L^{-1}$) was at the lower end of this range, and had an obvious impact on dissolved oxygen concentrations, with reductions in the solvent control relative to the non-solvent controls in both microcosms and mesocosms. The solvent did not appear to affect community structure, as illustrated by the close association of the controls and solvent control in the RDA, particularly in the microcosms. Separation of the solvent control and controls was evident in the mesocosm RDA, as the solvent may have had a slight impact on several groups such as copepodites and *Bosmina longirostris*.

Solvent effects have been observed before in microcosms. Triethylene glycol has been shown to produce acidic conditions after approximately three weeks resulting in losses of *Daphnia* (Taub 1989), and acetone also lowered pH (Kersting 1995) and dissolved oxygen (Taub and Crow 1978) in microecosystems and microcosms. Recommendations for solvent concentrations are primarily for single species laboratory tests and are well below concentrations causing toxic effects. However, at these concentrations solvents may directly, or indirectly, affect the results of multispecies tests. The following chapter presents the results of a study undertaken to determine whether lower ethanol concentrations reduce dissolved oxygen in microcosms to the same extent.

Mesocosms vs microcosms

This study was undertaken to establish whether community-level responses to a toxicant in small site-specific laboratory microcosms would be similar to those in more complex *in situ* mesocosms. Although environmental realism is enhanced in mesocosm experiments,

they are costly and time consuming to undertake. To my knowledge the mesocosm experiment described in this chapter is the first of its kind in New Zealand, but prospects for future studies may be limited due to resource consent restrictions. The overall similarity of response in the microcosms and mesocosms in this study suggests that the microcosm protocol may have potential, especially as a preliminary site-specific assessment of toxicant effects at the community level. Rather than undertaking a series of single species laboratory tests, which presumably necessitate laboratory culture, and may ultimately be just as expensive and time consuming as multispecies tests, a microcosm test such as this could be used to identify sensitive species present in a particular community. If required, futher tests could then use these species to develop water quality criteria.

Conclusions

1. Multivariate analysis of changes in community composition suggests that both systems responded to PCP in a similar manner.
2. Copepod sensitivity was similar in both systems, although response was delayed in the microcosms.
3. Dissolved oxygen concentrations responded similarly to the solvent and PCP in both systems.
4. Changes in community structure unrelated to PCP were observed in the microcosms with overall declines in rotifers and phytoplankton.
5. Problems inherent in mesocosm studies (expense, time, resource consent) are reduced considerably with small laboratory microcosms.
6. Control over initial uniformity in community composition was improved in the microcosms with the use of a composite water sample.
7. Improvements in microcosm design, and extention of the monitoring period, may have

been achieved with an extended pre-treatment phase and reinoculation from a stock culture, but this would have been at the expense of increased costs and time taken to complete the experiment.

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CHAPTER FIVE

Ethanol as a solvent carrier in microcosms and mesocosms: Effects on dissolved oxygen and pH in microcosms

Abstract

The effect of a toxicant solvent carrier (95% ethanol) on dissolved oxygen and pH in small naturally derived laboratory microcosms containing 1.6 L of lake water was investigated. Single applications of ethanol were added to the microcosms to give concentrations of 8, 16, 32, 63, 94, and 125 $\mu L.L^{-1}$ respectively, and effects were monitored over a period of 15 days. Dissolved oxygen and pH declined in all treatments after seven and five days respectively. The greatest decreases occurred in the four highest concentrations. After day eleven a recovery towards pretreatment levels and the non-dosed controls was observed in the two lowest concentrations.

Keywords: solvent effects; ethanol; microcosms; pH; dissolved oxygen

Introduction

Water miscible organic solvents are used as carriers of water insoluble test substances in aquatic toxicity tests because of their ability to dissolve a wide variety of substances and their low toxicity. Triethylene glycol (TEG), dimethyl sulfoxide (DMSO), dimethyl formamide (DMF), acetone, and alcohol (methanol and ethanol) are commonly used solvents. In general, test guidelines suggest that the concentration of organic solvents should not exceed 0.1 (OECD) or 0.5 (HMSO) $mL.L^{-1}$ of test solution (McKim 1985, Parrish 1985), yet the guidelines provide no rationale for their limit (Mank and Swigert 1996). Petrocelli (1985) suggests that the maximum concentration of solvent used in chronic toxicity tests should be less than 1/1000 of the juvenile fathead minnow 96 h LC50 value, and should not exceed 0.5 $mL.L^{-1}$.

In laboratory microcosm and *in situ* mesocosm experiments investigating the effect of pentachlorophenol (PCP) on planktonic community composition (Chapters 4 and 6), it became obvious that the solvent carrier (95% ethanol) was having an obvious effect on dissolved oxygen concentrations. However, the solvent did not appear to adversely affect planktonic community composition. The concentration of ethanol in the microcosms and mesocosms was 125 $\mu L.L^{-1}$. Reductions in dissolved oxygen concentration in all but the non-solvent controls were attributed primarily to the ethanol. Consequently, a laboratory microcosm experiment was undertaken to establish whether the use of lower ethanol concentrations would affect oxygen concentrations to the same extent.

Methods

A 15-day laboratory microcosm experiment was undertaken using water collected from Lake Rotomanuka on the afternoon of 24 February 1997. Water was pumped into 20 L buckets from a depth of two metres using a diaphragm pump. The *in situ* temperature and dissolved oxygen concentrations at two metres were 23°C and 85% saturation respectively. On return to the laboratory the lake water was combined and mixed thoroughly using a secchi disk, before being added to the microcosms (2 L glass jars). Oxygen concentration, pH and conductivity of the composite water were 103% saturation, 7.14 and 165 $\mu S.cm^{-1}$ respectively.

Appropriate amounts of ethanol were added to the microcosms prior to addition of 1.6 L of lake water to give replicated concentrations of 0, 8, 16, 32, 63, 94, and 125 $\mu L.L^{-1}$ respectively. The microcosms were randomly positioned under a bank of fluorescent lights (Sylvania GRO-LUX F18W/GRO) in a temperature controlled laboratory (22 ± 2°C) with a photoperiod of 14L:10D. Light intensity at the water surface was 17.5 $\mu E.m^{-2}.s^{-1}$. The microcosms were covered with plastic petri dishes to reduce evaporation.

Dissolved oxygen and pH measurements were taken each afternoon on days 1 to 5, 7 to 11, and 15. Dissolved oxygen was measured in the undisturbed water column, the microcosms were then stirred gently and a sample removed for pH measurement. Samples were then returned to their respective microcosms. The microcosms were also stirred on days when samples were not taken.

Results

The mean temperature in the microcosms during the 15 day experiment was 24°C (± 1.2). The pH initially increased in all treatments and remained steady before starting to decline between days five and eight in all of the ethanol treatments compared to the controls, with the biggest decreases at the four highest concentrations (32 to 125 $\mu L.L^{-1}$) (Fig. 14a).

A recovery back to pretreatment values occurred between days 11 and 15 in the lower concentrations (8 and 16 $\mu L.L^{-1}$), though values were still lower than the controls. Mean control pH (± 1 S.D.) was 7.5 (± 0.15).

Dissolved oxygen concentrations exhibited a similar response (Fig. 14b). Concentrations fluctuated slightly then started to decline between days seven and eight. Rapid declines occurred in all of the ethanol treated microcosms between days seven and ten, and continued in the four higher treatments to give final oxygen concentrations of < 30% saturation by day 15. After day eleven a recovery was observed in the 8 and 16 $\mu L.L^{-1}$ ethanol treatments as oxygen concentrations rose to ≥ 65% saturation. Mean control dissolved oxygen was 101 (± 7.7)% saturation. Values for pH and dissolved oxygen in replicate treatments were within 5% of the mean.

Discussion

Declines in pH and oxygen have been reported previously following the use of solvent carriers in microcosm studies. In laboratory microecosystems dosed with chlorpyrifos dissolved in acetone (66 $\mu L.L^{-1}$ acetone), Kersting and van Wijngaarden (1992) observed a decrease in pH relative to the controls after the disappearance of chlorpyrifos from the systems. Slight changes in oxygen concentration were also observed. As a result Kersting (1995) conducted further tests to verify the solvent effect and observed a decrease in pH by approximately two units. Recovery to pretreatment levels took several months.

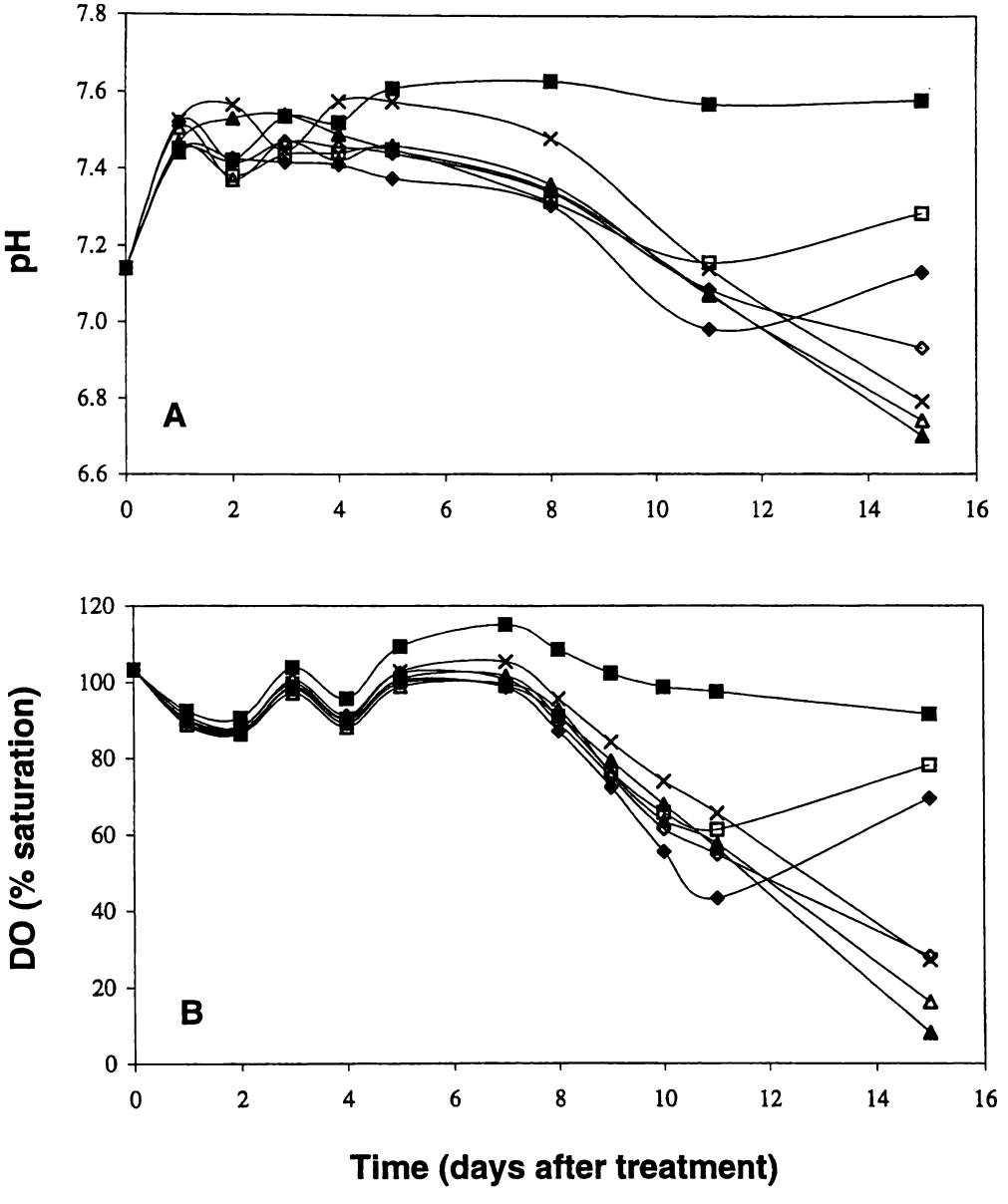

Figure 14. Changes in pH (A) and dissolved oxygen (B) in microcosms treated with ethanol. Values are the mean of two replicates for each treatment. (control (■), 8 (□), 16 (◆), 32 (◇), 63 (▲), 94 (△), 125 (x) $\mu g.L^{-1}$ ethanol).

Taub (1984, 1989) reported similar decreases in pH with triethylene glycol as the solvent in Standardised Aquatic Microcosms. Taub postulated that decreases in pH may have been the result of acidic degradation products. CO_2 production during metabolic breakdown of the solvent would also lower the pH, and result in concomitant oxygen declines (Kersting 1995). The lower pH observed in the present study was probably due to the latter as both pH and oxygen declined.

Rayburn and Fisher (1996) have suggested a maximum allowable concentration of 1 $g.L^{-1}$ ethanol for developmental toxicity tests with the grass shrimp (*Palaemonetes pugio*). They calculated average four day and twelve day LC50 values of 12.07 and 3.63 $g.L^{-1}$ ethanol respectively. They also observed eye malformations following embryonic exposure of grass shrimp to ethanol. The concentrations of ethanol added to the microcosm and mesocosm experiments described in Chapters 4 and 6 were considerably less than those causing deformity and mortality in Rayburn and Fisher's study, therefore it is unlikely that the planktonic community suffered directly as a result of the ethanol. Resultant decreases in oxygen may have contributed indirectly to the toxic effects of PCP on the planktonic community, as Taub and Crow (1978) concluded that *Daphnia* mortality in microcosms treated with 0.1 $mL.L^{-1}$ acetone was an indirect effect due to decreased oxygen concentrations resulting from metabolism of the acetone, rather than direct toxicity. However, increased sensitivity of the planktonic community to PCP during the winter mesocosm experiment (Chapter 6), in conjunction with high oxygen concentrations (> 77% saturation), would tend to suggest that changes in oxygen concentration did not unduly influence PCP toxicity.

The use of solvent carriers may also result in interactions between the solvent and toxicant, as was observed by Calleja and Persoone (1993). The addition of ethanol reduced the toxicity of diazepam to *Artemia salina* larvae whereas toxicity to *Daphnia magna* neonates was increased by a factor of 1.5. In sublethal tests with the naupliar stages of three

freshwater copepods (Chapter 2), naupliar survival in the solvent controls was improved by almost 50% compared to that of the non-solvent controls in tests with the calanoid *Calamoecia lucasi*. Although only speculative, it is possible that ethanol addition provided an organic substrate for increased bacterial production and thus, provided an additional food source for the nauplii. Whether PCP toxicity was enhanced or reduced in the naupliar tests because of the use of a solvent is unknown however. A similar effect on community composition was observed in the microcosm (Chapter 4) and mesocosm experiments (Chapter 6). The lower PCP treatments and solvent controls diverged from the non-solvent controls in an opposite direction to the higher PCP treatments suggesting the possibility of a stimulatory effect on the planktonic community.

Ethanol concentrations added to the laboratory microcosms (Chapter 4) and *in situ* mesocosms (Chapter 6) were the same as the highest concentration used in this study. The use of lower concentrations did not markedly reduce the magnitude of the dissolved oxygen and pH declines although they were reversed in the two lowest treatments after day eleven, and values had recovered almost to pretreatment and control levels by day fifteen. Thus reducing the amount of solvent in the microcosm and mesocosm experiments would not have appreciably diminished the solvent effect as the experiments were only conducted for 12 and 20 days respectively.

The results from this study have shown that ethanol induces changes in ecosystem functioning at very low concentrations, suggesting that its use as a solvent carrier in community-level tests should be limited.

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CHAPTER SIX

Seasonal variation in community-level responses of aquatic mesocosms dosed with pentachlorophenol

Abstract

Seasonal variations in planktonic community response to pentachlorophenol (PCP) were studied in four mesocosm experiments between the winter of 1996 and autumn of 1997 using a regression design with seven unreplicated treatments ranging from 4 to 121 $\mu g.L^{-1}$ PCP, a solvent control and replicated controls. Mesocosms (860 L) in Lake Rotomanuka were dosed with single applications of technical grade PCP (nominally 86%) dissolved in ethanol, and mixed to ensure an even distribution of PCP with depth.

Temperature, pH, conductivity, dissolved oxygen, chlorophyll *a*, zooplankton and phytoplankton abundance were monitored on days 0, 2, 4, 8, 12, 16, and 20. The effects of treatment and time on plankton species composition in each seasonal experiment were analysed using redundancy analysis (RDA). Monte Carlo permutation tests were used to identify significant treatment effects on each sample day. Daily EC50 estimates were calculated using total copepod % change in abundance.

There was no significant difference in community composition between pre-treatment mesocosms, and the replicated controls throughout each experiment. RDA identified treatment effects at 24 $\mu g.L^{-1}$ PCP in summer, 36 $\mu g.L^{-1}$ PCP in spring and autumn, and 54 $\mu g.L^{-1}$ PCP in winter. With the exception of *Cryptomonas*, which responded positively to PCP, all other taxa were most sensitive in winter and spring, with copepods showing the highest sensitivity. Copepod 48 h EC50 values ranged from 47 to 90 $\mu g.L^{-1}$ in spring and summer respectively. Chronic values ranged from 4.3 to 36 $\mu g.L^{-1}$ PCP in spring and summer.

Keywords: mesocosm; community-level response; seasonal sensitivity; multivariate analysis; copepods; PCP; solvent effects; New Zealand

Introduction

Temperate aquatic communities undergo qualitative, predictable seasonal shifts in algal and zooplankton abundance, and limiting factors such as nutrients, temperature and light (Sommer et al. 1986; Lampert 1987). Such changes are likely to affect the manner in which communities respond to toxicants because of differences in species assemblages and physiological states (Taub 1997), and the importance in timing of a toxicant addition on planktonic communities has been shown in a number of studies. The timing of copper and streptomycin additions to laboratory microcosms affected community sensitivity, and responses appeared to be strongly influenced by developmental stage, species density, and associated differences in water chemistry at the time of addition (Kindig et al. 1983; Swartzman et al. 1990; Taub et al. 1991). Carbaryl addition, at different stages in the seasonal cycle, also induced distinct recovery patterns in experimental pond zooplankton communities (Hanazoto and Yasuno 1987, 1990). In addition to variable predation pressures, Hanazoto and Yasuno (1990) emphasized the significance of population trends at the time of treatment on zooplankton recovery in their experimental ponds.

Compared with Australian and northern temperate lakes, and in contrast to the regular seasonal changes summarised in the PEG-model (Sommer et al. 1986), New Zealand zooplankton communities exhibit very little predictable seasonality in total abundance, or in the relative importance of major groups (Burns 1991). Consequently, the magnitude of seasonal changes in biomass are reduced and abundance cycles exhibit little similarity either between years or between lakes. Because of the low species diversity of New Zealand's crustacean zooplankton, the same species are often present throughout the year. Studies on calanoid copepod (Chapman 1973; Chapman and Green 1987; Burns 1992) and cladoceran (Forsyth and James 1991; Greenwood 1993) population dynamics in New Zealand lakes have shown that they generally breed throughout the year. However, clutch

sizes are small indicating that populations are food limited for much of the time (Chapman and Green 1998).

These features of New Zealand lakes provide a unique opportunity to investigate the modifying effect of season on planktonic community responses to a toxicant. Without the confounding effects of major shifts in species assemblages, any differences in community sensitivity to a toxicant would be more likely to result from changing abiotic conditions, such as temperature, or nutritional status. Such changes could be expected to alter physiological states and hence sensitivity to toxicant stressors.

In situ mesocosms have not been used previously in New Zealand toxicity studies and nothing is known about seasonal variations in the sensitivity of freshwater communities to toxicants. This study was undertaken to test the hypothesis that seasonality is important in determining the response of a planktonic community to PCP. To investigate this, four separate seasonal mesocosm experiments were conducted in Lake Rotomanuka between the winter of 1996 and the autumn of 1997. The direct effects of the toxicant on community structure were investigated over a period of 20 days and compared between seasons using univariate and multivariate procedures.

Methods

Study site

Lake Rotomanuka (37°55'S, 175° 19'E) is a small lake (surface area 136,730 m^2) situated approximately fifteen kilometres south of Hamilton, New Zealand. The lake is surrounded by farmland and is protected from prevailing westerly winds making it an ideal site for mesocosm deployment. It is the deepest (maximum and mean depths are 8.7 and 5.0 m respectively; Lowe and Green 1987) and the clearest of the Waikato lakes with secchi depths ranging from 2.25 to 4.8 m (Green 1989). Etheredge (1983) found that the lake had a mesotrophic or eutrophic dinoflagellate community dominated by *Peridinium* species.

Lake Rotomanuka normally stratifies in summer with a thermocline at 3 to 4 m, and has a deoxygenated hypolimnion.

In situ mesocosms

Mesocosm experiments were conducted in winter (August 1996), spring (October 1996), summer (January, February 1997) and autumn (April 1997). Ten mesocosms were anchored in a row at the eastern end of the lake in approximately 6 m of water, positioned 2 m apart in a north-south orientation to avoid shading by adjacent mesocosms at low sun angles. Each mesocosm was made from 100 μm clear polyethylene tubing sealed at the bottom end with wooden battens, and attached to a float made from a galvanised steel tube (extending 240 mm above the water surface) with a polystyrene collar to provide buoyancy (Fig. 5; Chapter 4). Protective covers made from 4.5 mm clear acrylic (perspex) prevented dilution by rainwater and fouling by birds. The polyethylene bags were 3 m deep with an internal diameter of 0.6 m to give a total volume of approximately 860 L. New bags were used in each experiment, and two days prior to adding PCP were filled with lake water pumped from a depth of 2 m using a diaphragm pump.

The experiments employed a regression design (Liber et al. 1992) with seven unreplicated treatments. Three controls were used in the winter and spring experiments, but only two in the summer and autumn experiments to include a solvent control when it became apparent the solvent was having an affect on oxygen concentrations. A technical formulation of PCP (nominally 86%, Aldrich Chemical Co. Milwaukee, WI) was used and contained TeCP (10% w/w) as the major contaminant (Chapter 3). Nominal PCP concentrations of 4, 10, 24, 54, 121, 272 and 608 $\mu g.L^{-1}$ were applied to the mesocosms as single applications on day 0 for the first (winter) experiment. Based on the results of single species laboratory tests and LC50 values reported in the literature, the concentrations were within the range where significant biological responses occur. The concentration range was reduced to

nominal PCP concentrations of 4, 10, 24, 36, 54, 81, and 121 $\mu g.L^{-1}$ for the later experiments when it became obvious that significant biological effects occurred at a concentration of 121 $\mu g.L^{-1}$ PCP. The random assignment of concentrations to mesocosms varied for each experiment.

Chemical analysis of water samples to determine initial PCP concentrations and rates of decay were undertaken during the spring experiment only. The results of these analyses are presented in Chapter 3, and suggest that predicted nominal concentrations were close to measured concentrations. Furthermore, the calculated half-life for PCP of 21 days (Chapter 3) also supports the use of nominal concentrations during the course of this study. Appropriate amounts of the PCP formulation were dissolved in 100 mL of 95% ethanol, followed by dilution in 15 L of lake water before being added to each mesocosm. 100 mL of 95% ethanol was also added to the solvent control. To ensure an even distribution of PCP in the water column, each mesocosm was mixed using a secchi disk.

The mesocosms were sampled from a boat on days 0, 2, 4, 8, 12, 16 and 20, between the hours of 10.00 and 13.00. Temperature and dissolved oxygen were measured at three depths (surface, middle and bottom) in each mesocosm and the daily mean for each treatment calculated. Conductivity was measured at the surface. Light intensity was measured on two occasions during the autumn experiment at 0.5 m depth intervals in the water column with a quantum light meter equipped with a LI-COR 2π sensor. Two depth-integrated water column samples were collected and pooled from each mesocosm using a 3 m length of PVC pipe (80 mm I.D.). Samples for pH, chlorophyll *a* and phytoplankton analyses were taken from the combined sample, before filtering the water through a 40 μm mesh plankton net to concentrate the zooplankton. Zooplankton and phytoplankton samples were preserved with 10% formalin and Lugol's iodine respectively. The remaining water was returned to its respective mesocosm. On completion of each experiment the polyethylene bags were split allowing the contents to mix with surrounding lake water.

Sample analysis

Zooplankton samples were resuspended with distilled water, and made up to known volumes in a measuring cylinder. Appropriate dilutions were chosen to give counts of 100 to 200 individuals of the dominant taxa per subsample. Counts of ~100 individuals have been calculated to reduce subsampling coefficients to ±10% (Cryer 1983 and papers therein). After thorough mixing 5 mL subsamples were transferred to a gridded perspex counting tray, mounted on the movable stage of a model MZ12 Leica stereomicroscope. One subsample was counted per sample. Cryer (1983) has shown that subsamples drawn from a sample were random. Copepods were separated according to genus and life stage (nauplii, copepodites, adults). Rotifers were identified to species level according to Shiel (1995).

