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NATURAL VARIABILITY OF ZOOPLANTON AND PHYTOPLANKTON IN OUTDOOR AQUATIC MICROCOSMS

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I dedicate this thesis to
Professor J. Schwoerbel
with respect and gratitude

TABLE OF CONTENTS

Index of Figures

Index of Tables

Abbreviations

ANOSIM	Analysis of Similarities
CLASSIC	Community Level Aquatic System Studies Interpretation Criteria
cov	Coefficient of variation
EAC	Ecologically acceptable concentration
EC	European Community
EC50	Effective Concentration (50%)
E.P.A.	Environment Protection Agency
Fig.	Figure
HARAP	Higher-tier Aquatic Risk Assessment for Pesticides
MDS	Multidimensional Scaling
n.a.	Not applicable
NOEC	No Observed Effect Concentration
PEC	Predicted Environmental Concentration
PRC	Principal Response Curve
SCP	Scientific Committee on Plants
sd	Standard deviation
Tab.	Table
TER	Toxicity: Exposure Ratio

Preface: Field Studies in Aquatic Ecological Risk Assessment

Field studies in aquatic ecotoxicology are gaining more and more importance in the overall ecological risk assessment of xenobiotics (Maund *et al.*, 1999). The purpose of these field studies is to evaluate the potential for unacceptable effects of e.g. plant protection products, to ecosystems at the population or community level of organization. The resulting data are used for a more indepth ecological risk assessment, where the toxicity of a chemical is compared to the probability of exposure under natural environmental conditions.

In the European Union, registration requirements for pesticides are laid down in European Council Directive 91/414/EEC (EC Directive, 1991). Directive 91/414/EEC provides a tiered testing sequence for the production of ecotoxicological information. Thus field studies are conditionally required when triggered by results of acute and chronic laboratory studies. In the United States, regulatory testing for the environmental fate and effects of plant protection products is conducted under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and the Toxic Substances Control Act. FIFRA requirements include aquatic toxicity information, to be generated under a tiered testing sequence.

While in Europe, aquatic multispecies tests are increasingly used to assess the fate and effects of pesticides, the U.S. Environmental Protection Agency (EPA) dropped requirements for aquatic mesocosm studies for pesticides in 1992, due to difficulties to interpret these studies, a policy decision, which has been discussed controversially (Clements & Kiffney,1994; Shaw & Kennedy, 1996). In practice, data are still submitted to U.S. E.P.A. as supporting information.

Exclusive reliance on single species toxicity tests for prediction of environmental safety has been criticized for years (e.g., Cairns, 1983; Pontasch, 1989). One opinion is that uncertainty in environmental risk assessment can be substantially reduced by optionally incorporating tests that directly examine those properties of ecosystems that are object of protective legislation (e.g., Perry *et al.*, 1988; Huber, 1997; HARAP, 1999).

Recently, European authorities considered that the results of field trials might be used in regulatory risk assessments without the application of an uncertainty factor (HARAP, 1999) or with a factor close to one (CLASSIC, 2001, Scientific Committee on Plants, 2000) provided that the studies have been properly designed, executed, analyzed and interpreted. However, in order to fulfill these requirements, strong background data for aquatic model ecosystems are needed to properly set up a study, to define meaningful endpoints and to select adequate statistical procedures.

The present work aims to contribute to develop these background data and assist in the actual discussion of these issues by assessing the natural variability of aquatic outdoor microcosms.

1 INTRODUCTION

1.1 ECOLOGICAL RISK ASSESSMENT PROCEDURE

Disturbances of aquatic ecosystems may be caused by human activities like habitat destruction, or by toxic substances entering the water body via industrial effluents, accidental spills or use of plant protection products. Plant protection products may enter aquatic ecosystems by spray-drift, leaching or surface runoff from nearby treated agricultural land.

Since risk is a function of hazard and exposure, one part of ecological risk assessment seeks to estimate the exposure of non-target organisms by estimating the amount of pesticide, that is likely to reach freshwater ecosystems on the basis of its physicochemical properties and its use pattern (PEC, predicted environmental concentration). On the other hand, hazard assessment aims to detect the threshold amount of pesticide that does not cause harm to aquatic ecosystems, usually described as NOEC (No-Observed-Effect Concentration), the concentration at which no effect is observed compared to the control system. Additionally, the concept of the Ecologically Acceptable Concentration (EAC) is currently under investigation for ecosystem testing. Exposure at the EAC would result in some transient effect, which is fully reversible in the ecosystem (HARAP 1999, CLASSIC 2001). The toxicity:exposure ratio (TER) gives the quotient of both, hazard and exposure estimates and provides a convenient expression of quantitative risk, which can then be used for regulatory decision-making (e.g. Uniform Principles, decision-making criteria).

For pesticides, testing requirements include the generation of acute and chronic toxicity data with aquatic invertebrates, fish, algae and aquatic macrophytes, as well as bioaccumulation studies for lipophilic (log P>3) chemicals (first tier). Effect and no-effect concentrations from these studies are then compared to estimates of predicted exposure concentrations to generate risk quotients, i.e. the toxicity to exposure ratio already mentioned above (TER). According to the Uniform Principles, further evaluation, for example by outdoor microcosm tests is required, if the TER for acute toxicity and exposure is <100, or for chronic toxicity and exposure is <10 (EC, 1997).

As the preliminary risk characterization is based on laboratory tests which usually do not simulate degradation of the active substance, it is regarded to be very conservative in the sense that risks to the environment from normal uses of individual compounds should be lower than predicted. A more realistic insight into effects caused by a compound under investigation is obtained by studies conducted in aquatic indoor or outdoor model ecosystems because exposure and responses of the organisms are more similar to natural systems.

Several types of outdoor aquatic model ecosystems have been described (Graney *et al.*, 1994; Hill *et al.*, 1994). They vary in size and design. Water volume of model ecosystems varies from 1 to 15 m^3 (microcosms) and goes up to 500 m^3 (mesocosms). The design of the testing facility determines the level of replication available for a study. Mainly two different systems exist.

A testing facility may consist of a set of separate identical tanks, which are all connected to a mixing tank. In this case, homogeneous starting conditions are gained by water circulation via the mixing tank. Alternatively, a testing facility may consist of a big basin as a "host" for identical enclosures. In this case enclosures are equivalent to isolated tanks and are placed into the host basin shortly before test start to provide similar starting conditions, which can also be enhanced by additional water circulation.

Aquatic model ecosystems are regarded as surrogates for existing ecosystems and are considered to mimic real-world conditions (Howick *et al.*, 1992). They are also seen as model ecosystems, which take a position in between laboratory and real-world conditions, but adequately represent "natural" multi-trophic systems (Belanger, 1997). In any case, model ecosystems should be self-sustaining, "mature" or equilibrated (Seitz, 1994; Howick *et al.*, 1992). These properties may be difficult to measure and are probably never fully achieved.

The problem is partially circumvented in ecotoxicology by comparing ecosystem function and structure of disturbed (treated) to undisturbed (control) microcosms. Usually, a set of microcosms is used, which is built, inoculated and maintained in such a way that microcosms develop as similarly as possible. Ecosystem structure and function are investigated before and after application of the test material, and measurements in the treatment groups are compared with those in the control.

However, all microcosm/ mesocosm systems are subject to an inherent temporal and spatial variability. It was a major goal of the present work to critically discuss the available methods to minimise the error related to this variability and to contribute to an adequate interpretation of the data obtained in microcosm studies.

1.2 WHAT ARE WE TRYING TO PROTECT?

Different opinions exist about the goal, which is served by ecological risk assessment, i.e. *what exactly should be protected*. According to Directive 91/414/EEC (EC Directive, 1991), the "safety of a plant protection product to the environment" needs to be demonstrated. In this case, the environment concerned is the "edge of the field", i.e. small surface waters, ditches etc. directly adjacent to a treated field (while the EU Water Framework Directive covers quality requirements for large bodies of surface water). The annexes to Directive 91/414/EEC specify a tiered battery of test systems by which the lack of "unacceptable effects" must be demonstrated. However, the borderline between unacceptable and acceptable impacts of pesticides to the aquatic environment remains nebulous.

In a more general context, the "integrity of an ecosystem" or "ecosystem health" is mentioned as a goal of environmental protection. The definition of the term "ecosystem integrity" implies underlying ecosystem properties, such as ecosystem stability, resilience, or recovery (Cairns, 1992).

In environmental toxicology, ecosystem stability is seen as a property of ecological systems and defined as the ability of the ecosystem to return to an equilibrium state once it has been disturbed (Dewey *et al.*, 1994; Johnson *et al.*, 1994). This stability-recovery paradigm is also part of the U.S. and E.U. risk assessment framework.

The capacity of an ecosystem to recover after perturbation by xenobiotic contaminants depends on many factors, including the persistence and bioavailability of the toxicant, the life-history attributes of organisms, and the proximity and location of recolonization sources (Fairchild *et al.*, 1992). The speed at which an ecosystem returns to equilibrium after a perturbation is a measure of its resilience; a highly resilient system would thus return to equilibrium rapidly after a disturbance (DeAngelis, 1992). Following this stability-recovery paradigm, a full recovery would have to be demonstrated in ecotoxicological field tests in order to prove the "ecologically acceptable concentration" (EAC), as defined in the CLASSIC workshop (CLASSIC, 2001).

However, the stability-recovery paradigm has also been criticized in ecology and ecotoxicology. For example, Matthews *et al.* (1996) state that the equilibrium model of community organization, which asserts that species abundances will be stable through time as a result of biotic interactions, and that the system will return to the equilibrium point following a disturbance, is rarely applicable. In their opinion, communities are unique products of their etiologies. The implication of this hypothesis on environmental toxicology would be that almost all environmental events leave lasting effects. Also according to Connell and Sousa (1983), non-equilibrium conditions appear to be the rule rather than the exception in many ecosystems. If these hypotheses hold true, the political ecological goal of preservation of natural systems necessarily would involve the paradox that *we seek to preserve systems that change constantly*.

This non-static character is not only a property of the ecosystems which we seek to protect. It is also in the property of the microcosm test system, which is employed as a model for higher-tier risk assessment. This stresses the need for the quantification of the natural temporal variability in microcosms, i.e. the seasonal or year-to-year variability.

In the present study, the natural or ´system-inherent´ temporal variability of zooplankton and phytoplankton communities was investigated in 12 microcosms over a time period of 5 months. Year-to-year variability of the zooplankton community was calculated for three subsequent years. Temporal variability is specifically addressed in chapter 3.1.4 and is also discussed in the next chapter.

1.3 NATURAL VARIABILITY

Natural communities are characterized as being
(a) spatially heterogeneous at any scale of resolution (Sousa, 1984).
(b) dynamic systems, with population densities and relative abundances of species changing with time. Even local extinctions of populations of certain species from natural communities are commonplace (Connell *et al.*, 1983).

Spatial heterogeneity is a characteristic feature of natural systems. It is defined as the complexity and variability of system properties (e.g., nutrients, species abundance, patch mosaics) in space. A mosaic of patches due to spatial discontinuities in the distributions of populations can be observed in ecological systems at all scales (Sousa, 1984; Pickett & White, 1985). However, variability can be different at different scales (Jackson, 1994). Spatial heterogeneity may be related to heterogeneous environmental conditions as well as to population dynamics.

Traditionally, competitors found separated in space are interpreted as a result of species specialization within spatially heterogeneous environments. More recently, it has been shown that the spatial metapopulation structure also arises self-generated on an intrinsically homogenous substrate (Hassel *et al.*, 1994).

At the temporal scale, aquatic environments are inherently and continuously variable. Impacts of environmental fluctuations upon structure and function of the affected community can be identified on different time scales. For example, this is the case for phytoplankton response to changes of light conditions, as rapid transitions of phytoplankton to shade for a few seconds caused by the passage of clouds, alternations between day and night, day-to-day variations in cloud cover, or changes of the incident angle of sun rays due to seasonal variability (Reynolds, 1990). Seasonal variability of external factors, mainly light and nutrient availability, forms the basis for seasonal succession of plankton in fresh waters, as implemented in the PEG-model (Sommer *et al.*, 1986).

Spatial and temporal variability in outdoor microcosms have caused considerable difficulty to risk assessors in the past. Interpreting the ecological significance of effects measured in mesocosms was stated to be complicated by the complexity and variability of ecosystem studies in general (Clement and Kiffney, 1994), and due to ecological factors influencing the outcome of perturbations under field conditions (Maund *et al.*, 1997).

In the present work, spatial and temporal variability of zooplankton and phytoplankton communities were investigated in aquatic outdoor microcosms. In order to achieve a high level of replication, 12 ponds were used to estimate the spatial variability (inter-replicate variance). Temporal variability was investigated for the eight sampling events during 1997. Moreover, zooplankton samples from the years 1996, 1997 and 1998 were compared to describe the year-to-year variability.

The investigation of natural variability in the present study was aimed at defining a normal operating range for the testing facility used. Definition of such a system-inherent standard level of variability would allow a proper choice of replication. The observed system-inherent variability of zoo- and phytoplankton communities is summarized and discussed in chapters 3.1, 3.2, 3.5 and 3.6.

1.4 ECOSYSTEM DISTURBANCE

According to Pickett and White (1985) a disturbance is any relatively discrete event in time that disrupts ecosystem, community or population structure and changes resources, substrate availability, or the physical environment. They distinguish two kinds of disturbance, destructive events and environmental fluctuation, and classify disturbances as endogenous or exogenous, i.e. factors responsible for community change to be found within the community or outside the community, respectively.

Different theories exist about how ecosystems react to disturbance and how species diversity or richness are affected (e.g., Pickett & White, 1985, Lawton & Brown, 1993; Vitousek & Hopper, 1993; Pimm, 1984; Tilmann & Downing, 1994; Sousa, 1984):

(a) The diversity-stability hypothesis holds that more diverse ecosystems are more likely to contain some species that can thrive during a given environmental perturbation and thus compensate for competitors that are reduced by that disturbance. This view thus predicts that biodiversity should promote resistance of ecosystem function to disturbance.
(b) In contrast, the species-redundancy hypothesis asserts that many species are so similar that ecosystem functioning is independent of diversity as long as major functional groups are represented.
(c) The intermediate disturbance hypothesis states that species richness will be greatest in communities experiencing some intermediate level of disturbance.
(d) One more hypothesis relates disturbance frequency to species richness, saying that where disturbance recurs more frequently than the time required for competitive exclusion, richness should be maintained.

Generally, the diversity-stability hypothesis seems to form the basis for interpretation of most ecotoxicological field studies. The use of diversity indices in ecological risk assessment is based on the assumption that the number of taxa and the evenness with which individuals are distributed among the taxa are both reduced in stressed aquatic communities, resulting in lower richness and diversity index values. However, this might not always be true as can be seen from hypotheses (b), (c) and (d). Thus, it remains unclear whether species diversity really is a meaningful endpoint for ecosystem integrity.

A change in natural exogenous factors such as wind, light and precipitation may directly or indirectly impose changes to communities in the aquatic environment, i.e. by wind-induced mixing, light-limitation or surface run-off events.

One aim of the present thesis was to investigate and quantify the impact of such exogenous factors on aquatic communities. Imposing exogenous ´natural´ disturbance factors to three groups of ponds was expected to affect community structure of aquatic communities in the treated groups when compared to the control group. In the present work, three exogenous factors were selected to simulate natural disturbance: light, mixing regime and surface run-off. These ´natural´ disturbances were then contrasted with an ´anthropogenic disturbance´, the contamination with the well-studied insecticide diazinon. The experimental approach for all four treatments is outlined in the following paragraphs. Results are presented and discussed in sections 3.3, 3.4, 3.7 and 3.8.

Light/ Shadow

Habitats of aquatic organisms are subject to persistent environmental variation, which is mostly brought about by solar energy, either directly (e.g., variation with time of day or season) or indirectly (changes in atmospheric pressure, wind, evaporation etc.). Solar energy is of special importance for phytoplankton, which is not only exposed to temporal, but also to spatial variability of light conditions as the light gradient varies within the water column. Different phytoplankton species show different ability to respond to changing light conditions (e.g., Reynolds, 1990). Biological responses to changing light conditions in the water column include migration of motile algae to avoid photo-inhibition or to increase the photosynthesis rate as well as chromatic adaptation to exploit particular habitats.

In the treatment group SHADOW, the extent to which shading of microcosms would induce shifts in community composition of phytoplankton, and indirectly also zooplankton, was investigated in comparison to control microcosms.

Mixing/ Turbulence

Changes in phytoplankton community structure may be a result of changes in the physical structure of the water column caused by externally imposed (allogenic) physical perturbations, e.g. temporary wind-induced mixing. In frequently mixed lakes, autogenic succession (i.e. community response to a stable physical environment) is frequently overridden by abrupt allogenic alterations in the physical environment (Carrick *et al.*, 1993; Schelske *et al.*, 1995; Agbeti, 1997). This is consistent with the observation that changes in species composition of phytoplankters can be the consequence of reduced turbulence, which was also reported for microcosms (Lundgren, 1985; Eppley, 1978).

Therefore, in the treatment group TURBULENCE, the extent to which turbulent mixing would affect patterns in plankton distribution, plankton dynamics and finally community structure of phytoplankton and zooplankton was investigated in comparison to the control group.

Runoff/ Turbidity

Turbidity is a naturally occurring disturbance factor for aquatic ecosystems. Soil particles may enter the aquatic ecosystem via surface runoff and transiently increase turbidity in the water column. The runoff scenario is frequently used in aquatic ecotoxicology for regulatory testing of plant protection products (Hill *et al.*, 1994). Further, research has shown that turbidity may also arise from suspended sediments which cause significant changes in community composition of zooplankton (Koenings *et al.*, 1990; Kirk *et al.*, 1990, Jack *et al.*, 1993).

Besides soil particles, nutrients or contaminations with plant protection products can enter aquatic ecosystems via surface runoff in the vicinity of land with intensive agriculture. Interaction of nutrient loading and pesticide application were studied in detail by Van Donk (1995), Brock (1995) and Cuppen (1995) and were not included in the present work.

However, as is has been shown that suspended particles at a certain concentration disturb aquatic communities, it is important to know whether soil particles applied to simulate a “run-off event” in ecotoxicological microcosm studies *per se* have the potential to cause changes in community structure of phyto- and zooplankton and thus prevent adequate interpretation of pesticide effects.

In the present work, the extent to which two simulated runoff events and the consequent transient turbidity in the treatment group RUNOFF would affect community structure of phytoplankton and zooplankton in the treatment group was investigated in comparison to the control group.

Diazinon

In contrast to the above ´natural disturbance factors´, one group of model ecosystems was exposed to an ´anthropogenic disturbance factor´, i.e., a pesticide. Effects of the pesticide on the microcosm were compared to effects caused by naturally occurring exogenous disturbance factors.

The broad-spectrum organophosphate insecticide diazinon was chosen as test substance because a large data-base is available for this substance. Diazinon was tested in laboratory and field studies and has been shown to be highly toxic to many aquatic invertebrates including crustaceans, molluscs and aquatic insects (Giddings, 1992). It is slightly toxic to algae. Diazinon disrupts acetylcholine signal transduction in neuronal and neuro-muscular synapses. The compound acts as an inhibitor of acetylcholine-esterase by its stable covalent binding to a serine-moiety in the active center of the enzyme.

In the present work, diazinon was applied at a concentration which was expected to result in effects on zooplankton and insects as well as having secondary effects on phytoplankton. The test concentration was chosen so that recovery of the communities after degradation of the test substance was possible.

1.5 WHAT CONSTITUTES AN EFFECT?

Any disturbance of an ecosystem will result in some reaction of the communities or the physicochemical properties of the system, which might or might not be measurable. For ecotoxicological testing, it is crucial to address these reactions as measurement endpoints when setting up a study. In theory, two types of endpoints are distinguished (Suter, 1990):

(a) assessment endpoints, i.e. formal expressions of the actual environmental value that is to be protected
(b) measurement endpoints, i.e. the expression of an observed or measured response to the impact.

Definition of assessment endpoints in the risk assessment procedure involves scientific, political and economic judgements. As mentioned above, possible endpoints might be “maintaining ecosystem integrity” “maintaining biodiversity” (no species extinction), “maintaining functionality” (biomass production), “protecting the habitat from degradation” or “protecting rare or endangered species” (Maund *et al.*, 1997).

The choice of measurement endpoints in aquatic field studies largely depends on the usage pattern of the plant protection product to be tested (e.g., herbicide, insecticide) or the properties of a certain chemical. Measurement endpoints usually include overall functional characteristics of ecosystems (nutrient turnover, pH, dissolved oxygen etc.). However, functional characteristics have been criticized as not always being sensitive ecosystem stress indicators (Woin, 1998). Measurement endpoints such as population densities and community composition of zooplankton, phytoplankton, benthic invertebrates are investigated in most micro-/ mesocosm studies, while periphyton and microorganisms are measured less frequently (Brock and Budde, 1994). However, all mentioned structural measurement endpoints suffer from undesirable variability in model ecosystem studies, which may result in decreased sensitivity (e.g., Amman *et al.,* 1997).

Consequently, natural variability of the ecosystem can potentially mask effects of a test chemical or other human impact. This has to be taken into consideration when planning an outdoor microcosm study, selecting the endpoints to be observed and when choosing the statistical methods to evaluate measurements.

The effect of natural variability on the sensitivity of statistical tools and thus its influence on selecting the appropriate endpoints is discussed in chapters 3.2, 3.4, 3.6 and 3.8 of the present work.

Currently available guidance recommends the study of community composition or taxonomic richness on the lowest practical taxonomic level for most groups of organisms (SETAC, 1992a, SETAC, 1992b). Measurement of community responses to stress, however, becomes more difficult as system complexity increases and the approach causes considerable taxonomic problems and financial effort, which might not always be justified. Two questions remain open in the actual discussion (Maund *et al.,* 1999, Warwick *et al.*, 1990). First, do all species matter for the function of ecosystems or is there enormous redundancy (functional and structural redundancy)? Second, do all individuals need to be identified to the species level or are there cases where taxonomic resolution at a higher hierarchy level is sufficient?

There is evidence for functional redundancy, for example as reported by Kersting *et al.* (1997). In field ecosystem studies, the "dissolved oxygen-pH-alkalinity-conductivity syndrome" was shown to be a sensitive indicator for temporary effects on ecosystem metabolism. Functional redundancy, i.e. replacement of one organism by another within a functional unit, can mask structural changes to a certain extent.

Considerable redundancy in the species that characterize the community composition was shown for the marine macrobenthos by Clarke and Warwick (1998). If species within a taxonomic group reacted in similar ways to disturbances, analysis at taxonomic levels higher than that of species revealed patterns very similar to the full species analysis (Clarke and Warwick, 1998; Warwick, 1993). Gradients of change in community structure were shown to be still detectable or even to become more obvious at higher taxonomic resolution levels than the species level (Olsgard *et al.*, 1997). However, aggregation levels that do not alter the perceived pattern of impact may not be the same for different communities in the marine macrobenthos (Somerfield & Clarke, 1995). Further studies of taxonomic resolution are needed in different communities and in different environments before any general recommendations regarding optimum taxonomic levels can be given (Olsgard *et al.*, 1997).

In the present work, the question of taxonomic resolution was addressed for the zooplankton and phytoplankton communities using graphical and multivariate statistical tools, see chapter 3.3.8 and 3.7.8.

1.6 OBJECTIVES

The investigation of community structure and ecosystem function – be it in research or in regulatory testing - depends on sophisticated test design and statistical tools which enable meaningful data interpretation (HARAP, 1999, CLASSIC, 2001).

Problems encountered in ecological research cannot be avoided in ecotoxicological practice. There is a need for basic research to properly address the ecological problems of temporal, spatial and functional variability in order to define the limits of ecologically acceptable impacts of human activity. The present work is intended to contribute to this area of research by its objectives already mentioned in previous paragraphs, which can be summarized as follows.

(1) Variability of population densities in outdoor microcosms occurs naturally at a temporal as well as at a spatial scale. In ecotoxicological field testing, it has caused considerable confusion in the context of endpoint selection and data interpretation. This might be overcome by a clear definition of a normal operating range for the testing facility used, which would allow proper enumeration of replicates. Therefore, the key questions to be answered in the present thesis are:

What is the range of the system-inherent spatial and temporal variability of freshwater communities in outdoor microcosms?

Is it comparable to variability in natural ecosystems?

How should test designs be optimized in order to minimize errors related to inherent variability?

(2) The level of replication needs to be related to the population densities found. Some taxa occur at very low densities while others show consistently high densities. Low densities and zero-counts pose statistical problems and difficulty in the interpretation. These difficulties may be overcome by a high number of replicates or by grouping of data on higher taxonomic levels. Consequently, it was asked:

What is the relevance of taxonomic resolution for accurate data interpretation of zoo- and phytoplankton community data?

Is taxonomic identification down to the species level needed for every individual or is redundant information being produced?

(3) One of the key questions in ecotoxicology testing is the selection of adequate and efficiently measurable endpoints. Choice of endpoints is related to the kind and extent of reaction of communities to disturbances. Reactions to different kinds of disturbances were investigated in the present work, posing the following questions.

To what extent is the system-inherent natural variability under investigation influenced by natural disturbance factors?

How can effects of plant protection products best be detected and interpreted?

To what extent can effects of natural disturbances mask treatment effects in outdoor studies with plant protection products?

2 MATERIALS AND METHODS

2.1 MICROCOSMS

2.1.1 Definition

By definition, aquatic model ecosystems with less than 15 m^3 are called microcosms (SETAC guidance document, 1994). In the present study, microcosms are also referred to as ´tanks´ or ´ponds´.

2.1.2 Guidelines

Requirements for the European risk assessment procedure including Aquatic Model Ecosystem Studies are given in EU Council Directive 91/414/EEC and the related Guidance Document on Aquatic Toxicology (EC Directive, 1991; EC Guidance Document, 2000). Practical guidance for conducting and interpreting aquatic outdoor studies is given in SETAC guidance documents (SETAC, 1992a; SETAC, 1992b) and by the summaries of two expert meetings held in 1998 and 1999 (HARAP, 1999; CLASSIC, 2001). Aquatic Model Ecosystems and their use in ecological risk assessment were also summarized in two books edited by Graney *et al.* (1994) and Hill *et al.* (1994).

2.1.3 Testing facility

The 28 aquatic microcosms used for this study were located at the outdoor test site of Novartis Crop Protection AG (now Syngenta) in 8260 Stein, Switzerland (Figure 2-1). Each microcosm had a diameter of 300 cm, a sediment layer of 15 cm, and a water column of 120 cm, resulting in a volume of about 10 m^3. The microcosm walls were made of black poly-ethylene (HD-PE). All microcosms were connected to a mixing tank, allowing an exchange of water and plankton between the microcosms via the mixing tank. A natural pond, established in 1995, served as a source for water and plankton for the mixing tank (Figure 2-2).

2.1.4 Inoculum

All microcosms were filled with 10 cm of topsoil from a nearby field with known agricultural history. The topsoil was covered with a 5 cm-layer of sediment from a natural pond. The sediment served as a source for invertebrates, zoo- and phytoplankton. It was therefore not sieved and handled with care. Well water and pond water was used to fill the microcosms. Organisms were also introduced by connecting the microcosms to the mixing tank and the natural pond, respectively. Continuous circulation of the water phase was allowed for 7 months before test start in 1997.

Macrophytes were planted about 7 months before test start. Sprouts of *Myriophyllum verticillatum, Chara spec. and Potamogeton crispus* were obtained from a commercial supplier (Hydrobaumschule Alfred Forster, CH-3207 Golaten, Switzerland). Prior to test start, the percent cover of the different species was determined.

2.1.5 Water circulation

During the pre-exposure phase, full circulation of the water was allowed between all subsystems (Figure 2-2). Two days before the exposure phase started, the microcosms were disconnected from the mixing tank and pond and remained isolated throughout the exposure and post-exposure phases.

Figure 2-1. Microcosms, Syngenta AG, Stein, Switzerland.

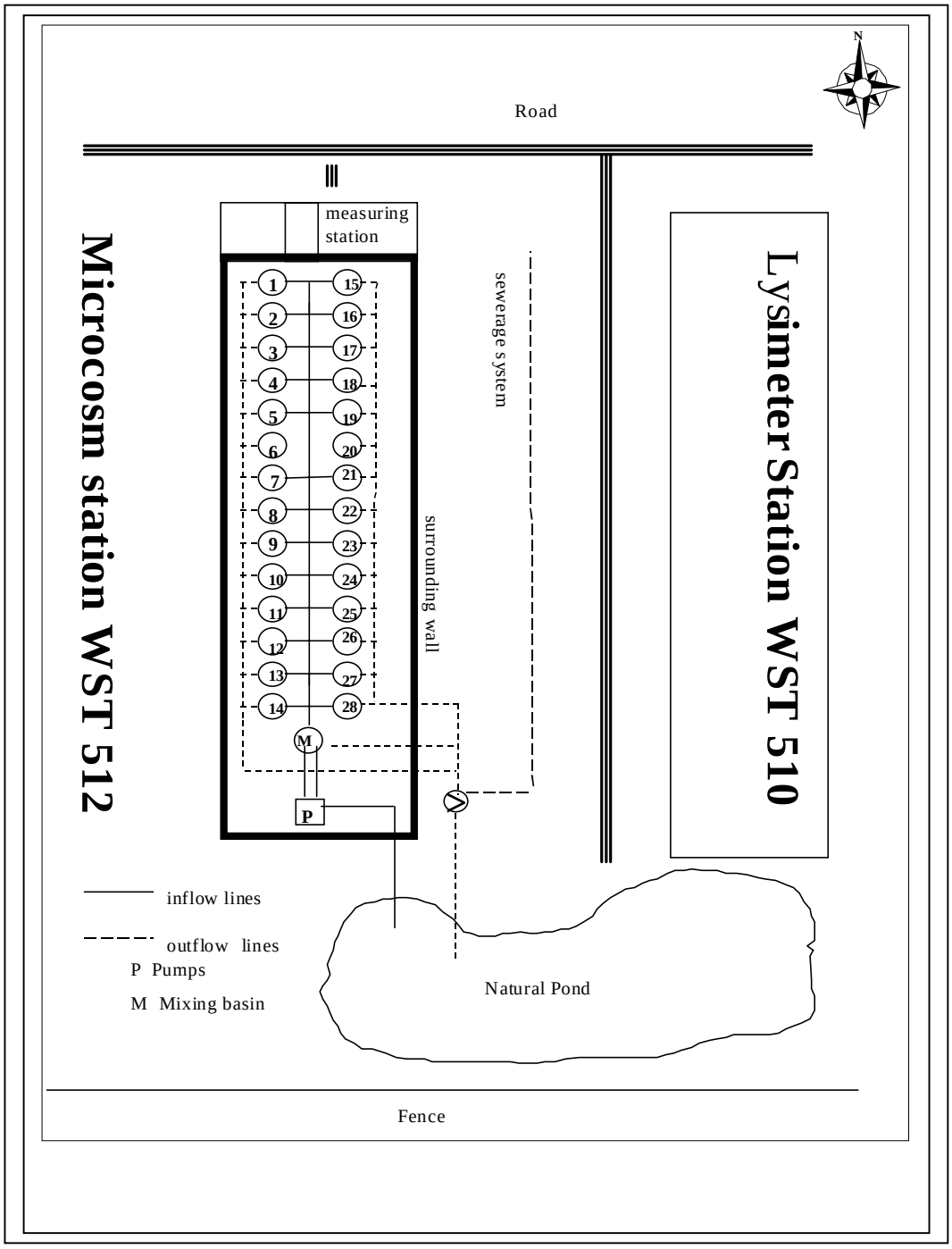


Figure 2-2. Testing facility (a) and schematic view of tank connection (b).

2.2 STUDY DESIGN

2.2.1 Investigation of Natural Variability in the CONTROL group

Quantification of natural variability was addressed by investigating a set of 12 microcosms from June through October 1997. This set of microcosms is further on addressed as "CONTROL group". The comparatively high number of replicates for the control group was chosen in order to achieve robust and statistically relevant background data.

All CONTROL ponds had very similar starting conditions as they were filled with the same substrate and inoculum. Moreover, the water was circulated between the ponds and a mixing pond for a period of 7 months before the observation period started.
The observation period was characterized by three phases, the pre-exposure phase, the exposure phase and the post-exposure phase. As described above, during the pre-exposure phase, all ponds were connected to a mixing pond and water was permanently circulated within the system. During the exposure and post-exposure phase, the microcosms were disconnected from the mixing system.
Permanent water circulation during the pre-exposure phase was expected to cause homogeneous conditions and thus lead to a low natural variability within the CONTROL group. Disconnecting the ponds from the mixing system and thus creating isolated systems was expected to result in an increased system-inherent variability within the control group during the course of the second and third phase of the study.

Emphasis was put on the investigation of spatial and temporal variability of structural parameters within the CONTROL group. Temporal variability was determined on the basis of 8 sampling events. Spatial variability was defined as the variability measured within a group of replicate microcosms on each individual sampling occasion. Various measures were used to describe the natural variability: the coefficient of variation, the precision level related to the standard error, the mean:variance relationship, the relationship of zero-count to mean, the Shannon-Wiener diversity index and dominance curves.

The terms "natural variability" and "system-inherent variability" were synonymously used to describe the naturally occurring variability in the CONTROL group. The variability in the CONTROL group was later on compared with the variability occurring in the four treatment groups where specific, "natural" or anthropogenic disturbance factors were imposed.

2.2.2 Disturbance Factors

Imposing exogenous disturbance factors to three groups of ponds was expected to result in changes of community structure of aquatic communities in the treated groups when compared to the CONTROL. In the present work, three exogenous factors were selected to simulate natural disturbance: light, mixing regime and turbidity and in contrast to the natural disturbance, an insecticide was applied to simulated anthropogenic disturbance. This was achieved by

a) constantly changing the light conditions by shading a group of 4 microcosms for a period of four weeks (referred to as treatment SHADOW),
b) constantly mixing the water-column in 3 microcosms for a period of four weeks (referred to as treatment TURBULENCE),

c) simulation of two surface run-off events within the period of 4 weeks in four microcosms (referred to as treatment RUNOFF).
d) application of the insecticide diazinon to 4 microcosms on test day 0, to achieve a nominal initial concentration of 16µg/L in water (referred to as treatment DIAZINON).

2.2.3 Light/ Shadow

Light conditions were manipulated in four of the microcosms by installing white plastic tents. These had a diameter of 300 cm and a height of 200 cm. The light intensity was thereby permanently reduced during the 4-week exposure period. Light intensity was measured twice during the study period at the water surface at four different spots of each tank, on the water surface at the outer border of the ponds.

Table 2-1 shows that the exposure to sunlight was reduced in the shaded ponds to about 50-60%. Distribution of light intensities was homogenous on overcast days (see August 8). On sunny days, about 20% of the water surface of the shaded ponds was exposed to direct sunlight. Additional measurements were therefore performed also 50 cm from the border which showed that the major part of the water surface had homogeneous light conditions.

Table 2-1. Light intensity measured on the water surface.

	July 24 (14:00) ´sunny´ E_d (0) ($\mu Em^{-2}s^{-1}$)	**August 8 (15:00) ´overcast´ E_d (0) ($\mu Em^{-2}s^{-1}$)**
Pond 1, South	800 (a)	195
Pond 1, East	665	186
Pond 1, North	626	202
Pond 1, West	775 (a)	206
Pond 2, S	800 (a)	291
Pond 2, E	760	243
Pond 2, N	680	259
Pond 2, W	800 (a)	265
Pond 15, South	780 (b)	308
Pond 15, East	760	254
Pond 15, North	660	297
Pond 15, West	780 (c)	303
Pond 16, S	800 (d)	370
Pond 16, E	750	280
Pond 16, N	600	331
Pond 16, W	800 (e)	325
Control	1770	675
Control	1930	620
Control	2000	625
Control	1980	625

Note: During sunny days, about 20% of the surface of the treated ponds were exposed to direct sunlight. Values mentioned under (a)-(e) were measured in the middle of the ponds, while light intensities at the outer border of the pond were 1900 (a), 1700 (b), 1920 (c), 1800 (d) and 1980 (e). Light intensity was measured using a Quantum Meter LI 190 SB (400-700 nm) and a LI 185 B sensor of Bachofer, D-Reutlingen.

2.2.4 Mixing/ Turbulence

Permanent turbulence throughout the 4-weeks exposure period was created in four microcosms by installing 2 pumps per microcosm. Turbulence was created in the horizontal as well as in the vertical direction as water was sucked from about 25 cm above the sediment surface and pumped to a level of about 15 cm below the water surface. In this treatment group, one of the four microcosms developed a leak during the exposure phase and was excluded from the study.
Turbulence was measured using a flowmeter as described in Biehle (1996). Impulses counted were transformed into cm/s. The resulting values (m/s) measured on one occasion in all three ponds are shown in Table 2-2 and were found in a range of 10-82 cm/s. The measurement locations are displayed in Figure 2-3. Legend: Pump (P) and measurement locations (1) – (6).

Table 2-2. Current velocities

	Current velocities (cm/s)		
Location	pond 3	pond 7	pond 17
1	82	74	82
2	82	77	82
3	10	10	10
4	12	14	13
5	11	10	10
6	21	40	25

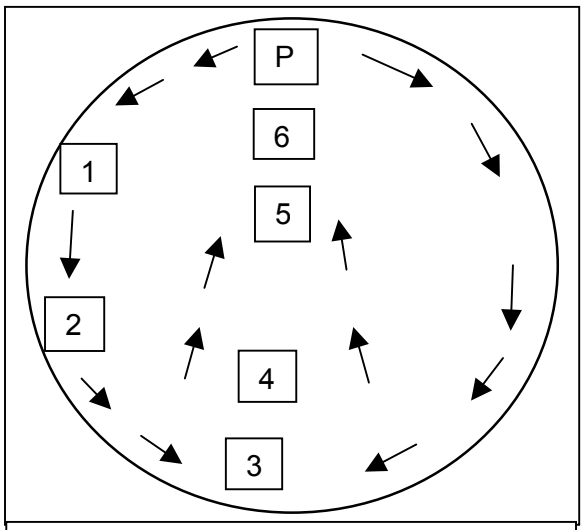


Figure 2-3. Turbulence Measurement

Legend: Pump (P) and measurement locations (1) – (6).

2.2.5 Surface Runoff

The potential disturbance of the plankton community by application of fine particles was investigated in the present work by simulating two surface runoff events and the consequent import of soil particles into the microcosms. In order to exclude physicochemical interactions of the substrate with the water column and to avoid import of nutrients or organic matter into the microcosms, inert washed and finely ground quartz sand was used instead of soil.

The amount of particles added to the microcosms was chosen according to reports of surface run-off events in the area of Basel, Switzerland, as described by Seiberth (1997) and Schaub (1997), where surface runoff (4.88 L/m^2 and 0.97 kg/m^2) resulted in a sediment load of 199 g/L. With an anticipated dilution factor of 1000, this corresponds to a load of about 200 mg/L sediment in the investigated microcosms. In the present study, surface runoff was simulated by application of 2000 g of quartz sand to each of a group of 4 microcosms (10 cbm water), resulting in a load 200 mg/L. The quartz sand was applied twice during the exposure phase, i.e. on day 0 and day 14 and consequent visible turbidity remained for about two days.

The exact particle size distribution of the applied quartz sand is given in Table 2-3. Particle size distribution was measured at Novartis Crop Protection, Basel using a CILAS 715 particle size detector. About 5% of the applied sand had particle sizes below 2 µm and 50% of the sand had particle sizes below 48 µm. The most frequently encountered particle size class was 128 µm (26% of the total volume). The maximum particle size measured was 192 µm.

Table 2-3. Particle size distribution of quartz sand

Particle size (µm)	<1	<2	<3	<12	<48	<96	<128	<192
Of total (%)	1.9	5.1	7.9	25.8	51.4	65.4	91.3	100

2.2.6 Diazinon

Diazinon has been tested in laboratory and field studies and has been shown to be highly toxic to many aquatic invertebrates (Table 2-4), including crustaceans and aquatic insects. It is slightly toxic to algae. Diazinon was tested in a mesocosm trial in 1992 (Giddings, 1992). The corresponding report served as the basis for choosing the appropriate diazinon concentration for the present test. In Gidding´s study, diazion was applied 6 times during a 2-month period using spray-drift and surface run-off simulations. Measured maximum concentrations were 2.4, 4.5, 9.2, 16 and 33 µg/l. Diazinon was reported to have half-life of about 5-12 days in the cited study. Plants (macrophytes, phytoplankton and periphyton) were generally insensitive to diazinon, except for Bacillariophyceae which showed consistent reductions in abundance at the highest treatment level.

However, many zooplankton and macroinvertebrate taxa were affected at levels of 9 to 33 µg/l. A few taxa, notably *Cladocera* and *Trichoptera*, were affected at even lower treatment levels. Most taxa recovered during the post-treatment period to control levels.

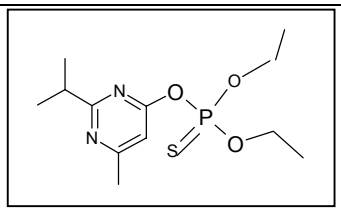
The application rate was chosen so that effects on primary and secondary consumers of the aquatic community were likely to occur, but a recovery of affected populations could be expected. A single application was made on July 11, 1997 to microcosms No. 4, 13, 14 and 21. To each microcosm, 160 mg of diazinon were added in order to achieve an initial nominal concentration of 16 µg/l. The diazinon sample used for the present study is described in Table 2-5. The results of analytical determinations of diazinon concentrations in the treated ponds as well as the analytical method are given in Appendix 3.

Table 2-4. Diazinon in ecotoxicological testing.

Species	Test Type	Effect Conc. (µg/L)	Reference
ALGAE			
Selenastrum capricornutum	7 d, EC50	6400	Hughes (1988)
Scenedesmus subspicatus	7 d, EC50	17´300	Hitz (1982)
Bacillariophyceae	Mesocosm, LOEC	25	Giddings (1992)
ROTIFERS			
Brachionus clyciflorus	24 h, EC50	29´200	Fernandez-Casalderrey *et al.* (1992)
	48 h, NOEC	8000	Snell *et al.* (1992)
Ploima	Mesocosm, LOEC	9.2 (r)	Giddings (1992)
Floscullariaceae	Mesocosm, LOEC	33 (r)	Giddings (1992)
CRUSTACEANS			
Daphnia magna	48 h, EC50	1.20	Dennis *et al.* (1979)
	21 d, NOEC	0.17	Suprenant (1988)
Daphnia pulex	48 h, EC 50	0.9	Sanders *et al.* (1966)
Simocephalus serratulus	48 h, EC50	1.4	Sanders *et al.* (1966)
Ceriodaphnia dubia	48 h, EC50	0.41	LeLievre (1991)
Cladocerans	Mesocosm,LOEC	2.4 (r)	Giddings (1992)
Copepoda	Mesocosm,LOEC	33 (r)	Giddings (1992)
INSECTS			
Diptera	Mesocosm,LOEC	9.2	Giddings (1992)
Ceratopogonidae	Mesocosm,LOEC	4.5	Giddings (1992)
Tanypodinae	Mesocosm,LOEC	2.4	Giddings (1992)
Ephemeroptera	Mesocosm,LOEC	33	Giddings (1992)
Odonata	Mesocosm,LOEC	16	Giddings (1992)

(r): densities recovered to control levels in the post-treatment period.

Table 2-5. Diazinon. Physico-chemical properties.

Structure of Diazinon	
Common Name	Diazinon
IUPAC name	O, O-Diethyl-O-(isopropyl-6-methyl-pyrimidin-4-yl)-thiophosphate
Emp. Formula	C_{12} H_{21} N_2 O_3 P S
CAS-number	333-41-5
Molecular Weight	304.4
Batch	AMS 140/105
Appearance	Colourless oil
Purity	99.4%
Water solubility	40 mg/L (20°C)
Boiling point	83°C
Pka	2.39
Log Pow (octanol/water coefficient)	3.95
Log Kom (soil sorption coefficient)	2.12
Transformation	Transformation in water by hydrolysis
Half Life in water	5-12 days (outdoor experiment)

2.2.7 Assignment of tanks to treatment groups

As outlined above, the 28 microcosms, were assigned to five groups (SHADOW, TURBULENCE, RUNOFF, DIAZINON and CONTROL). Table 2-6 gives detailed information about the number of replicates used per treatment group and the microcosms assigned to each group. A completely randomized assignment of microcosms to treatments was not feasible because of the following reasons: (a) the treatment group “shadow” had to be placed in a way that nearby microcosms were not influenced, and (b) the macrophytes had developed differently in the microcosms, in terms of percent cover and species abundance, although all microcosms had started with the same supply of plants. Therefore, macrophyte distribution was estimated in terms of percent cover and accordingly, the microcosms were classified and grouped into three classes, depending on the quality and quantity of macrophytes (Appendix 1). Each treatment group contained microcosms of at least two different size classes.

Table 2-6. Treatment groups and number of replicates per treatment group.

Treatment	Number of replicates	Tank no.
1) CONTROL	12	5, 6, 8, 9, 10, 11, 18, 20, 22, 24, 27, 28
2) SHADOW	4	1, 2, 15, 16
3) TURBULENCE	3	3, 7, 17
4) RUNOFF	4	19, 23, 25, 26
5) DIAZINON	4	4, 13, 14, 21

Note: Tank no. 12 was initially assigned to treatment group (3), but developed a leak during the exposure phase and was therefore excluded from the study.

2.2.8 Time schedule for treatments

The study period was divided into three phases: the pre-exposure or equilibration phase, the exposure phase and the post-exposure or recovery phase, see Table 2-7 for detailed information. As outlined above, during the pre-exposure phase, tanks were equilibrated by allowing permanent water circulation. Two days before start of the exposure phase, the water circulation was stopped and 28 isolated test units were created. Water circulation was not allowed during the exposure and post exposure phase, respectively. The exposure phase was set at a duration of 28 days. Treatments were performed either permanently (SHADOW, TURBULENCE) or by single or double applications (DIAZINON, RUNOFF). The exposure period was followed by a disturbance- or treatment-free 73 days post exposure phase.

Table 2-7. Time schedule for treatments.

	Pre-exposure **Day-24 to 0**	Exposure **Day 1 to 28**	Post-exposure **Day 28 to 101**
CONTROL	15.11.96 – 10.7.97	11.7.97- 8.8.97 (no treatment)	9.8.97- 20.10.97
SHADOW	15.11.96 – 10.7.97	11.7.97- 8.8.97 (permanently)	9.8.97- 20.10.97
TURBULENCE	15.11.96 – 10.7.97	11.7.97- 8.8.97 (permanently)	9.8.97- 20.10.97
RUNOFF	15.11.96 – 10.7.97	11.7.97- 8.8.97 (two applications: 11.7. and 25.7.97)	9.8.97- 20.10.97
DIAZINON	15.11.96 – 10.7.97	11.7.97- 8.8.97 (one application: 11.7.98)	9.8.97- 20.10.97

2.3 ENDPOINTS AND SAMPLING IN 1997

2.3.1 Endpoints: Choice of structural parameters

Phytoplankton and zooplankton were investigated in the present study, representing primary producers and primary or secondary consumers, respectively. Micro-organisms and protozoa were not investigated. There were no amphibians or fish in the microcosms. Structural parameters were investigated qualitatively (taxonomic identification to the lowest practical level) and quantitatively (individuals/ volume).

2.3.2 Time schedule for sampling of organisms

The time schedule for sampling of zooplankton and phytoplankton is given in Table 2-8. Taxonomic identification of samples was performed for days –24, 0, 4, 14, 16, 28, 80 and 101, with day 0 equal to the day of the start of the exposure phase.

Table 2-8. Time schedule for sampling of phytoplankton and zooplankton.

Day	Date	Study Phase
-24	16.6.97	Pre-exposure phase
0	10.7.97	
4	14.7.97	Exposure phase
14	24.7.97	
16	26.7.97	
28	7.8.97	
80	28.9.97	Post-exposure phase
101	19.10.97	

2.3.3 Sampling and Conservation of Phytoplankton and Zooplankton

Plankton samples consisted of mixed, depth-integrated samples. Plankton was collected using a polyethylene tube, 150 cm long and 5 cm in diameter. The tube was lowered to the sediment surface, then lifted several centimeters above the sediment surface to avoid sample contamination with sediment. The tube was closed with a plastic plug to collect a sample of the entire water column (depth-integrated). The water sample was transferred into a bucket. This procedure was repeated seven times at different locations in the microcosm. The eight samples were poured together and served as source for phyto- as well as zooplankton samples.

For the phytoplankton sample, half a liter of the mixed sample was transferred to a polyethylene flask and fixed with Lugol´s solution (Schwoerbel, 1994). For the zooplankton sample, the rest of the mixed sample was filtered through a 60 μm nylon sieve. The filtrate was transferred with about 60 mL of water to a 100 mL glass vessel and 30 mL of a sugar formalin solution were added (240 g sucrose/L formalin).

2.3.4 Measurement of Macrophytes

The percent cover in relation to the total surface area of the microcosms per macrophyte species was estimated three times (days –38, 36 and 80) during the study. No estimations were made regarding volume or weight of macrophytes. Results are shown in Appendix A.

2.3.5 Taxonomic identification and counting of zoo- and phytoplankton

Zooplankton and phytoplankton were in the majority of cases identified to the species level. Taxonomic identification was performed by the staff of the Technical University of Munich, using the taxonomic keys mentioned in chapter 6. Their help is gratefully acknowleged (chapter 7).

Zooplankton
Each zooplankton sample was filtered through a mesh of 54μm and the retained organisms were transferred into a counting chamber divided into 4 sections subdivided by a grid into four sectors. In general, 2 sectors were examined microscopically. The density of the total zooplankton and individual taxa in a sample was determined using the following equation: D = (number of individuals in 2 sectors) x 2/ (volume sampled).

Phytoplankton
Phytoplankton was counted using the sedimentation method (Schwoerbel, 1994). The sedimentation volume was for the majority of cases 50 mL and the area of sedimentation was 530.66 mm^2. Phytoplankton was counted on an area of 2.6 mm^2, equivalent to 0.5% of the total area and thus 0.5% of the sedimentation volume. In case of high algal density, the subsamples had a volume of 20mL or 10mL.

2.3.6 Functional parameters

Temperature, oxygen saturation, pH, conductivity and turbidity were measured regularly during the study. Measurements were performed in each pond about 10 cm below the water surface at the same time of the day (2 p.m. – 5 p.m.). Measurement results and measurement devices used are displayed in Appendix B.

2.4 ZOOPLANKTON SAMPLING IN 1996 AND 1998

The study described took part in 1997. Zooplankton sampling was also performed at regular intervals in 1996 and 1998. In 1996 and 1998, for the collection of zooplankton, an plankton net of 30 cm in diameter and a 55 μm mesh was used. Depth integrated samples from the whole water column were taken and preserved with ethyleneglycol (20-25 mL spiked with 6% formalin). The filtered water column corresponded to about 0.4 to 0.5 m^3.

2.5 DATA EVALUATION AND INTERPRETATION

2.5.1 Choice of Descriptive and Statistical Methods

For the present study, several descriptive approaches were used to analyze the natural variability of the control microcosms (a-f). Univariate and multivariate statistical approaches were employed when comparing the treatment groups to the control (f-h).

The diversity of methods was the basis for a comparison of the applicability of various methods to microcosm data sets. The following methods were used:

a) coefficient of variation;

b) mean: variance relationship;

c) level of precision;

d) maximum abundance;

e) diversity index (Shannon-Wiener index) for changes in community structure;

f) dominance curves;

g) community comparison index (Bray-Curtis similarity index) for changes in community composition;

h) univariate statistics (Dunnett´s test) for changes in density for individual taxa, the total abundance and also for diversity indices;

i) multivariate statistical techniques (non-metric Clustering, Multi-dimensional Scaling, Principal Response Curves method) for changes in community composition.

2.5.2 Coefficient of Variation

The coefficient of variation (cov) in percent was calculated as

cov = sd/ m * 100.

Mean (m), standard deviation (sd) and coefficients of variation were calculated to determine inter-replicate variability for zooplankton and phytoplankton species. COVs were also calculated for data grouped on higher taxonomic levels as well as for the species diversity.

2.5.3 Levels of Precision

The level of precision (p) was calculated for the control group according

p = SE/m

where m is the mean number of individuals and SE is the standard error calculated from 12 replicates. The standard error SE was calculated as ($SE=s/n^{0.5}$), where s is the standard deviation and n the number of replicates.

2.5.4 Variance: Mean Relationship

Inter-replicate variance (s^2) is usually well-correlated with the density of organisms in replicate samples (Downing, 1987). It rises as a power function of the mean (m):

$s^2 = am^b$

where a and b are constants fitted by least squares regression of log s^2 on log m. In the present study, Microsoft Excel (1999) was used for the least squares regression.

2.5.5 Maximum Density

In addition to the above-mentioned endpoints, year-to-year variability was also characterized by maximum density for each taxon. The maximum density refers to the highest density measure for a population in one microcosm on one sampling occasion. The maximum density per taxon and year was extracted from a cumulative list of all taxa found within the three-years period. Values were grouped according to max. abundance (<1, <10, <100, <1000). This also allowed the determination of taxa present or absent in the 1996, 1997 and 1998, when compared to the cumulative list.

2.5.6 Transformation of data for statistical analysis

In the present study, data were log-transformed, if not mentioned otherwise.

Univariate tests such as the Dunnett´s test rely on assumption of normality and constant variance across the groups. Usually these presumptions are not fulfilled for species occurring at low numbers. Therefore data were transformed using a logarithmic transformation, which has the effect of reducing right-skewness (in simple words: many zeros in one group and densities of 100/L or so in the other group) and stabilising the variance. Transformations play an entirely separate, but equally important, role in the clustering and ordination methods, that of defining the balance between contributions from common and rarer species in the measure of similarity of two samples. Using the log-transformation, rare species are given more weight and more abundant species are down-weighted.

2.5.7 Diversity Index

The diversity index gives a measure of the way in which the total number of individuals is divided up among different species. It reduces multivariate community data to a single index. The number of species present in a data set (species richness), as well as the distribution of individuals among the different species (evenness), contribute to the community diversity. In the present study, the Shannon-Wiener diversity index (H´) was calculated as a measure for diversity, with $[H' = - \sum_i p_i (\log p_i)]$, where p_i is the proportion of the total count arising from the *i*th species.

It is important to note that taxonomic identification could not always be performed at the species level, as will be explained in detailed in the “Results” section. The diversity index has therefore been applied to data sets including taxa identified at the genus or family level. This should be kept in mind when judging the sensitivity of this index in the present study.

2.5.8 Dominance Curves

Ranked species abundance curves (dominance curves) are a way of graphically presenting patterns of relative species abundances. They are based on the ranking of species (or higher taxa) in decreasing order of their importance in terms of abundance. The ranked abundances are expressed as percentage of the total abundance of all species. In the present study, the cumulative ranked abundances are plotted against the relevant species rank. Using this way of distributional representation, the most elevated curves have the lowest diversity.

2.5.9 Dunnetts´s Test

In the present study, the Dunnett´s test was used to test the null hypothesis that there are no differences between the treatment and the control groups (Dunnett, 1972). This requires the assumption that the data are normally distributed, and have constant variance across the groups. The significance level for the Dunnett´s test was set to 5%. In other words, treatment groups were assumed to be statistically different to the control when the hypothesis that "there are no differences between treatment and control groups" was rejected at a significance level of 5%. The software ECOS (ECOS, 1997) and the underlying software package SAS were used for the Dunnett´s test.

The Dunnett´s test was used as a univariate statistical tool to compare the total density data and the density data of abundant species of the four treatment groups with the control group. It is worth noting that test results were not shown for species data which did not meet the assumption of normality, i.e. species which occurred irregularly at very low densities.

The Dunnett´s test is appropriate for multivariate data, when the species abundance information is reduced to a single index, such as Shannon-Wiener diversity and when replicate samples exist. The Dunnett´s test was therefore also applied to diversity indices (indices of treatment groups vs. control group).

2.5.10 Matrices of Similarity Coefficients

For multivariate data analysis, similarity coefficients were calculated as a basis for Clustering and Multidimensional Scaling. Triangular matrices of similarity coefficients, in the present study Bray-Curtis similarity coefficients, were computed between every pair of samples. Bray-Curtis similarity coefficients were calculated (Bray and Curtis, 1957) using the PRIMER program (PRIMER, 1994). This coefficient is a simple measure to indicate how similar the abundance levels are for each species, averaged over all species. It is defined such that a value of 100% represents total similarity and 0% complete dissimilarity. The underlying similarity between the *jth* and *kth* samples, S_{jk}, is defined as

$$S_{jk} = 100 \{1 - \frac{\sum_{i=1}^{p} | Y_{ij} - Y_{ik} |}{\sum_{i=1}^{p} (Y_{ij} + Y_{ik})} \}$$

Where Y_{ij} represents the entry in the *ith* row and *jth* column of the data matrix, i.e. the abundance for the *ith* species in the *jth* sample. Similarly, Y_{ik} is the count for the *ith* species in the *kth* sample.

2.5.11 Hierarchical Clustering

The clustering starts from a triangular matrix of similarity coefficients. Samples are fused into groups and the groups into large clusters, starting with the highest mutual similarities, then gradually lowering the similarity level at which groups are formed. The process ends with a single cluster containing all samples. The cluster is represented by a dendrogram with one axis representing the full set of samples and the other axis defining the similarity level to which two samples or groups are considered to have fused.

The degree of similarity is given as Bray-Curtis similarity coefficients in percent. In the present study, cluster analysis was performed using the PRIMER program (PRIMER, 1994). Hierarchical clustering has proved to be a useful technique for delineating groups with distinct community structure. However, it is best used in conjunction with ordination, e.g. MDS or PCA, which are described below.

2.5.12 Non-metric Multi-Dimensional Scaling (MDS)

Multidimensional scaling (MDS) is an ordination method, which starts from a triangular matrix of similarity or dissimilarity coefficients. Similarity or dissimilarity values are ranked. If, for example sample 1 is more similar to sample 2 than to sample 3, MDS constructs a map or configuration of the samples attempting to satisfy all conditions imposed by the rank similarity matrix. Consequently, sample 1 will be placed closer on the map to sample 2 than to sample 3. The non-metric MDS algorithm is an iterative procedure, constructing the MDS plot by successively refining the positions of the points until they satisfy, as closely as possible, the dissimilarity relations between samples. A measure of goodness-of-fit is given with the *stress* value. Large scatter of the data leads to large stress values and indicates that sample relationships can not be compressed into a low number of dimensions. For detailed description of the method, see Kruskal and Wish (1978). In the present study, MDS analysis was performed and two-dimensional MDS ordinations were plotted using the MDS and CONPLOT procedures. Both are included in the PRIMER program (PRIMER, 1994).

2.5.13 Analysis of Similarity (ANOSIM)

In the ANOSIM test, non-parametric permutation tests (Mantel, 1967) are combined with a general randomization approach to the generation of significance levels, so called “Monte Carlo” tests (Hope, 1968; Clarke and Warwick, 1994). The ANOSIM test is based on rank similarities between samples in a triangular similarity matrix. If rw is defined as the average of all rank similarities among replicates within treatment groups, and rb is the average of rank similarities arising from all pairs of replicates between different treatment groups, then a suitable statistic is [$R = (rb – rw) / (M/2)$], where $M = n(n-1)/2$ and n is the total number of samples under consideration.

R will usually fall between 0 and 1, indicating the degree of discrimination between the treatments. If all replicates within sites are more similar to each other than any replicates from different sites, then R=1. If similarities between and within sites are on the same average, R is approximately zero. An R-value substantially less than zero, indicates similarities across different treatments being higher than those within treatments.

The R-statistic is a comparative measure of the degree of separation of treatment groups. Whether it is significantly different from zero is checked by re-computing the R-statistic under permutations of the sample labels, i.e. the labels are randomly reshuffled, R recalculated and the process repeated a large number of times. Under the hypothesis of “no differences between treatment groups” there will be little effect to the value of R if the labels (identifying which replicate belongs to which treatment group) are arbitrarily re-arranged as the replicates all belong to one group, if the hypothesis is true.

The significance level of the R-statistic is calculated by referring the observed value of R to its permutation distribution. If the calculated R is unlikely to have come from a simulated distribution (of the statistic R under the null hypothesis of “no treatment group differences”), there is evidence to reject the null hypothesis. If only t of the T simulated values of R are as large or larger than the observed R then the null hypothesis can be rejected at a significance level of 100 (t+1)/(T+1)%.

In the present study, the degree of separation of treatment groups was calculated using the ANOSIM procedure (“Analysis of Similarities”) implemented in the PRIMER program (PRIMER, 1994). The analysis of similarities was calculated for zooplankton and phytoplankton data.

2.5.14 Principal Response Curves (PRC)

The Principal Response Curves (PRC) method was described in detail by van den Brink and Ter Braak (1999). It is based on the ordination technique called partial redundancy analysis, which is a constrained form of Principal Component Analysis. The PRC technique uses dimension reduction to summarise all information on the investigated populations simultaneously, to elucidate treatment effects at the community level. The PRC method plots the first principal component of the treatment effects against time, expressing the treatment effect as deviations from the control treatment. As a result, the vertical axis of a PRC diagram contrasts each treatment with the control.

Associated with the PRC is a set of species weights (taxon weights). Species weights can be interpreted as the affinity of each species with the diagram. The higher the weight (positive or negative values) the higher the affinity of the species to the PRC curve. Positive species weights for PRCs in the positive range indicate an increase of this species in the treatment when compared to the control. The same is true for negative species weights for a PRC in the negative range. Contrarily, negative species weights for a PRC in the positive range or positive species weights for a PRC in the negative range indicate an increase of the corresponding species in the treatment when compared to the control.

The PRC analysis gives an estimation of the variance allocation inherent to the data set. The percent variation explained by time is given first. Of the remaining variance, the percent of variation explained by treatment effects, and from there the percent variation captured by the first PRC is calculated.

For the present study, the software package CANOCO for Windows was used. Principal Response Curves were established for zoo- and phytoplankton community data, allowing a graphical comparison the treated group with the control over time. Monte-Carlo permutation tests were used to evaluate which treatment groups were statistically different from the control for different time points.

In the present study, PRC calculations were performed for two different time frames

(a) for the entire study period (day-24 to day 101) with the intention to show similarity of the tanks before exposure start and a possible recovery during the post-exposure phase.

(b) exclusively for the exposure phase (day 4 to day 28), because excluding pre- and post exposure phase gives less weight to time related and more weight to treatment related variability and increases statistical power.

The results were displayed as

a) PRC diagrams, showing the regression coefficients plotted against the sampling day;
b) tables explaining the variance allocation and the significance of the PRC for the data set including all treatments, and for the data sets including each treatment level individually for different time spans;
c) tables showing the outcome of the Monte-Carlo permutations for analysis on a day-by-day basis.
d) lists of species weights. These show the affinity of single species to the corresponding PRC: the higher the species weight (positive or negative values), the more pronounced the actual response pattern of this species is likely to follow the PRC pattern.

2.5.15 Data Analysis on different taxonomic levels

In the present study, most of the organisms were identified to the species level. However, depending on the community composition, it might be sufficient to analyze data on a higher taxonomic level. Zooplankton data were therefore also grouped at the family, order and class level. The sums of densities of all species belonging to the corresponding taxonomic level served as data basis for further calculations:

a) The percent contribution of major zooplankton orders as well as major zooplankton species to the total zooplankton density was shown graphically.
b) Data grouped at the family and class level were used for PRC calculations and were compared to the PRCs calculated at the species level.

3 RESULTS AND DISCUSSION

3.1 SYSTEM-INHERENT VARIABILITY OF ZOOPLANKTON

3.1.1 Zooplankton Taxa

All zooplankton taxa identified from the depth-integrated water samples are listed in Table 3-1. Note that the zooplankton list is a cumulative list of taxa found in all microcosms throughout the study, thus including taxa from the control as well as the treatment groups.

Identification was primarily performed to species level. If this was not possible, only the genus or family was specified. A total of 38 zooplankton taxa were found, of which 17 were identified to the genus and 21 to species level. For the calculations, in case of unidentified species it was assumed that the identified taxon described a single species, and not several (e.g., *Asplanchna spec.*). Consequently, when analyzing the data on the "species level", the calculations were performed with a number of species set equivalent to the number of taxa if not mentioned otherwise.

Table 3-1. Identified zooplankton taxa.

Nemathelminthes				
Rotatoria	Ploimida	Asplanchnidae	*Asplanchna*	*spec.*
		Colurellidae	*Colurella*	*spec.*
		Keratellidae	*Keratella*	*cochlearis*
			Keratella	*quadrata*
		Lecanidae	*Lecane*	*spec.*
		Synchaetidae	*Polyarthra*	*spec.*
			Polyarthra	*vulgaris*
			Synchaeta	*spec.*
			Synchaeta	*oblonga* (1)
		Testudinellidae	*Testudinella*	*patina*
		Trichocercidae	*Brachionus*	*spec.* (1)
			Conochilus	*spec.*
			Trichocerca	*spec.*
		Trichotriidae	*Trichotria*	*pocillum*
			Trichotria	*spec.*
			Trichotria	*tetractis*
		Gastropodidae	*Ascomorpha*	*spec.*
Arthropoda				
Crustacea (Branchiopoda)				
	Cladocera	Chydoridae	*Acroperus*	*harpae*
			Alona	*spec.*
			Alonella	*nana*(1)
			Chydorus	*sphaericus*
			Graptoleberis	*testudinaria*
			Leydigia	*acanthoceroides*
			Pleuroxus	*uncinatus*

Legend: (1) The Table lists all taxa found in the control as well as in the treatment groups throughout the study. 4 species were not found in the control group.

Table 3-1 (cont.). Identified zooplankton taxa.

		Daphniidae	*Ceriodaphnia*	*quadrangula*
			Ceriodaphnia	*reticulata*
			Ceriodaphnia	*spec.*
			Daphnia	*longispina*
			Scapholeberis	*mucronata*[1]
			Scapholeberis	*spec.*
			Simocephalus	*vetulus*
		Macrothricidae	*Macrothrix*	*laticornis*
		Sididae	*Diaphanosoma*	*brachyura*
			Diaphanosoma	*spec.*
Crustacea (Copepoda)[2]				
	Calanoida	Diaptomidae	*Eudiaptomus*	*gracilis*
	Cyclopoida	Cyclopidae	*Eucyclops*	*spec.*
			Megacyclops	*spec.*
			Mesocyclops	*spec.*

Legend: (1) The Table lists all taxa found in the control as well as in the treatment groups throughout the study. 4 species were not found in the control group. (2) Calanoid copepods contributed with <0.1% to the total density of copepods. Eucyclops contributed with <0.5% to the total density of copepods. Different developmental stages (nauplii, copepodites, adults) of *Megacyclops spec.* and *Mesocyclops spec*. were grouped under the taxon Cyclopoida.

3.1.2 Interreplicate variability of Zooplankton

Total Density, Population Densities and Species Diversity

Mean values, standard deviations and coefficients of variation were calculated for zooplankton total density and species diversity in the control group. These values were calculated on the basis of 12 replicate ponds for 8 sampling events.

Mean values and standard deviations for total density and species diversity as well as the contribution of each control replicate to the variability in the control group are shown in Figure 3-1 and in Table 3-2.

The mean total zooplankton density for the 12 control tanks increased during the study period, reaching a mean value about 7 times higher on day 101 (October) when compared to day –24 (June). The corresponding average coefficient of variation (Table 3-2) went up from 28% to 64% during the study period.

Mean values for diversity (Figure 3-1) reached slightly higher values in July and August (days 4, 14, 16 and 28) when compared to June or September (day –24 and day 80) and reached the minimum in October (day 101). The variability for the mean species diversity, initially showing a coefficient of variation of 10% reached a level of 44% on day 101 (Table 3-2).

