

**FATE AND EFFECTS OF TRIALLATE
IN A PRAIRIE WETLAND**

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by

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ABSTRACT

MSc.

Toxicology

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FATE AND EFFECTS OF TRIALLATE IN A PRAIRIE WETLAND

Extensive use of pesticides in the Canadian prairies, where cropland is interspersed with wetlands, increases the potential for contamination of aquatic ecosystems. Triallate is a widely used herbicide for the control of wild oats and it is frequently detected in aquatic systems. Because triallate is toxic to aquatic organisms and has the potential to persist in sediment, its impact on natural systems needs to be evaluated. The purpose of this research was to determine the fate of triallate in wetlands and its effect on selected aquatic organisms representing different trophic levels. Four littoral enclosures were built in a prairie pond; each was divided in half creating paired treatment and control cells. Triallate was applied at a concentration of 250 µg/L, representing a worst-case contamination by runoff. Time-specific concentrations of triallate in water, sediment and biota were measured. Triallate disappeared quickly from the water and partitioned to the sediment. Volatilization and uptake by organisms may have contributed to decreasing water concentrations. Levels in water decreased sharply in the first 3 days following application coupled with rising sediment concentrations. Levels in amphipods and aquatic plants also increased. Effects of triallate were evaluated for phytoplankton, periphyton, zooplankton (*Daphnia pulex*) and amphipods (*Hyalella azteca*). Biomass of phytoplankton and periphyton was not affected by triallate.

Daphnia pulex and *Hyalella azteca* suffered reduced body size and reproduction.

However, some of the results were inconsistent among enclosures. Microcosm and laboratory experiments were performed to complement the field study and demonstrated the toxicity of triallate to phytoplankton and *D. pulex*. Sublethal effects of triallate were manifested by reduced body size and reproduction in *D. pulex* exposed to 125 µg/L of triallate. These findings agree with results obtained in the field study.

The present interim guideline (0.24 µg/L) for the protection of aquatic life appears to be adequate. In natural systems, triallate binds to dissolved and particulate organic matter and partitions quickly to sediment, reducing the amount present in solution and therefore the exposure to aquatic organisms. However, triallate persists in sediment for a longer time period and the toxicity of triallate associated with sediment has not been evaluated.

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1.0 GENERAL INTRODUCTION

Wetlands are an inherent part of the prairie landscape. In south central Saskatchewan, average wetland densities range from 20 to 28 ponds per km² (National Wetlands Working Group 1988). Wetlands fulfill important functions in hydrological processes, waste assimilation, ecosystem diversity and productivity, nutrient cycling and as wildlife habitat (National Wetlands Working Group 1988). Although all wetlands functions are important, wetlands are most often valued for their role in providing habitats for wildlife, and the prairie pothole region is of vital importance to many populations of breeding waterfowl (Stewart and Kantrud 1973). Because of intensification of agricultural land use coupled with drought, wetland habitats are often seriously degraded or eliminated (Turner *et al.* 1987, Johnson and Grier 1988). Therefore, the quality of remaining wetlands becomes increasingly important. There is little doubt that the extensive use of pesticides over the pothole region can substantially alter the capacity of the land to provide food resources and adequate cover for breeding birds (Sheehan *et al.* 1987) and other animals. There is significant potential for adverse impacts of herbicides on wetlands, so it is essential to study the behaviour and fate of herbicides in aquatic ecosystems, as well as their chronic effects on invertebrates (Sheehan *et al.* 1987).

Agricultural practices on the prairies rely heavily on the use of pesticides. Herbicides are used extensively and continuously on prairie crops while insecticide use

is more episodic. Between 1978 and 1986, application of broadleaf and wild oat herbicides in the prairie provinces increased by 40 and 55%, respectively, while the total area of cropland increased only by 11.5% (Forsyth 1989). In 1989, 3.295×10^6 kg of active ingredients of wild oat herbicides was applied in Saskatchewan (Lewis 1991). Triallate is one of the major herbicides used to control wild oats in the province, second in importance to trifluralin (Donald and Syrgiannis 1995).

Triallate is the common name for N,N-diisopropyl-thiocarbamate 2,3,3-trichloroallyl. It is a pre-emergence herbicide for the control of wild oats in wheat, barley, peas, canola, flax and mustard (Saskatchewan Agriculture and Food 1992). This herbicide was introduced to the Canadian market in 1962 by Monsanto Co. Commercial formulations of triallate are available under the trade names Avadex BW and Fortress. Triallate is an amber oil with a molecular formula of $C_{10}H_{16}Cl_3NOS$ and a molecular weight of 304.7 (Figure 1.1). It has a water solubility of 4 mg/L (at 25 °C), a log K_{ow} (octanol/water partition coefficient) of 4.6 and is volatile (vapour pressure, 27.5 mPa at 25 °C) (Kent *et al.* 1992). Triallate is applied in the spring or in the fall and is incorporated in the soil shortly after application, usually by harrowing (Saskatchewan Agriculture and Food 1992). A granular formulation of Avadex BW can be applied on the surface in the fall and incorporated in the soil the following spring. This treatment is intended to reduce soil erosion by removing the fall tillage operation.

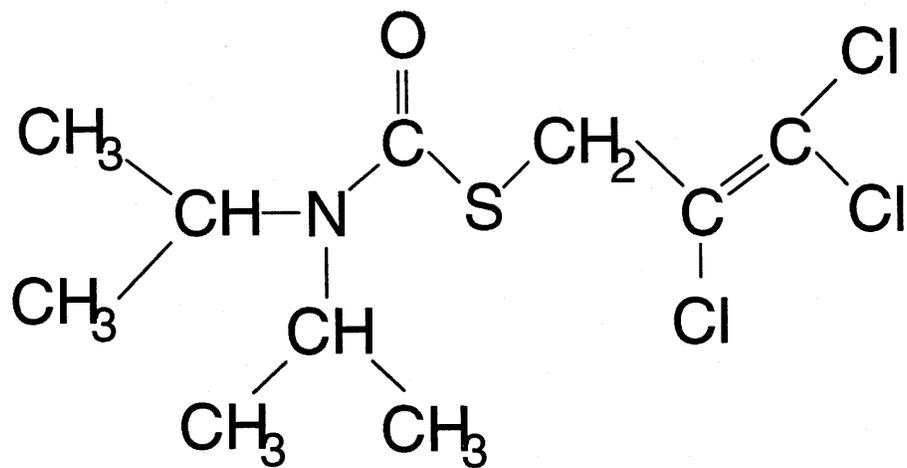


Figure 1.1. Molecular structure of triallate

The principal herbicidal action of triallate is either inhibition of cell elongation or expansion of the stem and leaf meristatic tissue; effects on the root tissue are less pronounced. Thiocarbamates also interfere with lipid formation, resulting in decreased epicuticular wax formation and thinner cuticle, increasing plant susceptibility to herbicides applied on foliage. Thiocarbamates also inhibit gibberellic acid synthesis which eventually affects cell elongation.

Triallate contamination of wetlands occurs by different processes: runoff, spray drift, and aeolian transport including deposition of herbicide-contaminated soil particles or atmospheric deposition of volatilized triallate. Triallate is readily transported by runoff (Waite *et al.* 1992). Loss of triallate by volatilization accounts for 17.6% of the amount applied in a single growing season in Saskatchewan with 50% of this loss occurring during the first 4-5 days (Grover *et al.* 1988a). The presence of airborne residues of triallate in both the central and southern regions of Saskatchewan was a consequence of the general use of this herbicide in both areas (Grover *et al.* 1988b). Atmospheric deposition of triallate accounts for 0.02% of the amount applied per hectare in the province (Waite *et al.* 1995).

Triallate has been found at varying concentrations in surface water, spring runoff water, sediment and biota on the Canadian prairies (Kent *et al.* 1992, Donald and Syrgiannis 1995). The fate of triallate entering the aquatic environment is not well understood. Because of the hydrophobic nature of triallate, rapid partitioning to particulate and dissolved organic carbon and sediment is expected (Kent *et al.* 1992). Triallate may possibly persist in sediment for a period of more than 1 year (Donald

and Syrgiannis 1995). Information on the fate and persistence of triallate in aquatic environments is scarce and incomplete.

Triallate is toxic to algae and aquatic invertebrates but toxicity to fish and vertebrates in general is less important (Kent *et al.* 1992). Aquatic macrophytes appear to be unaffected by triallate (Kent *et al.* 1992). Vertebrates have the ability to metabolize (via microsomal oxidases) and excrete triallate thus limiting the potential for bioaccumulation (Kent *et al.* 1992). Studies report bioconcentration factors in fish ranging from 150 to 838, and a study conducted by Monsanto Co. indicated a rapid elimination of triallate within 2 weeks at the end of the exposure period (Kent *et al.* 1992). I have found no report on the metabolism of triallate by invertebrates. *Daphnia* spp. and *Hyaella azteca* have the ability to metabolize certain organic contaminants (Landrum 1988). The mode of action of triallate on aquatic organisms is unknown.

Current triallate toxicity data are insufficient to establish a Canadian water quality guideline for the protection of freshwater aquatic organisms. An interim guideline of 0.24 µg/L has been established (Kent *et al.* 1992). Because triallate is toxic to aquatic invertebrates, is likely to be found in aquatic environments and has the potential to persist in sediments, it is essential to test its potential effects on aquatic systems.

Therefore, this research was designed to test the effects of the herbicide triallate on important aquatic organisms representing different trophic levels in the aquatic food chain. Specifically, the objectives were to determine: 1) fate of triallate in

water, sediment and biota; 2) effects of triallate on selected plants and invertebrates; and 3) direct effects of triallate on selected aquatic organisms in the laboratory.

The thesis is organized in 6 parts. In chapter 2, I describe the study area and general research methods. I address objective 1 (above) in chapter 3, by evaluating time-specific concentrations of triallate in water, sediment and biota. Effects of triallate on phytoplankton, periphyton, zooplankton (*Daphnia pulex*) and amphipods (*Hyaella azteca*) (objective 2) in mesocosms are evaluated in chapter 4. Microcosm (chapter 5) and laboratory experiments (chapter 6) were performed to complement the field experiment, and to study effects of triallate under controlled conditions (objective 3). Finally, a general discussion of my findings is presented in chapter 7.