Phytoplankton was counted using the sedimentation technique (Utermöhl 1958). Each sample was thoroughly mixed and a 50 mL aliquot sedimented for 24 h (Etheredge 1987). Samples were counted using an Olympus model IMT-2 inverted microscope. Larger taxa were counted during a scan of the entire basal plate at low magnification (x 60). Smaller, more numerous taxa were counted at higher magnifications (x 300; x 600) along diameter belt transects. To achieve 20% counting accuracy, an acceptable level for phytoplankton analyses (Utermöhl 1958, Hobro and Willén 1977), counting continued until each major species had been recorded at least 100 times (Etheredge 1987). The randomness of phytoplankton distribution on the basal plate of the sedimentation chamber, and in subsamples, was tested using Fisher's Index of Dispersion (Fisher 1948) (Appendix II). Texts consulted for species identification were Prescott (1978), Pridmore and Hewitt (1982), Croasdale and Flint (1986) and Etheredge (1983; 1987).

Chlorophyll a

As soon as possible after collection, water samples were filtered onto Whatman GF/C glass fibre filters (25 mm diameter), and stored at -20°C for no longer than one month. The filters were homogenised, and Chl. *a* extracted for 1 to 2 h at 4°C, using 10 mL of 90% acetone. The homogenate was centrifuged, and fluorescence of the supernatant measured before and after acidification with 2M HCl, using an Aminco SPF-500 spectrofluorometer set to 430 nm (excitation) and 665 nm (emission) (Strickland and Parsons 1968). Machine readings were calibrated using a chlorophyll *a* standard (Sigma Chemical Co. St. Louis, MO).

Chl. *a* concentration was calculated as:

$$\mu g/L = \left((unacidified - acidified) \times 0.4575 - 1.6353\right) \times \left(\frac{extract\ volume\ (mL)}{volume\ filtered\ (mL)}\right)$$

Multivariate analysis of treatment effects

Effects at the community level were analysed by redundancy analysis (RDA) using CANOCO version 3.1 (ter Braak 1988). RDA is based on a linear response model in which species abundance either increases or decreases monotonically in relation to environmental variables (ter Braak 1987). Initial analysis using detrended correspondence analysis (DCA) confirmed linearity of the data sets as the gradient lengths were less than three standard deviation units (see van Wijngaarden et al. 1995).

In contrast to principal components analysis (PCA), which takes into account all variance of a data set, RDA is constrained to the fraction of the total variance that is explained by the explanatory variables. In RDA, "PCP concentration" and "time," plus their interaction were combined as explanatory variables, thereby focussing on the variance attributed to treatment and time. There was an obvious solvent effect on dissolved oxygen concentrations between the controls and non-solvent control, so solvent (presence or absence) was used as a covariable to correct for differences between treatments which may

be attributed to its addition. To test for a treatment effect on species composition, data for each sampling day was tested separately for statistical significance using unrestricted Monte Carlo permutation tests. The Monte Carlo permutation test is a non-parametric test that randomly permutes the sampling data repeatedly in order to calculate a test statistic (van Wijngaarden et al. 1995).

The ordination results are presented as correlation biplots of species, sites and environmental variables using species-centred RDA in which each species is implicitly weighted by the variance of its abundance values. Species with high variance, often the abundant ones, therefore dominate the RDA solution, whereas species with low variance, often the rare ones, have only minor influence (ter Braak 1987). Euclidean scaling was used so that resulting ordination plots were optimal for interpreting distances between sites (ter Braak 1988). Species abundance was $\ln(1x + 1)$ transformed, and each treatment at each sampling time was considered to be a site.

EC50 calculation

Calculation of EC50 values used the method of Liber et al. (1992). The abundance of each species as well as total Crustacea, copepods, rotifers, and phytoplankton were plotted vs. time to determine whether dose-response relationships were apparent. In many instances, clear dose-response relationships were obscured by increases in abundance in the lower treatments relative to the controls, or overall declines in abundance of some taxa regardless of treatment level.

Copepods (predominantly *C. lucasi*) were prevalent in all four experiments and dose-response relationships were easy to identify. EC50 values for total copepod abundance were estimated by calculating their percent change in abundance relative to day 0 for each treatment, and plotting them as probit values vs. log PCP concentration. Daily EC50 estimates were determined from these graphs.

Results

Physicochemical parameters

Conductivity and pH were similar in all four experiments, while temperature varied with season (Table 13). Dissolved oxygen concentrations were lowest in autumn.

Table 13. Mean (± 1 S.D.) physicochemical parameters in the mesocosms.

	winter	spring	summer	autumn
temperature (°C)	11.3 (0.4)	17.3 (0.8)	22.6 (0.7)	18.4 (1.3)
DO (% saturation)	88.7 (6.4)	88.6 (12.5)	85.8 (22)	65.1 (27.7)
pH	7.1 (0.1)	7.4 (0.4)	7.4 (0.4)	7.1 (0.2)
Conductivity ($\mu S.cm^{-1}$)	171 (0.6)	160 (1.1)	160 (3.5)	155 (10.3)

Dissolved oxygen

With the exception of the winter experiment, oxygen concentrations decreased considerably in the highest PCP treatments (Fig. 15), with the most pronounced declines in autumn. Although there was a treatment effect in winter, it was only minor and oxygen did not fall below 77% saturation in the 121 $\mu g.L^{-1}$ PCP treated mesocosm. In summer and autumn a solvent effect was apparent, with decreased oxygen concentrations in the solvent control and lower PCP treated mesocosms.

Light conditions

In full sunlight there was a 77 to 85% reduction in light intensity between 0 to 0.5 m depth in the mesocosms compared to the lake, and under overcast conditions a 24 to 41% reduction (Table 14). At depths greater than 1 m, light intensity in the mesocosms was similar to that of the lake at the same depth.

Table 14. % reduction in light intensity in the mesocosms compared to the lake at the same depth.

	water column depth (m)				
conditions	surface	0.5	1.0	1.5	2.0
overcast	24	41	11	18	0
sunny	77	85	27	0	0

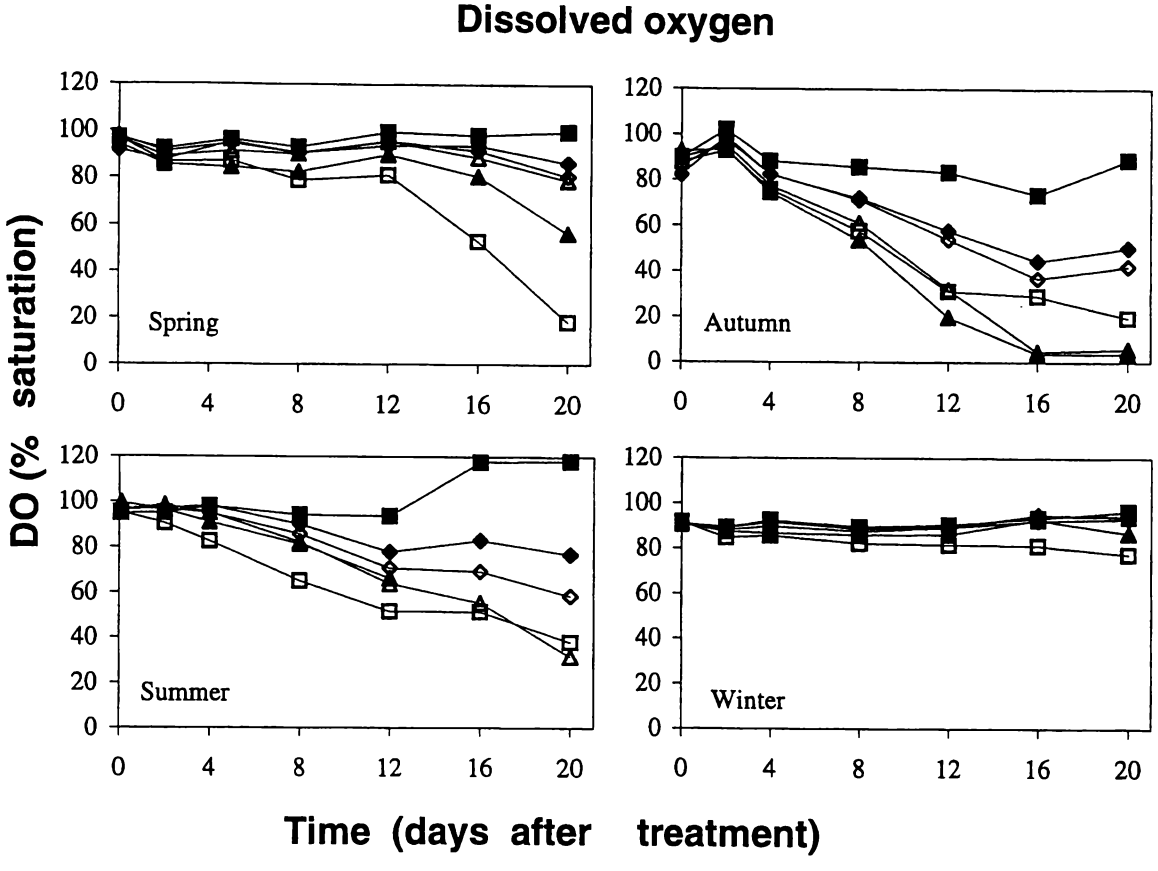


Figure 15. Changes in dissolved oxygen concentrations (% saturation) in seasonal mesocosm experiments. Not all treatments are shown so that general trends can be clearly identified. Data are missing for the 54 $\mu g.L^{-1}$ summer treatments on days 16 and 20 due to interference with the mesocosm, and are omitted for the 272 and 608 $\mu g.L^{-1}$ winter mesocosms. Control values are the mean of three replicates for winter and spring, and two replicates for summer and autumn. (■) control; (◆) solvent control in summer and autumn, and 4 $\mu g.L^{-1}$ in winter and spring; (◇) 10 $\mu g.L^{-1}$; (△) 36 $\mu g.L^{-1}$ in spring, summer and autumn, and 24 $\mu g.L^{-1}$ in winter; (▲) 54 $\mu g.L^{-1}$ and (□) 121 $\mu g.L^{-1}$ PCP.

Chlorophyll a

Chl. *a* concentration was reduced in the 121 $\mu g.L^{-1}$ PCP treatment in all experiments (Fig. 16). With the exception of winter, and to a lesser extent in spring, chl. *a* decreased in all mesocosms regardless of treatment. Concentrations increased again after day eight in the autumn controls.

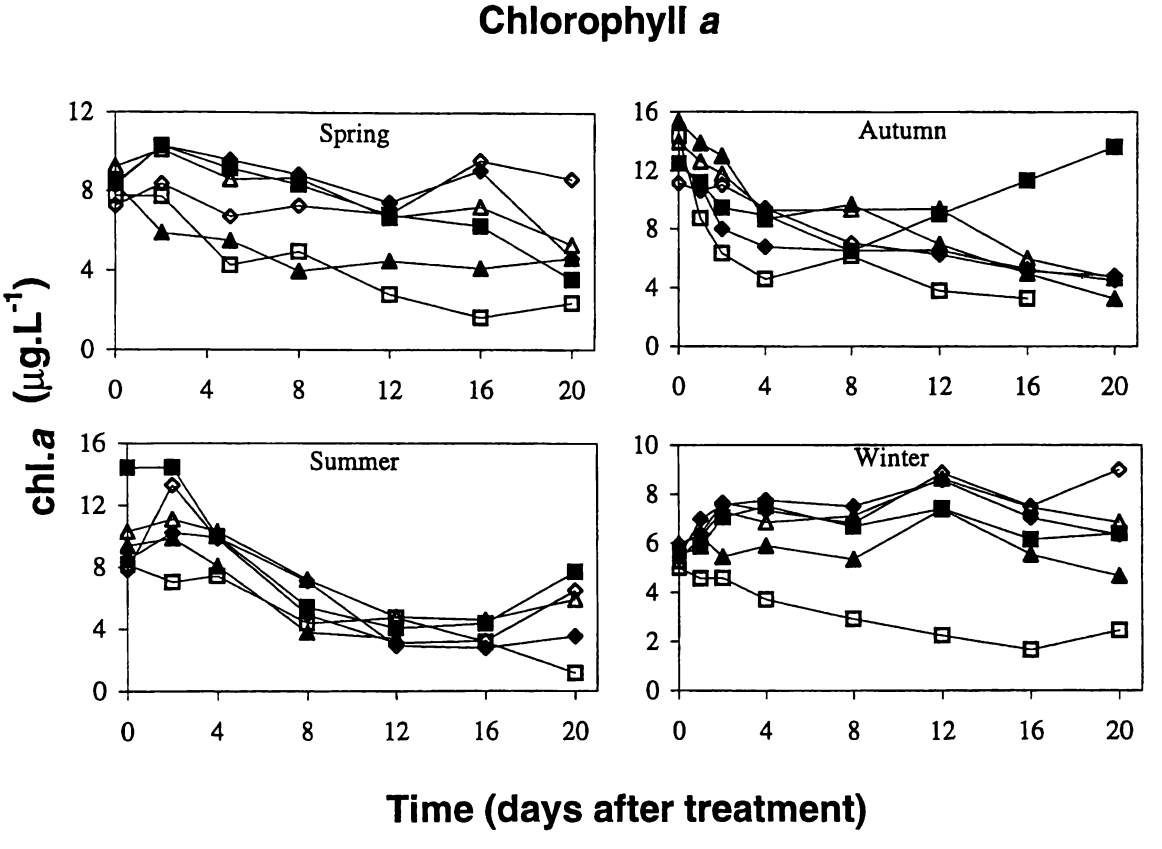


Figure 16. Changes in chlorophyll *a* concentration in seasonal experiments. See Figure 15 for explanation of data and symbols.

Phytoplankton

A pronounced treatment effect occurred in spring and to a lesser extent in winter, with an obvious reduction in phytoplankton in both the 54 and 121 $\mu g.L^{-1}$ PCP mesocosms after days two and four (Fig. 17). In summer, phytoplankton numbers remained stable before decreasing after day 12 in the 121 $\mu g.L^{-1}$ PCP treatment. A slight treatment effect in autumn was apparent in the highest concentration after day eight.

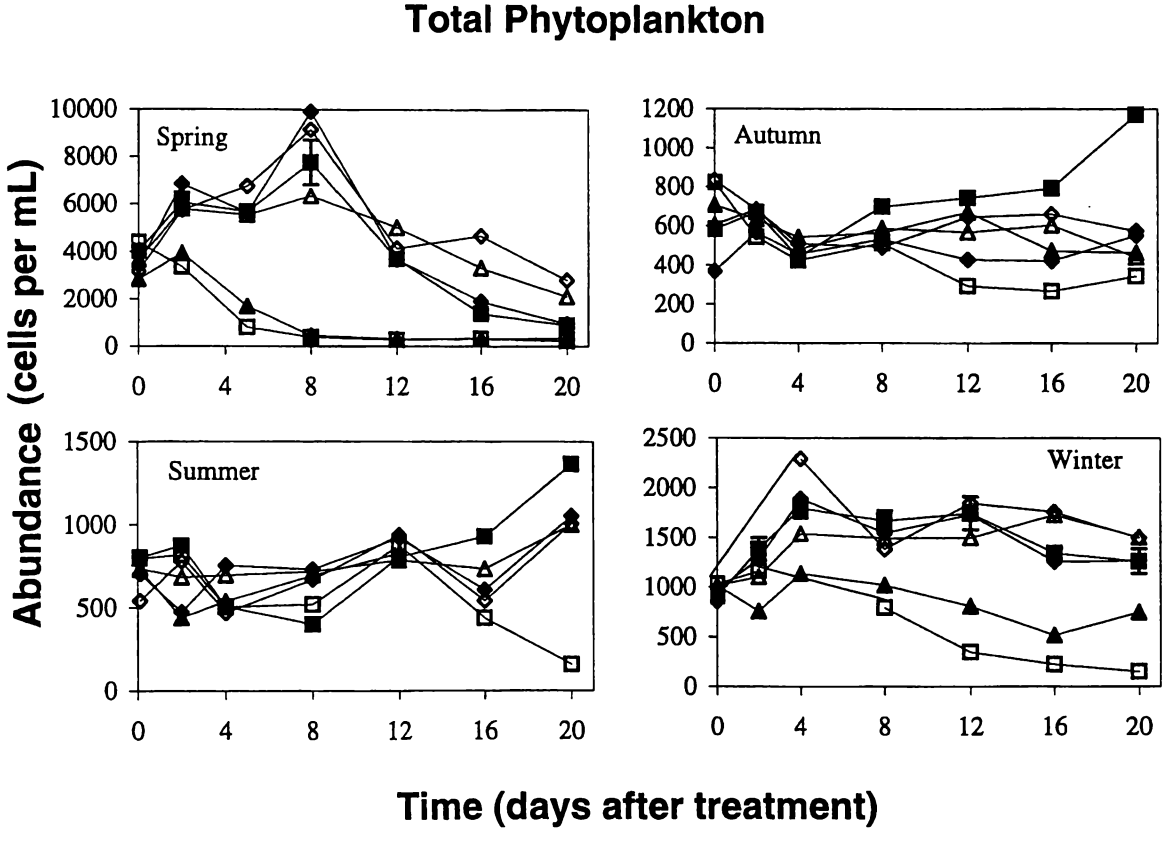


Figure 17. Changes in abundance of total phytoplankton in seasonal experiments. See Figure 15 for explanation of data and symbols.

Rotifers

Treatment effects were apparent in the 121 $\mu g.L^{-1}$ PCP mesocosms within two days in all experiments (Fig. 18). Numbers were also markedly reduced in the 54 $\mu g.L^{-1}$ PCP winter treatment. Overall declines in rotifers occurred during autumn regardless of treatment level.

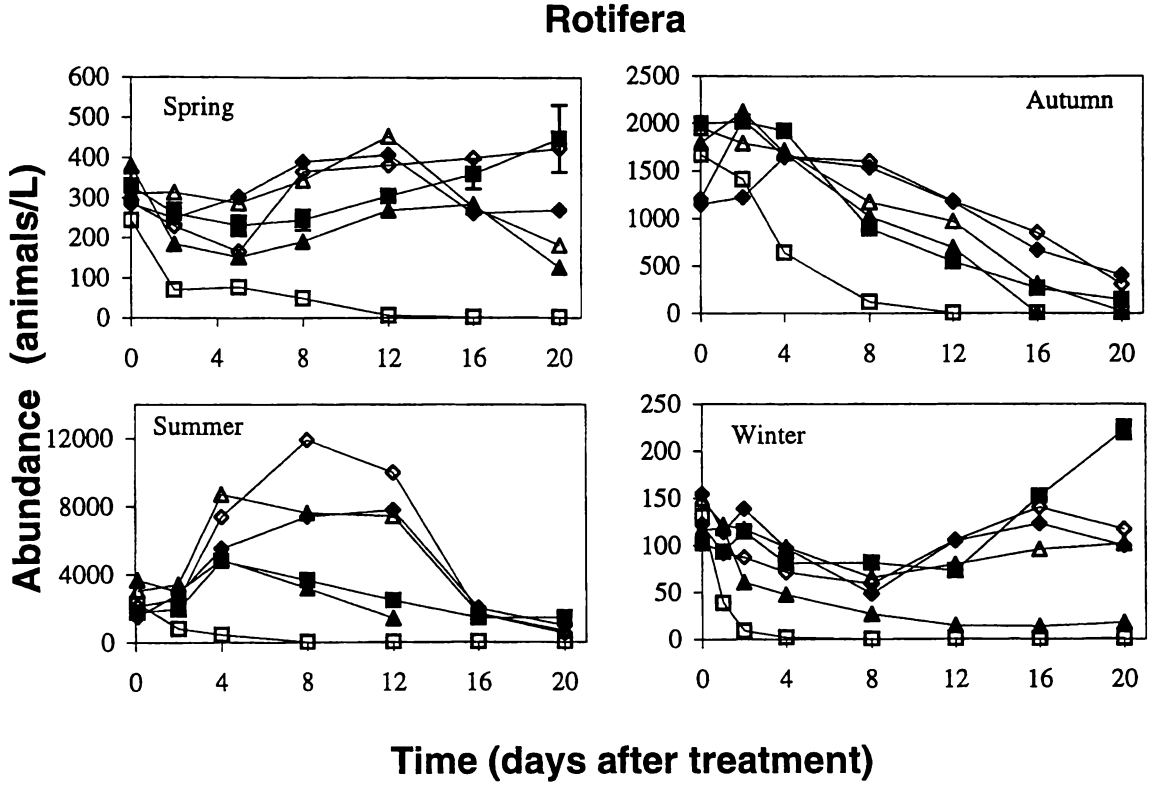


Figure 18. Changes in abundance of total Rotifera in seasonal experiments. See Figure 15 for explanation of data and symbols.

Crustacea

With the exception of summer, treatment effects on total Crustacea were apparent at 54 and 121 $\mu g.L^{-1}$ PCP (Fig. 19). In the 121 $\mu g.L^{-1}$ PCP treatments an effect was apparent within two days, but in the spring and autumn 54 $\mu g.L^{-1}$ PCP treatments the response was delayed and not as pronounced. In the highest winter treatments total Crustacea abundance began to increase again after day 12.

In summer, there was no obvious dose-response relationship and Crustacea abundance increased after day 16 in the 121 $\mu g.L^{-1}$ PCP treatment, this was due primarily to an increase in abundance of the cladoceran *Bosmina longirostris* (Appendix IV).

Copepod numbers decreased rapidly in the highest treatments (54 and 121 $\mu g.L^{-1}$ PCP) in winter, spring and autumn, whereas an obvious treatment effect was apparent only in the 121 $\mu g.L^{-1}$ PCP treatment in summer (Fig. 19). A delayed response was also apparent in the 36 $\mu g.L^{-1}$ autumn, winter and spring treatment. Variability between control mesocosms was greater in winter than spring.

EC50 values

EC50 values were estimated using total copepod % change in abundance relative to day 0 for those sample days on which abundance values spanned 50% (Fig. 20). Estimated EC50 values were highest in summer and lowest in winter and spring (Table 15). EC50 estimates did not change substantially after day four in autumn, winter and spring, or day eight in summer.

Table 15. Estimated EC50 values for total copepod % change in abundance.

	estimated EC50 values ($\mu g.L^{-1}$ PCP)					
	day 2	day 4	day 8	day 12	day 16	day 20
Spring	47	10[a]	9	-	4.3	7.5
Summer	90	85	55	52	36	-
Autumn	70	40	40	90	-	-
Winter	48	35	-	19[b]	26[b]	-

[a] sampled on day five due to bad weather on day four

[b] EC50 estimated as the highest concentration corresponding to 50% effect (see Fig. 20)

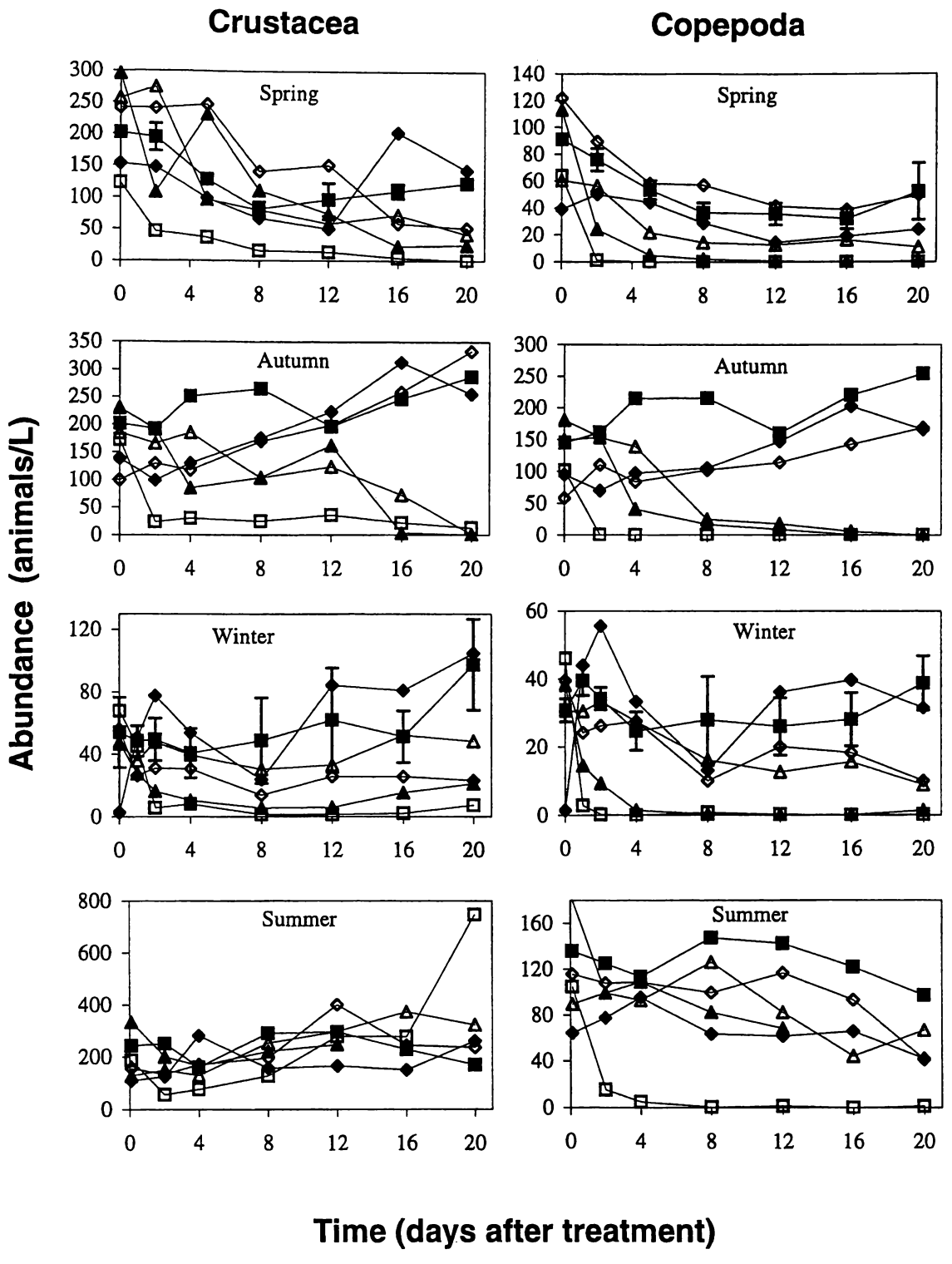


Figure 19. Changes in abundance of total Crustacea and Copepoda in seasonal experiments. See Figure 15 for explanation of data and symbols.