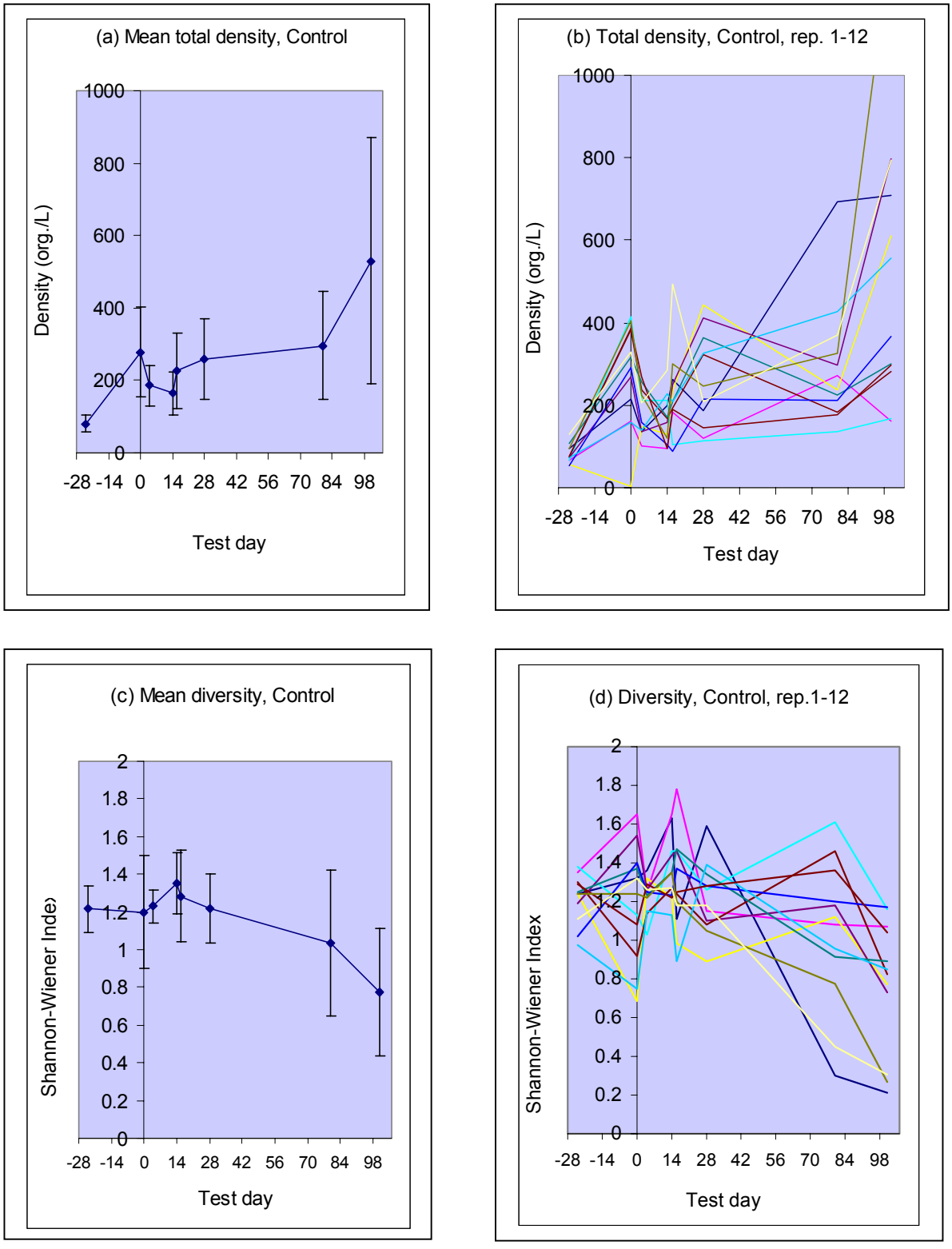


Figure 3-1. Total density (a, b) and species diversity (c, d) of zooplankton in the control group.

Table 3-2. Coefficients of variation for zooplankton in the control group

Coefficients of variation (%) in the control group								
	day -24	day 0	day 4	day 14	day 16	day 28	day 80	day 101
Diversity	10	25	7	12	19	15	37	44
Total density	28	45	30	36	46	43	51	64
K. quadrata	*39*	*57*	*74*	*56*	*60*	*71*	*76*	*104*
P. vulgaris	*57*	*n.a.*	*108*	*94*	*140*	*290*	*112*	*135*
D. longispina	*84*	*67*	*47*	*39*	*58*	*41*	*55*	*59*
S. vetulus	*181*	*157*	*346*	*346*	*274*	*168*	*94*	*160*
Cyclopoida	*34*	*52*	*51*	*54*	*66*	*37*	*45*	*91*

Coefficients of Variation for Zooplankton Taxa

Mean and coefficient of variation were calculated from 12 replicates for the density of each taxon present on sampling occasions 1 to 8. In the control group, 34 taxa were found throughout the duration of the study (Table 3-1), however not every taxon was found on every sampling occasion. The number of taxa present in at least one replicate on the respective test days varied from 10 to 21. Taxa which were absent from all 12 replicates on a certain test day were excluded from the evaluations presented in the following paragraphs. Therefore, the following results are based on 123 data points (n´=123). For example in Figure 3-2 each of the 123 data points represents the relationship of the coefficient of variation to the mean number of a certain taxon on a certain test day.

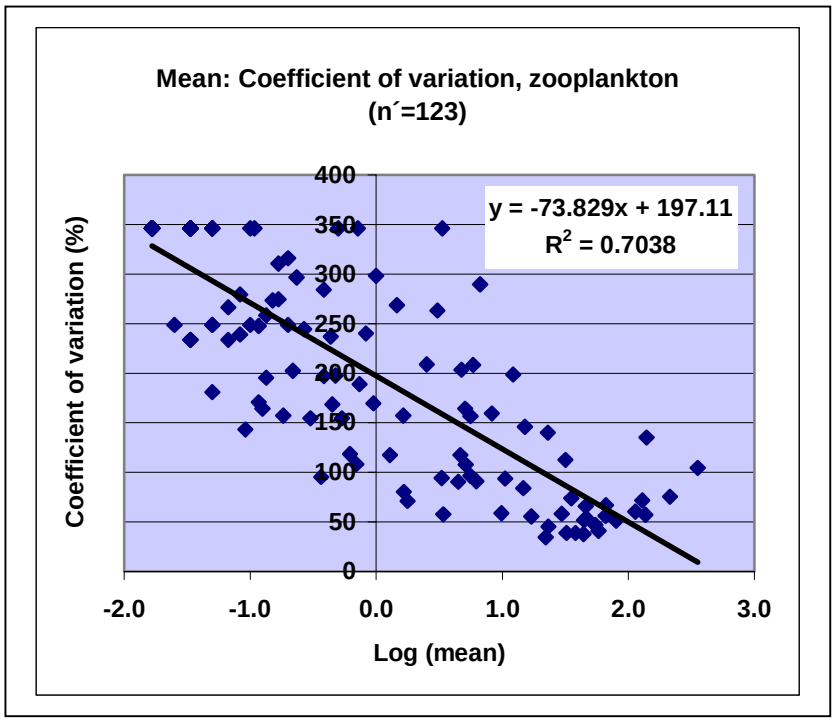


Figure 3-2. Mean: coefficient of variation for zooplankton in the control group.

Mean densities were clearly correlated with the coefficient of variation: the higher the mean, the lower the coefficient of variation (Figure 3-2). Mean total density ranged between 354 and 0.02 individuals/L. The corresponding coefficients of variation were found within a range of 34-346%.

It is important to notice that only about 19% and 23% of the taxa occurred with a mean density of 1-10/L or >10/L, respectively. For the majority of the data points (58%), mean densities were <1/L. As mentioned above, these numbers do not include total zero-counts (taxa not found in any replicate on a certain test day).

For 6% and 25% of the data points, coefficients of variation were <50% and <100%, respectively. For 48% of the data points, coefficients of variation were <200%. For the majority of data points (52%) coefficients of variation were >200%.

Coefficients of variation for populations of dominant zooplankton taxa were found at 39-104% for Ker*atella quadrata*, 57-290% for *Polyarthra vulgaris*, 39-84% for *Daphnia longispina*, 94-346% for *Simocephalus vetulus* and 34-66% for Cyclopoida (Table 3-2).

Mean: variance relationship for Zooplankton Taxa

Log (mean) and log (variance) were calculated for abundance of each taxon per sampling day, based on 12 replicate samples per test day. Taxa which were absent from all 12 replicates on a certain test day were excluded from the evaluations presented in the following paragraphs. Therefore, the following results are based on 123 data points (n´=123). For example in Figure 3-3 each of the 123 data points represents the relationship of the variance to the mean number of a certain taxon on a certain test day. The mean: variance relationship displayed in Figure 3-3 showed a highly significant correlation coefficient 0.97. The slope of the regression (b-value) was 1.60.

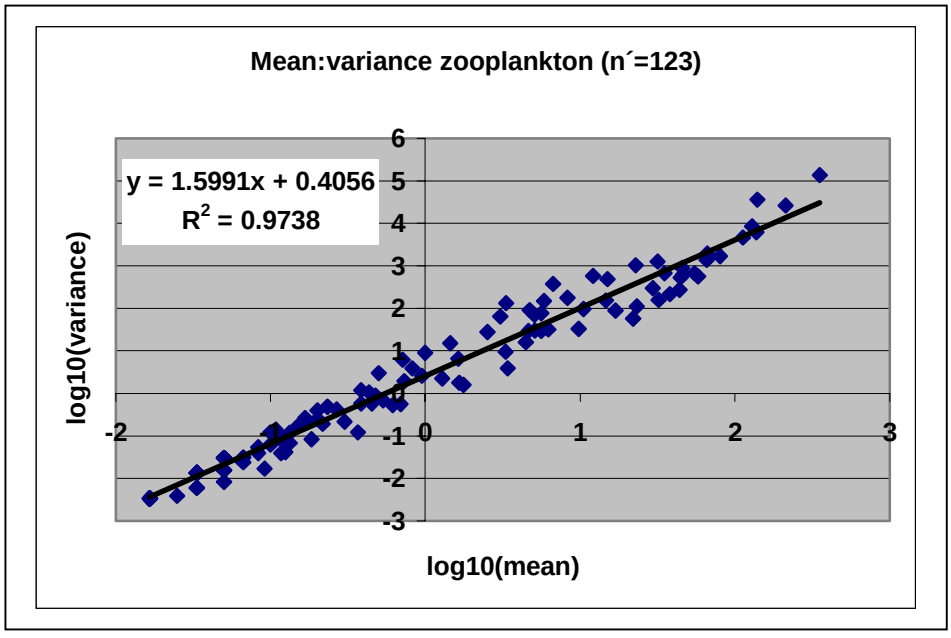


Figure 3-3. Mean: variance for zooplankton in the control group.

Mean: variance relationships were also calculated for the zooplankton data set for day -24, 0, 4, 14, 16, 28, 80 and 101. The corresponding slopes (b-values) ranged from 1.38 to 1.72 (Table 3-3). Correlation coefficients ranged from 0.93 to 0.98.

Table 3-3. Variance: mean relationship for zooplankton in 1997.

Mean:variance relationship for zooplankton in 1997								
	day -24	day 0	day 4	day 14	day 16	day 28	day 80	day -24
b	1,38	1,62	1,51	1,49	1,64	1,6	1,68	1,72
n´	11	14	18	21	19	13	10	17

n´= number of data points, i.e. number of taxa present on corresponding test day

Levels of precision for Zooplankton Taxa

The levels of precision (p) were calculated for all taxa and all sampling events on the basis of standard errors for 12 replicates in the control group. It could be shown that p is tightly correlated to the mean (m): the higher the mean, the lower the value of p (Figure 3-4).

It is important to notice that for the whole data set (n´= 123), precision levels of $\leq$ 0.2 were reached in only 18% of the cases and that precision levels always exceeded a value of 0.2 for mean densities below 1 individual/L.

As already mentioned above, mean densities exceeded 1 individual/L in 42% of the data points. Thereof, 43% were found at a precision level $\leq$0.2. For dominant taxa, precision levels below 0.2 were found in the majority of the cases (Table 3-4). Cyclopoida, *Daphnia longispina* and *Keratella quadrata* reached this precision level almost throughout the entire study period, while other rotifers and cladocerans usually exceeded a value of 0.2.

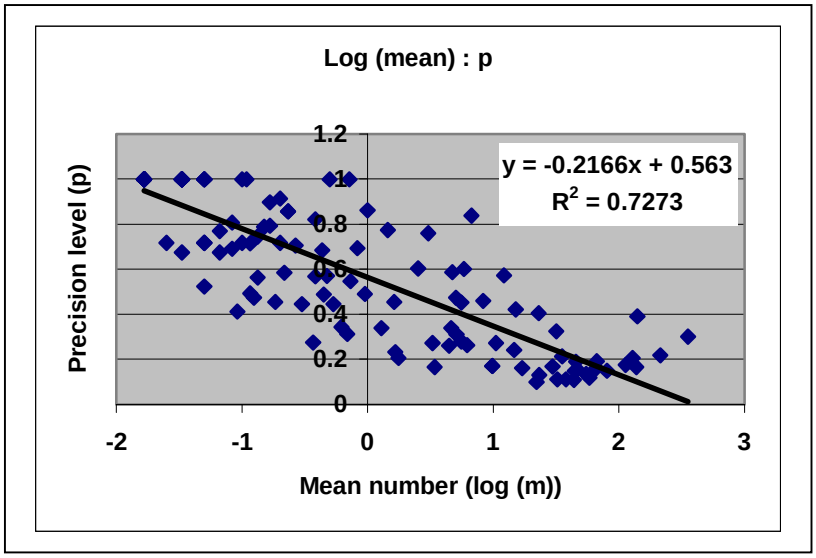


Figure 3-4. Mean: precision level for zooplankton in the control group.

Table 3-4. Levels of precision for zooplankton.

	Levels of precision (p)[1] for test days –24 to 101							
	-24	0	4	14	16	28	80	101
Cyclopoida	0.10	0.15	0.15	0.16	0.19	0.11	0.13	0.26
Daphnia longispina	0.24	0.19	0.14	0.11	0.17	0.12	0.16	0.17
Keratella quadrata	0.11	0.17	0.21	0.16	0.17	0.21	0.22	0.30
Polyarthra vulgaris	0.17	-	-	-	-	-	-	-
Polyarthra spec.	-	-	-	0.27	-	-	-	-
Sychaeta spec.	0.21	-	-	-	-	-	0.26	-
Asplanchna spec.	-	0.28	-	-	-	-	-	-
Simocephalus vetulus	-	-	-	-	-	-	0.27	-
Pleuroxus uncinatus	-	-	-	-	-	-	-	0.23
Leydigia acanthoceroides	-	-	-	-	-	-	-	0.28
No. of taxa[2]	11	14	18	21	19	13	10	17

Note (1): The level of precision (p) was calculated for all taxa and sampling days. The cut-off value for this table was set at $p \leq 0.30$. (2) The total number of taxa found in the samples on the respective test day.

Zero-Counts

From the cumulative list of 34 zooplankton taxa found in the control group, 23, 20, 16, 13, 15, 21, 24 and 17 taxa were not all represented in any of the control microcosms on test days –24, 0, 4, 14, 16, 28, 80 and 101, respectively. This is equivalent to 38-71% of the data points (n´= 123), (Figure 3-3).

Figure 3-5 displays the number of taxa with 0 zero-counts (taxon present in all 12 replicate ponds), 1-6 zero-counts (taxon not represented in up to 50% of the replicates) and 6-11 zero-counts (taxon not represented in >50% of the replicates).

On most test days, 60-70% of the taxa from the cumulative list were represented in <50% of the replicates. It is important to notice that only 10-25% of taxa were represented in all replicates on most of the test days.

The proportion of data points with 2 and less zero-counts (and a mean density of 10 individuals/L) was 32% (Figure 3-6). The proportion of data points with 6 and less zero-counts (and mean density of 1/L) was 41%. The corresponding coefficients of variation for ≤2 and ≤6 zero-counts, respectively, were 100% and 200% (Figure 3-7).

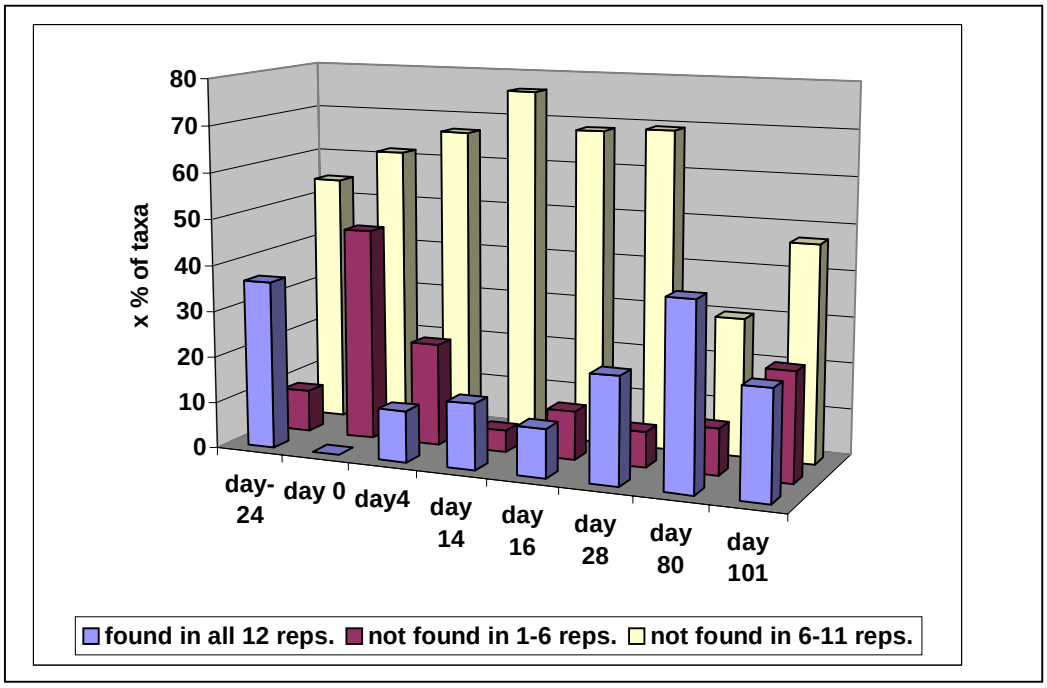


Figure 3-5. Zero-counts for zooplankton in the control group.

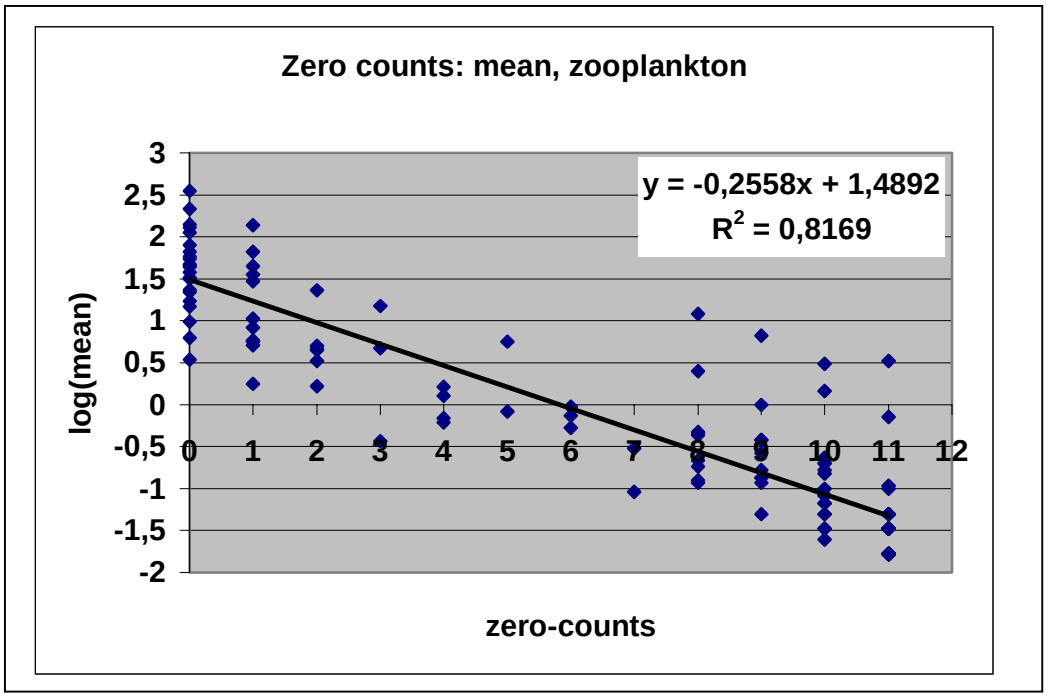


Figure 3-6. Zero-counts: mean number for zooplankton in the control group .

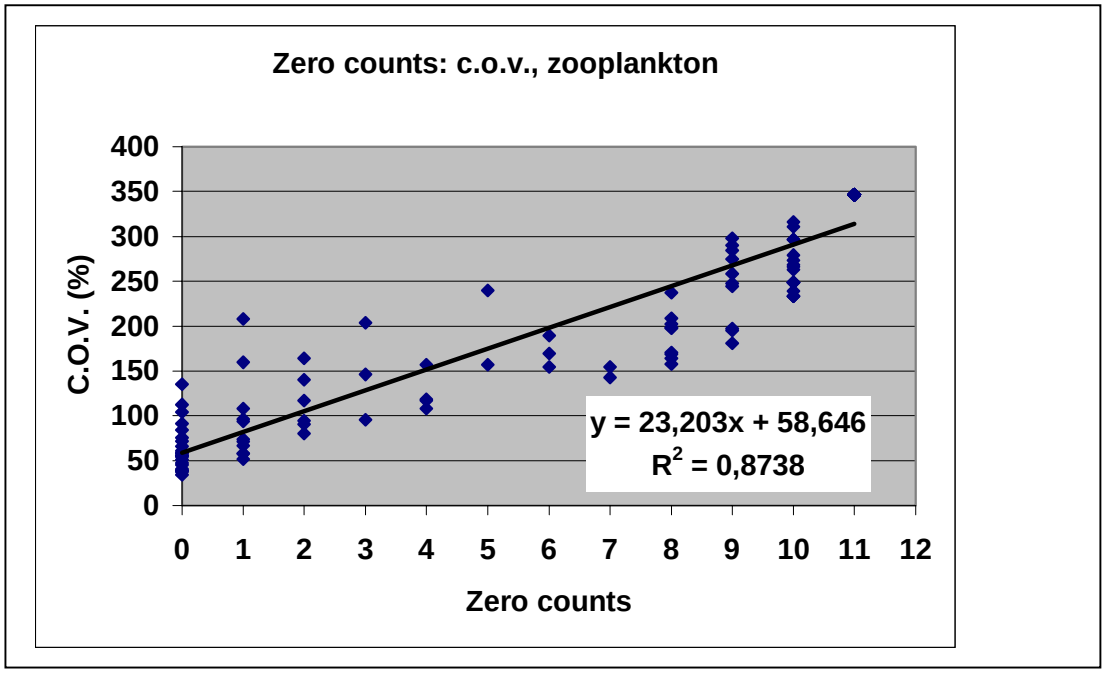


Figure 3-7. Zero-counts: coefficient of variation (c.o.v.) for zooplankton in the control group.

3.1.3 Seasonal Variability of Zooplankton

As already described above, the observed increase in total zooplankton density was accompanied by a decrease in species diversity. The shift in diversity can be explained by the dominance partition of single taxa (Figure 3-8). The zooplankton community in the control was dominated by 4-5 taxa on days-24 through day 28. An even more extreme dominance partition was observed for days 80 and 101, where the most abundant species contributed to about 70% to the total density. Moreover, Figure 3-8 shows that the dominance partitions of days –24, day 0, day 4, day 14, day 16 and day 28 were found within the same range, while the partition of days 80 and 101 strongly deviated from this range.

Mean densities and standard deviations calculated from the 12 control replicates for the dominant species are shown in Figure 3-9. *Daphnia longispina* and Cyclopoida reached their peak densities on days 0-28 (July-August), while rotifers peaked at the end of the test (day 101, October).

The dominance plot is best read in conjunction with the corresponding list of ranked species (Figure 3-8, Table 3-5). For example: for day 14, rank 1 was assigned to *Keratella quadrata*, which contributed with 40% to the total zooplankton density; for the same test day, rank 2 was assigned to Cyclopoida, which contributed with about 25% to the total density. The species ranking for the control group (Table 3-5) clearly shows that the communities were strongly dominated by the taxa *Keratella quadrata,* Cyclopoida, *Daphnia longispina* and *Polyarthra vulgaris,* which altogether formed over 90% of the total zooplankton density throughout the duration of the study.

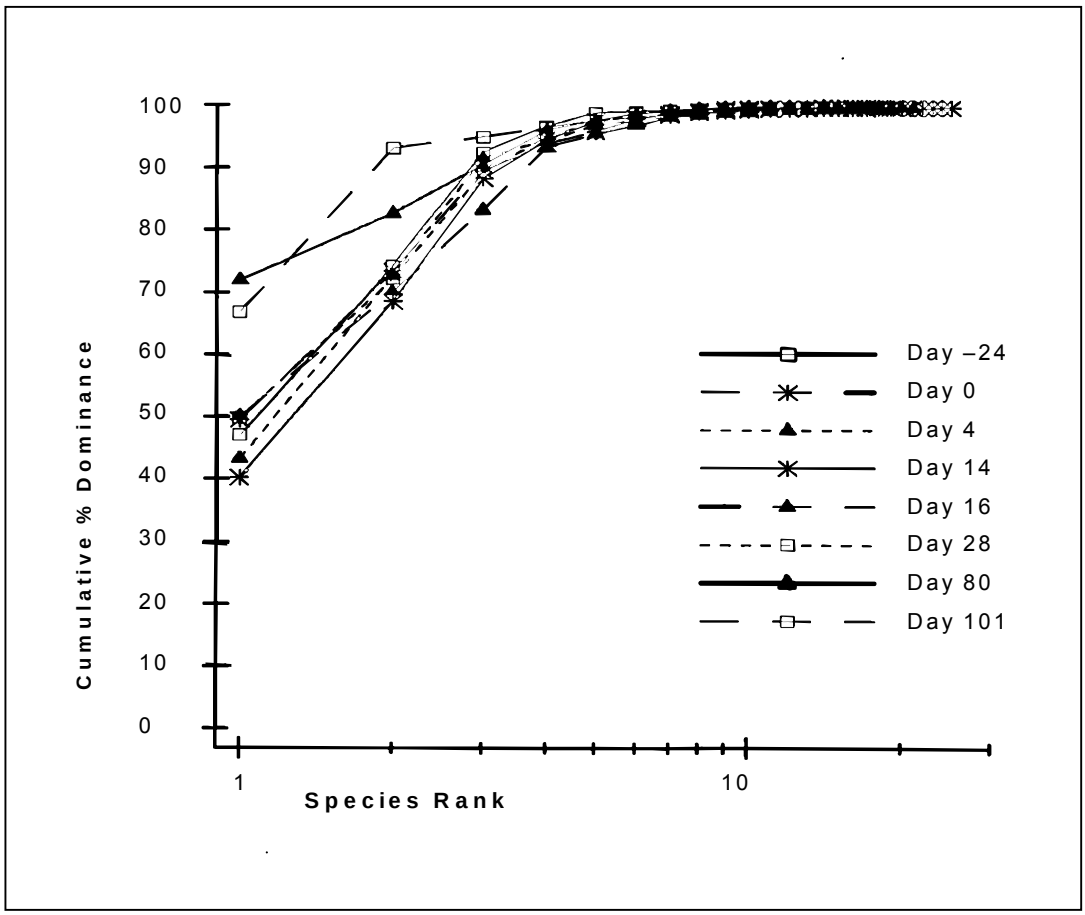


Figure 3-8. Dominance plot for zooplankton in the control group.

Table 3-5. Ranking of zooplankton taxa in the control group.

	Rank 1	Rank 2	Rank 3	Rank 4
day-24	*Keratella quadrata*	*Cyclopoida*	*Daphnia longispina*	*Polyarthra vulgaris*
day 0	*K. quadrata*	*D. longispina*	*Cyclopoida*	*Synchaeta oblonga*
day 4	*Cyclopoida*	*D. longispina*	*K. quadrata*	*P. vulgaris*
day 14	*K. quadrata*	*Cyclopoida*	*D. longispina*	*Polyarthra spec.*
day 16	*K. quadrata*	*Cyclopoida*	*D. longispina*	*P. vulgaris*
day 28	*K. quadrata*	*D. longispina*	*Cyclopoida*	*Polyarthra spec.*
day 80	*K. quadrata*	*P. vulgaris*	*Cyclopoida*	*D. longispina*
day 101	*K. quadrata*	*P. vulgaris*	*D. longispina*	*Simocephalus vetulus*

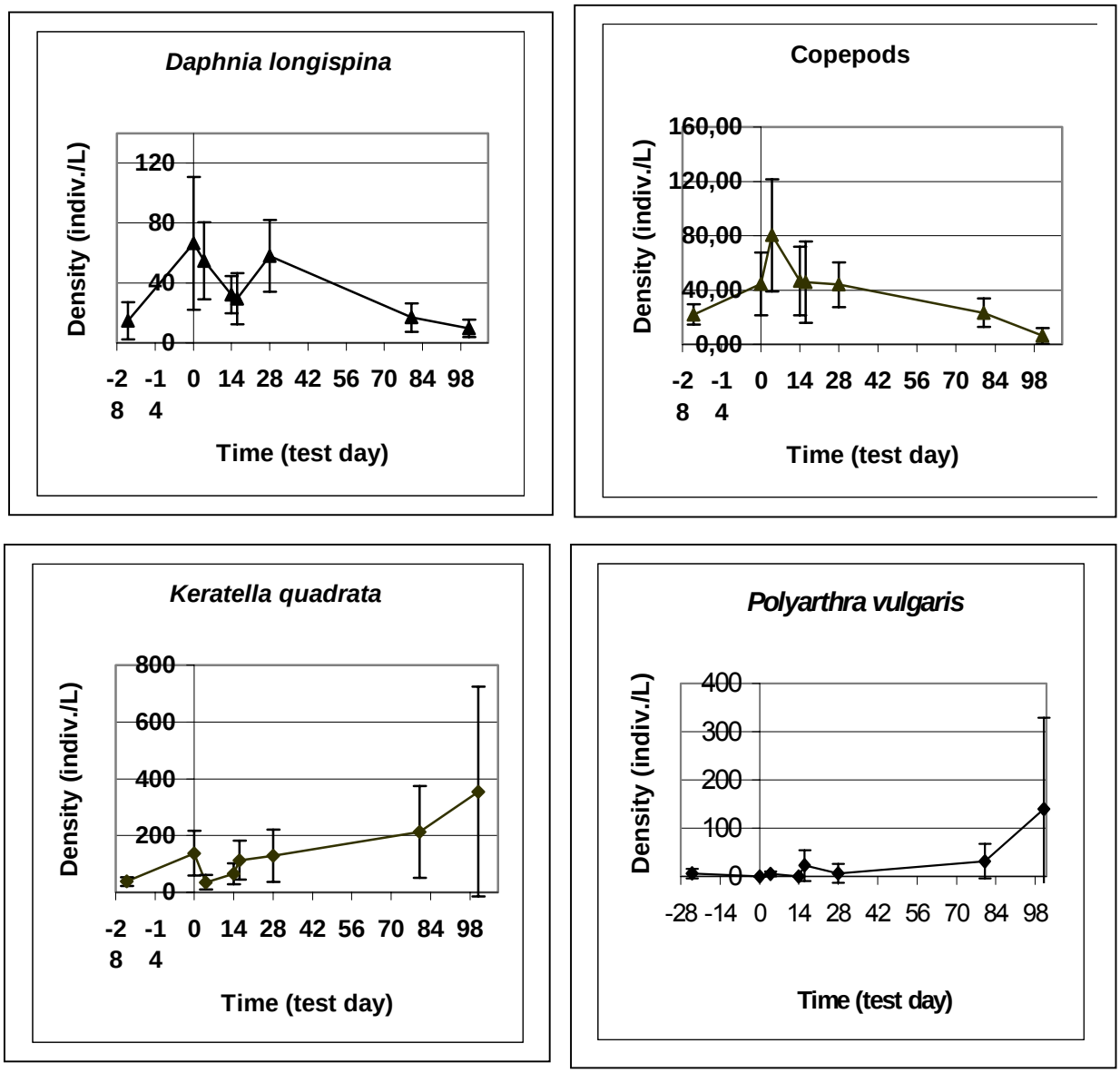


Figure 3-9. Dominant zooplankton taxa in the control group.

Mean: variance relationships were calculated for dominant zooplankton taxa on the basis of 12 replicates (n=12). Log 10 (s^2) was plotted against log 10 (M) for sampling days –24, 0, 4, 14, 16, 28 and 80 (n´=7). The resulting regressions are shown in Figure 3-10. Slopes of the mean:variance relationship (b-values) for dominant taxa ranged from 1.58 to 2.53, correlation coefficients were found in a range of 0.79 to 0.98.

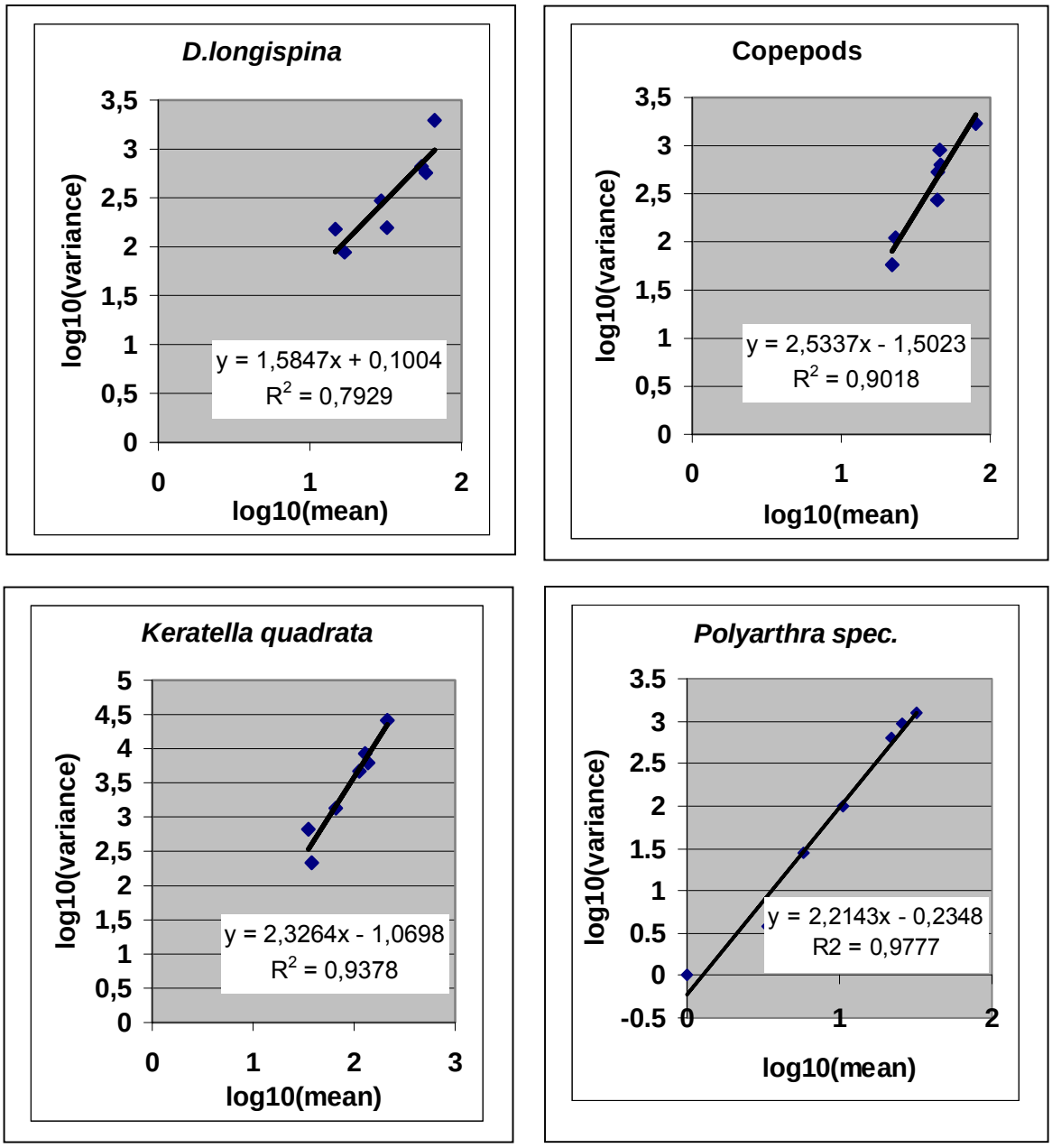


Figure 3-10. Mean: Variance for dominant zooplankton in the control group.

3.1.4 Zooplankton Year-to-Year Variability for 1996, 1997 and 1998

Maximum densities of taxa found in 1996, 1997 and 1998 are shown in Table 3-6. ´Maximum` density in this case refers to the highest population density measured in one of the replicates on the respective sampling day. The data are listed in descending order of densities found in 1997.

Table 3-6. Maximum densities per liter of zooplankton in control ponds. Cumulative list for 1997, 1996 and 1998.

Taxon	1996	1997	1998	*Taxon (cont.)*	1996	1997	1998
Keratella quadrata	174	655	842	*Trichotria pocillum*	0.00	0.40	0.00
Daphnia longispina	12.7	160	25.5	*Ceriodaphnia* spec.	0.00	0.40	2.88
Copepoda	41.7	155	146	*Trichocerca* spec.	0.04	0.40	0.77
Polyarthra vulgaris	26.3	117	518	*Trichotria tetractis*	0.00	0.40	0.00
Polyarthra spec.	0.00	78.0	0.00	*Trichotria* spec.	0.40	0.20	0.48
Synchaeta spec.	0.32	76.40	29.0	*Acroperus harpae*	1.28	0.20	0.00
Simocephalus vetulus	3.32	29.8	12.7	*Colurella* spec.	0.00	0.20	0.00
Asplanchna spec.	0.00	17.2	0.19	*Conochilus* spec.	0.00	0.20	0.00
Ceriodaphnia quadrangula	2.40	14.2	17.7	*Diaphanosoma* spec.	0.00	0.20	0.00
Ceriodaphnia reticulata	25.2	13.2	2.21	*Lecane* spec.	1.92	0.00	71.1
Pleuroxus uncinatus	1.92	4.80	4.52	*Alona costata*	1.96	0.00	12.9
Lecane monostyla	0.08	4.40	0.00	*Lepadella* spec.	0.00	0.00	6.73
Chydorus sphaericus	0.84	2.40	85.9	*Brachionus* spec.	0.00	0.00	0.19
Eudiaptomus gracilis	0.16	2.40	0.00	*Bosmina longirostris*	5.12	0.00	0.00
Scapholeberis mucronata	0.00	2.20	0.00	*Cypricercus affinis*	0.40	0.00	0.00
Graptoleberis testudinaria	0.00	1.20	0.10	*Colurella undinata*	0.28	0.00	0.00
Keratella cochlearis	0.00	0.80	0.10	*Lepadella patella*	0.16	0.00	0.00
Alona spec.	0.28	0.60	0.00	*Synchaeta pectinata*	0.12	0.00	0.00
Testudinella patina	0.00	0.40	0.00	*Scapholeberis* spec.	0.08	0.00	0.00

Legend: sorted in descending order of respective species according to density in 1997.

From the cumulative list for the years 1996, 1997 and 1998, 26%, 37% and 47% of the taxa were not at all found in 1996, 1997 and 1998, respectively (Table 3-7). It is important to notice that a large part of the remaining taxa (16-32%) occured at maximum densities below 1. Densities above 100/L were encountered in only 3-11% of the cases.

Furthermore, it is worth noting that taxa, which did not occur at all in one year were usually found at low densities in the following and/or precedent year (e.g. *Graptoleberis testudinaria, Lecane monostyla*, Table 3-6).

Variance: mean relationships were calculated for the three years. Resulting slopes (b-values) are shown for single taxa as well as the zooplankton community (Table 3-8). Resulting b-values were highly variable for the year-to-year comparison of the 5 dominant taxa. They are found in a range of 1.39-2.33 (*Keratella quadrata*), 1.65-2.54 (Copepoda), 1.1-2.11 (*Ceriodaphnia* spec.*),* 1.22-3.27 (*Daphnia longispina*) and 1.97-2.21 (*Polyarthra* spec.*).*

Resulting b-values were more homogenous for the whole zooplankton community and were found at 1.70, 1.74 and 1.89 for 1996, 1997 and 1998, respectively. Note that in this case ´community´ is based on densities of the 5 dominant zooplankters for each year.

Table 3-7. Zooplankton interannual variability of taxon presence/ absence and maximum densities.

Density	Percent of Taxa		
(Indiv./L)	1997	1996	1998
0	26	37	47
<1	32	32	16
1-10	16	18	11
11-100	16	11	18
>100	11	3	8

Table 3-8. Zooplankton variance: mean relationship for dominant taxa in 1996, 1997 and 1998.

	b-values for dominant taxa and ´community´		
	1997	1996	1998
Keratella quadrata[(1)]	2.33	1.39	1.49
Copepoda[(1)]	2.54	1.65	1.75
Ceriodaphnia spec.[(1)]	1.97	1.10	2.11
Daphnia longispina[(1)]	1.56	3.27	1.22
Polyarthra spec.[(1)]	2.21	1.97	2.16
´Community´[(2)]	1.74	1.70	1.89

(1): n´= 7 (7 sampling events/year)

(2): ´Community´ in this case consists of the 5 dominant taxa mentioned above with n´= 35 (7 sampling events x 5 dominant taxa).

3.2 DISCUSSION OF SYSTEM-INHERENT VARIABILITY OF ZOOPLANKTON

3.2.1 System-inherent variability of zooplankton in microcosms is comparable to variability in natural ecosystems

Interreplicate variability of population densities is rarely mentioned explicitly in literature. This is true for ´natural ecosystems´ as well as for model ecosystems. Downing *et al.* (1987) calculated a general mean:variance relationship from marine and freshwater zooplankton samples. Up to now it has not been shown to what extent this model applies also to data derived from outdoor microcosms. Due to the lack of information on variability in natural and model ecosystems, data were extracted and calculated for data from published studies as a basis for comparison with the variability in the present study (Table 3-9).

Table 3-9. Zooplankton variability.

Interreplicate variability

Taxon	cov (%)	b	System	Author
Cladocerans	35-82	-	Lake littoral	Vuille, 1991
	22-79	1.64[(a)]	Lake pelagial	Malone *et al.*, 1983
	50-100[(b)]	-	Indoor Microcosms	Van den Brink *et al.*, 1995
	71-120	2.0[(a)]	Mesocosms	Ali *et al.*, 1997
	39-84	1.56	Outd. Microcosms	Present study
Copepods	23-76	-	Lake littoral	Vuille, 1991
	57-76	1.45[(a)]	Lake pelagial	Malone *et al.*, 1983
	16-36[(b)]	-	Indoor Microcosms	Van den Brink *et al.*, 1995
	27-191	1.8[(a)]	Mesocosms	Ali *et al.*, 1997
	34-91	2.54	Outd. Microcosms	Present study
Rotifers	24-157	1.52[(a)]	Lake pelagial	Malone *et al.*, 1983
	11-24[(b)]	-	Indoor Microcosms	Van den Brink *et al.*, 1995
	39-104[(c)]	2.33	Outd. Microcosms	Present study
Zooplankton	-	1.53	Lake pelagial	Pinel-Alloul *et al.*, 1988
	28-64	1.60	Outd. Microcosms	Present study

Interannual variability

Taxon	cov (%)	b	System	Author
Cladocerans	23-165	-	Lake pelagial	Kratz *et al.*, 1987
Copepods	37-59	-	Lake pelagial	Kratz *et al.*, 1987
Rotifers	40-231	-	Lake pelagial	Kratz *et al.*, 1987

Legend: (a) b-values were calculated from extracted values; (b) coefficient of variation (cov) based on SE/m: can be expected to be lower compared to sd/m ; (c) *Keratella quadrata*.

Coefficients of variation

In the present study, coefficients of variation for dominant zooplankton populations were usually found at 35-75% and rarely exceeded 100% (e.g., *Keratella quadrata, Daphnia longispina*, Cyclopoida), while less abundant taxa in most of the cases exceeded coefficients of variation of 100% (e.g*.,* Simocephalus *vetulus*) (Table 3-2).

These findings correspond very well with variability found in natural ecosystems (Table 3-9), namely with zooplankton data from the littoral zone of Lake Biel (Vuille, 1991). Vuille (1991) reported coefficients of variation ranging between 23-76 for copepod species and 35-82 for cladoceran species. Others report coefficients of variation between 22-191% (based on standard deviation) and of 11-100% (based on standard error).

Thus, the interreplicate variability, measured as coefficient of variation, of zooplankton data of the present study in general lies well within the range of variability found in microcosm studies and in natural ecosystems.

Mean:Variance

Based on the mean:variance relationship, the slope (b) has been used as an index of spatial variability in natural aquatic ecosystems (Downing *et al.*, 1987; Pinel-Alloul *et al.*, 1988). The mean: variance relationship is described as $s^2 = aM^b$, where s^2 is the variance and M the average of randomly placed replicate population estimates.

Downing *et al.* (1987) derived a b-value of 1.85 in their common algorithm from 1189 sets of replicate samples of marine and freshwater zooplankton taxa. For individual populations they found b-values ranging from 0.97 to 3.69. Calculation of b-values according to the method used above for data extracted from published data gave b-values in the range of 1.5 to 2.0 (Table 3-9).

In the present study, the common algorithm for all zooplankton taxa revealed a b-value of 1.60 (Table 3-3), while b-values calculated for individual species ranged from 1.58 to 2.54 (Table 3-8). Thus, the interreplicate variability, measured as ´b´, of zooplankton data of the present study lies well within the range of variability published for natural ecosystems and outdoor mesocosms.

Zero-counts

As known for marine community data, zeros are the dominant entry for the large majority of species (Clarke, 1999). For example, in a marine benthic macrofauna study with 174 species and 39 samples, 69% of the entries were zeros (Gray *et al.,* 1990). No data on the proportion of zero-counts for zooplankton studies could be found in literature. Equally, it can be assumed that in ecotoxicological experiments the majority of replicates of an experimental condition may result in zero-counts for a particular species. Presumably, the presence or absence of a species is also strongly affected by seasonality and thus the duration of the observations.

In the present study about 81% of the entries were zero-counts (based on 3648 entries). It can be stated with some caution that this number reflects the situation in natural ecosystems, keeping in mind that the only available reference is related to marine benthic macrofauna (Gray *et al.* 1990).

Moreover, it was shown in the present work that the coefficient of variation increases and the mean decreases with the number of replicates representing a zero-count for a certain taxon (Figure 3-7, Figure 3-6). This results in a high variance:mean relationship for those species which are represented in the majority of replicates with a zero-count and thus in a low statistical power. This problem will be further discussed below.

3.2.2 Interreplicate variability was high for many small populations and therefore precision levels were not satisfactory for most taxa

For the investigated microcosms and the applied sampling design, the variation exceeded a coefficient of 100% for the majority (75%) of the taxa and population densities ranged below one individual per liter in most of the cases (58%). It was shown that the coefficient of variation (cov) is tightly correlated to the mean: the higher the mean, the lower the cov (Figure 3-2). Moreover, the coefficient of variation increased with increasing numbers of zero-counts in the group of replicate samples (Figure 3-7).

The high variability for low population densities combined with the fact that the majority of taxa occur at very low densities may result in statistical problems in an ecotoxicological test when comparing small populations in control to treatment groups. Therefore, for the evaluation of an ecotoxicological microcosm test it is of paramount importance to know which population densities allow a sound interpretation. Or even better, knowing the history of the microcosms, one could consider changing the sampling design to decrease the number of counts below a certain threshold before starting a study

Comparison with Downing *et al.* (1987)

To relate sampling design to the number of individuals per liter needed for a reasonable statistical evaluation, one can refer to Downing *et al.* (1987). They proposed a sampling design which relates the sample size and population density to the number of replicate samples needed for a certain precision level. The number of zooplankton samples (n´) necessary to obtain a required level of precision p (where p= SE/m and SE= $s/n^{0.5}$) can be calculated with the mean population density (m) and the sampler volume (V) as follows:

$$(n' = 0.745\ m^{-0.378}\ V^{-0.267}\ p^{-2}).$$

This algorithm implies that fewer replicate zooplankton samples are needed with increasing population density and sampler volume (Table 3-10).

To give an example: Table 3-10 suggests to use 5 replicates and a sample volume of 10 liters to achieve a precision level of p=0.2 for a mean population density of 10 individuals per liter. However, at this replication level statistically sound statements cannot be made for population densities below 10/L. Increasing the number of replicates and/or the sampler volume would lead to higher precision levels.

Table 3-10. The number of zooplankton samples necessary to obtain a precision of p= 0.2 (SE/m= 0.2) according to Downing *et al.* (1987).

	Volume of replicate sample			
Population density (indiv./L)	1 L	10 L	100 L	1000 L
1×10^{-4}	606	328	177	96
1×10^{-3}	254	137	75	40
1×10^{-2}	107	58	31	17
1×10^{-1}	45	24	13	7
1.0	19	10	6	3
1 x 10	8	5	3	2
1×10^{2}	4	2	2	2
1×10^{3}	2	2	2	2

As b-values of the investigated system were found well in the range of the b-values which were used as a basis for Downing´s table (Table 3-8, Table 3-10) it was assumed that Downing´s predictions apply to the present study. To verify this, calculations of precision levels were performed for all taxa and all sampling events (Table 3-4). In the present study, the sampler volume was 10L and the number of replicate microcosms used was 12. According to Table 3-10, a precision level of 0.2 can be predicted for taxa with population densities greater than 1/L.

It was shown that in the present study, only few taxa reached precision levels below 0.2 throughout the study period, namely Cyclopoida, *Daphnia longispina* and *Keratella quadrata* (Table 3-4). These 3 taxa consistently occur at densities, which definitely would allow a sound statistical evaluation.

In contrast, it was shown that the majority of the taxa usually exceeded a p-value of 0.2, which means that a sound statistical analysis would not be possible for these taxa. It is important to notice that only in 17% of the cases with densities from 1/L to 10/L, the precision level of 0.2 was reached. A much better situation was found for densities above 10/L, where the criterion of $p<0.20$ applied to 61% of the data points.

Thus, for the present study, Downing´s predictions (Table 3-10) apply to a minor part of the data sets. This finding might be related to the fact that the investigation of zooplankton in the present study was based on 123 data-sets, while Downing *et al.* (1987) refer to a much higher number of 1189 data-sets. Further, outliers were not eliminated in the present study with the intention to show the ´true natural variability´, while Downing´s calculations might be based partly or entirely on data-sets where outliers have been eliminated.

Summarising the above, for the investigated microcosms and the applied sampling design, the natural variability has a strong impact on the interpretability of the majority of findings for taxa occurring at densities below 10 individuals per litre at the precision level of p=0.2. The following published data confirm the results of the present study (Ammann *et al.,* 1997; Lozano *et al.*, 1992; Liber, 1992).

Low statistical power for low population densities

The lack of statistical power for taxa with low population densities has been described by Ammann *et al.* (1997) for a series of marine microcosm experiments. According to their calculations, 11 to 19 replicates would be required to detect a 50% decrease in the mean number of taxa which occurred at mean densities around 1/L (Poisson model, level of significance α=0.1). For mean densities greater than 10/L, 1 to 2 replicates would be sufficient to detect a 50% decrease. However, their publication also clearly shows the difficulty of detecting *small deviations* of the treated microcosms when compared to the control in an ecotoxicological test. To detect a reduction of the mean count of 20% would, according to Ammann *et al.* (1997), need 82 to134 replications (=ponds) when the mean counts are as low as 1/L. For mean numbers exceeding 10/L, one would still need 3 to 9 ponds.

Liber *et al.* (1992) used 3 replicates per treatment in a limnocorral system. Lowest measured macrozooplankton densities were about 10/10L. Based on the variance associated with the mean abundance data for macrozooplankton data they estimated that the smallest detectable percent decrease in total density was about 50% (for ANOVA and regression design, $p \leq 0.05$).

Lozano *et al.* (1992) also consider a 50% reduction in treatment zooplankton density compared to control density as significant in a study with littoral enclosures. They justify the cut-off limit of 50% by the fact that this magnitude of change corresponds to approximately 2.5 standard deviations based on a log10(N+1) density transformation. Lozano *et al.* (1992) recognize the high interreplicate variability for low population densities by including in their analysis only those populations that had five or more individuals per sampler in the controls.

From the cited studies it is evident that the selection of the size of response (e.g., a 20% or a 50% change) is critical for test design and data interpretation. From a pragmatic point of view, less precision might be acceptable to detect major changes in small populations. For large populations, higher precision levels can be applied due to the smaller natural variability, allowing the detection of small changes.

3.2.3 Inter-replicate variability within the control group increased through time

The inter-replicate variability measured as coefficient of variation for total density and species diversity was lowest during the pre-exposure phase (day-24) (Figure 3-1), which is due to the continuous water circulation between the microcosms and the mixing tank until test day –2.

A moderate increase of inter-replicate variability was shown for total density of zooplankton during days 0 through 28, after the isolation of the microcosms from the mixing tank. Coefficients of variation reached their maxima at the end of the study period (Table 3-2).

This trend was also observed for coefficients of variation calculated for dominant zooplankton taxa, i.e., *Keratella quadrata, Daphnia longispina* and Cyclopoida (Table 3-2).

In general, inter-replicate variability were much higher for taxa occurring at low densities, e.g., *Simocephalus vetulus* had coefficients of variation of 94-346% and therefore did not exhibit any relation of coefficient of variation to the disconnection of the microcosms from the mixing tank (Table 3-2).

Thus, the increase of inter-replicate variability can be related to an enclosure effect. The enclosure effect could be overcome by establishing a continued water inflow (and thus re-inoculation with zoo- and phytoplankton organisms) from the reservoir during exposure and post-exposure phase of the study. Continued water exchange would also have the advantage of simulating a more ´natural´ scenario as it would theoretically allow a re-colonization of the microcosms. However, the amount of water exchanged in treated ponds should not interfere with the maintenance of investigated product concentrations in an ecotoxicological test. Up to now, no limit value for a water exchange rate has been established in the existing guidance documents.

3.2.4 Zooplankton community structure was strongly dominated by few species throughout the study

The zooplankton community structure of the investigated microcosms was characterized as being strongly dominated by comparatively few taxa, namely by *Daphnia longispina,* Cyclopoida *(Meso- and Megacyclops), Keratella quadrata and Polyarthra vulgaris* (Table 3-5, Figure 3-8).

Due to the fact that the diversity index is not only based on species richness but also on their evenness, i.e. the distribution of individuals among the different species, the mean diversity index was comparatively low throughout the duration of the study (Figure 3-1). In the course of the study, increasing total density was accompanied by decreasing species diversity (Figure 3-1).

Changes in the dominance partition of zooplankton taxa (Figure 3-8) and the decreasing species diversity for the zooplankton community (Figure 3-1) could be linked to the clear dominance shift in the course of the study (Figure 3-9) namely to the significant increase in number of rotifers at test end (Table 3-5). Moreover, the decrease of the mean diversity index was influenced by 3 control replicates having very low indices (Figure 3-1).

In natural fresh water ecosystems population densities and species composition of the zooplankton fluctuate throughout the summer (Sommer *et al.*, 1986). For the present study, seasonal shifts in population densities (Figure 3-9) for cladocerans, Cyclopoida and rotifers can be explained for the three major groups as follows.

Cladocera

In the present study the species composition of Cladocerans was clearly dominated by *Daphnia longispina, Simocephalus vetulus* and *Ceriodaphnia* spec. Populations of other cladocerans were found at negligible numbers. This is in line with observations made by Sommer *et al.* (1986) that in unstocked ponds one or two species of large-bodied Daphnia are dominating, contrary to the situation in lakes where constant predation pressure from invertebrates and fish permits the co-existence of many herbivore species.

In lakes, peaks of juvenile and adult Daphniidae usually occur in May/ early June (Vuille, 1991; Einsle, 1987). As in the present study sampling started in mid-June it can be assumed that the yearly maximum Daphnia abundance was not captured by the applied sampling regime. This explains the low Daphnia numbers on test day-24 (June 16), which might be related to a `clear-water´ phase. The decline of Daphnia as of August 7 (day 28) might be related to an increase of inedible algae (Anabaena spec. (chapter 3.8).

For day 101 (October 97) when the mean population density of *Daphnia longispina* had dropped to about 10/L, two other cladocerans occurred at consistent but still low numbers: *Pleuroxus uncinatus* and *Leygidia acanthoceroides*. The latter was not found in the ponds on previous sampling events.

Copepoda

Species composition of Copepoda in the water column of the microcosms was strongly dominated by Cyclopoida. More than 99% of the Copepods caught June through September 1997 were identified as *Megacyclops* spec./ *Mesocyclops* spec., whereas the calanoid copepod *Eudiaptomus gracilis* occurred at very low numbers during the sampling period (June to October).

This finding is in line with seasonal changes found in the Mindelsee (Einsle, 1983; Einsle, 1969) showing that peak densities of *Eudiaptomus* are usually encountered in May and that summer populations of *Eudiaptomus* can be damaged by *Mesocyclops* to such an extent that only a few animals per litre survive.

In the present study, the number of cyclopoid copepods in the water column declines to a very low number in October 1997 (day 101). This can be related to the life cycle of *Megacyclops* spec./ *Mesocyclops* spec.: during winter only the copepodid stages IV and V are found in the benthos (e.g., Einsle, 1983).

Rotatoria

Species composition of Rotatoria in the water column of the microcosms was strongly dominated by *Keratella quadrata* and *Polyarthra vulgaris*, which represented more than 95% of the Rotatoria caught throughout the sampling period in 1997. The abundance of rotifers increased strongly as of test day 28, coinciding with a decrease of *Daphnia longispina* (Figure 3-9).

It is known that large cladocerans successfully compete with rotifers for food and that they mechanically interfere with *Keratella* (e.g., Gilbert, 1985). In the present study, the dominant role of rotifers in October 1997 may be related to the comparatively low numbers of *Daphnia longispina* in September and October 1997. Replacement of larger species of crustacean herbivores by rotifers has also been related to the fact that rotifers are less affected by interference with their food collecting apparatus which can be caused by some forms of inedible algae (Sommer *et al.*, 1986).

3.2.5 Investigation of the year-to-year variability showed weaknesses in the test design: many population densities close to zero

High interannual variability of crustacean production is known from natural ecosystems and confirms that the littoral ecosystem is versatile and unbalanced with low predictability and frequent changes in dominance within communities.

Kratz *et al.* (1987) report that the abundance distribution of copepods and cladocerans exhibited relatively greater variability among lakes than among years, suggesting that conditions specific to lakes are important in controlling these parameters. Vuille (1991) states that planktonic and epiphytic crustaceans in the littoral zone of lake Biel seem to be much more affected by unpredictable environmental factors like temperature variations than benthic communities. Moreover, changes in the composition of pelagic crustacean communities can be partly explained by immigration as well as disappearance of species (Einsle, 1983).

High interannual variability was also found for the present study (Table 3-6). Comparing the maximum population densities in the year in which the study was conducted to studies in the precedent and following years revealed the importance of method validation for the test system used. As can be seen from the table of maximum abundance, not every single taxon is found in every year (Table 3-6). However, taxa *absent* in one year often occur at low densities in the precedent or following year. It has to be emphasized that for the three years, most of the taxa were found at maximum population densities of <1/L (59-69%), few taxa at densities of 1-10/L and 10-100/L (12-24% and 12-17%), and very few at densities >100/L (3-7% of the taxa).

Apparently, the sampling method and the sampling volume chosen (1997: depth-integrated water column, 10L; 1996 and 1998: plankton net, 50L) resulted in very low species counts, close to the ´detection limit´ for the major part of the species. This suggests that taxa may erroneously be stated as being absent or as having disappeared from the system due to weaknesses of the sampling methodology. Low species counts can therefore be considered as being of limited value for the interpretation of a microcosm study. Ecologically relevant taxa, occurring at low densities pose a statistical problem, which should be taken into consideration when setting up a study.

3.3 DISTURBANCE EFFECTS ON ZOOPLANKTON

3.3.1 Coefficients of variation for zooplankton in the treatment groups

The treatment groups SHADOW, TURBULENCE, RUN-OFF and DIAZINON, consisted of 4, 3, 4 and 4 replicates, respectively. Population density and species diversity of all groups were compared to the control group.

The average total zooplankton densities (individuals/L) of each treatment group and the control, as well as the variability within the treatment groups shown as curves for each replicate, are displayed in Figure 3-11. In general, total densities increased throughout the study period. Densities for the treatments TURBULENCE and DIAZINON went up to about 1000 and 8000 individuals/L, respectively, while total densities for the treatments SHADOW and RUNOFF similar to the CONTROL never exceeded 1000 individuals/L.

The corresponding coefficients of variation (Table 3-11) were found within a range of 11 to 80%, except for the treatment DIAZINON on day 80 and 101 (120% and 103% respectively). Variability within groups was low and similar for all treatment groups during the pre-exposure phase on day-24 (14-30%).

Table 3-11. Coefficients of variation in the treatment groups and the control for zooplankton total density and species diversity.

Total density, Coefficients of variation (%)					
	SHADOW	TURBULENCE	RUNOFF	DIAZINON	CONTROL
day -24	14	27	30	19	28
day 0	28	68	18	29	45
day 4	43	53	36	61	30
day 14	35	25	80	57	36
day 16	27	68	28	35	46
day 28	49	34	46	43	43
day 80	34	59	11	120	51
day 101	32	50	56	103	64

Species Diversity, Coefficients of variation (%)					
	SHADOW	TURBULENCE	RUNOFF	DIAZINON	CONTROL
day -24	11	8	9	8	10
day 0	10	86	11	12	25
day 4	22	18	7	15	7
day 14	14	40	18	20	12
day 16	21	35	11	23	19
day 28	13	22	27	32	15
day 80	11	55	33	65	37
day 101	4	35	50	70	44

Note: results from the exposure phase are shaded (day 4 to 28).

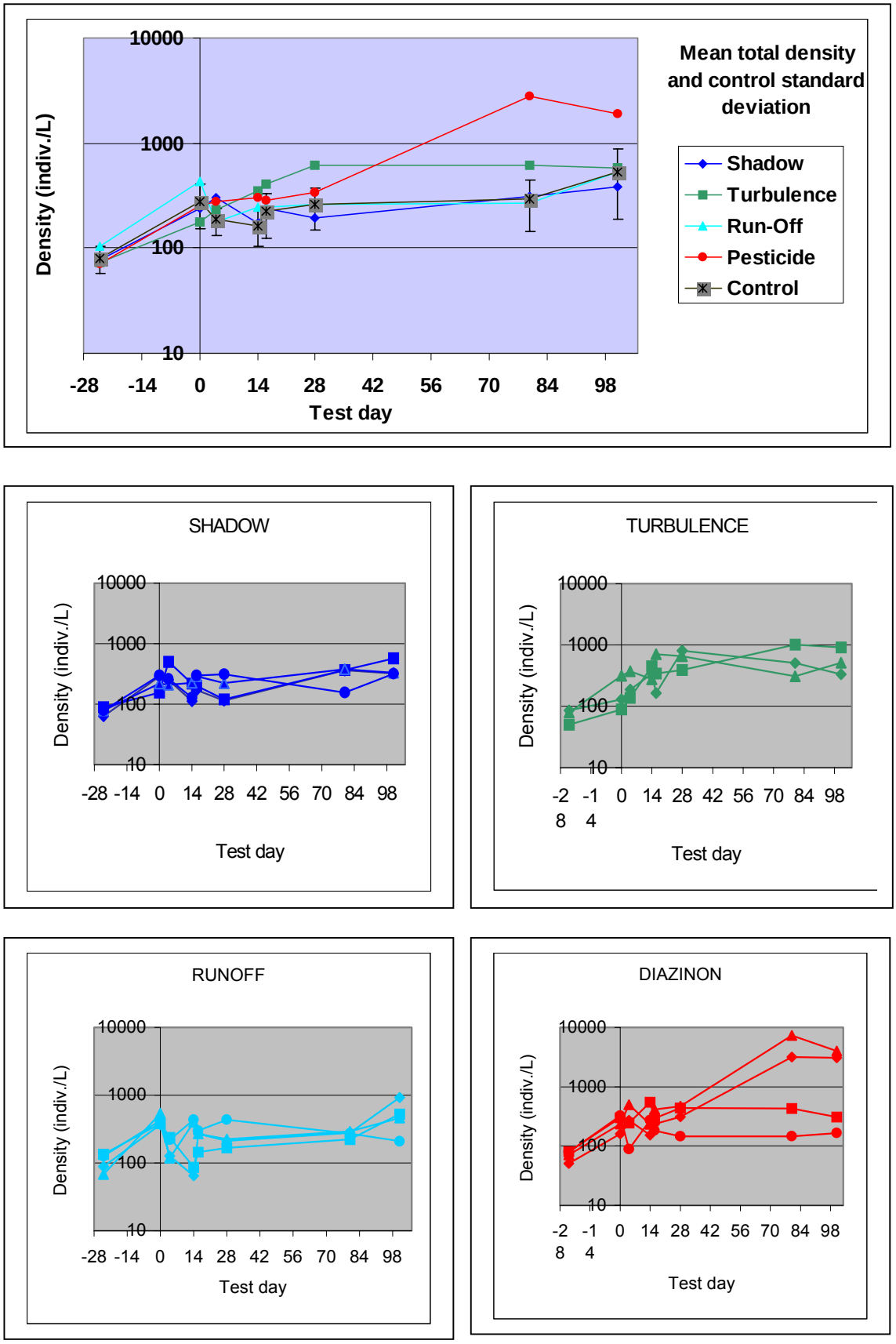


Figure 3-11. Mean zooplankton total densities for the treatments and the control and within variability for each treatment group.

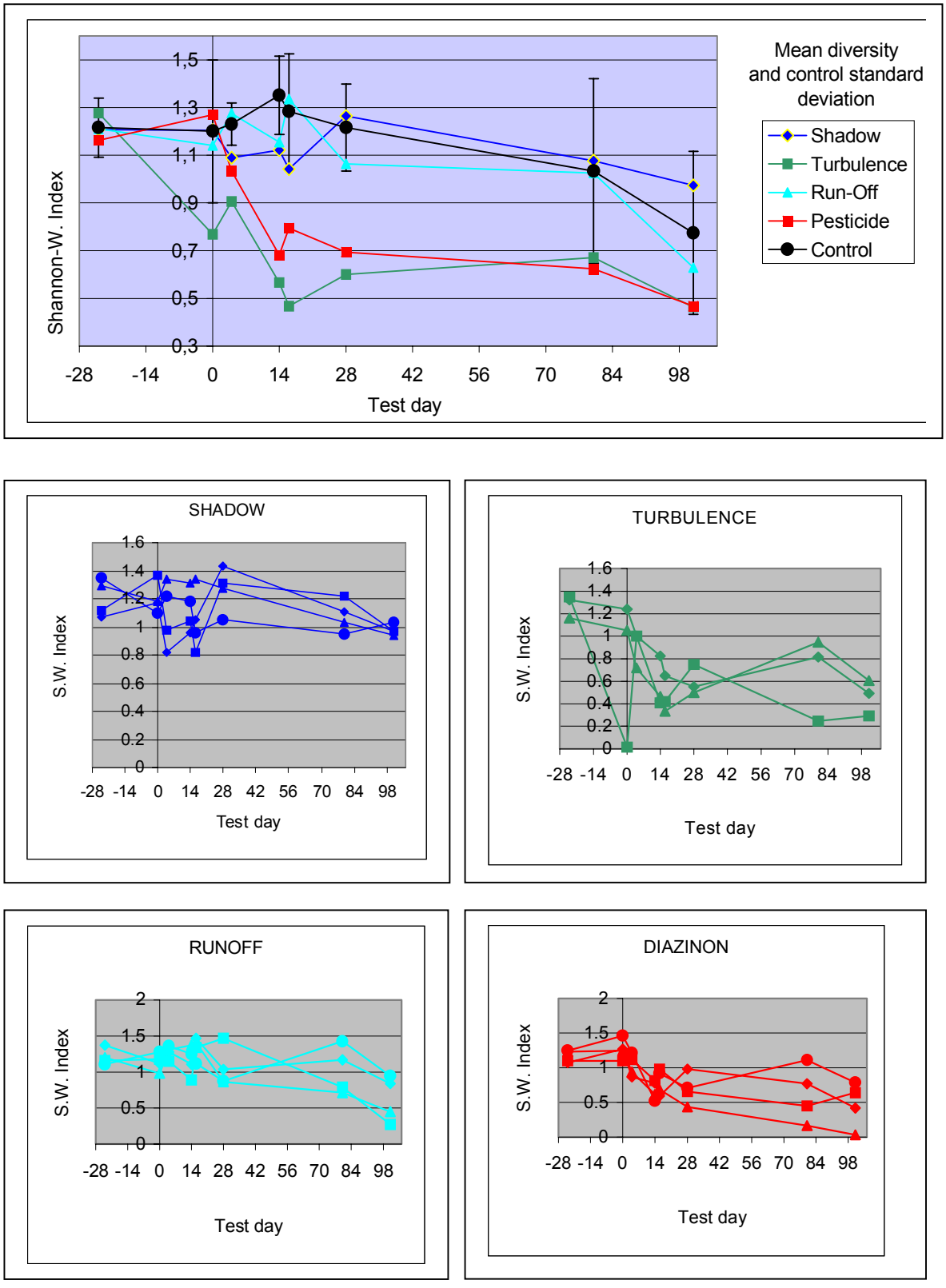


Figure 3-12. Mean zooplankton species diversity for the treatments and the control and within variability for each treatment group.