2.0 STUDY AREA

2.1 Gursky Pond

Field experiments presented in this thesis (chapters 3, 4 and 5) were conducted in a permanent prairie pond (52°08' N, 106° 07' W), hereafter, Gursky pond, located 4 km south of St. Denis, Saskatchewan. Animals, pond water and sediment used in the laboratory experiments (chapter 6) were also collected from this pond. Gursky pond has a surface area of 62 ha and a maximum depth of approximately 3 m. This pond is eutrophic (mean total phosphorus during spring overturn, 333 µg/L; maximum chlorophyll concentration, ~ 200 µg/L) and slightly alkaline (Figure 2.1). In the summer of 1993, temperature ranged from 11 to 20 °C and pH rose initially, dropped with heavy rains and then rose again (Figure 2.1). Dissolved oxygen ranged from 8 to 12 mg/L at the surface and from 5 to 8 mg/L at the bottom (Figure 2.1). Conductivity generally decreased from 0.868 mS/cm in June to 0.787 mS/cm in September (Figure 2.1).

2.2 Littoral Enclosures

Four littoral enclosures were built at randomly selected locations in the pond. Enclosures extended 20 m from shore into open water and included areas of emergent vegetation. They were 30 m wide and divided in half, creating paired treatment and control cells (i.e., each cell was 20 m x 15 m).

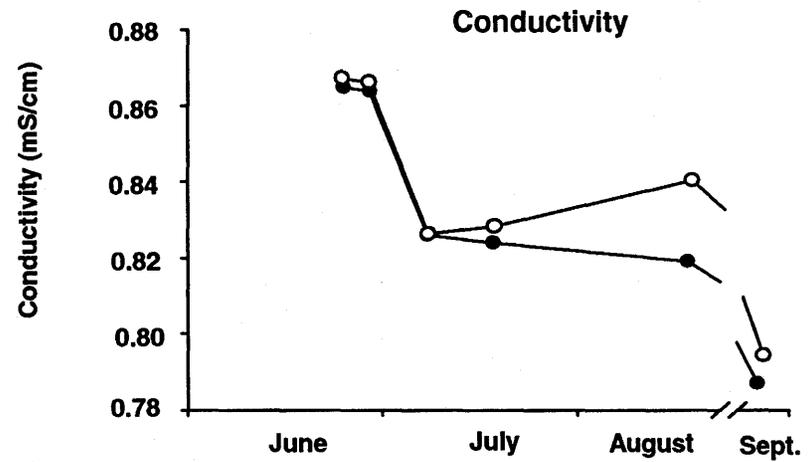
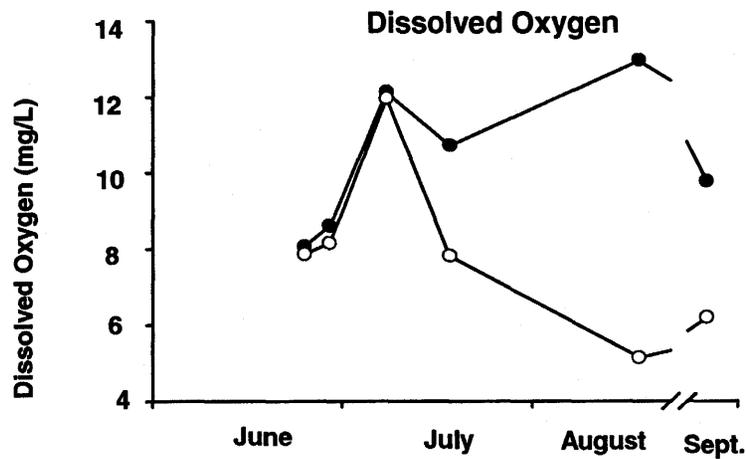
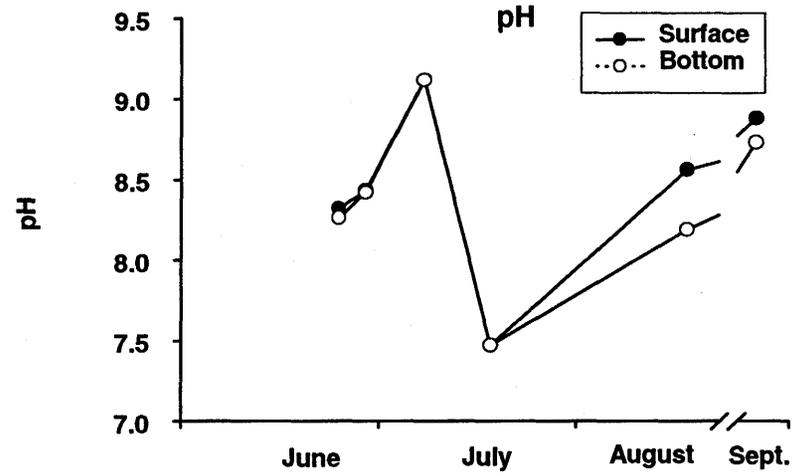
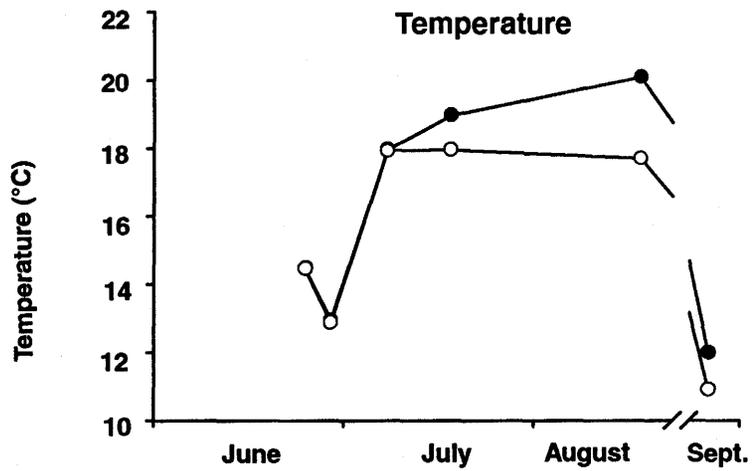


Figure 2.1. Seasonal trends in temperature, pH, dissolved oxygen and conductivity of the water in Gursky's pond, SK, during 25 June to 19 September, 1993. Measurements were made 20 cm below the water surface and 20 cm above the sediment (approximately 2 m below the surface).

Enclosures were made of clear polyethylene sheets (0.15 mm thickness), secured around a rope about 60 cm above the water surface and packed into the sediment with metal bars. Wooden posts were used to support the sides of the plastic (Figure 2.2).

2.3 Calculations of Triallate Dosing for Mesocosm Experiments

A worst case contamination by runoff was used as the scenario to calculate the dosage to the treatment mesocosms. A generic wetland with dimensions of 1 ha x 1 m deep within a 10 ha watershed was considered for calculations. Wauchope (1978) estimated that pesticide loss to runoff can reach 10% in a "catastrophic event" where rainfall occurs shortly after application or before soil incorporation. A 10% loss of herbicide applied at the maximum rate of application (2.2 kg ai/ha) (Saskatchewan Agriculture and Food 1992) gives a water concentration of triallate of 220 µg/L in the generic wetland. A test concentration of 250 µg/L was selected to approximate a worst-case contamination by runoff. This concentration is also within the range of triallate water concentration guidelines recommended for agricultural water uses including livestock watering (230 µg/L; Kent *et al.* 1992).

2.4 Triallate Application in Mesocosm Experiment

The amount of Avadex BW (Monsanto Agricultural Co.) required to obtain the target concentration of 250 µg/L active ingredient in each mesocosm was calculated based on the volume of individual mesocosms obtained by bathymetric survey.

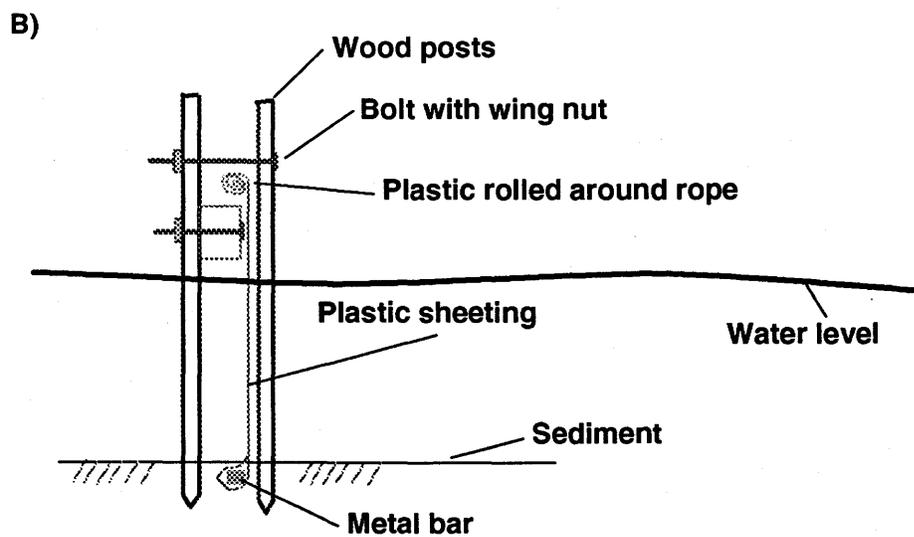
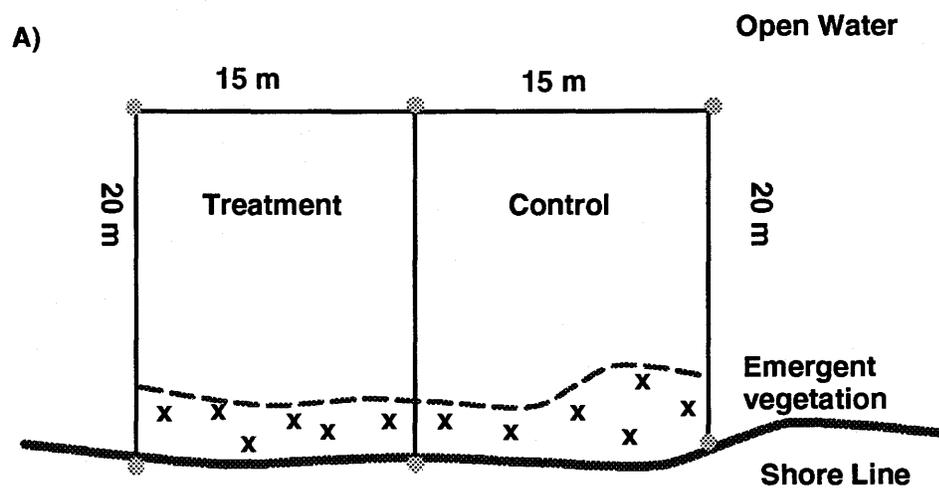


Figure 2.2. Diagram of mesocosm A) and diagram of enclosure wall B) used during experiments conducted at Gursky pond, SK, in 1993.