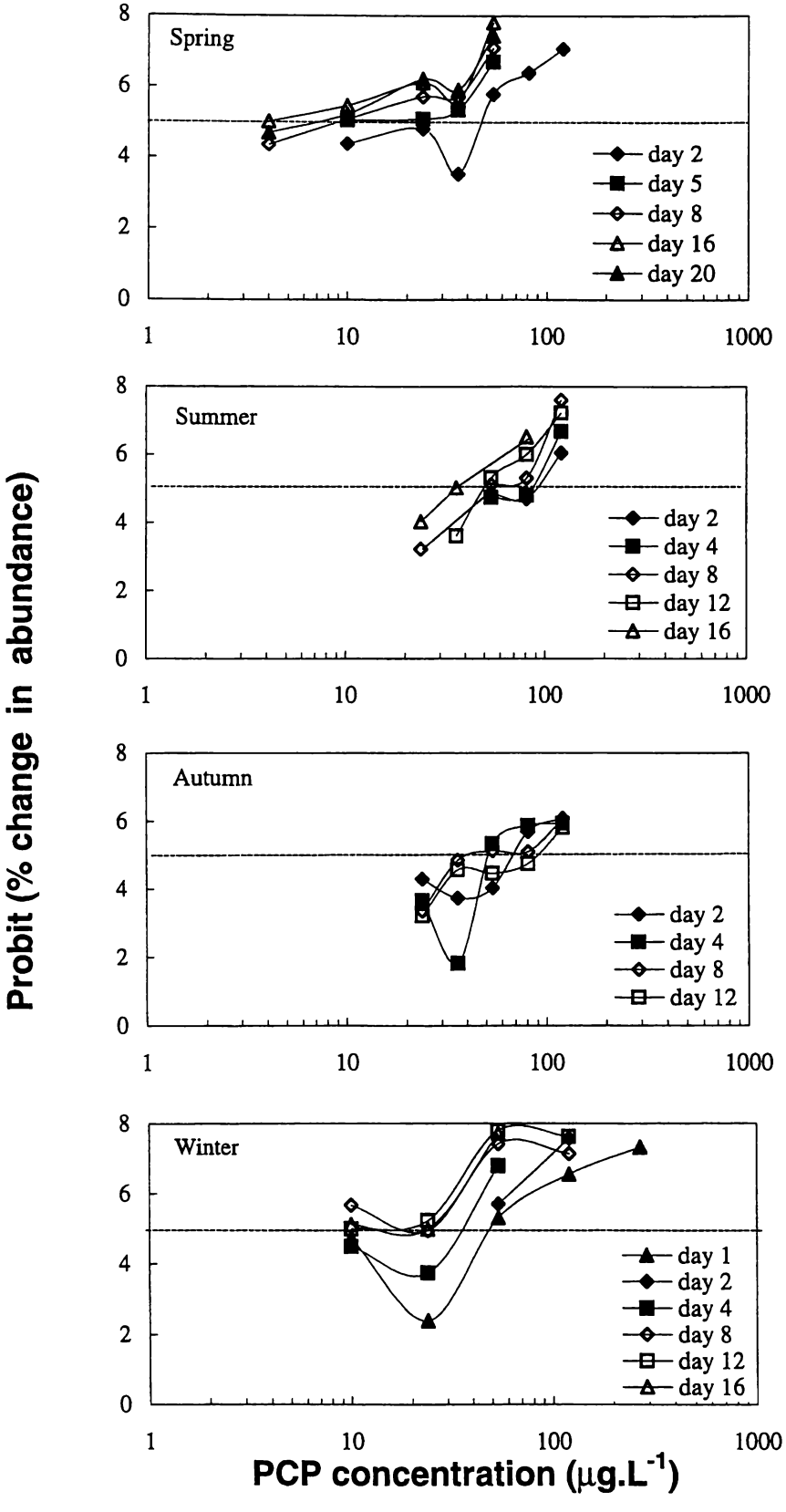


Figure 20. Probit plots for estimation of EC50 values for total copepod % change in abundance (compared to day 0) in seasonal mesocosm experiments on days when values spanned 50%.

Multivariate analyses

The RDA biplots (Figs. 21 to 24) summarise treatment and temporal effects on the planktonic community during the seasonal experiments. The goodness-of-fit of the axes constrained to the explanatory variables is indicated by the eigenvalues. The higher the eigenvalue, the more variation is explained by the axis (van Wijngaarden et al. 1995).

The eigenvalues for the first and second axes are presented in Table 16. Examination of the ordination plots suggests that, with the exception of winter, 'time' is the environmental variable associated with axis one, whereas 'treatment' is associated with axis two. In winter, 'time' is associated with the second axis indicating a weaker 'time' effect and a stronger treatment effect than in the other seasons.

Table 16. Eigenvalues for the first and second RDA axes for each seasonal experiment.

	axis 1	axis 2	% of total variance
Summer	38.5	13	51.5
Autumn	49.7	14.2	63.9
Winter	22.6	15.1	37.7
Spring	45.3	13.8	59.1

Monte Carlo permutation tests detected no significant difference in pre-treatment (day 0) community composition in the mesocosms in all four experiments. Significant ($p \leq 0.05$) treatment effects occurred on all post-treatment sampling days with the exception of day two ($p = 0.2$) in winter.

In the RDA biplots, samples with similar species composition lie close together while samples with dissimilar species composition lie far apart. The origin (centre) of a species ordination diagram represents the mean abundance of the individual species in all samples. Species closely associated with a site and present in higher than average abundance, occur on the same side of the origin, while sites on the opposite side of the origin from a species point contain less than average abundance of that species.

In Figs. 21 to 24, clustering of the mesocosms on day 0 (pre-treatment) indicates similar species composition at the start of each experiment, as confirmed by the permutation tests. With the exception of winter, shifts in the controls and lower PCP treated mesocosms from left to right indicate time vectors in this direction, while separation of the high and low PCP treated mesocosms between the upper and lower right quadrants indicates treatment effects. In winter, the time vector is along the second axis, and the treatments are distributed between the upper left and right quadrants. In all four experiments the control mesocosms remain closely associated for the duration suggesting relatively homogeneous community composition. The addition of a solvent control in summer (Fig. 21) and autumn (Fig. 22) identifies the possibility of a solvent effect as the solvent diverges slightly from the controls along with the lower treatments. This divergence appears to be a stimulatory rather than inhibitory effect as it occurs in the opposite direction to the higher treatments.

Shifts in community composition occurred with time and treatment. There was an overall reduction in the number of species in all mesocosms with time, primarily due to a decline in rotifer species diversity, although several species did increase in abundance. Increases in winter (Fig. 23) rotifers were due to *Trichocerca similis, Ascomorpha ovalis, Collotheca pelagica* and *Keratella cochlearis*, while *Polyarthra dolichoptera, C. pelagica* and *Synchaeta oblonga* increased in abundance in spring (Fig. 24). *Hexarthra mira* was the only rotifer to increase in abundance during summer, and in autumn all rotifers declined in abundance regardless of treatment level.

With the exception of *Bosmina longirostris*, which responded positively to PCP in summer, other zooplankton exhibited negative dose-response relationships, with declines in the higher treatments in all seasons. *Ceriodaphnia pulchella* increased in abundance in the controls and low treatments in summer and autumn, but was not present in winter and spring. With the exception of cyclopoid nauplii in autumn, copepod nauplii decreased in abundance with time, whereas copepodites and adults increased. Most phytoplankton

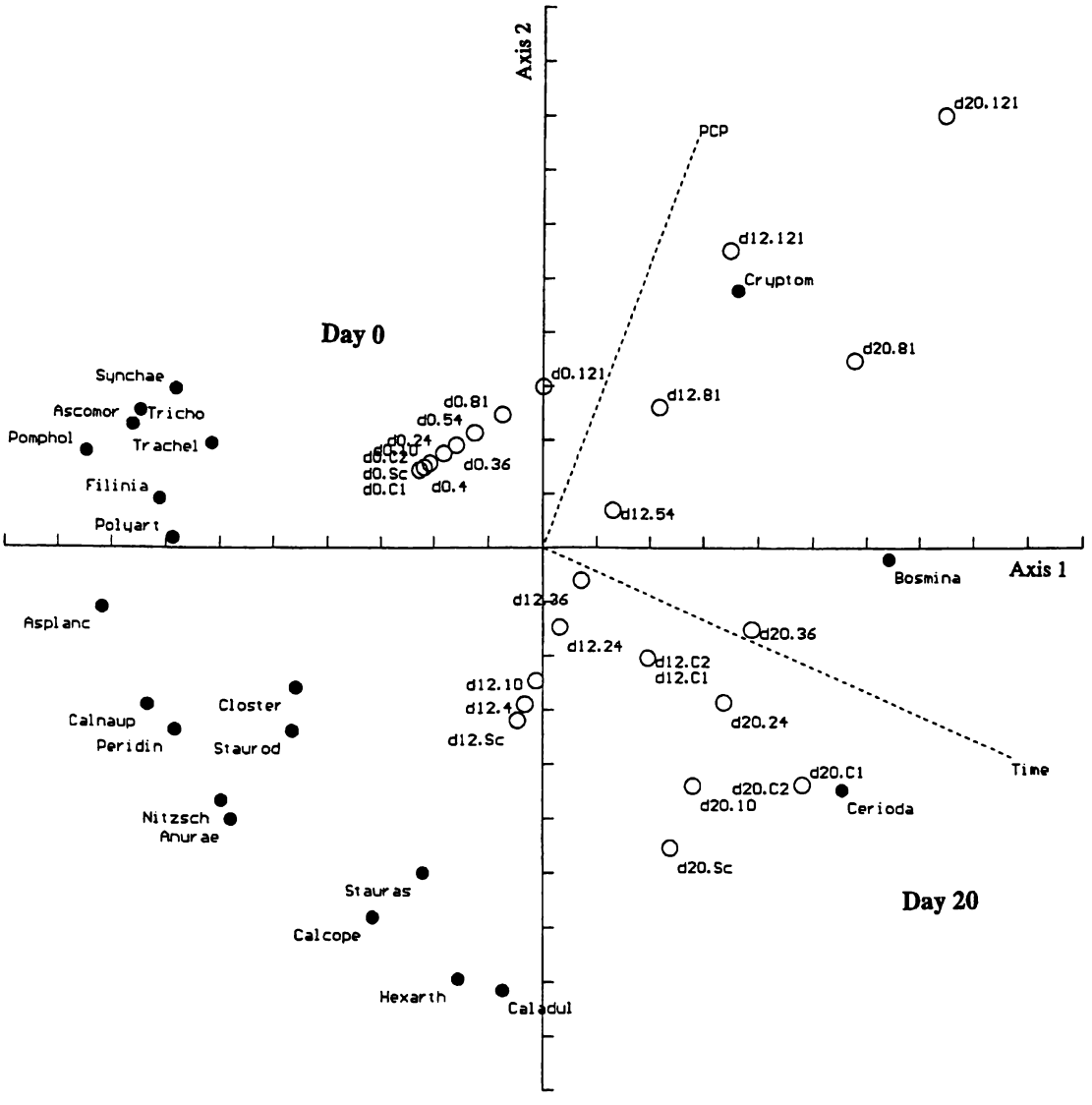
species generally decreased only in the highest treatments and the desmids, *Staurastrum* spp. and *Staurodesmus glaber* generally increased with time in the lower treatments and controls. *Cryptomonas* spp. were the only taxa to show a positive response to PCP in all four experiments, and are situated with the high treatments on the opposite side of the origin from the controls and other taxa. All other phytoplankton were either negatively affected by PCP, or occasionally, no dose-response relationships were observed.

Changes in abundance of zooplankton and phytoplankton taxa are presented in Appendices IV to VII.

Although somewhat subjective, community-level treatment effects can be distinguished from time and solvent effects at concentrations of 24 $\mu g.L^{-1}$ PCP in summer, and 36 $\mu g.L^{-1}$ PCP in spring and autumn in the RDA plots. Because of the extended concentration range in the winter experiment, fewer low concentrations meant that a treatment effect could not be identified below 54 $\mu g.L^{-1}$ PCP.

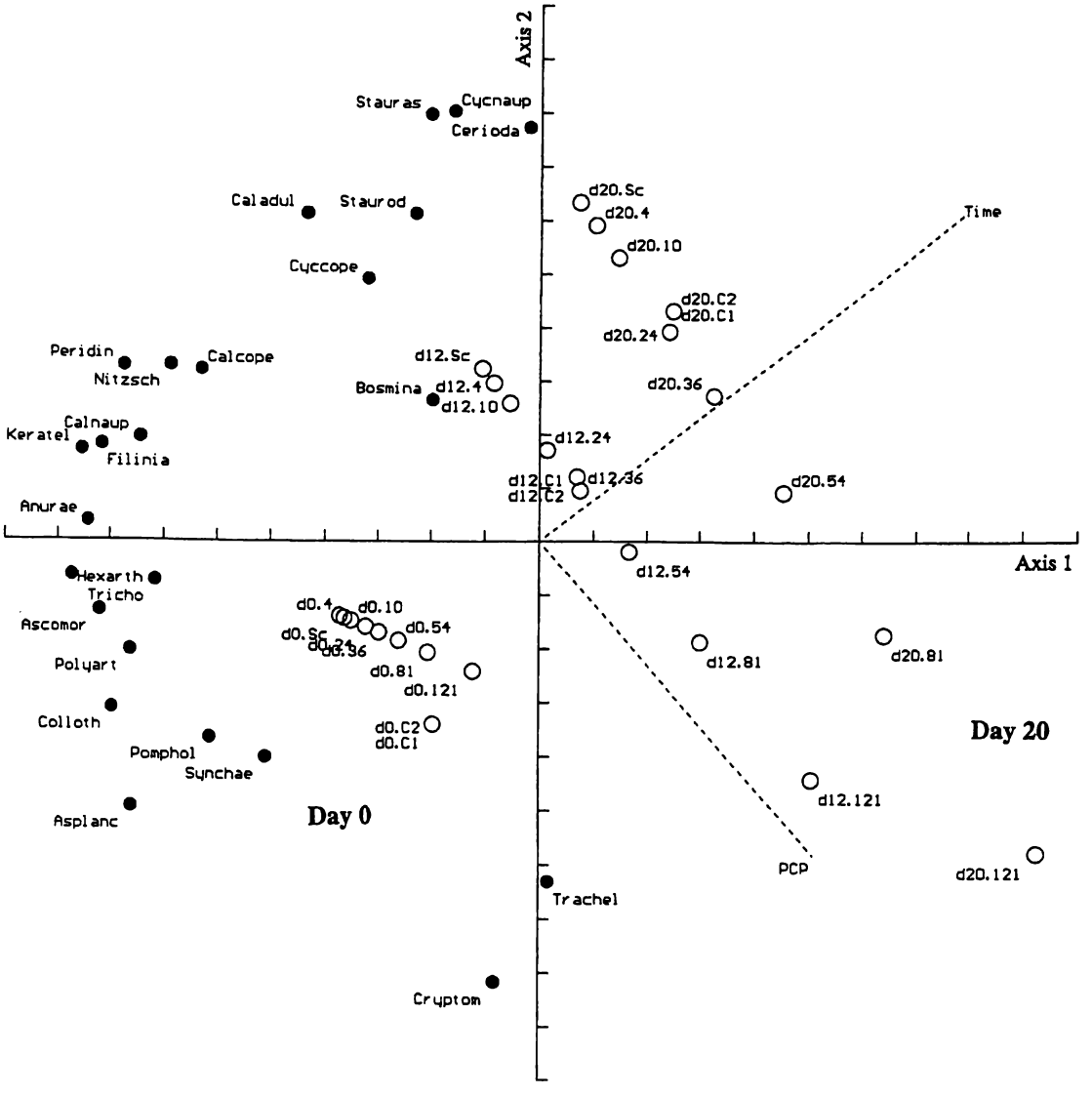
Following page:

Figure 21. Summer RDA biplot indicating effects of single PCP applications on mesocosm planktonic community structure. Species are shown as closed circles and sites as open circles. Site codes indicate sampling day followed by PCP concentration ($\mu g.L^{-1}$). Only site points for days 0, 12 and 20 are shown although all days were included in the analysis. Site points for days 2, 4, 8 and 16 are positioned intermediate to those shown. Only those species well explained by the ordination are shown, species in the centre of the diagram have been left out. RDA plots including site points for days 2, 4, 8, and 16 are shown in Appendix VIII.



Legend

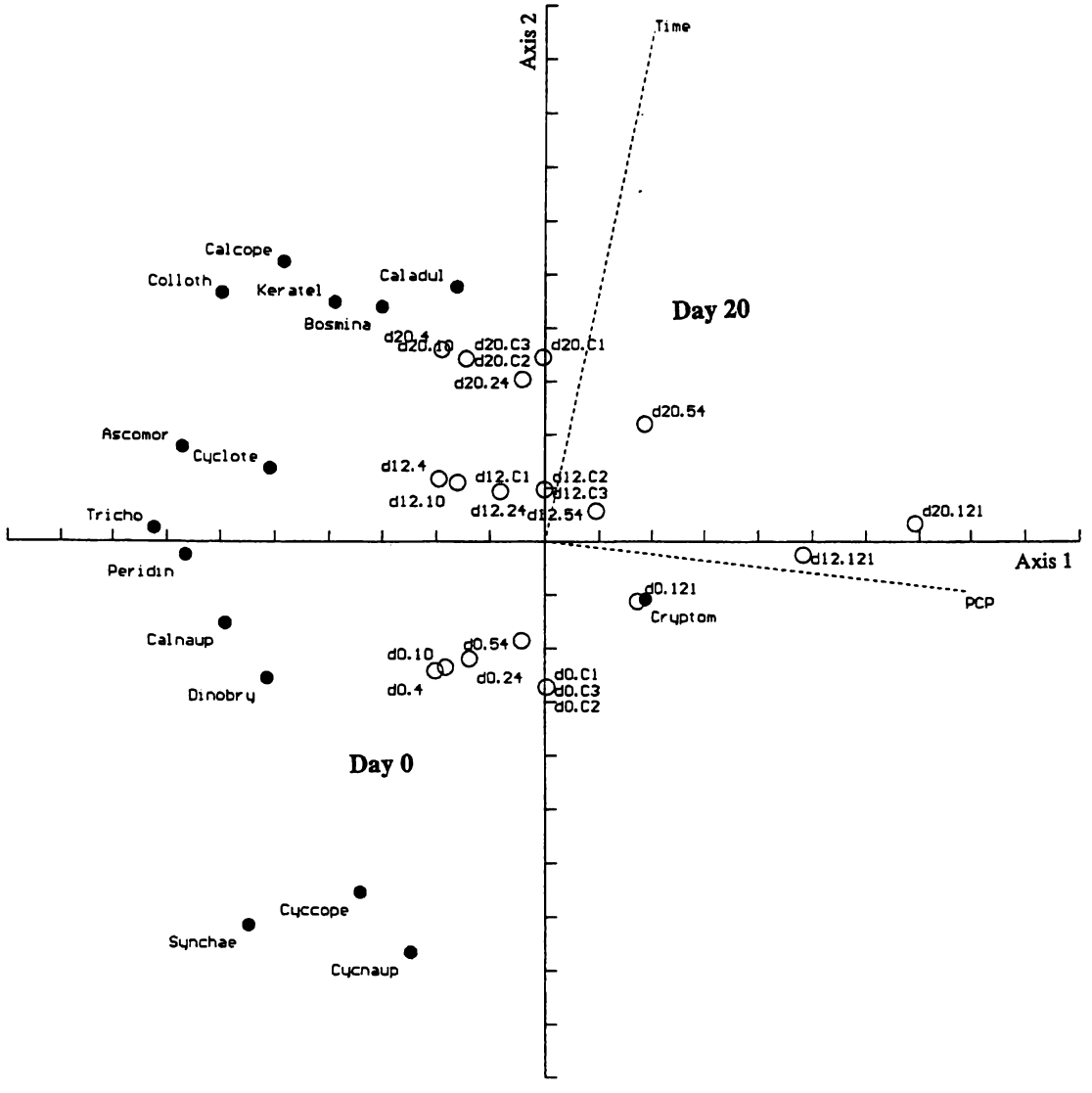
Crustacea	**Rotifers**	**Phytoplankton**
Calnaup = *Calamoecia* nauplii	Synchae = *Synchaeta oblonga*	Trachel = *Trachelomonas volvocina*
Calcope = *Calamoecia* copepodite	Tricho = *Trichocerca similis*	Stauras = *Staurastrum leptocladum* var. *insigne*
Caladul = *Calamoecia* adult	Ascomor = *Ascomorpha ovalis*	Staurod = *Staurodesmus glaber* var. *limnophilus*
Bosmina = *Bosmina longirostris*	Pomphol = *Pompholyx complanata*	Nitzsch = *Nitzschia acicularis*
Cerioda = *Ceriodaphnia pulchella*	Filinia = *Filinia longiseta*, *Filinia novaezealandiae*	Closter = *Closterium acutum* var. *variabile*
	Polyart = *Polyarthra dolichoptera*	Cryptom = *Cryptomonas erosa* *Cryptomonas ovata*
	Asplan = *Asplanchna priodonta*	Peridin = *Peridinium* spp.
	Anurae = *Anuraeopsis fissa*	
	Hexarth = *Hexarthra mira*	



Legend

Crustacea	**Rotifers**	**Phytoplankton**
Calnaup = *Calamoecia* nauplii	Synchae = *Synchaeta pectinata*	Trachel = *Trachelomonas volvocina*
Calcope = *Calamoecia* copepodite	Tricho = *Trichocerca similis*	Peridin = *Peridinium* spp.
Caladul = *Calamoecia* adult	Ascomor = *Ascomorpha ovalis*	Stauras = *Staurastrum* spp.
Bosmina = *Bosmina longirostris*	Pomphol = *Pompholyx complanata*	Nitzsch = *Nitzschia acicularis*
Cerioda = *Ceriodaphnia pulchella*	Hexarth = *Hexarthra mira*	Staurod = *Staurodesmus glaber* var. *limnophilus*
Cycnaup = Cyclopoid nauplii	Polyart = *Polyarthra dolichoptera*	Cryptom = *Cryptomonas ovata*
Cyccope = Cyclopoid copepodite	Asplan = *Asplanchna priodonta*	
	Anurae = *Anuraeopsis fissa*	
	Kerata = *Keratella cochlearis / tecta*	
	Colloth = *Collotheca ?pelagica*	