Figure 3-12 displays the average zooplankton diversity (Shannon-Wiener Index) for each treatment group and the control. Standard deviation bars are displayed for the control. The variability within the treatment groups is also shown as curves for each single replicate in Figure 3-12. Initially, Shannon-Wiener Indices of about 1.2 were found for all treatments. For the treatments TURBULENCE and DIAZINON, average species diversity dropped to values far below 1.0 during the exposure and post-exposure phase (day 0 to day 101). Average species diversity remained within a range of 1.0 to 1.3 for SHADOW and RUNOFF until day 28 and then also dropped to average values below 1.0 (day 80 and day 101).

From day –24 to day 28, the corresponding coefficients of variation (Table 3-11) varied between 7 to 40%, except for the treatment TURBULENCE on day 0 (86%). The high variation on day 0 in the TURBULENCE treatment was most likely due to a sampling error in one replicate (Figure 3-12); which was also supported by the fact that the coefficient of variation was again much lower (18%) on day 4. On day 80, coefficients of variation were 55% and 65% for the treatments TURBULENCE and DIAZINON, respectively, while they remained at 11% for SHADOW and at 33% for RUN-OFF. The lowest coefficients of variation throughout the study period were found for the treatment group SHADOW.

3.3.2 Dominant Zooplankton Taxa in Treatment Groups

As already noted for the control group, the zooplankton communities in all treatment groups were strongly dominated by few taxa throughout the duration of the study. This can be seen from the dominance plots, showing the percent contribution of each ranked taxon to the total density of the control and the four treatments for days -24, 16 and 101 (Figure 3-13). The dominance plot should be read in conjunction with the corresponding list of ranked species (Table 3-12). For example: for day 16, rank 1 for the pesticide treated ponds was assigned to Cyclopoida, which contributed with about 75% to the total zooplankton density. For the same test day, rank 2 was assigned to *Polyarthra vulgaris*, which contributed with about 10% to the total density.

Throughout the study period, the communities were in general dominated by the taxa *Keratella quadrata,* Cyclopoida*, Daphnia longispina, Polyarthra vulgaris, Polyarthra spec.* and *Synchaeta spec.* The taxa listed in Table 3-12 formed altogether over 97% of the total density for each treatment group counted on the corresponding sampling day (shown for days –24,16 and 101). Mean densities for the dominant species calculated for each treatment group and the control are shown in Figure 3-14.

When compared to the control, most obvious differences for dominant taxa were found for DIAZINON, where *Daphnia longispina*, being one of the most abundant species on day –24 and day 0 in both groups, had disappeared from the DIAZINON treated ponds on days 4 through 80. *Daphnia longispina* reappeared on day 101 in the DIAZINON treated tanks, however only with a density of 0.5 indiv./L (Figure 3-14). For the DIAZINON treated tanks, this resulted in an 80% dominance of Cyclopoida on days 14 to 28 and a 90% dominance of the species *Keratella quadrata* on days 80 to 101 as already seen in Table 3-12.

TURBULENCE treated tanks were also extremely dominated by *Keratella quadrata* on days 14 to 101, while copepod densities were comparatively low throughout the exposure phase (Figure 3-14).

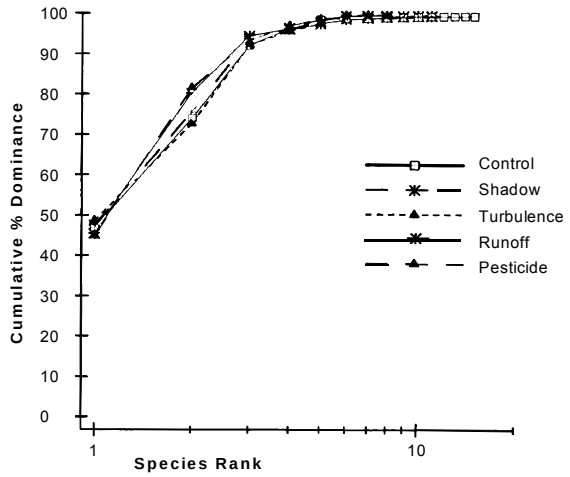
The treatments SHADOW and RUN-OFF showed dominance curves and species ranking very similar to the control curves throughout the duration of the study (Figure 3-13, Table 3-12).

Table 3-12. Ranking of species for all treatments on days –24, 0, 16, 28 and 101.

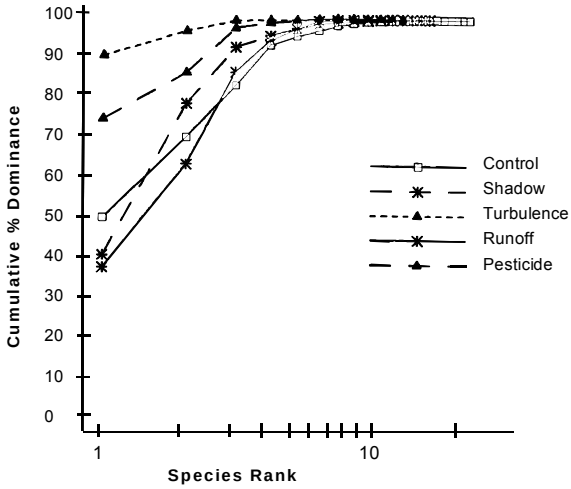
Day	Rank	Control	Shadow	Turbulence	Run-off	Diazinon
-24	*1*	*Keratella quadrata*	*K.quadrata*	*K.quadrata*	*K.quadrata*	*K.quadrata*
	2	Cyclopoida	Cyclopoida	Cyclopoida	Cyclopoida	Cyclopoida
	3	*Daphnia longispina*	*D. longispina*	*D. longispina*	*D. longispina*	*D. longispina*
	4	*Polyarthra vulgaris*	*P. vulgaris*	*P. vulgaris*	*P. vulgaris*	*P. vulgaris*
0	*1*	*K.quadrata*	*K.quadrata*	*K.quadrata*	*K.quadrata*	*K.quadrata*
	2	*D. longispina*	Cyclopoida	Cyclopoida	Cyclopoida	Cyclopoida
	3	Cyclopoida	*D. longispina*	*D. longispina*	*D. longispina*	*D. longispina*
16	*1*	*K.quadrata*	*Cyclopoida*	*K.quadrata*	*D. longispina*	Cyclopoida
	2	*Cyclopoida*	*K.quadrata*	*Cyclopoida*	*K.quadrata*	*P. vulgaris*
	3	*D. longispina*	*D. longispina*		Cyclopoida	*K.quadrata*
	4	*P. vulgaris*				
28	*1*	*K.quadrata*	*D. longispina*	*K.quadrata*	*K.quadrata*	Cyclopoida
	2	*D. longispina*	*K.quadrata*	*D. longispina*	*D. longispina*	*K.quadrata*
	3	Cyclopoida	Cyclopoida	Cyclopoida	Cyclopoida	*Polyarthra spec.*
	4	*Polyarthra spec.*	*Polyarthra spec.*			
101	*1*	*K.quadrata*	*K.quadrata*	*K.quadrata*	*K.quadrata*	*K.quadrata*
	2	*P. vulgaris*	*P. vulgaris*	*P. vulgaris*	*P. vulgaris*	*P.vulgaris*
	3	*D. longispina*	*D. longispina*	*Synchaeta spec.*	*D. longispina*	
	4	*Cyclopoida*	*Synchaeta spec.*			

Note: Cutoff at 97% cumulative abundance on the basis of untransformed data.
Note: Results from the exposure phase are shaded (day 16 and 28).

DAY -24



DAY 16



DAY 101

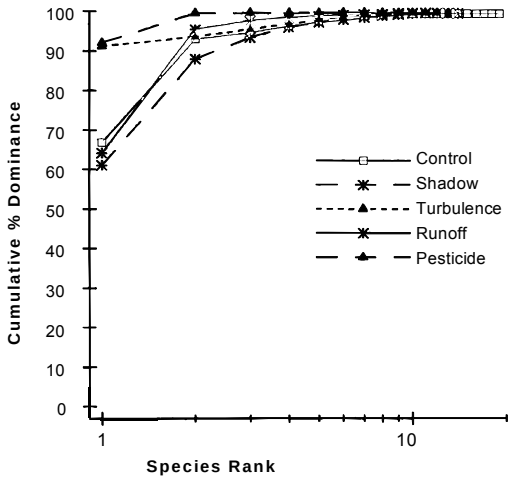


Figure 3-13. Dominance plots for CONTROL, SHADOW, TURBULENCE, RUN-OFF and DIAZINON on days –24, 16 and 101.

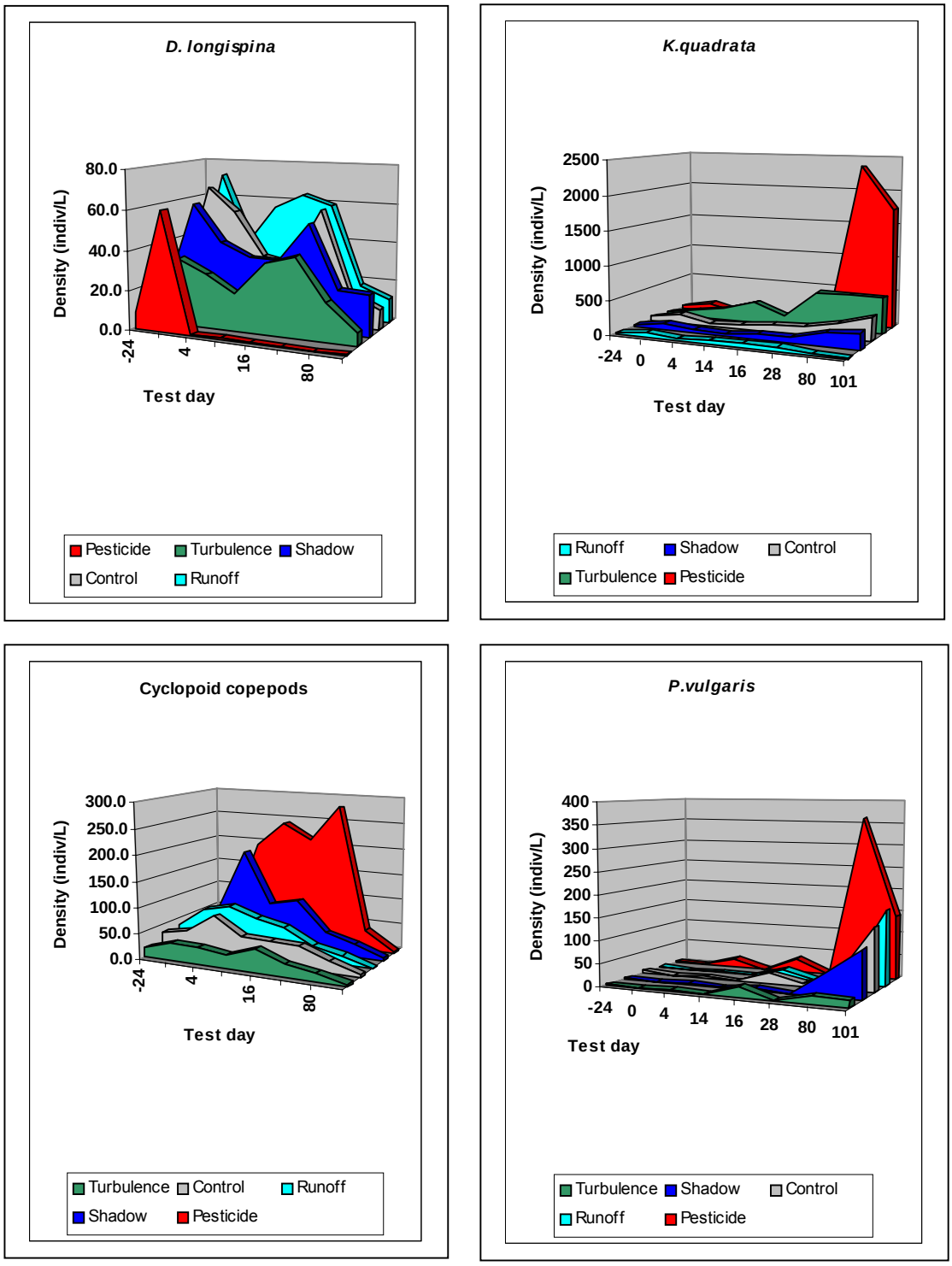


Figure 3-14. Mean densities for dominant taxa in the treatment control groups.

3.3.3 Univariate Testing for Effects on Zooplankton Density and Species Diversity

The Dunnett´s test was applied to the total density (individuals/L) and species diversity indices (Shannon-Wiener Index). Treatment groups were compared to the control group. Results of the Dunnett´s test are shown in Table 3-13.

Total density was significantly higher in treatments TURBULENCE and DIAZINON when compared to the control on day 28 and day 80, respectively. No differences to the control were found for total zooplankton density in the treatments SHADOW and RUNOFF.
Species diversity was significantly lower when compared to the control for treatments TURBULENCE and DIAZINON on days 4 through 28 and on days 14 through 28, respectively. No differences to the control were found for zooplankton species diversity in the treatments SHADOW and RUNOFF.

Table 3-13. Significant differences between control and treatment groups for total density and species diversity of zooplankton.

	Total density (log (counts/L))	Species Diversity (Shannon-Wiener Index)
Day -24	n.s.	n.s.
Day 0	n.s.	n.s.
Day 4	n.s.	Turbulence (-)
Day 14	n.s.	Turbulence (-), Diazinon (-)
Day 16	n.s.	Turbulence (-), Diazinon (-)
Day 28	Turbulence (+)	Turbulence (-), Diazinon (-)
Day 80	Diazinon (+)	n.s.
Day 101	n.s.	n.s.

Legend: values in the treatment group were not significantly (n.s.) or significantly higher (+) or lower (-) when compared to the control (Dunnett´s test, $p<0.05$).

The Dunnett´s test was also used to compare densities of dominant zooplankton taxa in the treatment groups to those in the control (Table 3-14). Significant differences between treatment groups and the control group were found for 8 zooplankton taxa, see also Figure 3-14 for graphical presentation for dominant species.

No differences between treatment and control groups were observed for the pre-exposure phase (day –24, day 0). All significant effects found during exposure and post-exposure phase could be attributed to the treatment groups DIAZINON and TURBULENCE. During the entire study period, no significant differences were found for dominant zooplankton taxa when comparing the treatments SHADOW and RUNOFF to the control.

Throughout exposure and post-exposure phase, *Daphnia longispina* occurred at significantly lower densities in the DIAZINON treated group when compared to the control.

Asplanchna spec. and Cyclopoida occurred at significantly higher densities when compared to the control during the exposure phase. On day 80 densities were significantly higher for *K.quadrata* and significantly lower for *Synchaeta spec.* in the DIAZINON treatment when compared to the control densities.

The treatment TURBULENCE showed a significant positive effect on the density of *P.uncinatus* during the exposure phase. On day 14 densities were significantly higher for *K.quadrata* and significantly lower for *Polyarthra spec.* in the TURBULENCE treatment when compared to the control densities.

Table 3-14. Significant differences between control and treatment groups for dominant zooplankton taxa.

	Day –24 / Day 0	Day 4	Day 14	Day 16	Day 28	Day 80	Day 101
Daphnia longispina	-	**D** (-)	**D** (-)	**D** (-)	**D** (-)	**D** (-)	**D** (-)
Pleuroxus uncinatus	-	-	-	**T** (+)	**T** (+)	-	**D** (-)
Cyclopoida	-	-	**D** (+)	**D** (+)	**D** (+)	-	-
Asplanchna spec.	-	**D** (+)	**D** (+)	-	**D** (+)	-	-
Keratella quadrata	-	-	**T** (+)	**D** (-)	-	**D** (+)	-
Lecane spec.	-	-	-	-	**D** (+)	-	-
Polyarthra spec.	-	-	**T** (-)	-	-	-	-
Synchaeta spec.	-	-	-	-	-	**D** (-)	-

Legend: values in the treatment groups Diazinon (D) and Turbulence (T), were significantly higher (+) or lower (-) when compared to the control (Dunnett´s test, $p<0.05$). Results from the exposure phase are shaded (day 4 to 28). Data were log-transformed.

3.3.4 Cluster Analysis for Zooplankton

Hierarchical clustering with group-average linking based on Bray-Curtis similarity matrices were performed for the zooplankton communities including all replicates of the four treatments and the control. Zooplankton densities (Y) for each taxon were log-transformed (Y´= log (Y+1)) before analysis, thereby giving more weight to rare and less weight to abundant taxa and attributing a very low weight to zero-counts.

Dendrograms for the hierarchical clustering are shown in Figure 3-15 for 4, 3, 4, 4 and 12 replicate samples of the treatment groups SHADOW, TURBULENCE, RUNOFF, DIAZINON and the controls, respectively. Note that the pre-treatment replicate samples were named according to the treatments they were assigned to in the exposure phase, although on day-24 and day 0, all 27 tanks remained untreated. Examples of dendrograms were shown for the pre-treatment, exposure and post-exposure phase, i.e. day –24, day 16 and day 101.

Analyses of pre-treatment samples showed that Bray-Curtis similarities were in general greater than 75% (day-24) and 65% (day 0). The dendrograms for the pre-treatment samples show that treatment replicates were not grouped into discrete clusters but were randomly distributed in different clusters.

This indicated that the treatments were assigned randomly to the 27 tanks and demonstrating that the ponds were quite similar to each other prior to the treatment.

During the exposure phase (day 4 to day 28), the treatments DIAZINON and TURBULENCE formed discrete clusters. Most obviously, clustering was found for the DIAZINON replicates on day 14 and day 16, where discrete clusters were formed at Bray-Curtis similarities of 70-80% (day 16, Figure 3-15).

While DIAZINON treated tanks cluster throughout the exposure period, the TURBULENCE replicates formed discrete clusters only on day 4, day 14 and day 16, but not on day 28. The treatments SHADOW and RUNOFF did not show distinguishable patterns throughout the exposure period.

Similarities were still comparatively high (>70%) for the samples taken on day 80 and day 101, i.e. 11-15 weeks after isolation of the tanks. On day 101, the four DIAZINON replicates are grouped into two clusters, one of them at a low similarity level. Replicates of the treatments SHADOW, TURBULENCE and RUNOFF and the control did not show distinguishable patterns throughout the post-exposure period.

Hierarchical clustering has proved a useful technique for delineating groups with distinct community structure. However, it is best used in conjunction with ordination. The next chapter shows the results of the ordination technique Multi Dimensional Scaling (MDS).

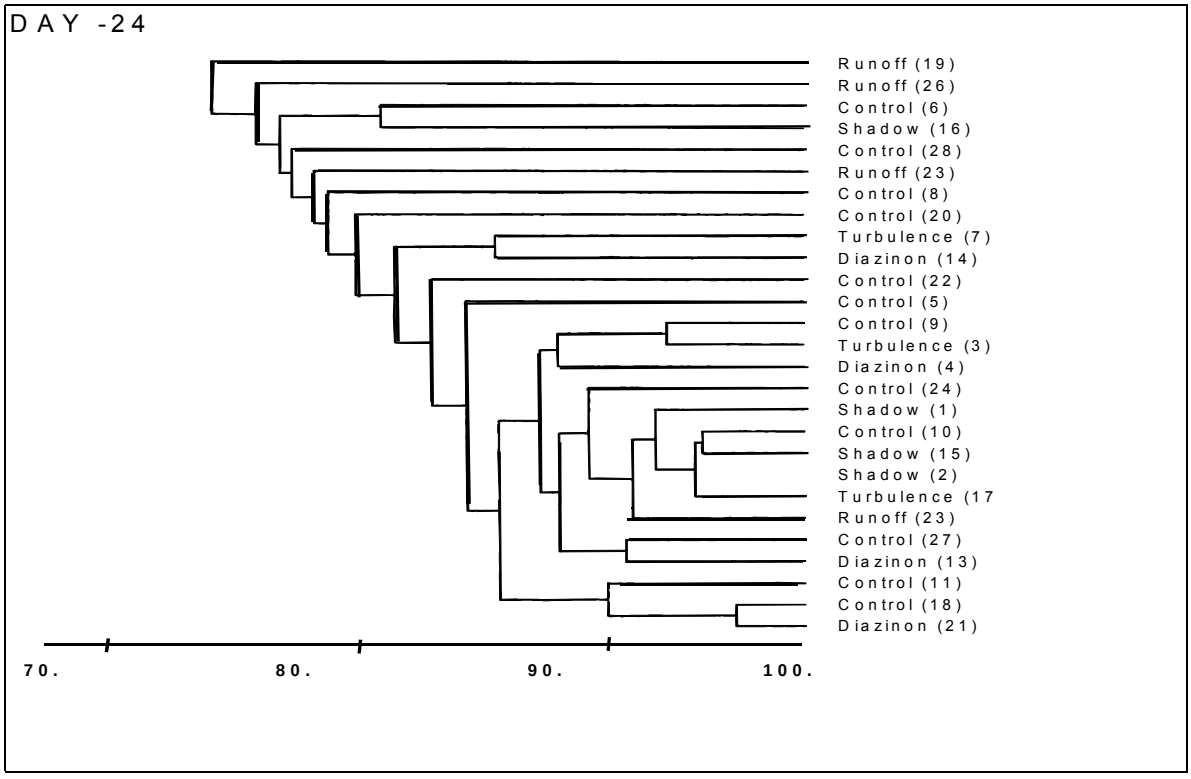
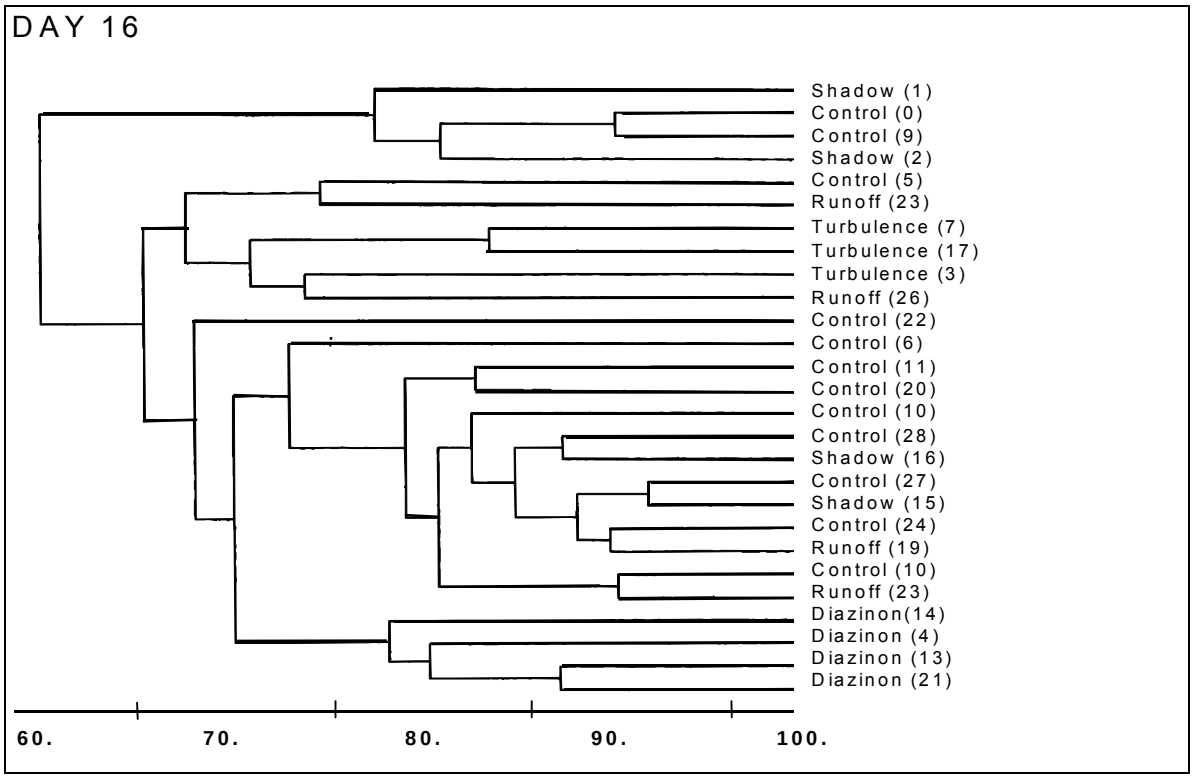


Figure 3-15. Dendrograms for hierarchical clustering of zooplankton counts for treatment and control groups for day-24, 16 and 101.



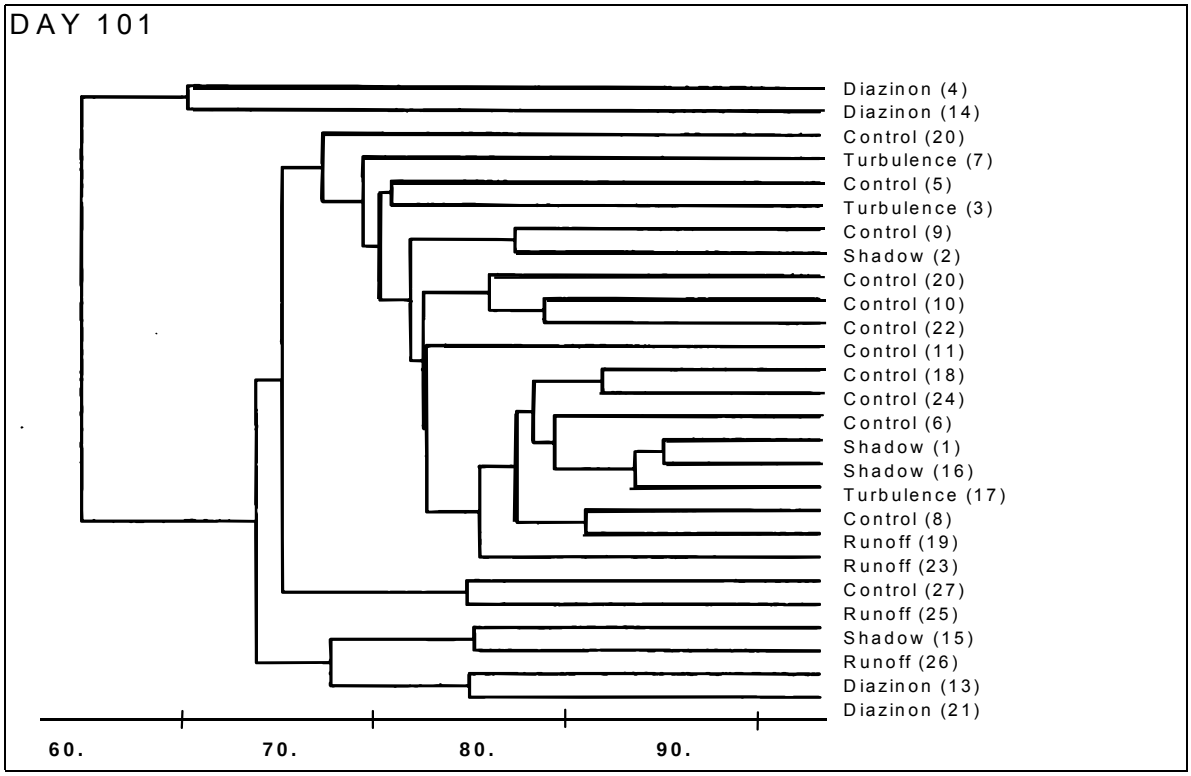


Figure 3-15 (cont.). Dendrograms for hierarchical clustering of zooplankton counts for treatment and control groups for day-24, 16 and 101.

3.3.5 Multi Dimensional Scaling For Zooplankton

Multi Dimensional Scaling (MDS) was performed for zooplankton data, based on a Bray-Curtis similarity matrix on log-transformed data. With MDS, a "map" of the samples is constructed based on a rank similarity matrix. Examples of MDS ordinations for the pre-treatment, exposure and post-exposure phase, i.e. for day –24, day 16 and day 101, are given Figure 3-16.

For pre-treatment samples (day –24 and day 0), no distinct formation of groups or gradation across the set of replicates could be seen from the MDS ordination. Groups were formed by superimposing clusters from Figure 3-15 at similarity levels of 70-80% for day –24. Distribution of the replicates over these groups did not show a clear pattern, thus indicating that the untreated replicates were randomly assigned to the treatments before exposure start.

During the exposure phase (day 4 to day 28), the replicates treated with DIAZINON formed discrete groups in the ordination diagram. Most obviously, grouping was found for all four of the DIAZINON replicates on day 14 and day 16. When superimposing clusters from Figure 3-15 at similarity levels of 60-80%, distinctive groups were found for DIAZINON treated replicates throughout the exposure period (day 16, Figure 3-16). The response for the treatment group TURBULENCE was much weaker, forming groups only on day 14 and day 16, but not on day 4 and day 28. The replicates of the treatments SHADOW and RUNOFF appeared to be randomly distributed over the remaining groups throughout the exposure period.

On day 101, the four DIAZINON replicates still formed discrete groups. Group formation was less obvious on day 101, as already detected with cluster analysis. Replicates of the treatments SHADOW, TURBULENCE and RUNOFF and the control appeared to be randomly distributed over the remaining groups throughout the post-exposure period.

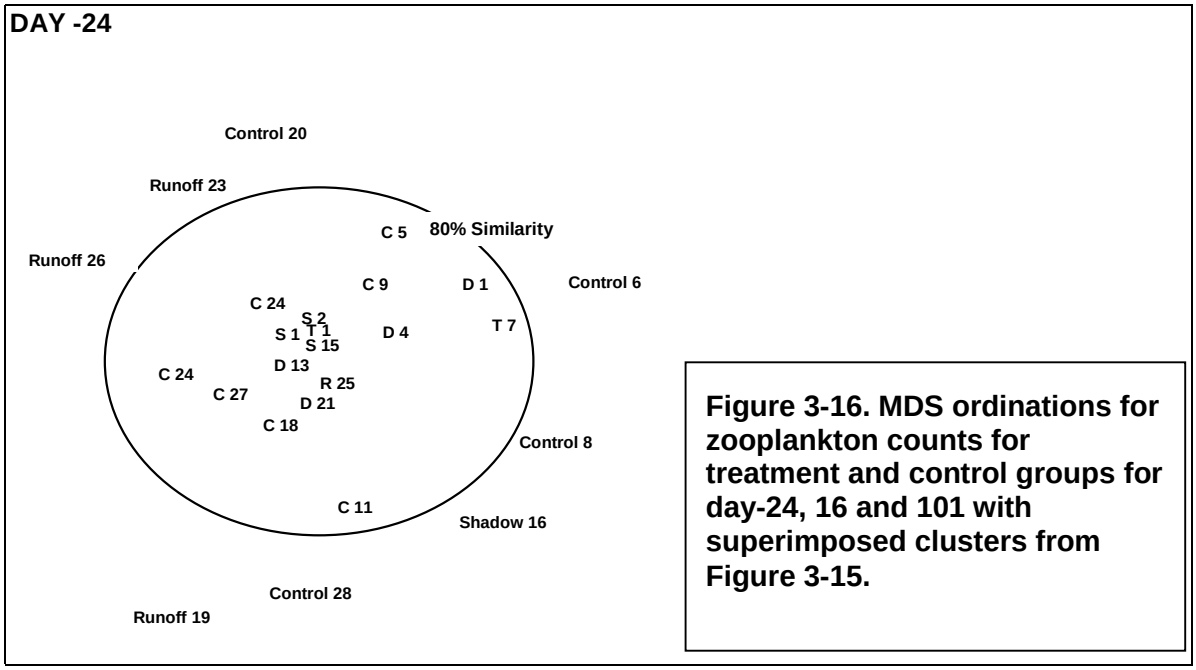


Figure 3-16. MDS ordinations for zooplankton counts for treatment and control groups for day-24, 16 and 101 with superimposed clusters from Figure 3-15.

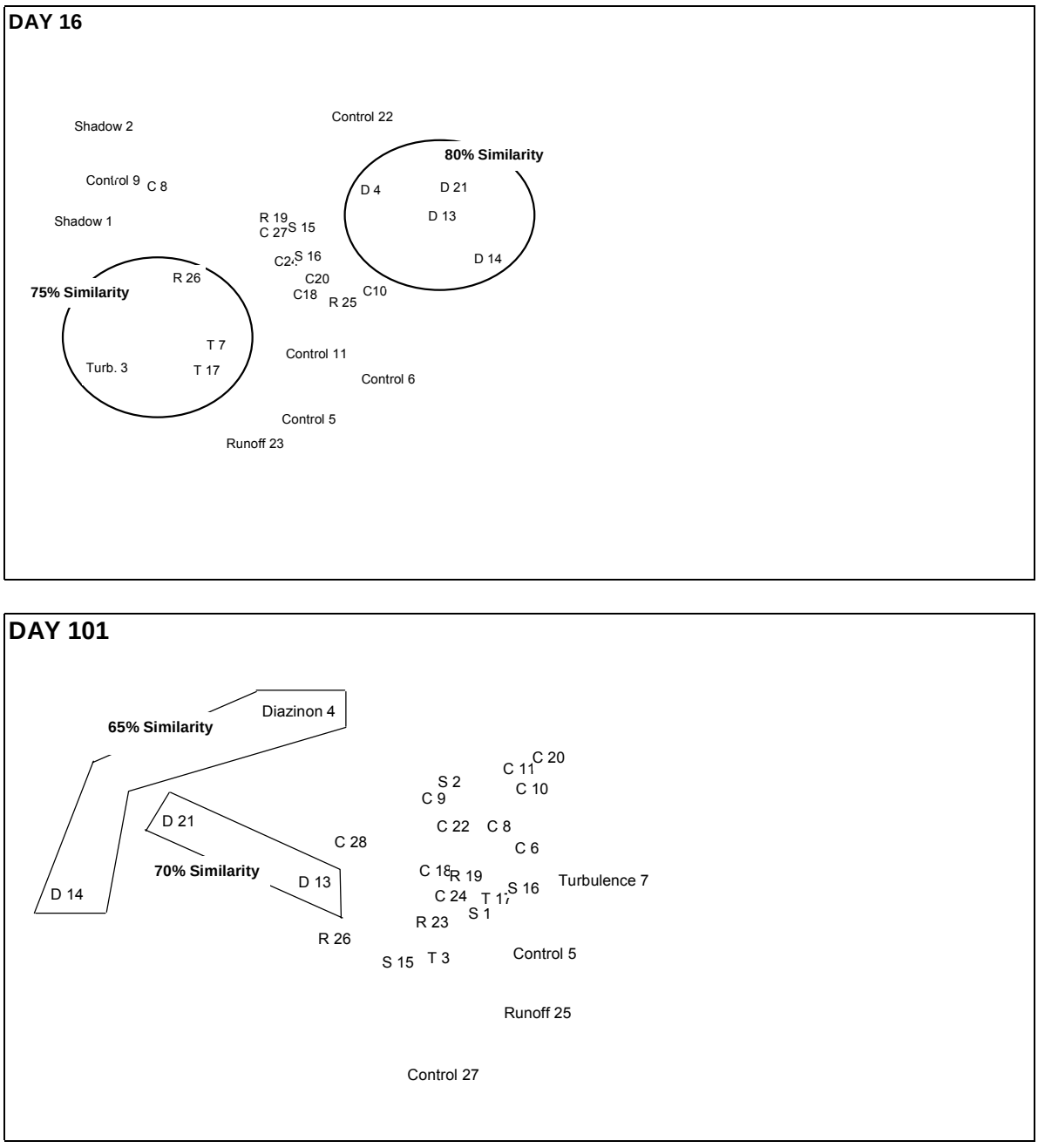


Figure 3-16 (cont.). MDS ordinations for zooplankton counts for treatment and control groups for day-24, 16 and 101 with superimposed clusters from Figure 3-15.

3.3.6 Analysis of Similarities for Zooplankton

The Analysis of Similarities (ANOSIM) test, a non-parametric permutation procedure, was applied to the same rank similarity matrix as was used for cluster and ordination methods, on the basis of log-transformed data. The null hypothesis assumes no differences between treatment groups and the control. Results are shown as R-values, reflecting the observed differences *between* treatment groups contrasted with differences among replicates *within* treatment groups. R is approximately 0 if the similarities between and within treatment groups are the same.

R is equal to 1 only if all replicates within a treatment group are more similar to each other than any replicates from different treatment groups. The significance of the R-values was calculated using a Monte-Carlo permutation procedure for every pair of treatment and control groups.

In Table 3-15, the shaded areas indicate statistically significant R-values. The DIAZINON treatment had significant effects on the zooplankton community on day 4 through day 101. Statistically significant differences were found when comparing the TURBULENCE treatment to the control for samples taken on days 14, 16 and 28.

DIAZINON treated samples differed significantly from the control group, but also from the other treatment groups, i.e. SHADOW, TURBULENCE and RUNOFF throughout the exposure and post-exposure period.

Treatments SHADOW and RUNOFF did not show significant differences to the control, except for a transient deviation on day 14. Significant differences were not found when comparing the treatments SHADOW, TURBULENCE and RUNOFF to each other.

Table 3-15. Analysis of Similarities for zooplankton.

R-values and significance								
	Day -24	Day 0	Day 4	Day 14	Day 16	Day 28	Day 80	Day 101
CONTROL / SHADOW	-0.25	0.11	0.05	0.25	0.07	0.16	-0.07	-0.02
CONTROL / TURBULENCE	-0.21	0.19	0.08	0.82	0.39	0.51	0.01	0.08
CONTROL / RUN-OFF	0.19	0.15	-0.04	0.30	-0.05	-0.17	0.06	0.13
CONTROL / DIAZINON	0.22	0.05	0.77	0.75	0.41	0.84	0.97	0.75
SHADOW / DIAZINON	0.03	0.19	0.65	0.59	0.57	0.68	1.00	0.67
TURBULENCE / DIAZINON	0.19	0.02	0.78	0.94	0.96	0.89	0.96	0.63
RUN-OFF / DIAZINON	0.08	0.10	0.63	0.57	0.67	0.84	0.92	0.54
SHADOW / TURBULENCE	0.09	0.06	0.17	0.22	0.28	0.35	-0.07	0.06
SHADOW / RUN-OFF	0.07	0.04	-0.18	-0.28	0.03	0.16	0.19	-0.09
TURBULENCE / RUN-OFF	0.11	0.26	0.19	0.37	0.17	0.43	0.13	-0.11

Note: Shaded areas indicate significant differences (Monte-Carlo permutations, p< 5%).

3.3.7 Principal Response Curves for Zooplankton

On the basis of Redundancy Analysis, Principal Response Curves (PRCs) were calculated and Monte-Carlo permutations were performed for the zooplankton community. Zooplankton data were log-transformed (Y´= log (Y+1), with Y equal to org./L) before analysis, thereby giving more weight to rare and less weight to abundant taxa. Principal Response Curves (PRCs) were calculated for the zooplankton community for treatment groups SHADOW, TURBULENCE, RUNOFF and DIAZINON individually. By calculating the PRCs individually for each treatment, emphasis was put on the detection of differences caused by each individual treatment in comparison to the control. Each data set included the control data and one of the other treatments SHADOW, TURBULENCE, RUNOFF or DIAZINON.

PRC Diagrams

The PRC diagrams for the treatments TURBULENCE, DIAZINON, RUNOFF and SHADOW are shown in Figure 3-17. All curves are very close to the control line (i.e., x-axes) during the pre-treatment phase (day –24 and day 0), indicating that the pre-treatment communities were similar.

Deviations from the control are clearly increased for the TURBULENCE treatment group on day 14 through day 28 and for the DIAZINON treatment group on day 4 through day 80. The PRC curves for TURBULENCE and DIAZINON drop to control level on day 80 and day 101, respectively. The treatments SHADOW and RUNOFF showed minor deviations from the control during the exposure and recovery phase.

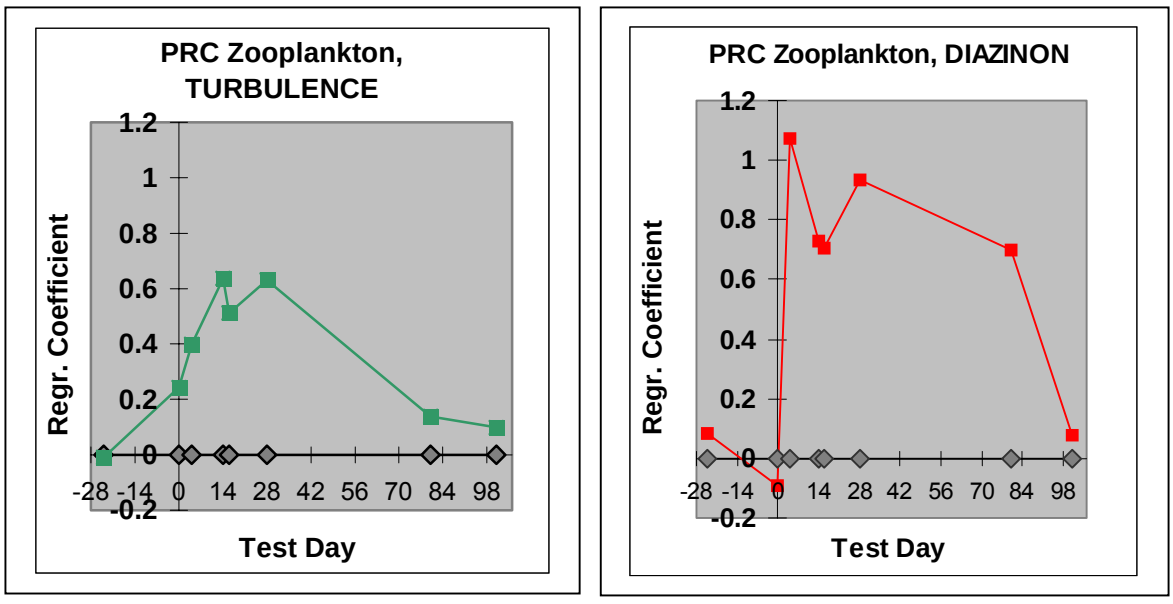


Figure 3-17. Principal Response Curves for zooplankton.

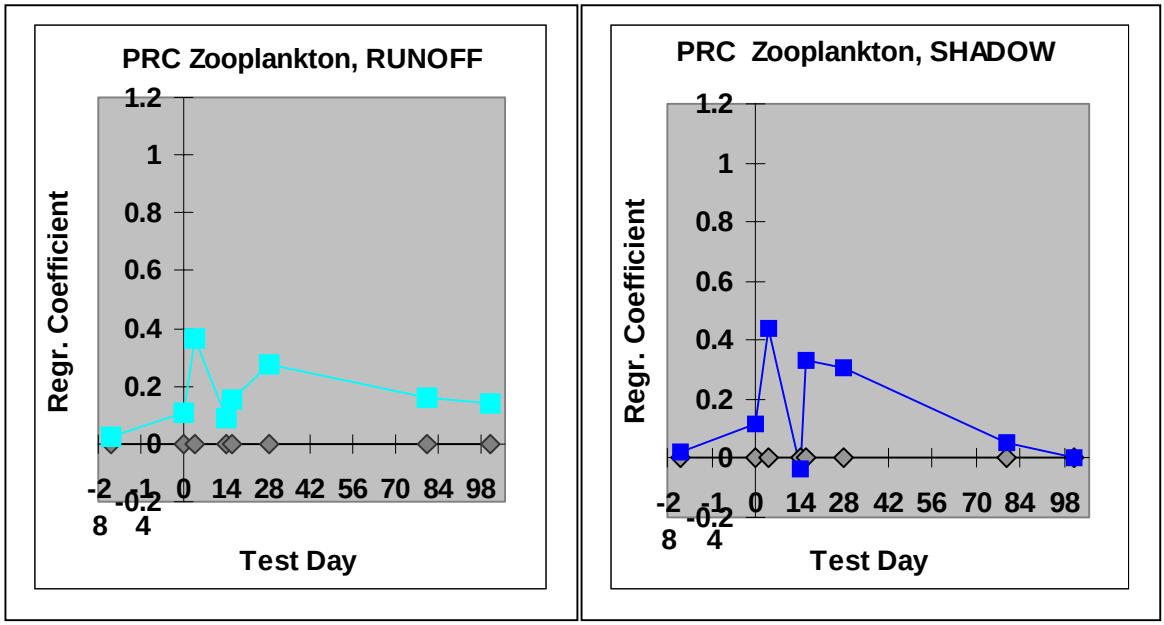


Figure 3-17 (cont.). Principal Response Curves for zooplankton.

Variance Allocation for entire study period and exposure phase

For all 4 PRC curves, variance allocations and significance tests (Monte-Carlo permutations) were calculated. Both calculations were performed for two different time frames:

- for the entire study period (day-24 to day 101) with the intention to show similarity of the tanks before exposure start and a possible recovery during the post-exposure phase.
- exclusively for the exposure phase (day 4 to day 28), because excluding pre- and post exposure phase gives less weight to time related and more weight to treatment related variability and increases statistical power.

The variance allocations for the PRCs calculated for the treatments SHADOW, TURBULENCE, RUNOFF and DIAZINON are shown in Table 3-16 and Table 3-17. When including data from the entire study period (day –24 to day 101), 49-55% of the total variance in the zooplankton data can be attributed to time. 3.2%, 5.7%, 2.4% and 13.0% of the variance can be attributed to the treatment regimes SHADOW, TURBULENCE, RUNOFF and DIAZINON, respectively. Thereof, 34%, 53%, 29% and 64% were captured by the corresponding PRCs. It is worth noting that the % variance attributed to treatment was very low and temporal variability was dominant.

When excluding the pre-treatment (day –24 and day 0) and the post-exposure (day 80 and day 101) data, 24-27% of the total variance in the zooplankton data can be attributed to time. With decreasing contribution of time, the percentage of variance attributed to treatment increased to 6.8%, 12.0%, 3.4% and 22.1% for the treatment regimes SHADOW, TURBULENCE, RUNOFF and DIAZINON, respectively. Thereof, 47%, 69%, 56% and 85% were captured by the corresponding PRCs (Table 3-17). It is worth mentioning that 2nd and 3rd PRCs were not significant (data not shown).

Table 3-16. Variance allocation of PRCs for zooplankton, all sampling events.

	% of variance accounted for by		% variance captured by first PRC	p-value of PRC
Data set (day-24 to day 101) for treatment groups	time	treatment regime		
SHADOW	55	3.2	34	0.40
TURBULENCE	53	5.7	53	0.007**
RUNOFF	55	2.4	29	0.88
DIAZINON	49	13.0	64	0.001**

Note: The percentages shown are the total variance which can be attributed to time and treatment regime, the % of variance captured by the first PRC and its significance (Monte-Carlo permutation tests, significant (**) with $p<0.05$).

Table 3-17. Variance allocation of PRCs for zooplankton, exposure phase.

	% of variance accounted for by		% of variance captured by first PRC	p-value of PRC
Data set (day 4 to day 28) for treatment groups	time	treatment regime		
SHADOW	24	6.8	47	0.18
TURBULENCE	24	12.0	69	0.003**
RUNOFF	27	3.4	56	0.73
DIAZINON	26	22.2	85	0.001**

Note: The percentages shown are the variance which can be attributed to time and treatment regime, the % of variance captured by the first PRC and its significance (Monte-Carlo permutation tests, significant (**) with $p<0.05$).

Significance

Monte-Carlo permutation tests for both time frames (day-24 to day 101 and day 4 to day 28) indicated that the PRC diagrams for TURBULENCE and DIAZINON displayed significant information (Table 3-16, Table 3-17).

More detailed information about the significance of treatment effects was gained by performing the Monte-Carlo permutation tests individually for each sampling date (Table 3-18). This analysis showed statistically significant deviation of the TURBULENCE treatment from the control for day 14 through day 28 ($p<0.05$). Further, the zooplankton community of the DIAZINON treated tanks was shown to be significantly different from to the control on day 4 through day 101, i.e. throughout the exposure and post-exposure phase. At first glance it seems surprising that the PRC was still significantly different for test day 101, when the curve had reached almost the control line (Figure 3-17). However, this finding was confirmed by all other statistical methods applied to the zooplankton data (Figure 3-15, Figure 3-16, Table 3-14, Table 3-15).

The PRCs for SHADOW and RUNOFF were not significant (Table 3-16, Table 3-17) and did not show any significant deviation from the control throughout the study period (Table 3-18).

Table 3-18. Significant differences between treatment groups and control group (Monte-Carlo permutations for individual test days).

	Zooplankton							
	d -24	d 0	d 4	d 14	d 16	d 28	d 80	d 101
SHADOW	0.76	0.71	0.19	0.14	0.37	0.14	0.58	0.82
TURBULENCE	0.25	0.29	0.24	0.003	0.04	0.03	0.43	0.54
RUNOFF	0.12	0.58	0.37	0.53	0.95	0.95	0.70	0.27
DIAZINON	0.93	0.60	0.001	0.001	0.001	0.001	0.001	0.001

Legend: significant with $p<0.05$ (shaded) or not significant with $p>0.05$. Data analysis at the species level.

Affinity of taxa to PRCs

Species weights (Table 3-19) indicate the affinity of single zooplankton taxa to the PRC. Species weights for the TURBULENCE treatment revealed a strong positive correlation for the species *Keratella quadrata* and *Pleuroxus uncinatus*. The species *Polyarthra spec., Polyarthra vulgaris, Synchaeta spec.* and *Daphnia longispina* were negatively correlated to the PRC, indicating that they occurred at lower densities in the TURBULENCE treatment when compared to the control.
Species weights for the DIAZINON treatment revealed a strong positive correlation for the species *Asplanchna spec.,* Cyclopoida and *Lecane spec.* The species *Keratella quadrata* and *Daphnia longispina* were negatively correlated to the PRC, indicating that they occurred at lower densities in the DIAZINON treatment when compared to the control.

Species scores for the treatments SHADOW and RUNOFF were not included in Table 3-19 because the corresponding PRCs for SHADOW and RUNOFF did not indicate any significant deviation from the control (Table 3-16, Table 3-17, Table 3-18).

Table 3-19. Zooplankton, species weights for the treatment groups.

TURBULENCE	
Taxon	Weight
Keratella quadrata	2.6
Pleuroxus uncinatus	1.7
Cyclopoida	-1.2
Daphnia longispina	-1.2
Synchaeta spec.	-3.1
Polyarthra spec.	-3.5

DIAZINON	
Taxon	Weight
Asplanchna spec.	2.0
Cyclopoida	1.4
Lecane spec.	1.4
Keratella quadrata	-1.0
Daphnia longispina	-5.9

Note: Only the species with a weight of ≥ 1.0 or ≤ -1.0 with the diagrams are displayed. The species weights show the affinity of single species to the PRC: the higher the species weight (positive or negative values), the more pronounced the actual response pattern of this species is likely to follow the PRC pattern.

3.3.8 Analysis of Zooplankton at different taxonomic levels

3.3.8.1 PRCs for Zooplankton at 3 taxonomic levels

Principal Response Curves for zooplankton were calculated for the species, family and class level. All sampling events (day-24 to day 101) and all treatments (Control, SHADOW, TURBULENCE, RUNOFF and DIAZINON) were included into one data set for the PRC calculations. Species weights and variance allocations were calculated for a reduced time frame with higher significance, i.e. for the exposure phase only.

All diagrams look very similar but differ in the scale of the regression coefficient (Cdt) (Figure 3-18). It is worth noting that the magnitude of Cdt seems to correlate with increasing taxonomic levels. All curves show a low deviation from the control level (zero line) for all treatment groups during the pre-treatment phase. For all three taxonomic levels, the treatment DIAZINON is strongly deviating from the control while the SHADOW, TURBULENCE and RUNOFF curves deviate only slightly from the control line. For all hierarchy levels, the DIAZINON curve reaches its maximum on day 4 and approaches the control level again on day 101.

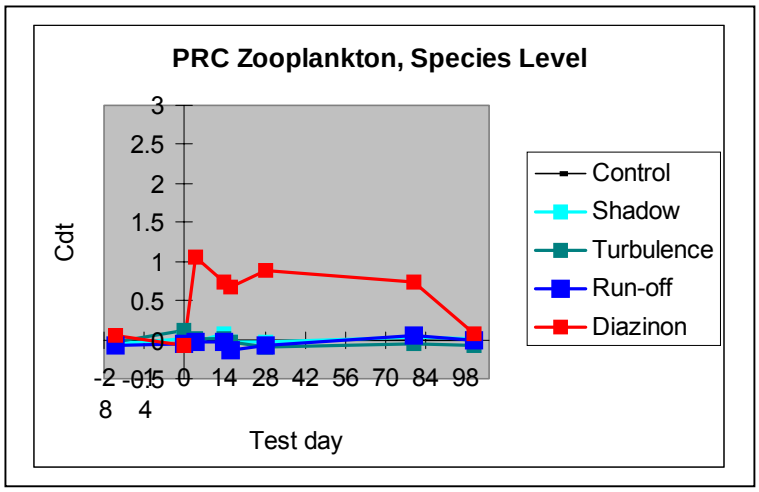
The variance allocation for the PRCs is shown in Table 3-20. Pre-treatment (day –24 and day 0) and post-exposure (day 80 and day 101) data were excluded from the calculations. It is worth noting that the % variance accounted for by treatment regime increases with the hierarchy level: while the treatment regime for the PRC calculated for data at the species level accounts for 27% of the variance, 54% of the variance can be allocated to the treatment regime when the data are grouped on the class level. The corresponding % variance allocated to time (seasonal variability) decreases from 20% to 6% (Table 3-20).

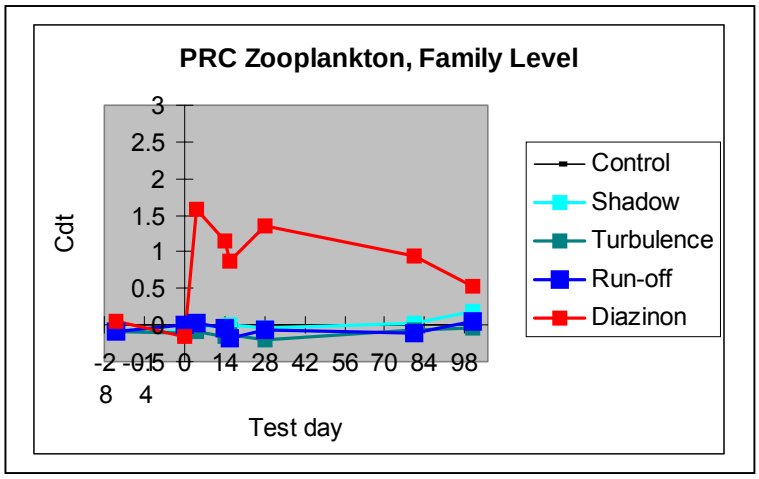
This indicates that the seasonality of species occurring at low numbers largely contributes to the temporal variance of the data set. The positive effect for the statistical power when grouping data at high hierarchical levels is evident from Table 3-20: the higher the hierarchical level, the lower the influence of seasonality on the data evaluation.

Table 3-20. Variance allocation of zooplankton data, grouped on different taxonomic levels.

	% of variance accounted for by		% variance capt. by first PRC	p-value of PRC
Data set including all treatments (day 4 to 28)	time	treatment regime		
SPECIES level	20	27	52	0.001**
FAMILY level	8	40	63	0.001**
CLASS level	6	54	75	0.001**

Note: The percentages shown are the total variance which can be attributed to time and treatment regime, the % of variance captured by the first PRC and its significance (Monte-Carlo permutation tests, significant (**) with $p<0.05$).





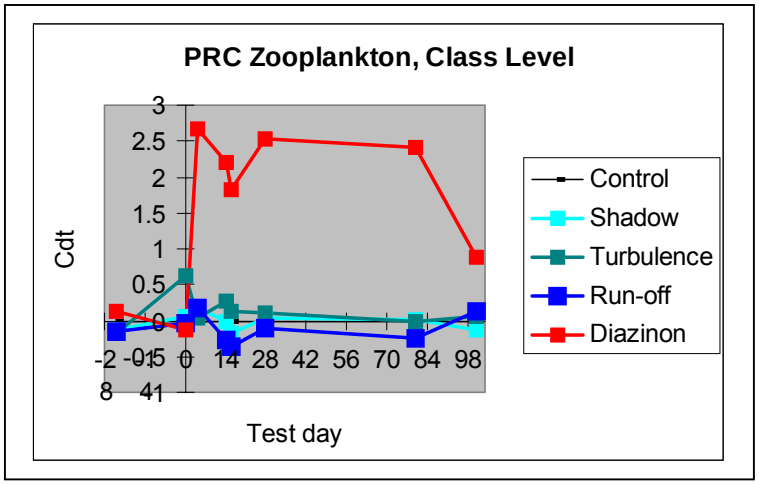


Figure 3-18. Principal Response Curves for zooplankton in the treatments, calculated on the species, family and class level.

The Monte-Carlo permutation tests indicated that the PRC diagrams for zooplankton grouped on all 3 hierarchy levels displayed significant information (Table 3-20). Monte-Carlo permutation tests were also performed individually for each sampling date (Table 3-21).

For data grouped at the family level, this analysis showed statistically significant deviations of the TURBULENCE treatment from the control for day 14 through day 28, as was already shown for the species level (Table 3-18). Additional to the significant deviations detected for the species level, the TURBULENCE treatment showed a significant deviation from the control also on day 0, when grouped on the class level. Furthermore, the zooplankton community of the DIAZINON treated tanks was shown to be significantly different from to the control on day 4 through day 101, i.e. throughout the exposure and post-exposure phase. Thus the results for data grouped on the family level showed the same pattern as for species level (Table 3-18).

The treatments RUNOFF and SHADOW did not show significant deviations from the control throughout the study period for data grouped on the family level, as already shown for analysis at the species level.

Table 3-21. Monte-Carlo permutations for zooplankton at the family level.

Zooplankton, FAMILY level								
	d -24	d 0	d 4	d 14	d 16	d 28	d 80	d 101
SHADOW	0.56	0.89	0.39	0.85	0.62	0.29	0.48	0.65
TURBULENCE	0.91	0.04	0.18	0.006	0.004	0.02	0.24	0.66
RUNOFF	0.17	0.51	0.84	0.62	0.45	0.65	0.23	0.72
DIAZINON	0.81	0.59	0.001	0.001	0.001	0.001	0.001	0.001

Note: permutations were calculated for each single sampling event on the basis of the PRCs calculated for a data set containing all treatment groups. Legend: significant with $p<0.05$ (shaded) or not significant with $p>0.05$.

Species weights, as listed in Table 3-22, show a high negative weight for Daphniidae, Brachionidae and Chydoridae, indicating that densities for these groups were lower in the DIAZINON treatment, when compared to the control. The taxa Asplanchnidae and Synchaetidae had high positive weights, indicating an increase of abundance for these groups in the DIAZINON treatment when compared to the control. Thus the decrease of *Daphnia longispina* is very well reflected in the species weights of Daphniidae and Crustacea (Branchiopoda). The increase of *Asplanchna* spec. finds its equivalent on the family level (Asplanchnidae), which is however not reflected on the class level (Rotatoria). This is due to the overall negative weight of the Brachionidae which also belong to this class.

It is important to note that the species weight given for the Brachionidae and Rotatoria is misleading when applied to test days 80 and 101. *Keratella quadrata*, which contributed with about 90% to the total zooplankton density on test day 101 (Table 3-12, Figure 3-13) in the treatment group DIAZINON was shown to occur at clearly higher numbers in this

respective group when compared to the control (Figure 3-14). However, considerable inter-replicate variability for the density of Keratella quadrata was observed in the treatment group DIAZINON for test days 80 and 101, which can be most likely related to an enclosure effect as homogeneity of data was better earlier in the study (Figure 3-21).

Table 3-22. Zooplankton, taxon weights for data grouped on the family and on the class level.

FAMILY	
Taxon	Scores
Asplanchnidae	1.57
Synchaetidae	1.11
Copepoda	1.05
Lecanidae	0.99
Chydoridae	-0.61
Brachionidae	-1.29
Daphniidae	-3.61

CLASS	
Taxon	Scores
Crustacea (Copepoda)	0.60
Rotatoria	-0.17
Crustacea (Branchiopoda)	-2.06

3.3.8.2 Contribution of orders and dominant species to total zooplankton density

The zooplankton community in the present study was dominated by few species in terms of population densities. The contribution in percent of each major taxon (identified to the species level except for cyclopoid copepods) to the total zooplankton density counted in each treatment group is presented in Figure 3-19. The contribution of major orders to total zooplankton density is shown in Figure 3-20.

The important role of the taxa *Daphnia longispina, Keratella quadrata* and Cyclopoida becomes obvious from Figure 3-19. These taxa altogether form more than 90% of the total density. As each of these taxa belongs to a different order, it is not surprising that the corresponding orders show curves very similar to the curves based on major species (Figure 3-19, Figure 3-20).

While the diagrams for treatments RUNOFF and SHADOW are very similar to the control, TURBULENCE and DIAZINON treatments differ strongly from the control. Beginning on test day 4 until test end, *Keratella quadrata* (Ploimida) is the very dominating taxon encountered in the TURBULENCE treated ponds, while *Daphnia longispina* and Cyclopoida play a minor role when compared to the control. *Daphnia longispina* (Cladocera) is almost entirely eliminated from the DIAZINON ponds as of test day 4. At the same time, Cyclopoida increased rapidly in number and become the dominant taxon until test day 80 in the DIAZINON treated ponds.

The percent contribution of species to the total density looks quite similar for all treatment groups and the control group on test day 101 (Figure 3-19). However, it is important to note that absolute numbers for *Keratella quadrata* were considerably higher in the treatment group DIAZINON when compared to the control and that within variability in this treatment group had considerably increased during the post-exposure phase (Figure 3-14, Figure 3-21).

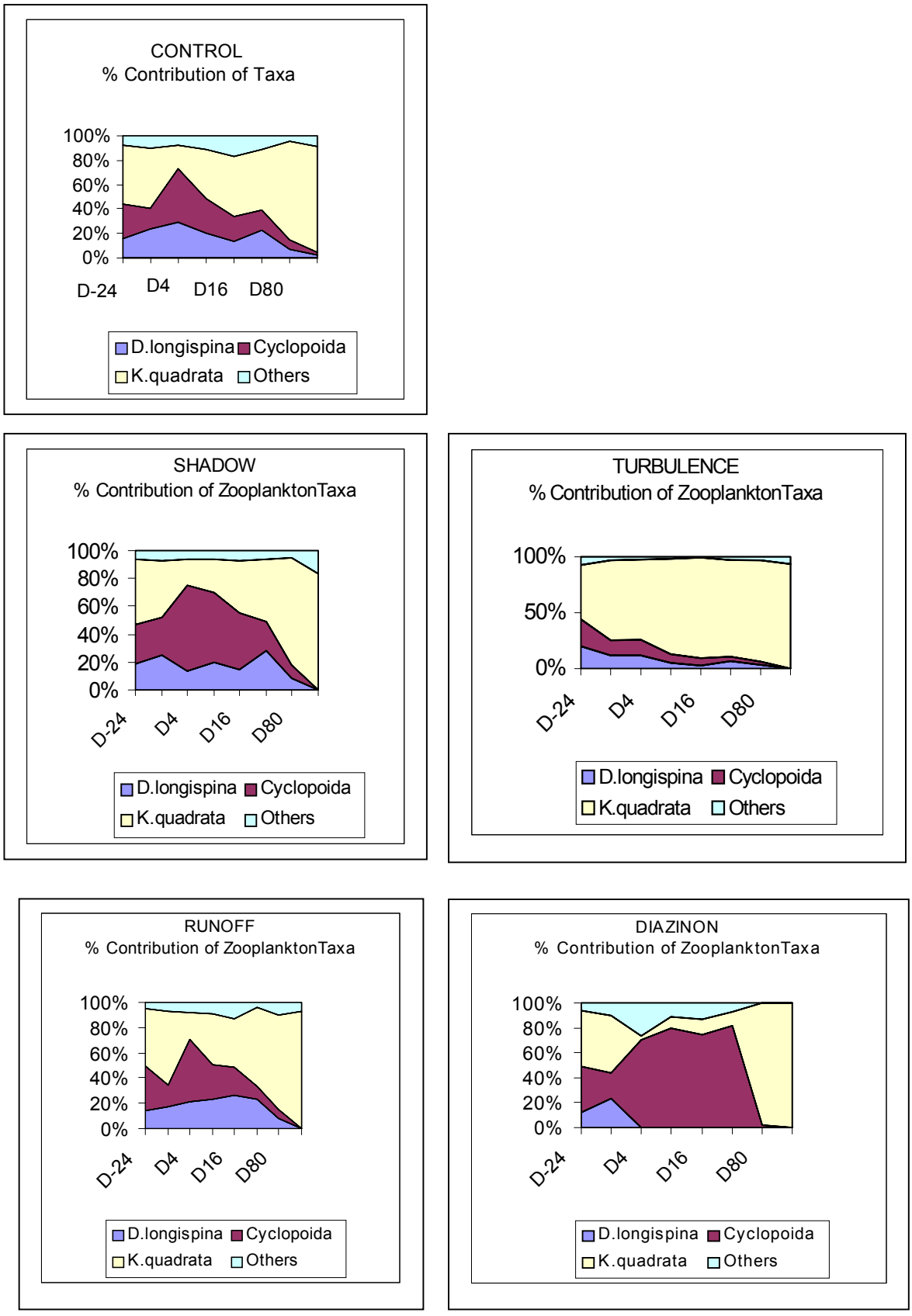


Figure 3-19. Contribution of major taxa to total zooplankton density in the control and the treatments.

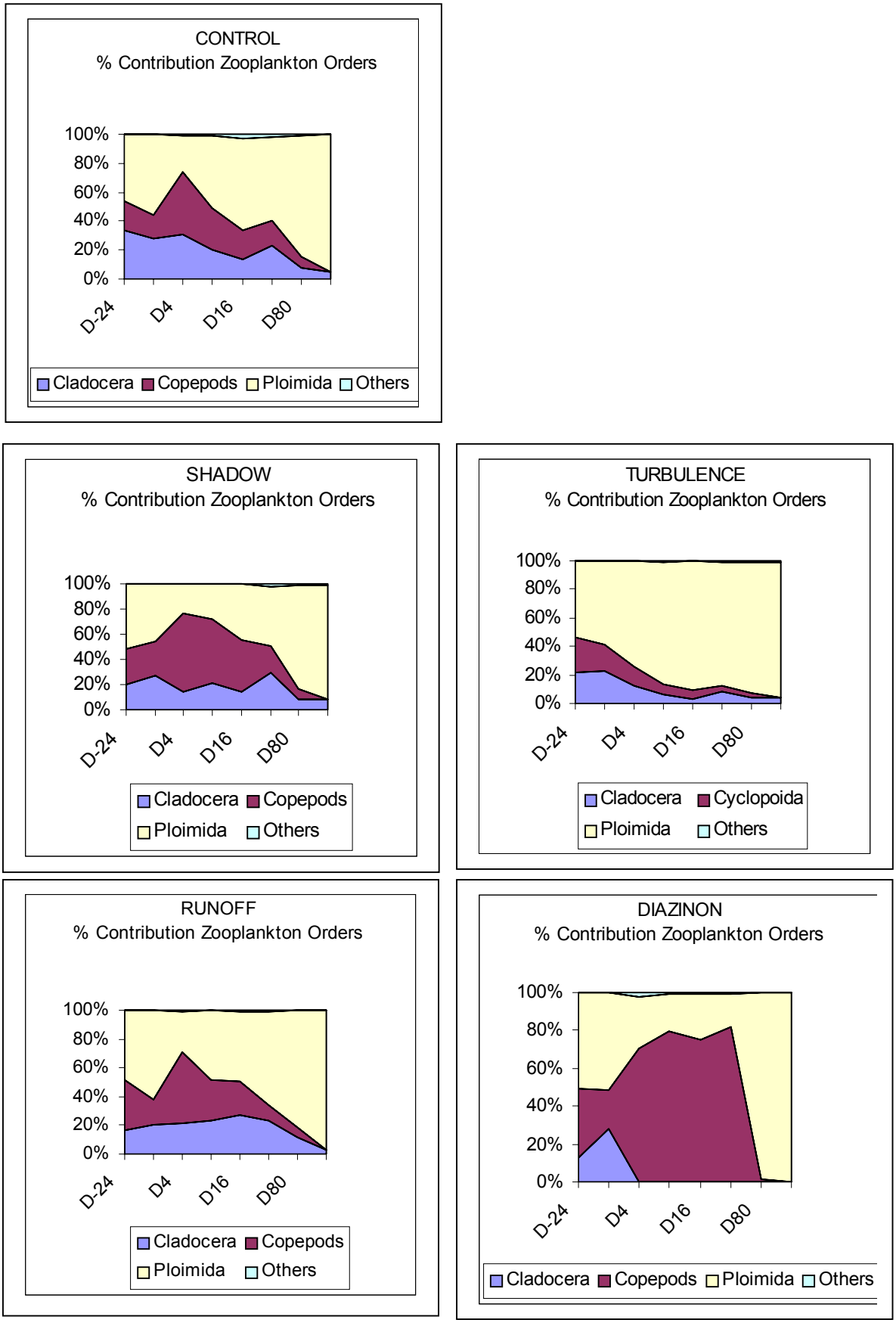


Figure 3-20. Contribution of major orders to total zooplankton density.