Avadex BW (400g triallate/L) was added to a 3.8 L hand-held pressurized sprayer with 50 ml of acetone, 12 g (dry weight) of fine sediment (previously collected from the pond) and filled with pond water. The entire contents of the sprayer were applied uniformly 1-2 cm below the water surface over the entire treatment area. Control enclosures received acetone only in a soil slurry delivered in another backpack sprayer used only for control cells. Acetone was chosen as a carrier solvent because of its relatively low toxicity compared to other solvents (Landrum 1982). The application was done from 2 boats, one for treatment cells and one for control cells. Triallate was applied on 19 June 1993.

3.0 FATE OF TRIALLATE IN MESOCOSMS

3.1 Introduction

Modern agriculture relies heavily on the use of agrochemicals such as pesticides and fertilizers. The proximity of agricultural land to wetlands in the Canadian prairies facilitates entry of these compounds into the aquatic environment where they have the potential to disrupt these systems. Ecological effects of a xenobiotic in any system depends on the compound's inherent toxicity as well as its bioavailability. Knowledge of the fate of toxicants that enter wetlands is important to identify the intensity, duration, and location where adverse effects can be expected.

Monitoring studies in the province of Saskatchewan frequently detect triallate in aquatic environments (Waite *et al.* 1992, Donald and Syrgiannis 1995), reflecting the widespread use of this herbicide. There are different routes through which triallate can enter the aquatic environment: runoff from fields, spray drift, aerial deposition (Waite *et al.* 1995), and wind deposition of soil particles to which triallate is adsorbed. Triallate is readily transported in spring runoff (Waite *et al.* 1992). Triallate adsorbs to soil particles but can be displaced by water and is also associated with the fine silt present in runoff. In soil, triallate adsorption is increased by high organic matter content (Mallawatantri and Mulla 1992). Several factors can interact to control runoff losses from the edge of fields including: the physicochemical properties of the pesticide, its formulation matrix, and

application method, the time interval between field application and a rainfall event, and the intensity and duration of the event (Huckins *et al.* 1986).

Information on the fate of triallate in the aquatic environment is scarce. In contrast, there has been considerable research on the fate of triallate in soil and the results of these studies are useful to understand processes and factors affecting triallate distribution and degradation.

The half-life of triallate in water reportedly ranges from 3 to 15 days (Kent *et al.* 1992). Triallate distribution in the environment is influenced principally by its hydrophobic nature ($\log K_{ow} = 4.6$ and $\log K_{oc}$ ranges between 3.3 and 3.53). In aqueous solution, triallate binds strongly onto soil particles and the degree of adsorption is correlated with organic matter content of the soil (Smith 1970), a phenomenon which greatly influences the distribution of triallate. After entering water, triallate is expected to bind to particulate matter such as suspended solids and aquatic organisms and to adsorb to the organic fraction of sediment. It is therefore expected that triallate should disappear fairly rapidly from the aqueous phase. In monitoring studies, triallate is measured more frequently in sediment than in water (Therrien-Richards and Williamson 1987, Donald and Syrgiannis 1995) although triallate is present in spring runoff and in surface water during annual application periods (Waite *et al.* 1992).

Donald and Syrgiannis (1995) studied the occurrence of pesticides in 19 prairie lakes in Saskatchewan. They found triallate in a large proportion of sediment and biota samples, but did not detect it in any water samples (limit of detection of triallate in water was 0.01 $\mu\text{g/L}$). Triallate was present in 39% of sediment samples with mean and

maximum concentrations of 10.17 and 31 ng/g dry weight, respectively. Triallate was detected in 54% of crustacean zooplankton samples with a mean of 5.64 ng/g wet weight and a maximum concentration of 10.2 ng/g wet weight. Zooplankton samples included *Artemia salina* and one or more species of *Branchinecta*, *Leptodiaptomus*, *Hesperodiaptomus*, or *Daphnia*. Similarly, Therrien-Richards and Williamson (1987) conducted a study of the La Salle River, Manitoba. They did not detect triallate in surface water but found triallate in 43% of sediment samples at concentrations ranging from 16.9 to 119 ng/g. Triallate was not detected in the aquatic macrophyte *Myriophyllum* sp., but it was present in fish at a maximum concentration of 9.2 ng/g.

Information on the fate of triallate in aquatic systems comes mainly from monitoring studies of environmental concentrations of triallate in natural systems and predictions based on the chemical properties of triallate. The lack of a controlled experiment where a known amount of triallate would be added to the aquatic system and monitored over time motivated this research. Therefore, my primary objective was to measure simultaneously the time-specific concentrations of triallate in water, sediment and selected biota to assess the dissipation of triallate from the water and its accumulation in sediment and biota. Crustaceans of the order Amphipoda, represented by the species *Gammarus lacustris* and *Hyalella azteca*, were used for triallate analysis since amphipods were present in large numbers in the pond. These epibenthic animals are in close contact with both water and sediment. The aquatic macrophyte *Potamogeton richardsonii* was chosen as a representative plant species because of its abundance and the relative ease of obtaining sufficient biomass for analysis.

3.2 Materials And Methods

A description of the mesocosms and application of triallate is provided in chapter 2.

3.2.1 Sampling

Water, sediment and biota samples were collected before and after treatment. Pre-treatment samples were collected on 28 April, 1993. Water and sediment samples were taken in duplicate 1 hour and 1, 2, 3, 6, 10, 20, 30, 60 and 90 days after triallate application. Sampling for biota began 2 days post-treatment. At each sampling period, water, sediment and biota samples were taken from every treatment cells but from only 2 of the control cells.

3.2.1.1 Water

Water samples were collected in 1 L clean glass bottles lowered approximately 30 cm underwater. Samples were preserved with 15 ml of chloroform to inhibit microbial degradation of the triallate and refrigerated at 4°C until analyzed.

3.2.1.2 Sediment

Initial sampling to evaluate background pesticide levels was performed at random locations using an Ekman dredge. Post-treatment sampling was done by using petri dishes filled with homogenized sediment. Prior to herbicide application, surface sediment (top 10 cm) was collected from the study pond, mixed thoroughly by hand and placed in petri dishes that were then individually placed on stands sitting on the pond sediment. At each sampling period, petri dishes were covered before being lifted and removed with the stand. Sediment samples were then transferred into new pre-cleaned glass jars and frozen

at -40 °C until analyzed. This technique reduced sampling variability because the same type and amount of sediment was submitted for analysis.

3.2.1.3 Amphipods

Amphipods were collected with a dip net, transferred to a sorting tray and sieved to remove other organisms. Amphipods were sorted by species, placed in new pre-cleaned glass jars and frozen at -40 °C until analyzed.

3.2.1.4 Aquatic Plants

The macrophyte *P. richardsonii* was sampled by removing (by hand) whole plants, but excluding roots. A sample consisted of approximately 10 plants. Plants were placed in new pre-cleaned glass jars and frozen at -40 °C until analyzed.

3.2.2 Residue Analysis

All samples were analyzed at the National Laboratory for Environmental Testing in Burlington, Ontario. Triallate and other neutral herbicides in water samples were extracted with dichloromethane, fractionated on deactivated florisil and concentrated before analysis. For sediments, neutral herbicides were ultrasonically extracted with 1:1 acetone:hexane solvent mixture. The extract was partitioned with water and back-extracted with dichloromethane. The combined extract was concentrated, cleaned and fractionated on a florisil column. Biota samples were extracted with dichloromethane using a Polytron homogenizer. The extract was dried using sodium sulphate, then concentrated to ~ 3 ml on a rotary evaporator. Clean up and fractionation were done on a florisil column. Two fractions from a 500 x 20 mm (i.d.) column packed with 10 g of florisil (10% by weight deactivated with water) were analyzed.

Dual capillary gas chromatographs equipped with electron capture detectors were used to quantitatively determine all neutral and hexachlorocyclohexane pesticides, except atrazine when an N-P detector was used. The identity of each peak in the sample extracts was assigned by comparison with the retention time of standard compounds analyzed individually under identical chromatographic conditions. Tentative confirmation on a retention basis was obtained using a second capillary column (different polarity). Concentrations were calculated against a microgram per liter level in-house calibration standard using an external standard calculation method and a single point calibration. The calibration table was updated every 5 injections. All positive detections were confirmed by gas chromatography-mass spectrometry, when necessary. Pesticide levels in sediment and biota were reported on a dry and wet weight basis, respectively, and a method spike and blank were analyzed every 10 to 12 samples to ensure that instruments were performing to specification. The limits of detection for triallate in water, sediment and biota samples were 0.01 µg/L, 0.2 ng/g dry weight and 2.0 ng/g wet weight, respectively.