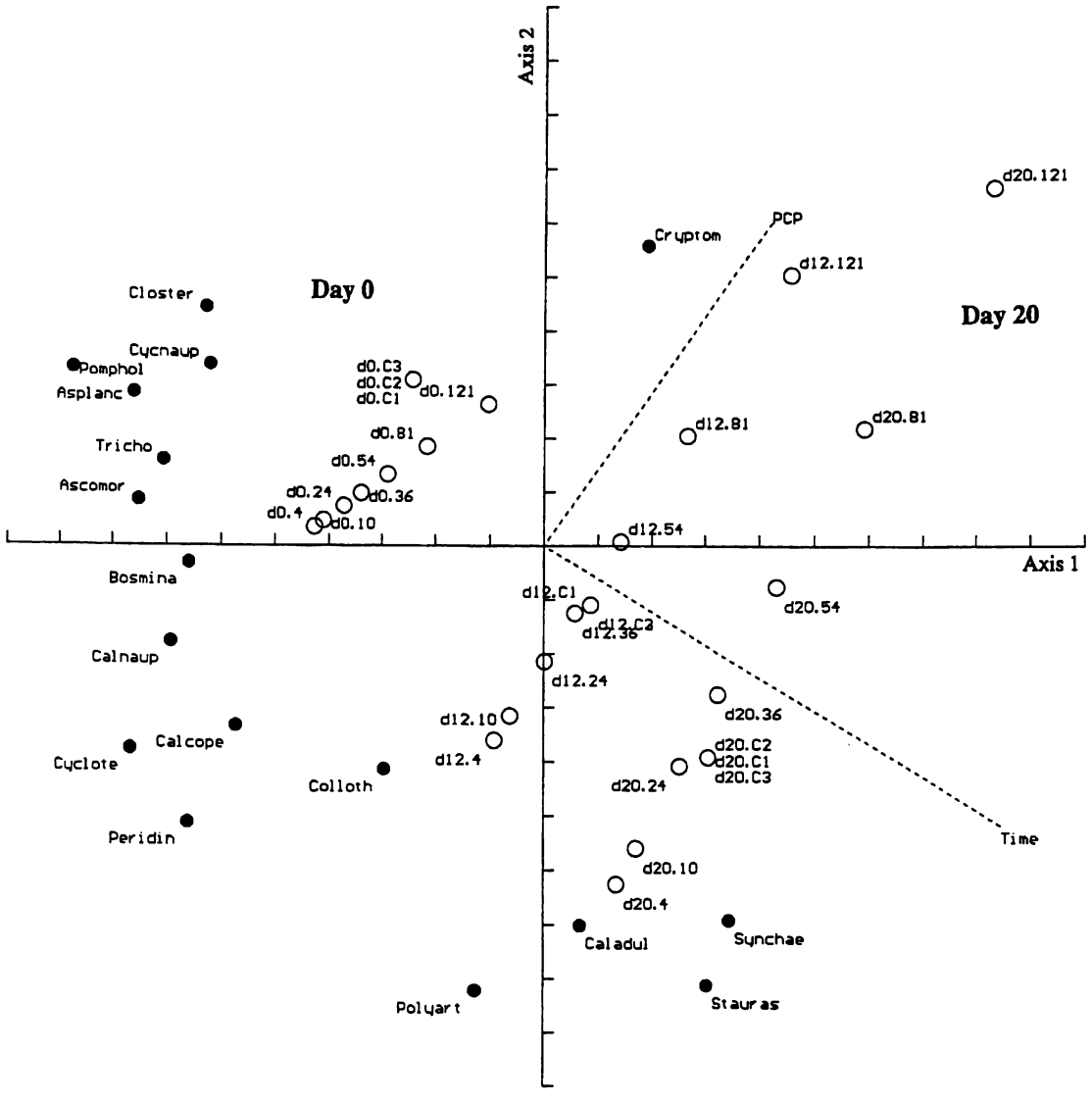
Figure 22. Autumn RDA biplot. See Figure 21 for explanation of data and symbols.



Legend

Crustacea	Rotifers	Phytoplankton
Calnaup = *Calamoecia* nauplii	Synchae = *Synchaeta pectinata*	Cryptom = *Cryptomonas erosa*
Calcope = *Calamoecia* copepodite	Colloth = *Collotheca ?pelagica*	Dinobry = *Dinobryon cylindricum*
Caladul = *Calamoecia* adult	Tricho = *Trichocerca similis*	Peridin = *Peridinium* spp.
Bosmina = *Bosmina longirostris*	Ascomor = *Ascomorpha ovalis*	Cyclote = *Cyclotella stelligera*
Cyccope = Cyclopoid copepodite	Keratel = *Keratella cochlearis*	
Cycnaup = Cyclopoid nauplii		

Figure 23. Winter RDA biplot. See Figure 21 for explanation of data and symbols.



Legend

Crustacea	Rotifers	Phytoplankton
Calnaup = *Calamoecia* nauplii	Synchae = *Synchaeta oblonga*	Peridin = *Peridinium* spp.
Calcope = *Calamoecia* copepodite	Tricho = *Trichocerca similis*	Cyclote = *Cyclotella stelligera*
Caladul = *Calamoecia* adult	Ascomor = *Ascomorpha ovalis*	Stauras = *Staurastrum gracile*
Bosmina = *Bosmina longirostris*	Pomphol = *Pompholyx complanata*	Cryptom = *Cryptomonas erosa*
Cycnaup = Cyclopoid nauplii	Colloth = *Collotheca ?pelagica*	Closter = *Closterium acutum* var. *linea*
	Polyart = *Polyarthra dolichoptera*	
	Asplan = *Asplanchna priodonta*	

Figure 24. Spring RDA biplot. See Figure 21 for explanation of data and symbols.

Discussion

Experimental design

It is inevitable that the isolation of components of an ecosystem will lead to changes in community composition as species respond to the changed abiotic environment, organic regimes and changing competition (Moverley et al. 1995). Such changes are an acknowledged feature of mesocosm studies, however the emphasis of ecotoxicological studies is not on differences between the enclosed community and that from which it was derived, but on treatment related differences between enclosures. Response to enclosure in the present study varied depending on species and season. Rotifers appeared to be most affected with overall declines in abundance observed for several species, especially during autumn.

In addition to abundance changes following enclosure, species distribution may also alter in response to changed circulation patterns and increased edge zones. Stephenson et al. (1984) investigated plankton spatial distribution in different sized enclosures and found that while the largest enclosures possessed distinctive edge zones with respect to zooplankton density, there were no distinct zonation patterns in the smallest enclosures (20 m^3). They attributed the lack of zonation in the smaller systems to the extension of the "edge zone" across the entire mesocosm. As the mesocosms in the present study were considerably smaller than those used by Stephenson et al. (1984), plankton distribution within the mesocosms was assumed to be homogeneous. The possibility of an edge effect was not disregarded however, so two samples were taken from each mesocosm (from the edge and centre) and pooled to ensure that a representative sample was obtained.

In the present study the use of an unreplicated experimental design employing seven treatment levels enhanced the ability to identify inter-season differences in community sensitivity. Ecotoxicological investigations usually employ replicated mesocosms to demonstrate that observed changes are caused by treatments and not natural variability.

However, if starting conditions are similar and abiotic conditions are maintained throughout the test, the number of replicates can be reduced (Heimbach 1994). In the present study, abiotic conditions in each experiment were similar between mesocosms, with the exception of dissolved oxygen, which displayed solvent and treatment related decreases. Community composition between the pretreatment mesocosms was not significantly different, and there was little variation between replicated controls throughout each experiment. Therefore, observed differences between the treated mesocosms were assumed to have been caused by PCP rather than natural variability.

Seasonal differences

Seasonality was clearly important in modifying PCP toxicity, with increased sensitivity apparent in winter and spring. With a few exceptions, dose-related trends were similar, and rotifers and phytoplankton were the most resilient of the taxonomic groups.

Of the two limnetic cladocerans in Lake Rotomanuka, *B. longirostris* is dominant with population maxima in spring and summer (Donald 1990), whereas *C. pulchella* is present only in low numbers. *Bosmina longirostris* exhibited a negative response to PCP in all but the summer experiment (Appendices IV to VII), during which, numbers increased dramatically after day 16 in the highest treatment (Appendix IV). Although present in considerably lower numbers, *C. pulchella* also began to increase after day 16 in the highest summer treatment (Appendix IV). *Ceriodaphnia pulchella* was also present in autumn and began to increase after day 12 in the highest treatment, whereas *B. longirostris* did not (Appendix V). Therefore, it appears that *B. longirostris* exhibits differential sensitivity depending on season, whereas *C. pulchella* did not, although it was only present in two experiments.

Copepods often dominate zooplankton communities numerically in New Zealand (Burns 1991). The calanoid copepod *C. lucasi* is found throughout the year in Lake Rotomanuka (Donald 1990), and was the dominant copepod in the mesocosms although densities were

lowest in winter. Marked decreases in copepods generally occurred following exposure to PCP concentrations greater than 36 $\mu g.L^{-1}$, with increased sensitivity in spring and winter, in agreement with laboratory tests using seasonally collected copepods (Chapter 2).

Rotifer densities were also considerably lower in winter and sensitivity was increased relative to the other seasons. With the exception of the highest concentration, treatment effects were not as clearly defined in summer and autumn due to overall declines in rotifers unrelated to PCP. Phytoplankton exhibited the most pronounced treatment effect during spring, although, following initial increases, density declined in all mesocosms. Treatment effects on phytoplankton were less pronounced in the other seasons, especially summer and autumn.

The seasonal variations observed in this study may be due to changes in nutritional status and metabolic rate, temperature or population trends. It has been suggested that planktonic communities in New Zealand may be food limited (Chapman 1973; Green 1976), which might explain the reduced tolerance of zooplankton to PCP in winter.

SAM are developed to simulate an early spring community with ample nutrients for algal growth, low algal abundance, and low grazing pressures in the initial stages (Taub 1997), followed by population growth and increasing grazing pressure. Thus SAM could be considered as analogous to spring communities undergoing rapid population increases and have shown increased sensitivity to toxicants when applied at an early developmental stage. Swartzman et al. (1990) and Taub et al. (1991) found that SAM were more responsive to copper added on day seven than on later days. Copper treatment on day seven eliminated *Daphnia* and delayed subsequent phytoplankton and zooplankton blooms. Little effect was observed following copper addition on day 14, and marked reductions occurred following treatment on day 21 but did not lead to total *Daphnia* elimination.

Temperature increases in spring could be expected to increase metabolic rates, and growth and production rates might also increase, although as mentioned previously, abundance

changes in New Zealand lakes do not conform to Northen Hemisphere models on seasonal cycles. It is possible therefore, that increased sensitivity in spring might be related to increasing temperature and metabolic rates, in contrast to reduced nutritional status in winter.

Seasonal differences in sensitivity were also apparent in the multivariate analyses, although identification of treatment effects was somewhat subjective. The inability to identify a treatment effect below 54 $\mu g.L^{-1}$ PCP in the winter experiment illustrated the importance of the appropriate choice of concentrations in regression design experiments. Results of the RDA were slightly contradictory of the general trends observed in individual species abundance. In contrast to EC50 estimates for summer which indicate increased resilience to PCP, the RDA suggested greater sensitivity. Overall, the ordination diagrams were beneficial in that effects identified at the community level are more readily comparable between experiments as they are not dependent on comparisons of individual taxa, which may fluctuate seasonally or have disjunct distributions. Furthermore, population dynamics may obscure treatment responses at the species level due to changes in abundance associated with growth and reproduction.

Cryptomonads

Cryptomonas spp. were the only taxa to exhibit a positive response to PCP regardless of season. Cryptomonads are generally present in low numbers throughout the year in planktonic communities, and have been implicated as an ecologically significant internal stabilising component with characteristics such as intermittent numerical dominance and effective pulse timing (Stewart and Wetzel 1986). Cryptophytes have been shown to increase in abundance during periods of decomposition following population declines of previously dominant algal species. Blinn and Stewart (cited in Stewart and Wetzel 1986) observed *Cryptomonas* increases following herbicide-induced reductions of the dominant chlorophyte and bacillariophyte populations in experimental ponds. Schauerte et al. (1982)

reported increases in heterotrophic flagellates, *Euglena acus* and *Trachelomonas hispida*, in experimental ponds treated with 1 and 5 $mg.L^{-1}$ PCP, although this may have been due to reduced grazing pressure by *Daphnia*. Following an initial increase in the highest summer treatment in the present study, *Cryptomonas* numbers declined possibly due to an increase in *B. longirostris*, which may have grazed on them.

Dissolved oxygen

Reductions in dissolved oxygen occurred in all experiments in response to both the solvent and PCP. The solvent effect was most pronounced during autumn and summer although oxygen concentrations were further reduced in the highest PCP treatments, probably because of reduced photosynthetic oxygen production and increased decomposition. Solvent effects have also been observed in laboratory microcosms (Taub and Crow 1978; Taub 1984, 1989; Kersting and van Wijngaarden 1992; Kersting 1995), with changes in oxygen concentration and pH attributed to bacterial metabolism of the solvent and acidic degradation products. There was no obvious solvent effect in winter and oxygen concentrations remained above 77% saturation despite a slight reduction in oxygen in the highest treatment. Low winter temperatures reducing bacterial metabolism and decomposition rates probably accounted for this. The high oxygen concentrations observed during winter, in combination with similar or increased sensitivity compared to the other seasons, suggests that effects observed during the other experiments can still be attributed primarily to PCP.

In contrast to dissolved oxygen, a stimulatory effect on community composition was apparent in the solvent and lower PCP treatments. In the RDA diagrams the solvent control (if present), 4 and 10 $\mu g.L^{-1}$ PCP mesocosms diverged from the controls in the opposite direction to the higher concentrations. The stimulatory effect of the solvent may have been related to an increase in bacterial production, which may have mitigated treatment effects at the lower concentrations by providing an additional food source. The solvent also

substantially increased periphytic growth on the enclosure walls relative to the non-solvent controls, particularly during the summer experiment when warm temperatures provided optimal growing conditions, so that by day 20 the excessive growth clogged the plankton net when collecting zooplankton samples.

Multispecies studies with PCP

Comparison of this study with previous multispecies studies using PCP are complicated by differences in experimental design, concentration ranges and data analysis. Outdoor experimental streams were dosed continuously with PCP at three levels (48, 144 and 432 $\mu g.L^{-1}$) for 12 weeks to evaluate a site-specific water quality criterion (Hedtke and Arthur 1985; Zischke et al. 1985; Yount and Richter 1986). Significant PCP effects occurred on fish, periphyton and system metabolism at all three dosing levels. Experimental pond studies have used only one (Schauerte et al. 1982; Crossland and Wolfe 1985) or two dosing levels (Fiend et al. 1988). Schauerte et al. (1982) applied a single doses of 1 $mg.L^{-1}$ PCP to replicate compartments in an experimental pond and observed total population declines of *Daphnia pulex* and *Nitzschia acicularis* within three and eleven days respectively. Dissolved oxygen concentrations also decreased. Significant treatment effects on rotifers and cyclopoid copepods occurred only at the higher concentration of 0.3 $mg.L^{-1}$ PCP in a subdivided artificial pond dosed continuously for eight weeks by Fiend et al. (1988). There was no significant effect of PCP on zooplankton or any direct effect on fish survival or growth in three replicate ponds dosed continuously for 30 days with 62.5 $\mu g.L^{-1}$ PCP by Crossland and Wolfe (1985). However they reported possible long-term effects on the snail population (*Lymnaea stagnalis*), and indirect effects on fish were observed when the death and decay of filamentous algae depressed oxygen concentrations causing fish deaths.

The use of a regression design in the current study increased the ability to detect treatment effects; consequently sensitivity was increased considerably when compared to previous

studies. For example, reductions in copepod abundance and changes in community composition were identified at concentrations as low as 10 $\mu g.L^{-1}$, and between 24 and 36 $\mu g.L^{-1}$ PCP respectively. Of those studies described above, similar sensitivity was recorded in the experimental streams dosed with PCP. However, dosing was continuous and the authors were unable to determine whether differences between the control and low-dosed stream were related to PCP or inter-stream variation (Hedtke and Arthur 1985). Although dosing was not continuous in the present study, chemical analyses in spring would tend to suggest that PCP was relatively persistent in the mesocosms with a calculated half-life of 21 days (Chapter 3). Therefore exposure would have occurred over an extended period, providing conditions analogous to a continuous dosing regime.

Conclusions

The use of a regression design mesocosm experiment employing seven unreplicated concentrations, and the combination of univariate and multivariate analyses resulted in the detection of population and community-level effects at considerably lower PCP concentrations than have been reported previously. Changes in community composition were identified at concentrations as low as 24 and 36 $\mu g.L^{-1}$ PCP. Furthermore, similar community composition in the replicated control mesocosms demonstrated that the changes could be attributed to PCP rather than natural variability. Seasonal variations in sensitivity to PCP were observed in crustacean, rotifer and phytoplankton abundance, with increased sensitivity in winter and spring. Phytoplankton and rotifers were more tolerant than the crustaceans, with copepods showing least resilience to PCP.

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CHAPTER SEVEN

Discussion

Effects at different levels of biological organisation

Single species aquatic toxicity tests have been criticised as an unreliable method for predicting responses at higher levels of biological organisation. However, studies undertaken to determine the predictive abilities of tests at different levels of complexity provide conflicting evidence. Often the only information gained may be a confirmation of laboratory test data (Shaw and Kennedy 1996), or an indication that single species tests are over- rather than under-protective (Crane 1997). For example, Lewis (1986) concluded that laboratory toxicity data was generally conservative in nature when comparing the effects of surfactants on enclosed phytoplankton communities and standard algal toxicity tests. However, the degree of similarity was highly dependent on the laboratory test species and the chosen endpoint. Similarly, Pratt et al. (1989) found that microcosm tests were generally less sensitive in detecting effluent toxicity than were acute and chronic single species tests using fathead minnows and daphnids. Pond derived microcosms and artificial ponds treated with oil displayed similar changes in community metabolism and zooplankton populations, and results were comparable to the 28-day LOEC for *D. magna* offspring production (Giddings and Franco 1985). Safe exposure levels determined from the community-level experiments were also accurately predicted from *D. magna* 48 h LC50s when a conservative application factor of 0.03 was applied. Conversely, Lampert et al. (1989) found that enclosure experiments with natural communities were far more sensitive to atrazine than were *Daphnia* in acute and sublethal tests.

Comparative studies such as those just described have not been undertaken previously in New Zealand so nothing is known about the predictive abilities of laboratory tests using indigenous species.

In the current study the responses of planktonic organisms exposed to PCP in toxicity tests at different levels of biological organisation were compared to determine the predictive abilities of each test. With exceptions, copepod LC50 values from laboratory tests were comparable to acute responses in the microcosms and mesocosms (Fig. 24). Copepods and rotifers were reduced or nearly eliminated in the highest microcosm and mesocosm treatments within two to four days after PCP addition, with a slight delay in response in the microcosms. Similarly, effective concentrations (EC50s) derived from changes in total copepod abundance (predominantly *C. lucasi*) in the microcosms and mesocosms were within the range of calculated chronic values from the sublethal copepod laboratory tests. Community-level treatment effects in the microcosms and mesocosms were identified at concentrations of 24 to 36 $\mu g.L^{-1}$ PCP, slightly higher than the chronic value calculated for *C. lucasi* but lower than those of *B. delicata* and *M. leuckarti*. However, the high control mortality in the *Calamoecia* naupliar tests created a potential confounding factor and would normally invalidate them although, as suggested by Green et al. (1996), high levels of natural mortality would tend to suggest the likelihood of increased sensitivity to toxicants. Therefore, in this instance, community-level responses could have been predicted with reasonable accuracy by the sublethal tests undertaken in the laboratory using *C. lucasi*.

Furthermore, laboratory tests using seasonally collected *C. lucasi* provided a reasonably accurate representation of seasonal variation in PCP toxicity to the planktonic community in the mesocosms, with good correspondence between laboratory and mesocosm values, especially in summer and autumn (Table 17).

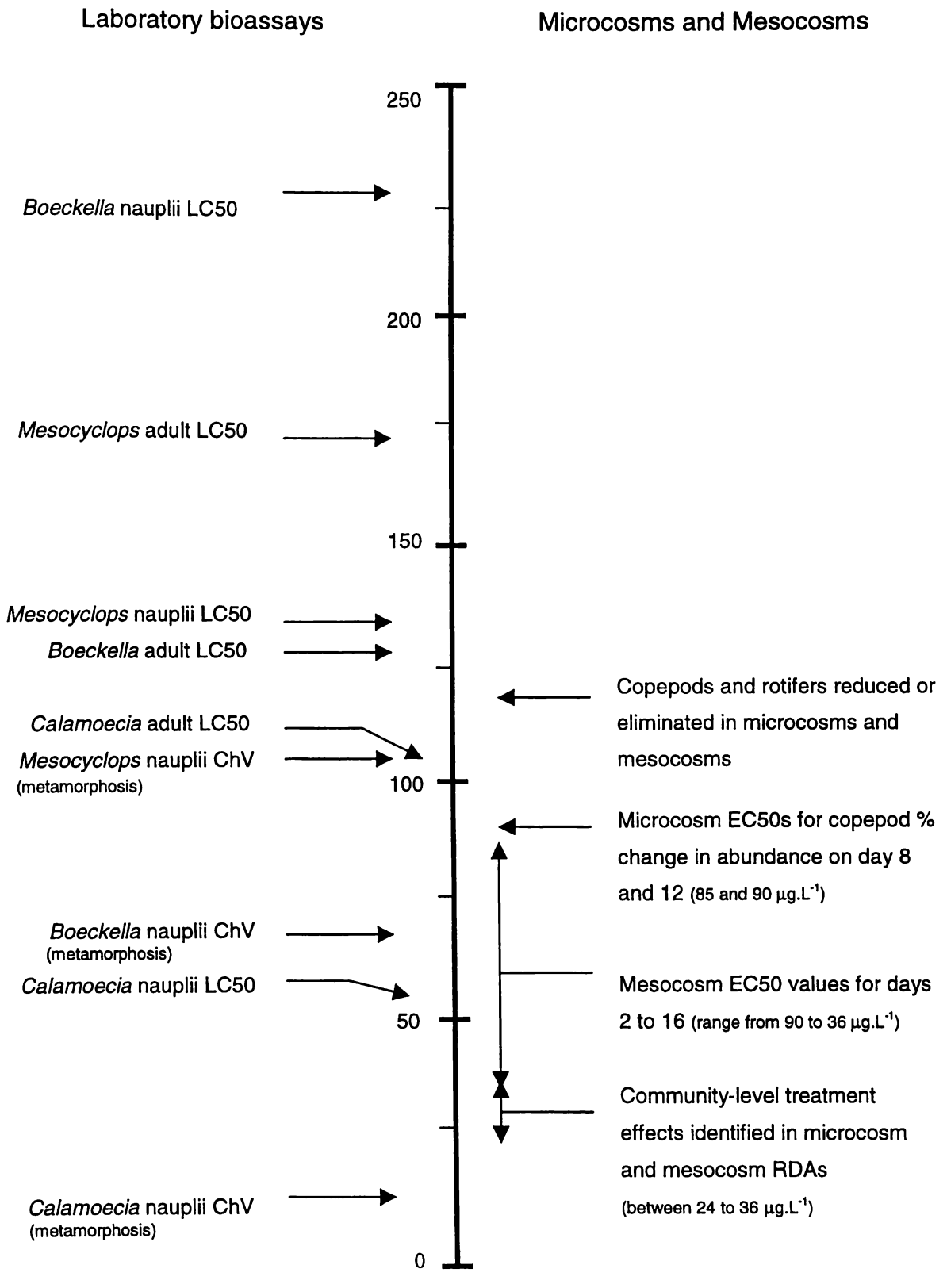

Figure 24. Comparison of microcosm and mesocosm responses with results from acute and sublethal laboratory copepod bioassays with PCP. Mesocosm responses are from the summer experiment. Central axis depicts PCP concentration ($\mu g.L^{-1}$). Test temperatures were 22 ±1°C (copepod bioassays) and ~23 ± 1°C (microcosms and mesocosms).