3.4 DISCUSSION OF DISTURBANCE EFFECTS ON ZOOPLANKTON

3.4.1 The Zooplankton community structure was clearly affected by the application of DIAZINON and did not recover by test end

Statistical Evidence

During the exposure and post-exposure phase, species diversity was reduced for the treatment group DIAZINON as shown with decreasing Shannon-Wiener indices and with high cumulative dominance for ranked species, when compared to the control group (Figure 3-12, Figure 3-13). This reduction was confirmed by the results of the Dunnett´s test (Table 3-13). Detailed univariate statistical analysis of the community showed that *Daphnia longispina, Keratella quadrata, Asplanchna* spec. and Cyclopoida were shown to play an important role for the detection of differences between the CONTROL and the DIAZINON treated ponds.

Several multivariate statistical methods were used for the comparison of treatment groups with the control. Changes in the zooplankton community structure for DIAZINON treated microcosms when compared to the control group during the exposure phase and post-exposure phase were shown using the Cluster and MDS methods (Figure 3-15,). These findings were confirmed using the Analysis of Similarities and the Principal Response Curves method (Table 3-15, Figure 3-17).

The Principal Response Curves method showed not only the change in community structure but also the contribution of single species to differences between the DIAZINON treated ponds and the control group. Changes in community structure were dominated by the almost complete disappearance of *Daphnia longispina* from day 4 through day 101 as shown by the PRC method and the Dunnett´s test. The species *Keratella quadrata* was present at lower densities in the DIAZINON treated ponds when compared to the controls during the exposure phase, while *Asplanchna* spec. and Cyclopoida showed higher densities when compared to the control (Table 3-19). These PRC results were also confirmed by the univariate Dunnett´s test (Table 3-14).

DIAZINON treated ponds were still significantly different from the control ponds throughout the post-exposure phase on days 80 and 101 (Figure 3-17; Table 3-18). This could be related to the dominant role of *Daphnia longispina,* which continued to occur at very low numbers until test end and did not reach control level (Figure 3-14, Table 3-14). Furthermore, numbers of *Keratella quadrata* showed significantly higher numbers in the Diazinon treated ponds when compared to the control group during the post-exposure phase (Figure 3-14, Table 3-14).

Population Responses

In an outdoor mesocosm study with diazinon (Giddings, 1992), which was used as reference study to the present investigation, the LOEC for cladocerans was 2.4 µg/L, 9.2µg/L and 33 µg/L for cladocerans, Ploimida and copepods, respectively (Table 2-4).

Thus, it could be assumed that cladocerans and Ploimida in the present study would be affected by the concentration measured in the present study (<16 µg/L), while effects on copepods were not expected to occur.

In the present study, diazinon concentrations measured in water in three of the treated ponds averaged 15 µg/L two hours after application on test day 0. In these ponds, concentrations had decreased to 2.1 µg/L by test day 28 (Table 3-23). In one of the four ponds, Diazinon concentrations were considerably higher (pond 13, Table 3-23). However, the zooplankton community in pond 13 underwent highly similar changes as the 3 ponds with lower concentrations as shown in the dendrograms and the MDS ordinations (Figure 3-15, Figure 3-16). Moreover, population densities of major zooplankton taxa in pond 13 were within the range of ponds 4, 14 and 21, as shown in Figure 3-21. This suggests there was a sampling or analytical error.

Table 3-23. Diazinon concentrations in ponds 4, 13, 14 and 21 on test day 0 (2 hours after application), day 14 and day 28.

	Concentration (µg/L)			
	Pond 4	Pond 13 [(a)]	Pond 14	Pond 21
Day 0	15.2	42.2	15.3	15.5
Day 14	4.57	8.27	5.67	4.05
Day 28	2.18	5.12	2.73	1.34

(a) Pond 13 was excluded from the calculation of the mean. See Appendix C for full data.

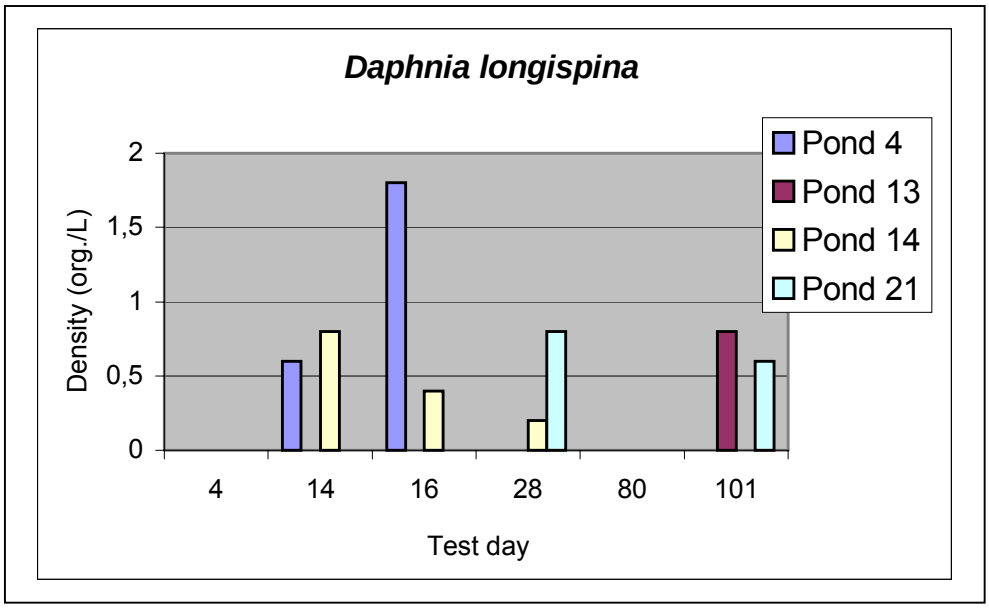
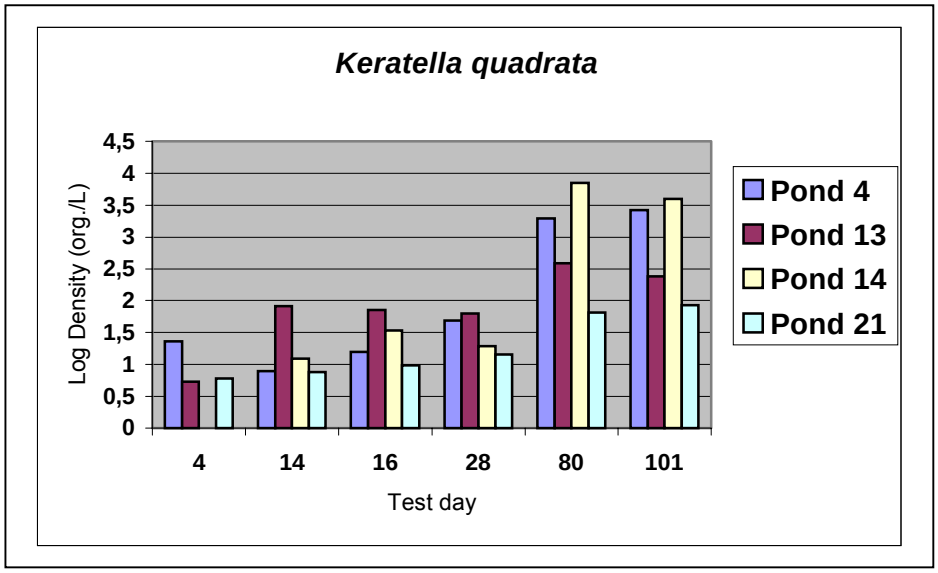


Figure 3-21. Densities of major taxa in the four Diazinon treated ponds.



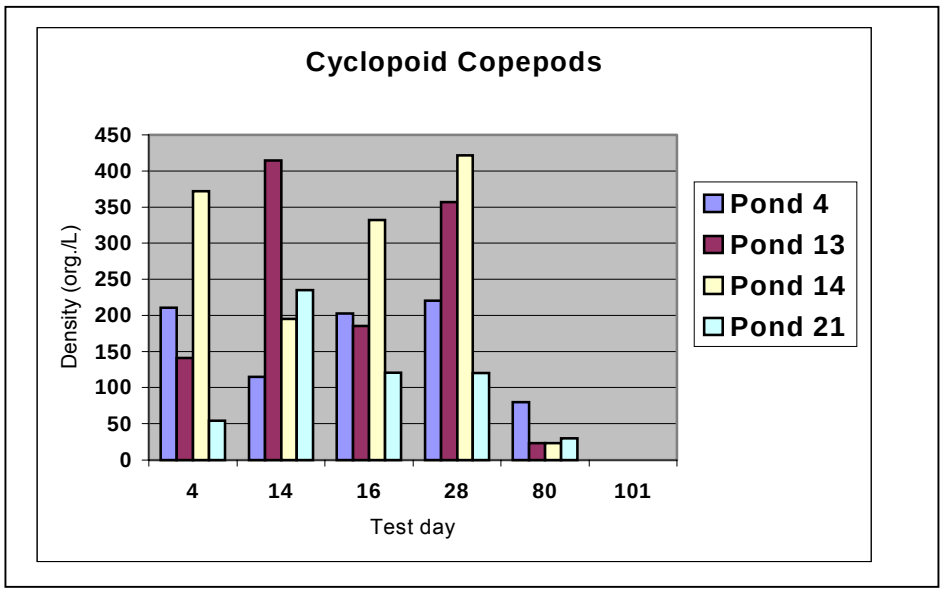


Figure 3-21 (cont.). Densities of major taxa in the four Diazinon treated ponds.

Daphnia longispina

In the present study, *Daphnia longispina* had decreased from a density of about 60/L to zero within a four-day period after Diazinon application. This can clearly be related to the fact that the LOEC as described by Giddings (1992) was exceeded in the present study during the exposure phase (Table 3-23).

Four weeks after Diazinon application, concentrations had dropped from initially 15 µg/L to about 2 µg/L. The measured concentration was thus below the LOEC (Giddings, 1992) as of test day 28. It was therefore assumed that recovery of *Daphnia* populations would take place during the post-exposure phase, starting on day 28. Recovery had been observed in the reference study, where Daphnia has recovered to control level about 42 days after the last application at actual concentrations during the exposure phase of 2.4 – 33 µg/L.

In the present study, *Daphnia longispina* was found at average densities below 1/L during the post-exposure phase (day 80 and 101). Average numbers of *Daphnia longispina* in the control group were 17/L and 10/L for days 80 and 101, respectively. Thus, although populations were not completely erased in the Diazinon treated ponds, it cannot be stated that Daphnia populations had recovered by test end.

Several possible explanations can be given for the discrepancy of results of the present study and the reference study. Firstly, the affected cladoceran species were not equivalent in the two studies. While Giddings (1992) reports *Alona* spec., *Chydorus* spec. and *Simocephalus* spec. being the most strongly affected species, *Daphnia longispina* was shown to be the dominant and most affected species in the present study.

Secondly, the two studies differed in terms of temporal coincidence of treatment and life cycle of the respective species. While Giddings (1992) applied Diazinon in June and the corresponding recovery phase started end of June, in the present study the ponds were treated in mid-July. The recovery phase in the present study thus had a comparatively late starting point, i.e. only in mid-August. At this time of the year, already the control numbers of *Daphnia longispina* had started to drop due to its life cycle. A possible explanation for the weak recovery of Daphnia in the present study may thus be the coincidence of low numbers due to the life cycle of this species and the chosen time of application/recovery.

Thirdly, the weak recovery of *Daphnia longispina* might also be related to a shift in algal composition, which was observed in the Diazinon treated ponds as of day 14. Algae inedible or moderately edible for *Daphnia* dominated the DIAZINON treated ponds during the recovery phase (chapter 3.7), which probably has contributed to the sustained suppression of *Daphnia longispina* after Diazinon concentrations had dropped to the reported levels.

Although recovery could not be shown by the end of the present study in autumn 1997, it seems highly probable that *Daphnia longispina* would have appeared again in the treated ponds in spring 1998. As shown by Giddings (1992) Diazinon disappears not only from water but also from the sediment within 100 days. Therefore, it seems very likely that resting stages of *Daphnia longispina*, which are known to be less susceptible to environmental stress and usually occur at high numbers in the sediment, would have enabled the build-up of new populations in the next season.

Rotifers (Ploimida)

Diazinon also affected *Keratella quadrata,* which showed significantly lower numbers in the treated ponds than in the control on test day 16 (Figure 3-14). However, the rotifer Asplanchna spec., also belonging to the order Ploimida, did not follow this pattern and showed higher numbers in the treated ponds when compared to the control during the exposure phase (Table 3-14). Increases in rotifer populations following insecticide application have been documented (Lozano *et al.*, 1992; van Donk *et al.*, 1995) and it is generally concluded that such an increase is due to a release from competition resulting from reductions in cladoceran populations. A possible explanation for this effect not having been detected in the study by Giddings (1992) is the minor contribution of *Asplanchna* populations to the total density of Ploimida. Also in the present study, Ploimida numbers were always dominated by *Keratella quadrata* (Table 3-12).

During the post-exposure phase (day 80 and 101), *Keratella quadrata* recovered in the Diazinon treated ponds and even exceeded numbers measured in the control ponds (Figure 3-14). This can be related to the Diazinon concentrations, which had dropped below the LOEC (Giddings, 1992) by test day 14. Additionally, the decrease of copepodite and adult cyclopoid copepods, which predate on rotifers (Lang, 1997), might have contributed to the increase of rotifers (Figure 3-22).

Copepods

In the present study, copepods were not directly affected by Diazinon application. Copepod numbers rapidly increased after treatment and showed significantly higher numbers in the treated ponds when compared to the control (Figure 3-14, Figure 3-19, Table 3-14). As juvenile cyclopoid copepods (nauplii) may compete with cladocerans for algae (Lang, 1997), the observed increase of Cyclopoida in the present study can be explained as a secondary effect due to the drastic reduction of *Daphnia longispina* and thus elimination of a competitor by application of Diazinon.

By test day 80, copepod numbers had strongly decreased in the Diazinon treated as well as in the control ponds (Table 3-14), which can be related to the seasonalit of the life cycle of *Megacyclops* and *Mesocyclops*.

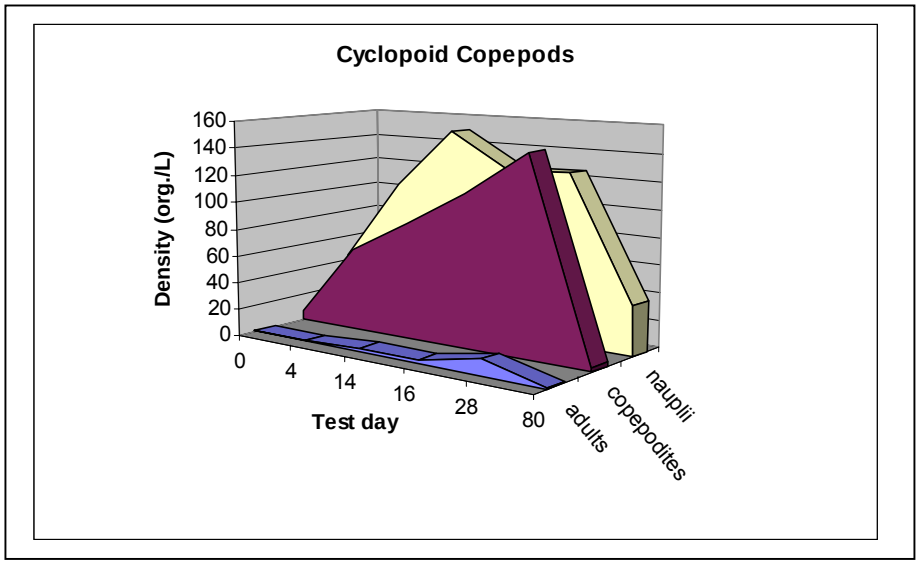


Figure 3-22. Cyclopoid copepods: nauplii, copepodites and adults in Diazinon treated ponds during the exposure phase.

Phytoplankton

As a secondary effect, the decrease of *Daphnia longispina* was followed by a shift in algal composition and an increase of total algal density, which may be attributed to reduced grazing pressure by *Daphnia longispina* due to the Diazinon application (Figure 3-23, Figure 3-42, p.153). As mentioned above, the shift in algal composition probably has contributed to the sustained suppression of Daphnia longispina after Diazinon concentrations had dropped to the reported levels.

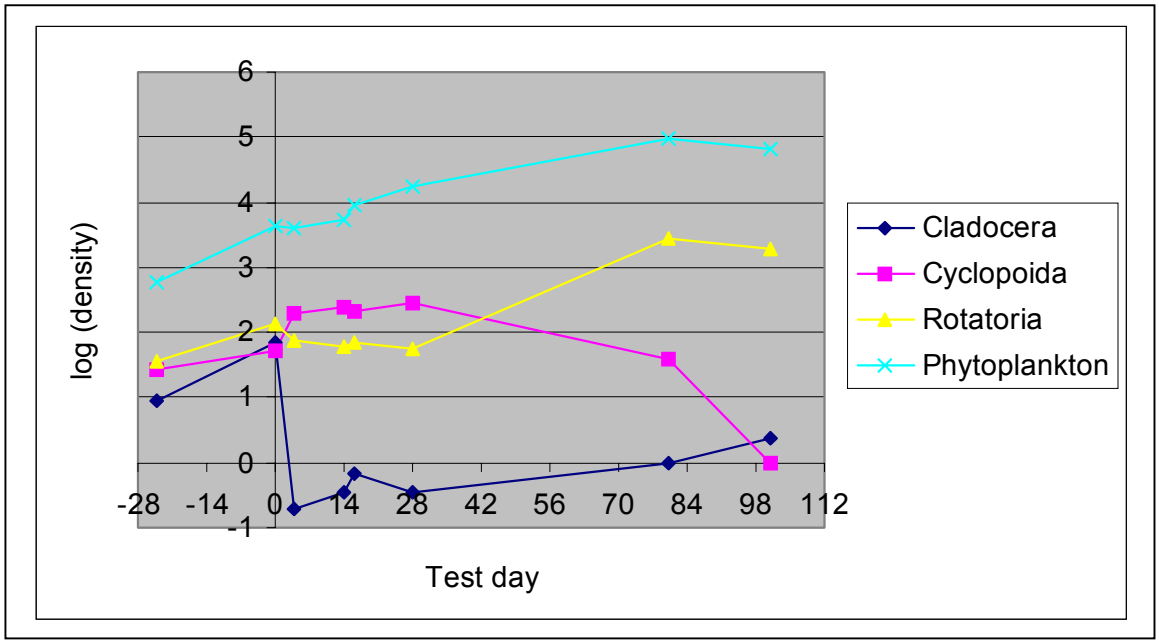


Figure 3-23. Zooplankton and phytoplankton densities before and after Diazinon application on day 0.

3.4.2 The Zooplankton community was affected by the TURBULENCE treatment but recovered to control level by test end

Statistical Evidence

During the exposure and post-exposure phase, species diversity was reduced for the treatment group TURBULENCE as shown with decreasing Shannon-Wiener indices and with high cumulative dominance for ranked species (Figure 3-12, Figure 3-13). The zooplankton community was dominated by *Keratella quadrata* and Cyclopoida. Univariate statistical analysis showed that, *Pleuroxus uncinatus, Keratella quadrata and Polyarthra* spec. played an important role in detecting differences between the CONTROL group and TURBULENCE treated ponds (Table 3-13).

Several multivariate statistical methods were used for the comparison of treatment groups with the control. Applying the Cluster, MDS and PCA methods indicated to some extent deviations of the TURBULENCE treatment group from the control group (Figure 3-15, Figure 3-16). More obvious, the differences between the TURBULENCE treatment and the control group could be shown using the Analysis of Similarities method and the Principal Response Curves method (Table 3-15, Figure 3-17). These differences were found to be statistically significant for days 14, 16 and 28. Changes in the zooplankton community structure for the TURBULENCE treatment group could be linked, using the PRC method, to an increase of *Keratella quadrata* and *Pleuroxus uncinatus* and a decrease of Cyclopoida, *Daphnia longispina, Polyarthra* spec., *Synchaeta* spec. and *Polyarthra vulgaris* when compared to the control (Table 3-19).

The Principal Response Curves showed a recovery of the zooplankton community for the TURBULENCE treatment group when compared to the control as from day 80 (Figure 3-17, Table 3-18). This finding was supported by the results of the Dunnett´s test, which showed none of the populations in the TURBULENCE treatment to be significantly different from the control on days 80 and 101 (Table 3-14).

Population responses

The comparatively low numbers of cladocerans and the significant increase of *Keratella quadrata* can be related to changed physico-chemical conditions and the availability of edible algae in the TURBULENCE treated ponds. As a reaction to turbulent mixing, turbidity was clearly increased in the treated microcosms from day 10 through day 46. Up to 20 nephleometric turbidity units (NTU) were measured in the TURBULENCE group, while in the control group turbidity remained below 5 NTU (Appendix B).

Turbidity could be partly attributed to the high density of phytoplankton measured in the TURBULENCE treated ponds (Figure 3-35). While phytoplankton densities remained high from test day 28 through day 80, turbidity decreased rapidly when the pumps were turned off on day 28 (Appendix B). These differences could be explained by the additional presence of suspended solids. Although suspended particles were not measured, suspended sediments and also periphyton were likely to occur in water due to the high current velocity and the installation of pumps close to the sediment.

The availability of edible algae was reduced in the TURBULENCE treated ponds (chapter 3.8). While small edible algae such as *Cryptomonas erosa/ovata* and *Chroomonas acuta* showed significantly lower numbers, moderately edible algae such as *Oocystis parva* and *Planktosphaeria gelatinosa* showed significantly higher numbers in the TURBULENCE group when compared to the control (Table 3-37). However, these changes can be expected to affect rotifers and crustaceans similarly and therefore do not justify the change in community composition, e.g., *Keratella quadrata* and juvenile *Daphnia* species consume algal cells in the 0.2 –25 μm size range, and adult *Daphnia* species and copepod nauplii consume algae up to a size of 60 μm (Lang, 1997; Gilbert, 1985).

Therefore, it seemed more likely that the decrease of crustaceans and increase of *Keratella quadrata* was due to the presence of suspended particles. It is known that suspended particles may cause significant changes in community composition of zooplankton (Koenigs *et al.,* 1990). The presence of suspended particles in natural ecosystems, such as turbid lakes favors rotifers over cladocerans because they interfere with ingestion of algae by non- selective zooplankton filter-feeders. Other zooplankton such as rotifers are not greatly suppressed by suspended particles, presumably because of their more selective feeding mechanisms (Jack *et al.*, 1993; Kirk *et al.*, 1990).

As stated above, turbidity decreased rapidly to almost control level, when the pumps were turned off on day 28 (Appendix B). With the disturbance factors being eliminated, the zooplankton community in the TURBULENCE group reached control levels during the post exposure phase (Figure 3-15, Figure 3-16, Figure 3-17).
As the zooplankton community composition was strongly dominated by the taxa *Keratella quadrata, Daphnia longispina* and Cyclopoida at test end, recovery can be explained by population responses of these three taxa. This recovery was related to the temporal

coincidence of the post-exposure phase timing and the life cycle of *Daphnia longispina* and Cyclopoida. Similar to the population developments in the TURBULENCE group, densities of both taxa had decreased in the control (Figure 3-14). Moreover, in the treated as well as in the control group, *Keratella quadrata* was the dominant species at test end.

3.4.3 The community structure was NOT affected by SHADOW

All statistical methods show that the treatment SHADOW did not significantly affect the zooplankton community structure or the abundance of individual zooplankton species.

Shading of the ponds might affect the phytoplankton composition and then, in a secondary effect also the zooplankton. In absence of detectable effects on the phytoplankton community (chapters 3.7 and 3.8) in the present study, it is plausible that also zooplankton communities remained unaffected.

3.4.4 The community structure was NOT affected by RUNOFF

All statistical methods showed that the treatment RUNOFF did not significantly affect the zooplankton community structure or the abundance of individual zooplankton species.

In natural lakes and reservoirs, suspended particles are a prominent feature and can have pronounced effects on zooplankton communities. Suspended clays and silts are known to interfere with ingestion of algae by non-selective zooplankton filter-feeders, and cladoceran abundance may be reduced in communities with high turbidities (Koenigs *et al.*, 1990). Other zooplankton such as rotifers are not greatly suppressed by suspended particles, presumably because of their more selective feeding mechanisms: Thus, the presence of suspended particles should favor rotifers over cladocerans (Kirk *et al.*, 1990).

In the present study, “runoff events” were simulated according to available geological data from the region in which the microcosm studies were performed (Seiberth, 1997; Schaub, 1997). A load of about 200 mg/L quartz sand was applied to the investigated microcosms. About 5%, 25% and 50% of the applied sand had particle sizes below 2, 12 and 48 µm, respectively, while the maximum particle size measured was 192 µm. The quartz sand was applied twice during the exposure phase, i.e. on day 0 and day 14 and consequent visible turbidity remained for about two days.
Applying univariate and multivariate statistics on the zooplankton data, the described scenario did not result in measurable effects on the abundance of any single species nor on the community structure (Table 3-14, Figure 3-17). This result can be interpreted as follows.

1. Duration of Exposure
Suspended solids (clay) have been shown to affect zooplankton communities at concentrations of 20 and 50 mg/L with an average particle size of 1.3 µm (Jack *et al.*, 1993). Jack *et al.* maintained these concentrations for twelve days, with daily, thorough stirring of the enclosures to reduce settling of the clay. Effects on Daphnia pulex were first detected after four days of continuous exposure. The duration of the disturbance was less extended in the present study (2 days).

2. Particle Size & Concentration

Kirk *et al.* (1990) have shown that 50-100 mg/L of suspended clay with a particle size of <2µm caused large reductions in the population growth rates of cladocerans but not of rotifers. The unchanged pattern of rotifers and cladocerans in the present study can therefore be related to the particle size distribution of the quartz sand used and the fact that only 2% of the applied sand (i.e. 4 mg/L) had a particle size of <2µm (Table 2-3, p.27).

3. Resulting Recommendation

In absence of clear guidelines, decisions on the amount of soil particles and the frequency of application for the simulation of runoff events in ecotoxicological field studies are taken on a case-by-case basis. From the present study and corresponding literature, it seems advisable to keep the turbidity restricted to no longer than 3 days and the suspended particles concentration below 20 mg/L in order to avoid side-effects on the zooplankton community through suspended particles, and to come to a meaningful assessment of effects of plant protection products in a microcosm test. It should also be taken into account that particle sizes around 2 µm have a maximum effect on non-selective filter feeders, and can lead to a shift from cladocerans to rotifers or selective herbivores.

3.4.5 Univariate and multivariate statistical methods lead to similar results

For the study design and the test system used in the present study, univariate and multivariate statistical methods lead to similar results. Selection of single species for univariate tests was based on density and abundance patterns, running the risk of missing rare but important species, while multivariate tools made use of the entire data set.

However, the output of some multivariate methods, such as dendrograms and MDS ordinations were found to be difficult to interpret (Figure 3-15, Figure 3-16). It appears as a result of the comparative work undertaken that the PRC method offers the most comprehensive way of data presentation because multivariate data are reduced to an univariate graphical presentation (Figure 3-17).

An advantage of the PRC method when compared to the Cluster or MDS ordinations clearly is the combination of displaying graphically in a comprehensive way the difference in community composition between a treatment and the control. The PRC method additionally allows for composing the corresponding lists of species weights, which indicates decreases or increases of species in the treatment when compared to the control.

Furthermore it appears to be advisable to crosscheck population effects as detected with the PRC method with a univariate test (e.g., Dunnett´s test). For example, population density of *Keratella quadrata* was shown to be statistically significantly lower in the DIAZINON group on day 16 and significantly higher on day 80 when compared to the control (Dunnett´s test, Table 3-14). This was confirmed by the negative species weight for *Keratella quadrata* when calculating PRC species weights for the exposure phase (PRC, Table 3-19).

It is important to notice that effects on *Keratella quadrata* were not detectable when calculating the PRC species weights for the entire study period due to the combination of the decrease of K.quadrata in the exposure phase and its increase in the post exposure phase.

Therefore, the criticality of the selection of an appropriate time-frame must be considered as a potential draw-back of the principle of PRC species weights: if the time-frame is not chosen correctly, effects may become undetectable.

3.4.6 Diazinon effects were detectable despite natural variability and changes due to natural disturbance

The system-inherent zooplankton variability was affected by the treatments SHADOW and DIAZINON, not by TURBULENCE and RUNOFF. In terms of interreplicate variability for total density and diversity (measured as coefficients of variation) the treatment groups behaved in general similar to the control during the pre-exposure and exposure phase (Table 3-11). No clear temporal trend was observable during these test phases, with the variation going up and down within a range of 14-80%. However, clear trends could be observed for the post-exposure phase when the within variability was lower for SHADOW and higher for DIAZINON when compared to the control, while TURBULENCE and RUNOFF behaved similar to the control.

Despite the natural variability measured in the control ponds, all multivariate methods enabled the detection of DIAZINON effects on the zooplankton community when comparing the treated group with the control (Figure 3-15, Figure 3-17).

Furthermore, zooplankton communities in DIAZINON treated ponds were also shown to be significantly different from all other treatment groups. Even though the TURBULENCE treatment had significantly affected the zooplankton community, DIAZINON effects were still detectable when comparing these two treatment groups, using Analysis of Similarities (Table 3-15).

This finding was confirmed using the PRC method. Further to the detection of effects caused by individual treatments (Figure 3-17), the PRC was applied to the whole data set, including the control and all 4 treated groups. By including all treatments in one calculation, emphasis was put on the comparison of pesticide effects to “naturally” occurring changes. The corresponding PRC diagram (Figure 3-24) showed a clear distinction of the Diazinon group from the “naturally disturbed” groups.

In conclusion, it appears that pronounced effects of a plant protection product on zooplankton community composition can be well detected despite naturally occurring interreplicate and seasonal variability, and that even simulated natural disturbances do not affect the ability to detect such effects.

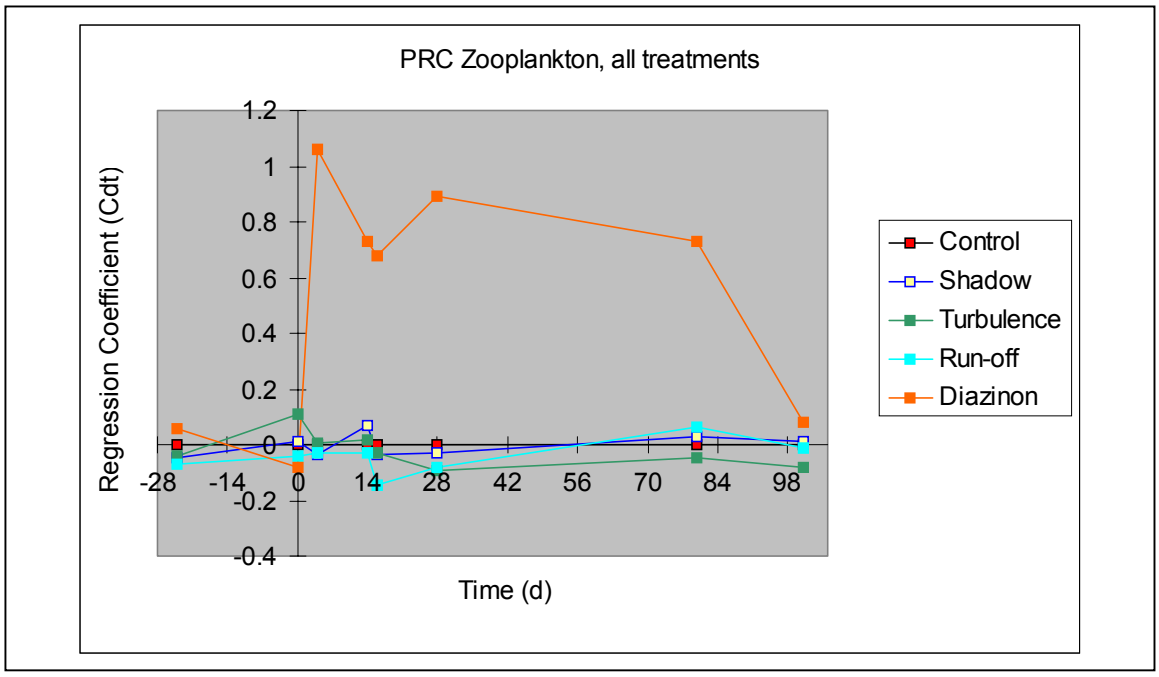


Figure 3-24. Principal Response Curves for zooplankton in the treatments calculated from one data set.

3.4.7 Taxonomic levels higher than the species level were sufficient for interpretation of the present study

Data were analyzed at different taxonomic levels, i.e. species, family, order and class. Two different methods were used, one being a graphical presentation of the percent contribution of each group to the total density and the other being the multivariate statistical method PRC (Figure 3-18, Figure 3-19, Figure 3-20). With both methods, it was shown that:

a) A change of taxonomic hierarchy level did not significantly influence the outcome of the graphical presentations and statistical evaluations;
b) This robustness was clearly related to the strong dominance of a few zooplankton taxa in the present data set. This finding was also supported by the dominance plot (Figure 3-13).

In a literature review by Brock and Budde (1994) it was shown that taxonomic identification is usually performed on the species or genus level when investigating changes in aquatic community composition. However, published data as well as the present investigation clearly indicate that zooplankton communities are in most cases dominated by few species and that this dominance partition is usually very well reflected when grouping zooplankton on higher taxonomic levels (Table 3-24, Figure 3-19, Figure 3-20).

Table 3-24. Dominance of zooplankton taxa in aquatic microcosm studies.

Contribution of major groups to total zooplankton [(a)]	Contribution of dominant taxa to major group [(b)]	Publication
Cladocera (5%) Copepoda (20%) Rotatoria (75%)	*Daphnia galeata* (80%) n.a.[(c)] *Polyarthra sp.* (80%)	Van Donk *et al.* (1995)
Cladocera (<2%) Copepoda (31-49%) Rotatoria (49-67%)	*Daphnia galeata* (60%) n.a. *Keratella quadrata* (>80%)	Van den Brink *et al.* (1995)
Cladocera (20-30%) Copepoda, ad. (5%) Copepoda, immat. (20-30%) Rotatoria (20-30%)	*Daphnia galeata* (>80%) *S. oregonensis*[(d)] (>80%) n.a. *Keratella cochlearis* (>90%)	Liber *et al* (1992)
Cladocera (28 %) Copepoda (16 %) Rotatoria (56 %)	*D. longispina* (86%) n.a. *Keratella quadrata* (89%)	Present study, control group, day 0

(a) For published data, % contribution was estimated from Figures; (b) occuring on > 50% of the sampling events; (c) n.a.= not applicable; (d) Skistodiaptomus oregonensis

Consistently, less than 5 zooplankton species appear to strongly dominate the zooplankton community in terms of their contribution to the total density. All remaining species contribute with very low numbers to the total density, but are also represented at very low absolute numbers.

Taking into account that low population densities result in low prediction levels, depending on the sampling volume, the number of replicates and the number of zero counts (chapter 3.2.2), one runs the risk of producing statistically useless and ecologically meaningless information when identifying all organisms down to the species level.

In published studies, the fact that low population density resulted in low prediction levels was taken into account by different measures:

- only those populations that had five or more individuals per sample in the controls were included in the analysis by Lozano *et al.* (1992);
- only the taxa present in more than 10% of all samples were considered as important in a study by van den Brink *et al.* (1995);
- species-level identification was used as data basis for analysis of community structure using multivariate statistical tools (van den Brink & Ter Braak, 1999)
- species data were grouped at higher taxonomic levels resulting in higher numbers, less statistical variability and increased power to detect effects (HARAP, 1999).

Given the fact that data of less abundant species are apparently in many cases not included in the data analysis or only when grouped on a higher taxonomic level, it seems worth questioning the need for taxonomic identification down to the species level. Collecting data at the species level during the study and grouping them later on for above-mentioned reasons into higher taxonomic groups for interpretation can hardly be rationalized in view of limited resources.

Also, indices derived from data sets based on taxonomic identification to the species level, such as species richness and diversity, were experienced as non-sensitive indicators of structural changes in ecotoxicological microcosm testing by most researchers (CLASSIC workshop, 2001).

In conclusion, performing identification down to the lowest hierarchical level in every case does not seem reasonable from a scientific nor an economic point of view. It has been shown in the present study that such information may be redundant, depending on the dominance partition or even useless if numbers of minor species are too low to allow to test for statistical significance.

In the light of the ongoing biodiversity discussion, regulatory authorities tend to generally prefer identification on the lowest taxonomic level for microcosm studies. Considering the results of the present study, it seems advisable to choose the taxonomic level on a case-by-case basis, taking full account of the main species/taxa of concern and the known dominance partition of taxa in the system used. This should enable taxonomic identification in a microcosm study being fixed on a meaningful level at the very beginning of a study and to use available resources at its best.

3.5 SYSTEM-INHERENT VARIABILITY OF PHYTOPLANKTON

3.5.1 Phytoplankton Taxa

The phytoplankton taxa identified in the present study are listed in Table 3-25. Note that the list is a cumulative list of taxa found in all microcosms throughout the study in 1997, including taxa from the control as well as the treatment groups.

Taxonomic identification was performed at the species level or genus level. A cumulative number of 151 taxa were found throughout the study, of which 40 were identified to the genus and 101 to species level, respectively. For the calculations, in case of unidentified species it was assumed that the identified taxon described an individual species, and not several (e.g., *Characium* spec.). Consequently, when analyzing the data on the "species level", the calculations were performed with a number of species set equivalent to the number of taxa.

Table 3-25. Phytoplankton. Identified taxa.

Chlorophyta			
Chlorophyceae			
Chlorococcales	Characiaceae	*Sykidion*	*praecipitans*
	Chlorococcaceae	*Ankyra*	*ancosa*
		Ankyra	*judayi*
		Characium	*acuminatum*
		Characium	*angustatum*
		Characium	spec.
		Chlorella	*ellipsoidea*
		Chlorella	*vulgaris*
		Choriocystis	spec.
		Hydrianum	*coronatum*
		Monoraphidium	*arcuatum*
		Monoraphidium	*circinale*
		Monoraphidium	*contortum*
		Monoraphidium	*griffithii*
		Monoraphidium	*komarkovae*
		Monoraphidium	*minutum*
		Planktosphaeria	*gelatinosa*
		Pseudoquadrigula	spec.
		Tetraedron	*caudatum*
		Tetraedron	*minimum*
		Tetraedron	*triangulare*
		Tetraedron	*trigonum*
	Hydrodictyaceae	*Pediastrum*	*boryanum*
		Pediastrum	*tetras*
	Oocystaceae	*Ankistrodesmus*	*acicularis*
		Ankistrodesmus	*fusiformis*
		Ankistrodesmus	*spiralis*
		Kirchneriella	spec.
		Oocystis	*naegelii*
		Oocystis	*parvula*
		Oocystis	spec.

Table 3-25 (cont). Phytoplankton. Identified taxa.

	Palmellaceae	*Sphaerocystis*	*schroeteri*
	Radiococcaceae	*Coenochloris*	spec.
	Scenedesmaceae	*Coelastrum*	*microsporum*
		Crucigeniella	*rectangularis*
		Scenedesmus	*aculeolatus*
		Scenedesmus	*acutus*
		Scenedesmus	*dimorphus*
		Scenedesmus	*ecornis*
		Scenedesmus	*longispina*
		Scenedesmus	*quadricauda*
		Scenedesmus	*sempervirens*
		Scenedesmus	spec.
		Scenedesmus	*tenuispina*
		Tetrachlorella	*ornata*
Oedogoniales	Oedogoniaceae	*Oedogonium*	spec.
Tetrasporales	Chlorangiaceae	*Chlorophysema*	spec.
	Hormotilaceae	*Hormotilopsis*	spec.
	Oocystaceae	*Treubaria*	*schmidlei*
		Treubaria	*setigera*
	Tetrasporaceae	*Apiocystis*	*brauniana*
		Paulschulzia	*pseudovolvox*
Ulotrichales	Ulotrichaceae	*Binuclearia*	spec.
Volvocales	Chlamydomonaceae	*Carteria*	spec.
		Chlamydomonas	spec.
		Sphaerellopsis	*fluviatilis*
	Phacotaceae	*Phacotus*	*lendneri*
	Polyblepharidaceae	*Nephroselmis*	*olivacea*
	Volvocaceae	*Pandorina*	*morum*
		Volvox	*aureus*
Conjugatophyceae			
Chaetophorales	Coleochaetaceae	*Coleochaete*	spec.
Desmidiales	Desmidiaceae	*Closterium*	*leibleinii*
		Staurastrum	spec.
Zygnematales	Desmidiaceae	*Cosmarium*	*botrytis*
		Cosmarium	*praemorsum*
		Cosmarium	*reniforme*
		Cosmarium	*humile*
		Cosmarium	*meneghinii*
	Zygnemataceae	*Spirogyra*	spec.
Chrysophyta			
Bacillariophyceae			
Pennales	Achnanthaceae	*Achnanthes*	*affinis*
		Achnanthes	*minutissima*
		Cocconeis	*placentula*
	Cymbellaceae	*Amphora*	spec.
		Amphora	*veneta*
		Cymbella	*affinis*
		Cymbella	*microcephala*
		Cymbella	spec.
	Epithemiaceae	*Epithemia*	*zebra*
	Fragilariaceae	*Fragilaria*	spec.
	Gomphonemaceae	*Gomphonema*	*angustatum*
		Gomphonema	*lacustris*
		Gomphonema	spec.

Table 3-25 (cont). Phytoplankton. Identified taxa.

		Naviculaceae	*Gyrosigma*	*acuminatum*
			Navicula	*cryptocephala*
			Navicula	*radiosa*
			Navicula	*seminulum*
			Navicula	spec.
		Nitzschiaceae	*Nitzschia*	*flexa*
			Nitzschia	*acicularis*
			Nitzschia	*gracilis*
			Nitzschia	*palea*
Chrysophyta	**Chrysophyceae**			
	Chromulinales	Chromulinaceae	*Chromulina*	*freiburgensis*
			Chromulina	*minima*
			Chromulina	*minuta*
			Chromulina	*parvula*
			Chromulina	spec.
			Chromulina	*sphaeridia*
			Chrysochromulina	*parva*
			Chrysochromulina	spec.
		Chrysococcaceae	*Chrysococcus*	*rufescens*
			Chrysococcus	spec.
	Monosigales	Monosigaceae	*Monosiga*	spec.
		Salpingoecaceae	*Salpingoeca*	spec.
	Ochromonadales	Dinobryaceae	*Kephyrion*	spec.
		Ochromonadaceae	*Ochromonas*	*nana*
			Ochromonas	*pinguis*
			Ochromonas	spec.
			Ochromonas	*variabilis*
			Ochromonas	*miniscula*
			Ochromonas	*sphagnalis*
		Synuraceae	*Mallomonas*	*radiata*
			Mallomonas	spec.
	Xanthophyceae			
	Mischococcales	Characiopsidaceae	*Characiopsis*	spec.
			Peroniella	*planctonica*
Cryptophyta				
	Cryptophyceae			
	Cryptomonadales	Cryptochrysidaceae	*Chroomonas*	*acuta*
		Cryptomonadaceae	*Cryptomonas*	*erosa/ovata*
			Cyathomonas	spec.
			Cyathomonas	*truncata*
		Katablepharidaceae	*Katablepharis*	*ovalis*
Cyanophyta				
	Cyanophyceae			
	Chroococcales	Chroococcaceae	*Chroococcus*	*minutus*
			Chroococcus	spec.
			Chroococcus	*turgidus*
			Dactylococcopsis	*raphidioides*
			Dictyosphaerium	spec.
			Dinobryon	*sertularia*
			Gomphosphaeria	*lacustris*
			Rhabdoderma	*Lineare*
	Nostocales	Nostocaceae	*Anabaena*	*planctonica*
			Anabaena	spec.
			Anabaena	*variabilis*

Table 3-25 (cont). Phytoplankton. Identified taxa.

	Nostocales	Nostocaceae	*Anabaena*	*planctonica*
			Anabaena	spec.
			Anabaena	*variabilis*
		Rivulariaceae	*Gloeotrichia*	spec.
	Oscillatoriales	Oscillatoriaceae	*Lyngbia*	*limnetica*
			Lyngbia	spec.
			Oscillatoria	*lacustris*
			Oscillatoria	*limnetica*
			Oscillatoria	*limosa*
			Oscillatoria	*planctonica*
			Oscillatoria	*rosea*
			Oscillatoria	spec.
			Oscillatoria	*tenuis*
			Oscillatoria	*tenuispina*
Euglenophyta				
	Euglenophyceae			
	Colaciales	Colaciaceae	*Colacium*	spec.
	Euglenales	Euglenaceae	*Euglena*	*clavata*
			Euglena	spec.
			Euglena	*viridis*
			Trachelomonas	spec.
Dinophyta				
	Dinophyceae			
	Dinokontae	Gymnodiniaceae	*Gymnodinium*	spec.
		Peridiniaceae	*Peridinium*	spec.

3.5.2 Interreplicate Variability of Phytoplankton

Species/Taxa present

In the control group, a total of 131 taxa occurred during the study period. Thereof, ten species were found on all sampling occasions: *Ankyra judayi, Planktosphaeria gelatinosa, Tetraedron minimum, Carteria* spec., *Chlamydomonas* spec. (Chlorophyta)*; Nitzschia palea, Chromulina sphaeridia* (Chrysophyta)*, Oscillatoria rosea* (Cyanophyceae)*; Chroomonas acuta* and *Cryptomonas erosa/ovata* (Crytophyta)*.* The number of taxa found in the individual control ponds on a given date varied among ponds and over time (Figure 3-25). The average number of taxa declined from 25 on test day -24 (June 16, 1997) to 11 on test day 101 (October 19, 1997). The maximum number of taxa detected in a control pond was 39, the minimum number was 7.

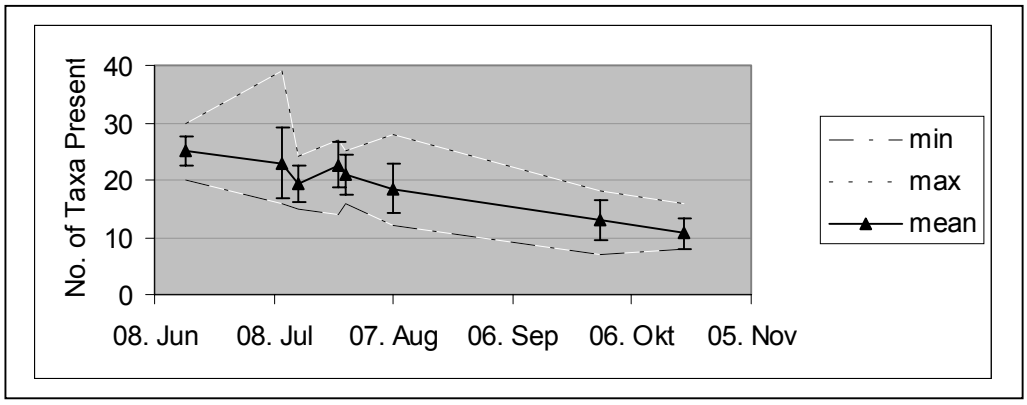


Figure 3-25. Number of phytoplankton taxa present in individual ponds.

Total Density, Population Densities and Species Diversity

Mean total density, mean species diversity (Shannon-Wiener Index) and the respective standard deviations and coefficients of variation were calculated for the phytoplankton in the control group. These values were calculated on the basis of 12 replicate ponds for 8 sampling events. Figure 3-26 displays mean values and standard deviations for total density and species diversity, as well as the contribution of individual control replicates to the variability in the control group. Corresponding coefficients of variation are given in Table 3-26.

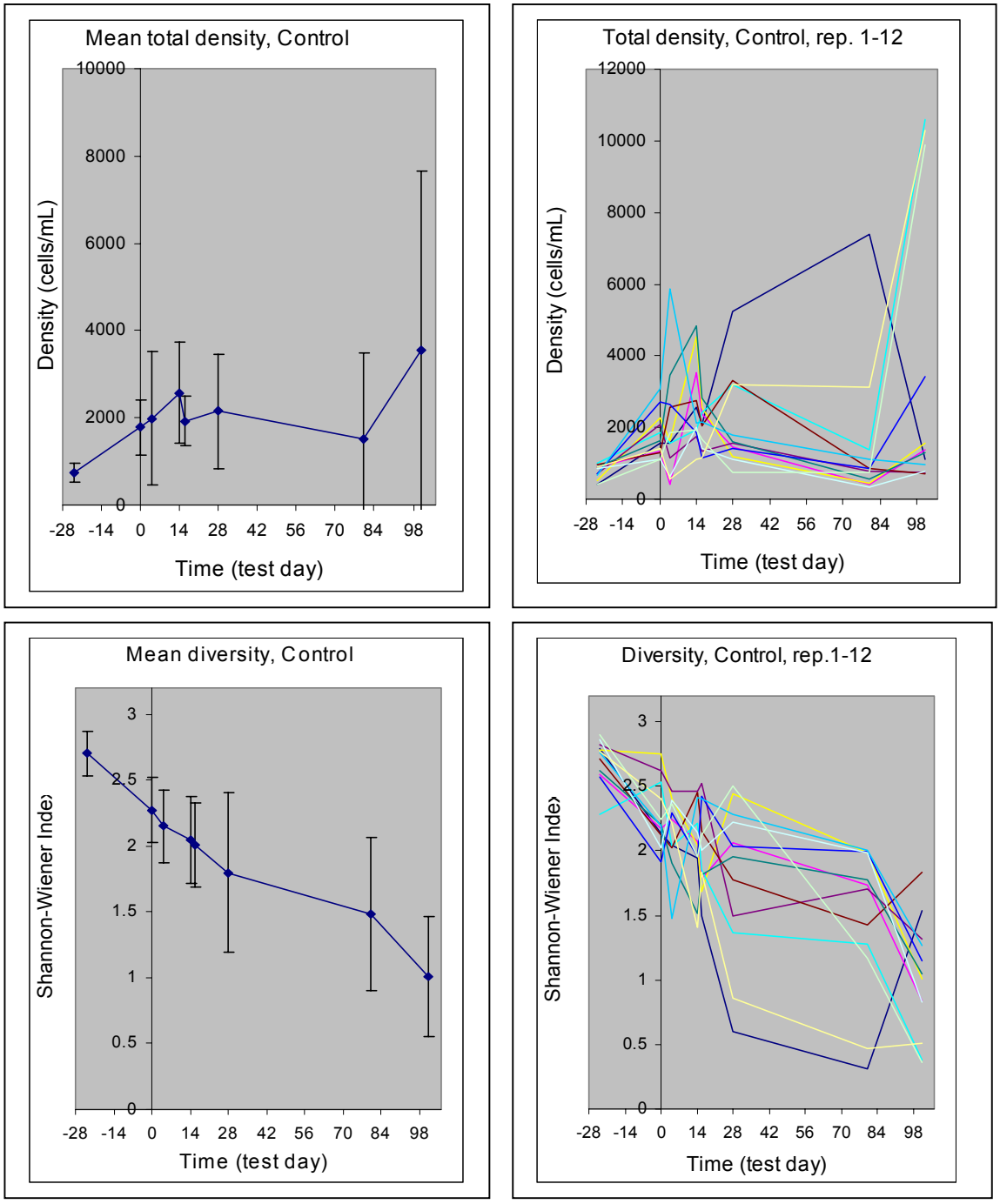


Figure 3-26. Phytoplankton total density and species diversity, control group.

The mean total Phytoplankton density increased during the study period, reaching a value of about the fourfold on day 101 (October) when compared to day –24 (June). The corresponding average coefficient of variation went up from 29% on day-24 to 133% on day 80, indicating an increasing variability within the control group over time (Table 3-26). However, during the exposure phase (day 4 to day 28), coefficients of variation did not exceed 77%. The mean value for species diversity (Figure 3-26) initially found at a Shannon-Wiener index of 2.7 steadily decreased during the study period and reached its minimum in October (day 101) with a value close to 1.0. The spatial variability for the mean species diversity, initially showing a coefficient of variation of 6%, climbed to 45% on day 101 (Table 3-26).

For individual taxa, coefficients of variation were found in the range of 31-346% throughout the study (Table 3-26). However, none of the individual taxa followed the seasonal pattern shown for coefficients of variation for total density.

Table 3-26. Coefficients of variation in the control group for Phytoplankton total density, species diversity and for density of individual taxa.

	Coefficients of variation (%) for phytoplankton in the control group							
	day -24	day 0	day 4	day 14	day 16	day 28	day 80	day 101
Species Diversity	**6**	**11**	**13**	**16**	**16**	**34**	**39**	**45**
Total density	**29**	**35**	**77**	**45**	**30**	**61**	**133**	**115**
Chroomonas acuta	*55*	*17*	*117*	*52*	*73*	*108*	*83*	*50*
Cryptomonas erosa/ovata	*74*	*50*	*53*	*77*	*93*	*85*	*91*	*90*
Crucigeniella rectangularis	*n.a.*	*121*	*201*	*111*	*126*	*171*	*n.a.*	*n.a.*
Oocystis naegelii	*244*	*31*	*89*	*65*	*76*	*112*	*161*	*n.a.*
Volvox aureus	*n.a.*	*n.a.*	*245*	*346*	*233*	*253*	*211*	*167*
Anabaena variabilis	*346*	*129*	*92*	*84*	*93*	*86*	*280*	*n.a.*
Lyngbia spec.	*n.a.*	*99*	*207*	*110*	*102*	*93*	*154*	*n.a.*
Oscillatoria rosea	*145*	*95*	*193*	*210*	*152*	*206*	*243*	*346*

Mean: Variance Relationship for Phytoplankton

The mean:variance relationship for the entire phytoplankton data set for the control group on days –24 through day 101 is shown in Figure 3-27. Log 10 (s^2) was plotted against log 10 (M) for 131 data pairs (n´=131). The resulting slope (b-value) was 1.96, the corresponding correlation coefficient was 0.89. The mean (M): variance (s^2) relationship was also calculated separately for all test days on the basis of 12 replicates (n=12) per test day. The slopes (b-values) derived from the regression analysis for the 8 sampling dates are shown in Table 3-27. Slopes ranged from 1.73 to 2.04 for sampling days –24 through 101, correlation coefficients (r^2) ranged from 0.85 to 0.91.

Table 3-27. Mean:variance, phytoplankton, individual test days.

	day -24	day 0	day 4	day 14	day 16	day 28	day 80	day 101
b	1.89	1.73	2.04	2.00	1.97	2.04	1.97	2.04
r^2	0.85	0.86	0.91	0.91	0.89	0.91	0.86	0.87

Legend: Slope (b) and correlation coefficient (r^2).

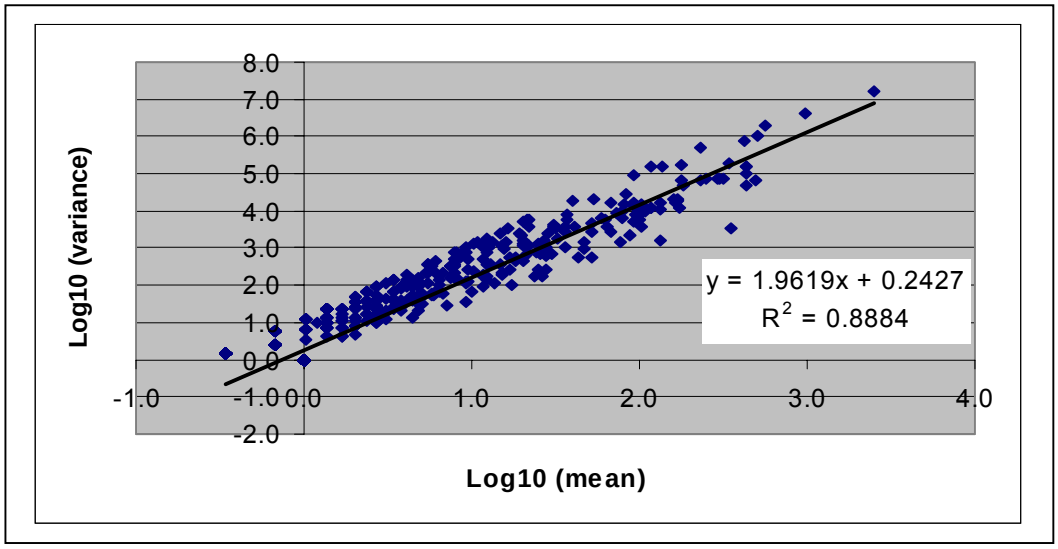


Figure 3-27. Mean: Variance relationship phytoplankton, all test days.

Levels of Precision for Phytoplankton

The levels of precision (p) were calculated for all taxa and all sampling events on the basis of standard errors for 12 replicates in the control group. It could be shown that the precision level is the more favorable the higher the correlated mean (Figure 3-28). It is important to note that the correlation coefficient for this relationship was very low: Therefore the curve indicates rather a trend than a clear correlation.

For the whole data set (n´= 441 data points), precision levels of $\leq$ 0.2 were reached in only 5% of the cases and that precision levels always exceeded a value of 0.2 for mean densities below 10 individuals/L. Mean densities exceeded 10 individuals/L in 29% of the cases. Thereof, 15% were found at a precision level $\leq$0.2.

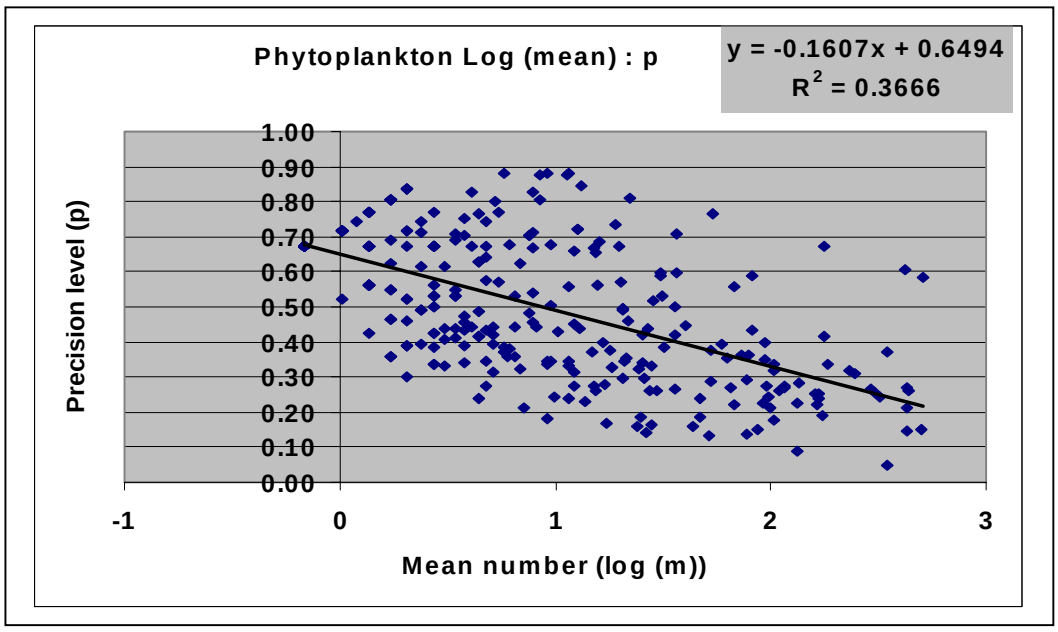


Figure 3-28. Mean: Precision level for phytoplankton.

Zero-Counts for Phytoplankton

The number of zero-counts plays a significant role in data interpretation. Population densities equivalent or close to zero occur naturally and may be related to seasonality or sampling design. Inter-replicate zero-counts were summarized for days –24, 0, 4, 14, 16, 28, 80 and 101 as number of species representing zero counts in x percent of the replicates in Figure 3-29. Calculation of zero-counts was based on the cumulative species list for the control ponds (n= 131). Zero counts were shown to be the rule rather than the exception. The minority of findings could be based on representation of a certain phytoplankton species in all the 12 replicate samples. For example, only 4% of all phytoplankton species (n=131) which occurred during the study were found in all 12 replicate samples taken on day 0 (Figure 3-29). The correlation between the number of zero-counts and the corresponding variation coefficient is displayed in Figure 3-30. Coefficients of variation always exceeded 100% with >4 zero counts (out of 12 replicates).

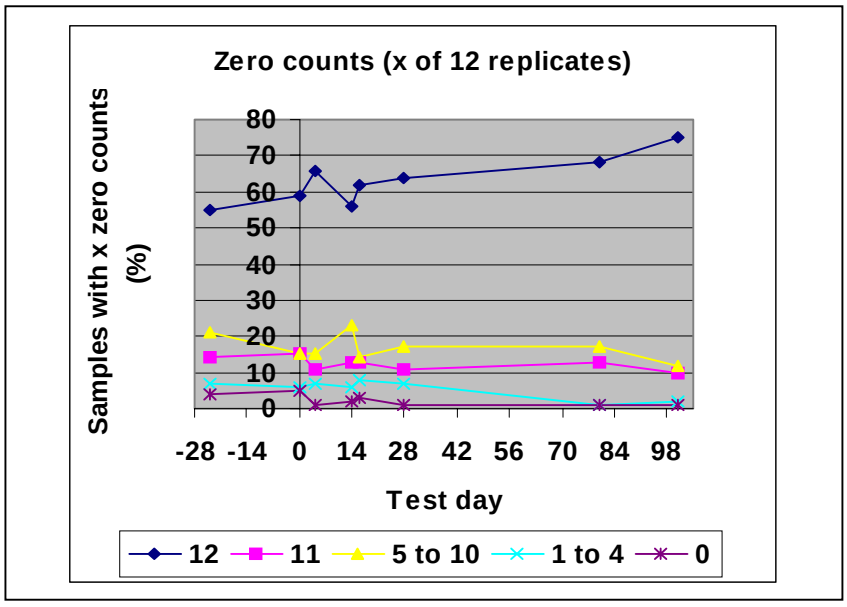


Figure 3-29. Number of zero-counts for x % of all species.

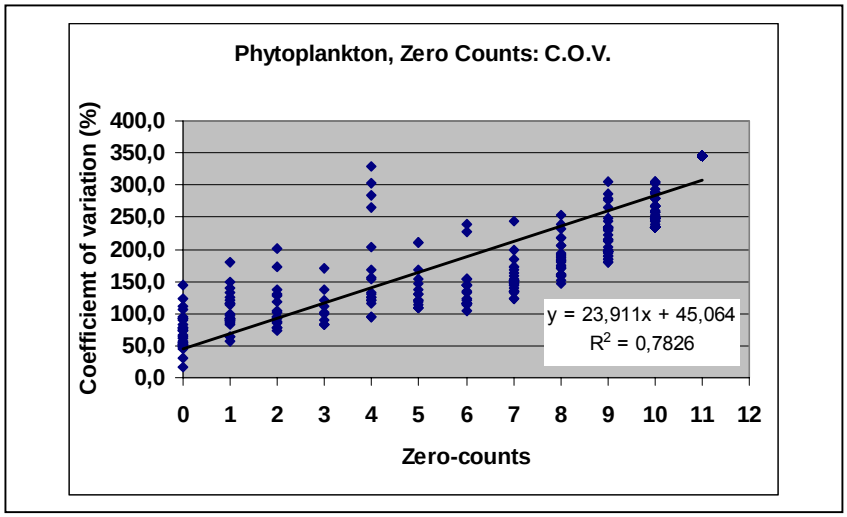


Figure 3-30. Number of zero-counts: coefficient of variation.

3.5.3 Seasonal Variability of Phytoplankton

At the beginning of the study, many small to medium sized populations contributed to the total phytoplankton density. At test end, 4 species represented about 97% of the total density. Data are presented as dominance curves (Figure 3-31), to be read in conjunction with the list of ranked dominant species per sampling event (Table 3-28).

On day-24, many taxa contributed with small numbers to the total phytoplankton density and the corresponding curve starts at a very low level of cumulative % dominance. For example, the most dominant species (rank 1= Kirchneriella spec.) contributed with 10 % to the total density (Figure 3-31). Dominance curves were more elevated for days 0 to 28, when diversity had slightly decreased. The most extreme partition was found for day 101, where 4 taxa cumulatively represented about 97% of the total density, namely *Volvox aureus*, *Chroomonas acuta*, *Cryptomonas erosa/ovata* and *Oscillatoria tenuispina* (Table 3-28). Mean densities for the dominant species are shown in Figure 3-33.

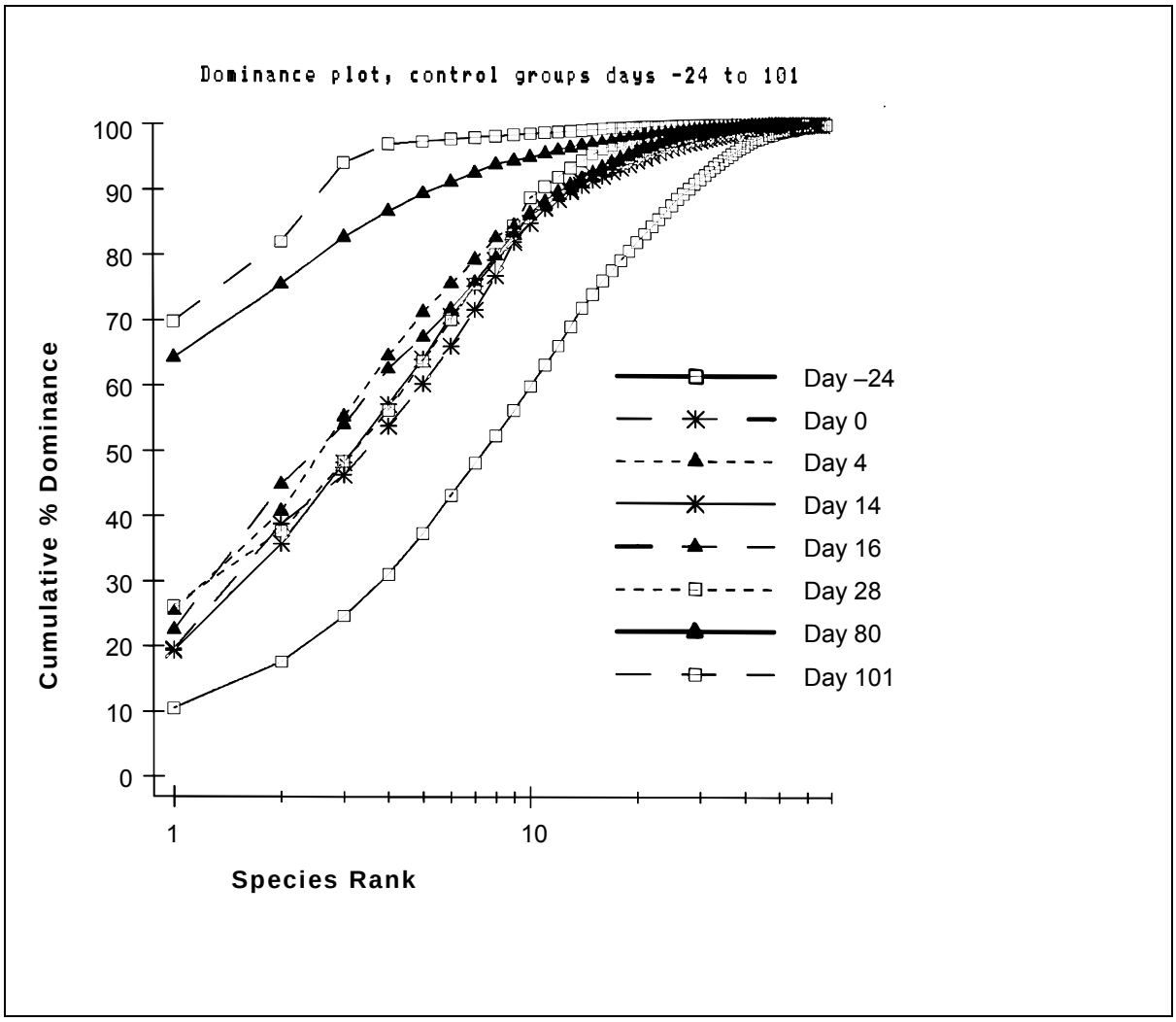


Figure 3-31. Dominance plot for Phytoplankton in the control group.