3.3 Results

3.3.1 Water

Triallate and other herbicides were absent from pre-treatment samples. Following triallate application, concentrations in treatment cells decreased rapidly from a mean of 238.6 ± 18.1 SE µg/L to a mean of 2.5 ± 1.0 SE µg/L within 10 days (Figure 3.1A). Concentration of triallate was below 1.2 µg/L in all control samples except on day 3 when levels rose to a mean value of 6.95 ± 0.74 SE µg/L . This increase may be due to

the close proximity of control and treatment cells, and the volatility of triallate; triallate vapours from treated cells may have been deposited in adjacent control cells. No other neutral herbicides were detected in any of the water samples.

3.3.2 Sediment

Pre-treatment samples indicated low levels of triallate in the sediment (mean value of 11.4 ± 0.8 SE ng/g). No other neutral herbicides, organochlorines or PCBs were detected. Post-treatment samples showed a sharp increase in triallate levels on day 2 to a mean level of 6188 ± 3082 SE ng/g and a gradual decrease to a mean level of 1408 ± 438 SE ng/g at 30 days post-application (Figure 3.1B). Results from post-treatment samples were highly variable. Triallate was measured only at very low levels in control water samples but it was detected in sediment samples at suspiciously high levels.

Contamination of the samples is suspected and duplicates have been submitted for further analysis. The herbicide diallate was present in a large proportion (73%) of samples at a mean concentration of 49.5 ± 4.8 SE ng/g and the herbicide trifluralin was detected in 45% of samples at a mean concentration of 2.8 ± 0.2 SE ng/g.

3.3.3 Amphipods

Pre-treatment levels of triallate in amphipods were low with a mean value of 6.52 ± 2.56 SE ng/g wet weight. Triallate concentration increased dramatically 2 days post-application, with levels reaching 18000 ± 3100 SE ng/g, and then decreasing to a mean value of 4608 ± 1152 SE ng/g after 6 days and to 144.38 ± 48.85 ng/g after 30 days (Figure 3.2A). Levels in amphipods were highly variable, particularly on day 6 post-treatment.

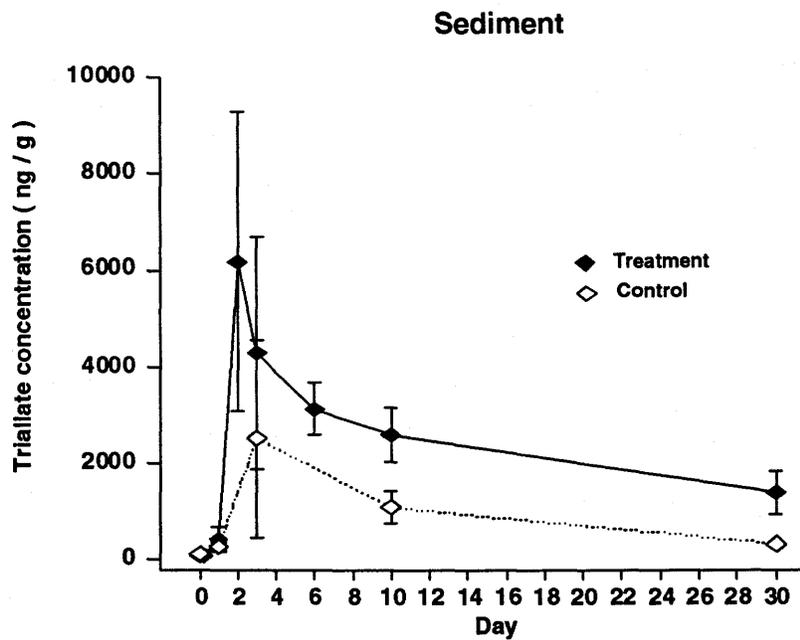
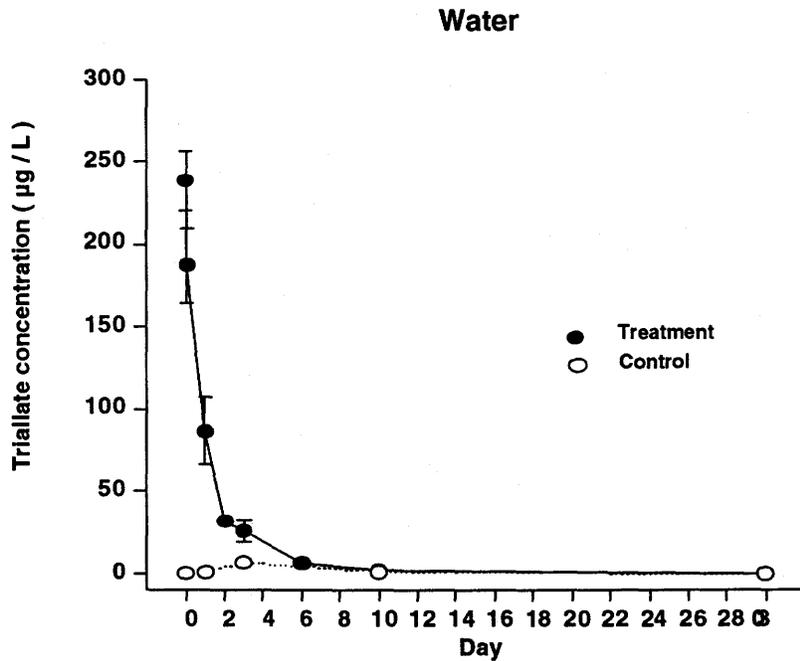


Figure 3.1. Triallate concentrations in water ($\mu\text{g/L}$) and sediment (ng/g dry weight) with time. Values are mean concentrations from the 4 enclosures (or 2 enclosures for control samples) with standard errors.

Levels of triallate in control samples were elevated, especially 2 days after application with a mean concentration of 4990 ± 1130 SE ng/g. Levels decreased to a mean value of 1538 ± 548 SE ng/g after 6 days and to 49 ± 19 SE ng/g after 30 days. The herbicide trifluralin was also found in a large proportion (62%) of the samples at a mean concentration of 2.9 ± 0.3 SE ng/g. The herbicide diclofop-methyl was present in only 2 samples (7.7%) taken 30 days post-treatment and had a mean concentration of 18.95 ± 6.95 SE ng/g.

3.3.4 Aquatic Plants

No pre-treatment samples of macrophytes were taken and only a limited number of post-treatment samples have been analyzed at this time. Triallate concentration was 90.7 ng/g (only 1 sample was analyzed) 1 day after application and increased to a mean value of 161.9 ± 64.1 SE ng/g after 6 days (Figure 3.2B). Levels decreased to a mean of 47.9 ± 10.3 SE ng/g and 37.1 ± 15.3 SE ng/g after 20 and 30 days, respectively. Triallate concentrations in control samples were 115 ng/g (only 1 sample was analyzed) 1 day after application and 15.2 ± 0.7 SE ng/g after 30 days. The herbicides diclofop-methyl and barban were both detected in one sample taken in enclosure 1A (control) 30 days post-treatment. The concentrations measured were 12.2 ng/g for both herbicides.

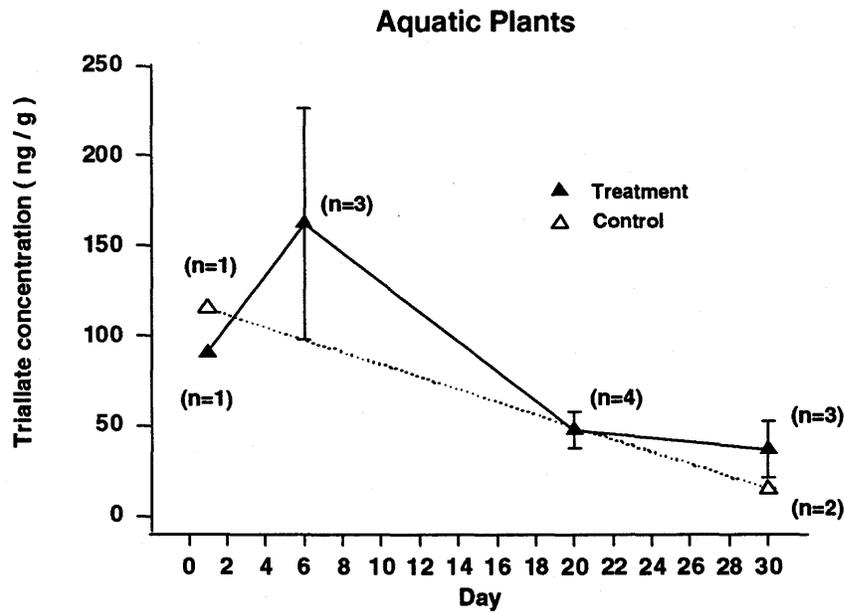
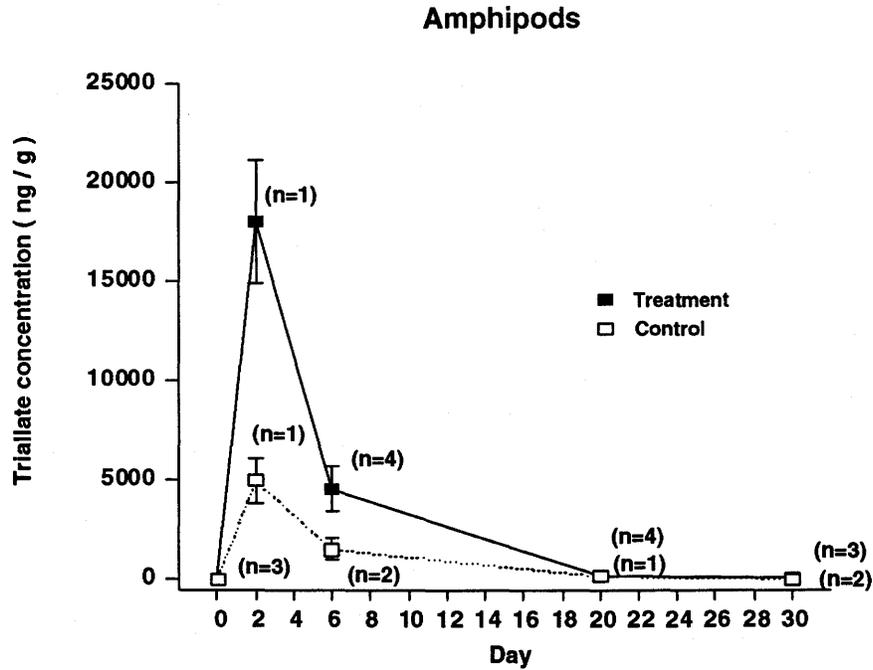


Figure 3.2. Triallate concentrations in amphipods (ng/g wet weight) and the aquatic plant *Potamogeton richardsonii* (ng/g wet weight) with time. Values are mean concentrations with standard errors. The number of enclosures sampled is shown in parentheses.