Table 17. Comparison of laboratory LC50 values ($\mu g.L^{-1}$ PCP) using seasonally collected *C. lucasi* and mesocosm copepod EC50 values ($\mu g.L^{-1}$ PCP).

	laboratory 48 h LC50 values	mesocosm 48 h EC50 values
Summer	106	90
Autumn	82	70
Winter	70	48
Spring	71	47

Selection of species for use in laboratory bioassays

There is a continuing need to identify ecologically relevant species that may have potential for routine use in laboratory bioassays, especially in countries where ecotoxicology is still a developing discipline. Furthermore, aquatic communities may often differ from those of North America and Europe where many of the current test protocols have been developed. The emphasis on developing standardised toxicity tests and the concomitant restrictions this places on the number of species used routinely reduces the value of many tests, with the already difficult task of field validation complicated further when the test species is not present in the receiving system. The importance of using the same species in laboratory tests as are found in the field has been emphasised regularly (e.g. Rand and Petrocelli 1985; Cairns 1993; Chapman 1995; Koivisto 1995).

Many internationally recognised standardised tests are of little relevance in New Zealand where aquatic communities are characterised by high levels of endemism and the absence or low diversity of many taxonomic groups found elsewhere (Forsyth and Lewis 1987). Cladocerans are the most commonly used toxicity test species worldwide (Persoone and Janssen 1998), whereas copepods often dominate planktonic communities numerically in New Zealand (Burns 1991). Unlike marine and estuarine copepods, which are used regularly in toxicity tests because of, among other things, their ecological importance (Widdows 1998), freshwater copepods have been largely ignored. The results from this study have shown that copepods were the most sensitive component of the planktonic

community, and with the exception of *C. lucasi* they adapted well to laboratory culture conditions. The variation in sensitivity between the three species also illustrates the necessity of testing a range of species present in the community under investigation to increase the accuracy of environmental effect predictions. Moreover, as noted by Slooff et al. (1986), LOECs tend to decrease with an increasing amount of toxicity data because of the considerable variation in sensitivity to toxicants that exists between species (Blanck 1984; Mayer and Ellersieck 1988).

Identification of effects in microcosms and mesocosms

The extensive use of hypothesis testing and the restrictions this places on experimental design is being increasingly questioned. In particular the use of NOECs and LOECs as the basis for estimating no-effect levels in single species (Hoekstra and van Ewijk 1993; Chapman and Caldwell 1996) and multispecies (Landis et al. 1997) toxicity tests has been criticised. Instead, it has been proposed that point-estimates (i.e. effective concentrations) and a regression-based approach (Moore and Caux 1997) may be better applied for estimating low toxic effects in single species tests. Similarly, the application of linear and non-linear regression techniques to determine point estimates from multispecies data is becoming more widespread.

Several mesocosm studies have used regression techniques to assess the effects of toxicants on planktonic communities (see Liber et al. 1992; Giddings et al. 1994; Thompson et al. 1994; Faber et al. 1998a,b). Liber et al. (1992) selected the linear portion of the dose response curve and using inverse regression calculated NEC values. Similarly, Giddings et al. (1994) applied linear regression to the portion of the exposure gradient where the response was strongest to identify a response threshold (EC0), although in several instances the regression line did not accurately reflect the dose-response relationship and was positioned inappropriately. Thompson et al. (1994) and Faber et al.

(1998a,b) applied both linear and non-linear regression analysis to abundance data to interpolate EC20 and EC50 values.

In the current study a line was drawn between the data points depicting copepod change in abundance and EC50 values were estimated where the line bisected the 50% response. Stimulation in the lower treatments meant the data violated the assumptions of linearity so linear regression was inappropriate. Omission of these data would have improved linearity and has been recommended (Streibig 1980; cited in Brain and Cousens 1989). It was also decided that the calculation of NEC values using inverse regression according to the method of Liber et al. (1992) would not contribute significantly to the study as EC50 and NEC estimates obtained by Liber et al. (1992) were very similar.

A move away from hypothesis testing and increasing use of point estimates based on dose response relationships may improve the predictive abilities of multispecies tests. However, given the complex nature of multispecies data it seems obvious that univariate techniques are still fundamentally inappropriate and incapable of identifying the majority of community-level treatment effects. Instead, multivariate analyses help maximise the ecological information from microcosm and mesocosm studies as they analyse data sets in their entirety. The resultant ordination plots depict the integrated response of the community to a toxicant, enhancing the interpretation of differences between treatments.

In this study, interpretation of treatment effects from the multivariate analyses were somewhat subjective, but as noted by Giddings et al. (1994), conservative interpretation can support the presumption that effects occurred even though they may not be demonstrated statistically.

PCP concentrations and rates of decay in the mesocosms

The mesocosm experiments conducted during this study were undertaken primarily to measure the ecological effects of PCP on the planktonic community. However, whenever

possible, it is advantageous to measure concentrations of the test chemical in the water (and any other system compartments) to confirm test concentrations, quantify the chemical exposure associated with the observed ecological responses, and to investigate rates of decay (Giddings 1994).

Unfortunately, logistical and financial constraints and initial problems with the analyses meant it was only possible to measure PCP concentrations in the spring mesocosm experiment. The results of the chemical analyses indicated that PCP concentrations were close to predicted nominal concentrations and that the rate of decay during the spring experiment was relatively slow, with a calculated half-life of 21 days. This was within the range found during other studies but was higher than expected. The slow decay rate was probably due to a reduction in light intensity in the mesocosms, which can be attributed to the mesocosm design with the high collar, perspex covers and large surface to volume ratio. PCP concentrations and decay rates in the other seasonal mesocosm experiments are unknown but it was assumed they were similar to those in the spring experiment.

Actual PCP concentrations were not determined for the microcosm experiment and it is not known whether decay rates were similar to those in the mesocosms. The similarity of responses to PCP in the microcosms and mesocosms would tend to suggest that concentrations were probably similar.

Mesocosm experiments - problems encountered

Initially, the mesocosm experiments were planned for Lake Rotorua, a larger lake (surface area ~80 km^2) in the central North Island. However, weather conditions on the lake were not conducive to the deployment and long-term maintenance of mesocosms in the lake, and after several disastrous attempts it was decided to relocate to a smaller more sheltered lake. Although the weather conditions on Lake Rotomanuka did not present major problems, the duck-shooting season (May to July) did. Following the premature and unforseen

termination of the first experiment (autumn) by duck shooters, the winter and repeat autumn experiments had to be planned to avoid the shooting season.

The use of a solvent carrier also presented problems, although reductions in dissolved oxygen resulting from the ethanol addition did not appear to have a negative impact on community composition. The solvent also increased the amount of periphytic growth on the enclosure walls, particularly in the summer experiment when temperatures were higher.

Finally, when undertaking mesocosm experiments involving the addition of toxicants, the often protracted resource consent application process in New Zealand needs to be taken into consideration and time allocated during the initial planning stages of a scientific investigation to obtain discharge consents (a regulatory requirement of the RMA, 1991).

Conclusions

To date aquatic ecotoxicity studies in New Zealand have focussed on identifying native species for use in single species laboratory tests (see Hickey 1995 and papers therein). Microcosms and *in situ* mesocosms have not been used previously in ecotoxicological studies in New Zealand and nothing was known about community-level toxicant effects on planktonic organisms or the predictive abilities of different toxicity tests.

Single species laboratory toxicity tests are, and will continue to be, the most frequently used toxicity test method. They provide essential information on toxicant effects at the organism level and test protocols can be standardised with relative ease, a necessity in the regulation process. They are also simple and economical to undertake on a routine basis. The three copepod species used in this study were all relatively sensitive to PCP and were reasonably amenable to laboratory culture, although further work is needed to improve the culture performance of *Calamoecia lucasi*. Time to metamorphosis proved to be a very sensitive and economical sublethal test endpoint. Metamorphosis is frequently used as a metric in copepod developmental and life history studies (e.g. Jameison 1986; Xu and

Burns 1991) but has not been used previously as an endpoint in toxicity tests. Instead, chronic toxicity tests with estuarine copepods have generally used test endpoints spanning full life cycle and reproductive conditions, requiring a prolonged exposure duration and considerable expenditure in terms of human resources. Delays in the onset of reproduction and time to peak offspring production have been noted in several studies at toxicant concentrations that reduce rates of population increase. Hence, time to metamorphosis may provide an equally sensitive test endpoint but without the extended test duration required for life cycle studies.

Microcosms provide an intermediate between single species tests and mesocosms in terms of complexity and environmental realism. The microcosm protocol developed in this study provided a cost-effective multispecies test that displayed similar community-level responses to those observed in the mesocosms. However, the small size of the microcosms limited the test duration, and high mortality rates unrelated to treatment level were a major confounding factor. Incorporation of aspects of other microcosm test procedures may improve the microcosm test design for future studies. Despite these shortcomings, the microcosms provided a quick and economical test at a higher level of biological organisation than the single species tests. Furthermore, it accurately identified copepods as an important and sensitive component of the aquatic community.

At the next level, mesocosms provide the opportunity to assess toxicant effects under more realistic environmental conditions, but at the expense of reduced control and repeatability. They are also more expensive to undertake in terms of financial cost and human resources. The mesocosms used in this study were small compared to those used in many overseas studies, with a consequent magnification of enclosure effects such as declines in species abundance and conspicuous periphytic growth on the walls. However, they were reasonably economical to undertake and community-level treatment effects were identified

at very low PCP concentrations suggesting the feasibility of the design for use in future studies.

The similarity of the responses between the different toxicity tests in this study confirmed the predictive abilities of the laboratory tests using freshwater copepods and the application of microcosm and mesocosm tests for identifying community-level toxicant effects in New Zealand aquatic communities. Whenever possible, a combination of laboratory and field investigations incorporating both single species and community-level assessments would provide a greater understanding of the environmental effects of toxicants.

It is hoped that, in addition to expanding the data available on the relative sensitivities of New Zealand freshwater species, this study has contributed to reducing some of the uncertainty associated with extrapolating toxicant effects from the laboratory to the field in our freshwater ecosystems.

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APPENDIX I

Distribution of zooplankton in the composite water used to fill the laboratory microcosms

The random distribution of zooplankton in the composite water used to fill the laboratory microcosms was tested using Fisher's Index of Dispersion (Fisher 1948) as described by Lund et al. (1958), using the formula:

$$\chi^2 = \frac{\Sigma(X - \overline{X})^2}{\overline{X}}$$

Five subsamples (4 L) were taken from the composite sample and Crustacea and rotifer numbers counted in each. Samples were resuspended with distilled water (20 mL for Crustacea and 60 mL for rotifers), and 5 mL subsamples transferred to a gridded perspex counting tray, mounted on the movable stage of a model MZ12 Leica stereomicroscope. The limiting value of χ^2 ($p = 0.05$) is 9.49 with four degrees of freedom.

The calculated χ^2 values indicate that the Crustacea were distributed randomly amongst the subsamples, whereas, four of the rotifer species were not (Table 1).

Table 1. χ^2 values of the mean for zooplankton (No. per L) in five subsamples.

Crustacea	mean (No. per L)	χ^2
Bosmina longirostris	195	2.516
Calanoid nauplii	80.5	5.186
Calanoid adults	19.8	3.573
Calanoid copepodites	50	4.222
Cyclopoid copepodites	3.7	1.49
Cyclopoid nauplii	12.2	1.831
Rotifers		
Keratella cochlearis	17.6	12.254
Hexarthra mira	850	35.995
Trichocerca similis	153	50.406
Polyarthra dolichoptera	45.9	5.671
Ascomorpha ovalis	35.4	2.799
Filinia spp.	7.6	22.561
Pompholyx complanata	148	8.961
Asplanchna priodonta	17.5	8.543
Other rotifers	8.6	5.185

APPENDIX II

Statistical analysis of the sedimentation technique for counting phytoplankton

The random distribution of phytoplankton in subsamples and on the basal plate of the sedimentation chamber was tested using Fisher's Index of Dispersion (Fisher 1948) as described by Lund et al. (1958), using the formula:

$$\chi^2 = \frac{\Sigma(X - \overline{X})^2}{\overline{X}}$$

To determine whether subsamples were randomly drawn from a sample, five subsamples (60 mL) were taken from a 500 mL Lake Rotomanuka sample and sedimented for 24 h. The five most common taxa were counted from a single transect of the basal plate in each subsample. The limiting value for χ^2 ($p = 0.05$) is 12.59 with six degrees of freedom.

Counts were made of five of the most common taxa in each of seven transects from a 60 mL Lake Rotomanuka sample after a sedimentation time of 24 h. The limiting value of χ^2 ($p = 0.05$) is 9.49 with four degrees of freedom.

The calculated χ^2 values indicate that the phytoplankton were distributed randomly amongst the subsamples (Table 1), and on the basal plate (Table 2).

Table 1. Phytoplankton counts (cells.mL^{-1}) and χ^2 values in five subsamples.

sample	*Peridinium*	*Mougetia*	*Staurastrum*	*Ceratium*	*Trachelomonas*
1	89	65	39	7	39
2	83	64	25	6	30
3	92	56	31	5	33
4	92	59	34	7	38
5	84	67	32	5	45
mean	88	62	32	6	37
χ^2	0.93	1.33	2.68	0.57	3.85

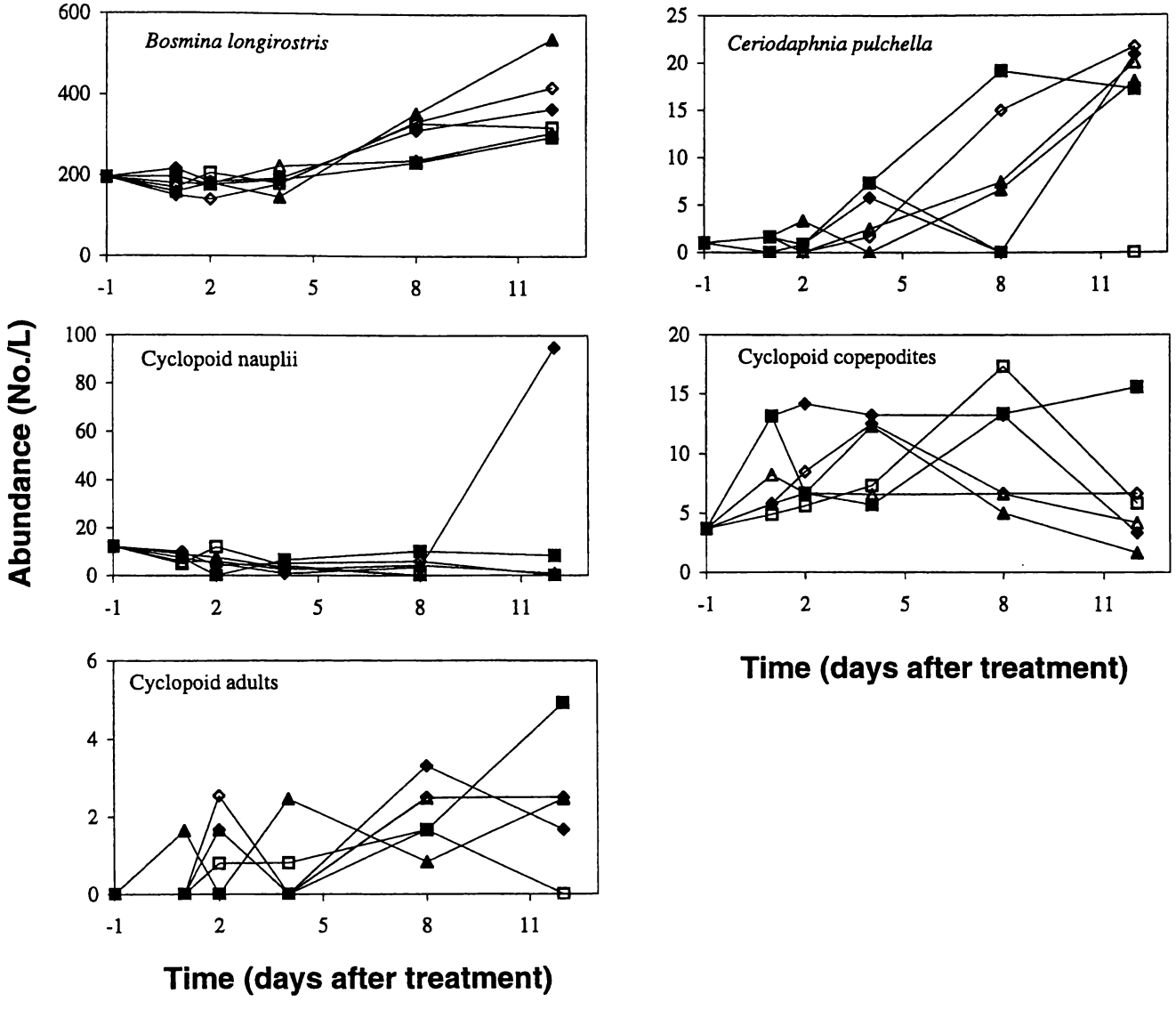
Table 2. Phytoplankton counts (cells.mL^{-1}) and χ^2 values in seven transects of the basal plate.

transect	*Peridinium*	*Mougetia*	*Staurastrum*	*Ceratium*	*Trachelomonas*
1	96	52	34	6	28
2	117	61	26	4	35
3	108	46	20	3	31
4	108	59	23	5	27
5	98	38	13	6	22
6	108	50	21	5	27
7	114	59	20	8	29
mean	107	52	22	5	29
χ^2	3.21	7.73	11.00	2.17	3.11

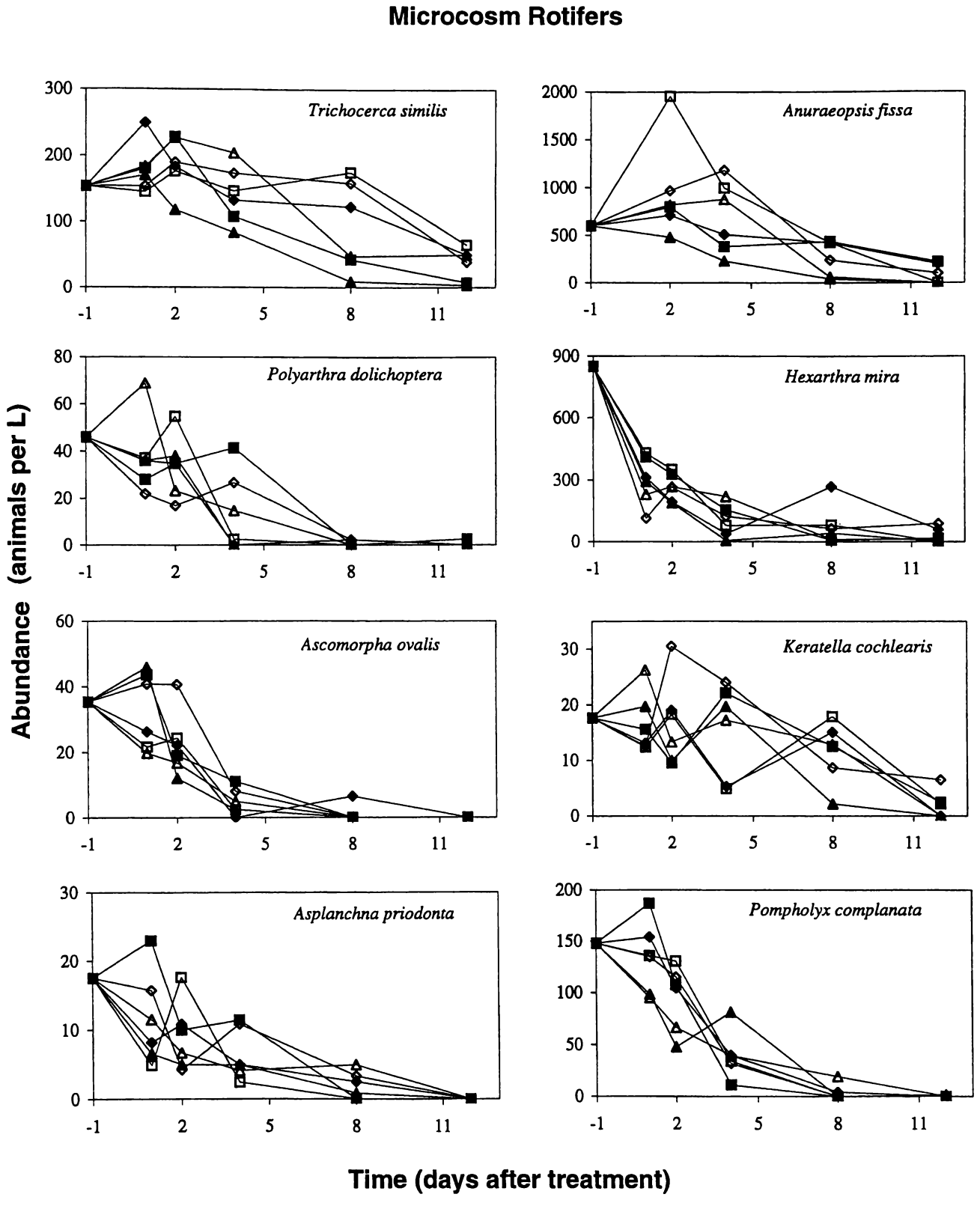
Appendix III

Graphs showing changes in abundance of Crustacea, Rotifer and Phytoplankton species in the laboratory microcosms (from Chapter Four).

Microcosm Crustacea

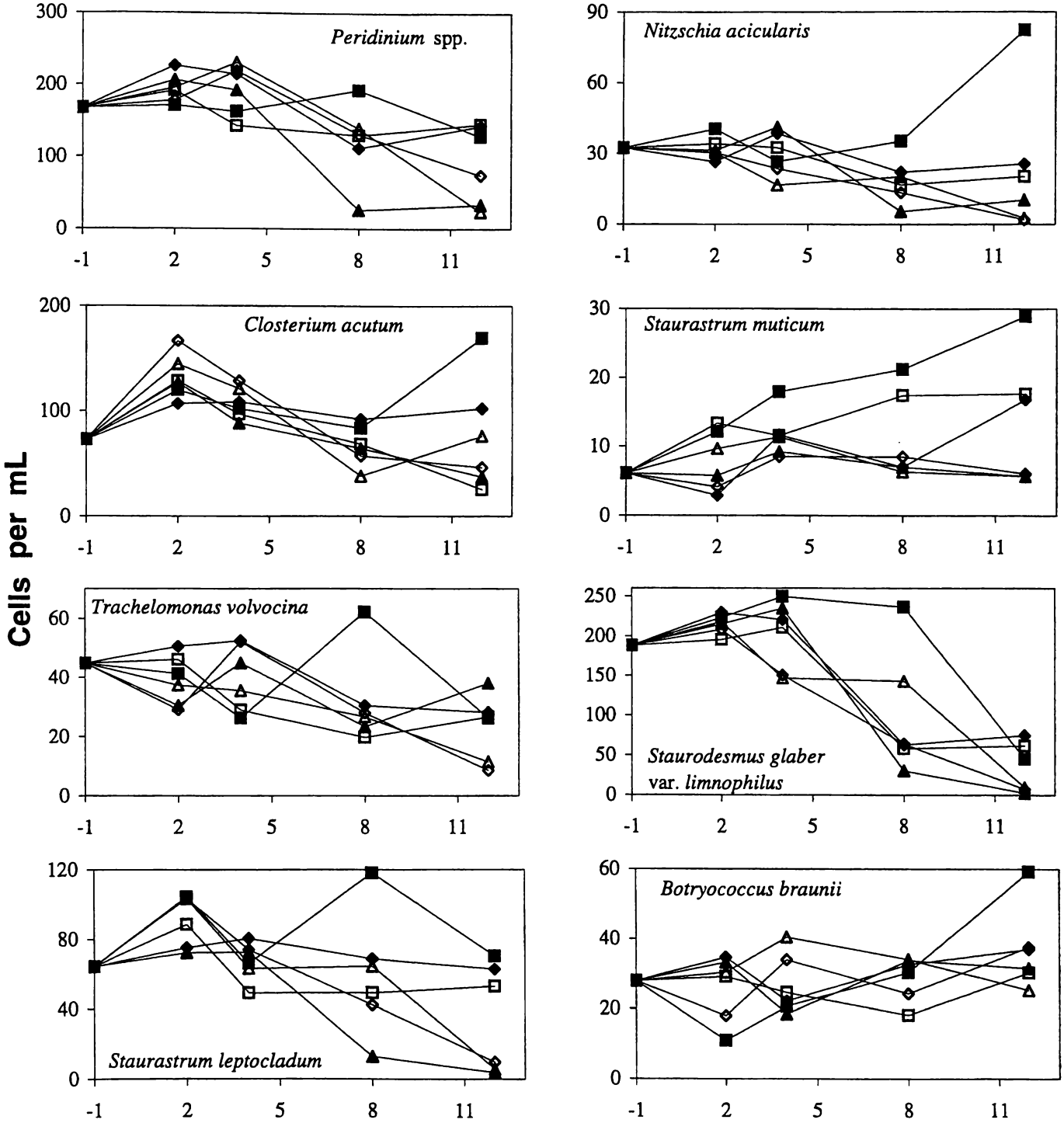


[control (■), solvent control (□), 10 (◇), 36 (◆), 54 (△)and 121(▲) $\mu g.L^{-1}$ PCP]