Table 3-28. Ranking of species according to their contribution to the total density in the control group for all test days.

	Rank 1	Rank 2	Rank 3	Rank 4	% of total [a]
day-24	*Kirchneriella* spec.	*Nitzschia gracilis*	*Oscillatoria tenuispina*	*Nitzschia palea*	31
day 0	*Chroomonas acuta*	*Anabaena variabilis*	*Lyngbia* spec.	*Oocystis naegelii*	54
day 4	*Crucigeniella rectangularis*	*O.naegelii*	*A.variabilis*	*C.acuta*	67
day 14	*C.acuta*	*Oscillatoria rosea*	*A.variabilis*	*C.rectangularis*	57
day 16	*C.acuta*	*A.variabilis*	*Volvox aureus*	*O.naegelii*	63
day 28	*V.aureus*	*C.acuta*	*Ankyra ancosa*	*Chlamydomonas* spec.	56
day 80	*V.aureus*	*C.acuta*	*Cryptomonas erosa/ovata*	*Chromulina minuta*	87
day 101	*V.aureus*	*C.erosa/ovata*	*C.acuta*	*O.tenuispina*	97

(a) The last column: cumulative contribution of the 4 mentioned taxa to the total density.

The contribution of major phytoplankton classes to the total phytoplankton density and thus the seasonal shifts in contribution of these classes is shown in Figure 3-33. Chlorophyceae, Cyanophyceae and Cryptophyceae were the dominant classes in terms of density. Chlorophyceae dominated the phytoplankton community throughout the study period, namely at test end, October 19, when they reached almost 80% of the total algal density. The contribution of Cyanophyceae to the total phytoplankton decreased during the study period, from initially 15% to about 5% at test end. Cryptophyceae contributed with 5-20% throughout the study period, while Bacillariophyceae contributed to a minor extent to the total density (Figure 3-33).

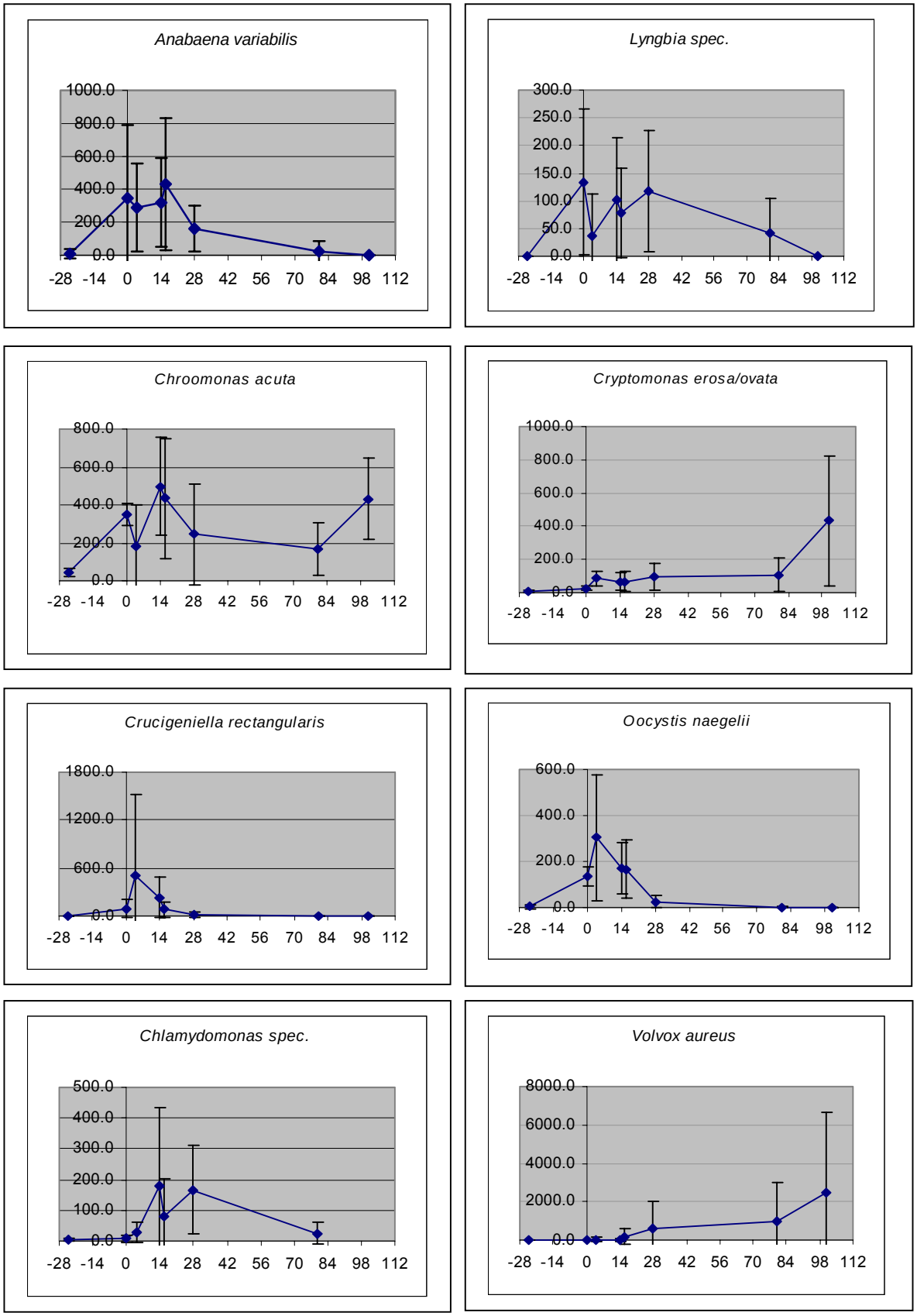


Figure 3-32. Mean densities for abundant Phytoplankton taxa in the control group.

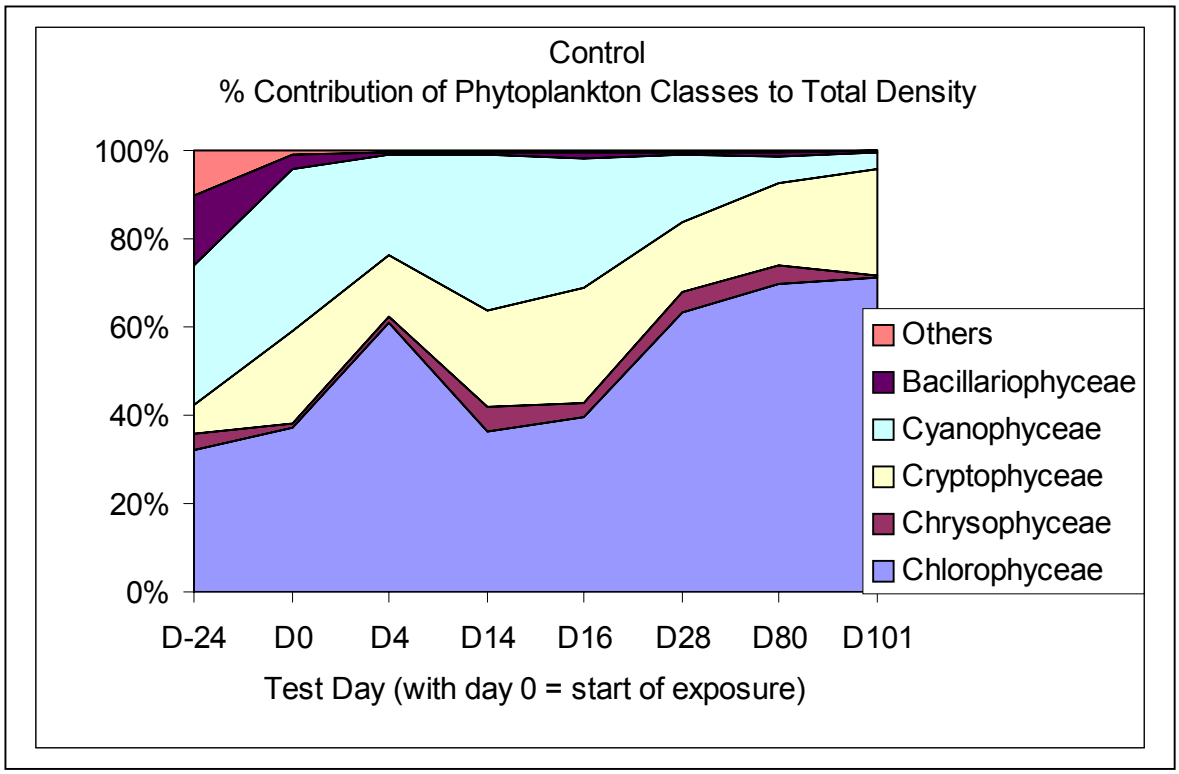


Figure 3-33. Percent contribution of major phytoplankton classes to total phytoplankton density in the control goup.

3.6 DISCUSSION OF SYSTEM-INHERENT VARIABILITY OF PHYTOPLANKTON

3.6.1 Phytoplankton inter-replicate variability was dominated by the number of zero counts

Coefficients of Variation

During the pre-exposure phase, a continuous water exchange between all ponds via a mixing tank had been applied. During this test phase, inter-replicate variability measured as coefficient of variation for total density and species diversity was very low (day-24) (Table 3-26). The high degree of similarity of phytoplankton communities in all microcosms has also been shown using hierarchical clustering of the data (Figure 3-39). This suggests that the applied test design proved useful in establishing highly similar conditions in all microcosms. Establishing ponds without additional water circulation was reported to result in low similarity of phytoplankton communities in such isolated test systems (Rosenzweig & Buikema, 1994).

A moderate increase of inter-replicate variability was shown for total density of phytoplankton during days 0 through 28, after disconnecting the microcosms from the mixing tank. Coefficients of variation for total density and species diversity reached their maxima at the end of the study period (Table 3-26). This increase in variation occurred about 80 days after the ponds had been disconnected from water circulation and thus had represented isolated test systems. It can be concluded that the increase of variability was related to an “enclosure effect”. As already stated in chapter 3.2, the enclosure effect could be overcome by establishing a continued water inflow (and thus re-inoculation with zoo- and phytoplankton organisms) from the reservoir during exposure and post-exposure phase of the study. Continuous or pulsed water exchange would also have the advantage of simulating a more ´natural´ scenario as it would allow a re-colonization of the microcosms.

Coefficients of variation for individual taxa did not follow the general temporal trend observed for the total density. For individual taxa, coefficients of variation were found in the range of 31-346% throughout the study (Table 3-26). Similar to zooplankton species, it seemed that also for many phytoplankton species, low mean numbers were correlated with high coefficients of variation. For example, *Anabaena variabilis* had lowest coefficients of variation on days 0 through 28 (Table 3-26) and this was related to high population densities on these days, as shown in Figure 3-32. In conclusion it can be stated that there was no clear trend that phytoplankton interreplicate variability was generally lower for taxa which occur at higher densities. In this respect the phytoplankton behaved clearly different when compared to the zooplankton (Figure 3-2).

Mean:Variance

Mean to variance relationships for phytoplankton and zooplankton showed unexpectedly high similarity (Figure 3-27, Figure 3-3), despite the differences in scale. Phytoplankton population densities ranged from 0.3 to 4200 cells/ml and zooplankton from 0.01 to 800 organisms/L.

However, as differences in density ranges are not reflected in the slope of the regression curve, it seems plausible that the slope of both curves was similar. The slope of the mean to variance relationship was 1.96 for phytoplankton and 1.59 for zooplankton. Considering that Downing *et al.* (1987) found a b-value of 1.85 for their zooplankton algorithm, the b-value for phytoplankton of the present study (1.96) is approaching even more the "general" mean to variance relationship of zooplankton.

The mean to variance relationship has been frequently used as an index of spatial variability in natural ecosystems (Downing *et al.*, 1987; Pinel-Alloul *et al.*, 1988). Due to the amazingly high similarity of b-values for phytoplankton and zooplankton in the present study, the sensitivity of this index may be questioned.

Precision levels

For the present study, a trend could be shown for the relationship between precision levels and the mean. Precision levels were negatively correlated to the mean, indicating that the precision level improves with increasing population densities (Figure 3-28). It is important to note that the correlation coefficient of the regression curve was very low (r^2= 0.36) and that therefore the curve indicated a trend rather than a clear correlation.

Results from the present study indicate that, similar to the zooplankton, also the phytoplankton populations can show high inter-replicate variability, with precision levels exceeding a p-value of 0.2 in the majority of cases (Figure 3-28). Precision levels always exceeded a value of 0.2 for mean densities below 10 individuals/L. Precision levels were somewhat more reliable for mean densities exceeding 10 individuals/L, but also if this requirement was fulfilled, precision levels of p=0.2 were exceeded in 85% of the cases.

In comparison with published data, precision levels in the present study were fairly low. For example, precision levels have been calculated by Rosenzweig & Buikema (1994) for phytoplankton in experimental ponds. Using 3 ponds per treatment as a basis for their calculations, the precision levels for phytoplankton classes ranged between $\pm$75% and $\pm$143%. The high variance between ponds in this study was most likely due to the test design, not allowing water exchange in the development phase of phytoplankton.

Zero-counts

Inter-replicate variability was strongly dominated by the number of zero-counts (Figure 3-29). The number of zero counts was calculated for each species and each sampling event. The correlation between zero-counts and coefficient of variation gave a clear indication for a relation between those two measures. The curve revealed that for all data points based on 5 and more zero counts among the 12 replicates, coefficients of variation always exceeded 100% (Figure 3-30). It is interesting to note that the described phytoplankton curve was almost identical to the one established for zooplankton, with slopes of 23,2 and 23,9, respectively (Figure 3-7, Figure 3-30).

Zero-counts appear to be the result of the seasonality of phytoplankton species and their short generation times. Seasonality has been reported in large number of studies (e.g. Sommer *et al.,* 1986), also revealing the high number of zero-counts encountered in research on natural ecosystems (e.g., Agbeti *et al.,* 1997).

3.6.2 Consequences for the test design

For the present study, using the described sampling and counting methods and a set of 12 replicate ponds, the number of zero-counts clearly dominated the measured interreplicate variability. From the observations made in the present study, it seems advisable not to rely on taxa, which occur in less than 50% of the replicates on a certain sampling day.

As shown in the present study, precision levels were more reliable when based on mean population densities exceeding a “threshold” of 10 individuals/mL. This implies that interpretation of changes in populations occurring at lower numbers can not be stated to be statistically sound.

Similar to the conclusions drawn for zooplankton (section 3.2.2) it seems advisable for the phytoplankton taking into account the importance of zero counts and the requirement of exceeding the described “threshold”, and adapting the sampling design and counting method accordingly when setting up a microcosm study. For example, it might prove useful to use a larger sampling volume, a larger sub-sample or a bigger counting area to avoid zero counts.

3.6.3 Changes in phytoplankton composition dominated by seasonality

Species rich in summer and less diverse in autumn

Phytoplankton is known to be species rich in summer due to reduced grazing pressure after the clear water phase (Sommer *et al.*, 1986). In the present study, the phytoplankton community structure was characterized as being highly diverse in summer (June-July). Of the 131 taxa identified, many contributed with small numbers to the total phytoplankton density during summer (Figure 3-31).

The dominance partition changed heavily during the study, with a few species dominating the community at test end, in autumn (Figure 3-31, Table 3-28). The dominance partition of phytoplankton (Figure 3-31) corresponded well with the diversity indices (Figure 3-26). Diversity was shown to reach lowest values on day 80 and day 101, when dominance plots showed most elevated curves. The mean number of taxa found in the control ponds had clearly decreased in autumn when compared to summer samplings (Figure 3-25).

Studies on inter-replicate variability are rarely found in literature. However, also for natural ecosystems, a strong dominance of the phytoplankton community by 5-10 species has been reported (Karentz & Smayda, 1998).

Seasonal interaction of phytoplankton and zooplankton

It was assumed that temporal changes in the phytoplankton community composition detected in the present study were largely related to natural seasonal succession, e.g. due to grazing by zooplankton and temperature variation.

As will be discussed in this section, the phytoplankton composition at large followed the pattern described for natural seasonal succession observed in lakes. The seasonal switching in size from small to large algae and from large to small herbivores was described as part of the Plankton Ecology Group (PEG) model (Sommer *et al.*, 1986). According to the PEG model, grazing on small-celled phytoplankton is followed by a shift to large-celled algae species, which are inedible for zooplankton. Dominance of large-celled algae may lead to reductions of larger zooplankton, e.g. non-specific filter feeders. Larger species of crustacean herbivores are then replaced by smaller species and by rotifers.

The phytoplankton community in the present study followed the seasonality described above. In summer (July 10 to August 7, day 0-28), small celled edible Cryptophyceae (e.g. *Chroomonas acuta*) and moderately edible Chlorophyceae (e.g. *Oocystis naegelii*) showed high densities (Figure 3-32, Table 3-29). This food availability was positively correlated with the number of crustaceans (Figure 3-34).

For test days 80 and 101 (September 28 and October 19), the increase in total phytoplankton density was related to the significant increase in *Volvox aureus* (Figure 3-26, Figure 3-32). *Volvox aureus* is often too large to be grazed by copepods and cladocerans (Hurlbert, 1975). Thus the dominant numbers of algae inedible to filter feeders in autumn partly explain the coinciding decrease of crustaceans (Figure 3-34). It is assumed that decreasing temperatures (Appendix B) further contributed to the decline of crustaceans in autumn. The decrease of crustacean herbivores was followed by an increase of rotifers. Rotifers increased rapidly in numbers due to their short generation time after day 28 (Figure 3-34). In the present study, the release from grazing pressure showed positive effects on populations of small edible algae in autumn. The yearly maximum density of *Chroomonas acuta* was measured on test day 101 (October 19) and also *Cryptomonas erosa/ovata* showed comparatively high numbers on this day (Figure 3-38)).

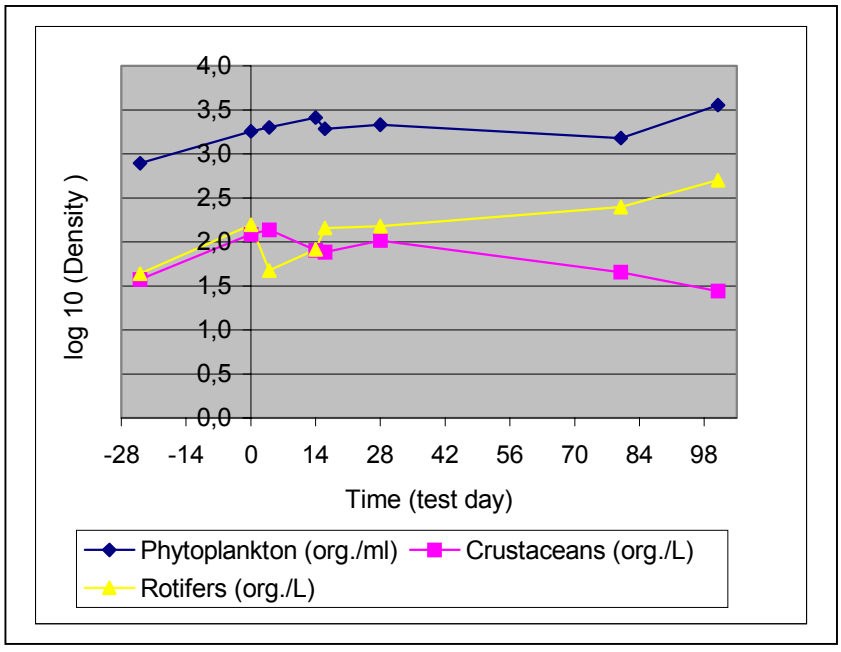


Figure 3-34. Seasonal interaction phyto- and zooplankton.

Table 3-29. Algae as prey for rotifers and crustaceans (Lang ,1997)

1) small edible single cells, 5-30 µm	*e.g. Cryptomonas ovata*
2) small, coccale, edible algae, 2-10 µm	*e.g. Chlorella* spec.
3) moderately edible single cells, 10- 200 µm	*e.g. Oocystis* spec.
4) moderately edible, colony-forming or filamentous cells, 10 –100 µm	*e.g. Bacillariophyceae*
5) filamentous algae, inedible for most zooplankton species, 5-80 µm	*e.g. Anabaena* spec.

3.7 DISTURBANCE EFFECTS ON PHYTOPLANKTON

3.7.1 Coefficients of variation for Phytoplankton in treatment groups

The average total phytoplankton densities (cells/mL) for each treatment group and the control are displayed in Figure 3-35 (top). For the control group, standard deviation bars are indicated. Figure 3-35 also shows the inter-replicate variability within the treatment groups, with each curve representing one replicate pond. The treatment groups SHADOW, TURBULENCE, RUN-OFF and DIAZINON, consisted of 4, 3, 4 and 4 replicates, respectively. The corresponding coefficients of variation are shown in Table 3-30.

Mean total densities for the treatments TURBULENCE and DIAZINON went up to about 70'000 and 100'000 cells/mL, respectively, while mean total densities for the treatments SHADOW and RUNOFF rarely exceeded 10´000 cells/mL.
Inter-replicate variability within the treatment group was comparatively low for the treatments SHADOW and DIAZINON, reaching variation coefficients of 14% to 75% and 25% to 71%, respectively (Table 3-30, Figure 3-35). The corresponding coefficients of variation for the treatments TURBULENCE and RUNOFF were found within a range of 35% to 140% and 9% to 170%, respectively and they were in general much higher for days 14 to 101 when compared to days –24 to 4 (Table 3-30, Figure 3-35).

Table 3-30. Coefficients of variation for phytoplankton total density and diversity in the treatment groups and in the control.

	Coefficients of variation for total phytoplankton density (%)				
	SHADOW	TURBULENCE	RUNOFF	DIAZINON	CONTROL
day -24	14	35	9	25	29
day 0	75	40	80	71	35
day 4	31	10	49	28	77
day 14	22	115	63	41	45
day 16	37	35	132	48	30
day 28	66	94	167	36	61
day 80	54	140	170	69	133
day 101	21	112	26	40	115

	Coefficients of variation for phytoplankton diversity(%)				
	SHADOW	TURBULENCE	RUNOFF	DIAZINON	CONTROL
day -24	12	4	1	3	6
day 0	9	13	15	22	11
day 4	12	10	6	15	13
day 14	17	56	12	13	16
day 16	32	8	35	13	16
day 28	6	26	72	18	34
day 80	12	54	54	21	39
day 101	14	71	22	29	45

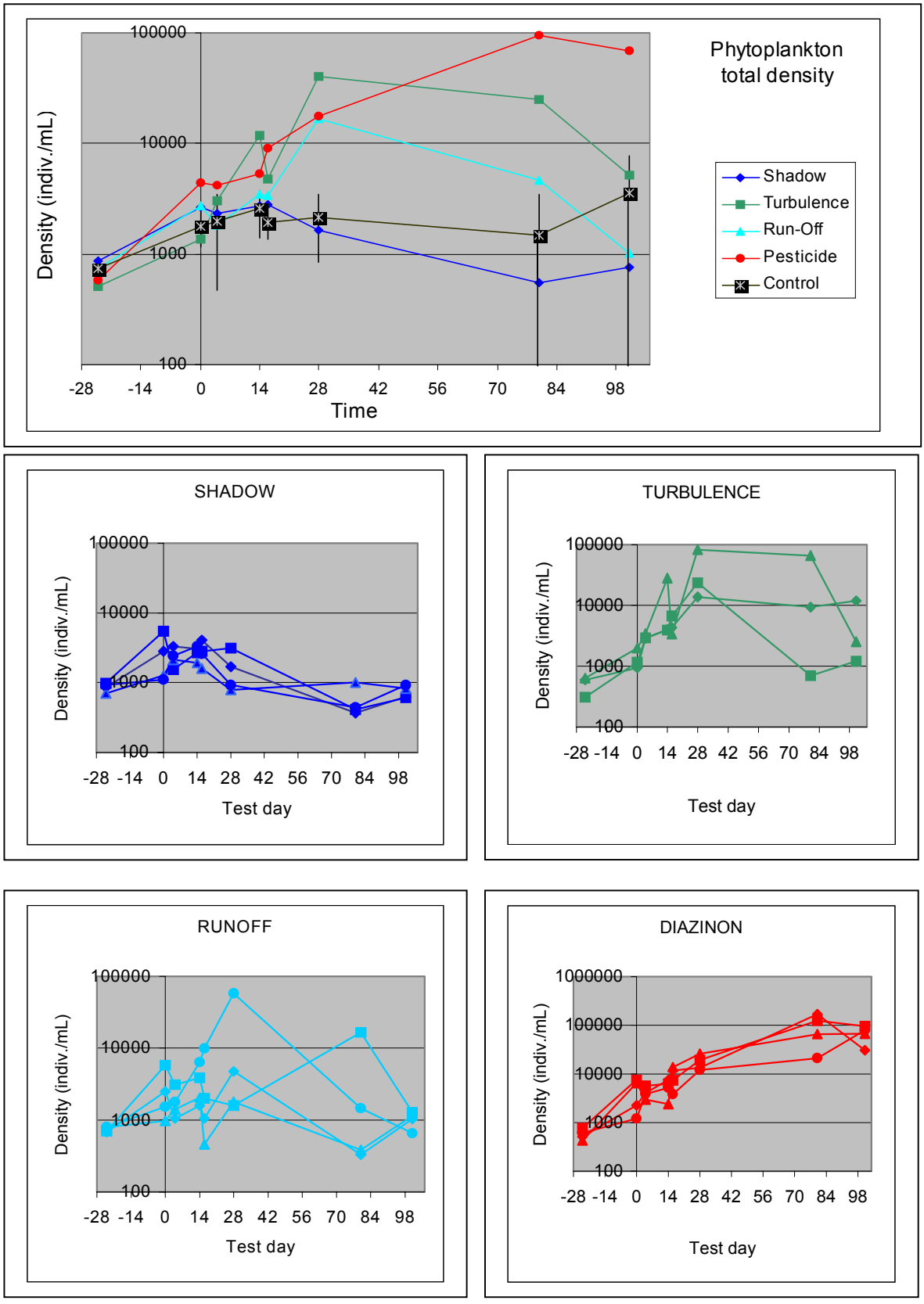


Figure 3-35. Phytoplankton, total density. Mean values for the treatments and the control and interreplicate variability for each treatment group.

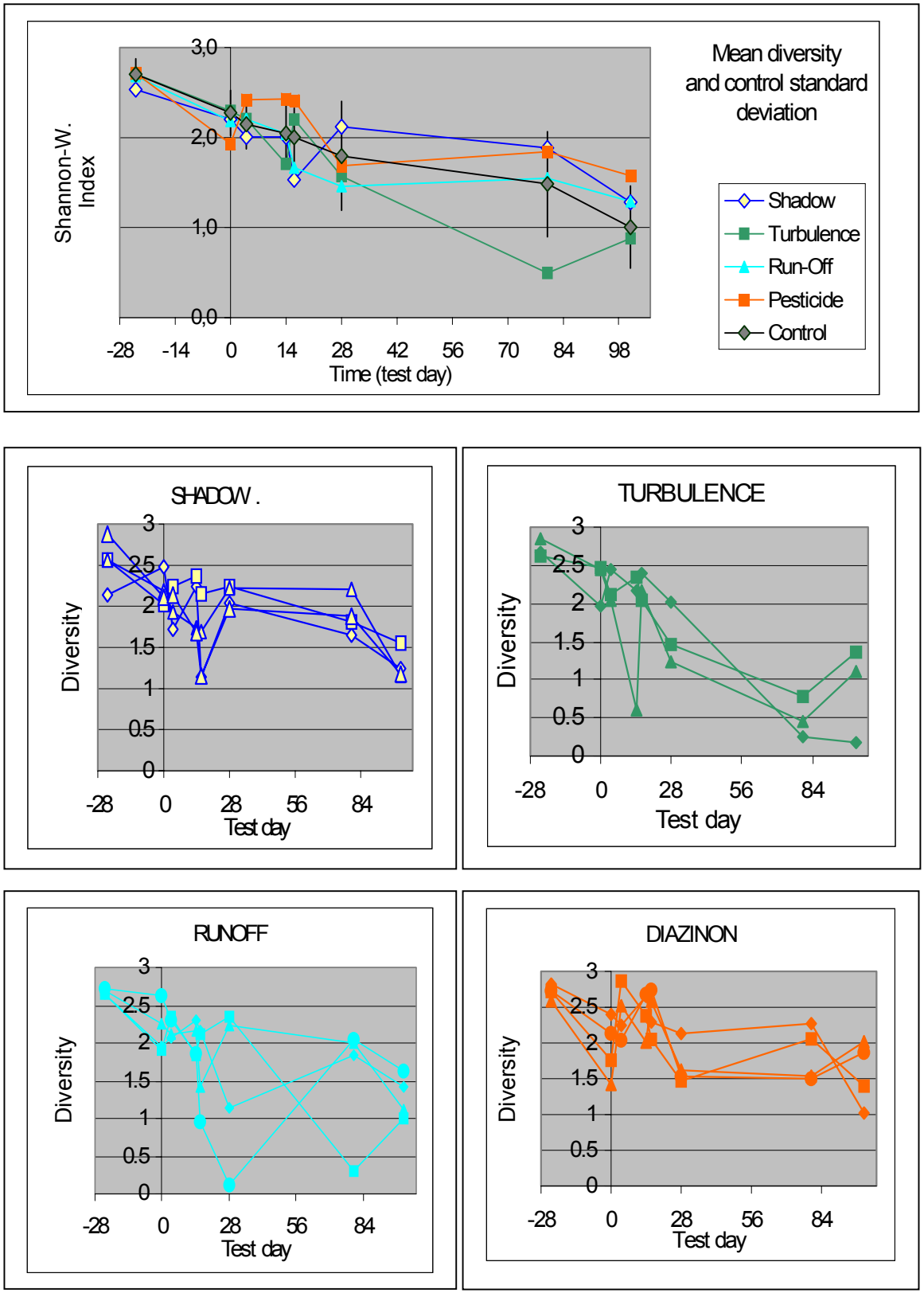


Figure 3-36. Phytoplankton, diversity. Mean values for the treatments and the control and interreplicate variability for each treatment group.

Figure 3-36 displays the average phytoplankton diversity (Shannon-Wiener Index) of each treatment group and the control including standard deviation bars for the control group. Moreover, it shows the inter-replicate variability within the treatment groups: each curve represents a replicate pond. For the pre-exposure phase, average Shannon-Wiener Indices for all treatments were found in a range of 2.0 to 2.5. Diversity remained at indices of 1.5 to 2.5 for the treatment group SHADOW during the exposure phase (except for day 16) and slightly decreased at test end (day 101). The phytoplankton community of DIAZINON treated microcosms showed high diversity throughout the study period (values of 1.5 to 2.8, except for day 101).

Coefficients of variation for the pre-exposure phase were found within a range of 1% to 22% for all treatment groups (Table 3-30). Variability within the treatment group was comparatively low for the treatments SHADOW and DIAZINON during exposure and post-exposure phase, reaching variation coefficients of 6% to 32% and 3% to 29%, respectively. The corresponding coefficients of variation for the treatments TURBULENCE and RUNOFF were much higher: 8% to 71% and 6% to 72%, respectively.

3.7.2 Dominant Phytoplankton Taxa

Phytoplankton communities in all treatment groups showed a high diversity during the pre-exposure phase. In the dominance plot, the 10 most abundant taxa cumulate to 60% of the total density on day –24 in all treatment groups and the control (Figure 3-37). The corresponding ranking of dominant taxa is listed in Table 3-31. Compared to pre-exposure samplings, phytoplankton communities showed lower diversity on days 14, 16 and 28. The treatment group RUNOFF had the most elevated dominance curve, indicating the lowest diversity, while the control and the DIAZINON groups still showed a comparatively high diversity (day 16, Figure 3-37). Dominance partition for days 80 and 101 showed elevated curves for the treatments TURBULENCE and RUNOFF, indicating that these groups were strongly dominated by few taxa, while dominance curves for SHADOW and DIAZINON still indicated a high species diversity until test end (day 80, Figure 3-37).

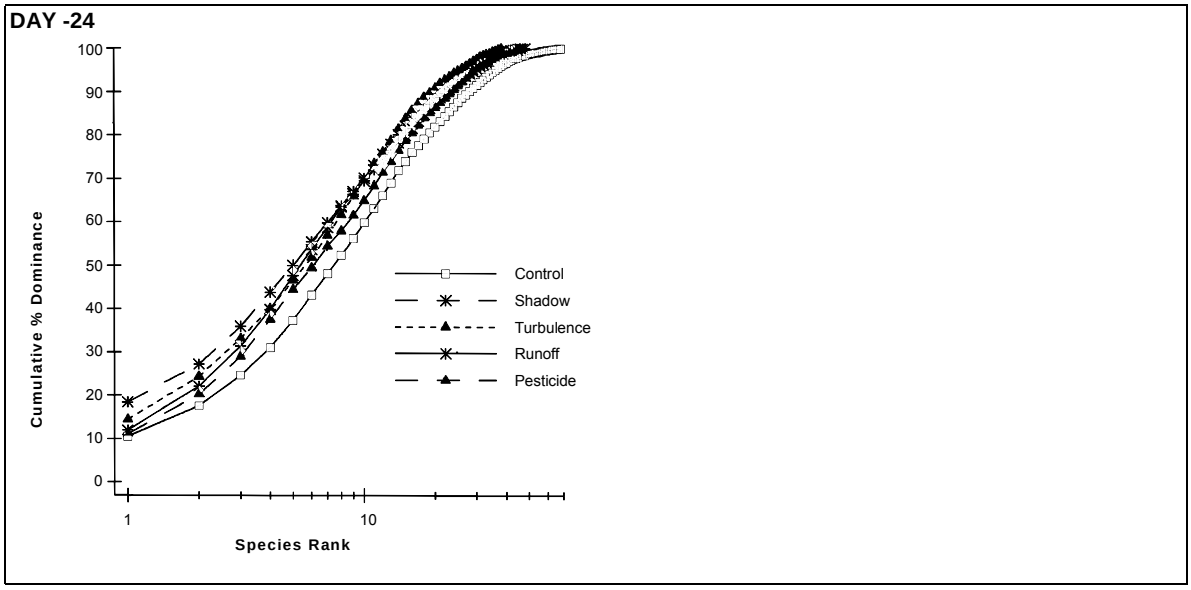
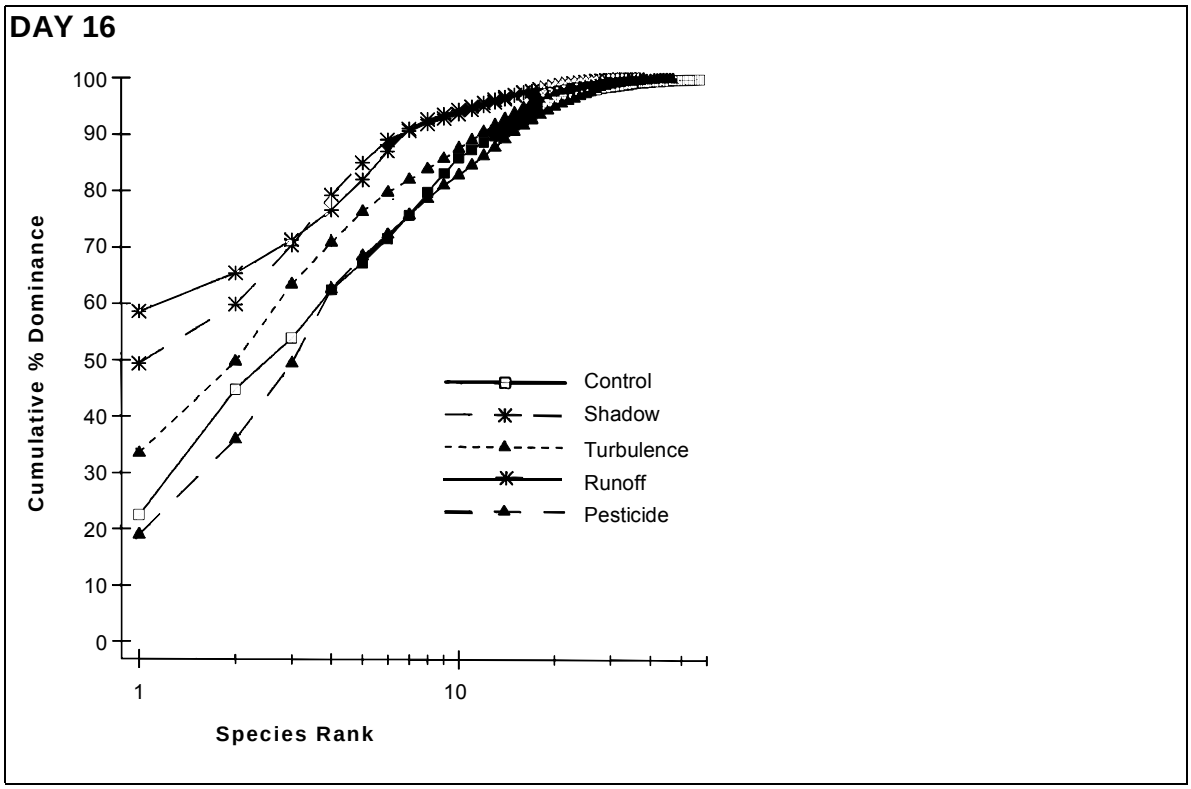


Figure 3-37. Dominance plots phytoplankton day -24, 16 and 80.



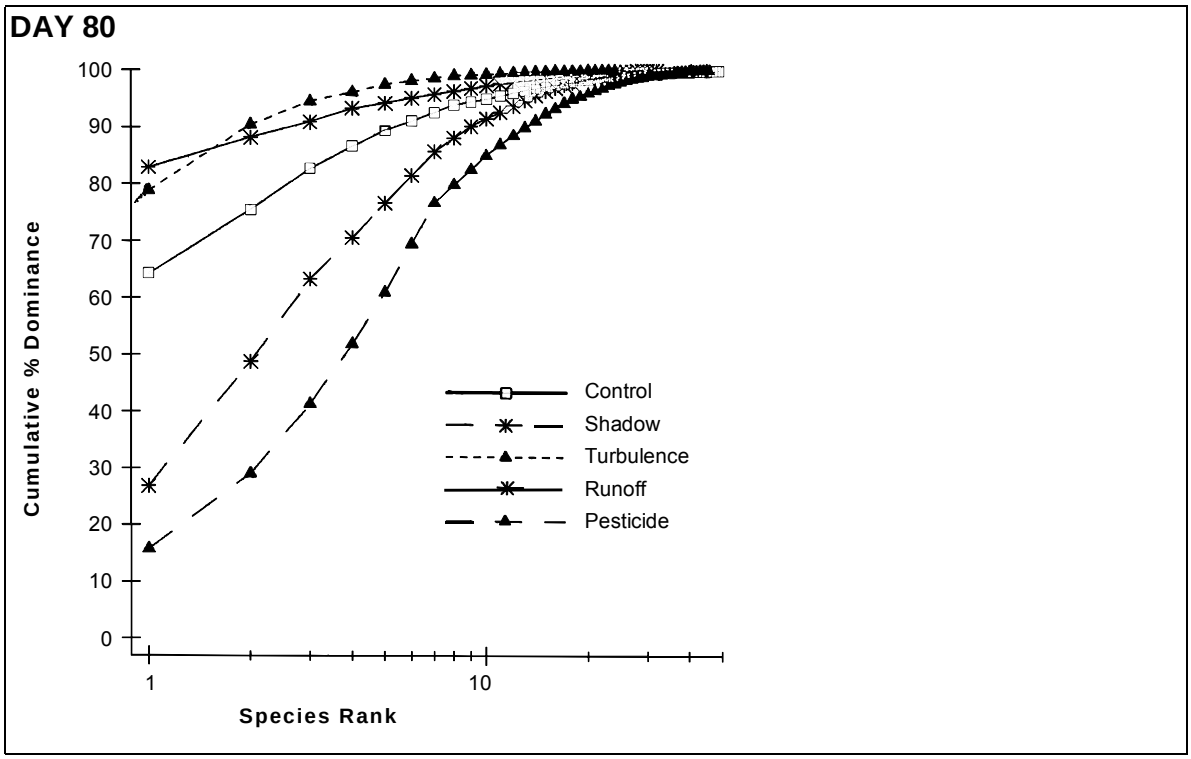


Figure 3-37 (cont.): Dominance plots phytoplankton, day -24, 16 and 80.

Table 3-31. Ranking of phytoplankton taxa for all treatments on days –24, 0, 14, 28 and 101.

Day	SHADOW	TURBULENCE	RUN-OFF	DIAZINON
-24	*Kirchneriella* spec.	*Kirchneriella* spec.	*Kirchneriella* spec.	*Kirchneriella* spec.
	Oscillatoria rosea	*Chroomonas acuta*	*Chlorella vulgaris*	*C. acuta*
	C. acuta	*Nitzschia gracilis*	*C. acuta*	*Nitzschia palea*
	N. palea	*N. palea*	*N. gracilis*	*Sphaerocystis schroeteri*
0	*C. acuta*	*C. acuta*	*Anabaena variabilis*	*A. variabilis*
	Oocystis naegelii	*O. naegelii*	*C. acuta*	*O. naegelii*
	Lyngbia spec.	*Lyngbia* spec.	*O. naegelii*	*C. acuta*
	A. variabilis	*A. variabilis*	*Lyngbia* spec.	*Crucigeniella rectangularis*
14	*C. rectangularis*	*C. rectangularis*	*Oocystis parvula*	*A. variabilis*
	O. naegelii	*O. parvula*	*C. rectangularis*	*P. gelatinosa*
	C. acuta	*Planktosphaeria gelatinosa*	*C. acuta*	*Monoraphidium circinale*
	Lyngbia spec.	*O. naegelii*	*A. variabilis*	*Gomphonema lacustris*
28	*C. acuta*	*O. parvula*	*O. parvula*	*M. circinale*
	O. rosea	*M. circinale*	*C. acuta*	*A. variabilis*
	Lyngbia spec.	*P. gelatinosa*	*A. variabilis*	*P. gelatinosa*
	O. parvula	*M. minutum*	*Lyngbia* spec.	*G. lacustris*
101	*C. acuta*	*Cryptomonas erosa/ovata*	*C. erosa/ovata*	*M. minutum*
	C. erosa/ovata	*Oscillatoria tenuis*	*Volvox aureus*	*Scenedesmus tenuispina*
	O. tenuis	*C. acuta*	*C. acuta*	*N. palea*
	Cocconeis placentula	*N. palea*	*O. tenuis*	*Monoraphidium contortum*

Furthermore, mean densities were calculated for each treatment group and the control for the most dominant species (Figure 3-38). Standard deviations for the control group were already displayed in Figure 3-32. When compared to the control, most obvious differences for dominant species were found for the treatment groups DIAZINON (*Chroomonas acuta, Anabaena variabilis, Oocystis naegelii, Cryptomonas erosa/ovata, Chlamydomonas* spec.) and TURBULENCE (*Chroomonas acuta, Volxox aureus, Cryptomonas erosa/ovata*). For the RUNOFF and SHADOW treatment, obvious differences to the control group were observed for *Volvox aureus* and *Crucigeniella rectangularis*, respectively.

However, due to the high phytoplankton diversity, differences between groups are difficult to explain using this way of graphical presentation. A set of at least 30 taxa would be needed to cover the most important differences between each pair of treatment group and the control, respectively. Therefore, the contribution of individual taxa to differences between groups will be shown in the following chapters using univariate and multivariate methods.

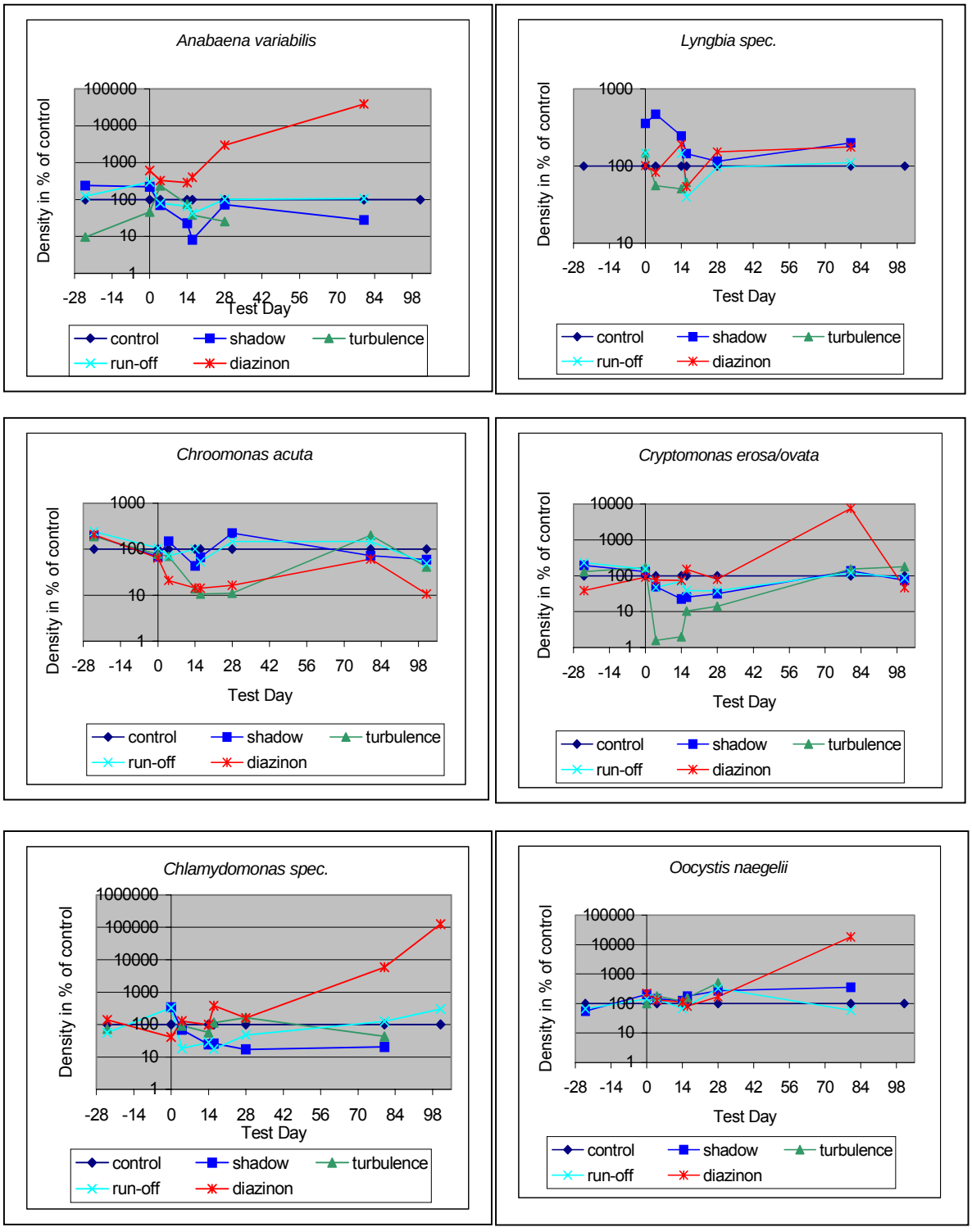


Figure 3-38. Mean densities for abundant phytoplankton taxa in the treatments SHADOW, TURBULENCE, RUN-OFF, DIAZINON and the control.

3.7.3 Univariate Testing for Effects on Phytoplankton Taxa

The Dunnett´s test was used to compare densities of all taxa in the treatment groups to those in the control. Table 3-32 lists all taxa, which deviated significantly from the control at least once during the study period for one or several treatment groups. Shaded areas indicate the presence of significant differences to the control of a treatment on two or more consequent sampling days.

During the pre-exposure phase (day –24 and day 0), the densities for few taxa were significantly higher in the treatment groups TURBULENCE, SHADOW and DIAZINON, respectively, when compared to the control *(Cocconeis placentula, Chlorophysema* spec.*, Monoraphidium contortum, Oocystis naegelii, Oocystis parva, Sphaerosystis schroeteri*. However, none of these taxa showed densities significantly different from the control during the exposure or post-exposure phase. During the exposure and post-exposure phase many taxa regularly occurred at higher densities in the treatments when compared to the control. When comparing the TURBULENCE treatment to the control, significantly higher numbers were found on two or more consequent sampling days for the taxa *Nitzschia palea, Monoraphidium circinale, Oocystis parva, Planktosphaeria gelatinosa, Scenedesmus aculeolatus* and *Tetraedron minimum*. The species *Chroomonas acuta* and *Crypromonas erosa/ovata* occurred at lower densities in the TURBULENCE treatment when compared to the control. Note that all significant deviations were found for the exposure phase, none for the post-exposure phase.

The treatment DIAZINON consistently affected densities for the following taxa: *Nitzschia gracilis, Nitzschia palea, Monoraphidium circinale, Monoraphidium komarkovae, Oocystis parva, Paulschulzia pseudovolvox, Planktosphaeria gelatinosa, Tetraedron minimum, Gomphosphaeria lacustris, Lyngbia limnetica* and *Peridinium* spec. (higher densities in the treatment), and *Chroomonas acuta* (lower densities in the treatment). Note that all significant deviations were found for the exposure and post-exposure phase, except for the taxa *Gomphosphaeria lacustris* and *Lyngbia limnetica.* For the treatment groups SHADOW and RUNOFF, no consistent differences to the control group could be shown for data analyzed at the species level during exposure and post-exposure phase.

Table 3-32. Significant differences between phytoplankton population densities between control and treatment groups (Dunnett´s test, p<0.05).

		day						
		-24	0	4	14	16	28	80
Bacillariophyceae								
Achnanthes	*minutissima*	o	o	T+	o	o	o	o
Cocconeis	*placentula*	T+	o	o	o	o	o	o
Navicula	*radiosa*	o	o	T+	o	o	o	o
Nitzschia	*gracilis*	o	o	D+, T+	D+	D+	D+	D+
Nitzschia	*palea*	o	o	D+	D+, T+	D+, T+	D+, T+	D+

Note: Density data were log-transformed before applying the Dunnett´s test.
Legend: values in the treatment groups, i.e. Shadow (S), Turbulence (T), Runoff (R) and Diazinon (D), being significantly higher (+) or lower (-) compared to the control. Shaded areas indicate the presence of significant differences to the control of a treatment on two or more consequent sampling days. Table continues on next page.

Table 3-32 (cont.). Significant differences between phytoplankton population densities between control and treatment groups (Dunnett´s test, p<0.05).

		day						
		-24	0	4	14	16	28	80
Chlorophyceae								
Ankyra	*ancosa*	o	o	T+	o	o	o	o
Ankyra	*judayi*	o	o	o	o	o	T-	o
Binuclearia	spec.	o	o	D+	o	o	o	o
Chlamydomonas	spec.	o	o	o	o	o	o	D+
Chlorophysema	spec.	T+	o	o	o	o	o	o
Choriocystis	*cf.minos*	o	o	o	T+	o	o	o
Kirchneriella	spec.	o	o	o	o	T+, D+	o	o
Monoraphidium	*arcuatum*	o	o	o	D+	o	o	o
Monoraphidium	*circinale*	o	o	D+	D+, T+	D+, T+	D+, T+	D+
Monoraphidium	*contortum*	S+	o	o	o	o	o	D+
Monoraphidium	*komarkovae*	o	o	D+	D+	D+	o	D+
Monoraphidium	*minutum*	o	o	T+	o	o	T+	o
Oocystis	*naegelii*	o	S+, D+	o	o	o	o	o
Oocystis	*parva*	o	T+	o	T+	T+	T+, D+	o D+
Paulschulzia	*pseudovolvox*	o	o	D+	o	D+	D+	D+
Crucigeniella	*rectangularis*	o	o	o	o	S+	o	o
Pediastrum	*tetras*	o	o	T+	o	o	T+	o
Phacotus	*lendneri*	o	o	T+	o	o	o	o
Planktosphaeria	*gelatinosa*	o	o	o	T+, D+	T+, D+	T+, D+	D+
Pseudoquadrigula	spec.	o	o	o	o	T+	o	o
Scenedesmus	*aculeolatus*	o	o	o	D+	T+, D+	T+	o
Scenedesmus	*ecornis*	o	o	R+	o	o	o	o
Scenedesmus	*longispina*	o	o	o	o	o	o	D+
Scenedesmus	*sempervirens*	o	o	o	o	o	o	D+
Scenedesmus	*tenuispina*	o	o	o	T+	o	T+	D+
Sphaerellopsis	*fluviatilis*	o	o	D+	o	o	o	D+
Sphaerocystis	*schroeteri*	D+	o	o	o	o	o	o
Sykidion	*praecipitans*	o	o	o	o	o	o	D+
Tetraedron	*caudatum*	o	o	o	o	o	D+	o
Tetraedron	*minimum*	o	o	D+	T+, D+	T+, D+	T+, D+	D+
Tetraedron	*trigonum*	o	o	o	o	o	o	T+
Treubaria	*schmidlei*	o	o	o	o	o	o	D+
Chrysophyceae								
Chromulina	*minuta*	o	o	T+	o	o	o	D-
Mallomonas	*radiata*	o	o	o	o	D+	o	T+
Ochromonas	*variabilis*	o	o	o	D+	o	o	o

Note: Density data were log-transformed before applying the Dunnett´s test.
Legend: values in the treatment groups, i.e. Shadow (S), Turbulence (T), Runoff (R) and Diazinon (D), being significantly higher (+) or lower (-) compared to the control. Shaded areas indicate the presence of significant differences to the control of a treatment on two or more consequent sampling days. Table continues on next page.

Table 3-32 (cont.). Significant differences between phytoplankton population densities between control and treatment groups (Dunnett´s test, p<0.05).

		day						
		-24	0	4	14	16	28	80
Conjugatophyceae								
Cosmarium	*praemorsum*	o	o	S+	o	o	o	o
Cryptophyceae								
Chroomonas	*acuta*	o	o	o	T- D-	T-, D-	D-	D-
Cryptomonas	*erosa/ovata*	o	o	T-	o	T-	T-	D+
Oochromonas	*sphagnalis*	o	o	T+	o	o	o	o
Cyanophyceae								
Anabaena	*planctonica*	o	o	o	D+	o	o	o
Anabaena	*variabilis*	o	o	o	o	S-	D+	o
Chroococcus	*minutus*	o	o	o	o	T+, D+	o	o
Dictyosphaerium	spec.	o	o	o	o	o	o	D+
Gloeotrichia	spec.	o	o	o	o	o	o	T+
Gomphosphaeria	*lacustris*	o	o	D+	D+	D+	o	o
Lyngbia	*limnetica*	o	o	o	D+	D+	o	o
Lyngbia	spec.	o	o	o	o	o	T-	o
Oscillatoria	*tenuis*	o	o	R+	o	o	o	o
Dinophyceae								
Gymnodinium	spec.	o	o	D+	o	o	D+	o
Peridinium	spec.	o	o	o	D+	D+	D+	D+

Note: Density data were log-transformed before applying the Dunnett´s test.
Legend: values in the treatment groups, i.e. Shadow (S), Turbulence (T), Runoff (R) and Diazinon (D), being significantly higher (+) or lower (-) compared to the control. Shaded areas indicate the presence of significant differences to the control of a treatment on two or more consequent sampling days.

3.7.4 Cluster Analysis for Phytoplankton Communities

Hierarchical clustering with group-average linking based on Bray-Curtis similarity matrices were performed for the phytoplankton communities including all replicates of the four treatments and the control. Phytoplankton densities (Y) were log-transformed (Y´= log (1Y+1)) before analysis, thereby giving more weight to rare species and less weight to abundant species. Dendrograms for the hierarchical clustering are shown in Figure 3-39 for 4, 3, 4, 4 and 12 replicate samples of the treatment groups SHADOW, TURBULENCE, RUNOFF, DIAZINON and the controls, respectively. Note that the pre-treatment replicate samples were named according to the treatments they were assigned to in the exposure phase, although on day-24 and day 0, all 27 tanks remained untreated. Examples of dendrograms were shown for for the pre-treatment, exposure and post-exposure phase, i.e. day 0, day 16 and day 101 (Figure 3-39). Numbers given in the plots indicate the microcosm numbers.

Analyses of pre-treatment samples showed that Bray-Curtis similarities were in general greater than 60%. The dendrograms for the pre-treatment samples show that treatment replicates were not grouped into discrete clusters but were randomly distributed in different clusters, thus indicating that the treatments were assigned randomly to the 27 tanks (in spite of the restrictions described in the Materials and Methods chapter).

During the exposure phase, phytoplankton in the treatment groups DIAZINON (day 14 to 28) and TURBULENCE (day 4 to day 28) formed discrete clusters. Most obviously, clustering was found for the DIAZINON replicates on day day 16, where a discrete cluster was formed at Bray-Curtis similarities of 65%. The treatments SHADOW and RUNOFF did not show distinguishable patterns throughout the exposure period.

Similarities decreased during the study period from initial Bray-Curtis similarities of greater than 60% to general similarities of 40% (day 101, Figure 3-39). The treatment group DIAZINON forms a very discrete cluster on day 101 when it is related to the rest of the microcosms on a similarity level of only 20%. This clearly indicates that the phytoplankton community in the DIAZINON treated group has developed differently when compared to the control. Replicates of the treatments SHADOW, TURBULENCE and RUNOFF and the control did not show distinguishable patterns throughout the post-exposure period.

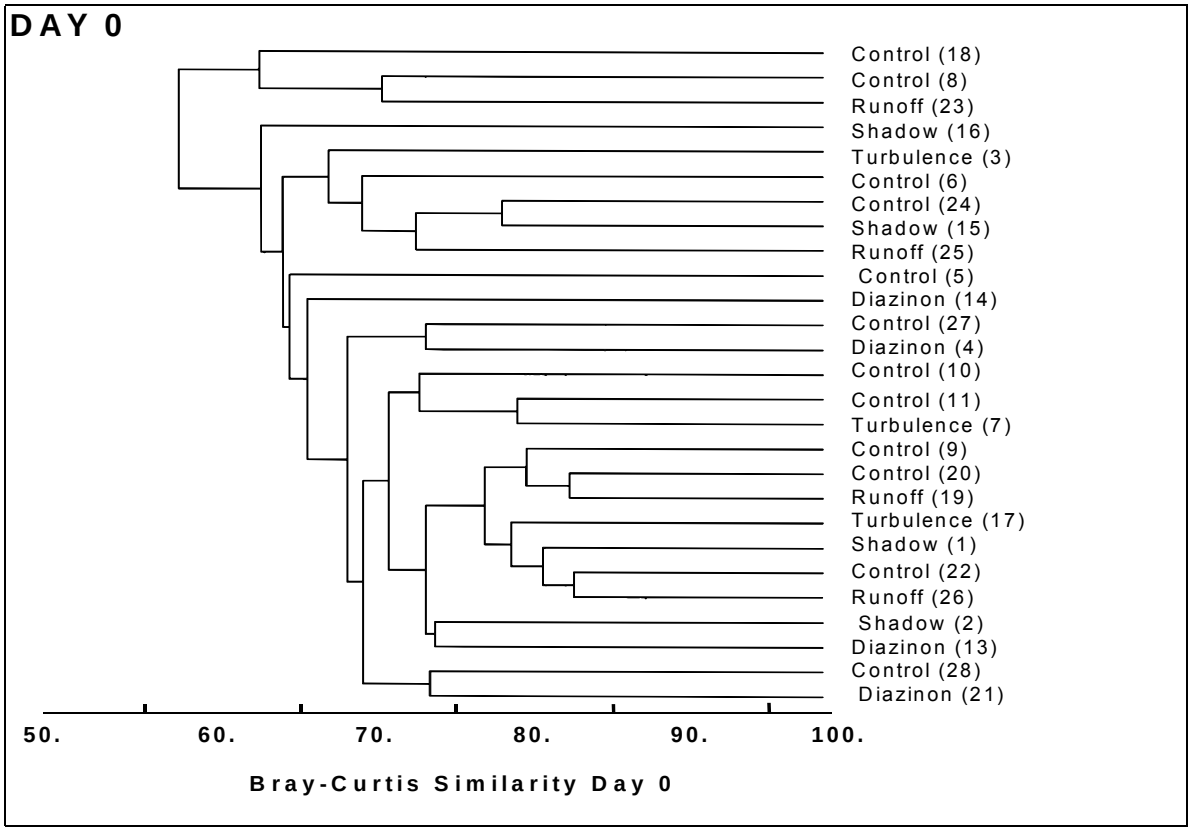
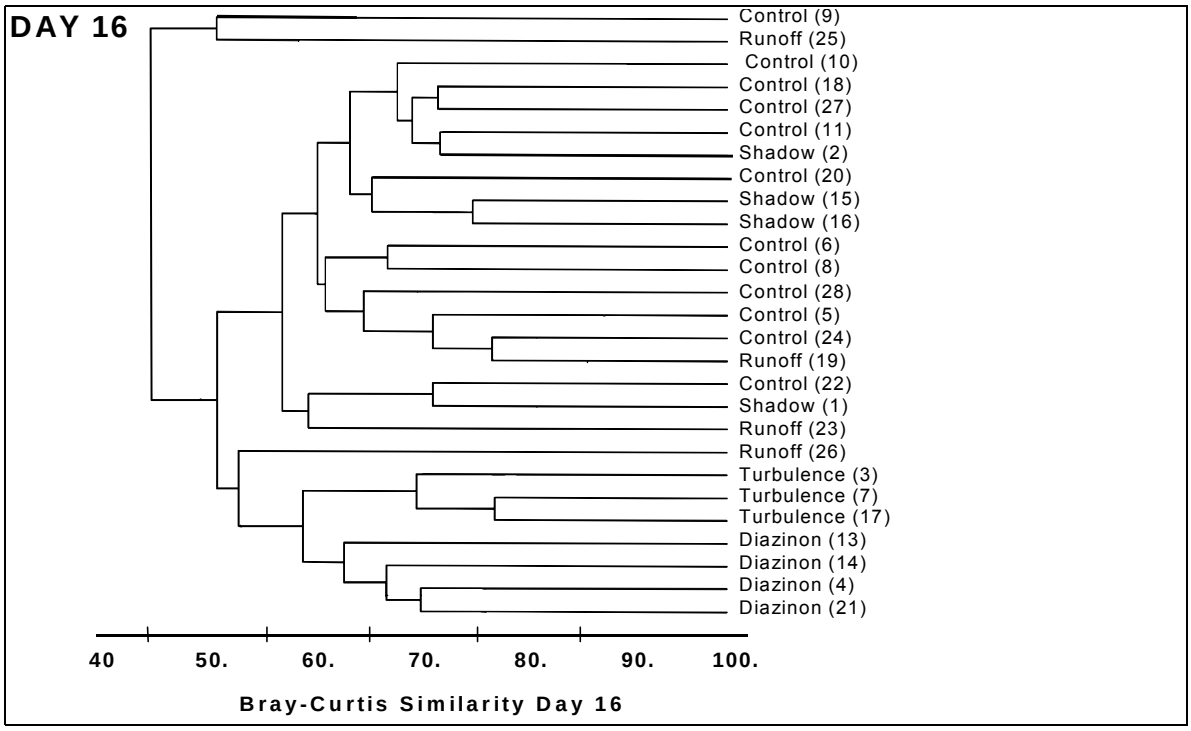


Figure 3-39. Dendrograms for hierarchical clustering phytoplankton communities in the control and treatment groups for day 0, 16 and 101.



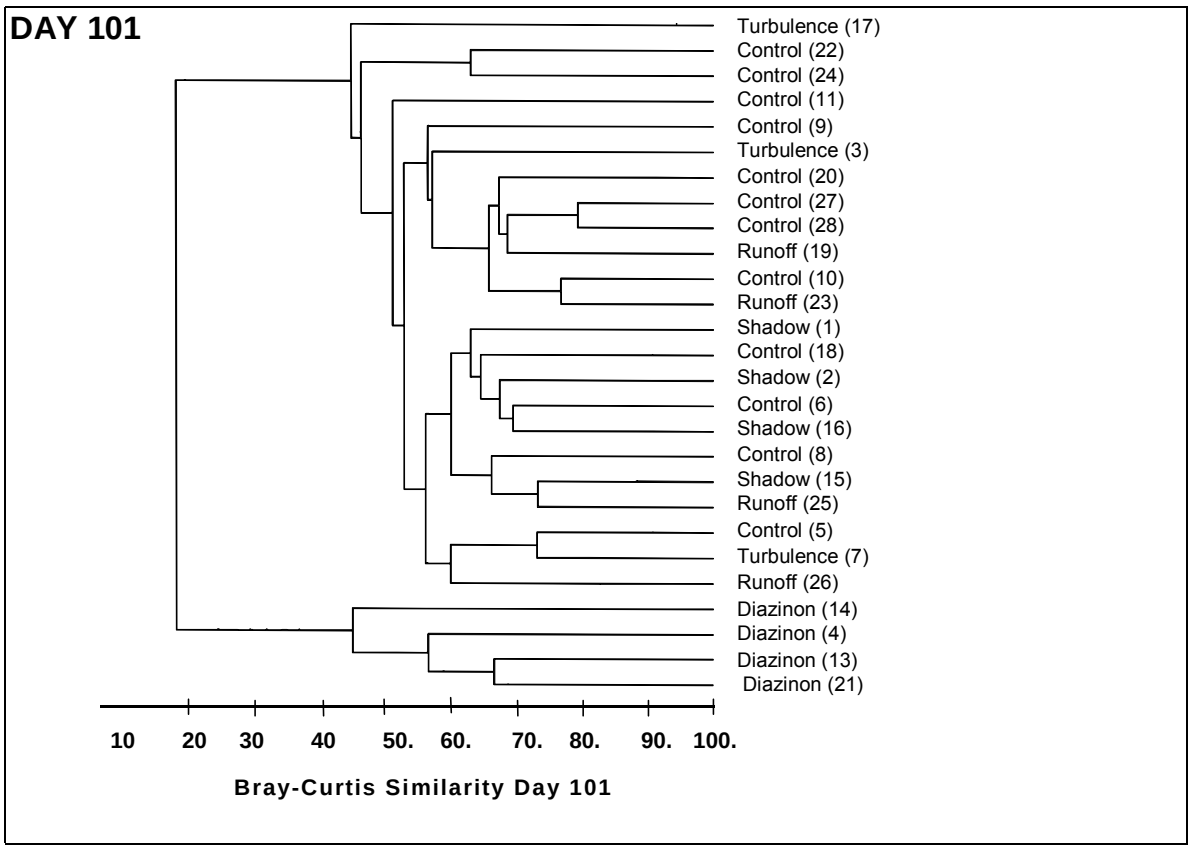


Figure 3-39 (cont.). Dendrograms for hierarchical clustering phytoplankton communities in the control and treatment groups for day 0, 16 and 101.

3.7.5 Multi Dimensional Scaling for Phytoplankton Communities

Multi Dimensional Scaling (MDS) was performed for phytoplankton densities, based on a Bray-Curtis similarity matrix on log-transformed data. With MDS, a "map" of the samples is constructed which attempts to satisfy all the conditions imposed by the rank similarity matrix. Examples of MDS ordinations for the pre-treatment, exposure and post-exposure phase, i.e. for day –24, day 16 and day 101, are given in Figure 3-40. Note that the pre-treatment replicate samples were named according to the treatments they were assigned to in the exposure phase, although on day-24 and day 0, all 27 tanks remained untreated.