3.4 Discussion

3.4.1 Distribution of Triallate

The level of triallate applied in this study represents a worst case contamination event by runoff in a 'standard' permanent pond (see chapter 2). The concentrations reported here are therefore much higher than the levels of environmental contamination in natural systems reported by others (Therrien-Richards and Williamson 1987, Waite *et al.* 1992, Donald and Syrgiannis 1995). However, background levels measured in pre-treatment samples of sediment and amphipods are in agreement with the concentrations they reported.

Dissipation of triallate from the water column was very rapid. Two main processes, volatilization and partitioning to the sediment, contribute to the disappearance of triallate from the water; other processes such as hydrolyzation or photodecomposition appear to be unimportant (Kent *et al.* 1992). Although I did not measure volatilization of triallate, this process was certainly responsible for some loss from the system as there was a strong smell of triallate immediately after application. Muir (1991) predicted that rapid volatilization of triallate would occur from shallow waters based on its high transfer coefficient. Partitioning of triallate to the sediment is clearly demonstrated by a sharp rise in sediment concentration of triallate 2 days after application coupled with a considerable decrease in the water concentration (Figure 3.1). Triallate levels in sediment remained elevated for at least 30 days and possibly longer. Bioaccumulation of triallate was another process contributing to a decrease of triallate in water. Triallate levels were elevated in amphipods and to a lesser extent, in the aquatic plant *P. richardsonii*.

Donald and Syrgiannis (1995) suggest that triallate may persist in lake sediment for a year or more based on its presence in dry lake sediments that had not received any input for over a year. Persistence in sediment would not be surprising since approximately 25% of soil-applied triallate carries over from one growing season to the next in the prairies (Smith 1971) but the mechanisms of soil and sediment persistence might be very different.

After initial rapid loss by volatilization, microbial degradation is the main process responsible for the disappearance of triallate in soil (Anderson 1981). Adsorption and desorption are important in soil because these processes bring triallate in the solution phase and make it available for biodegradation (Anderson 1981). Physical separation of the herbicide and microorganisms is the main factor limiting the rate of microbial degradation of triallate (Anderson and Domsch 1980).

Microbial degradation of triallate in the aquatic environment is not well understood. McKercher and Thangudu (1982) observed an increased retention of triallate in flooded soils, suggesting that anaerobic conditions in sediment are not favourable for microbial degradation. Anaerobic conditions do not necessarily occur in the upper layer of sediment where most of the triallate is probably found. Sediments experience steep vertical gradients of oxygen over distances of millimeters to centimeters (Burton 1991). Benthic algae create vertical distributions of oxygen in the sediment and this gradient can be altered by epibenthic and benthic organisms enabling oxygen to penetrate more deeply into sediments (Burton 1991). Moreover, microbial organisms adapt rapidly to different habitats and microbial degradation of certain compounds is

possible under anaerobic conditions (Atlas and Bartha 1991). However, formation of bound residue in the sediment may make triallate unavailable to microorganisms. In soil, triallate degradation occurs in the solution phase (Anderson 1981). Anderson and Domsch (1980) report the biodegradation of bound residue of a closely related herbicide diallate. Nonetheless physical separation between microorganisms and herbicide impairs biodegradation (Anderson and Domsch 1980). These phenomena rather than the inability of microorganisms to degrade triallate, may be responsible for the persistence of this herbicide.

Soil microorganisms are not unique in their ability to degrade triallate; pelagic aquatic bacteria can also utilize this herbicide. Waiser (1995) demonstrated the utilization of triallate by aquatic bacteria after the addition of nutrients (nitrogen and phosphorus) in a microcosm experiment with a natural bacterial community from an oligotrophic lake. Bacterial numbers, rates of glucose uptake as well as thymidine incorporation increased due to the presence of a readily available carbon source, triallate. Bacteria required nutrient addition to utilize the herbicide as a source of carbon and triallate addition alone did not stimulate bacterial growth or metabolism. Likewise, Johnson (1986) observed no differences in microbial processes in the hydrosol after triallate exposure to triallate concentrations of 10, 100 and 1000 $\mu\text{g/L}$ in a microcosm.

3.4.2 Level of Exposure to Aquatic Organisms

One of the objectives of this study was to relate the biological effects (described in chapter 4) to concentrations of triallate to which organisms were exposed. The target water concentration of 250 $\mu\text{g/L}$ was nearly reached and levels were fairly consistent

among the 4 enclosures. Exposure from water was important in the first few days only since triallate disappeared quickly from the water reaching a mean concentration of 86.8 ± 20.2 SE $\mu\text{g/L}$ after 1 day and 32.1 ± 0.7 SE $\mu\text{g/L}$ after 2 days.

Triallate partitioned to sediment and concentrations were very high 2 days after application (6188 ± 3082 SE ng/g) and remained high one month after application (1408 ± 438 SE ng/g). Levels of triallate measured in sediment are questionable, however, because of very high variability and the presence of triallate in control samples at fairly high concentrations. Only very low levels of triallate were measured in control water samples on one sampling day (day 3) suggesting possible contamination of the sediment samples. Although precise levels of triallate measured in sediment are questionable, it is clear that sediments are an important sink for triallate in aquatic systems.

Triallate-contaminated sediments potentially constitute an important source of exposure for benthic organisms. Chronic effects could be important since triallate levels in sediment were elevated for an extended time period. Bioavailability of triallate associated with sediments is not known. Adsorption and desorption processes in sediment determine the amount of triallate released back to pore water. Generally, it is the portion of a toxicant present in solution that is available for uptake by organisms and responsible for toxicity; bound residues are not bioavailable (Nebeker *et al.* 1984, Di Toro *et al.* 1991). However, ingestion of chemical-contaminated organic material in sediment or in water may constitute another route of exposure.

Triallate concentrations in amphipods were high. Levels measured 6 days after application were twice as high as concentrations measured in *H. azteca* exposed to a

similar concentration (220 µg/L) of triallate in small limnocostracans (Arts *et al.* 1995).

Because amphipods are in close contact with both water and sediment, both sources may contribute to their total exposure to triallate. The relative contribution of triallate present in water and in sediment was not studied, however.

Other herbicides were also measured in sediment and biota. Diallylate and trifluralin were found in a large proportion of the sediment samples. In comparison to the high level of triallate, the presence of these herbicides is probably insignificant. Trifluralin was also present at low levels (2.9 ± 0.3 SE ng/g) in a large number of amphipod samples, but diclofop-methyl was present in only 2 samples. Levels of triallate were, however, many times higher than concentrations of these other herbicides.

3.5 Conclusion

Knowledge of the fate of a contaminant after it enters a wetland is important to understand potential impacts on such a system. The herbicide triallate is widely used in the Canadian prairies, and is known to contaminate the environment, yet little was known about the fate of triallate in natural aquatic systems. I found that dissipation of triallate from the water was very rapid within the first 2 days and after 10 days triallate was present only at a low concentration (mean = 2.5 ± 1.0 SE µg/L). Volatilization and partitioning to the sediment are two main processes contributing to the disappearance of triallate from water. Levels of triallate in sediment were extremely high and remained elevated for at least one month; however, there was large variation and triallate was detected in control samples suggesting the possibility of contamination of samples. Therefore, these results need to be viewed with caution. Uptake of triallate by

amphipods was high (mean concentration 2 days after application is 18000 ± 3100 SE ng/g), but again the presence of triallate in control samples indicates possible contamination. Levels in the aquatic macrophyte *P. richardsonii* were lower than levels measured in amphipods with a mean concentration of 161.9 ± 64.1 SE ng/g after 6 days. Exposure of aquatic organisms to residues in water is of short duration while triallate in the sediment remains elevated for an extended period.

Overall, the dissipation curve of triallate in water will be particularly pertinent when evaluating potential exposure of planktonic organisms. Unfortunately, levels of triallate in sediment and biota must be considered with caution.

4.0 EFFECTS OF TRIALLATE ON SELECTED ORGANISMS IN MESOCOSM EXPERIMENTS

4.1 Introduction

The impact of widespread use of pesticides on environmental quality is a serious concern. This is particularly true on the Canadian prairies where remnants of natural habitats and wetlands are closely interspersed with extensive areas of cropland. Triallate is a frequently used and ubiquitous herbicide for control of wild oats in the prairie pothole region that often finds its way into wetlands (Waite *et al.* 1992, Kent *et al.* 1992, Donald and Syrgiannis 1995).

Triallate is toxic to a variety of aquatic organisms. Adverse effects have been reported for algae, zooplankton, chironomids and fish, but aquatic macrophytes were not affected by triallate (Kent *et al.* 1992). *Daphnia magna* and *Ceriodaphnia dubia* are particularly sensitive to triallate, demonstrating a 48 h LC50 of 57 and 12 µg/L, respectively (Kent *et al.* 1992). In the latter species, reproduction was reduced (by 59%) at a concentration of 2.4 µg/L. Fishes and vertebrates in general are more tolerant (Kent *et al.* 1992).

Laboratory bioassays are a logical first step in toxicity evaluation, providing an indication of the potential toxicity of a compound. To understand ecological effects of a toxicant, however, it is imperative to examine effects on multiple species from different trophic levels. Studies of complex systems are more realistic since they allow for biotic

interactions that occur in nature. In addition, when natural processes occur, such as bioconcentration of toxicants in food, adsorption by sediments or microbial degradation, then standard laboratory tests may be poor predictors of ecosystem effects because exposure to the toxicant is different (Cairns and Pratt 1989). Another reason for covering more than one trophic level is that effects not apparent at one level (e.g. primary producers) because of high variance and (or) low treatment effect, might be more important at another level further up the food chain. In complex natural systems, indirect effects of herbicides may be more important than direct effects and therefore non-target organisms may be better indicators of herbicide stress (Lampert *et al.* 1989).