[control (■), solvent control (□), 10 (◇), 36 (◆), 54 (△)and 121(▲) $\mu g.L^{-1}$ PCP]

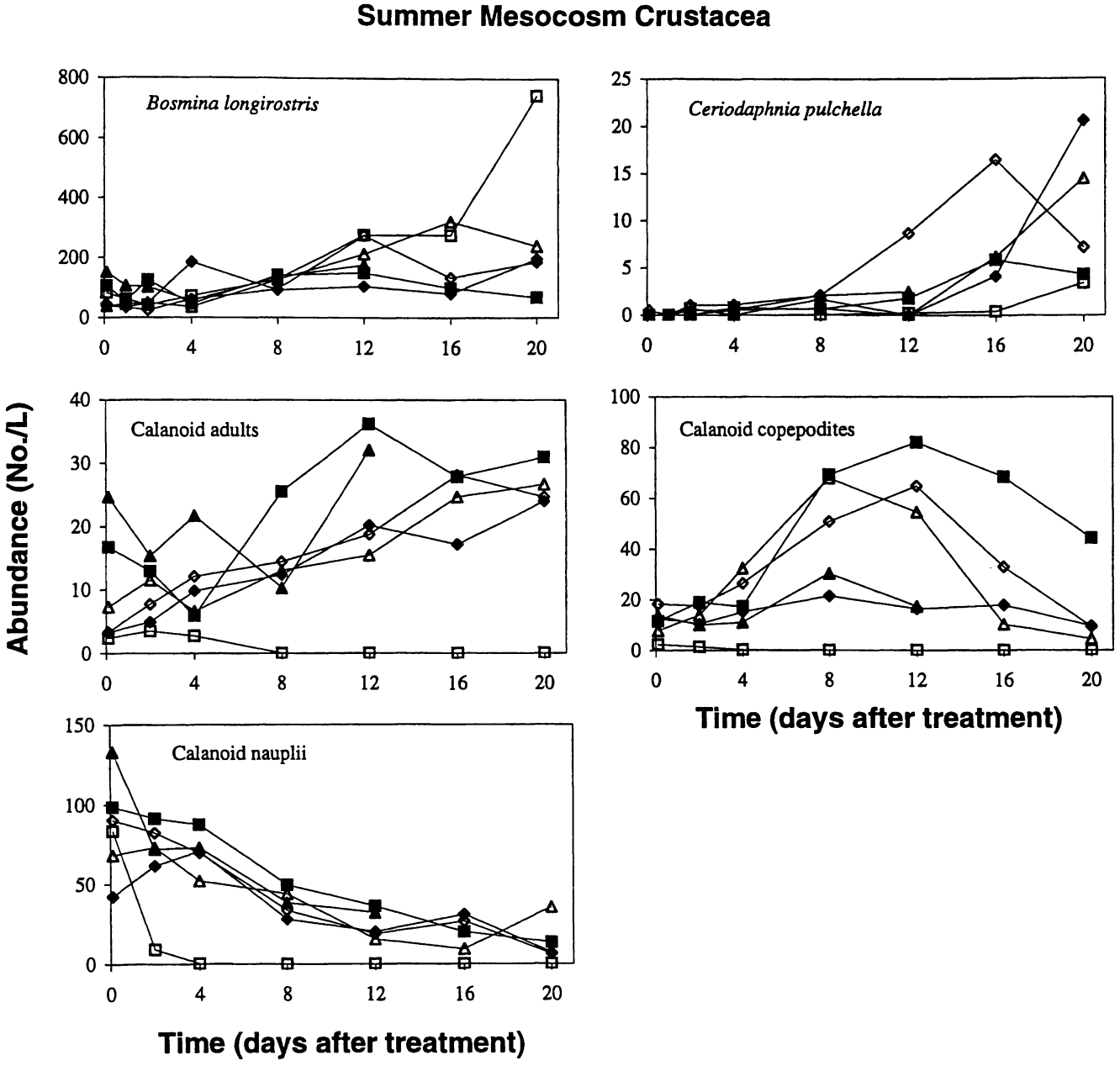
Microcosm Phytoplankton



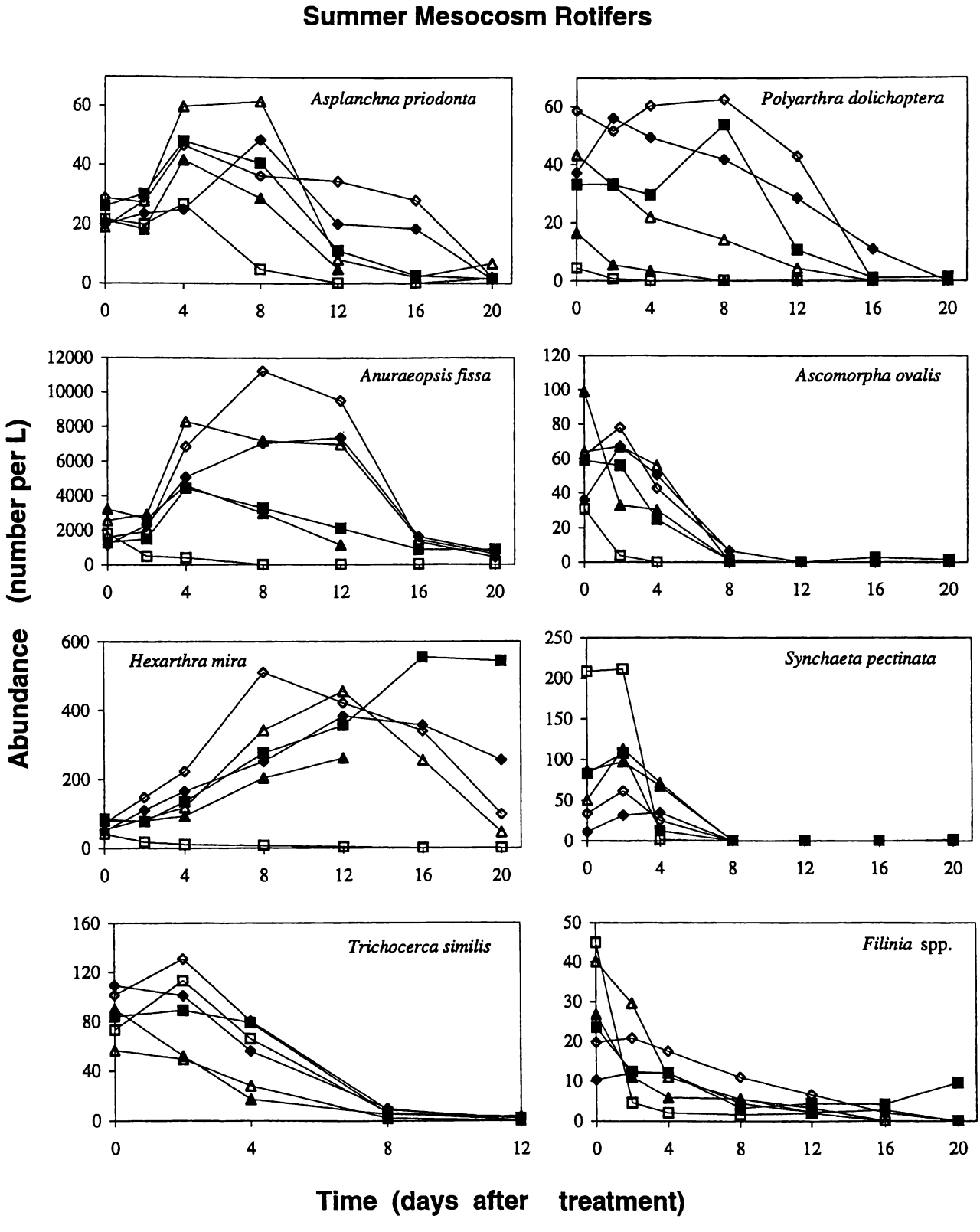
[control (■), solvent control (□), 10 (◇), 36 (◆), 54 (△)and 121(▲) $\mu g.L^{-1}$ PCP]

Appendix IV

Graphs showing changes in abundance of Crustacea, Rotifer and Phytoplankton species in the summer mesocosms (from Chapters Four and Six).



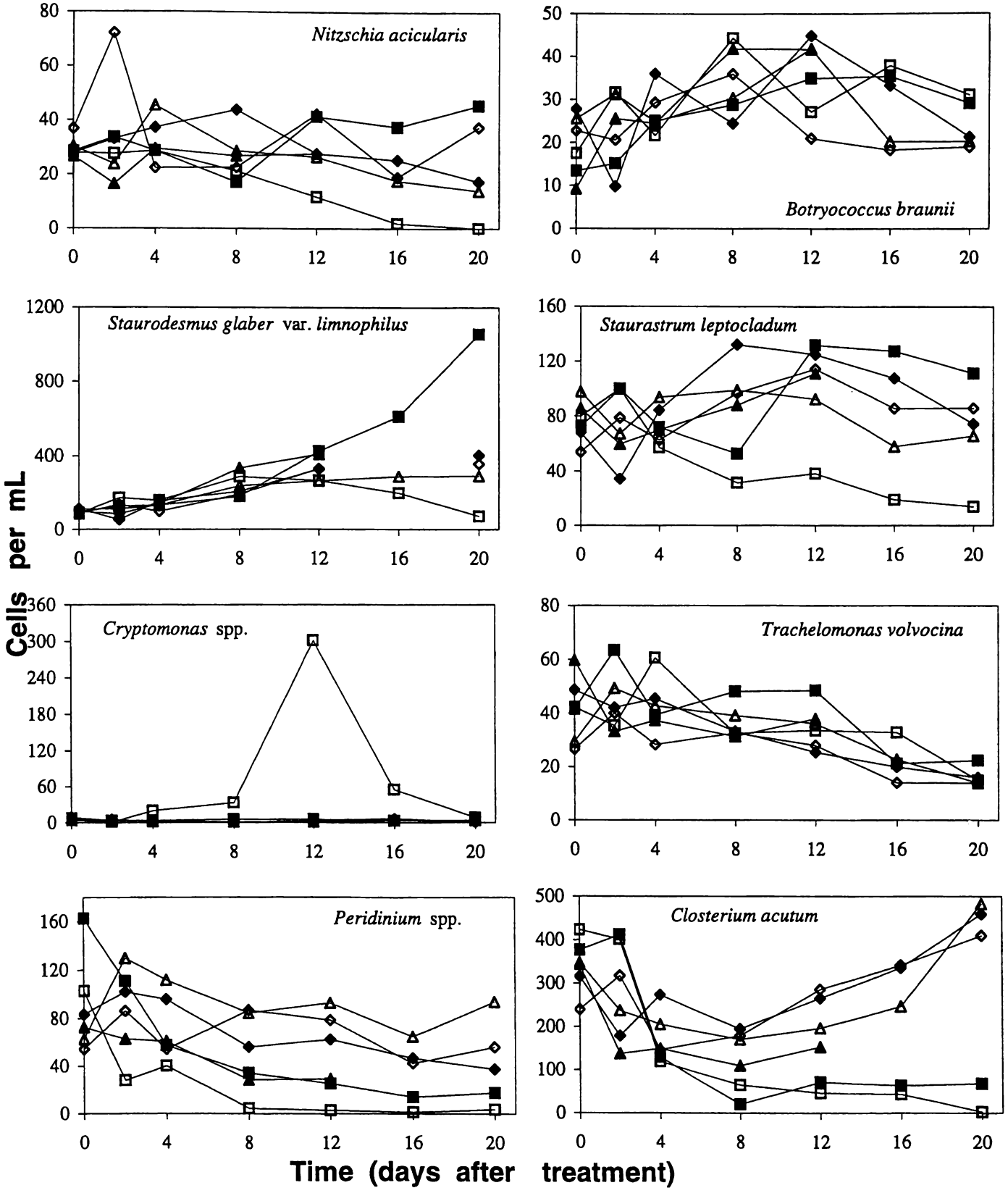
[control (■), solvent control (◆), 10 (◇), 36 (△), 54 (▲)and 121(□) $\mu g.L^{-1}$ PCP]



[control (■), solvent control (◆), 10 (◇), 36 (△), 54 (▲)and 121(□) $\mu g.L^{-1}$ PCP]

Summer Mesocosm Phytoplankton

[control (■), solvent control (◆), 10 (◇), 36 (△), 54 (▲)and 121(□) µg.L^{-1} PCP]

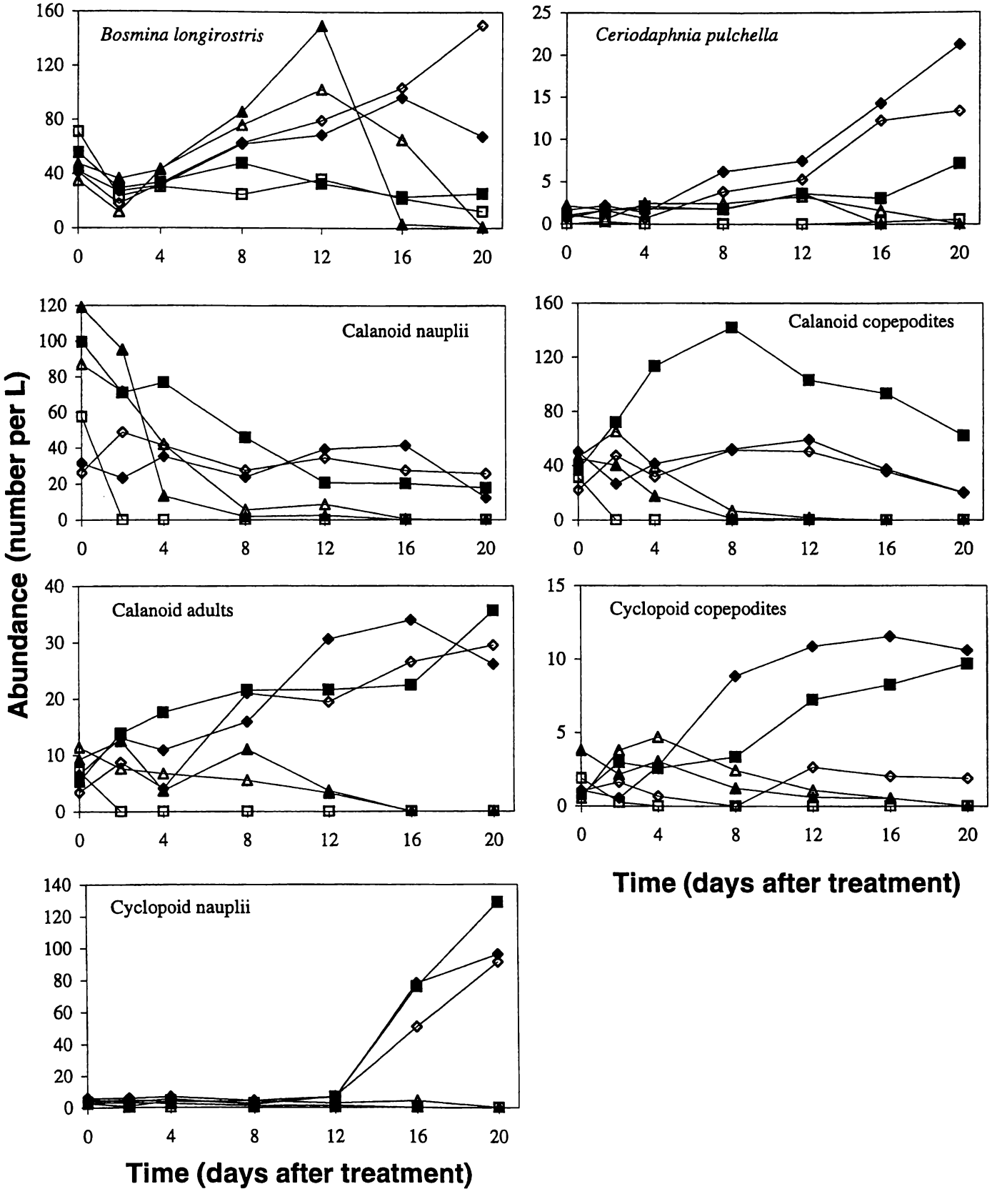


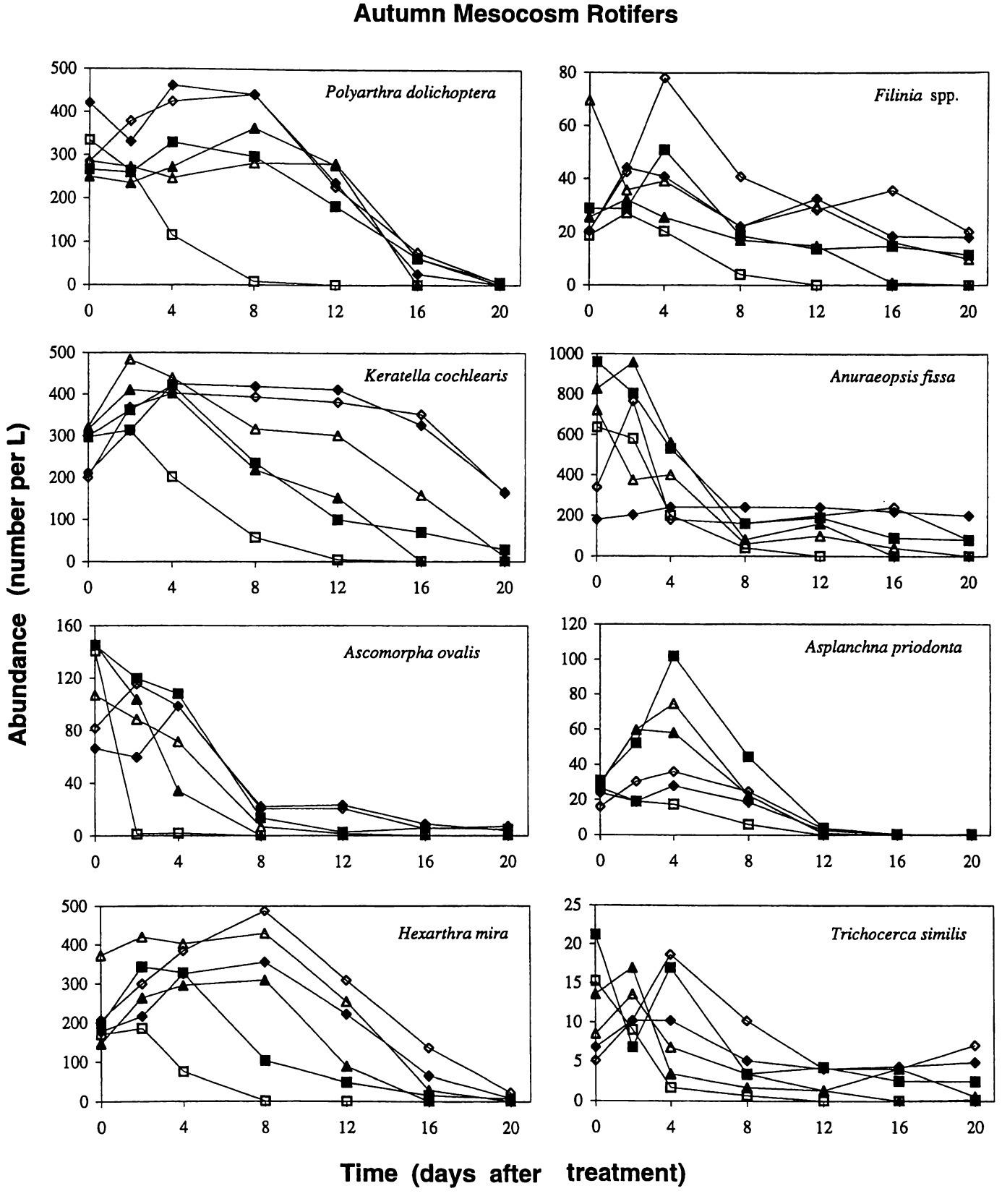
Appendix V

Graphs showing changes in abundance of Crustacea, Rotifer and Phytoplankton species in the autumn mesocosms (from Chapter Six).

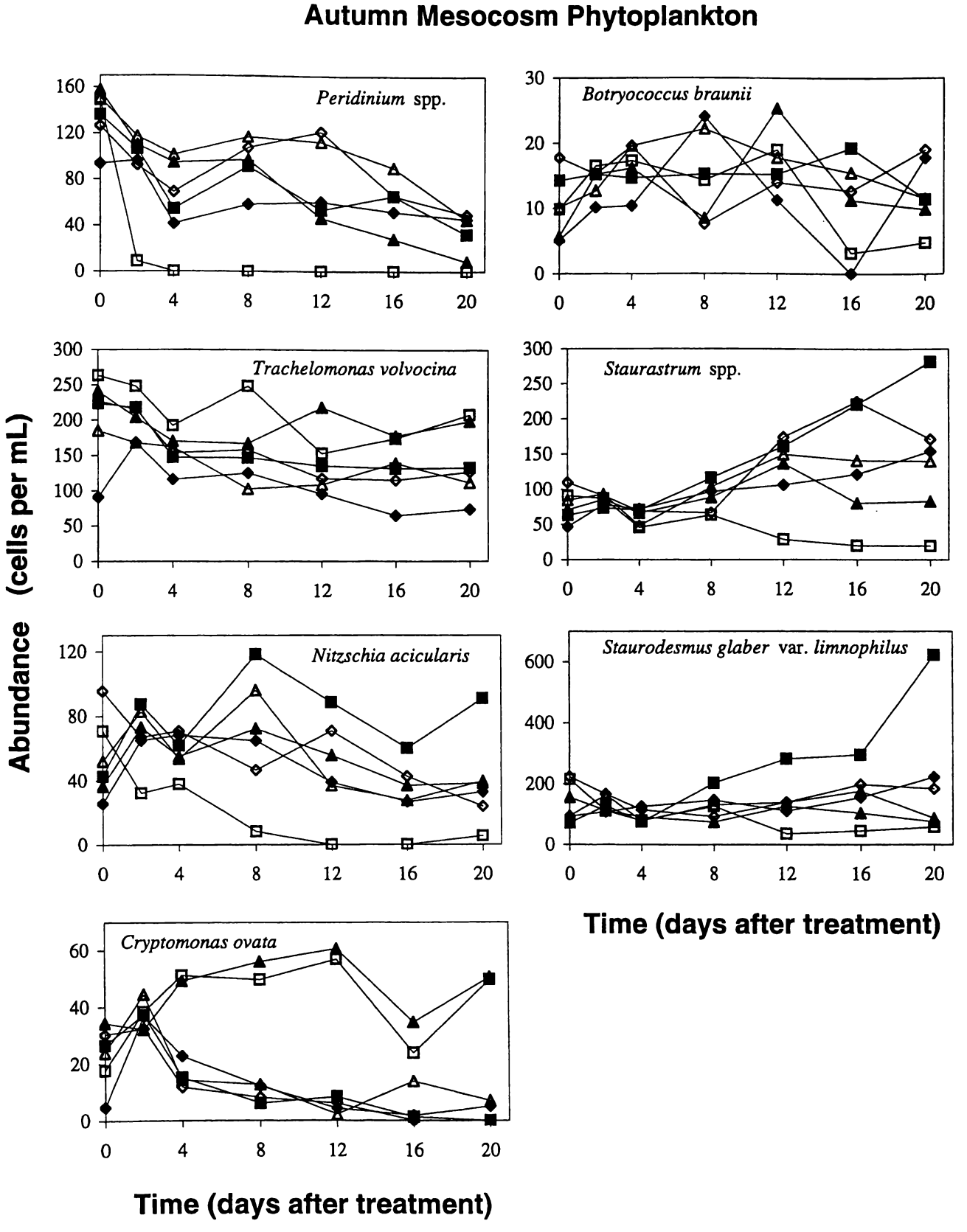
Autumn Mesocosm Crustacea

[control (■), solvent control (◆), 10 (◇), 36 (△), 54 (▲) and 121(□) µg.L^{-1} PCP]