For pre-treatment samples (day –24 and day 0) no distinct formation of groups or gradation across the set of replicates could be seen from the MDS ordination.
During the exposure phase (day 4 to day 28), the replicates treated with DIAZINON formed discrete groups in the ordination diagram. Most obviously, grouping was found for all four of the DIAZINON replicates on days 14, 16 and 28. When superimposing clusters from Figure 3-39, distinctive groups were found for DIAZINON treated replicates throughout the exposure period. The response for the treatment TURBULENCE was clearly to be seen from MDS ordinations for days 14, 16 and 28. The replicates of the treatments SHADOW and RUNOFF appeared to be randomly distributed over the remaining groups (also containing control replicates) throughout the exposure period.
On days 80 and 101, the four DIAZINON replicates still formed a discrete group. Group formation was even more obvious than during the exposure phase, as already observed with Cluster-analysis. Replicates of the treatments SHADOW, TURBULENCE and RUNOFF and the control appeared to be randomly distributed over the remaining groups throughout the post-exposure period.

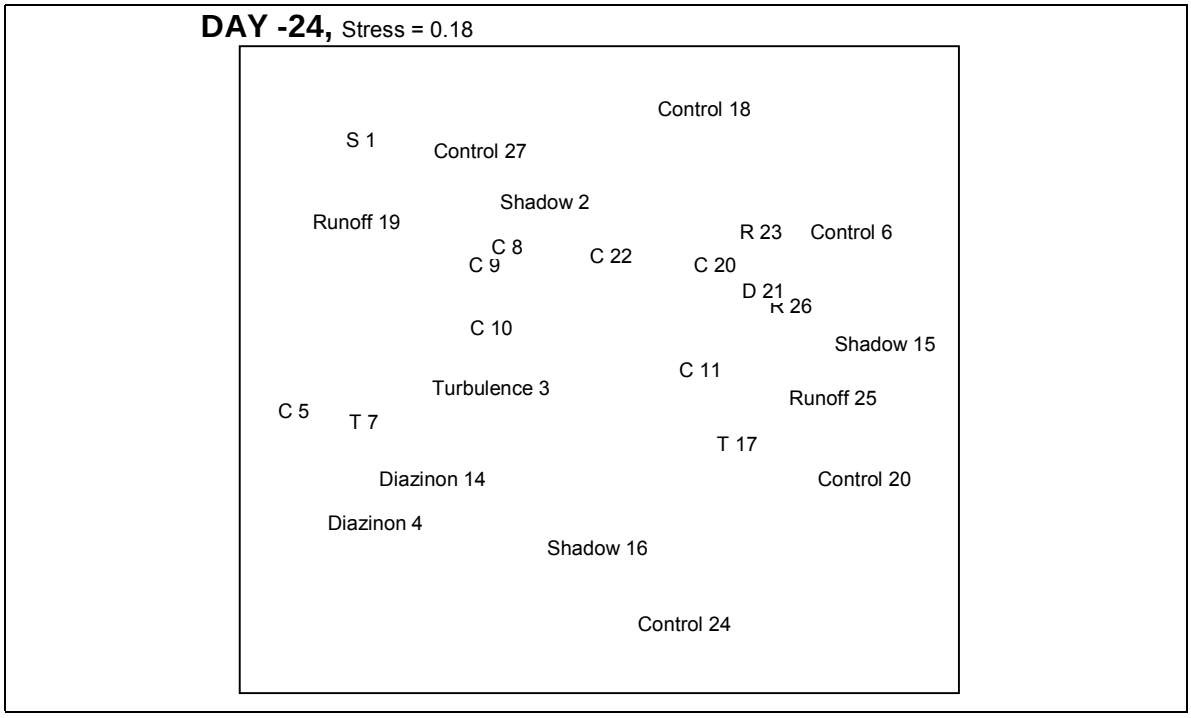
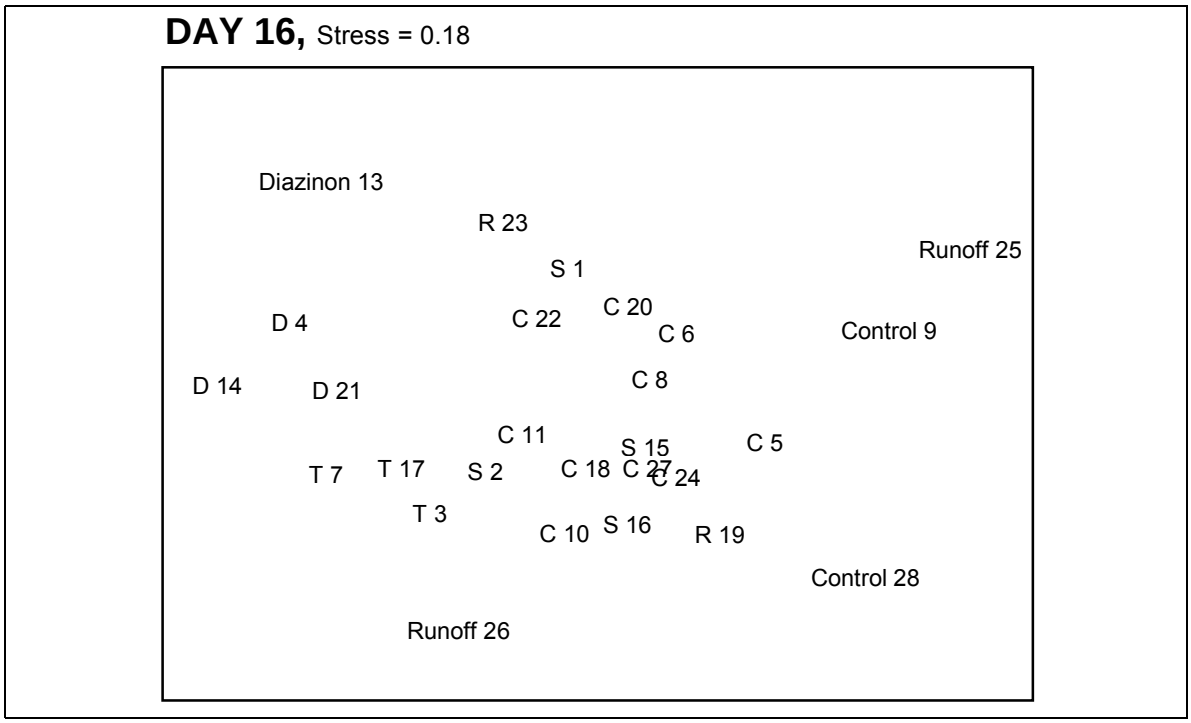


Figure 3-40. Multi Dimensional Scaling for phytoplankton communities in the control and treatment groups for day –24, 16 and 101.



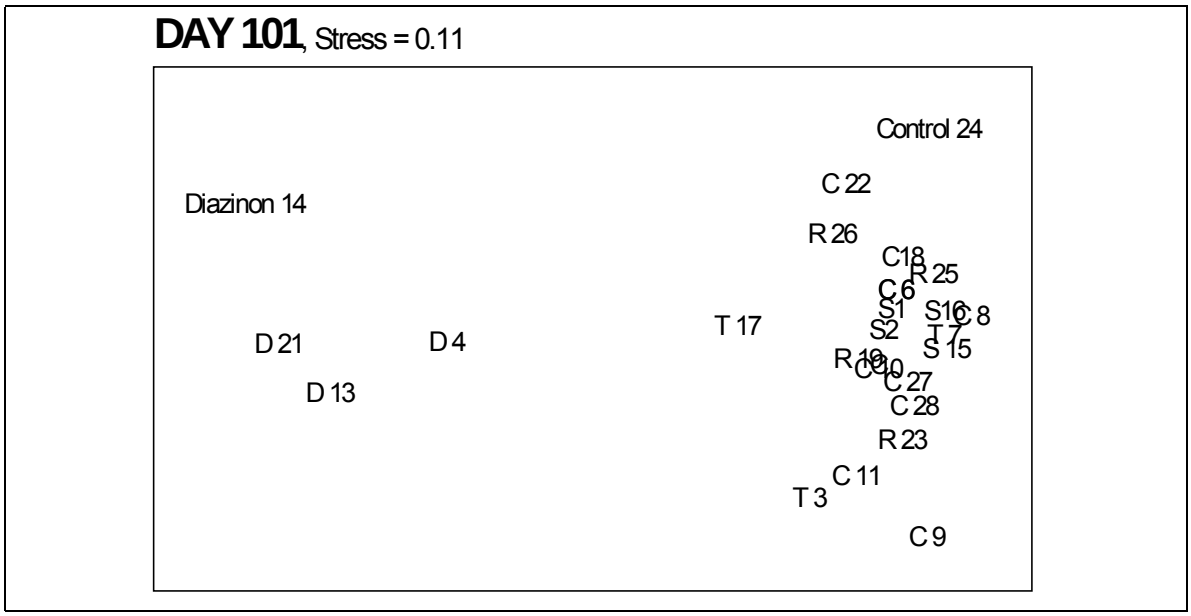


Figure 3-40 (cont.). Multi Dimensional Scaling for phytoplankton communities in the control and treatment groups for day –24, 16 and 101.

3.7.6 Analysis of Similarities for Phytoplankton Communities

Results of the Analysis of Similarities (ANOSIM) test are shown in Table 3-33. The shaded areas indicate statistically significant R-values. Statistically significant differences were found when comparing the control group to the TURBULENCE and the DIAZINON treatment for samples taken on days 16 to 28 and 14 to 101, respectively.

For the DIAZINON treatment group, significant effects on the phytoplankton community were shown for day 4 through day 101 when compared to the treatment groups SHADOW and RUNOFF, and for days 14 to 101 when compared to the TURBULENCE treatment group. R-values for the comparisons of the DIAZINON group with the other treatment groups were close to or even equal to one for days 80 and 101. This showed that for these sampling events all replicates within the DIAZINON treatment group were more similar to each other than to any replicates from different treatment groups.

The treatment groups SHADOW and RUNOFF did not show significant differences to the control group throughout the observation period. Significant differences were observed for the comparison of SHADOW and TURBULENCE treated ponds on days 4, 28 and 101.

Table 3-33. Analysis of Similarities for phytoplankton data (R-values). Statistically significant values are shaded (Monte-Carlo permutations, p< 5%).

Analysis of Similarities for Phytoplankton (R-values and significance)

	Day-24	Day 0	Day 4	Day 14	Day 16	Day 28	Day 80	Day 101
CONTROL / SHADOW	-0.01	-0.10	-0.31	-0.13	-0.02	0.09	-0.28	-0.11
CONTROL / TURBULENCE	-0.07	-0.11	0.02	0.03	0.48	0.92	0.29	0.20
CONTROL / RUN-OFF	-0.10	-0.16	-0.12	-0.25	0.36	0.22	0.24	-0.04
CONTROL / DIAZINON	0.14	-0.01	0.26	0.34	0.81	0.65	0.96	0.99
SHADOW / DIAZINON	-0.17	-0.18	0.75	0.63	0.96	0.81	1.00	1.00
TURBULENCE / DIAZINON	-0.11	-0.3	0.44	0.59	0.63	0.98	0.85	1.00
RUN-OFF / DIAZINON	0.13	-0.12	0.67	0.63	0.66	0.58	0.57	1.00
SHADOW / TURBULENCE	-0.17	-0.17	0.78	0.48	0.74	1.00	0.33	0.63
SHADOW / RUN-OFF	-0.08	-0.20	0.20	-0.17	0.04	-0.16	0.15	0.16
TURBULENCE / RUN-OFF	0.06	-0.17	0.39	0.50	0.19	0.57	-0.06	-0.02

Note: statistically significant differences between groups are shaded.

3.7.7 Principal Response Curves for Phytoplankton Communities

Principal Response Curves (PRCs) were calculated and Monte-Carlo permutations were performed for the zooplankton community. Taxon densities were log-transformed (Y´= log (Y+1)) before analysis, thereby giving more weight to rare and less weight to abundant taxa. PRCs were calculated for the phytoplankton community for treatment groups SHADOW, TURBULENCE, RUNOFF and DIAZINON individually. By calculating the PRCs individually for each treatment, emphasis was put on the detection of differences caused by each individual treatment in comparison to the control. Each data set included the control data and either the treatment SHADOW or TURBULENCE or RUNOFF or DIAZINON.

The PRC calculations for these data sets were performed for two different time frames, i.e. the entire study period (day-24 to day 101) and for the exposure phase (day 4 to day 28). It was expected that the latter would have a positive effect on the power of the test.

PRC Diagrams

PRCs are shown in Figure 3-41. All curves were very slightly deviating from the control level during the pre-treatment phase (day –24 and day 0). Deviations from the control clearly increased for the TURBULENCE treatment group on day 4 through day 28 and for the DIAZINON treatment group on day 4 through day 101.

The Principal Response Curve for TURBULENCE reached control level on day 80, while the PRC for DIAZINON at this point in time was still significantly different to the control. For the treatments SHADOW and RUNOFF, deviations from the control increased slightly during the exposure phase (day 4, 14, 16 and 28) and decreased to control level on day 101.

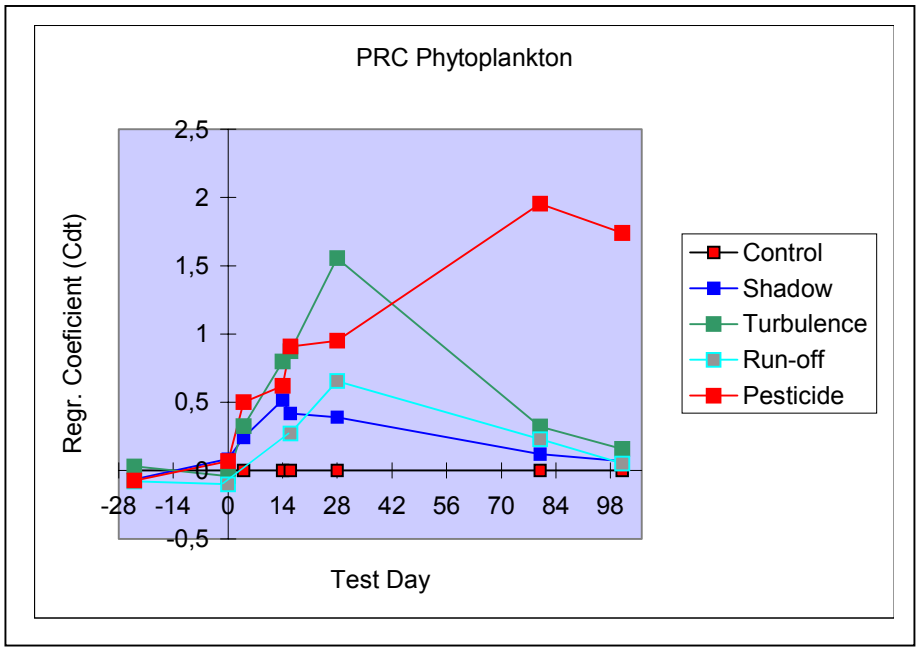


Figure 3-41. Principal Response Curves, phytoplankton.

Variance Allocation for Entire Study Period and Exposure Phase

The variance allocations for the PRCs calculated for the treatments SHADOW, TURBULENCE, RUNOFF and DIAZINON are shown in Table 3-34 and Table 3-35.

When including data from the entire study period (day –24 to day 101), 26% to 41% of the total variance in the phytoplankton data can be attributed to time. 4.5%, 12%, 3.3% and 26.0% of the variance can be attributed to the treatment regimes SHADOW, TURBULENCE, RUNOFF and DIAZINON, respectively. Thereof, 40%, 65%, 45% and 66% were captured by the corresponding PRC.

When excluding the pre-treatment (day –24 and day 0) and the post-exposure (day 80 and day 101) data, less variance was attributed to time and more to treatment. Then, 7.9 to 10% of the total variance in the phytoplankton data was attributed to time and 7.7%, 23%, 7.8% and 26% of the variance can be attributed to the treatment regimes SHADOW, TURBULENCE, RUNOFF and DIAZINON, respectively. Thereof, 58%, 74%, 51% and 78% were captured by the corresponding PRCs (Table 3-35).

Table 3-34. Variance allocation of phytoplankton, all sampling events.

Treatment group	% of variance accounted for by time	treatment regime	% of variance captured	p-value of PRC
SHADOW	41	4.5	40	0.042 **
TURBULENCE	36	12	65	0.028 **
RUNOFF	38	3.3	45	0.064
DIAZINON	26	25	66	0.001 **

Note: The percentages shown are the total variance which can be attributed to time and treatment regime, the % of variance captured by the first PRC and its significance (Monte-Carlo permutation test, significant (**) with $p<0.05$).

Table 3-35. Variance allocation for phytoplankton, exposure phase.

Treatment group	% of variance accounted for by time	treatment regime	% of variance captured	p-value of PRC
SHADOW	9.3	7.7	58	0.034 **
TURBULENCE	8.4	23	74	0.003 **
RUNOFF	10	7.8	51	0.087
DIAZINON	7.9	26	78	0.001 **

Note: The percentages shown are the total variance which can be attributed to time and treatment regime, the % of variance captured by the first PRC and its significance (Monte-Carlo permutation test, significant (**) with $p<0.05$).

Significance

The Monte-Carlo permutation tests for both time frames (day-24 to day 101 and day 4 to day 28) indicated that the PRCs for SHADOW, TURBULENCE and DIAZINON displayed significant information. The PRC for RUNOFF was not significant (Table 3-34, Table 3-35).

Detailed information about the significance of treatment effects was gained by performing the Monte-Carlo permutation tests individually for each sampling date (Table 3-36). This analysis showed statistically significant deviation of the TURBULENCE treatment from the control for day 4 through day 28 ($p<0.05$). Further, the Phytoplankton community of the DIAZINON treated tanks was shown to be significantly different from to the control on day 4 through day 101, i.e. throughout the exposure and post-exposure phase.

The treatments RUNOFF and SHADOW did not show any significant deviations from the control throughout the study period (Table 3-36).

Table 3-36. Results of significance tests for phytoplankton, species level.

	Phytoplankton							
	d -24	d 0	d 4	d 14	d 16	d 28	d 80	d 101
SHADOW	0.74	0.46	0.40	0.13	0.10	0.16	0.52	0.31
TURBULENCE	0.84	0.99	0.02	0.006	0.003	0.003	0.12	0.33
RUNOFF	0.77	0.80	0.43	0.32	0.18	0.13	0.47	0.75
DIAZINON	0.30	0.48	0.001	0.001	0.001	0.001	0.001	0.001

Note: Monte-Carlo permutations were performed for each single sampling event, on the basis of the PRCs calculated individually for each treatment.
Legend: significant with $p<0.05$ (shaded).

Affinity of Taxa to PRCs

Species weights in Table 3-37 show the affinity of single phytoplankton species to the PRC, calculated for the exposure phase. Species weights for the TURBULENCE treatment revealed a strong positive correlation for *Monoraphidium circinale, Oocystis parva, Planktosphaeria gelatinosa, Nitzschia palea* and *Monoraphidium minutum*, indicating that these species occurred at higher densities than in the control. The species *Oscillatoria rosea, Anabaena variabilis, Lyngbia spp., Chroomonas acuta* and *Cryptomonas erosa/ovata* were negatively correlated to the PRC, indicating that they occurred at lower densities in the TURBULENCE treatment when compared to the control.

Species weights for the DIAZINON treatment revealed a strong positive correlation for *M. circinale, Gomphonema lacustris, Tetraedron minutum, P. gelatinosa* and *Paulschulzia pseudovolvox*. The species *C. acuta, Chromulina minuta* and *Volvox aureus* were negatively correlated to the PRC, indicating that they occurred at lower densities in the DIAZINON treatment when compared to the control.

Differences between the SHADOW treatment and the control were mainly due to *Crucigeniella rectangularis and Lyngbia spp.* (increased numbers) and to *Cryptomonas erosa/ovata* and *Anabaena variabilis* (decreased numbers). It is important to note that the percent variance, which could be related to the SHADOW treatment regime was very low and that Monte-Carlo tests did not indicate significant deviations for the SHADOW treatment: Therefore the analysis of species weights should be interpreted with reservation and respective data are not listed in Table 3-37. As there were clearly no indications for significance of the PRC for the treatment RUNOFF, species weights for the RUNOFF were not mentioned in Table 3-37.

Table 3-37. Phytoplankton, species weights for the treatments SHADOW, TURBULENCE and DIAZINON.

TURBULENCE		DIAZINON	
Taxon	Weight	Taxon	Weight
Monoraphidium circinale	3.7	*Monoraphidium circinale*	4.0
Oocystis parva	3.3	*Gomphonema lacustris*	3.4
Planktosphaeria gelatinosa	2.9	*Tetraedron minutum*	3.3
Nitzschia palea	2.9	*Planktosphaeria gelatinosa*	2.5
Monoraphidioum minutum	2.6	*Paulschulzia pseudovolvox*	2.4
Tetraedron minimum	2.5	*Nitzschia palea*	2.3
Ssenedesmus aculeolatus	2.3	*Peridinium* spec.	2.1
Scenedesmus tenuispina	2.3	*Lyngbia limnetica*	2.0
Lyngbia spec.	-1.3	*Scenedesmus aculeolatus*	2.0
Chroomonas acuta	-2.1	*Anabaena variabilis*	2.0
Cryptomonas erosa/ovata	-2.1	*Ankyra ancosa*	-1.1
		Chromulina minuta	-1.1
		Chroomonas acuta	-1.4

Note: Only the species with a weight of $\geq$2.0 or <-1.0 with the diagrams are displayed. The species weights show the affinity of single species/taxon to the PRC: the higher the species weight the more pronounced the actual response pattern of this species is likely to follow the PRC pattern.

3.7.8 Phytoplankton Data Analysis on different taxonomic levels

3.7.8.1 Percent contribution of Phytoplankton orders and major species to total density

The contribution in percent of major classes to the total phytoplankton density counted in each treatment group is presented in Figure 3-42.

Chlorophyceae played a dominant role in the control and all treatment groups during the exposure phase (day 4 to day 28). During the post-exposure phase, Chlorophyceae clearly dominated the treatment groups TURBULENCE, DIAZINON and RUNOFF. Chlorophyceae played a minor role in the SHADOW treatment group during the post-exposure phase.

Cryptophyceae were the most dominant class in the SHADOW treatment group on days 28, 80 and 101 contributing with up to 80% to the total phytoplankton density in these ponds. This treatment group thus showed a clearly distinct pattern when compared to the control during the end of the exposure and all the post exposure phase. Cryptophyceae contributed with about 15% to the total phytoplankton density in the control throughout the exposure and post-exposure phase. In all other treatment groups Cryptophyceae played a minor role throughout the study period.

Cyanophyceae dominated the DIAZINON treated ponds during the pre-exposure and exposure phase (day 0 to day 28), contributing with up to 60% to the total phytoplankton density. This class was also represented in the control group and the SHADOW treated group throughout the study period, however rarely exceeding 30% of the total density. Cyanophyceae played a minor role in the TURBULENCE treatment.

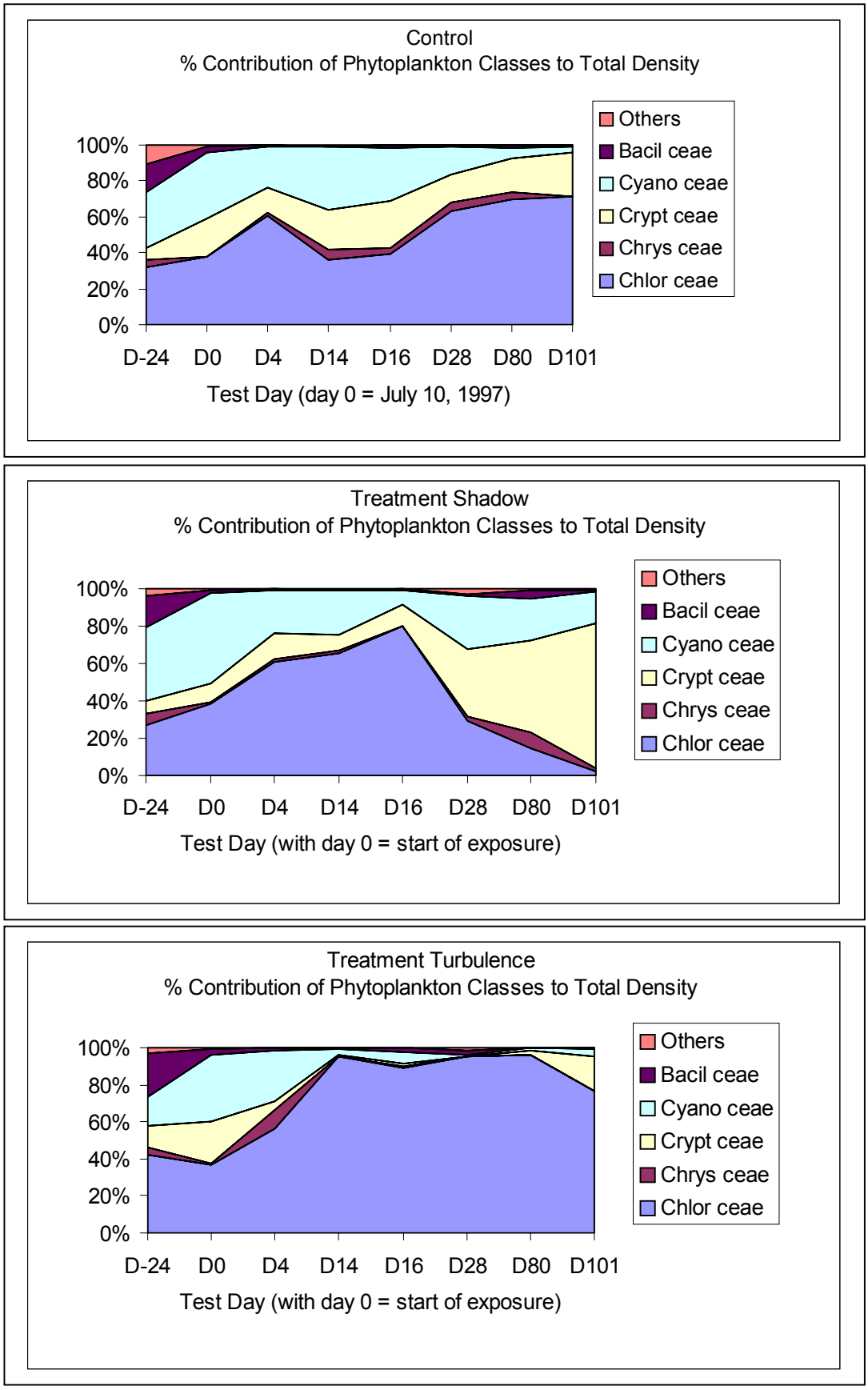
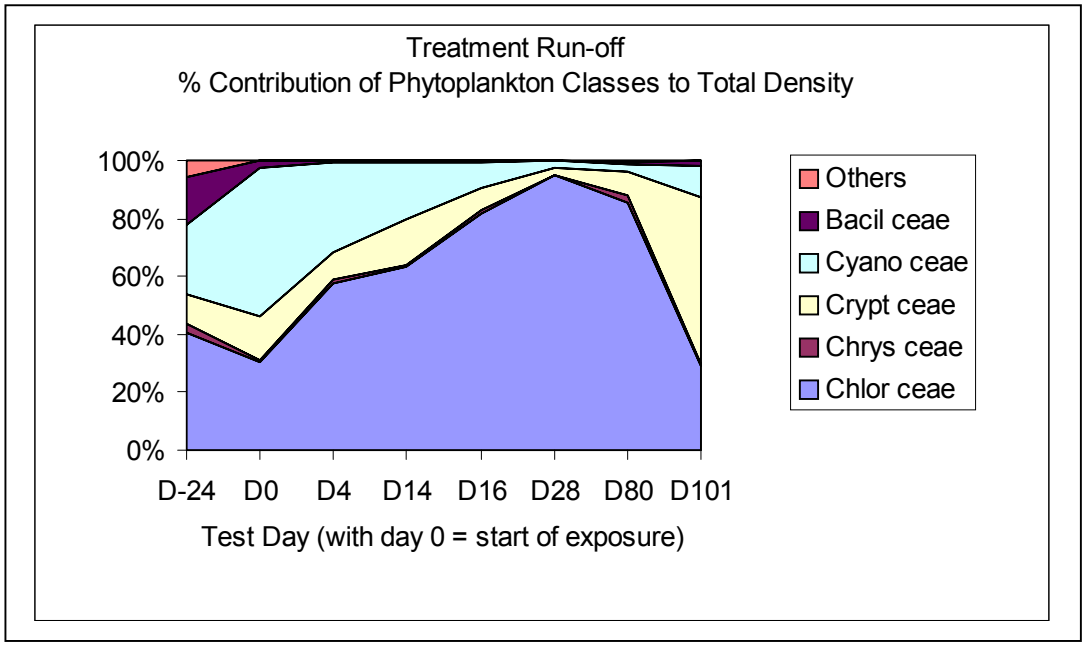


Figure 3-42. Contribution of major classes to total phytoplankton density in the control and the treatments.



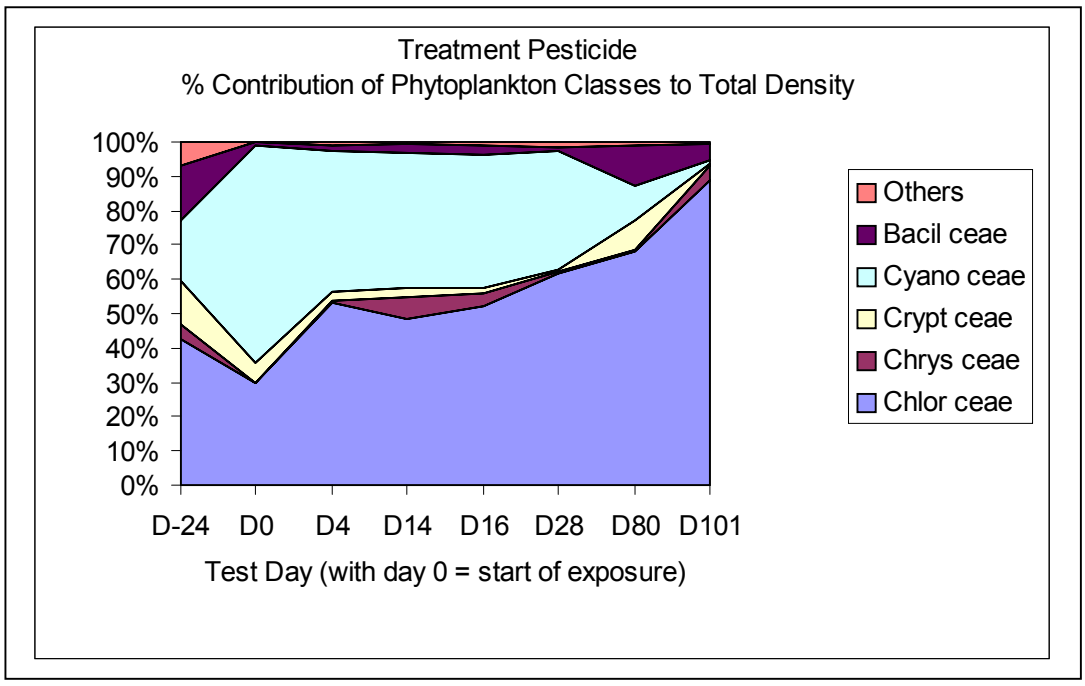
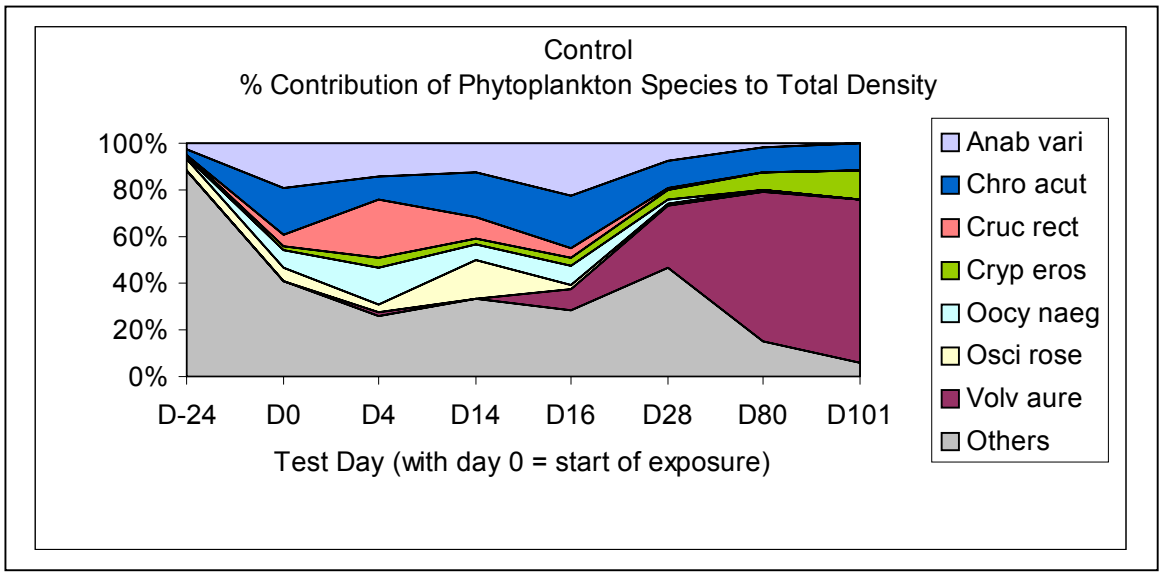


Figure 3-42 (cont.). Contribution of major classes to total phytoplankton density in the control and the treatments.

Legend Fig. 3-42: Bacillariophyceae (Bacil ceae), Cyanophyceae (Cyano ceae), Cryptophyceae (Crypt ceae), Chrysophyceae (Chrys ceae), Chlorophyceae (Chlor ceae).

The contribution in percent of major species to the total phytoplankton density counted in each treatment group is presented in Figure 3-43. *Crucigeniella rectangularis* and *Oocystis naegelii* contributed significantly to the total phytoplankton density during the exposure phase (day 4 to day 28) in the control and in all treatment groups. *Chroomonas acuta* is contributing with consistently high numbers to the total density in the control and the treatment groups SHADOW and RUNOFF throughout the study period.

Anabaena variablilis played a major role in DIAZINON treated ponds and was represented to a limited extent also in all other groups. Very clearly, *Volvox aureus* became the dominant species in the control and TURBULENCE group during the post exposure phase, while the density of this species remained negligible in all other treatment groups. The contribution of species not mentioned individually in this figure ("others"), indicates a clear shift in community composition for the TURBULENCE and DIAZINON treated groups when compared to the control.



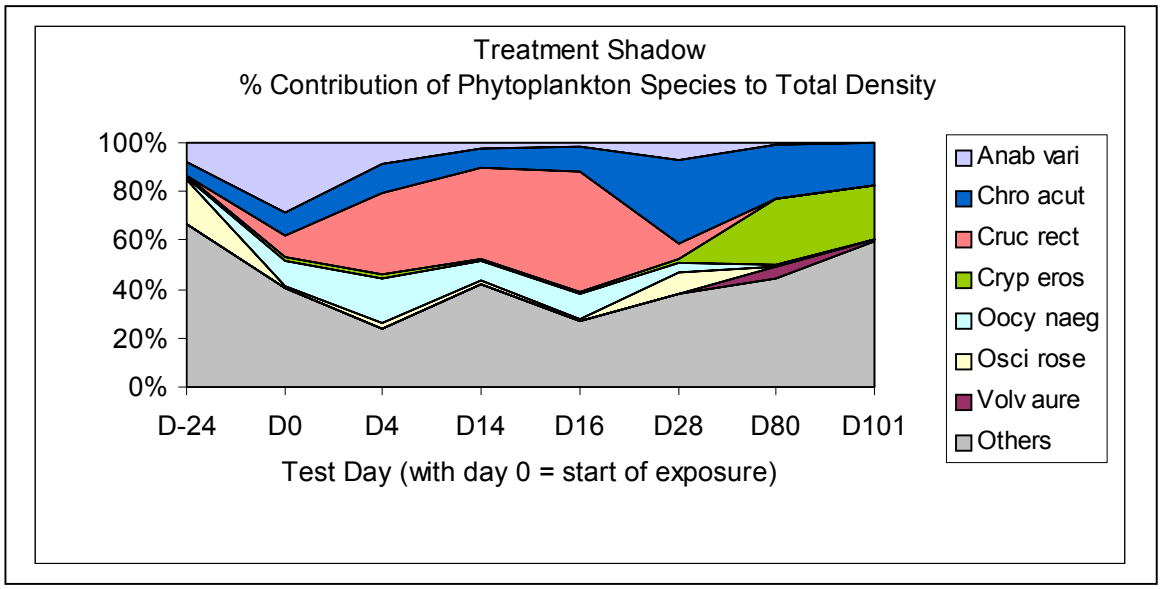
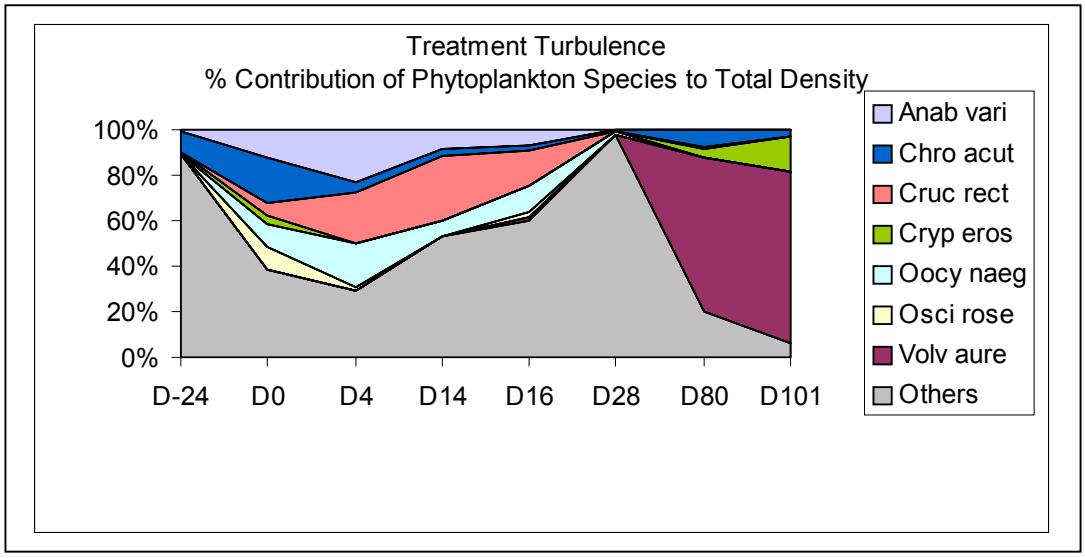
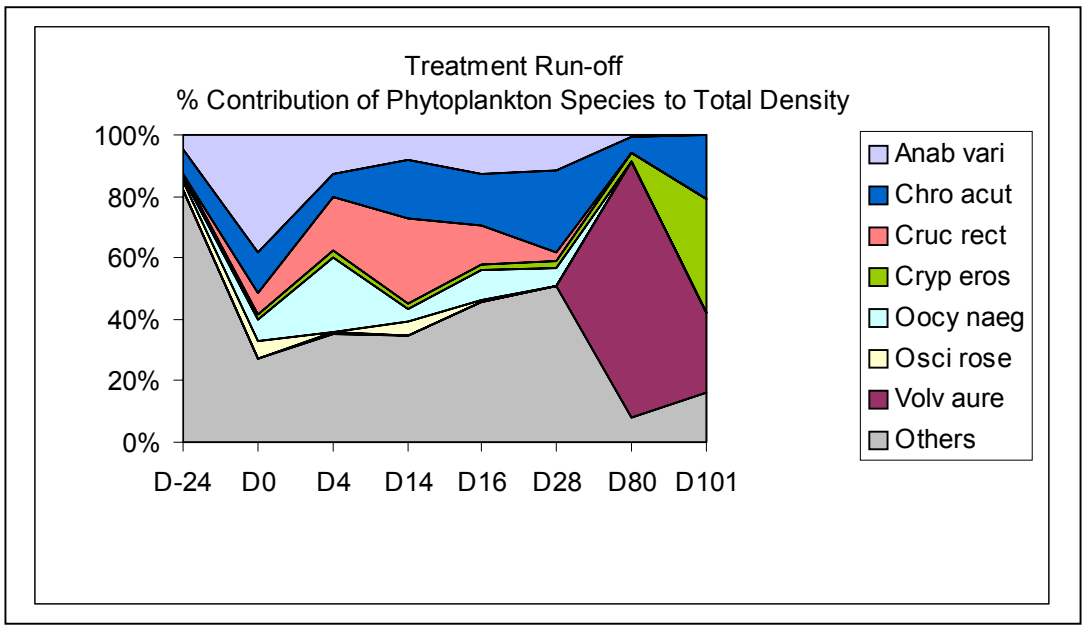


Figure 3-43. Contribution of major species to total phytoplankton density in the control and the treatments.





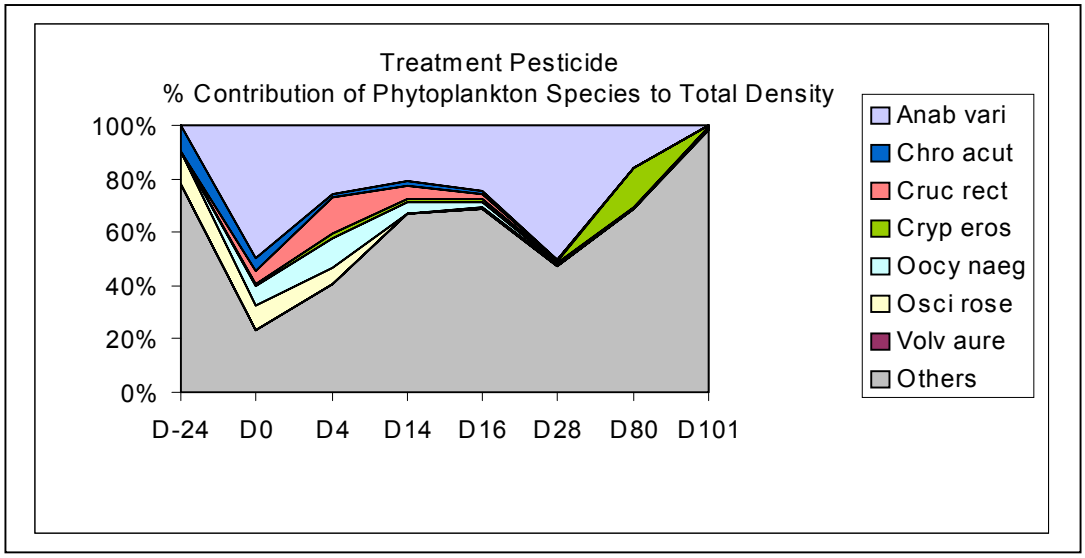


Figure 3-43 (cont.). Contribution of major species to total phytoplankton density in the control and the treatments.

3.7.8.2 Principal Response Curves for Various Taxonomic Levels of Phytoplankton

Principal Response Curves for SHADOW, TURBULENCE, RUNOFF and DIAZINON were calculated for data grouped on the species, family and class level (Figure 3-44). All sampling events (day-24 to day 101) were included into one data set for the PRC calculations. Species weights and variance allocations were calculated for a reduced time frame with expected higher significance, i.e. for the exposure phase only.

For all three taxonomic levels, the treatments DIAZINON and TURBULENCE are strongly deviating from the control during the exposure phase. For all hierarchy levels, the TURBULENCE curve reaches its maximum on day 28 and approaches the control level again on day 80. The DIAZINON treatment resulted in maximum values on day 80, and the curves for all hierarchy levels did not reach control level until test end.

RUNOFF curves only slightly deviated from the zero line (control), none of the values were shown to be significantly different from the control (see Monte-Carlo test below). The same is true for the SHADOW treatment. However it is worth noting that data grouped on the class level resulted in a negative curve for the SHADOW treatment, while all other taxonomic groups showed positive curves.

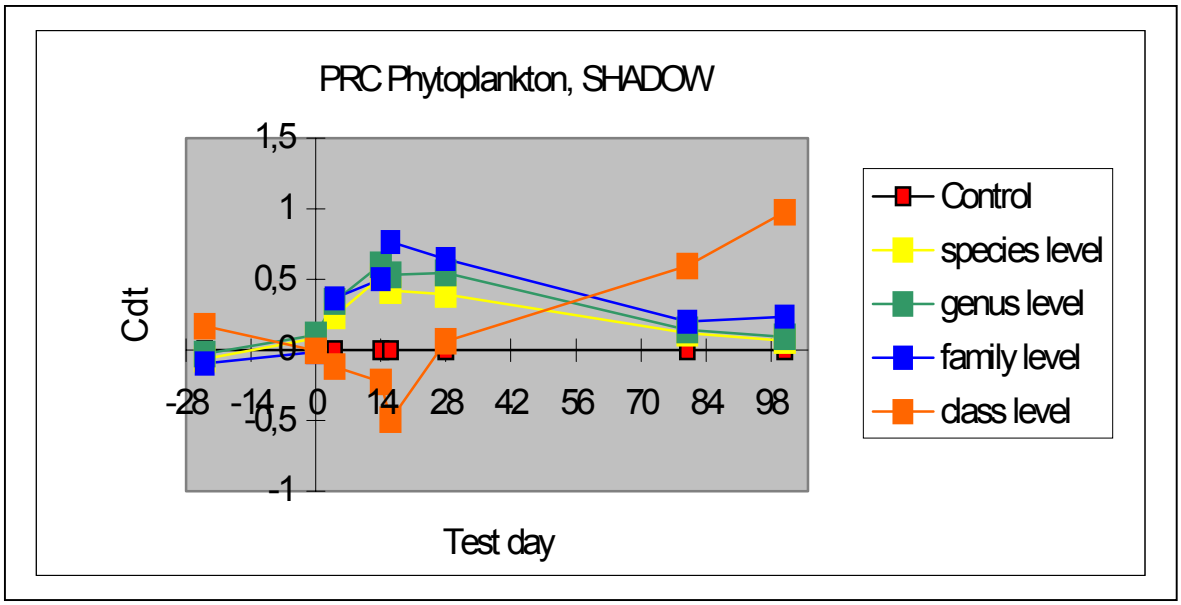
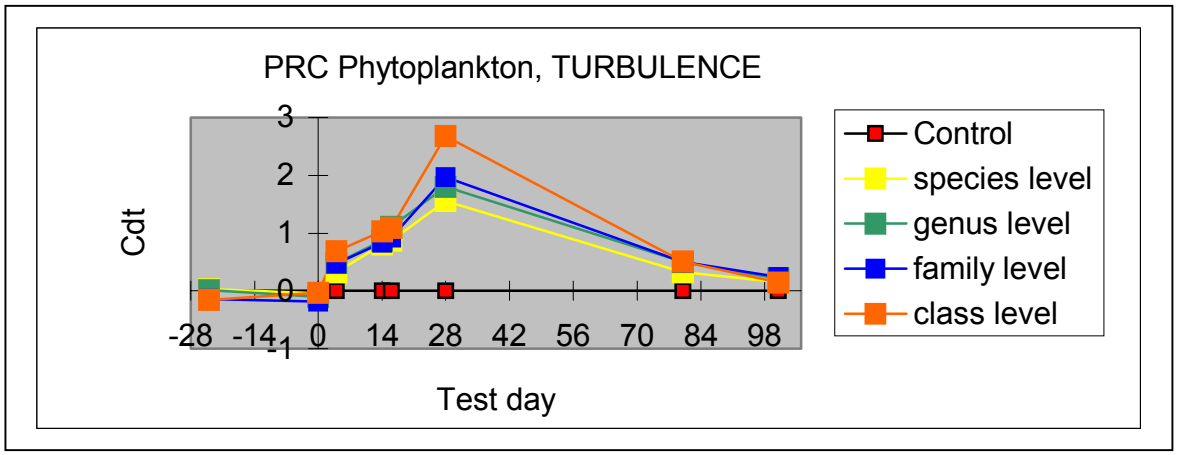
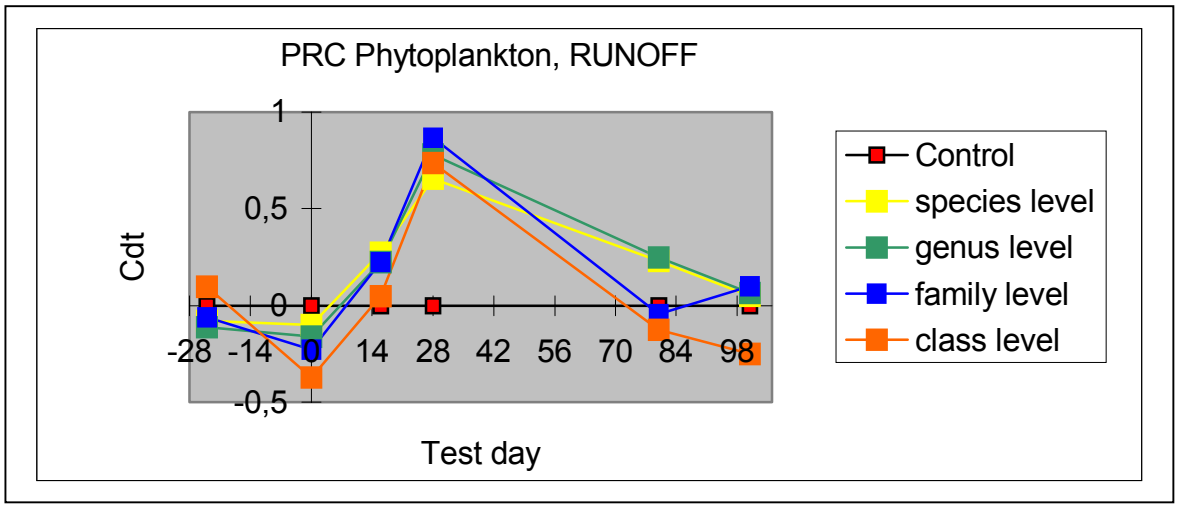


Figure 3-44. Principal Response Curves for phytoplankton grouped at the species, family and class level in the treatment groups SHADOW, TURBUENCE, RUNOFF and DIAZINON.





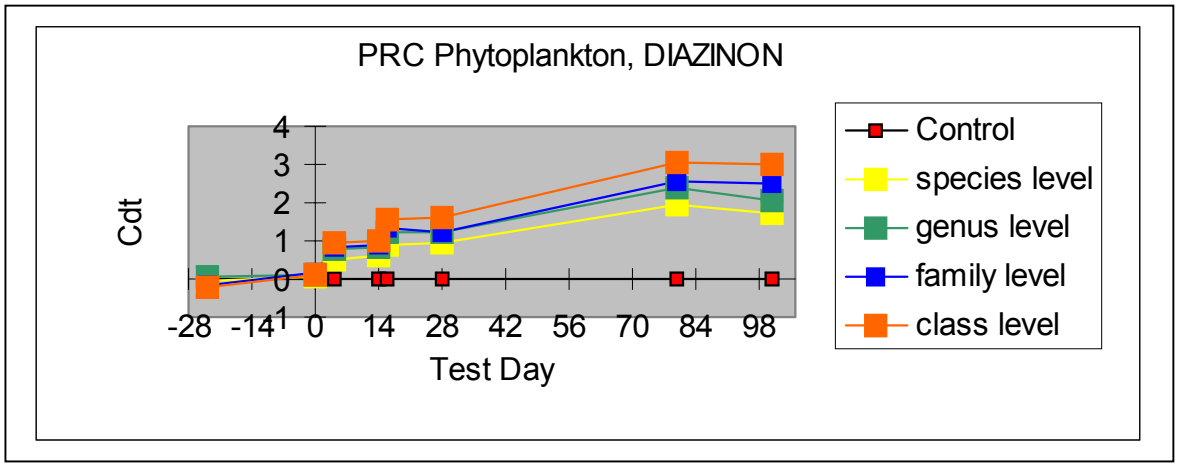


Figure 3-44 (cont.). Principal Response Curves for phytoplankton grouped at the species, family and class level in the treatment groups SHADOW, TURBUENCE, RUNOFF and DIAZINON.

Significance

The variance allocation for the PRCs is shown in Table 3-38. PRCs were calculated for the data set including all treatments and the control for the exposure phase only. Of the total variance, 7%, 6% and 2% could be attributed to time and 32%, 31% and 40% of the variance could be attributed to the treatment regime for the data grouped on species, family and class level, respectively. The corresponding PRC diagrams captured 46, 47 and 64% of the variance. Monte-Carlo permutation tests indicated that the PRC diagrams for phytoplankton grouped on the species, family and class levels displayed significant information.

Table 3-38. Variance allocation of phytoplankton data, grouped at the species, family and class level.

	% of variance accounted for by time	treatment	% of variance by first PRC	p-value of PRC
SPECIES level	6.6	32.2	46	0.001**
FAMILY level	6.1	30.7	47	0.001**
CLASS level	1.9	39.5	64	0.001**

Note: The percentages shown are the total variance which can be attributed to time and treatment regime, the % of variance captured by the first PRC and its significance (Monte-Carlo permutation test, significant (**) with $p<0.05$).

When grouped on the species, family or class level Monte-Carlo permutation tests (Table 3-39) showed statistically significant deviations from the control for the TURBULENCE and DIAZINON treatments. The treatments RUNOFF and SHADOW did not show significant deviations from the control throughout the exposure period for data grouped on the species, family and class level.

When grouped at the species level Monte-Carlo permutation tests showed statistically significant deviation of the TURBULENCE treatment from the control for day 14 through day 28 ($p<0.05$). The TURBULENCE treatment deviated significantly from the control for day 14 through day 28 ($p<0.05$) for data grouped on the family level and for day 4 through day 28 when the data were grouped on the class level.

The Phytoplankton community of the DIAZINON treated tanks was shown to be significantly different from to the control on day 4 through day 101, i.e. throughout the exposure and post-exposure phase for all hierarchy levels. The treatments RUNOFF and SHADOW did not show significant deviations from the control throughout the study period.

Table 3-39. Results of Monte-Carlo permutation tests for phytoplankton data grouped on the species, family and on the class level.

Phytoplankton, SPECIES level				
	day 4	day 14	day 16	day 28
SHADOW	0.40	0.13	0.10	0.16
TURBULENCE	0.02	0.006	0.003	0.003
RUNOFF	0.43	0.32	0.18	0.13
DIAZINON	0.001	0.001	0.001	0.001

Phytoplankton, FAMILY level				
	day 4	day 14	day 16	day 28
SHADOW	0.364	0.304	0.022	0.103
TURBULENCE	0.058	0.007	0.003	0.003
RUNOFF	0.706	0.580	0.554	0.088
DIAZINON	0.001	0.001	0.001	0.001

Phytoplankton, CLASS level				
	day 4	day 14	day 16	day 28
SHADOW	0.601	0.758	0.207	0.270
TURBULENCE	0.003	0.034	0.003	0.003
RUNOFF	0.722	0.475	0.888	0.311
DIAZINON	0.006	0.002	0.001	0.001

Note: permutations were calculated for each single sampling event on the basis of the PRCs calculated for each treatment group seperately. Legend: significant with $p<0.05$ (shaded) or not significant with $p>0.05$.

Species/ Taxon Weights

Species/ Taxon weights were calculated for each treatment separately and for the hierarchy levels species, family and class (Table 3-40). Given the fact that Principal Response Curves were not shown to be significant for the SHADOW and RUNOFF treatments at any point in time (Table 3-39), species weights for these treatments were not included in Table 3-40.

For the treatment group TURBULENCE, a high negative weight was found for *Cryptomonas erosa/ovata* and *Chroomonas acuta*. Correspondingly, the weight for the class Cryptophyceae also showed a negative value. As the corresponding PRC-curves for the species as well as the class level are in the positive range, the negative weights for these taxa indicate a decrease in number when compared to the control. This finding is supported by Figure 3-42. *Monoraphidium circinale, Oocystis parva* and *Planktosphaeria gelatinosa* showed high positive weights indicating higher population densities for these species in the TURBULENCE treated ponds when compared to the control. Correspondingly, the Chlorophyceae also show a positive weight. When correlated with the corresponding PRC-curve, this indicates an increase of Chlorophyceae in the TURBULENCE treatment during the exposure and post-exposure phase when compared to the control.

These findings were confirmed for test days 14, 16 and 28 using the Dunnett´s test and the Monte Carlo permutation test (Table 3-32, Table 3-39). Both tests showed the three species to have significantly higher densities in the TURBULENCE treatment when compared to the CONTROL. This is also supported by the fact that Chlorophyceae contributed with >80% to the total phytoplankton density on days 14 through 80 (Figure 3-42).

For the treatment DIAZINON, a high negative weight was found for *Chroomonas acuta*. Correspondingly, the weight for the class Cryptophyceae also showed a negative value. As the corresponding PRC-curves for the species as well as the class level are in the positive range during the exposure phase, the negative weights for these taxa indicate a decrease in number when compared to the control. This finding is supported by Figure 3-42. Using the Dunnett´s test these findings were also confirmed for days 14 through 28 for the species level (Table 3-32) and the Monte Carlo permutation test for days 4, 14, 16 and 28 on the species, family and class level (Table 3-39). Both tests showed the three species to have significantly lower densities in the DIAZINON treatment when compared to the CONTROL.

Monoraphidium circinale, Tetraedron minimum and *Planktosphaeria gelatinosa* showed high positive weights indicating higher population densities for these species in the DIAZINON treated ponds when compared to the control. Correspondingly, the Chlorophyceae also showed positive weights. When correlated with the corresponding Principal Response Curve, this indicates an increase of Chlorophyceae in the DIAZINON treatment during the exposure and post-exposure phase when compared to the control. All findings were confirmed for test days 4 through 28 using the Dunnett´s test and the Monte Carlo permutation test (Table 3-32, Table 3-39). Both tests showed the three species to have significantly higher densities in the DIAZINON treatment when compared to the CONTROL. This is supported by the fact that Chlorophyceae contributed with >80% to the total phytoplankton density in the DIAZINON treatment group on days 14 through 80 (Figure 3-42).

Moreover, the Cyanophyceae played an important role in the DIAZINON treated ponds. *Gomphosphaeria lacustris* and *Lyngbia limnetica* showed high positive weights (Table 3-22) indicating that these species occurred at higher densities in the DIAZINON treatment when compared to the control during the exposure phase. These findings were confirmed by the significance tests (Table 3-32, Table 3-39) and the fact that Cyanophyceae contributed with about 30% to the total phytoplankton density on days 0 through 28 (Figure 3-42).

For the treatment SHADOW the taxon weights on the class level showed a positive weight for Cryptophycae (data not shown). The corresponding PRC-curve was negative until day 28 and positive for the post exposure phase, indicating that Cryptophyceae were lower in number when compared to the control for test days 4 through 28 but higher on test days 80 and 101(Figure 3-44). It should be noted that none of the deviations from the SHADOW treatment to the CONTROL could be proven to be significant (Table 3-39).

Table 3-40. Species/Taxon weights for phytoplankton community during the exposure phase, for data grouped on the species, family and class level.

TURBULENCE

Class		Family		Species	
Bacillariophyceae	1.68	Nitzschiaceae	2.66	Monorphidium circinale	3.7
Chlorophyceae	1.36	Chlorococcaceae	2.30	Oocystis parva	3.3
Cyanophyceae	-0.39	Scenedesmaceae	2.21	Planktoshaeria gelatinosa	2.9
Cryptophyceae	-1.59	Oocystaceae	2.20	Nitzschia palea	2.9
		Chroococcaceae	1.04	Monoraphidium minutum	2.6
		Nostocaceae	-0.76	Tetraedron minimum	2.5
		Oscillatoriaceae	-1.59	Oscillatoria rosea	-0.7
		Cryptochrysidaceae	-2.11	Anabaena variabilis	-0.8
				Lyngbia spec.	-1.3
				Chroomonas acuta	-2.1
				Cryptomonas erosa/ovata	-2.3

DIAZINON

Class		Family		Species	
Dinophyceae	1.66	Chroococcaceae	2.77	Monorphidium circinale	4.0
Bacillariophyceae	1.41	Nitzschiaceae	2.18	Gomphonema lacustris	3.4
Cyanophyceae	1.34	Tetrasporaceae	2.02	Tetraedron minimum	3.3
Chlorophyceae	1.10	Volvocaceae	-0.67	Planktoshaeria gelatinosa	2.5
Cryptophyceae	-0.74	Cryptochrysidaceae	-0.85	Paulschulzia pseudovolvo	2.4
				Nitzschia palea	2.3
				Peridinium spec.	2.1
				Lyngbia limnetica	2.0
				Scenedesmus acutus	2.0
				Anabaena variabilis	1.9
				Volvox aureus	-0.8
				Ankyra ancosa	-1.1
				Chroomonas acuta	-1.4

Note: The species weights show the affinity of single species to the PRC: the higher the species/ taxon weight the more pronounced the actual response pattern of this species/ taxon is likely to follow the PRC pattern. Calculation of species/ taxon weights was performed on the basis of exposure data (day 4 to 28). Different colors indicate to which class the species or family belongs to, e.g. green for Chlorophyceae.

3.8 DISCUSSION OF DISTURBANCE EFFECTS ON PHYTOPLANKTON

3.8.1 Phytoplankton community structure was clearly affected by the application of DIAZINON and did not recover by test end

Statistical Evidence

Several multivariate statistical methods were used for the comparison of treatment groups with the control. Changes in the phytoplankton community structure for DIAZINON treated microcosms when compared to the control group during the exposure phase and post-exposure phase were shown using the Cluster and MDS methods (Figure 3-39, Figure 3-40). These findings were confirmed using the Analysis of Similarities and the Principal Response Curves method (Table 3-33, Figure 3-41).

Changes in community structure during the exposure phase were dominated by the significant increase in density of 10 species as shown by the list of species weights (Table 3-37), namely *Nitzschia gracilis, N. palea (*Bacillariophyceae), *Monoraphidium circinale, Paulschulzia pseudovolvox, Planktosphaeria gelatinosa, Scenedesmus aculeolatus, Tetraedron minimum* (Chlorophyceae), *Lyngbia limnetica, Anabaena variabilis* (Cyanophyceae) and *Peridinium* spec. (Dinophyceae). The species *Chroomonas acuta* was present at lower densities in the DIAZINON treated ponds when compared to the controls during the exposure and post-exposure phase. All results were confirmed by the results of the Dunnett´s test (Table 3-32).

DIAZINON treated ponds were still significantly different from the control ponds throughout the post-exposure phase on days 80 and 101 (Figure 3-41). The principal response curves, the MDS and the cluster analysis clearly indicated that the phytoplankton community in the DIAZINON treated ponds did not reach control level by test end (Figure 3-41, Figure 3-40, Figure 3-39). The population density of *Chroomonas acuta* continued to be significantly lower in the treated ponds when compared to the control ponds. Significantly higher population densities were found in DIAZINON treated ponds for the species *Nitzschia gracilis, N. palea (*Bacillariophyceae), *Monoraphidium circinale, Oocystis parva, Paulschulzia pseudovolvox, Planktosphaeria gelatinosa, Scenedesmus* spec.*, Tetraedron minimum* (Chlorophyceae), and *Peridinium* spec. (Dinophyceae).

During the exposure and post-exposure phase, species diversity was increased for the treatment group DIAZINON as shown with increasing Shannon-Wiener indices and with low cumulative dominance for ranked species, when compared to the control group (Figure 3-36, Figure 3-37).

Population Responses

In the reference outdoor mesocosm study (Giddings, 1992) algal species were in general not affected, except for the class Bacillariophyceae, which had a LOEC of 25 µg/L (Table 2-4). In single species laboratory studies, green algae were affected at levels of 6.4 – 17.3 µg/L Diazinon.

Thus, it could be assumed that phytoplankton in the present study would not be directly affected with measured concentrations usually below 16 µg/L, Table 3-23, Table 2-4).

Cyanophyceae

The Cyanophyceae were the predominant class on test day 0 in the DIAZINON treated ponds and their contribution to the total density remained high when compared to the control group throughout the duration of the study (Figure 3-42). Although none of the individual species showed significantly higher numbers in the DIAZINON treatment group during the pre-exposure, it is worth noting that filamentous algal blooms had occurred before Diazinon application (Table 3-32 & Appendix A). This class being represented with such high numbers and being in general inedible for filter-feeders may have contributed to the sustained suppression of *Daphnia longispina* (Figure 3-23).

Chlorophyceae

The Chlorophyceae were the predominant class during the exposure and post-exposure phase in the DIAZINON treated ponds. The dominance of Chlorophyceae was also observed in the control group (Figure 3-42). However, numbers were significantly increased for a series of species in the DIAZINON treatment group during the exposure and the post-exposure phase when compared to the control (Table 3-32). None of the green algae showed significantly lower numbers when compared to the control during exposure and post-exposure phase.

Bacillariophyceae

The contribution of Bacillariophyceae to the total algal density in the DIAZINON treated ponds was low when compared to the control group (Figure 3-42). This was linked to the strong dominance of Chlorophyceae and Cyanophyceae in DIAZINON treated ponds throughout he exposure and post-exposure phase.
Bacillariophyceae were not affected at the applied test concentration of 16µg/L, which is well in line with Giddings´ study (1992). Two species belonging to this class were found at significantly higher numbers when compared to the control during the exposure and post-exposure phase (*Nitschia gracilis, N. palea,* Table 3-32).

Cryptophyceae

The contribution of Cryptophyceae to the total algal density in the DIAZINON treated ponds was low when compared to the control group (Figure 3-42). This was linked to the strong dominance of Chlorophyceae and Cyanophyceae in DIAZINON treated ponds throughout he exposure and post-exposure phase.
Population densities of *Chroomonas acuta* were significantly lower in the DIAZINON treated ponds when compared to the control during the exposure and post-exposure phase. As significant effects were not observed before day 14, and taking into account the short generation times of algae, it seems highly unlikely that this effect can be linked to an algal toxicity of DIAZINON. Rather, the strong dominance of Chlorophyceae and Cyanophyceae has lead to a weak representation of this species in DIAZINON treated ponds, while in the control ponds *Chroomonas acuta* remained one of the major species throughout the study period (Table 3-28, Table 3-31).

Conclusion

In the present study, the phytoplankton community has reacted to DIAZINON application with overall increased algal density and increased species diversity during the exposure and post-exposure phase.

Both changes can be interpreted as a secondary effect related to the drastic reduction of *Daphnia longispina* in DIAZINON treated ponds and the resulting reduction of grazing pressure for phytoplankton. Although not negatively affected in terms of densities measured for individual populations (except for one out of 131 species) and species diversity, the phytoplankton community had significantly changed after DIAZINON application. The community structure was still significantly different to the control at test end.

The results of the present study clearly display the draw-back of analyzing microcosm data on the population level only, and the added value of analyzing data on the community level. As can be seen from recent publications, the method of community analysis has become a common tool for the evaluation of microcosm studies (Sibley *et al.*, 2001; Maund *et al.*, 1999). This appears to be a consequence of expert workshops, where the use of multivariate statistical tools for community analysis was highly recommended (HARAP 1999, CLASSIC 2001).

3.8.2 Phytoplankton community was affected by the TURBULENCE treatment but recovered to control level by test end

Statistical Evidence

Several multivariate statistical methods were used for the comparison of the treatment group TURBULENCE with the control. Changes in the phytoplankton community structure for TURBULENCE treated microcosms when compared to the control group during the exposure phase were shown using the Cluster and MDS methods (Figure 3-39, Figure 3-40). These findings were confirmed using the Analysis of Similarities and the Principal Response Curves method (Table 3-33, Figure 3-41). The phytoplankton community in the TURBULENCE treatment group reached control level during the post-exposure phase and can thus be stated to have recovered by test end.

Changes in phytoplankton community structure during the exposure phase were dominated by the significant and consistent increase in density of 8 species as shown by the list of species weights (Table 3-37), namely *Nitzschia palea (*Bacillariophyceae), *Monoraphidium circinale, M. minutum, Planktosphaeria gelatinosa, Scenedesmus aculeolatus, S. tenuispina, Tetraedron minimum* and *Oocystis parva* (Chlorophyceae). The species *Chroomonas acuta* and *Cryptomonas erosa/ovata* was present at lower densities in the TURBULENCE treated ponds when compared to the controls during the exposure phase. All results were confirmed by the results of the Dunnett´s test (Table 3-32).

Phytoplankton communities in TURBULENCE treated ponds were no longer significantly different from the control ponds throughout the post-exposure phase on days 80 and 101 (Figure 3-41).

The principal response curves, the MDS and the cluster analysis clearly indicated that the phytoplankton community in the TURBULENCE treated ponds reached control level by test end (Figure 3-41, Figure 3-40, Figure 3-39). None of the species affected during the exposure phase continued to be significantly different in the treated ponds when compared to the control ponds during the post-exposure phase.