Toxicity of triallate to aquatic organisms has been demonstrated in laboratory and microcosm experiments, but its effects in natural systems are largely unknown. The goal of this research was to determine the impact of triallate contamination on the aquatic system by measuring effects on a number of organisms representing different trophic levels. Phytoplankton, periphyton, zooplankton (*Daphnia pulex*), and amphipods (*Hyaella azteca*) were selected for evaluation in a mesocosm experiment. A brief description of these organisms is presented below.

Phytoplankton refers to algae suspended in the water column. These photosynthetic primary producers are of vital importance in aquatic systems since they form the base of aquatic food chains. Importance of algae in nutrient cycling and energy flow prescribes their inclusion in assessments of the stability and balance of aquatic ecosystems (Stevenson and Lowe 1986). Moreover, phytoplankton responds quickly to changes in water quality (Stevenson and Lowe 1986).

Periphyton represents a community of algae, bacteria, fungi and microscopic animals attached to various substrates. For instance, periphyton grows on rocks, sediment, sand, aquatic plants and artificial substrates including glass, plastic and ceramic tiles. Periphyton, together with phytoplankton, is the foundation of many food chains in aquatic systems. Ubiquity, sensitivity to environmental perturbation and high turnover rate are some characteristics responsible for the increased use of periphyton in environmental assessment (Napolitano *et al.* 1994). Moreover, natural and artificial substrates supporting complex periphytic assemblages can be collected and transported easily with little disturbance, and thus they are useful for toxicity studies.

The animal components of freshwater plankton, the zooplankton, are dominated by 3 major groups: the rotifers, and 2 subclasses of the Crustacea, the Cladocera and Copepoda. Most are 0.2 to 3.0 mm in length but some are as small as 0.1 mm. Zooplankton are primary consumers which feed on algae, bacteria and detritus. They are very important in aquatic food chains, being a major link between primary producers and vertebrate predators, but some species of zooplankton are carnivorous.

D. pulex was the dominant crustacean zooplankton in Gursky's pond in summer, 1993. The body of this cladoceran is covered by a bivalve cuticular carapace that attains a maximum length of about 3.0 mm. *Daphnia* are non-selective filter feeders, consuming algae, bacteria, fungi, protozoans and organic debris. Reproduction is parthenogenetic for the greater part of the year in most habitats. Females produce eggs which develop into parthenogenetic females without fertilization. The number of eggs produced varies from 2 to 40 (Pennak 1989). Eggs are deposited in a transparent brood chamber, a cavity

dorsal to the body and delimited by the carapace. The egg has a central yolk droplet which consists mainly of triacylglycerol (Goulden and Henry 1984) and is used for nutrition during development. Egg development occurs in the brood pouch and proceeds through 5 distinctive development stages (Threlkeld 1979). The lipid droplet remains intact in the first stage (which accounts for ~30 % of total development time) but the egg changes in later stages, with the embryo becoming distinct and growing into a miniature form of the parent. Young are released at molting. One clutch of eggs is normally released into the brood chamber during each adult instar. *D. pulex* has 3 to 4 juvenile instars and 15 to 20 adult instars with the duration of each adult instar being highly variable, although about 2 days under optimum conditions (Pennak 1989). An adult can therefore produce several hundred progeny in a lifespan under favourable temperature conditions and food supply. Lifespan is approximately 42 days at 18 °C under good conditions (Pennak 1989). Growth is not continuous in *Daphnia* but occurs in a stepwise fashion at the end of each instar. Four events occur in rapid succession at the end of each adult instar: release of young from the brood pouch, molting, rapid increase in size, and deposition of a new clutch of eggs into the brood pouch all occurring in a matter of minutes to a few hours (Pennak 1989). *Daphnia* spp. are popular test organisms since they are easily cultured as parthenogenetic clones, they have a short life cycle, and their eggs, which are carried in a transparent brood pouch, can be easily removed, counted and measured.

H. azteca is a small amphipod (2 to 10 mm) that inhabits permanent lakes, ponds and streams and is particularly abundant in prairie potholes where densities exceeding 10

000 animals/m² are common (de March 1981). *H. azteca* is an epibenthic animal that hides and feeds mainly on the bottom sediment but becomes more active swimming at night. The upper 2 cm of sediment constitutes the limit of vertical distribution of *H. azteca* in sediment (Wetzel 1983). *H. azteca* is detritivorous as it feeds mainly on particulate animal and plant remains but also grazes periphyton attached to macrophytes and other substrates. It is also a deposit-feeder, consuming epibenthic algae and bacteria and other organic material from ingested sediment particles (Hargrave 1970). Growth, density and body size of *H. azteca* are influenced by the quantity of epipelagic algae and sediment microflora (Wetzel 1983). Obligatory sexual reproduction occurs in amphipods. Reproduction begins when water temperature reaches 20 °C and up to 5 generations can be produced in a season. The male carries the female on its back and they remain in amplexus for 1 to 7 days until the female molts (Pennak 1985). Copulation takes place within 1 day after molting. Eggs and later the live young are carried in a brood pouch in the female's abdomen, also called a marsupium. Eggs in the female can be seen both in the ovary and in the marsupium. At each mating the female molts and releases young from the previous mating. Mature females can produce 1-30 young at each molt and the number produced is related to female size (Pennak 1985). *H. azteca* has a minimum of nine instars to maturity, with the first five comprising the juvenile stage; its life cycle lasts less than a year (Pennak 1985). It is frequently used in sediment toxicity testing (Burton 1991). *H. azteca* also is an important food source for fish, waterfowl, wading birds, salamanders and larger invertebrates.

The organisms selected to evaluate the impact of triallate represent different trophic levels; primary producers, primary consumers and a detritivore associated mainly with sediment. Measures of biomass of phytoplankton and periphyton, and measures of size and fecundity of *D. pulex* and *H. azteca*, are the main endpoints used to evaluate effects. In addition to measures of survival, measures of fecundity are important to predict the impact of a toxicant at the population and community level. The effects on size (growth) and energetic state are useful measures of effects because these properties can be related to fecundity (Giesy and Graney 1989). For these reasons, considerable effort was made to study the fecundity of *D. pulex* and *H. azteca* and to evaluate energy allocation to *D. pulex* offspring with measures of egg size and egg lipid content. The total amount of lipid energy that a female allocates to reproduction, maternal lipid investment (Arts and Sprules 1988), was also used to study the effect of triallate on energetic states of *D. pulex*.

4.2 Materials and Methods

A description of the mesocosms and details of application methods for triallate is provided in chapter 2.

4.2.1 Sampling Schedule

To study biological effects, samples were collected before and after treatment. Pre-treatment samples were taken 2 and 3 days before triallate application and post-treatment samples were taken 1, 2, 3, 6, 10, 20, 30, 60 and 90 days after triallate application. Sampling for zooplankton started on day 2 and amphipod samples were first collected 3 days post-treatment. Measurements of population parameters were

performed for all samples of phytoplankton, but only on selected samples of zooplankton and amphipods; these were selected on the basis of the life-cycle of the each organism and likelihood of observing effects.

4.2.2 Physicochemical Characteristics

Physical parameters such as temperature, pH, dissolved oxygen and conductivity were monitored over time in the mesocosms and in the pond using an automatic analyzer (Surveyor 5, Hydrolab Corp. Austin, Texas).

4.2.3 Phytoplankton

Phytoplankton biomass was estimated using size-fractionated chlorophyll analysis. Sequential filters with pore sizes of 1.2 μm , 20 μm and 30 μm were used to partition the biomass of algae into different size groups. Edible algae (1.2-20 μm and 20-30 μm) were retained on 1.2 and 20 μm filters while non-edible algae (> 30 μm) were retained on the 30 μm filter. Phytoplankton samples were collected with 1 L bottles lowered approximately 30 cm underwater. Samples were kept cool in the dark and a measured sample volume (250 ml) was filtered within 12 h of collection. Filters were frozen at -40 $^{\circ}\text{C}$ and analyzed at a later date. Chlorophyll analysis was performed separately on each fraction and total chlorophyll *a* was obtained by adding the three fractions. Chlorophyll *a* was extracted with 90% ethanol and quantified using the fluorometric method of Nusch (1980).

4.2.4 Periphyton

Periphyton growth on an artificial substrate was estimated from chlorophyll *a* concentration and ash free dry mass measurement. In each enclosure, 3 ceramic tiles (20

x 20 cm) were placed on an adjustable support. Tiles were kept 10 cm under the water surface and periphyton was sampled every two weeks. Periphyton was scraped off the tile with a sharp blade within a standard area (28.3 cm²). Periphyton samples were frozen at -40 °C and kept in the dark until analyzed. Periphyton samples were resuspended in 20 ml of deionized water and 2 aliquots of 2 ml were taken and filtered on separate GFC filters: one for chlorophyll analysis, the other for ash free dry mass determination.

Chlorophyll analysis was described by Nusch (1980) and ash free dry mass determination followed Aloï (1990).

4.2.5 *Daphnia pulex*

Zooplankton samples were collected using a 30 L Schindler trap. Three to five samples were taken in each enclosure and pooled to obtain a sufficient number of animals ~300 *D. pulex*. Samples were preserved with saturated sugar formaldehyde solution (8% formaldehyde).

Gravid females were measured for body length, egg-free body weight, clutch size, mean egg volume (per female), mean lipid volume (per female), and maternal lipid investment (MLI). MLI represents the lipid energy reserve transferred from the mature female to the eggs at the time that they are deposited in the brood chamber. Thirty gravid females with stage 1 eggs were used for measurements when possible.

Zooplankton samples taken at pre-treatment, 2, 6, 10, 20 and 30 days post-treatment were analyzed.