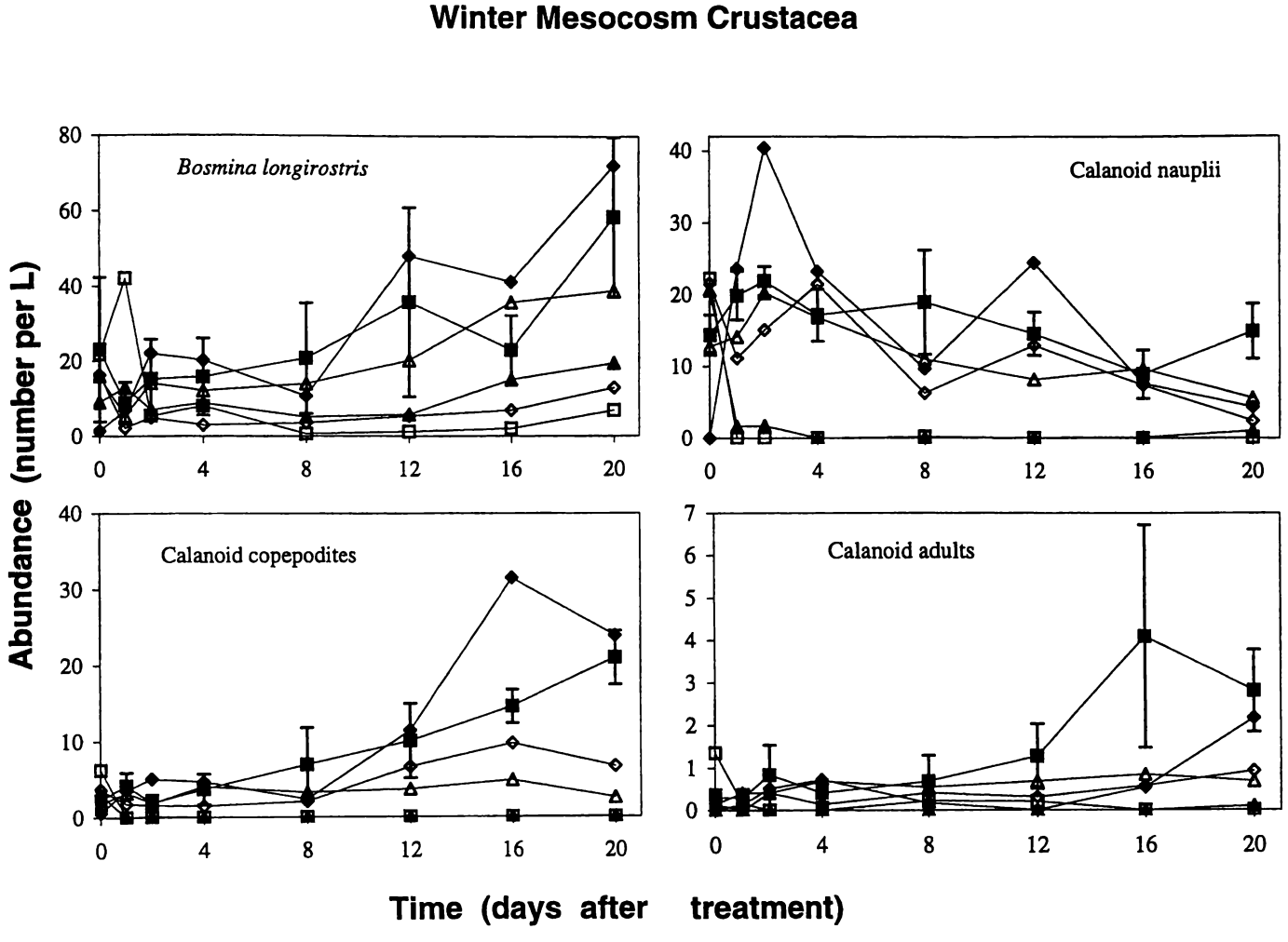
[control (■), solvent control (◆), 10 (◇), 36 (△), 54 (▲) and 121(□) $\mu g.L^{-1}$ PCP]



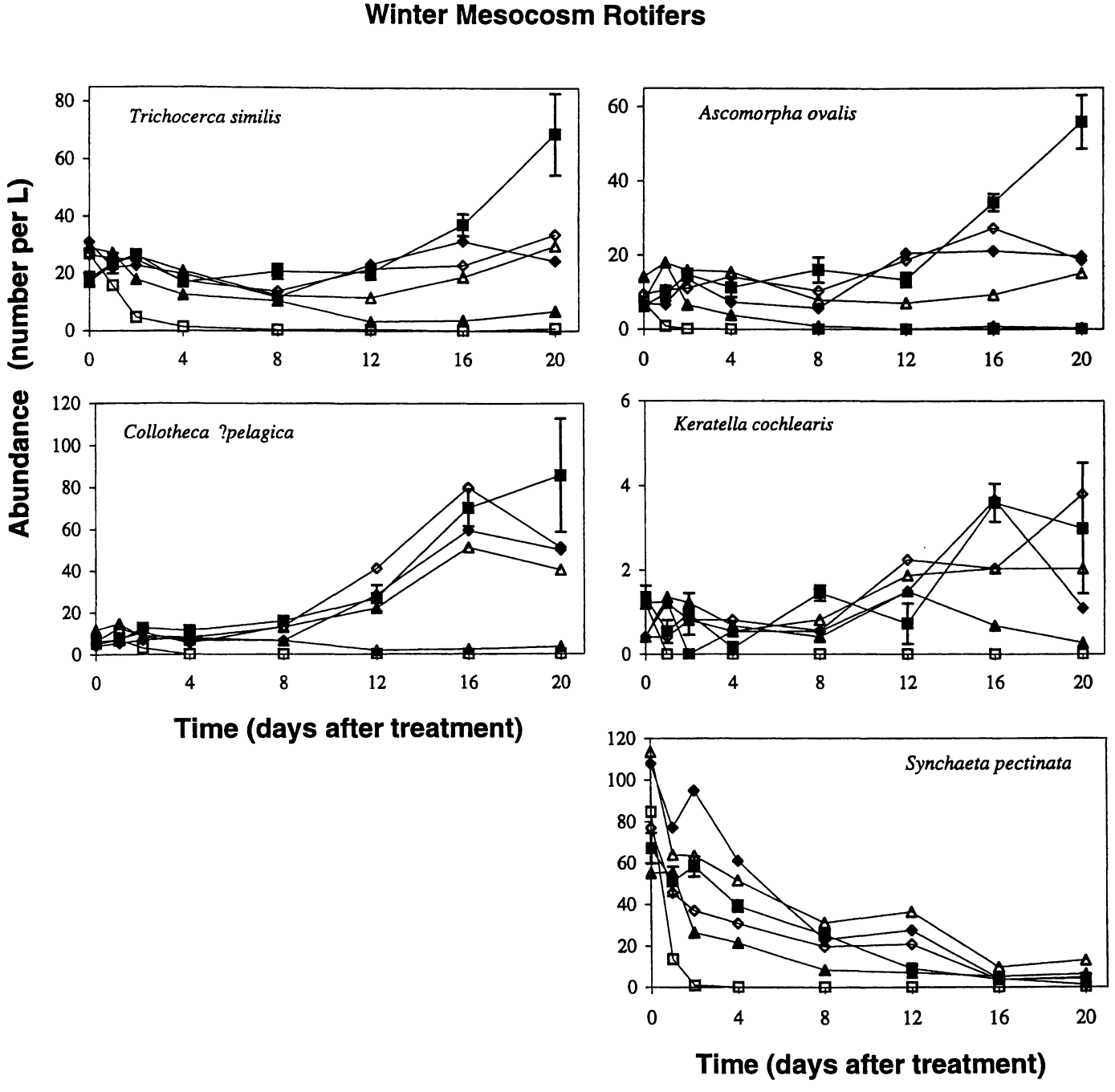
[control (■), solvent control (◆), 10 (◇), 36 (△), 54 (▲) and 121(□) $\mu g.L^{-1}$ PCP]

Appendix VI

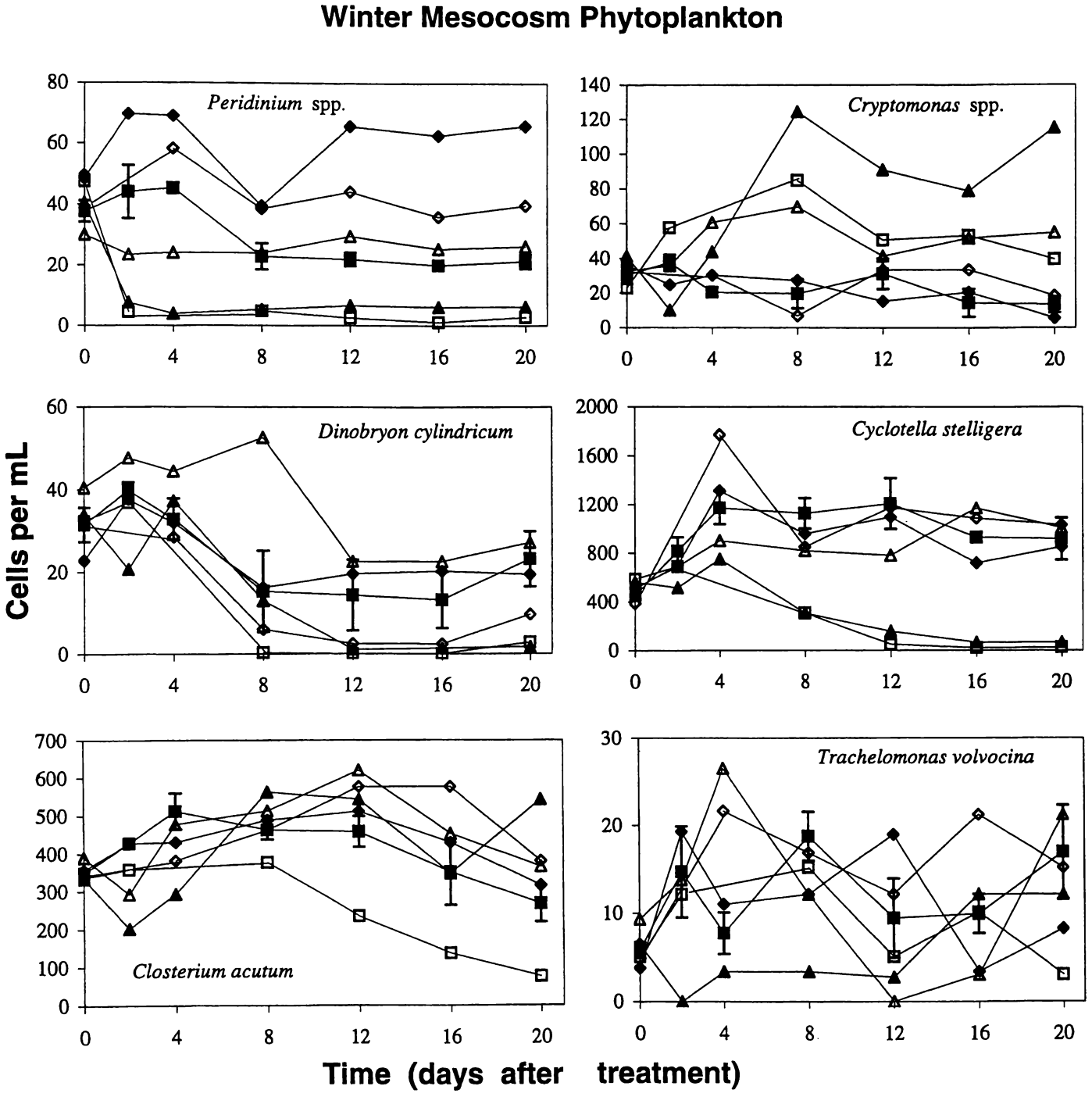
Graphs showing changes in abundance of Crustacea, Rotifer and Phytoplankton species in the winter mesocosms (from Chapter Six).



[control (■), 4 (◆), 10 (◇), 24 (△), 54 (▲) and 121(□) $\mu g.L^{-1}$ PCP]



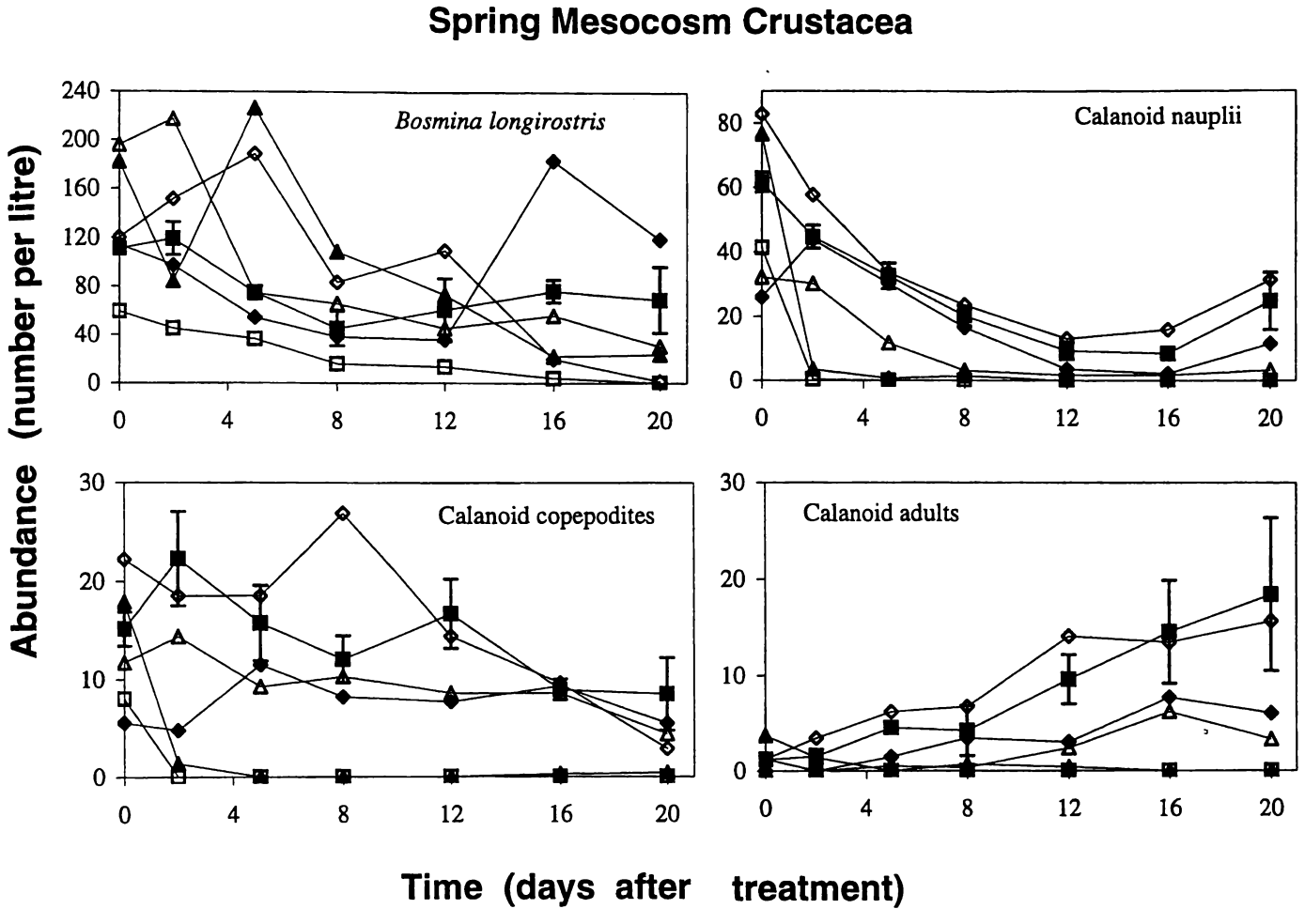
[control (■), 4 (◆), 10 (◇), 24 (△), 54 (▲) and 121(□) $\mu g.L^{-1}$ PCP]



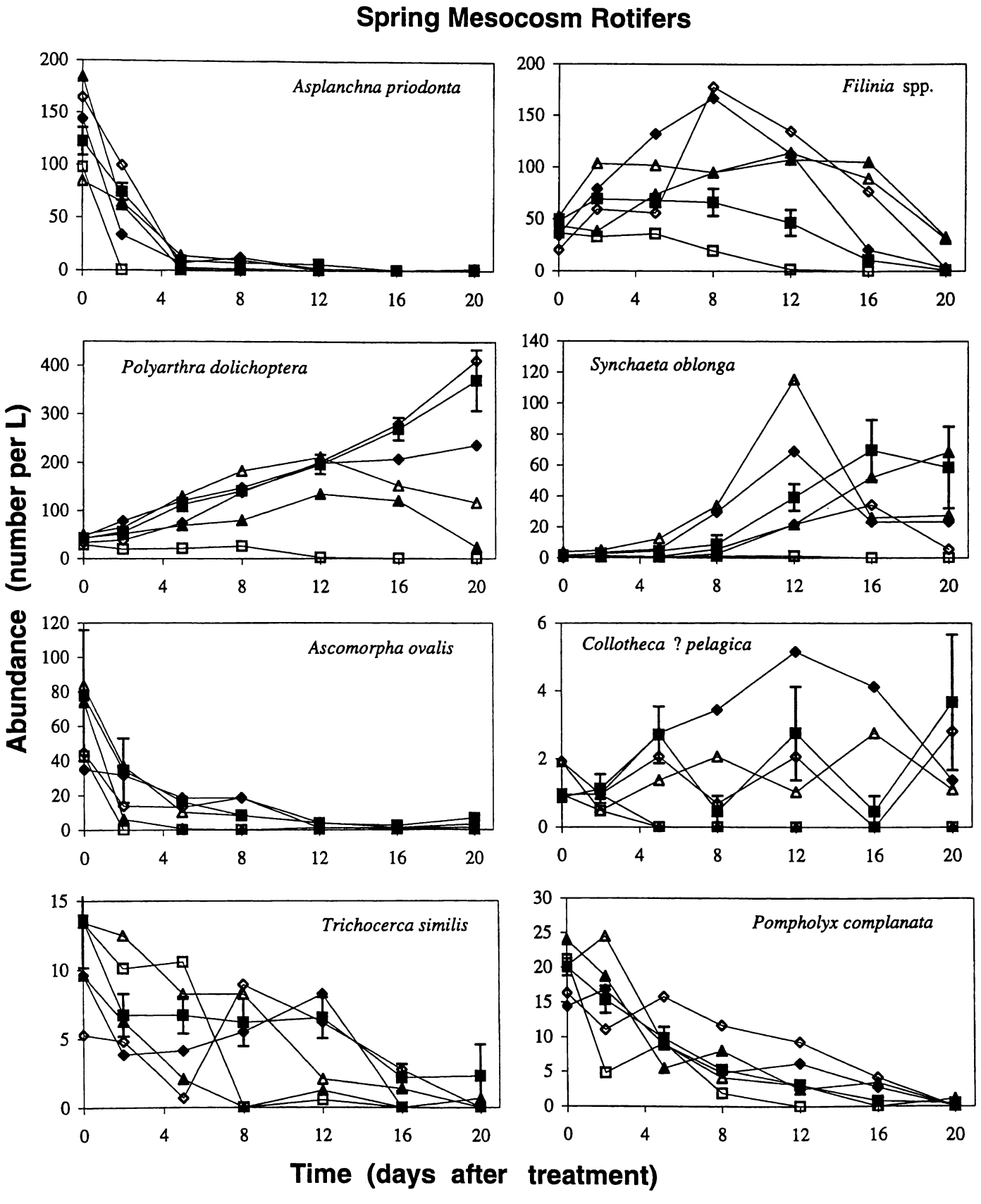
[control (■), 4 (◆), 10 (◇), 24 (△), 54 (▲) and 121(□) $\mu g.L^{-1}$ PCP]

Appendix VII

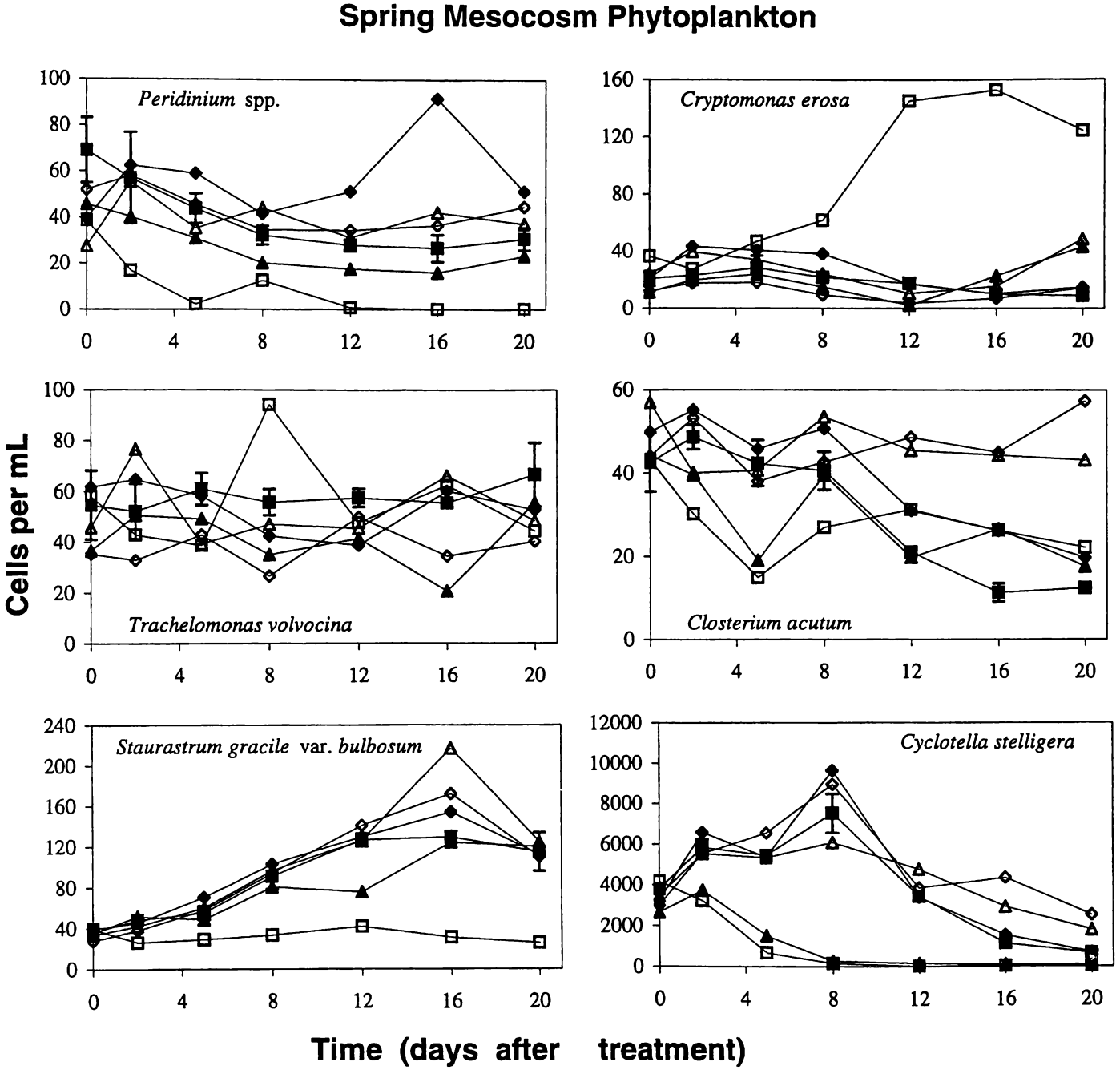
Graphs showing changes in abundance of Crustacea, Rotifer and Phytoplankton species in the spring mesocosms (from Chapter Six).