Community Response

Changes in the turbulence climate of lakes were reported to cause shifts in phytoplankton community composition and succession (e.g., Berman & Shteinman, 1998). In the present study, the simulation of turbulence induced significant changes in community composition. Shifts in community composition can be a result of improved nutrient availability due to enhanced water movements (cf. Schwoerbel, 1999). In the present study, turbulence resulted in an increased total density of phytoplankton.
The TURBULENCE treatment favored the growth of Chlorophyceae over Cyanophyceae and Cryptophyceae. This is in line with findings from nutrient enrichment studies for which significant changes in phytoplankton community structure were reported, with green algae increasing more than any other group in terms of relative abundance (Vanni, 1987; van Donk, 1995).

Chlorophyceae

The Chlorophyceae were the predominant class during the exposure and post-exposure phase in the TURBULENCE treated ponds. The dominance of Chlorophyceae was also observed in the control group (Figure 3-42). However, numbers were significantly higher for a series of species in the TURBULENCE treatment group during the exposure and the post-exposure phase when compared to the control (Table 3-32). During the post exposure phase, *Volvox aureus* was the predominant green alga, representing over 95% of the total density of Chlorophyceae. None of the green algae showed significantly lower numbers when compared to the control during exposure and post-exposure phase.

Cryptophyceae

The contribution of Cryptophyceae to the total algal density in the TURBULENCE treated ponds was negligible when compared to the control group throughout the exposure and post-exposure phase (Figure 3-42). Population densities of *Chroomonas acuta* and *Cryptomonas erosa/ovata* were significantly lower in the TURBULENCE treated ponds when compared to the control during the exposure phase. However, these species had recovered to control level by test end (Figure 3-38, Table 3-32).

Bacillariophyceae

The contribution of Bacillariophyceae to the total algal density in the TURBULENCE treated ponds was low when compared to the control group for the exposure and post-exposure phase (Figure 3-42). *Nitzschia palea* was found at significantly higher numbers when compared to the control during the entire exposure phase, but reached control level by test end (Table 3-32). None of the Bacillariophyceae species occurred at lower numbers in the treatment when compared to the control.

Conclusion

In the present study, the phytoplankton community has reacted to TURBULENCE treatment with overall increased algal density during the exposure phase. Although not negatively affected in terms of densities measured for individual populations except for two out of 131 species, the phytoplankton community had significantly changed during TURBULENCE treatment. The community structure had recovered during the post-exposure phase and reached control level at test end. As stated before for the DIAZINON treatment group, the results for the TURBULENCE treated group again emphasize the added value of analyzing data on the community level.

3.8.3 Phytoplankton community structure was NOT significantly affected by SHADOW

Lack of Statistical Evidence

None of the statistical methods used could prove the phytoplankton community in the treatment group SHADOW to be significantly different from the control during the exposure phase (Table 3-32, Table 3-33, Figure 3-39, Figure 3-40, Figure 3-41).
However, a trend could be shown for the total density of Cryptophyceae for the post-exposure phase. The contribution of Cryptophyceae to the total density in the SHADOW treatment group clearly increased during the post exposure phase (Figure 3-42). This trend could be confirmed using the principal response curves method (Figure 3-44), however, this change not being statistically significant.

Community Response

Different phytoplankton species show different ability to respond to changing light conditions be it migration of motile algae to avoid photo-inhibition or light-limitation, or chromatic adaptation to exploit particular habitats (Reynolds, 1990). According to recent competition theory, the species with the lowest “critical light intensity” should be the superior light competitor (Huisman *et al.*, 1999). Therefore, it was assumed that shading of a group of microcosms in the present study and thus reduction of the light intensity incident upon the water surface would induce changes in phytoplankton community composition.

In the present study, light intensity at the water surface was reduced to 30-40% of the intensity measured at the surface of control ponds (Table 2-1). Minimum measured light intensity at the water surface in the treatment group was 195 µE m^{-2} s^{-1}. Light saturation is known to occur for most phytoplankton species in the range of 60-100 µE m^{-2} s^{-1} (Harris, 1978). Thus, taking into account reflection at the water surface and light attenuation in the water column, limiting light conditions might have occurred on cloudy days in the treatment group SHADOW, however on sunny days the light limitation points were most likely exceeded for all species.

It is assumed that in the present study, changes imposed to the treatment group SHADOW were not strong enough or not sufficiently extended (on a temporal scale) to induce detectable changes to the phytoplankton community.

The observed trend for increased numbers of Cryptophyceae during the late exposure and the post-exposure phase, although not statistically significant, might possibly be explained by the motility of these phytoflagellates and their related ability to respond to changing light conditions.

3.8.4 Phytoplankton community structure was NOT significantly affected by RUNOFF

None of the statistical methods used could prove the phytoplankton community in the treatment group RUNOFF to be significantly different from the control during the exposure phase (Table 3-32, Table 3-33, Figure 3-39, Figure 3-40, Figure 3-41).
The contribution of Chlorophyceae was slightly more pronounced during the exposure phase in the RUNOFF treated groups when compared to the control group (Figure 3-42). This trend was not statistically significant.

Suspended solids influence water transparency and light intensity in the water column. They can alter productivity of the phytoplankton through particle effects on light penetration and scatter (Grobelaar, 1985; Kirk, 1985).
In the present study, a load of about 200 mg/L quartz sand was applied twice to the investigated microcosms. By using washed quartz sand, nutrient enrichment and consequent effects on the phytoplankton were avoided. Related turbidity was negligible, never exceeding 8 NTU and disappeared within 2 days after application (Appendix B). In the control group, turbidity ranged between 3 and 5 NTU during the exposure phase.
It is assumed that in the present study, changes imposed to the treatment group RUNOFF were not strong enough or not sufficiently extended on a temporal scale to induce detectable changes to the phytoplankton community.

3.8.5 How to combine univariate and multivariate statistical methods

For the study design and the test system used in the present study, univariate and multivariate statistical methods lead to similar results. Selection of single species for univariate tests was based on density and abundance patterns, running the risk of missing rare but important species, while multivariate tools made use of the entire data set. However, the output of some multivariate methods, such as dendrograms and MDS ordinations were found to be difficult to interpret (Figure 3-39, Figure 3-40).

It appears as a result of the comparative work undertaken that the Principal Response Curves Method (PRC) method offers also for phytoplankton the most comprehensive way of data presentation because multivariate data are reduced to an univariate graphical presentation (Figure 3-41). An advantage of the PRC method when compared to the Cluster or MDS ordinations clearly is the combination of displaying graphically in a comprehensive way the difference in community composition between a treatment and the control. Further, the PRC method allows for composing the corresponding lists of species weights, which indicates decreases or increases of species in the treatment when compared to the control.

As already discussed for zooplankton (paragraph 3.4.5) it is generally advisable to crosscheck population effects as detected with the PRC method with a univariate test. For the phytoplankton data all results of the PRC list of species weights could be confirmed using the Dunnett´s test (Table 3-32, Table 3-37).

3.8.6 Diazinon effects on phytoplankton were detectable despite natural variability and changes due to natural disturbance

Inter-replicate variability was low for the treatments SHADOW and DIAZINON when compared to the control throughout the entire study duration (Figure 3-35). Within variability in the treatment groups TURBULENCE and RUNOFF increased during the course of the study and was in the majority of the cases higher than in the control group (Table 3-30). Inter-replicate variability of the control group showed a similar temporal trend.

Despite the natural variability measured in the control ponds, all multivariate methods enabled the detection of DIAZINON effects on the zooplankton community when comparing the treated group with the control (Figure 3-39, Figure 3-40, Figure 3-41).

Further, phytoplankton communities in DIAZINON treated ponds were also shown to be significantly different from all other treatment groups. Even though the TURBULENCE treatment had significantly affected the phytoplankton community, DIAZINON effects were still detectable when comparing these two treatment groups, using Analysis of Similarities (Table 3-33). This finding was confirmed using the PRC method. Further to the detection of effects caused by individual treatments (Figure 3-41) the PRC was applied to the whole data set, including the control and all 4 treated groups. By including all treatments in one calculation, emphasis was put on the comparison of pesticide effects to “naturally” occurring changes. The corresponding PRC diagram (Figure 3-45) showed a clear distinction of the Diazinon group from the “naturally disturbed” groups.

In conclusion, it appears that pronounced effects of a plant protection product on phytoplankton community composition can be well detected despite naturally occurring inter-replicate and seasonal variability, and that even natural disturbances do not affect the ability to detect such effects.

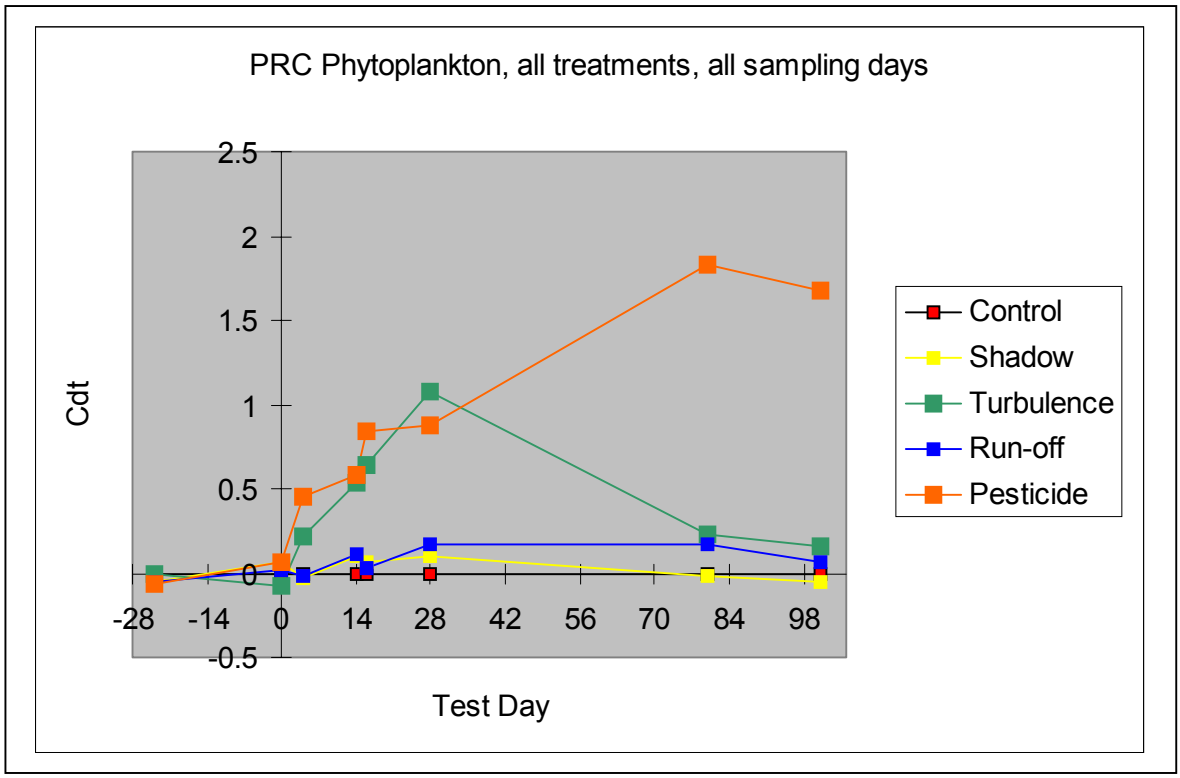


Figure 3-45. Principal Response Curves for phytoplankton in the treatments calculated from one data set.

3.8.7 Taxonomic levels higher than the species level not sufficient for interpretation of the present study

Data were analyzed on different taxonomic levels, i.e. species, family, order and class. Two different methods were used, one being a graphical presentation of the percent contribution of each group to the total density and the other being the multivariate statistical method PRC (Figure 3-42, Figure 3-43, Figure 3-44).

The phytoplankton community was characterised as species rich and variation between treatment groups in community composition were considerable. The dominance partition was less pronounced for the phytoplankton communities when compared to the zooplankton. Seasonal variability played an important role in phytoplankton community composition while populations of dominant zooplankton species were shown to occur throughout the study. The contribution of individual species to phytoplankton classes was highly variable due to seasonality (Figure 3-42, Figure 3-43). However, it could be shown that 4 to 10 species dominated the total phytoplankton density on each individual sampling event (Table 3-31, Figure 3-37).

When calculating Principal Response Curves for data grouped at 3 different taxonomic hierarchical levels, significance increased for Principal Response Curves calculated on the family and class level when compared to the species level (Table 3-38):

Curves remained very similar with the exception for the SHADOW treatment, where grouping data on the class level resulted in a curve totally different from the species and family level (Figure 3-44).

As phytoplankton communities were species richer than zooplankton communities and also underwent significant changes depending on the season it seems, at first glance, advisable to analyze phytoplankton data at the species level. However, as already demonstrated for the zooplankton, also the phytoplankton data interpretation suffers from high standard deviations and the high frequency of zero-counts encountered per species and sampling event. Therefore, also for phytoplankton data, one runs the risk of producing statistically useless and ecologically meaningless information when identifying all organisms down to the species level.

Brock *et al*. (1994) reviewed the use of specific structural parameters to characterize the responses of phytoplankton in 57 micro- and mesocosm studies. In about 35% of the experiments that paid attention to the phytoplankton, efforts were made to identify the organisms sampled to the genus or species level. Usually, investigations were based on dominant taxa only or data were aggregated to a higher taxonomic level.

The present study has shown that this approach, as used in the past, is reasonable and scientifically justifiable. Due to the high number of zero-counts observed for phytoplankton, coupled with high seasonal variability, changes for individual populations can in the majority of cases not be proven to be statistically significant. On the other hand, with the present study, it could also be shown that effects on the ecosystem can be reliably detected using community analysis methods. These methods however rely on taxonomic identification to a low hierarchical level.

Therefore, as stated for the zooplankton, it seems advisable to choose the taxonomic level for the phytoplankton on a case-by-case basis, taking full account of the main species/taxa of concern and the known dominance partition of taxa in the system used. This should enable taxonomic identification in a microcosm study being fixed on a meaningful level at the very beginning of a study and to use available resources at its best.

4 CONCLUSIONS

The present study has shown that in the investigated outdoor aquatic microcosms

- The range of system-inherent variability of zooplankton was comparable to the variability in natural ecosystems (chapter 3.2).
- Phytoplankton variability was dominated by seasonality, similar to natural ecosystems (chapter 3.6).
- The natural variability of zooplankton and phytoplankton was dominated by the high prevalence of zero-counts (chapters 3.2 and 3.6).
- The variance of zooplankton and phytoplankton population densities was tightly correlated to the mean (chapters 3.2 and 3.6).
- Weak natural disturbances such as simulation of shading and runoff events did not significantly affect zooplankton and phytoplankton communities (chapters 3.4 and 3.8).
- Intensive natural disturbances such as simulation of turbulent mixing significantly affected zooplankton and phytoplankton communities (chapters 3.4 and 3.8).
- Community recovery from this disturbance could be observed (chapters 3.4 and 3.8).
- Pesticide effects were best detected using multivariate statistics in combination with univariate methods and graphical presentation (chapters 3.4, 3.8).
- Secondary effects on the ecosystem due to pesticide application could be observed (chapter 3.8).
- Pesticide effects were detectable despite changes due to simulated natural disturbance (chapters 3.4, 3.8).
- Taxonomic resolution at higher hierarchical levels was satisfactory for data interpretation depending on the dominance partition of species (chapters 3.4, 3.8).

On the basis of the results of the present study, the following recommendations are made on how to take into account the natural variability for the test design and data evaluation of ecotoxicological testing in outdoor aquatic microcosms.

Recommendation 1: Check distribution

For data sets where the variance of species counts increases with the mean value, Kennedy *et al.* (1999) recommended using an evaluation based on the log-linear model. The log-linear model may be appropriate for such data because it assumes the variance to be some fixed constant multiplied by the mean (Poisson distribution). Based on this model, power functions can be computed to determine observable differences at a certain precision level and to determine which percent decrease in the treatment compared to the control will be detected as statistically different. The approach taken by Kennedy *et al.* (1999) allows for estimating sample sizes required to achieve desired levels of statistical power.

An increasing variance of species counts with the mean could also be shown for the zooplankton and phytoplankton species counts investigated in the present study, despite the comparatively high number of 12 control replicates.

Therefore, the findings of Kennedy *et al.* (1999) can be confirmed and it can be considered as useful to check the mean: variance distribution in the investigated testing facility when designing an ecotoxicological microcosm test, especially for those species which are considered important and tend to occur at low numbers.

Recommendation 2: Determine required levels of detectable change

The system-inherent variance in combination with the applied precision level determines the smallest detectable percent decrease in population density (Kennedy *et al.*, 1999; Amman *et al.* 1997; Liber *et al.*, 1992; Lozano *et al.*, 1992).

The lack of statistical power for taxa, which occur with low densities has been described by Ammann *et al.* (1997) for a series of marine microcosm experiments. According to their calculations, 11 to 19 replicates would have been required to detect a 50% decrease in the mean number of taxa with mean densities around 1/L (Poisson model, level of significance α=0.1). For mean densities greater than 10/L, 1 to 2 replicates would be sufficient to detect a 50% decrease. However, their publication also clearly shows the difficulty of detecting *small deviations* of the treated microcosms when compared to the control in an ecotoxicological test. To detect a reduction of the mean count of 20% would, according to Ammann *et al.* (1997), need 82 to134 replications (=ponds) when the mean counts are as low as 1/L. For mean numbers exceeding 10/L, one would still need 3 to 9 ponds.

Similarly, Liber *et al.* (1992) estimated the smallest detectable percent decrease in total density being about 50% for species occurring at densities >1/L, based on the variance associated with the mean abundance data for macrozooplankton (ANOVA and regression design, $p \leq 0.05$). Also Lozano *et al.* (1992) consider a 50% reduction in treatment zooplankton density compared to control density as significant in a study with littoral enclosures. They justify the cut-off limit of 50% by the fact that this magnitude of change corresponds to approximately 2.5 standard deviations based on a log10(N+1) density transformation. Lozano *et al.* (1992) recognize the high inter-replicate variability for low population densities by including in their analysis only those populations that had five or more individuals per sampler in the controls. These observations were confirmed by the present study.

In the pertinent case, for the investigated microcosms and the applied sampling design, the natural variability also had a strong impact on the interpretability of the majority of findings, namely for taxa occurring at densities below 10 individuals per litre when applying a precision level of p=0.2 to detect statistical differences between the treatment and the control group.

Therefore, based on the findings in the present study and taking into account experience from published microcosm studies it seems advisable to determine a realistic size of response and the corresponding level of precision required to detect the change when setting up a microcosm study. It seems that the size of response and the statistically detectable reduction needs to be adapted to the feasibility when setting up a regulatory microcosm study.

Recommendation 3: Use non-metric multivariate tools

The problem of distributional assumptions and related problems with statistical power can partly be circumvented using non-metric multivariate statistical tools (Clarke, 1999; Kedwards *et al.*, 1999). A fully multivariate analysis such as ´non-metric multidimensional scaling´ does not require distributional assumptions and exploits the information on the specific composition and abundance of each sample, taking into account also those species which occur at low numbers and thus can pose statistical problems when analysed individually. Moreover, multivariate methods such as the Principal Response Curves method have the advantage of reflecting the community response and the contribution of individual populations to changes in the community response when compared to a control group (van den Brink and Ter Braak, 1999).

The similarity-based multivariate methods ´non-metric multidimensional scaling´ and ´hierarchical clustering´ have also been applied successfully to the zooplankton and phytoplankton data in the present study. However, the Principal Response Curves method (PRC) was shown to be the most comprehensive way of displaying community response. Detection of significant effects using the PRC method was shown to depend on the selected time-frame for statistical analyses. It seems therefore advisable to take into account this critical point when analysing community data and to cross-check PRC results with univariate statistical tests or graphical presentation.

Recommendation 4: Group data into ´time-windows´

The problem of weak precision levels for single taxa which can occur due to low densities or low replication numbers can be overcome by grouping data according to ´time-windows´ (Knauer *et al.,* 2000). When evaluating an ecotoxicological test with a high number of sampling events during the exposure phase, it may be justified to group data of taxa occurring at several consequent sampling events. Knauer *et al.* have shown that the statistical power of a test by this means can reach significant levels for grouped data.

For the present study, grouping of samples from the control ponds according to ´time-windows´ did not increase precision levels. However, it cannot be excluded that precision levels would have increased if more sampling events had been available. In general it therefore seems advisable to group data according to time-windows, if appropriate, i.e. depending on the sampling schedule.

Recommendation 4: Group data on higher taxonomic levels

Ferraro & Cole (1990) suggested that grouping animals to higher taxa may dampen natural variability in faunal patterns, improving the ability of subsequent analyses to assess small pollution impacts. Grouping of data on higher taxonomic levels may be justified if the investigated community exhibits high structural redundancy. The latter can be remarkably high due to the resilience or compensation potential within an assemblage (e.g. Warwick *et al.*, 1990). Clarke & Warwick (1998) describe a method for quantifying structural redundancy in ecological communities, which is based on the extraction of subsets of species which have multivariate response patterns similar to that of the whole community.

For the present study, structural redundancy of zooplankton was shown using the Principal Response Curves method. However, due to the underlying dominance partition of the zooplankton community (i.e. the strong dominance of one taxon within each order), statistical power did not increase significantly when grouping data according to higher taxonomic levels, although the attribution of time and treatment to the total variance became more distinct at taxonomic levels higher than species. In contrast, statistical power increased for the more diverse phytoplankton community, when grouping data at the class level.

Therefore, it may be appropriate to group data at taxonomic levels higher than the species level to increase the statistical power of the applied tests but also to display changes, which are not observable when considering only individual populations.

Recommendation 5: Check zero-counts

Zero-counts may occur as a result of a ´low detection limit´ for the respective species in a microcosm study. This may be related either to an inefficient sampling strategy or to the fact that the species is indeed represented at very low numbers in the investigated microcosms, e.g. due to its seasonality.

It was shown in the present study that the mean values for population densities are negatively correlated to the number of replicates representing a zero-count. If the majority of replicates of an experimental condition are zero-counts for a particular species and the mean reaches a certain limit-value, this may result in severe interpretational and statistical problems. For the zooplankton community in the present study, statistically sound data interpretation was possible only for about 17% of the data points with densities from 1/L to 10/L, while 61% of the data were reliable for taxa represented at densities above 10/L. Further, statistically sound data interpretation was impossible for species which were present in less than 50% of the replicates at a certain sampling event, due to related high variation and low mean values.

For an ecotoxicological study with 3-4 replicates it seems therefore advisable to interpret results with great caution for those species which occur at mean densities below 10/L and are represented in less than 50% of the replicates at a certain time point.

Recommendation 6: Adjust sampling design

If statistically sound data are needed for species which are usually found near the critical limit values as described above, one might need to consider changing the sampling strategy and changing the design for counting the samples. This may include increasing the sampling method and volume as well as the minimum numbers counted per species and sample (e.g., Downing *et al.*, 1987; Persaud & Yan, 2001).

Recommendation 7: Check influence of seasonality and secondary effects on potential for population recovery

Temporal variability of population densities plays a critical role for the potential for recovery of affected zooplankton and phytoplankton communities.

Populations of *Daphnia longispina* had not recovered in the present study several weeks after diazinon application, while recovery had been observed in a previous mesocosm study (Giddings, 1992) at a similar test concentration. The two studies differ in terms of temporal coincidence of treatment and life cycle of the respective species. While Giddings (1992) applied diazinon in June and the corresponding recovery phase started end of June, in the present study the ponds were treated in mid-July. The recovery phase in the present study thus had a comparatively late starting point, i.e. only in mid-August. At this time of the year, already the control numbers of *Daphnia longispina* had started to drop due to its life cycle. A possible explanation for the weak recovery of *Daphnia* in the present study may thus be the coincidence of low numbers due to the life cycle of this species and the chosen time of application/recovery.

Further, the weak recovery of *Daphnia longispina* might also be related to a shift in algal composition, which was first observed in the diazinon treated ponds 14 days after application. It is assumed that changes in phytoplankton community composition were partly due to the elimination of the *Daphnia longispina*, and thus the shift in algal composition can be interpreted as a secondary effect. Algae inedible or moderately edible for *Daphnia* dominated the diazinon treated ponds during the recovery phase which, in addition to the life-cycle effect, has probably contributed to the sustained suppression of *Daphnia longispina* after diazinon concentrations had dropped to the levels below the reported LOEC.

It should be kept in mind that in natural systems, due to water exchange, re-inoculation or dilution effects, recovery is likely to occur faster than in isolated microcosm systems. Therefore, secondary effects, which are likely to be due to the static character of the microcosms might need a follow-up experiment to allow truly the determination of a realistic environmentally acceptable concentration (EAC). Such an approach may include simulating a moderate re-inoculation of aquatic outdoor microcosms.

Final Remarks

Overall, the added value of using microcosm tests when compared to laboratory testing is very obvious. It is an effective test system to detect changes to the community structure of aquatic ecosystems and allows the investigation of secondary effects.

There are some limitations concerning the detection and interpretation of effects and recovery for species occurring at low population densities, which is due to the number of replicates which can be set up in a microcosm study, but also to the seasonality of phytoplankton and zooplankton species. As shown in the present study, these difficulties can be overcome, e.g., using the appropriate statistical tools or grouping the data at higher taxonomic levels.

However, it must be kept in mind that statistics can provide the tools to detect changes in population densities or community composition, but can never replace expert judgment. Expertise in ecology is required when interpreting detected effects and estimating the relevance of different intensities of change. However, expert judgment strongly depends on the state-of-the-art in ecological research.

Natural variability, although undesirable statistically, *is* a characteristic property of natural ecosystems. If the intention is to operate microcosms that are similar to nature this suggests that natural variability will also be a feature of such surrogate ecosystems. Natural variability of species in time and space has the advantage that it increases “stochasticity”, and allows communities to adapt to natural - and anthropogenic - changes of environmental conditions. According to recent hypotheses in ecological research this results in a dynamic process, with natural communities changing constantly on a temporal and spatial scale (e.g., Matthews *et al.*, 1996). It is the opinion of the author that accepting this hypothesis of constant change and integrating it into ecotoxicology will be the major challenge for scientists and regulators in future.

5 SUMMARY

5.1 ABSTRACT

Increasing scrutiny on the ecological risk assessment of xenobiotics brings about an increasing demand for refined, higher tier investigations, which evaluate potential effects on aquatic ecosystems at the population or community level of organization. However, the use of test systems such as microcosms and mesocosms meets considerable difficulty in the regulatory context due to unresolved questions related to the design and interpretation of these complex studies and the natural variability of the populations sampled.

The present study attempts to make a contribution to resolve these questions. An array of 28 aquatic outdoor microcosms in Stein, Switzerland was available for the investigation. Twelve microcosms were left untreated to investigate the inherent temporal and spatial variability of the test system. The remaining 16 microcosms were subjected to controlled disturbances to assess "natural" (run-off, shadow, turbulence) or xenobiotic (diazinon) effects on the system-inherent variability and to compare these "natural" disturbances to those related to the specific biological activity of a model plant protection product.

Key objectives of the investigation were to optimize the design of micrososm studies with a view to minimize errors related to inherent variability and to identify the optimal taxonomic resolution for accurate data interpretation.

It was found that the range of system-inherent variability in microcosms was similar to the variability in natural ecosystems and that the variance of population densities was tightly correlated to the mean. Among the non-metric multivariate statistical tools implemented, the Principal Response Curves method (PRC) appeared to be the most comprehensive way of displaying community response, however, the sensitivity critically depended on the selection of an appropriate time-frame for the analysis.

Natural variability had a strong impact on the interpretability of the majority of findings, namely for taxa occurring at densities below 10 individuals per litre and those, which were represented in less than 50% of the samples at a given time point. If statistically sound data are needed for particular species occurring at these critical limits, specific sampling and counting strategies should be considered. The grouping of samples according to ´time-windows´ did not increase precision levels in the pertinent study, but may be promising if a more frequent sampling schedule is used. Grouping data at higher taxonomic levels did increase the statistical power of the analysis, in particular for the phytoplankton community. The assessment at higher taxonomic levels can also display changes, which are otherwise not observable.

The influence of seasonality and secondary effects must be carefully considered in the interpretation of data from static microcosm systems. A specific follow-up experiment simulating a moderate re-inoculation may be suitable to determine more realistic environmentally acceptable concentrations (EAC) of xenobotics in aquatic ecosystems.

5.2 ZUSAMMENFASSUNG

Die zunehmende Verschärfung von Auflagen zum Nachweis der Umweltverträglichkeit von Fremdstoffen bringt eine zunehmende Nachfrage nach Untersuchungen in aquatischen Systemen auf der Ebene der Populationen und Lebensgemeinschaften mit sich. Im regulatorischen Kontext stösst die Bewertung solcher Mikrokosmos- und Mesokosmos-Studien jedoch auf Schwierigkeiten, da Fragen zum optimalen Aufbau und zur Interpretation dieser komplexen Studien offen sind, die insbesondere die Unterscheidbarkeit von Effekten von der natürlichen Variabilität der untersuchten Populationen betreffen.

Die vorliegende Untersuchung leistet einen Beitrag zur Klärung einiger dieser Fragen. Es stand eine Anlage aus insgesamt 28 Mikrokosmen in Stein (Syngenta AG, Schweiz) zur Verfügung. Zwölf dieser Teiche blieben unbehandelt, um die inhärente, zeitliche und räumliche Variabilität des Systems zu untersuchen. Die verbleibenden 16 Teiche wurden kontrollierten Störungen ausgesetzt, um den Einfluss „natürlicher“ (Eintrag von Schwebstoffen, Beschattung, Turbulenz) oder xenobiotischer (Insektizid) Faktoren auf die inhärente Variabilität zu untersuchen, und „natürliche“ Störungen mit solchen zu vergleichen, die auf die spezifische biologische Aktivität einer Modellsubstanz zurückzuführen sind.

Zweck der Untersuchung war es insbesondere, einen Beitrag zur Optimierung der Planung solcher Studien zu leisten, indem Wege zur Minimierung möglicher Fehler aufgrund inhärenter Variabilität aufgezeigt werden. Der Einfluss der gewählten taxonomischen Auflösung der Daten auf deren statistische Auswertbarkeit wurde ebenfalls untersucht.

Die inhärente Variabilität der untersuchten Mikrokosmen bewegte sich im Rahmen der Variabilität, die auch für natürliche Systeme berichtet wird, wobei die Varianz der beobachteten Populationsdichten eng mit deren Mittelwert korreliert war. Unter den angewandten nicht-metrischen, multivariaten statistischen Methoden erschien die Methode der „Principal Response Curves“ (PRC) als besonders gut geeignet, Effekte auf Lebensgemeinschaften darzustellen, wobei allerdings die Sensitivität der Analyse stark von der Auswahl eines geeigneten Zeitrahmens abhing.

Die natürliche Variabilität beeinflusste die Interpretierbarkeit der Ergebnisse stark, vor allem für Taxa, deren Abundanz unter 10 Individuen pro Liter lag und solchen, die in weniger als der Hälfte der entnommenen Proben gefunden wurden. Für Spezies mit Abundanzen in diesem kritischen Bereich, sollten spezifische Probenahme-Strategien angewendet werden, um statistisch zuverlässige Daten zu erhalten. Die Gruppierung von Daten aus einzelnen Probenahmen in grössere Zeitfenster hatte im vorliegenden Fall keinen Einfluss auf die Präzision der Analysen, könnte sich aber als sinnvoll erweisen, wenn häufigere Probenahmen verfügbar sind. Dagegen beeinflusste eine Gruppierung der Funde auf höherer taxonomischer Ebene die statistische Auswertung vor allem des Phytoplanktons erheblich. Die Betrachtung höherer taxonomischer Ebenen kann in manchen Fällen Veränderungen deutlich machen, die sonst unbeobachtet bleiben.

Die kritische Betrachtung der Ergebnisse unter ökologischen Gesichtspunkten ist zur sinnvollen Interpretation statischer Mikrokosmen-Versuche unabdingbar, da saisonale und sekundäre Veränderungen auftreten. Zur endgültigen Bestimmung ökologisch verträglicher Eintragsgrenzen kann es notwendig werden, in einem Nachfolge-Experiment auch moderate Re-inokulationen zu untersuchen.

6 REFERENCES

Agbeti, M.D.; Kingston, J.C.; Smol, J.P.; Watters, C.: Comparison of phytoplankton succession in two lakes of different mixing regimes. - Arch. Hydrobiol. 140, 37-69 **(1997).**

Ali, A.; Rui-De, X.; Alam S.K.: Ecotoxicological effects of abamectin (MK-936) on natural populations of selected invertebrates in man-made ponds. – Med. Entomol. Zool 48 (3), 233-241 **(1997).**

Ammann, L.P.; Waller, W.T.; Kennedy, J.H.; Dickson, K.L.; Foster, L.M.: Power, sample size and taxonomic sufficiency for measures of impact in aquatic systems. – Environ. Toxicol. Chem. 16 (11), 2421-2431 **(1997).**

Belanger, S.E.: Literature review and analysis od biological complexity in model stream ecosystems: Influence of size and experimental design. – Ecotox. Env. Safety 36, 1-16 **(1997).**

Berman, T.; Shteinman, B.: Phytoplankton development and turbulent mixing in lake Kinneret (1992-1996). – J. Plankton Res. 20, 709-726 **(1998).**

Biehle, G.: Untersuchungen zur Hydrodynamik, Biomechanik, Morphologie und Funktionsanatomie von Wassermoosen am Beispiel von *Fontinalis antipyretica*. Diplomarbeit, Albert-Ludwigs-Universität Freiburg im Breisgau **(1996).**

Brock, T.C.M.; Roijackers, R.M.M.; Rollon, R.; Bransen, F.; Van der Heyden, L.: Effects of nutrient loading and insecticide application on the ecology of Elodea-dominated freshwater microcosms: 2. Responses of macrophytes, periphyton and macroinvertebrate grazers. - Arch. Hydrobiol. 13(1), 53-74 **(1995).**

Brock, T.C.M.; Budde, B.J.: On the choice of structural parameters and endpoints to indicate responses of freshwater ecosystems to pesticide stress. In: *Freshwater Field Tests for Hazard Assesment of Chemicals.* Hill, I.A.; Heimbach, F.; Leeuwangh, P.; Matthiesen, P. *(eds.)*. CRC Press Inc., Boca Raton, Florida **(1994).**

Cairns, J. Jr.: The threshold problem in ecotoxicology. - Ecotoxicology1, 3-16 **(1992).**

Cairns, J. Jr.: Are single species tests alone adequate for estimating hazard? – Hydrobiologia 100, 87-94 **(1983).**

Calow, P.: Ecotoxicology: what are we trying to protect? – Environ. Toxicol. Chem. 13(10),1549 **(1994).**

Caquet, T.; Lagadic, L.; Jonot, O.; Baturo, W.; Kilanda, M.; Simon, P.; Le Bras, S.; Echaubard, M.; Ramada, F.: Outdoor experimental ponds (mesocosms) designed for long-term ecotoxicological studies in aquatic environment. - Ecotoxicol. Environ. Safety 34(2),125-133 **(1996).**

Carrick, H.J.; Aldridge, F.J.; Schelske, C.L.: Wind influences phytoplankton biomass and composition in a shallow, productive lake. - Limnol.Oceanogr. 39, 1179-1192 (1993).

Clarke, K.R.: Nonmetric multivariate analysis in community level ecotoxicology. - Environ. Toxicol. Chem. 18 (2),118-127 **(1999).**

Clarke, K.R.; Warwick, R.M.: Quantifying sructural redundancy in ecological communities. – Oecologia113, 278-289 **(1998).**

Clarke, K.R.; Warwick, R.M.: Similarity based testing for community pattern: The two-way layout with no replication. - Mar. Biol. 118, 167-176 **(1994).**

Clarke, K.R.; Warwick, R.M.: Change in Marine Communities: An approach to statistical analysis and interpretation. Plymouth Marine Laboratory, Plymouth, UK, 144 pp. **(1994 b).**

CLASSIC Community Level Aquatic System Studies - Interpretation Criteria (CLASSIC). Society of Environmental Toxicology ad Chemistry Europe (SETAC-Europe), Brussels, Belgium **(2001).**

Clements, W.H.; Kiffney, P.M.: Assessing contaminant effects at higher levels of biological organization. - Environ. Toxicol. Chem. 13, 357-360 **(1994).**

Connel, J.H.; Sousa, W.P.: On the evidence needed to judge ecological stability or persistence. - American Naturalist 121, 789-824 **(1983).**

Cuppen, J.G.M.; Gylstra, R.; Van Beusekom, S.; Budde, B.J.; Brock, T.C.M.: Effects of nutrient loading and insecticide application on the ecology of Elodea-dominated freshwater microcosms: 1.Responses of macroinvertebrate detritivores, breakdown of plant litter and final conclusions. - Arch. Hydrobiol. 134(2), 157-177 **(1995).**

DeAngelis, D.L.: Dynamics of nutrient cycling and food webs. Chapman & Hall, London (1992).

Day, K.E.; Kaushik, N.K.; Solomon, K.R.: Impact of fenvalerate on enclosed freshwater planktonic communities and on *in situ* rates of filtration of zooplankton. - Canad. J. Fish. Aquat. Sci. 44, 1714-1728 **(1987).**

Dennis, W.H.:Degradation of diazinon by sodiumhypochlorite. Chemistry and aquatic toxicity. - Environm. Sci. & Techn. 13 (5), 594-598 **(1979)**.

Dewey, S.L, deNoyelles, F. Jr.: On the use of ecosystem stability measurements in ecological effects testing. In: *Aquatic Mesocosm Studies In Ecological Risk Assessment.* IR.L. Graney, J.H. Kennedy and J.H. Rodgers Jr. (eds.), CRC Press Inc. Boca Raton, Florida (1994)

Downing, J.A.: Spatial heterogeneity: evolved behaviour or mathematical artefact? – Nature 323, 255-257 **(1986).**

Downing, J.A.; Perusse, M.; Frenette, Y.: Effect of interreplicate variance on zooplankton sampling design and data analysis. - Limnol. Oceanogr. 32, 673-680 **(1987).**

Dunnett, C.W.: A Multiple Comparisons Procedure for Comparing Several Treatments with a Control. - Journal of the American Statistical Association (50), 1096-1121 **(1955)**.

Dunnett, C.W.: Pairwise Multiple Comparisons in the Homogeneous Variance, Unequal Sample Size Case. - Journal of the American Statistical Association (75), 789-795 **(1980)**.

EC Guidance Document on Aquatic Ecotoxicology in the frame of the Directive 91/414/EEC. Commission Document N° 8075/VI/97 rev 7 of 08.07.2000. - DG Health and Consumer Protection, Brussels **(2000).**

EC Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. Official Journal L230 of 19.08.1991, **(1991).**

EC Uniform Principles for evaluation and authorisation of plant protection products. - Annex VI to Directive 91/414/EEC concerning the placing of plant protection products on the market **(1997).**

Einsle, U.: Long term changes in planktonic associations of crustaceans in Lake Constance and adjacent waters and their effects on competitive situations. – Hydrobiologia 106, 127-134 **(1983).**

Einsle, U.: Populationsdynamische und synökologische Studien am Crustaceen-Plankton zweier Kleinseen. - Beitr. Naturk. Forsch. 28, 53-73 **(1969).**

Eppley, R.W.; Koeller, P.; Wallace, G.T.: Stirring influences on the phytoplankton species composition with enclosed columns of coastal sea water. - J. Exp. Mar. Biol. Ecol. 32, 219-239 **(1978).**

Fairchild, J.F.; La Point, T.W.; Zajicek, J.L.; Nelson, M.K.; Dwyer, F.J.; Lovely, P.A.: Population-, community- and ecosystem-level responses of aquatic mesocosms to pulsed doses of a pyrethroid pesticide. - Environ. Toxicol. Chem. 11, 115-129 **(1992).**

Fernandez-Casalderrey, A.; Ferrando, M.D.; Andreu-Moliner, E.: Acute toxicity of several pesticides to the rotifer *Brachionus calyciflorus*. - Bull. Envir. Contam. Toxicol. 48, 14-17 **(1992).**

Ferraro, S.P.; Cole, F.A.: Taxonomic level sufficient for assessing pollution impacts on the Southern California Bight macrobenthos- revisited. - Environ. Toxicol. Chem. 14, 1031-1040 **(1995).**

Ferraro, S.P.; Cole, F.A.: Taxonomic level and sample size sufficient for assessing pollution impacts on the Southern California Bight macrobenthos. – Mar. Ecol. Prog. Ser. 67, 251-262 **(1990).**

ECOS. Statistical package for the evaluation of microcosm studies. Novartis Crop Protection AG, Basel **(1997).**

Fliedner, A.; Klein, W.: Effects of Lindane on the planktonic community in freshwater microcosms. - Ecotoxicol. Environ. Safety 33 (3), 228-235 **(1996).**

Friesen, K.J.; Segstro, M.D.; Muir, D.C.G.; Webster, G.R.B.: Effect of the input pathway on the distribution of 1,2,4,7-Pentachlorodibenzo-p-Dioxin in an aquatic mesocosms. - Environ. Toxicol. Chem. 14 (11), 1921-1929 **(1995).**

Giddings, J.M..: Aquatic mesocosm test for enviromental fate and ecological effects of Diazinon. Unpublished study report (1992).

Gilbert, J.J.: Competition between rotifers and *Daphnia*. – Ecology 66,1943-1950 **(1985).**

Gillespie, W.B., Rodgers, J.H. Jr.; Crossland, N.O.: Effects of a non-ionic surfactant (C14-15 AE-7) on aquatic invertebrates in outdoor stream mesocosms. - Environ. Toxicol. Chem. 15(8), 1418-1422 **(1996).**

Graney, R.L; Kennedy, J.H.; Rodgers, J.H. Jr. (eds.), *Aquatic Mesocosm Studies In Ecological Risk Assessment.* CRC Press Inc. Boca Raton, Florida (1994).

Gray, J.S.; Clarke, K.R.; Warwick, R.M.; Hobbs, G.: Detection of initial effects of pollution on marine benthos: an example from the Ekofisk and Eldfisk oilfields. – Mar. Ecol. Prog. Ser. 66, 285-299 **(1990).**

Gregg, W.W.; Rose, F.L.: Influences of aquatic macrophytes on invertebrate community structure. – Hydrobiologia 128(1), 45-56 **(1985).**

Grobelaar, J.U.: Phytoplankton productivity in turbid waters. - J. Plankton Res. 7, 653-663 **(1985).**

Hambright, K.D.: Can zooplanktivorous fish really affect lake thermal dynamics? - Arch. Hydrobiol. 130(4), 429-438 **(1994).**

HARAP: Guidance Document on Higher-tier Aquatic Risk Assessment for Pesticides (HARAP). Eds: Campbell PJ, Arnold DJS, Brock TCM, Grandy NJ, Heger W, Heimbach F, Maund SJ, Streloke M. Society of Environmental Toxicology ad Chemistry Europe (SETAC-Europe), Brussels, Belgium (1999).

Harris, G.P.: Photosynthesis, productivity and growth: The physiological ecology of phytoplankton. - Arch. Hydobiol. Beih.10, 1-171 **(1978).**

Hassell, M.P.; Comins, H.N.; May, R.M.: Species coexistence and self-organizing spatial dynamics. - Nature 370, 290-292 **(1994).**

Hastings, A.; Higgins, K.: Persistence of transients in spatially structured ecological models.- Science 263, 1133-1136 **(1994).**

Havens, K.E.: Experimental perturbation of a freshwater plankton community: a test of hypothesis regarding the effects of stress. – Oikos 69(1), 147-153 **(1994).**

Hill, I.A.; Heimbach, F.; Leeuwangh, P.; Matthiesen, P. *(eds.)*: Freshwater Field Tests for Hazard Assesment of Chemicals. CRC Press Inc., Boca Raton, Florida **(1994).**

Hitz, H.R: Report on the Growth Inhibition of Algae (Scenedesmus Subspicatus). – unpublished study report, Ciba-Geigy, Basel, 1982.

Hope, A.C.A.: A simplified Monte Carlo significance test procedure. - J. R. Statist. Soc. Ser. 30, 582-598 **(1968).**

Howick, G.L.; Giddings, J.M.; DeNoyelles, F.; Ferrington, L.C. Jr.; Kettle, W.D.; Baker D.: Rapid establishment of test conditions and trophic-level interactions in 0.04-hectare earthen pond mesocosms. - Environ. Toxicol. Chem. 11(1), 107-114 **(1992).**

Huber, W.: Künstliche Testsysteme zur Bewertung von PSM: Herbizide in der aquatischen Umwelt. - UWSF - Z. Umweltchem. Ökotox. 9(6), 355-359 **(1997).**

Hughes, J.S.: The Toxicity of Diazinon Technical to Selenastrum capricornutum. - unpublished study report, 1988.

Hurlbert, S.H.: Secondary effects of pesticides on aquatic ecosystems. – Residue Reviews 58, 81-148 (1975).

Jack, J.D.: The effect of clays on a freshwater plankton community: An enclosure experiment. - Arch. Hydrobiol. 127 (3), 257-270 **(1993).**

Jackson, J.B.C.: Community unity? – Science 264, 1412-1413 **(1994).**

Joern, A.: In defense of whole-community bioassays for risk assessment. - Environ. Toxicol. Chem. 15, 407-409 **(1996).**

Johnson, M.L.; Huggins, D.G.; deNoyelles, F. Jr.: Structural equation modelling and ecosystem analysis. In: *Aquatic Mesocosm Studies In Ecological Risk Assessment.* IR.L. Graney, J.H. Kennedy and J.H. Rodgers Jr. (eds.), CRC Press Inc. Boca Raton, Florida (1994).

Karentz, D., Smayda, T.: Temporal patterns and variations in phytoplankton community organization and abundance in the Narrangasett Bay during 1959-1980. – J. Plankton Res. 20, 145-180 **(1998).**

Kedwards, T.J.; Maund, S.J.; Chapman, P.F.: Community level analysis of ecotoxicological field studies: 1. Biological monitoring. - Environ. Toxicol. Chem. 18 (2), 149-157 **(1999).**

Kennedy, J.H.; Ammann, L.P.; Waller, W.T.; Warren, J.E.; Hosmer, J.H.; Cairns, S.H.; Johnson, P.C.; Graney, R.L.: Using statistical power to optimize sensitivity of analysis of variance designs for microcosms and mesocosms. - Environ. Toxicol. Chem.18 (2), 113-117 **(1999).**

Kersting, K.; Van Den Brink, P.J.: Effects of the insecticide Dursban 4E (active ingredient chlorpyrifos) in outdoor experimental ditches: Responses of ecosystem metabolism. - Environ. Toxicol. Chem. 16 (2), 251-259 **(1997).**

Kirk, J.T.O.: Effects of suspensoids (turbidity) on penetration of solar radiation in aquatic ecosystems. – Hydrobiologia 125, 195-208 **(1985).**

Kirk, K.L.; Gilbert, J.J.: Suspended clay and the population dynamics of planktonic rotifers and cladocerans. – Ecology 71 (5), 1741-1755 **(1990).**

Knauer, K.; Rufli, H.; Gonzalez-Valero, J.: Importance of the experimental design of mesocosm studies. SETAC Europe Congress, Brighton **(2000).**

Koenigs, J.P.; Burkett, R.D.; Edmundson, J.M.: The exclusion of limnetic cladocera from turbid glacier-meltwater lakes. – Ecology 71 (1), 57-67 **(1990).**

Kratz, T.K.; Frost, T.M.; Magnuson J.J.: Inferences from spatial and temporal variability in ecosystems: long-term zooplankton data from lakes. - American Naturalist 129(6), 830-846 **(1987).**

Kruskal, J.B.; Wish, M.: Multidimensional Scaling. Sage Publications, Beverly Hills, California **(1978).**

Lang, M.M.: Das Nahrungsnetz im Pelagial des Bodensees: eine Analyse auf der Basis der Theorie der Nahrungsnetze. Konstanzer Dissertationen, Hartung-Gorre Verlag, Konstanz **(1997).**

Lawton, J.H.; Brown, V.K. In: Biodiversity and ecosystem function, Schulze, E.D.; Mooney, H.A. (eds.), Springer Verlag Berlin (1993).

Landis,W.G.; Matthews, R.A.; Matthews, G.B.: The layered and historical nature of ecological systems and the risk assessment of pesticides. - Environ. Toxicol. Chem. 15(4), 432-440 **(1996).**

LeLievre, M.K.: (Diazinon Technical) Acute Toxicity to Ceriodaphnids (Ceriodaphnia dubia) under Static Conditions. – Unpublished study report, Ciba-Geigy, Basel **(1991).**

Li, H.; Reynolds, J.F.: A simulation experiment to quantify spatial heterogeneity in categorical maps. – Ecology 75 (8), 2446-2455 **(1994).**

Liber, K.; Kaushok, N.K.; Solomon, K.R.; Carey, J.H.: Experimental designs for aquatic mesocosm studies: a comparison of ANOVA and REGRESSION design for assessing the impact of tetrachlorophenol on zooplankton populations in limnocorrals. - Environ. Toxicol. Chem. 11 (1), 61-77 **(1992).**

Lowe, R.L.; Guckert, J.B.; Belanger, S.E.; Davidson, D.H.; Johnson, D.W.: An evaluation of periphyton community structure and function on tile and cobbe substrata in experimental stream mesocosms.- Hydrobiologia 328, 135-146 **(1996).**

Lozano, S.J.; O´Halloran, S.L.; Sargant, K.W.: Effects of Esfenvalerate on aquatic organisms in littoral enclosures. - Environ. Toxicol. Chem. 11, 35-47 **(1992).**

Lundgren, A.: Model ecosystems as a tool in freshwater and marine research. - Arch. Hydrobiol. Suppl. 70, 157-196 (1985).

Malone, B.J.; McQueen, D.J.: Horizontal patchiness in zooplankton populations in two Ontario kettle lakes. – Hydrobiologia 99, 101-124 **(1983).**

Mantel, N,: The detection of disease: clustering and a generalized regression approach. Cancer Res. 27, 209-220 **(1967).**

Matthews, R.A.; Landis, W.G.; Matthews, G.B.: The community conditioning hypothesis and its application to environmental toxicology. - Environ. Toxicol. Chem. 15(4), 597-603 **(1996).**

Maund, S.; Chapman, P.; Kedwards, T.; Tattersfield, L.; Matthiessen, P.; Warwick, R.; Smith, E.: Application of Multivariate statistics to ecotoxicological field studies. - Environ. Toxicol. Chem. 18 (2), 111-112 **(1999).**

Maund, S.J.; Sherratt, T.N.; Stickland, T.; Biggs, J.; Williams, P.; Shillabeer, N.; Jepson, P.J.: Ecological considerations in pesticide risk assessment for aquatic ecosystems. - Pestic. Sci. 49, 185-190 **(1997).**

Maurer, A.B.: Ecological Science and statistical paradigms: at the threshold. – Science 279, 502-503 **(1998).**

Neill, W.E.: Induced vertical migration in copepods as a defence against invertebrate predation. – Nature 345, 524-525 **(1990).**

Odum, E.P.:Trends expected in stressed ecosystems. – BioScience 35, 419-422 **(1985).**

Olsgard, F.; Somerfield, P.J.; Carr, M.R.: Relationships between taxonomic resolution and data transformations in analyses of a macrobenthic community along an established pollution gradient. – Mar. Ecol. Prog. Ser. 149, 173-181 **(1997).**

Oviatt, C.A.; Perez, K.T.; Nixon, S.W.: Multivariate analyses of experimental marine ecosystems. - Helgoländer Wiss. Meeresunters. 30, 30-46 **(1977).**

Perez, K.T.; Morrison, G.M.; Lockie, N.F.; Oviatt, C.A.; Nixon, S.W.; Buckley, B.A.; Heltsche, J.F.: The importance of physical and biotic scaling to the experimental simulation of a coastal marine ecosystem. - Helgoländer Wiss. Meeresunters. 30,144-162 **(1977).**

Perry, J.A.; Toelstrup, N.H. Jr.: Whole ecosystem manipulation: a productive avenue for test system research? - Environ. Toxicol. Chem. 7, 941-951 **(1988).**

Persaud, A.D.; Yan, N.D.: Accounting for spatial variability in the design of sampling programmes for chaoborus larvae. – J. Plankton Res. 23 (3), 279-285 **(2001).**

Pimm, S.L.: The complexity and stability of ecosystems. - Nature 307, 321-326 **(1984).**

Pickett, S.T.A.; White, P.S.: The ecology of natural disturbance and patch dynamics. Academic Press Inc., Orlando, Florida (1985).

Pinel-Alloul, B.; Downing, J.A.; Perusse, M.; Codin-Blumer, G.: Spatial heterogeneity in freshwater zooplankton: variation with body size, depth and scale. – Ecology 69 (5), 1393-1400 **(1988).**

Pontasch, K.W.; Smith, E.P.; Cairns, J. Jr.: Diversity indices, community comparison indices and canonical discriminant analysis: interpreting the results of multispecies toxicity tests. - Water Res. 23, 1229-1238 **(1989).**

PRIMER Software Package. Plymouth Marine Laboratory, Plymouth, UK **(1994).**

Pusey, B.J.: The effects of pulsed application of chlorpyrfos on macroinvertebrate communities in an outdoor artificial stream system. - Ecotoxicol. Environ. Safety 27, 221-250 **(1994).**

Reynolds, C.S.: Temporal scales of variability in pelagic environments and the response of phytoplankton. - Freshwater Biology 23, 25-53 **(1990).**

Rodgers, J.H. Jr; Crossland, N.O.; Kline, E.R.; Gillespie, W.B. Jr.; Figueroa, R.A.; Dorn, P.B.: Design and construction of model stream ecosystems. - Ecotoxicol. Environ. Safety 33, 30-37 **(1996).**

Rosenzweig, M.S.; Buikema A.L. Jr.: Phytoplankton colonization and seasonal succession in new experimental ponds. - Environ. Toxicol. Chem. 13 (4), 599-605 **(1994).**

Sanders, H.O.; Cope, O.B.: Toxicities of several pesticides to two species of cladocerans. - Trans. Am. Fish Soc. 95, 165-169 **(1966).**

Schaub, D.: Beitrag der Bodenerosion zum diffusen Stoffeintrag in Oberflaechengewaesser der Schweiz. – Ingenieurbiologie 2, 4-6 **(1997).**

Schelske,C.L.; Carrick, H.J.; Aldridge, F.J.: Can wind-induced resuspension of meroplankton affect phytoplankton dynamics? - J. N. Amer. Benthol. Soc. 14, 616-630 **(1995).**

Schwoerbel, J.: Einfuehrung in die Limnologie. Gustav Fischer Verlag Stuttgart, 8. Aufl. (1999).

Schwoerbel, J.: Methoden der Hyrobiologie, UTB (1994).

SCP. Opinion of the scientific committee on plants regarding the inclusion of esfenvalerate in annex 1 of council directive 91/414/eec concerning the placing of plant protection products on the market. - Scientific Committee on Plants SCP/ESFEN/002-final. 6 April 2000, European Commission, Brussels **(2000).**

Seiberth, C.: Messung der DOC und POC Austraege ueber den Vorfluter des Einzugsgebietes Laenebachtal. Undergraduate Thesis, University of Basel, Switzerland,126 pp **(1997).**

SETAC Guidance Document on methodologies in outdoor aquatic field studies. Society of Environmental Toxicology ad Chemistry Europe (SETAC-Europe), Brussels, Belgium **(1994).**

SETAC Guidance Document on testing procedures for pesticides in freshwater static mesocosms. Report from the meeting held at Monks Wood Experimental Station, Huntingdon, UK, 3-4 July, 1991. Society of Environmental Toxicology ad Chemistry Europe (SETAC-Europe), Brussels, Belgium **(1992a).**

SETAC Proceedings of a workshop on aquatic microcosms for ecological assessment of pesticides (Wintergreen, Virginia, USA, October 1991) SETAC Foundation for environmental education and the RESOLVE program of the World Wildlife Fund **(1992b).**

Seitz, A.: The concept of ecological stability applied to aquatic ecosystems. In: *Freshwater Field Tests for Hazard Assesment of Chemicals.* Hill, I.A.; Heimbach, F.; Leeuwangh, P.; Matthiesen, P. *(eds.)*. CRC Press Inc., Boca Raton, Florida **(1994).**

Shaw, J.L.; Kennedy, J.H.: The use of aquatic field mesocosm studies in risk assessment. - Environ. Toxicol. Chem. 15, 605-607 **(1996).**

Sibley, P.K.; Harris, M.L.; Bestari, K.T.; Steele, T.A.; Robinson, R.D.; Gensemer, R.W.; Day, K.E.; Solomon, K.R.: Response of zooplankton communities to liquid creosote in freshwater microcosms. - Environ. Toxicol. Chem. 20 (2), 394-405 **(2001).**

Snell, T.W.; Moffat, B.D.: A two-day life-cycle test wit *Brachionus calyciflorus*. - Environ. Toxicol. Chem. 11, 1249-1257 **(1992).**

Somerfield, P.J.; Clarke, K.R.: Taxonomic levels in marine community studies revisited. – Mar. Ecol. Prog. Ser. 127, 113-119 **(1995).**

Sommer, U.; Gliwicz, Z.M.; Lampert, W.; Duncan, A.: The PEG-model of seasonal succession of planktonic events in fresh waters. – Arch. Hydrobiol. 106(4), 433-471 **(1986).**

Sousa, W.P.: The role of disturbance in natural communities. - Annual Rev. Ecol. Syst. 15, 353-391 **(1984).**

Stenalt, E.T.; Johansen, B., van Lillienskold, S.; Hansen, B.W.: Mesocosm study of *Mytilus edulis* larvae and postlarvae, including the settlement phase, exposed to a gradient of tributyltin. - Ecotoxicol. Environ. Safety 40, 212-225 **(1998).**

Stone, L.: Ecosystem resilience, stability and productivity. - American Naturalist 148 (5), 892-903 **(1996).**

Stone, L.; Weisburd, R.S.J.: Positive feedback in aquatic ecosystems. - Trends in Ecology and Evolution 7, 263-267 **(1992).**

Sugiura K.: A multispecies laboratory microcosm for screening ecotoxicological impacts of chemicals. - Environ. Toxicol. Chem. 11(9), 1217-1226 **(1992).**

Surprenant, D.C.: The Chronic Toxicity of 14C-Diazinon Technical to Daphnia Magna under Flow-Through Conditions. – Unpublished study report, Ciba-Geigy, Basel **(1988).**

Suter, G.W.: Endpoints for regional ecological risk assessments. - Environmental Management 14, 9-23 **(1990).**

Tilmann, D.; Downing, J.A.: Biodiversity and stability in grasslands. - Nature 367, 363-365 **(1994).**

Valone, T.J.; Brown, J.H.: Effects of competition, colonization, and extinction on rodent species diversity. – Science 267, 880-882 **(1995).**

Van den Brink, P.J.; Ter Braak, C.J.F.: Principal response curves: Analysis of time-dependent multivariate responses of biological community to stress. - Environ. Toxicol. Chem. 18, 138-148 **(1999).**

Van Den Brink, P.J.; van Donk, E.; Gylstra, R.; Crum, S.J.H.; Brock, T.C.M.: Effects of chronic low concentrations of the pesticides chlopyrifos and atrazine in indoor freshwater microcosms. – Chemosphere 31 (5), 3181-3200 **(1995).**

Van Den Brink, P.J.; Van Wijngaarden, R.P.A.; Lucassen, W.G.H.; Brock, T.C.M.; Leeuwangh, P.: Effects of the insecticide Dursban 4E (active ingredient chlorpyrifos) in outdoor experimental ditches: 2. Invertebrate community responses and recovery. -Environ. Toxicol. Chem. 15 (7), 1143-1153 **(1996).**

Van Donk, E.; Prins, H.; Voogd, H.M.; Crum, S.J.H.; Brock, T.C.M.: Effects of nutrient loading and insecticide application on the ecology of Elodea-dominated freshwater microcosms: 1. Responses of plankton and zooplanktivorous insects. - Arch. Hydrobiol. 133 (4), 417-439 **(1995).**

Van Wijngaarden, R.P.A.; Van Den Brink, P.J.; Oude Voshaar, J.H.; Leeuwangh, P.: Ordination techniques for analysing response of biological communities to toxic stress in experimental ecosystems. – Ecotoxicology 4, 61-77 **(1995).**

Vanni, M.J.: Effects of nutrients and zooplankton size on the structure of a phytoplankton community. – Ecology 68, 624 **(1987).**

Vuille, T.B.: Abundance, standing crop and production of microcrustacean populations (Cladocera, Copepoda) in the littoral zone of Lake Biel, Switzerland. - Arch. Hydrobiol. 123, 165-185 **(1991).**

Vitousek, P.M.; Hopper, D.U. In: Biodiversity and ecosystem function, Schulze, E.D.; Mooney, H.A. (eds.), Springer Verlag Berlin (1993).

Warwick, R.M.: Environmental impact studies on marine communities: pragmatical considerations. – Australian J. Ecol. 18, 63-80 **(1993).**

Warwick, R.M.; Platt, H.M.; Clarke, K.R.; Agard, J.; Robin, J.: Analysis of macrobenthic community structure in relation to pollution and disturbance in Hamilton Harbour, Bermuda. – J. Exp. Mar. Biol. Ecol. 138, 119-142 **(1990).**

Woin, P.: Short- and long-term effects of the pyrethroide insecticide fenvalerate on an invertebrate pond community. – Ecotox. Env. Safety 41, 137-156 (1998).

Taxonomic identification of zoo- and phytoplankton

BAYERISCHES LANDESAMT FÜR WASSERWIRTSCHAFT 1990: Biologische Gewässeranalyse in Bayern - Taxaliste der Gewässerorganismen. Informationsberichte Bayer. Landesamt für Wasserwirtschaft 4/90, Loseblattsammlung.

ENGELHARDT, W. 1989: Was lebt in Tümpel, Bach und Weiher. Franckh'sche Verlagsbuchhandlung, W. Keller & Co., Stuttgart. (Kosmos Naturführer). 13. Auflage

ETTL, H., GERLOFF, J., HEYNING, H., MOLLENHAUER, D. (Eds.) (1978-1999): Süßwasserflora von Mitteleuropa. (Begründet von A.Pascher), *Gustav Fischer Verlag, Stuttgart*:

FLÖßNER, D. 1972: Krebstiere, Crustacea; Kiemen- und Blattfüßer, Branchipoda, Fischläuse, Branchiura. In: Die Tierwelt Deutschlands, Teil 60. Gustav Fischer Verlag, Jena.

GERMAIN, H. 1981: Flore des diatomées. Eaux douces et saumatres du Massif Amoricain et des contrées voisins d'Europe occidentale. Collection "Faunes et Flores actuelles", Société Nouvelles des Éditions Boubée, Paris.

HERBST, H. V. 1976: Blattfußkrebse, Phyllopoden. Echte Blattfüßer und Wasserflöhe. Franckh Verlag, Stuttgart (Kosmos).

HUBER-PESTALOZZI, G. 1950: Das Phytoplankton des Süßwassers. Teil 3: Cryptophyceae, Peridineae. In: Die Binnengewässer, E. Schweizerbart, Stuttgart.

HUBER-PESTALOZZI, G. 1955: Das Phytoplankton des Süßwassers. Teil 4: Euglenophyceae. In: Die Binnengewässer, E. Schweizerbart, Stuttgart.

HUBER-PESTALOZZI, G. 1961: Das Phytoplankton des Süßwassers. Teil 5: Chlorophyceae. Volvocales. In: Die Binnengewässer, E. Schweizerbart, Stuttgart.

HUBER-PESTALOZZI, G. 1962a: Das Phytoplankton des Süßwassers. Teil 1: Blaualgen. Bakterien. Pilze In: Die Binnengewässer, E. Schweizerbart, Stuttgart.

HUBER-PESTALOZZI, G. 1962b: Das Phytoplankton des Süßwassers. Teil 2/1: Chrysophyceae In: Die Binnengewässer, E. Schweizerbart, Stuttgart.

HUBER-PESTALOZZI, G. 1962c: Das Phytoplankton des Süßwassers. Teil2/2: Diatomeae. In: Die Binnengewässer, E. Schweizerbart, Stuttgart.

HUBER-PESTALOZZI, G. 1972: Das Phytoplankton des Süßwassers. Teil 6: Chlorophyceae. Tetrasporales In: Die Binnengewässer, E. Schweizerbart, Stuttgart.

HUBER-PESTALOZZI, G. 1982: Das Phytoplankton des Süßwassers. Teil 8/1: Conjugatophyceae. Zygnematales, Desmidiales, excl. Zygnemataceae. In: Die Binnengewässer, E. Schweizerbart, Stuttgart.

HUBER-PESTALOZZI, G. 1983: Das Phytoplankton des Süßwassers. Teil 7/1: Chlorophyceae. Chlorococcales. In: Die Binnengewässer, E. Schweizerbart, Stuttgart.

HUSTEDT, F. 1965: Kieselalgen. Franckh Verlag, Stuttgart (Kosmos).

KIEFER, F. 1973: Ruderfußkrebse, Copepoden. Franckh Verlag, Stuttgart (Kosmos).

KIEFER,F. 1978: Freilebende Copepoda. Das Zooplankton der Binnengewässer, Teil 2. In: Die Binnengewässer, Bd. 26, E. Schweizerbart, Stuttgart.

KRAMER, K. 1986: Kieselalgen. Biologie, Baupläne der Zellwand, Untersuchungsmethoden. Franckh'sche Verlagsbuchhandlung, W. Keller & Co., Stuttgart. (Kosmos)

LÖFFLER, H. 1972: Ostracoda. Das Zooplankton der Binnengewässer, Teil 1. Bestimmungsschlüssel und Abbildungen. In: Die Binnengewässer, Bd. 26 1. Teil, 235 - 246. E. Schweizerbart Verlagsbuchhandlung, Stuttgart.

RUTTNER-KOLISKO, A. 1972: Rotatoria. Das Zooplankton der Binnengewässer, Teil 1, 99-234. In: Die Binnengewässer, Bd. 26, E. Schweizerbart, Stuttgart.

SKUJA, H. 1965: Grundzüge der Algenflora und Algenvegetation der Fjeldgegenden um Abisko in Schwedisch-Lappland. Nova Acta Regiae Societatis Scientiarum Upsaliensis, Ser. 4, Vol. 18. Nr.3.

STREBLE, H. und D. KRAUTER 1978: Das Leben im Wassertropfen. Franckh'sche Verlagsbuchhandlung, W. Keller & Co., Stuttgart. 4. Auflage.

VOIGT, M. und W. Koste 1978: Rotatoria. Die Rädertiere Mitteleuropas. Begr. von M. Voigt; neu bearbeitet von W. Koste. Gebrüder Bornträger, Berlin, Stuttgart. 2. Auflage.

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The skills of colleagues at Syngenta and at the Technical University of Munich greatly helped evaluating the vast amount of samples collected during the experimental phase of the study. Among those to appear on this page are certainly Ursula Davo and Eva Sandmann who performed the taxonomic identification of phyto- and zooplankton with great expertise. I thank Gabriele Thoma for sharing her experience and field & laboratory support, and Thorsten Behsen for performing the analytical analysis of Diazinon samples. I gratefully acknowledge Heinz Schmidli for statistical advice.

To the many colleagues and friends who were so generous in allowing their time, sharing their experience and offering their help, I have the pleasure to count Steve Maund, Jeff Giddings, Paul van den Brink and Theo Brock. Many thanks to Christian Verschueren and Pierre Urech who allowed me to take off some weeks to finalize my thesis while I was working for them.

Finally, my thanks go to my husband, children, parents and brothers who were a source of inspiration and always whole-heartedly committed to allow me the time to work on this thesis during the past years.

Appendix A: Macrophytes

Percent cover of macrophytes and presence of filamentous algae. July 10, 1997 (test day 0).

Pond	Class	% cover				Fil. Algae
		Potamogeton spec.	*Chara* spec.	*Myriophyllum* spec.	Total macrophytes	
1	L	6	12	0	18	o
2	H	34	6	0	40	xx
3	M	18	8	0	26	x
4	H	40	5	0	46	x
5	M	8	12	0	20	x
6	H	8	24	4	36	x
7	M	12	12	4	28	xx
8	M	12	8	0	20	o
9	M	2	12	8	22	x
10	L	2	10	0	12	o
11	M	16	8	8	32	x
12	H	35	8	0	43	xxx
13	L	4	4	0	8	xxx
14	L	4	4	0	8	xxx
15	L	4	8	0	12	xx
16	M	4	20	0	24	x
17	M	2	20	0	22	o
18	M	12	12	0	24	x
19	M	16	8	0	24	o
20	M	12	8	4	24	xx
21	L	2	12	0	14	o
22	M	8	12	0	20	o
23	H	0	8	24	32	x
24	H	36	12	0	48	o
25	H	24	8	0	32	o
26	L	8	2	0	10	x
27	L	6	6	0	12	x
28	L	12	2	0	14	o
Mean		12	10	2	24	

Legend:

Classification according to % total cover:
L: Low (<20%)
M: Medium (20-30%)
H: High (>30%)

Estimation of filamentous algae:
o: none
x: low abundance
xx: medium abundance
xxx: high abundance

Percent cover of macrophytes and presence of filamentous algae.
August 15, 1997 (test day 36).