A compound microscope was used for measurements of body size, clutch size, egg volume, and lipid volume. Body length was measured from the dorsal margin of the

carapace above the eye to the posterior tip of the carapace at the base of the tail spine.

Eggs from each female were removed from the brood chamber, counted, and a subsample of 10 eggs was measured for width, length and diameter of the lipid droplet. Volumes of eggs and lipid droplets were calculated from formulae for volumes of prolate spheroids and spheres, respectively. The volume of a prolate spheroid is calculated as:

$volume = \frac{4}{3}\pi\left(\frac{a}{2}\right)^2 * \left(\frac{b}{2}\right)$, where a=short axis and b=long axis of the spheroid. Mean egg

volume and mean lipid volume were determined for each female. Each female (eggs removed) was subsequently dried at 55 °C for 24 h and weighed on a microbalance (Cahn C-30) to the nearest 0.001 mg. MLI was calculated as the product of the average lipid volume of stage 1 eggs in the clutch and clutch size (Arts and Sprules 1988).

4.2.6 Amphipods

Amphipods were collected with a dip net, transferred to a sorting tray and placed in plastic jars. Samples were preserved in sugar formaldehyde solution (8% formaldehyde). Selected samples were sorted by species (*H. azteca* or *G. lacustris*), and measurements of body length, body weight and clutch size were performed on *H. azteca*. Thirty males and 30 gravid females were randomly selected and measured. Body length was measured using a digitizing pad aided by a computer program (SigmaScan, Jandel Co.) (Quigley and Lang 1989). Eggs were removed from the marsupium of females and counted. Females (eggs removed) were dried at 55 °C for 48 h and weighed individually on a microbalance (Cahn C-30) to the nearest 0.001 mg.

4.2.7 Statistical Analysis

Analysis of variance, multivariate analysis of variance and analysis of covariance were used (Zar 1984). Analyses were executed on the Statistical Analysis System (SAS Institute 1990).

4.3 Results

4.3.1 Physicochemical Characteristics

Temperature, pH, dissolved oxygen and conductivity were measured in the pond and monitored in each enclosure throughout the experiment. Triallate did not appear to alter the physicochemical characteristics of the water (Figure 4.1). ANOVA indicated that the parameters did not differ among enclosures, and most of the variation was associated with sampling date. Therefore, data from the 4 enclosures were pooled to produce a more powerful test of treatment effects. ANCOVA (with sampling date as a covariable) indicated that no differences existed between treatment and control groups for any of the parameters (Table 4.1).

4.3.2 Phytoplankton

Multivariate analysis of variance was used to assess simultaneously the effect of triallate, enclosure and sampling date on chlorophyll *a* concentration for 3 size fractions and total chlorophyll *a*. There were no significant differences between enclosures or treatments for any of the size fractions and for total chlorophyll *a*, and the interaction between enclosure and treatment was also non-significant. The only significant effect was sampling date and differences exist between sampling date for all fractions except the fraction retained on the 30 μm filter.

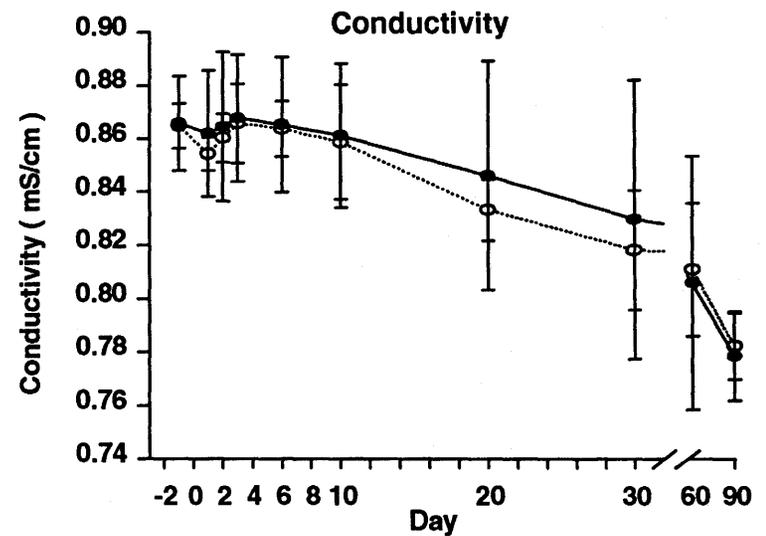
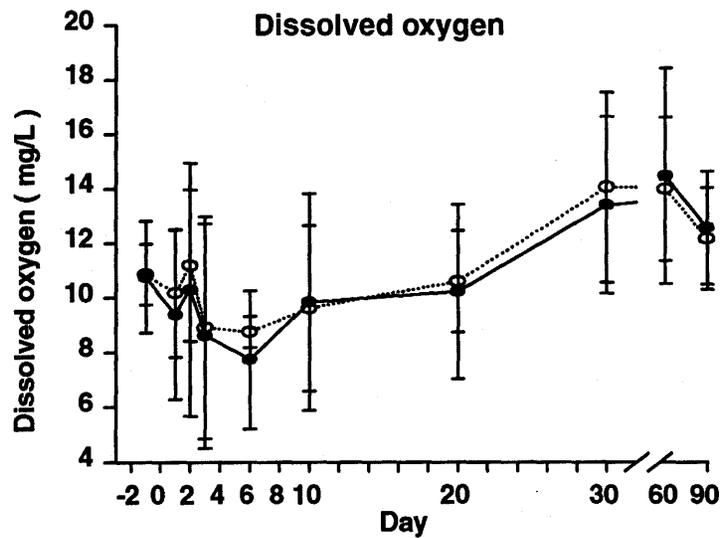
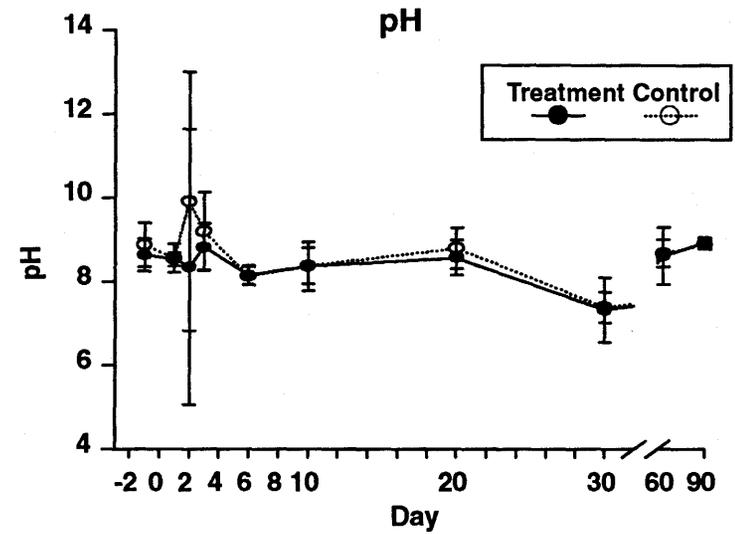
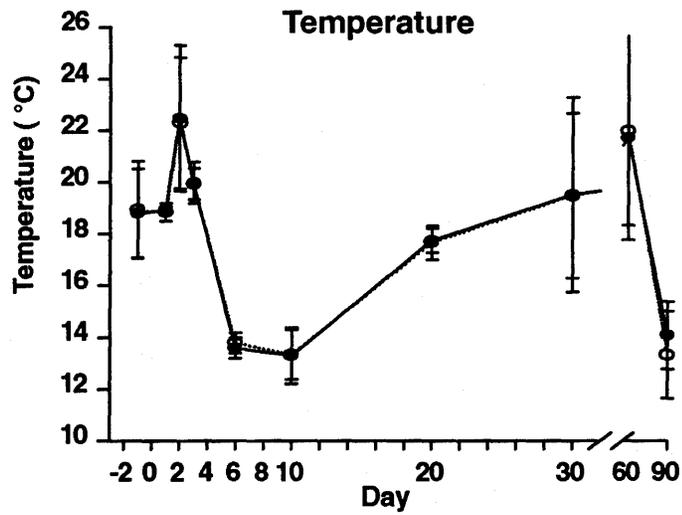


Figure 4.1. Physicochemical characteristics of the surface water for treated and control enclosures. Values are means of 4 enclosures with 95% confidence intervals. Treatment was applied on day 0 (19 June 1993).

Table 4.1. Results of ANCOVA for effect of treatment on temperature (Temp), pH, conductivity (Cond) and Dissolved oxygen (DO). Treatment is the main effect and sampling date (day) is the covariate. The interaction between treatment and sampling date was not significant. Shown are partial F-values for each parameter.

Source	DF	Temp	pH	Cond	DO
Treatment	1	0.14	1.42	0.37	0.29
Day	1	2.11	0.11	241.85***	17.00***
Error MS	77	12.52	0.72	0.01	5.18
R-Square		0.03	0.02	0.76	0.21

* P < 0.05, ** P < 0.01, *** P < 0.001; DF is degrees of freedom.

Following this analysis, samples from the four enclosures were pooled together to produce a more powerful test of treatment and sampling date effects on chlorophyll *a* concentration for the 3 fractions and total chlorophyll *a* (Table 4.2). Again, differences existed only for sampling date for all fractions except inedible algae (> 30 μm). I was unable to detect significant treatment effects, and the interaction of treatment and sampling date also was non-significant. Chlorophyll *a* concentrations for the 3 size fractions and for total chlorophyll *a* varied over time (Figures 4.2 and 4.3).

4.3.3 Periphyton

After 2 weeks growth, periphyton biomass was estimated from chlorophyll *a* concentration and ash free dry mass. Analysis of variance indicated significant differences between enclosures, and enclosure and sampling date interactions for both variables, so the analysis was done separately for each enclosure.

Because there were significant differences among sampling dates (for both variables), analysis of covariance was used with treatment as a main effect and sampling date as a covariable. Chlorophyll *a* concentration (Figures 4.4 and 4.5) and ash free dry mass (Figures 4.6 and 4.7) did not differ between treatments (Table 4.3).