[control (■), 4 (◆), 10 (◇), 36 (△), 54 (▲) and 121(□) $\mu g.L^{-1}$ PCP]



[control (■), 4 (◆), 10 (◇), 36 (△), 54 (▲) and 121(□) $\mu g.L^{-1}$ PCP]



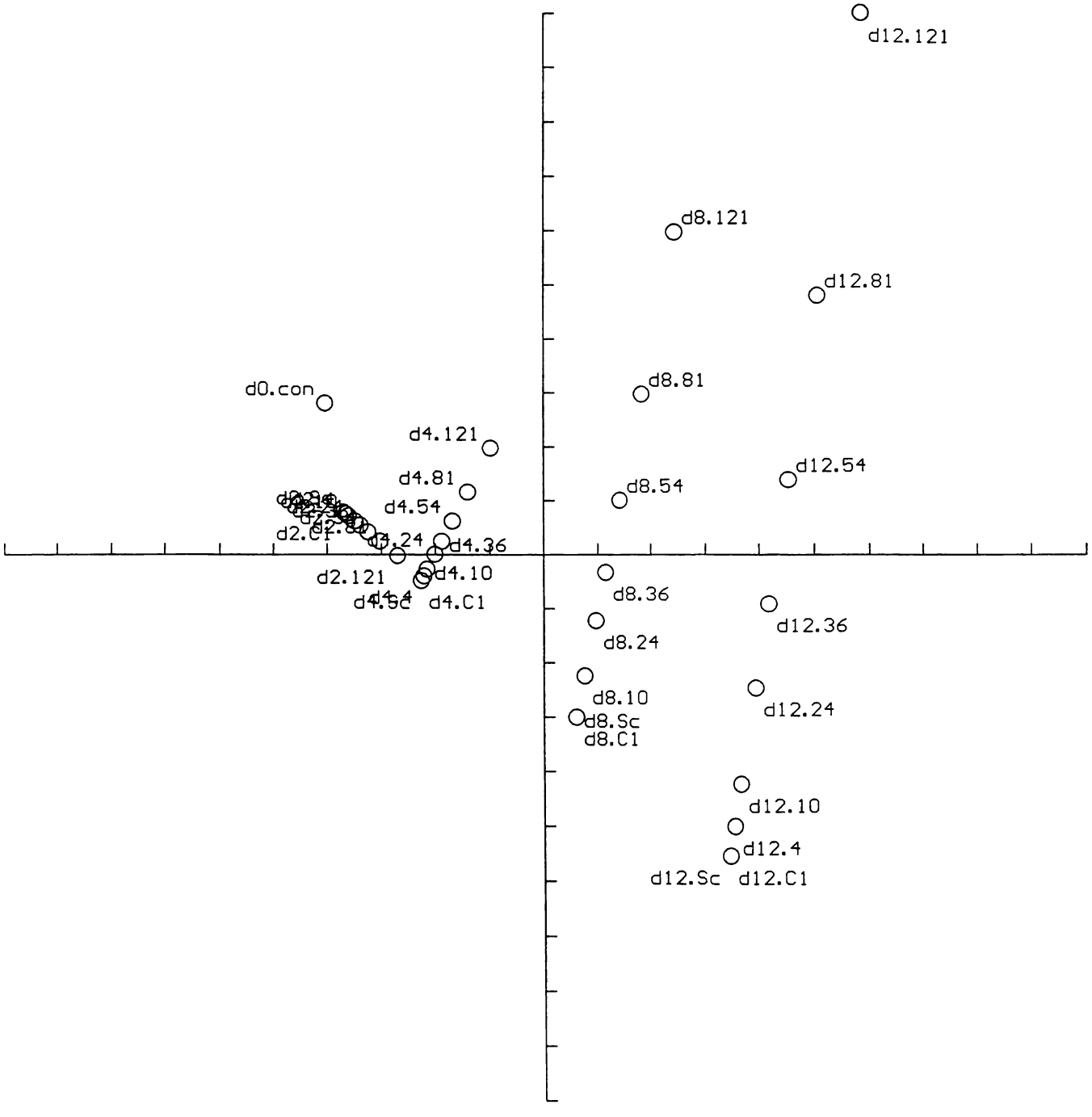
[control (■), 4 (◆), 10 (◇), 36 (△), 54 (▲) and 121(□) $\mu g.L^{-1}$ PCP]

Appendix VIII

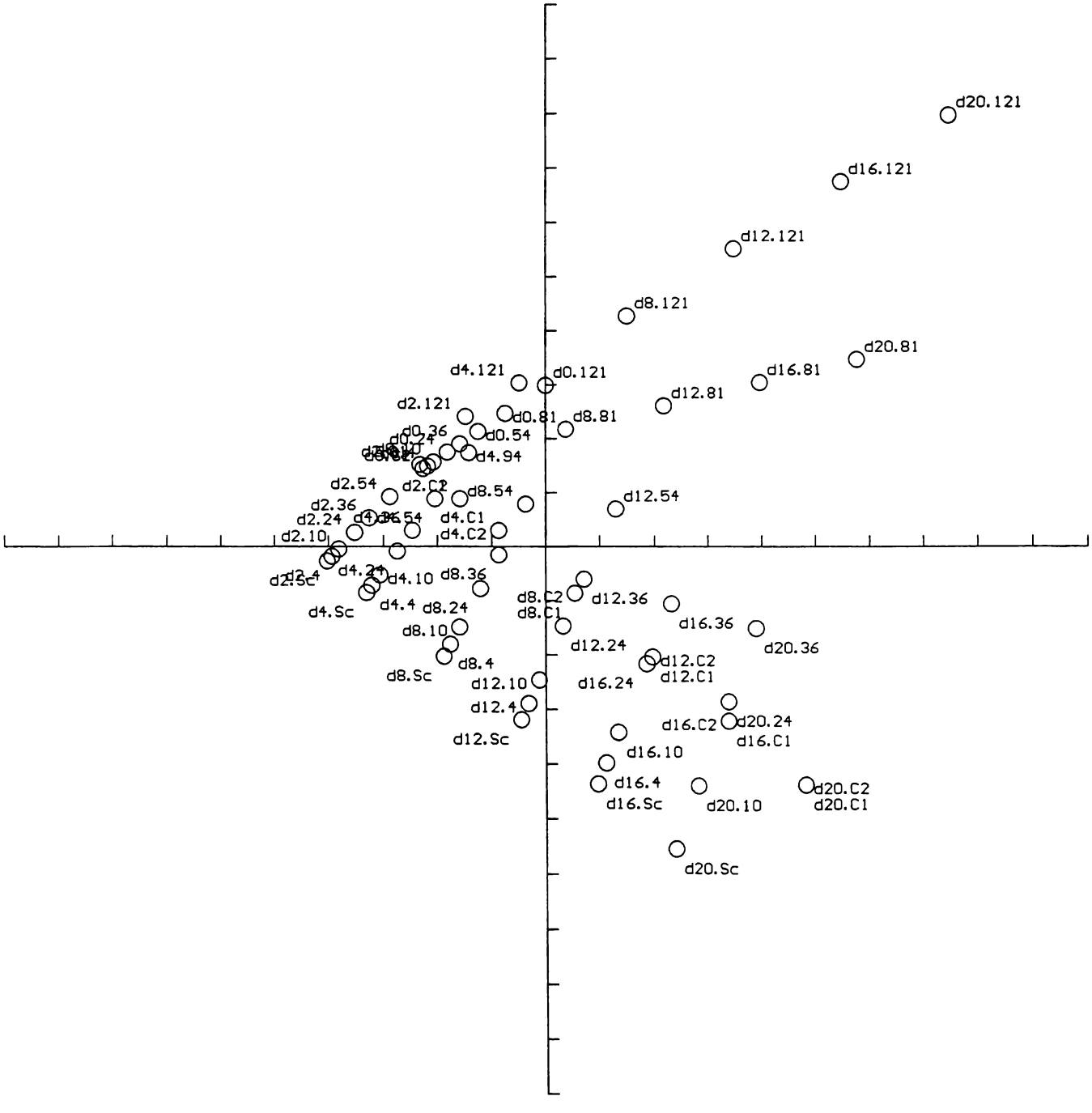
RDA site plots for microcosm and seasonal mesocosm experiments.
Site points for all sample days are shown (from Chapters Four and Six).

Sites are shown as open circles.
Site codes indicate sampling day followed by PCP concentration.

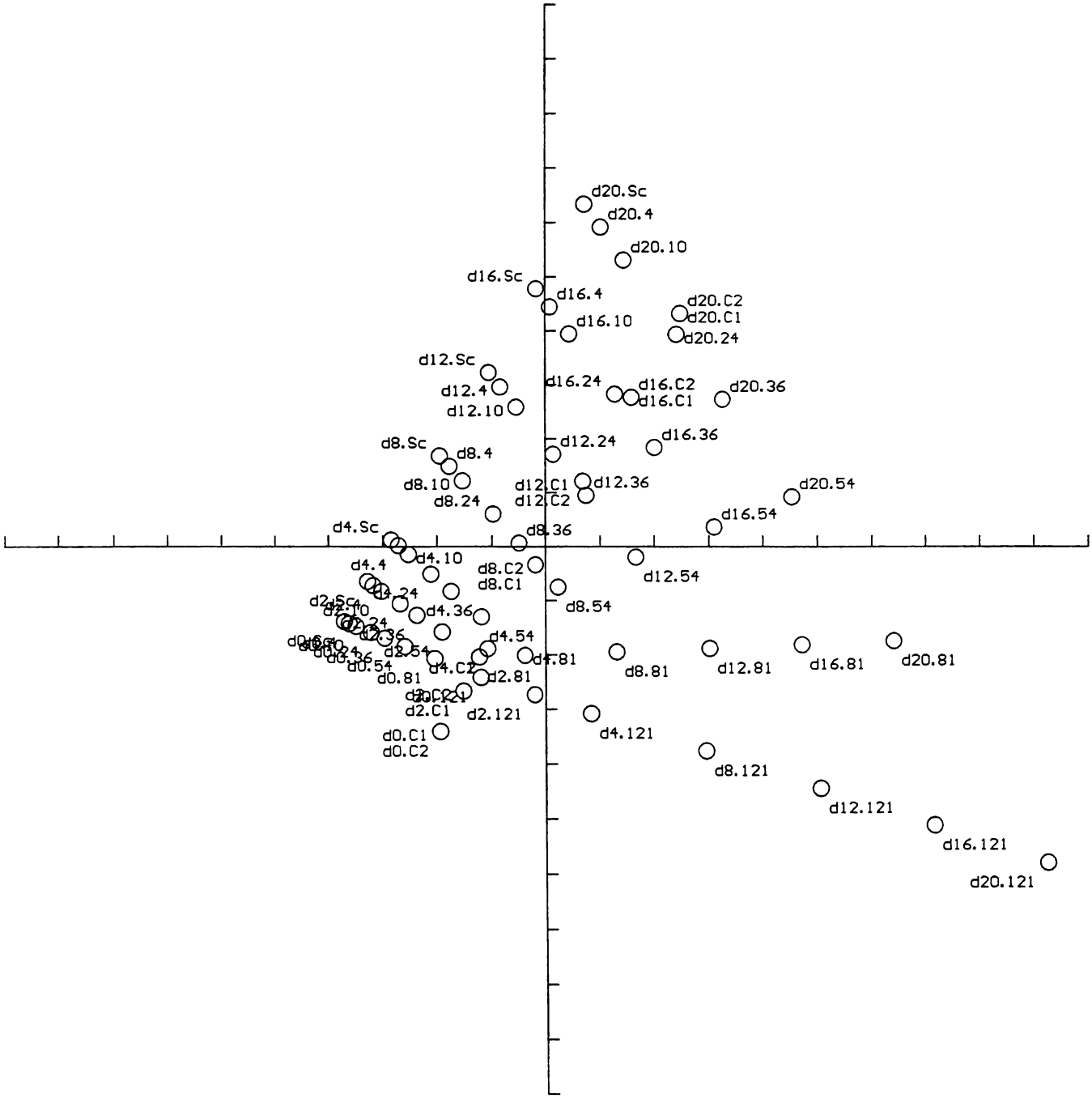
Microcosm RDA site plot



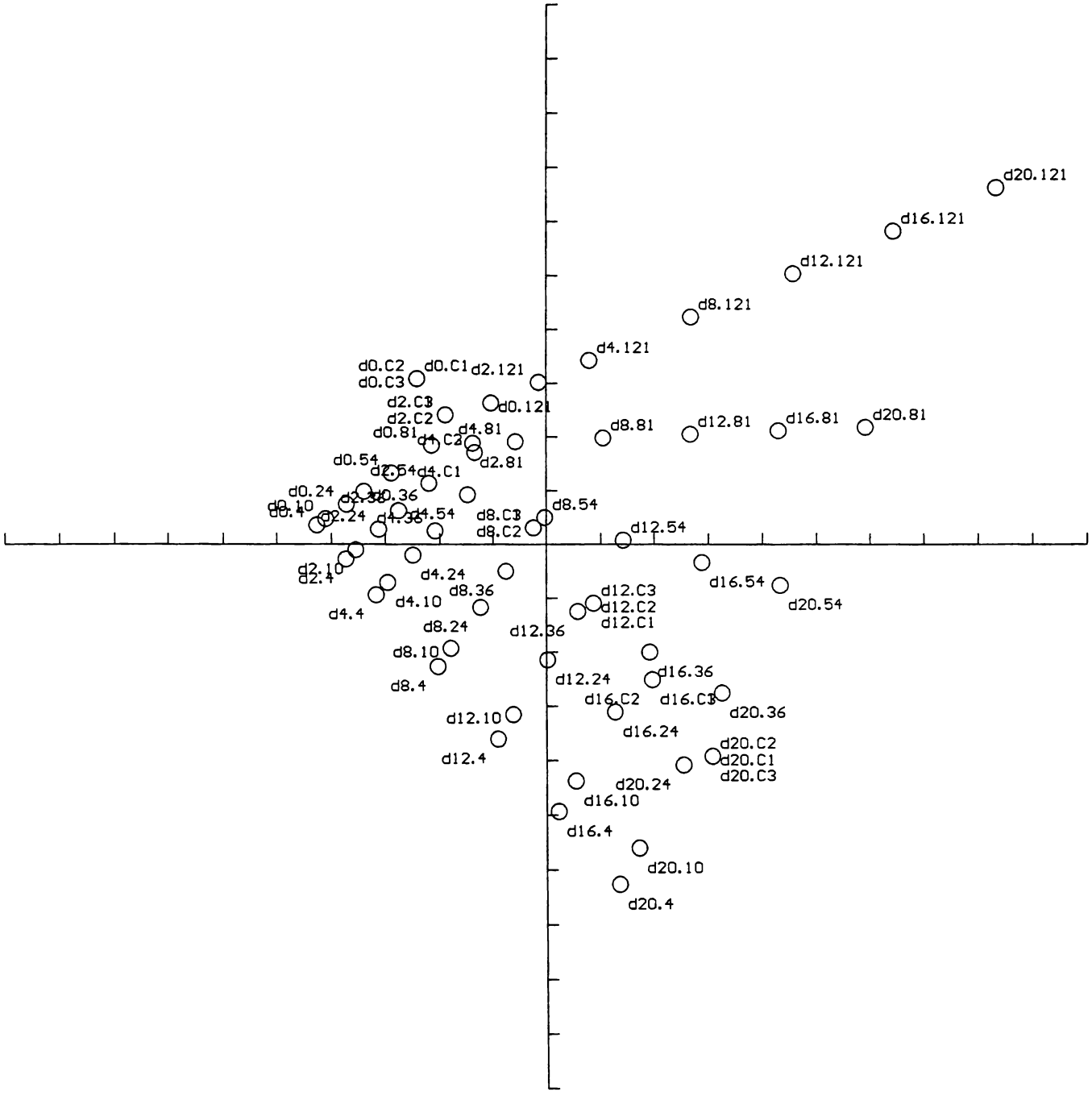
Summer mesocosm RDA site plot



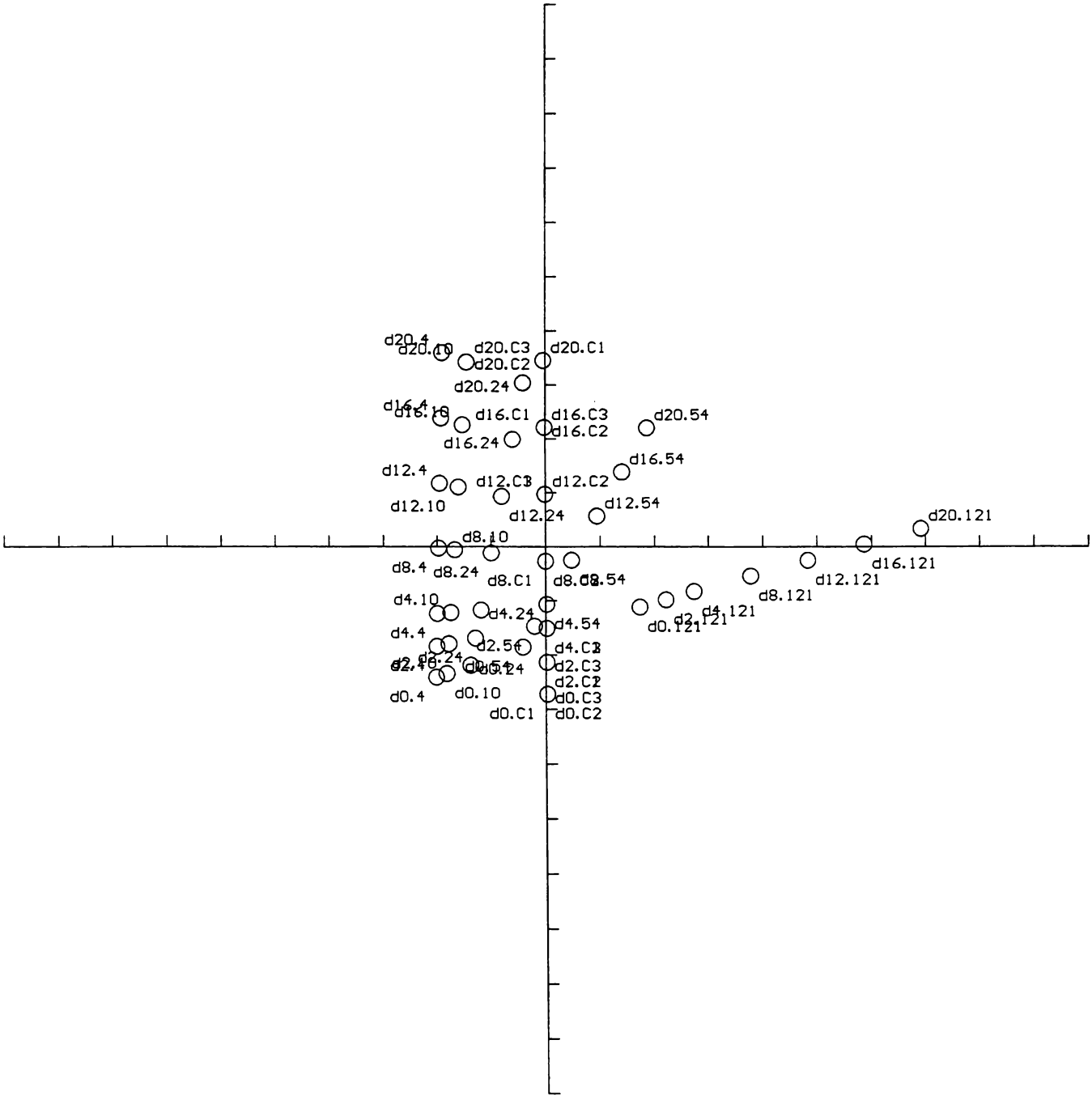
Autumn mesocosm RDA site plot



Spring mesocosm RDA site plot



Winter mesocosm RDA site plot



Appendix IX

Conference abstracts for poster and paper presentations
of work from this thesis.

InterSECT 96 - International Symposium on Environmental Chemistry and Toxicology.
Sydney, Australia 14th - 18th July 1996.
Abstract for poster presentation.

The use of Freshwater Copepods in Laboratory Toxicity Tests.

Kate J. Willis and John D. Green.

Copepods are abundant in both marine and freshwaters and are by far the most numerous of the Crustacea, and among the most common of all animals. They are of great importance in aquatic foodwebs and calanoid copepods frequently dominate zooplankton commnities in New Zealand. It is therefore suprising that freshwater copepods are not commonly used in laboratory toxicity tests.

The results from laboratory toxicity tests using two species of freshwater copepod will be presented. The filter feeding calanoid copepod, *Calamoecia lucasi*, occurs in the North Island of NZ and Australia. The carnivorous cyclopoid copepod, *Mesocyclops* cf. *leuckarti*, has a worldwide distribution and is relatively common in NZ lakes.

The sensitivity of juvenile and adult stages of *C. lucasi* and *M. leuckarti* from wild populations and laboratory cultures will be compared with representative cladocera using the reference toxicant pentachlorophenol. Culture methods for the two copepods will also be presented.

Fourth Annual Conference of the Australasian Society for Ecotoxicology.

Brisbane, Australia 17th - 19th July 1997.

Abstract for paper presentation.

A Comparative Investigation of the Effect of Pentachlorophenol on Planktonic Organisms Using Laboratory Microcosms and *in situ* Mesocosms.

Kate Willis* and John Green.

It is now widely accepted that a combination of laboratory and field investigations provide the best means for understanding the effects of toxicants on aquatic communities, however, the complexity, cost, and potential problems likely to be encountered when trying to undertake field experiments may make such studies prohibitively expensive.

We have undertaken a comparative investigation using *in situ* mesocosms (860 L) and laboratory microcosms (1.6 L) containing natural plankton assemblages from Lake Rotomanuka, Waikato, New Zealand and the toxicant pentachlorophenol (PCP).

Using a regression designed experiment, a single dose of PCP was applied to both mesocosms and microcosms. Nominal initial PCP concentrations were 5, 12, 28, 42, 63, 94, and 141 µg/L, in addition to a solvent and non-solvent controls. The experimental period was 12 days with sample collection on days 0, 1, 2, 4, 8, and 12. Three metre depth integrated tube samples were taken from the *in situ* mesocosms, whereas the laboratory microcosms were destructively sampled. On each sampling occasion, temperature, dissolved oxygen, pH, and conductivity were also recorded.

Qualitatively similar results were obtained from both the laboratory and field mesocosms. The physical conditions were similar throughout the experimental period. A gradual decrease in chlorophyll *a* concentration was observed in all experimental enclosures. A solvent effect was observed in both experiments in the form of progressively decreasing oxygen concentrations over time when compared to the non-solvent controls. This decrease in oxygen was more pronounced in the higher PCP concentrations. PCP toxicity appeared to be more marked in the field mesocosms. Changes in planktonic assemblages were complex reflecting alterations in species composition due to toxicity, solvent effects, grazing by herbivores and stresses associated with laboratory culture.

On the basis of these results we suggest that the *in situ* mesocosms were a more sensitive and realistic indictator of environmental susceptibility to this toxicant, however, the cost effectiveness of such studies will be further discussed in relation to the problems outlined above.

Society of Environmental Toxicology and Chemistry 18th Annual Meeting.
San Francisco, CA, USA 16th - 20th November 1997.
Abstract for paper presentation.

A Seasonal Investigation into the Effect of Pentachlorophenol on Planktonic Assemblages Using Lake Mesocosms.

K.J. Willis and J.D. Green.

We investigated the seasonal variability in the sensitivity of natural planktonic assemblages to the toxicant pentachlorophenol (PCP) in a small New Zealand lake, Lake Rotomanuka. Four experiments were conducted in winter, spring, summer, and fall. Ten mesocosms, constructed from 3 metre lengths of polythene tubing filled with 860 litres of lake water, were suspended from polystyrene floats anchored in the lake. Using a regression design, a single dose of PCP was applied to the surface water of each mesocosm and then mixed. Nominal PCP concentrations were 4, 10, 24, 36, 54, 81, and 121 $\mu g.L^{-1}$, in addition to a solvent and non-solvent controls. The experimental period was 20 days with sample collection on days 0, 1, 2, 4, 8, 12, 16, and 20. On each sampling occasion, temperature, dissolved oxygen, pH, conductivity, and chlorophyll *a* concentration were recorded. Depth-integrated water samples were collected using a 3 metre length of PVC pipe and analysed for zooplankton and phytoplankton species composition. A solvent effect was observed in the form of progressively decreasing oxygen concentrations over time. The decrease in oxygen was more pronounced in the higher PCP concentrations. Changes in planktonic assemblages were complex reflecting alterations in species composition due to seasonality, toxicity, solvent effects and grazing by herbivores.

Society of Environmental Toxicology and Chemistry 18th Annual Meeting.
San Francisco, CA, USA 16th - 20th November 1997.
Abstract for poster presentation.

Comparative Chronic Toxicity Tests Using Three Species of Freshwater Copepod From New Zealand.

K.J. Willis and N. Ling

Copepods are abundant in both marine and freshwaters and are by far the most numerous of the Crustacea, and among the most common of all animals. They are of great importance in aquatic foodwebs and calanoid copepods frequently dominate zooplankton communities in New Zealand. It is therefore suprising that freshwater copepods are not commonly used in laboratory toxicity tests. The results from chronic laboratory toxicity tests using three species of freshwater copepod will be presented. The filter feeding calanoid copepods, *Calamoecia lucasi* and *Boeckella delicata*, occur in the North Island of New Zealand. The carnivorous cyclopoid copepod, *Mesocyclops* cf. *leuckarti*, has a worldwide distribution and is relatively common in NZ lakes. All three species of copepod were collected from Lake Rotomanuka, and cultured in the laboratory. Using the reference toxicant pentachlorophenol (PCP), the sensitivity of two different life stages was compared for the three species. Naupliar stages were exposed until they moulted into the first copepodite stage, this transition being the test end-point. Time taken to moult and naupliar survival were recorded. Gravid females bearing their first clutch of eggs were exposed to PCP until three egg clutches were produced. Female survival and naupliar production were recorded for the duration of the test.