Pond	Class	*% cover*				Fil. Algae
		Potamogeton spec.	*Chara* spec.	*Myriophyllum* spec.	Total macrophytes	
1	M	4	25	0	29	x
2	L	0	12	0	12	x
3	T	T	T	T	T	T
4	T	T	T	T	T	T
5	H	0	38	0	38	xx
6	H	0	60	0	60	x
7	T	T	T	T	T	T
8	M	0	25	0	25	x
9	H	0	12	25	37	o
10	M	0	30	0	30	o
11	H	0	33	0	33	o
12	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
13	T	T	T	T	T	T
14	T	T	T	T	T	T
15	M	0	20	0	20	xx
16	M	0	20	0	20	xx
17	L	0	12	0	12	o
18	M	0	20	0	20	x
19	L	0	12	0	12	o
20	L	0	6	6	12	x
21	T	T	T	T	T	T
22	L	0	12	0	12	x
23	H	0	6	36	42	o
24	M	0	30	0	30	o
25	L	0	12	0	12	x
26	T	T	T	T	T	T
27	L	2	2	0	4	x
28	L	0	8	0	8	o
Mean		0,3	20	3	23	

Legend

Classification according to % total cover:
L: Low (<20%)
M: Medium (20-30%)
H: High (>30%)
T: turbid

Estimation of filamentous algae:
o: none
x: low abundance
xx: medium abundance
xxx: high abundance

Note:
n.a.: Pond 12 had developed a leak during the study and was therefore excluded from the evaluation.
T: Some ponds were highly turbid and plants were not visible.

Percent cover of macrophytes and presence of filamentous algae.
October 27, 1997 (test day 109).

Pond	Class	*% cover*				Fil. Algae
		Potamogeton spec.	*Chara* spec.	*Myriophyllum* spec.	Total macrophytes	
1	L	8	0	0	8	x
2	H	0	70	0	70	o
3	H	0	60	0	60	o
4	T	T	T	T	T	T
5	M	2	20	0	22	x
6	H	6	30	0	36	x
7	H	0	50	0	50	o
8	H	4	40	0	44	xx
9	H	0	50	0	50	o
10	H	0	50	0	50	o
11	H	0	80	0	80	o
12	na	na	na	na	na	na
13	T	T	T	T	T	T
14	T	T	T	T	T	T
15	M	0	30	0	30	xx
16	H	0	50	0	50	xx
17	H	0	80	0	80	xx
18	H	12	24	0	36	xxx
19	H	8	50	0	58	xx
20	H	4	40	0	44	o
21	T	T	T	T	T	T
22	H	0	60	0	60	o
23	H	0	60	0	60	o
24	H	0	50	0	50	xx
25	L	0	0	0	0	x
26	H	0	0	70	70	o
27	L	0	0	0	0	xx
28	L	0	8	0	8	x
Mean		2	39	3	44	

Legend

Classification according to % total cover:
L: Low (<20%)
M: Medium (20-30%)
H: High (>30%)
T: turbid

Estimation of filamentous algae:
o: none
x: low abundance
xx: medium abundance
xxx: high abundance

Note:
1) Pond 12 had developed a leak during the study and was therefore excluded from the evaluation.
2) Some ponds were highly turbid (T) and plants were not visible.

Development of macrophytes and filamentous algae in control and treatment groups.

		Macrophyte cover			Filamentous Algae		
Treatment	Pond	July 10	Aug 25	Oct 27	July 10	Aug 25	Oct 27
CONTROL	5	M	H	M	x	xx	x
CONTROL	6	H	H	H	x	x	x
CONTROL	8	M	M	H	o	x	xx
CONTROL	9	M	H	H	x	o	o
CONTROL	10	L	M	H	o	o	o
CONTROL	11	H	H	H	x	o	o
CONTROL	18	M	M	H	x	x	xxx
CONTROL	20	M	L	H	xx	x	o
CONTROL	22	M	L	H	o	x	o
CONTROL	24	H	M	H	o	o	xx
CONTROL	27	L	L	L	x	x	xx
CONTROL	28	L	L	L	o	o	x
SHADOW	1	L	M	L	o	x	x
SHADOW	2	H	L	H	xx	x	o
SHADOW	15	L	M	M	xx	xx	xx
SHADOW	16	M	M	H	x	xx	xx
TURBULENCE	3	M	M	H	x	T	o
TURBULENCE	7	M	M	H	xx	T	o
TURBULENCE	12	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
TURBULENCE	17	M	L	H	o	o	xx
RUNOFF	19	M	L	H	o	o	xx
RUNOFF	23	H	H	H	x	o	o
RUNOFF	25	H	L	L	o	x	x
RUNOFF	26	L	L	H	x	T	o
DIAZINON	4	H	H	T	x	T	T
DIAZINON	13	L	L	T	xxx	T	T
DIAZINON	14	L	L	T	xxx	T	T
DIAZINON	21	L	L	T	o	T	T

Legend

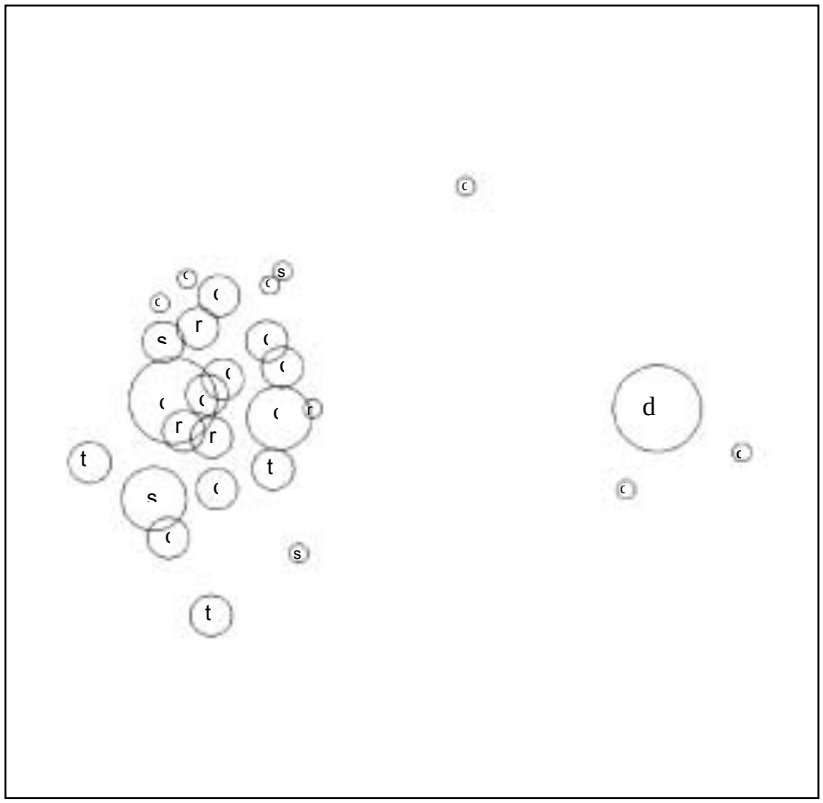
Classification according to % total cover:
L: Low (<20%)
M: Medium (20-30%)
H: High (>30%)
T: turbid

Estimation of filamentous algae:
o: none
x: low abundance
xx: medium abundance
xxx: high abundance

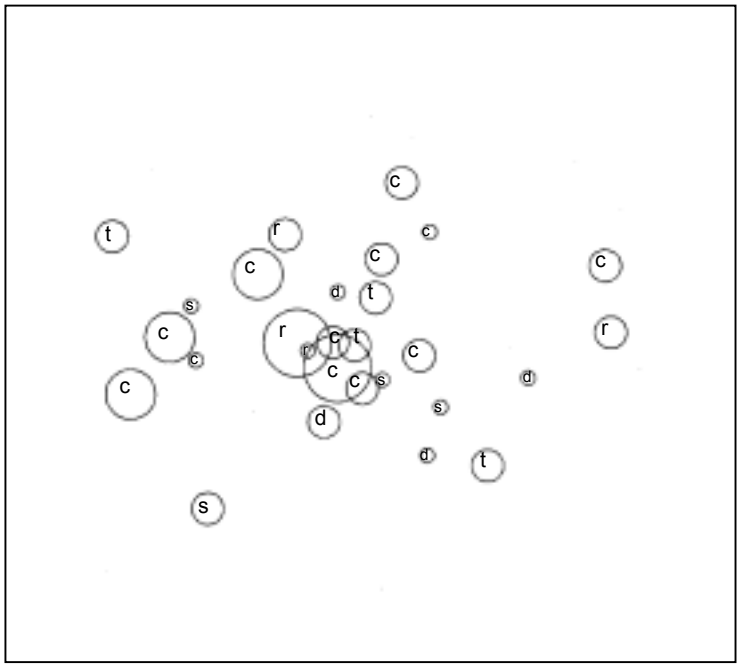
1) Pond 12 had developed a leak during the study and was therefore excluded from the evaluation.
2) Some ponds were highly turbid (T) and plants were not visible.

Note : the community structure of phytoplankton and zooplankton did not show any correlation to macrophyte cover, when superimposing the classification according to percent macrophyte cover to Multidimensional Scaling Plots for phytoplankton community on day 0 and for zooplankton on day 28.

Zooplankton Day 28



Phytoplankton Day 0

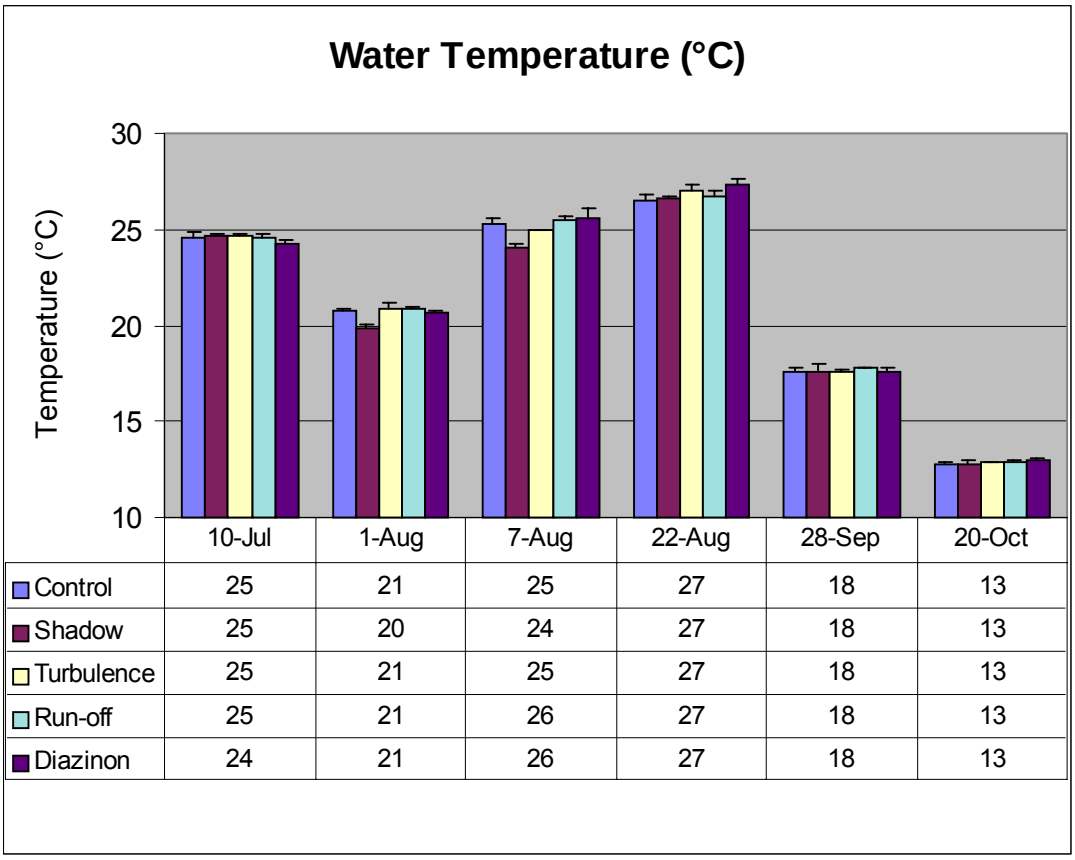


Appendix B: Physical-chemical Measurements

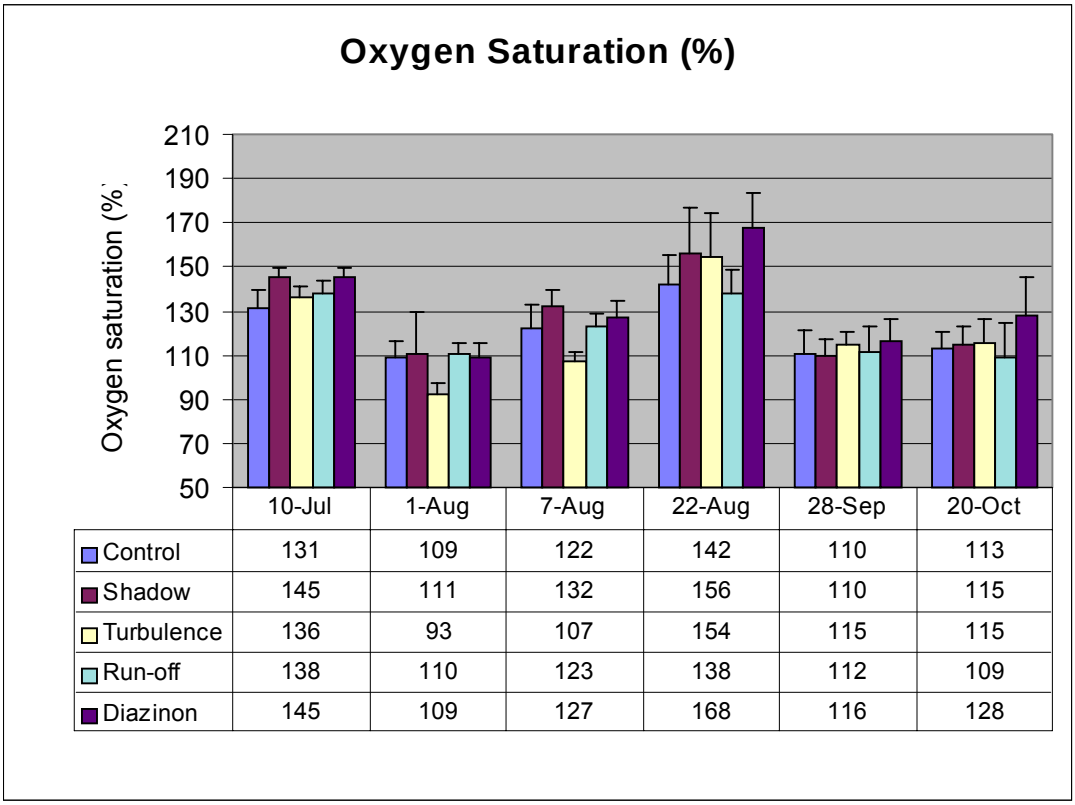
Measurement Devices

Parameter (unit)	Measurement device
Temperature (°C)	WTW OXI 323 dissolved oxygen- and temperature meter with a WTW Ox 325 probe.
Dissolved oxygen (%)	WTW OXI 323 dissolved oxygen- and temperature meter with a WTW Ox 325 probe. Dissolved Oxygen was measured as (mg/L) and transformed into % saturation, taking into account the measured temperature.
pH	WTW pH 96 meter with a WTW pH probe.
Conductivity (µS/cm)	WTW LF 330 conductivity meter with a WTW TeraCon 325 probe.
Light intensity ($\mu Em^{-2}s^{-1}$)	Quantum Meter LI 190 SB (400-700 nm) and a LI 185 B sensor of Bachofer, D-Reutlingen.
Turbidity (Nephleometric Turbidity Units, NTU).	Prozess-Trübungsmesser, Monitek Modell 160/31, Polyaqua AG, CH- Wiedlisbach.
Turbulence (cm/s)	Flowmeter, University of Freiburg, Germany. As described in Biehle, G.: Untersuchungen zur Hydrodynamik, Biomechanik, Morphologie und Funktionsanatomie von Wassermoosen am Beispiel von *Fontinalis antipyretica*. Diplomarbeit, Albert-Ludwigs-Universität Freiburg im Breisgau (1996).

Measurements

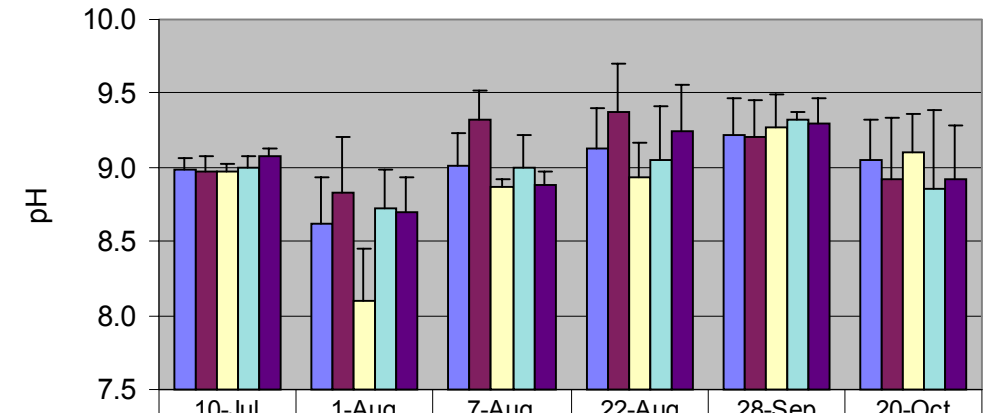


	10-Jul	1-Aug	7-Aug	22-Aug	28-Sep	20-Oct
Control	25	21	25	27	18	13
Shadow	25	20	24	27	18	13
Turbulence	25	21	25	27	18	13
Run-off	25	21	26	27	18	13
Diazinon	24	21	26	27	18	13



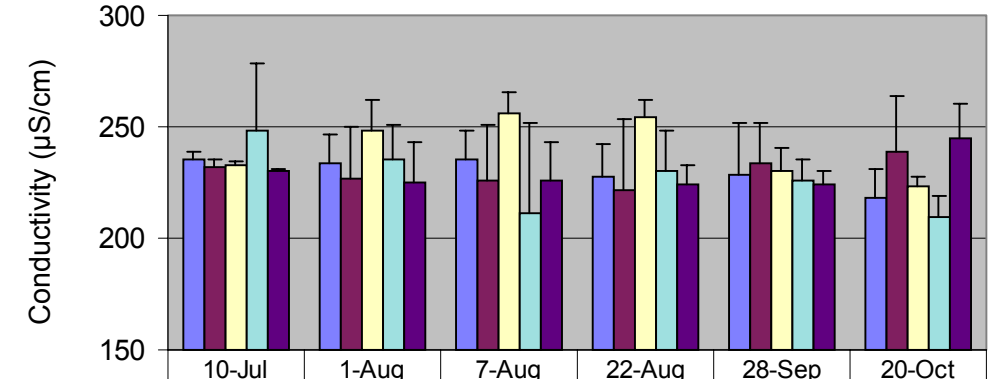
	10-Jul	1-Aug	7-Aug	22-Aug	28-Sep	20-Oct
Control	131	109	122	142	110	113
Shadow	145	111	132	156	110	115
Turbulence	136	93	107	154	115	115
Run-off	138	110	123	138	112	109
Diazinon	145	109	127	168	116	128

pH

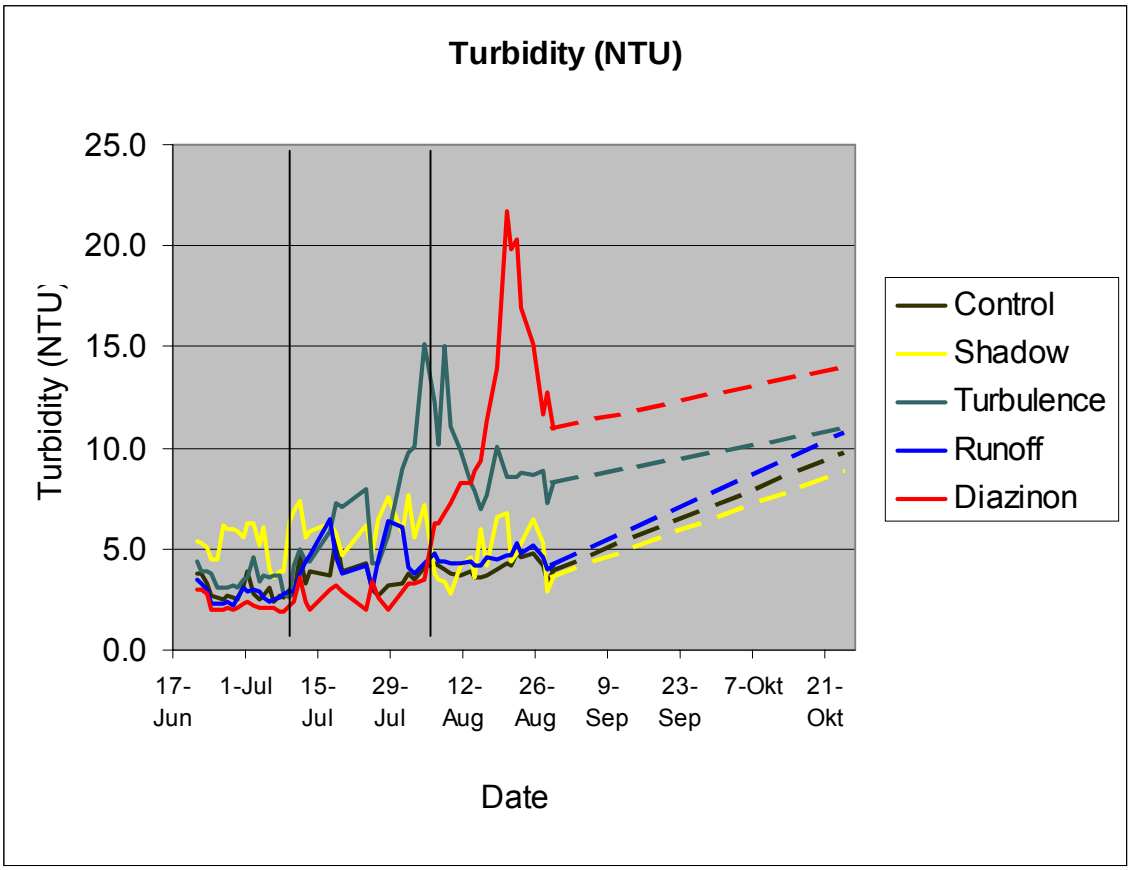


	10-Jul	1-Aug	7-Aug	22-Aug	28-Sep	20-Oct
Control	9.0	8.6	9.0	9.1	9.2	9.1
Shadow	9.0	8.8	9.3	9.4	9.2	8.9
Turbulence	9.0	8.1	8.9	8.9	9.3	9.1
Run-off	9.0	8.7	9.0	9.1	9.3	8.9
Diazinon	9.1	8.7	8.9	9.3	9.3	8.9

Conductivity (µS/cm)



	10-Jul	1-Aug	7-Aug	22-Aug	28-Sep	20-Oct
Control	235	234	236	227	228	218
Shadow	232	227	226	222	234	239
Turbulence	232	248	256	254	230	224
Run-off	248	236	212	231	226	210
Diazinon	230	225	226	224	224	245


Turbidity (NTU)
25.0
20.0
15.0
10.0
5.0
0.0
Turbidity (NTU)
17-Jun
1-Jul
15-Jul
29-Jul
12-Aug
26-Aug
9-Sep
23-Sep
7-Okt
21-Okt
Date
Control
Shadow
Turbulence
Runoff
Diazinon

APPENDIX C

ANALYTICAL RESULTS

Determination of Diazinon
in an aqueous medium of an ecotoxicological test

Table of Contents

1. Materials and Methods

A. Specimens	Received for analysis	Analyzed
Day 0, 14, 28	April 13-14, 1998	April 13-14, 1998

Storage of specimens : Frozen at -18 to -22 °C

B. Method

Analytical method: AM98-02 (effective from February 12, 1998)

Abstract of AM 98-02: 200ml of the specimens were extracted on a solid phase extrraction column and eluted with 2mL of acetonitrile. The extract was made up to 4mL with bidistilled water.
Analysis was performed on a HPLC-Backflush system, using two columns:

1. Nucleosil C18, µm, 10 x 4.6 mm, Bischoff No. 63021835
2. Supelcosil LC-ABZ Plus, 5µm, 250 x 2.1 mm, Supelco No. 5-7927

Deviation to method:

1. Only one injection per vial was made
2. Calculation was made with the PC100 HPLC Calculation Software, Themo Separation Products.

Recovery rates : The recoveries were performed right before or during the determination of the specimens.

Days 0 - 28	Diazinon = 105 %	(N = 2, RSD = 1.7 %)

LOD/ LOQ The LOD under this conditions is 0.3µg/L, the LOQ is 0.8µg/L

Abbreviations used

N:	Number of determinations
RSD:	Relative standard deviation
LOD:	Limit of detection
LOQ:	Limit of quantification

2. Results

2.1 Analysis of the test specimens

Pond 4 Sampling day [d]	Concentration determined Diazinon [ug/L]	Values corrected for recovery rate Diazinon [µg/L]	% of nominal concentration Diazinon [%]
0	14.5	15.2	95
14	4.36	4.57	29
28	2.08	2.18	14
Pond 13 Sampling day [d]	**Concentration determined Diazinon [ug/L]**	**Values corrected for recovery rate Diazinon [µg/L]**	**% of nominal concentration Diazinon [%]**
0	40.4	42.4	265
14	7.88	8.27	52
28	4.88	5.12	32
Pond 14 Sampling day [d]	**Concentration determined Diazinon [ug/L]**	**Values corrected for recovery rate Diazinon [µg/L]**	**% of nominal concentration Diazinon [%]**
0	14.6	15.3	96
14	5.4	5.67	35
28	2.6	2.73	17
Pond 21 Sampling day [d]	**Concentration determined Diazinon [ug/L]**	**Values corrected for recovery rate Diazinon [µg/L]**	**% of nominal concentration Diazinon [%]**
0	14.8	15.5	97
14	3.86	4.05	25
28	1.28	1.34	8

2.2 Analysis of the diluted application solution

Application solution	Concentration determined Diazinon [ug/L]	Values corrected for recovery rate Diazinon [µg/L]	% of nominal concentration Diazinon [%]
0	15.8	16.6	103

- Recoveries : see the following tables for the average recovery values.

3. Evaluation of the method

The analytical procedure was tested and confirmed with fortified specimens containing the test item Diazinon in dilution water. The results are summarized in the following table:

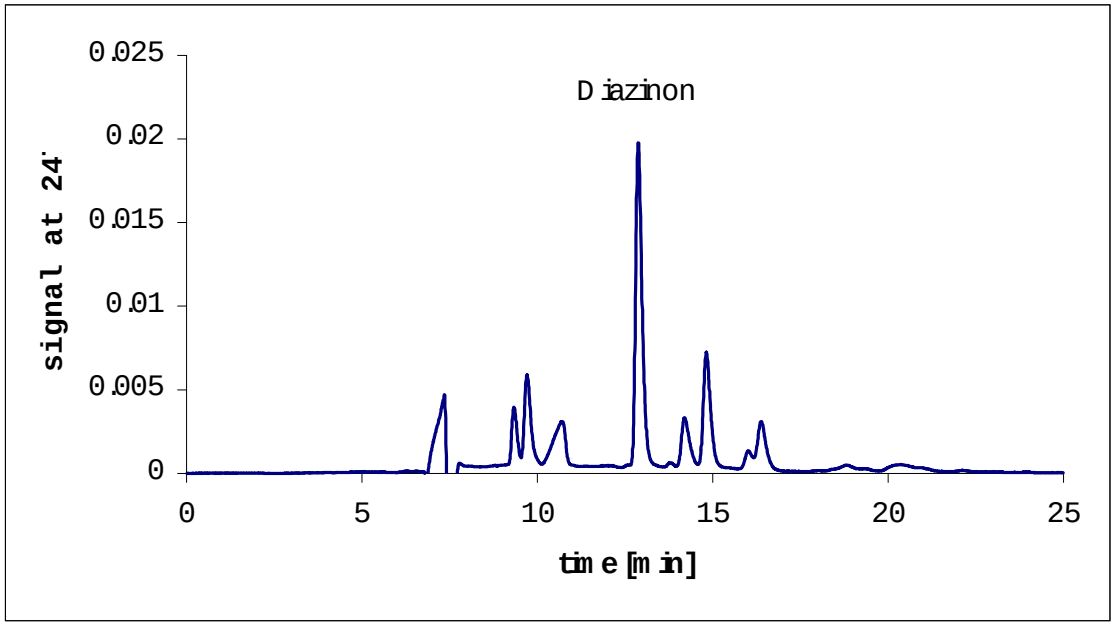
Concentration created [µg/L]	Concentration measured [µg/L]	Recovery rate in [%]
16	17.0	106
4	4.14	104

Resulting overall recovery rate : 105 % (N = 2, RSD = 1.7 %)

4. Chromatograms

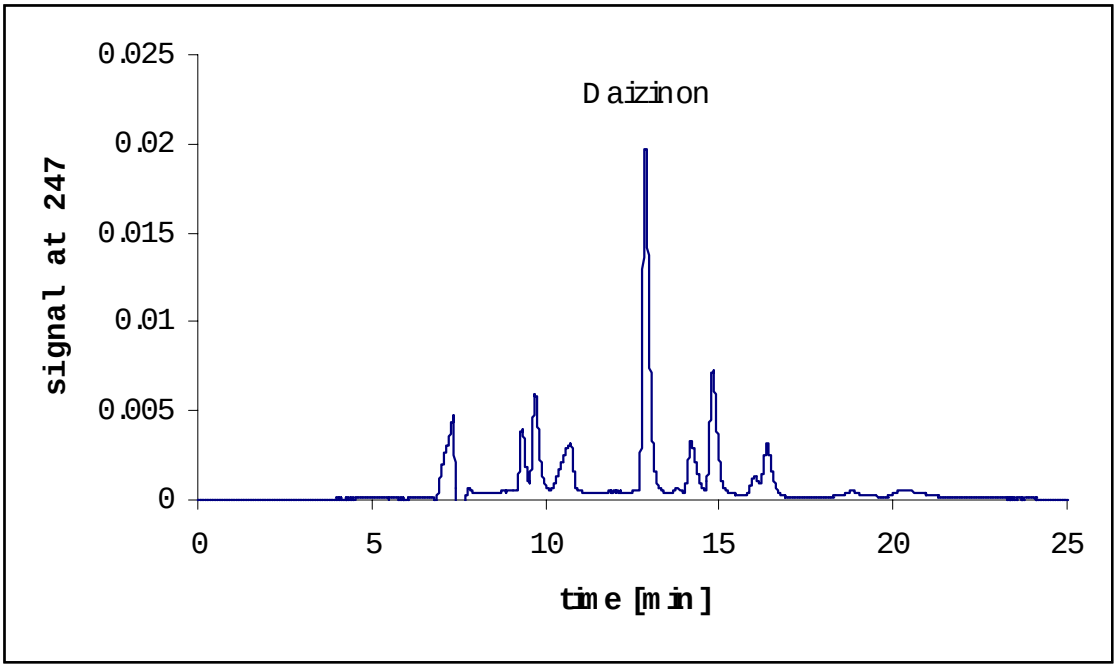
4.1 Chromatograms of the test item

(16 µg/L of Diazinon)



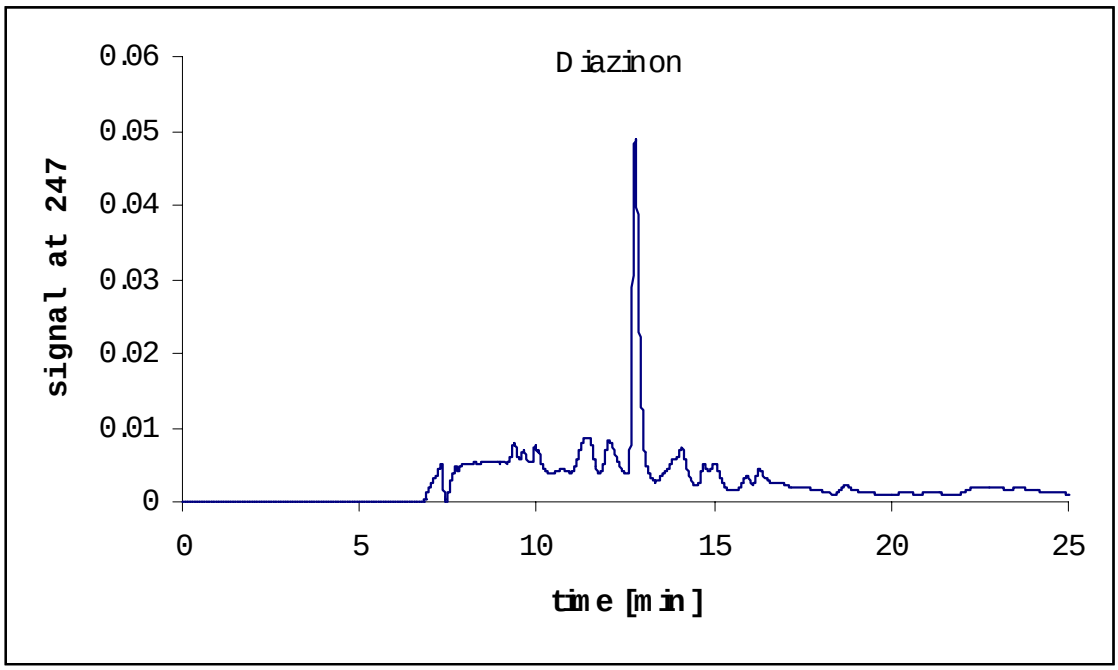
4.2 Chromatogram of the diluted applicationsolution solution

(32 µg/L of Diazinon)

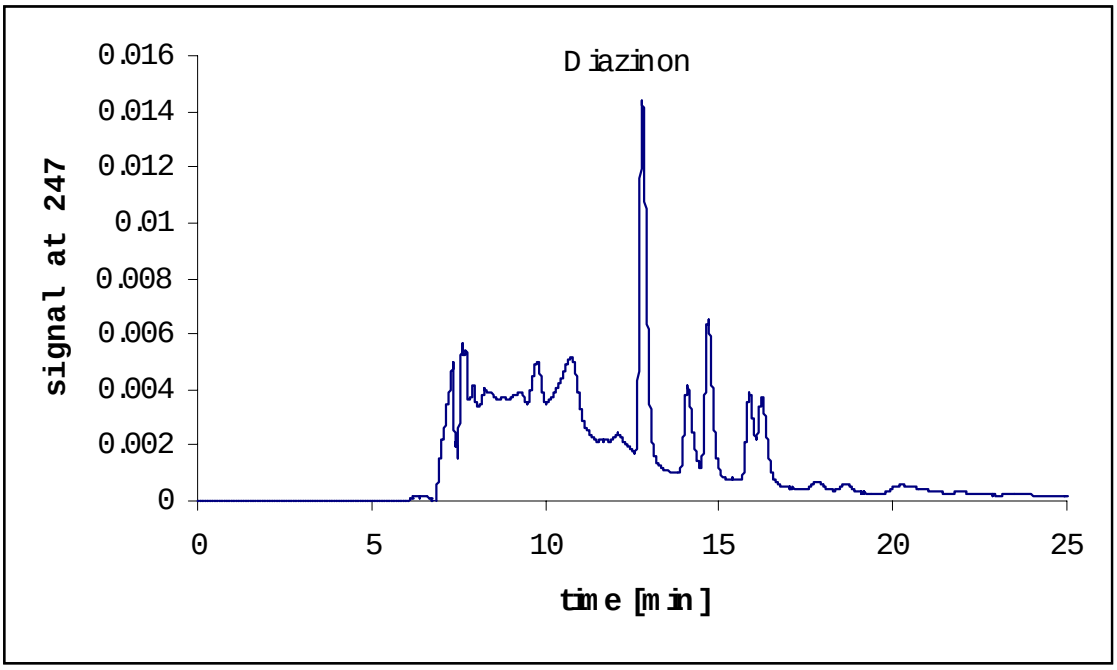


4.3 Chromatograms of specimens

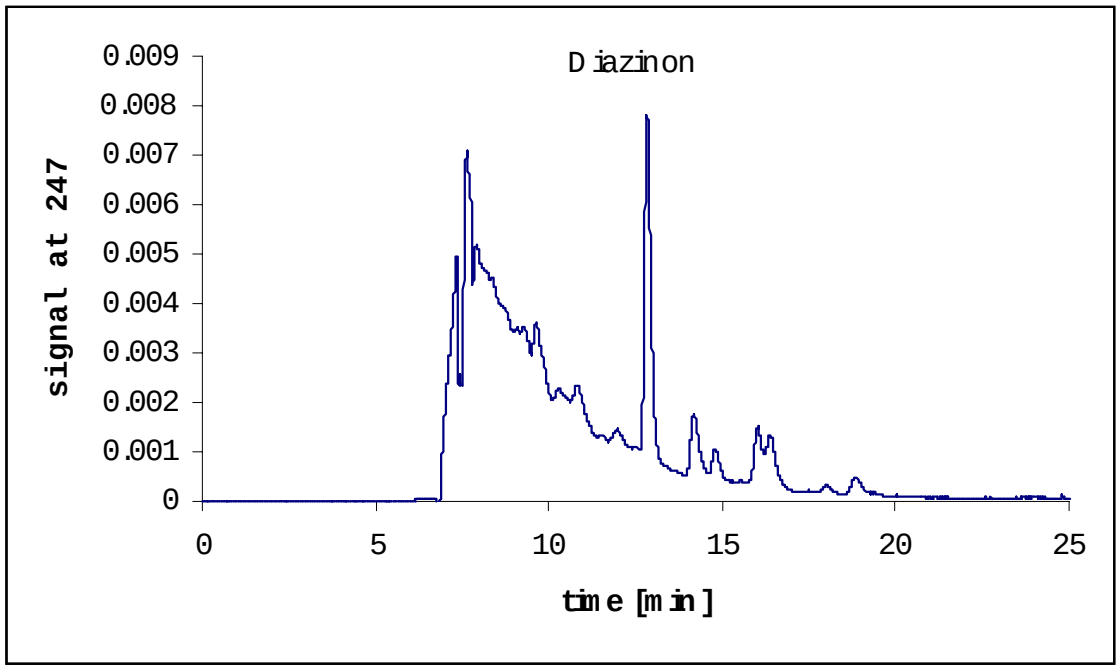
Chromatogram of a specimen at day 0
(Pond 4)



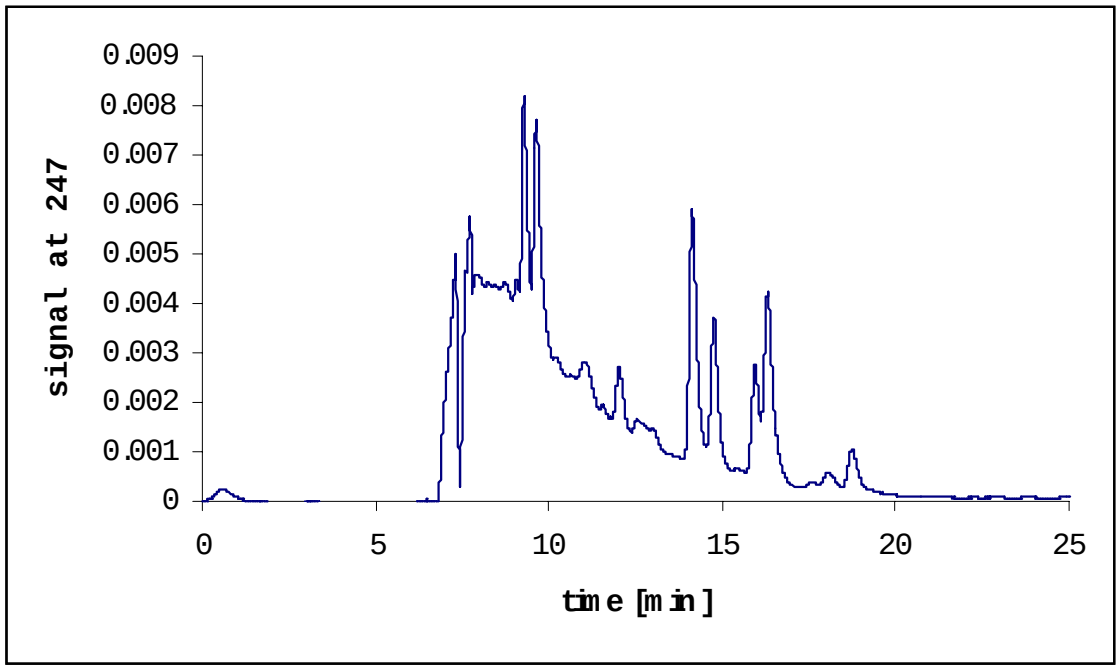
Chromatogram of a specimen at day 14
(Pond 4)



Chromatogram of a specimen at day 28
(Pond 4)



Chromatogram of a specimen at day 28
(Pond 8, Blank)



ANALYTICAL METHOD

Determination of G 24480 in water of an ecotoxicological test

Table of Contents

1. Scope

Determination of G 24480 (batch 601011) in test water of ecotoxicological tests by HPLC.

2. Principle

A high performance liquid chromatographic method (AM98-02) has been developed which separates G 24480 from test water excipients.
The sample is extracted through a solid phase columns and analyzed by HPLC :

Injection on system 1
Nucleosil C18, 5 µm, 10 x 4.6 mm
Injection volume : 1000 µL
Acetonitrile / bidistilled water : 20/80 (V/V)
Flow rate : 0.6 mL

Backflush after 5.0 min on system 2
Supelcosil LC-ABZ Plus, 5 µm, 250 x 2.1 mm
Acetonitrile/bidistilled water : 60/40 (V/V)
Flow rate : 0.3 mL
UV detection at 247 nm

2.3 Initial conditions after 15.0 min on system 1
Acetonitrile / bidistilled water : 20/80 (V/V)
Flow rate : 0.6 mL

The detection limit under these conditions is in the order of 0.0003 mg/L for G 24480.

3. Application

The method AM98-02 is valid for a final concentration range of 0.0008 - 0.002 mg/L for G 24480 (batch AMS 140/7). The sample determinations and the recoveries are carried out during the analysis of the test samples.
The method is suitably accurate, precise, specific and sensitive for the analysis of G 24480 in ecotoxicological test water.

4. Procedure

4.1. Reagents, solutions, equipment

Reagents

Bidistilled water (quartz apparatus)
Test water as used in each test
Acetonitrile : gradient grade, MERCK No. 1.0030

Solutions

Solvents for reference solutions:	Bidistilled water/acetonitrile	800 mL/200 mL
Mobile phase for HPLC system 1:	acetonitrile	200 mL
	bidistilled water	800 mL
Mobile phase for HPLC system 2:	acetonitrile	600 mL
	bidistilled water	400 mL

Equipment

HPLC-pump for system 1:	TSP P4000 or equivalent
HPLC-pump for system 2	TSP 8800 or equivalent
Injector :	TSP AS3000 with a Rheodyne injection valve and a 1000 µL loop
For the backflush switch on system 2	Motor Rheodyne valve MV-6 from Hengeller Analytik Instruments
HPLC column of system 1	Nucleosil C18, µm, 10 x 4.6 mm, Bischoff No. 63021835
HPLC column of system 2	Supelcosil LC-ABZ Plus, 5µm, 250 x 2.1 mm, Supelco No. 5-7927
Detector :	Variable wavelength detector Spectra System UV 3000
Lab. Computer :	TSP PC 1000
Solid phase extraction column:	Solid phase extraction microcolumns with filter (SPEC-PLUS-3ml-C18AR) Cat. No. 532-19-20

4.2. Sample- and reference solutions

4.2.1. Sample solution

A defined volume of the sample is passed through a preconditioned (preconditioning : flush with 10 mL acetonitrile and 10 mL bidistilled water) solid phase extraction column with a speed of about 2-3 mL/minute. The sample bottle and the measuring vessel are rinsed with about 10 mL bidistilled water. Both solutions are subsquently passed through the solid phase extration. The substance is eluted with maximum 2 mL of acetonitrile. The eluate is made up to the volume with bidistilled water. The final solution is analyzed with the described backflush system.

4.2.2. Reference solutions

At least 19 -30 mg (in duplicates) of G 24480 (batch : AMS 140/7) are weighed and dissolved in acetonitrile. From these stock solutions, reference solutions in the range of the test concentrations (related to the nominal concentrations) are prepared in the solvent for the reference solutions (see chapter : Reagents, solutions, equipment).

4.3. Injection sequence

The references and the samples are analyzed on the equilibrated HPLC system. The sequence is set to inject samples and references alternately (two injections per vial). An example of a chromatogram is given (see chapter : Data)

4.4. Operating conditions

Column 1:	Nucleosil C18, 5 µm (10 x 4.6 mm i.d.)
Column 2	Supelcosil ABZ Plus, 5µm, (250 x 2.1 mm i.d.)
Eluent for system 1:	acetonitrile / bidistilled water 20 / 80 (V/V)
Eluent for system 2:	acetonitrile / bidistilled water 60 / 40 (V/V)
Pump 1 :	Spectra P4000, flow rate 0.6 mL
Pump 2 :	SP 8800, flow rate 0.3 mL
Injection volume :	1000 µL
Wavelength :	247 nm
Analysis time :	25 min

4.5. Retention times

G 24480 : approx. 13 ± 1.0 min

4.6. Calculations

External Standard Method

4.6.1 G 24480 concentrations found in the sample

$$C_{S1} = \frac{(A_{S1} - B_1) \times K_D}{S_{R1}} \quad mg/L$$

Where :

- C_{S1} = G 24480 concentration (mg/L) found in the sample
- A_{S1} = Mean value of the G 24480 peak area (counts) from both injections of the sample
- B_1 = Y-intercept of the linear regression line of G 24480 reference solutions (counts)
- S_{R1} = Slope of the linear regression line of the G 24480 reference solutions (counts/mg/L)
- K_D = Dilution factor of the sample

4.6.2. G 24480 concentration corrected for the average recovery

$$C_{R1} = \frac{C_{S1} \times 100}{R_{A1}} \quad mg/L$$

Where :

C_{R1} = G 24480 concentration of the sample (mg/L) corrected for the G 24480 average recovery
C_{S1} = G 24480 concentration (mg/L) found in the sample
R_{A1} = G 24480 average recovery (%) obtained from the same calculation mode used for the samples

4.6.3. G 24480 percent of the nominal concentration

$$P_{E1} = \frac{C_{R1} \times 100}{C_{N1}} \quad mg/L$$

Where :

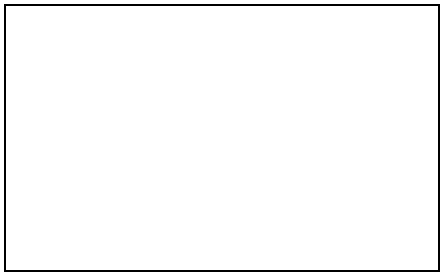
P_{E1} = Percent of the G 24480 nominal concentration in the sample
C_{N1} = G 24480 nominal concentration (mg/L) in the sample
C_{R1} = G 24480 concentration (mg/L) found in the sample corrected for the G 24480 average recovery

4.7. Conclusions

Apparatus and parameters are typical examples and may be changed, if required. Thus, the appearance of the chromatograms of the individual determinations may differ due to the conditions used (solvents, column, environment etc.). Any changes will be reported and explained in the raw data. Major (principal) changes must be noted in the report.

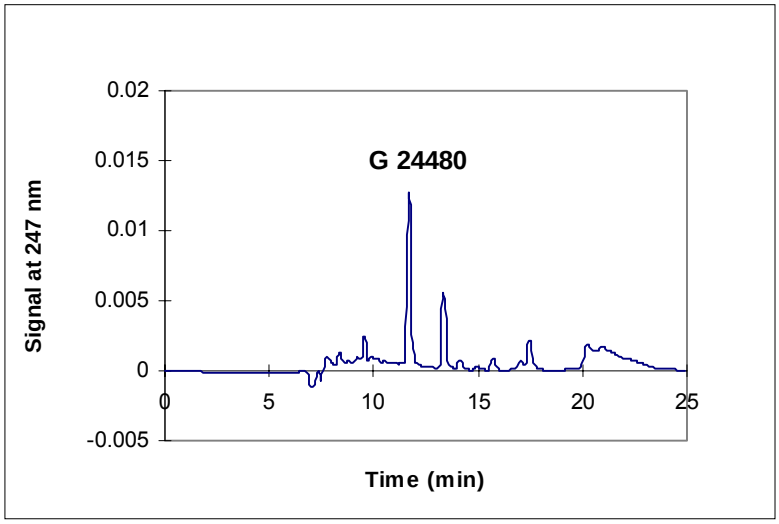
4.8. Data

Chemical stucture and Molecular Weight

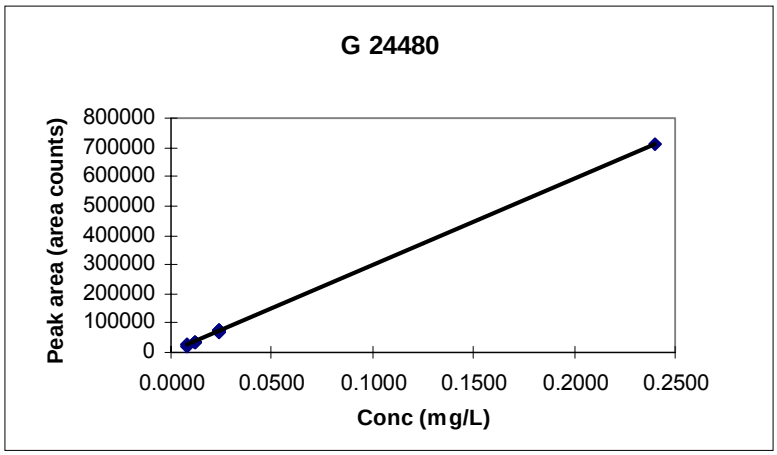


G 24480 **M = 304.4 g/mol**

Typical example of a G 24480 chromatogram, corresponding to about 0.0242 mg/L in reference solution

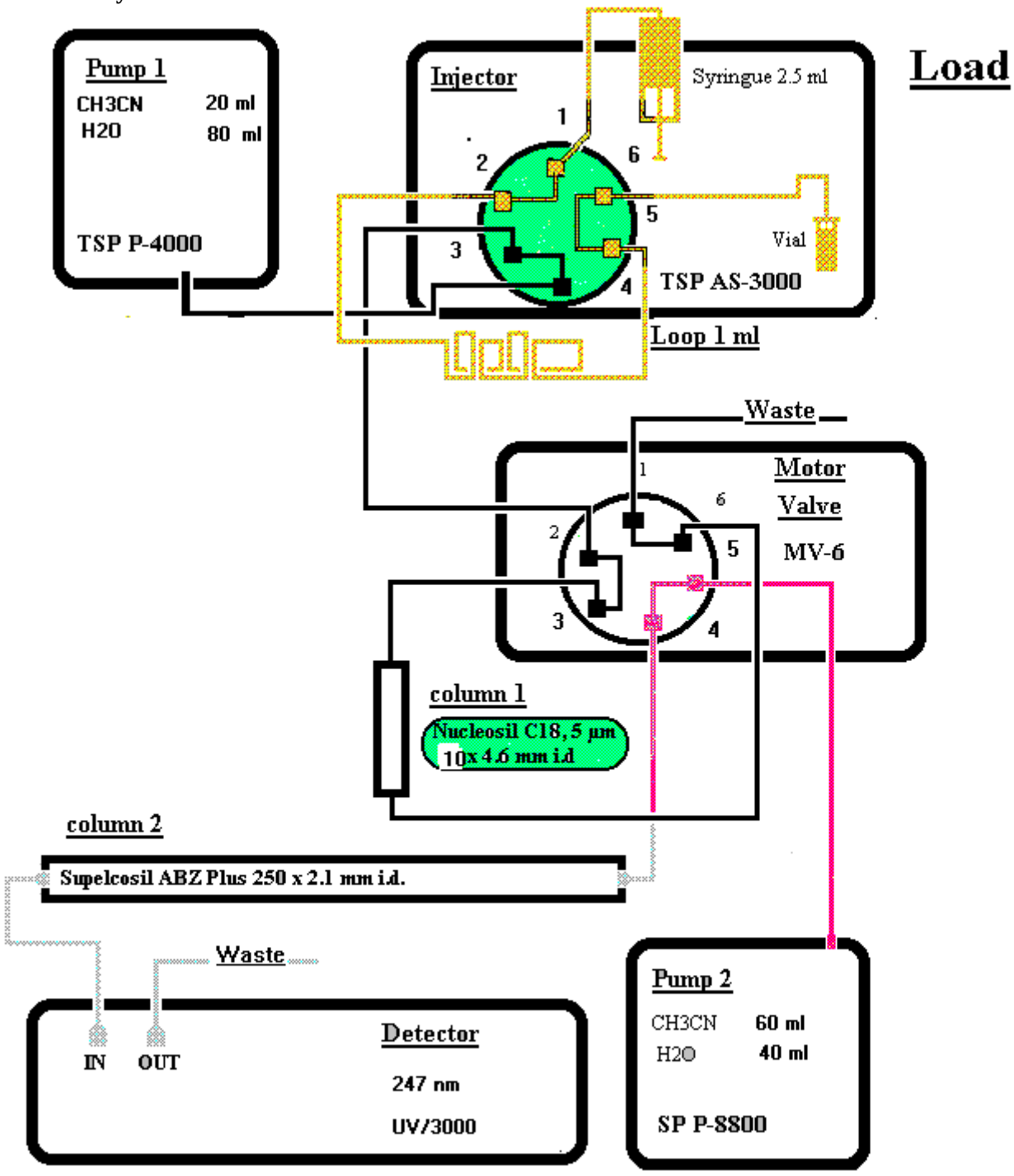


Linearity of G 24480 assay (batch : AMS 140/7)

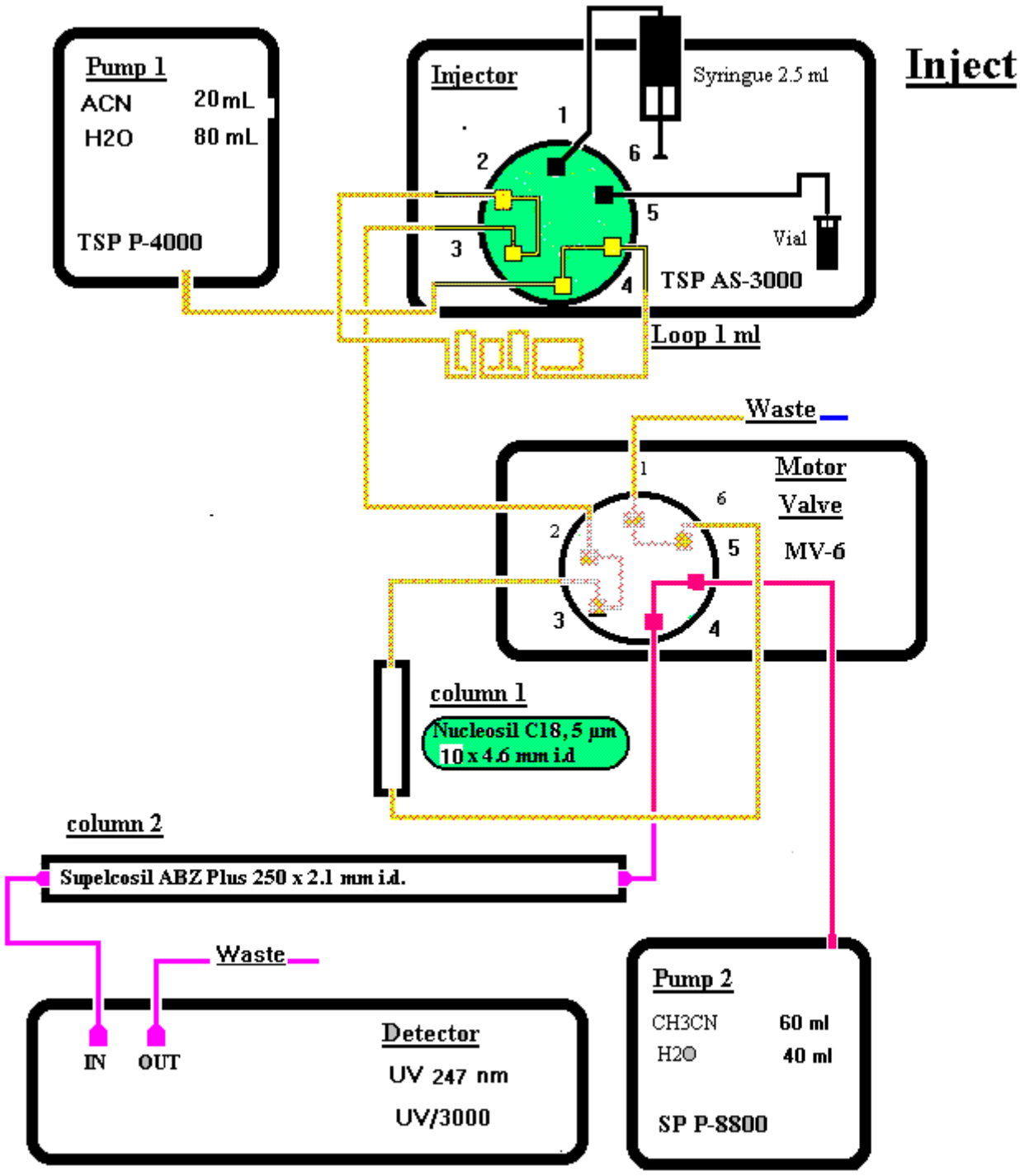


5. Backflush system

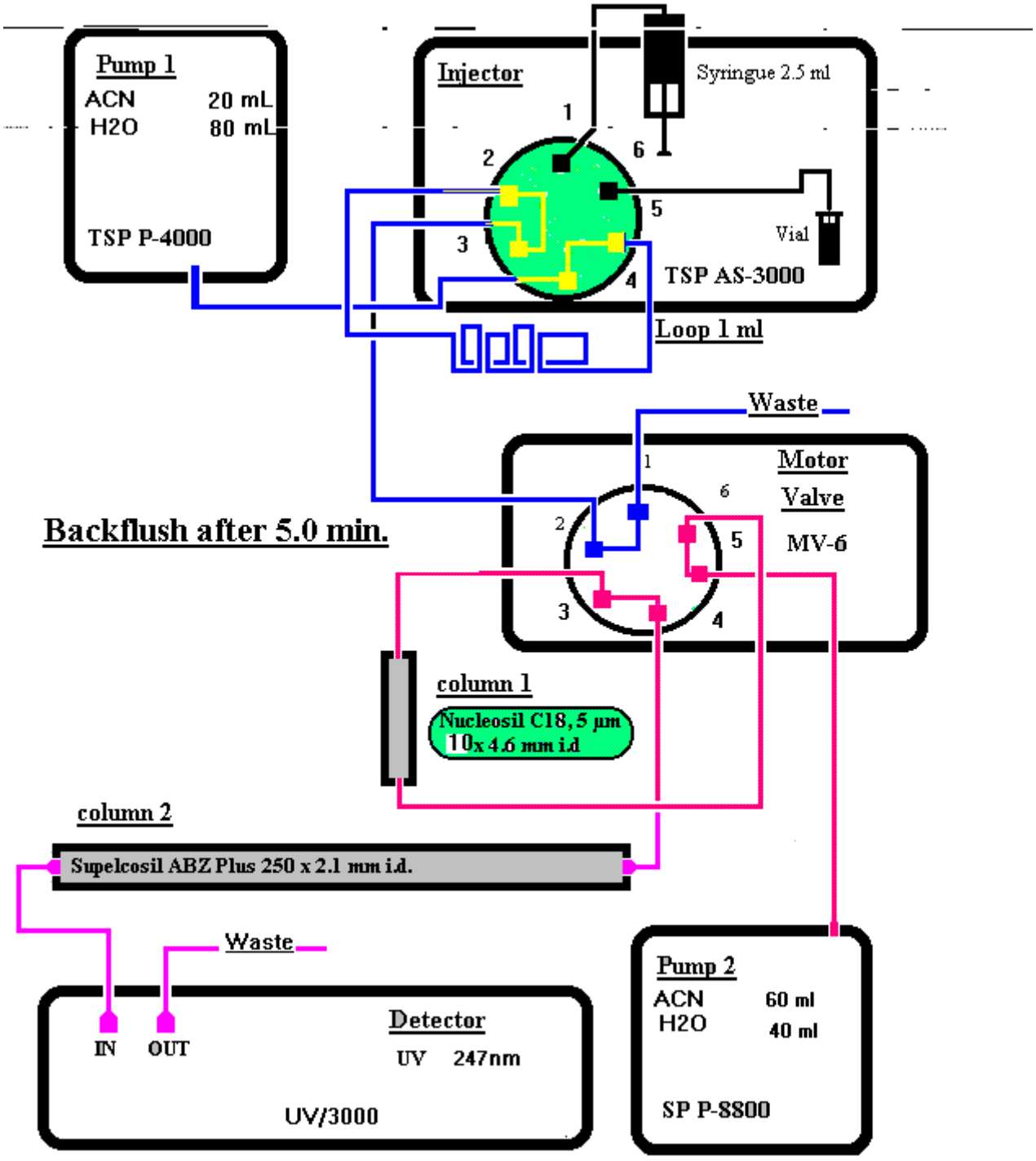
5.1 HPLC System : Position " Load '



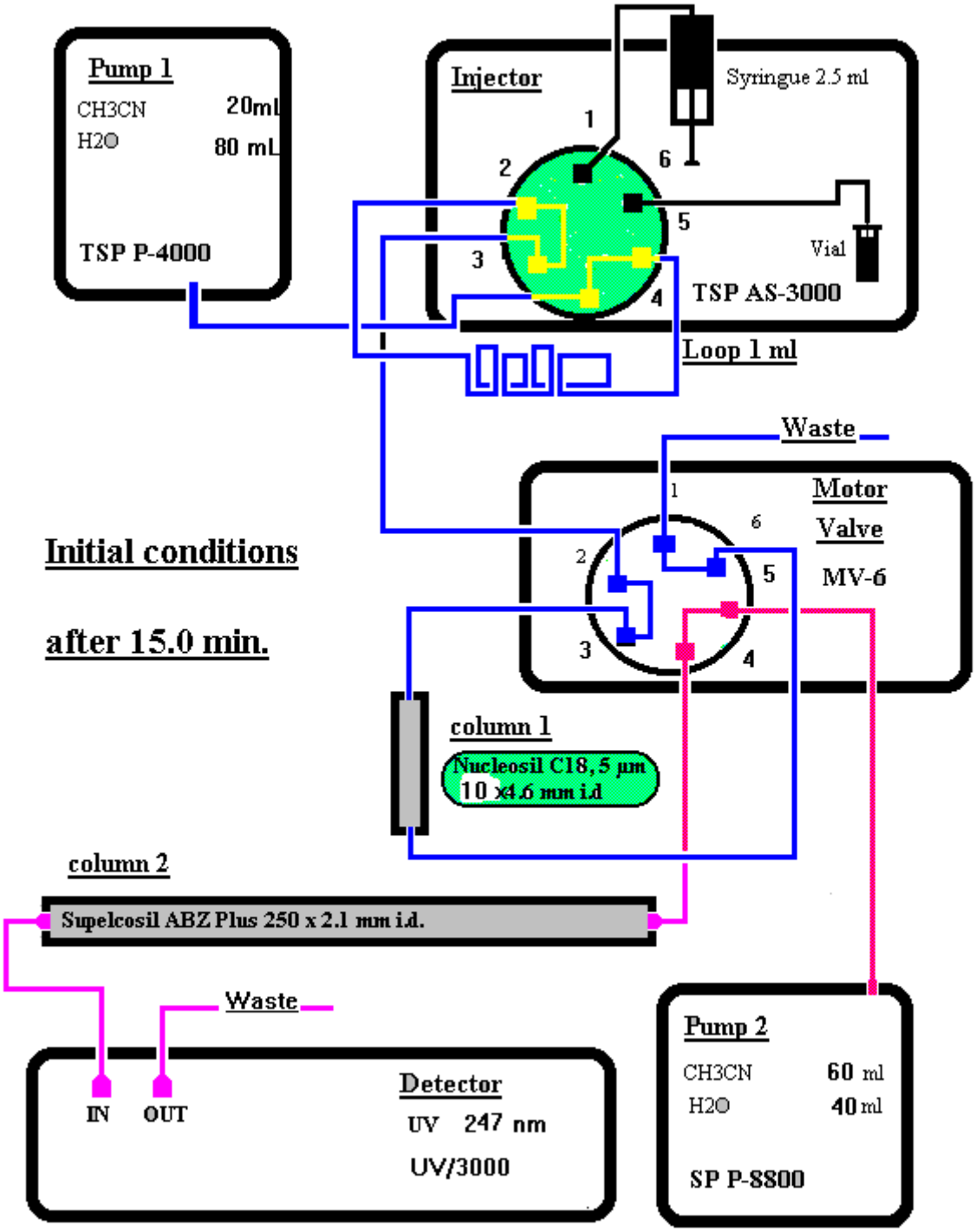
5.2 HPLC System : Position " Inject '



5.3 HPLC System : Position " BACKFLUSH ' AFTER 5.0 MIN.



5.4 HPLC System :" INITIAL CONDITIONS " AFTER 15.0 MIN.



CURRICULUM VITAE

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EDUCATION

1995 **Diploma (Dipl.-Biol.)**
Albert-Ludwigs-Universität, Freiburg i.Br., Germany
Microbiology, Limnology, Plant Physiology, Laws. Undergraduate Thesis: " Ecotoxic and genotoxic potential of industrial effluents".

PROFESSIONAL EXPERIENCE

01/2002 - TODAY **Manager, Policy & Office Coordination**
CropLife International, Brussels, Belgium

2001 **Manager, International Scientific & Regulatory Affairs**
CropLife International, Brussels, Belgium

1999 - 2000 **Manager, Science & Technology**
European Crop Protection Association, Brussels, Belgium

1997-1999 **Research Associate**
Springborn Laboratories (Europe) AG, Horn, Switzerland

1996-1997 **Consultant, aquatic & terrestrial ecoloxicological studies**
Novartis Crop Protection AG, Basel, Switzerland

1995 **Internship**
Product Safety, Terrestrial Ecotoxicology, Ciba -Geigy AG, Basel

1992 **Internship Fungicide Research & Development**
Sandoz AG, Agricultural Research Station, Witterswil, Switzerland

1991 **Internship Seed Research & Production**
Sandoz AG, Agricultural Research Station, Witterswil, Switzerland

1988, 1999 **Internship Herbicide Research & Development**
Ciba-Geigy AG, Agro Division, Stein, Switzerland

PRESENTATIONS AND PUBLICATIONS

1998 **SETAC (EUROPE) Annual Meeting, Bordeaux**
Platform Presentation. Maise S, Galicia HF, Gonzalez-Valero J, Huber W. : Community Structure of outdoor aquatic microcosms: natural variability and influence of abiotic factors.

1998 **IUPAC Conference, London**
Poster Presentation. Maise S, Galicia HF, Gonzalez-Valero J, Huber W.: Outdoor aquatic microcosm studies as tool for improved risk assessment of plant protection products.

1996 **International Review Conference, Cardiff, UK**
Poster presentation and Publication. Maise S, Candolfi MP, Neumann C, Vickus P and Mäder P.: A species Comparative Study: Sensitivity of *Aphidus rhopalosiphi, A. matricariae* and *A. colemani* (Hymneoptera: Aphidiidae) to Dimethoate 40 EC. Published by the Welsh Pest Management Forum, Cardiff, 1997.

1999 **CLASSIC – Workshop**
Invited expert to the workshop on Community Level Aquatic System Studies Interpretation Criteria, Fraunhofer Institute, Schmallenberg, Germany