Table 4.2. Results of MANOVA for the effect of treatment on chlorophyll *a* concentration for 3 size fractions and for total chl *a* in phytoplankton samples. Treatment and sampling date are the main effects. The interaction between treatment and sampling date was not significant. Shown are partial F-values for each fraction and for total chl *a*.

Source	DF	Phytoplankton size fractions			
		1.2 μm	20 μm	30 μm	Total Chl <i>a</i>
Treatment	1	0.15	0.19	0.88	0.74
Day	1	92.51***	12.45***	0	5.44*
Error MS	85	267.31	4.35	3967.99	4926.47
R-square		0.52	0.13	0.01	0.07

* P < 0.05, ** P < 0.01, *** P < 0.001; DF is degrees of freedom.

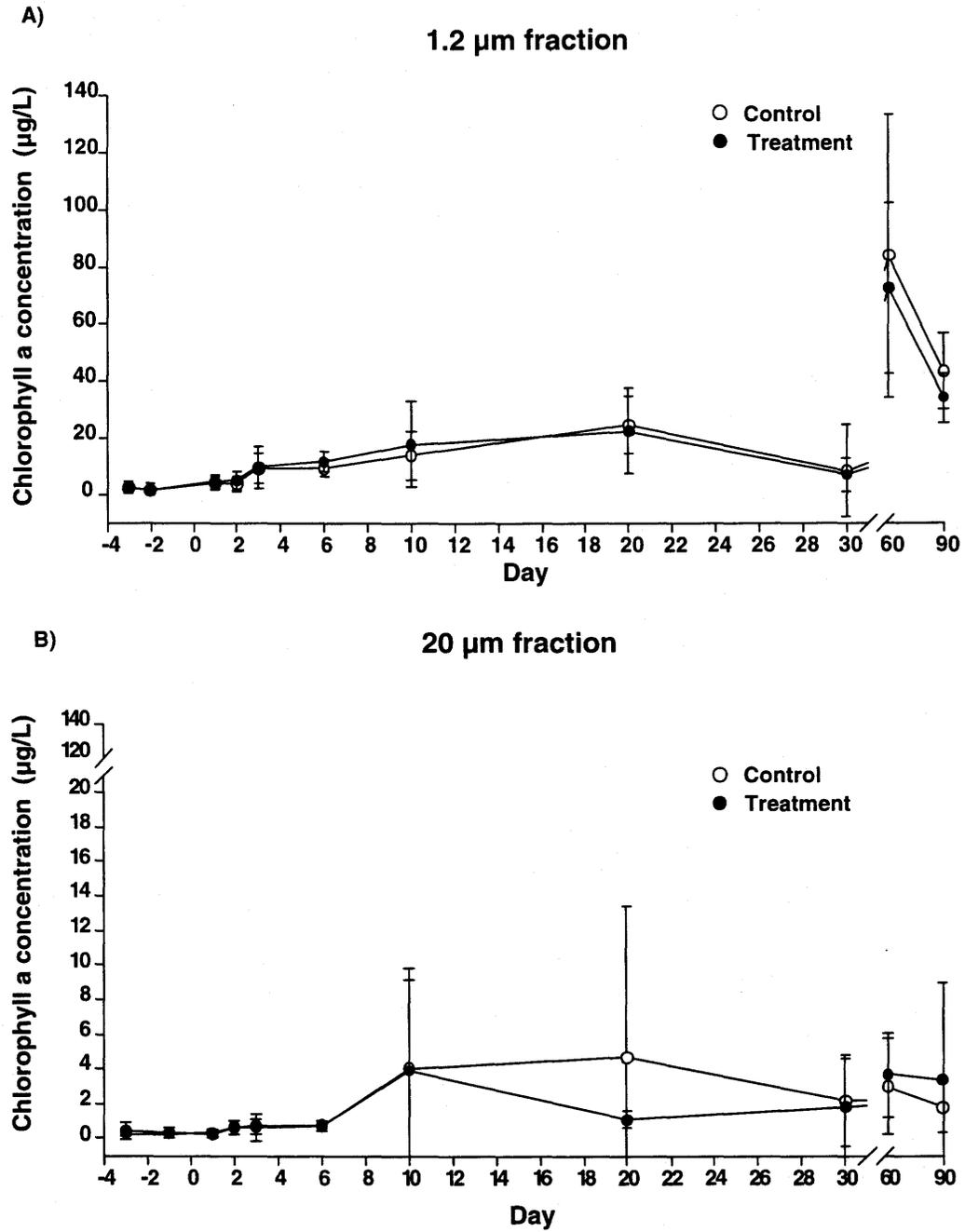


Figure 4.2. Chlorophyll *a* concentration for 2 size fractions of phytoplankton. Values are means from the 4 enclosures with 95% confidence intervals. A) 1.2 μm fraction and B) 20 μm fraction.

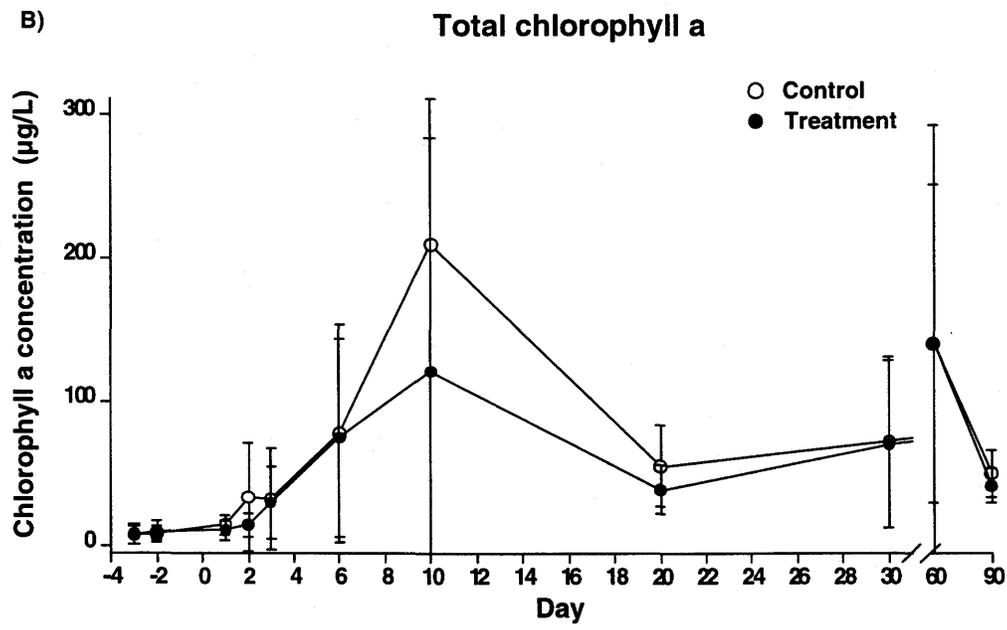
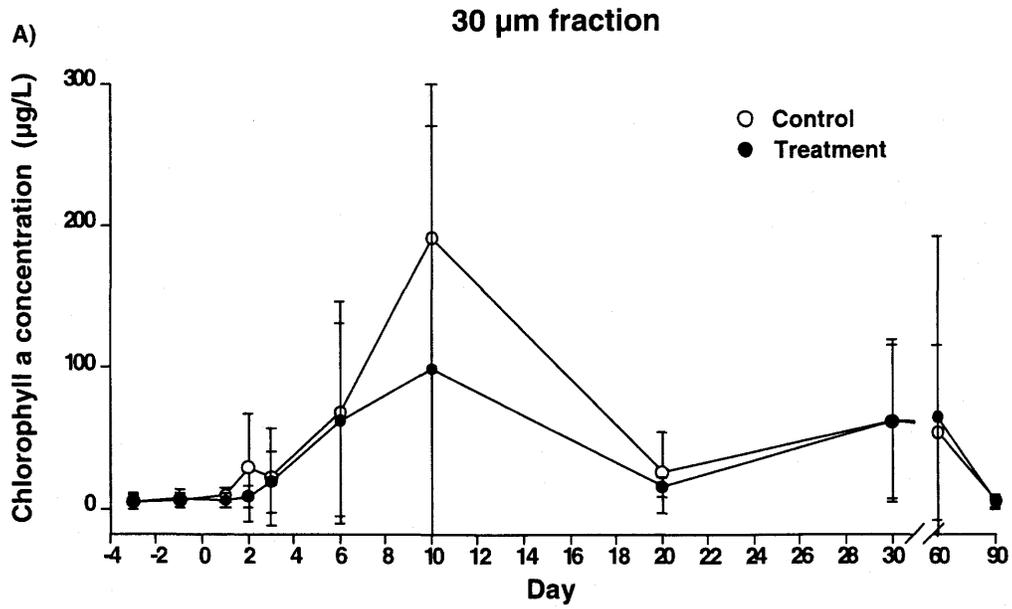


Figure 4.3. Chlorophyll *a* concentration for 30 μm size fraction of phytoplankton and total chlorophyll *a*. Values are means from the 4 enclosures with 95% confidence intervals. A) 30 μm fraction and B) total chlorophyll *a*. Scale is different from Figure 4.2.

Table 4.3. Results of ANCOVAs for the effect of treatment on chlorophyll *a* (Chl *a*) concentration and ash free dry mass (AFDM) of periphyton samples. Treatment is the main effect and sampling date (day) is the covariate. The interaction between treatment and sampling date was not significant. Shown are partial F-values.

Source	DF	<u>Enclosure 1</u>		<u>Enclosure 2</u>		<u>Enclosure 3</u>		<u>Enclosure 4</u>	
		Chl <i>a</i>	AFDM						
Treatment	1	1.31	0.01	0.92	0.76	1.91	0.49	1.67	1.58
Day	1	0.66	0.53	3.34	1.02	0.08	3.04	17.51***	7.10*
Error MS	27	0.57	0.01	0.73	0.01	0.01	0.01	1.29	0.05
R-square		0.07	0.02	0.12	0.06	0.06	0.12	0.42	0.25

* P < 0.05, ** P < 0.01, *** P < 0.001; DF is degrees of freedom.

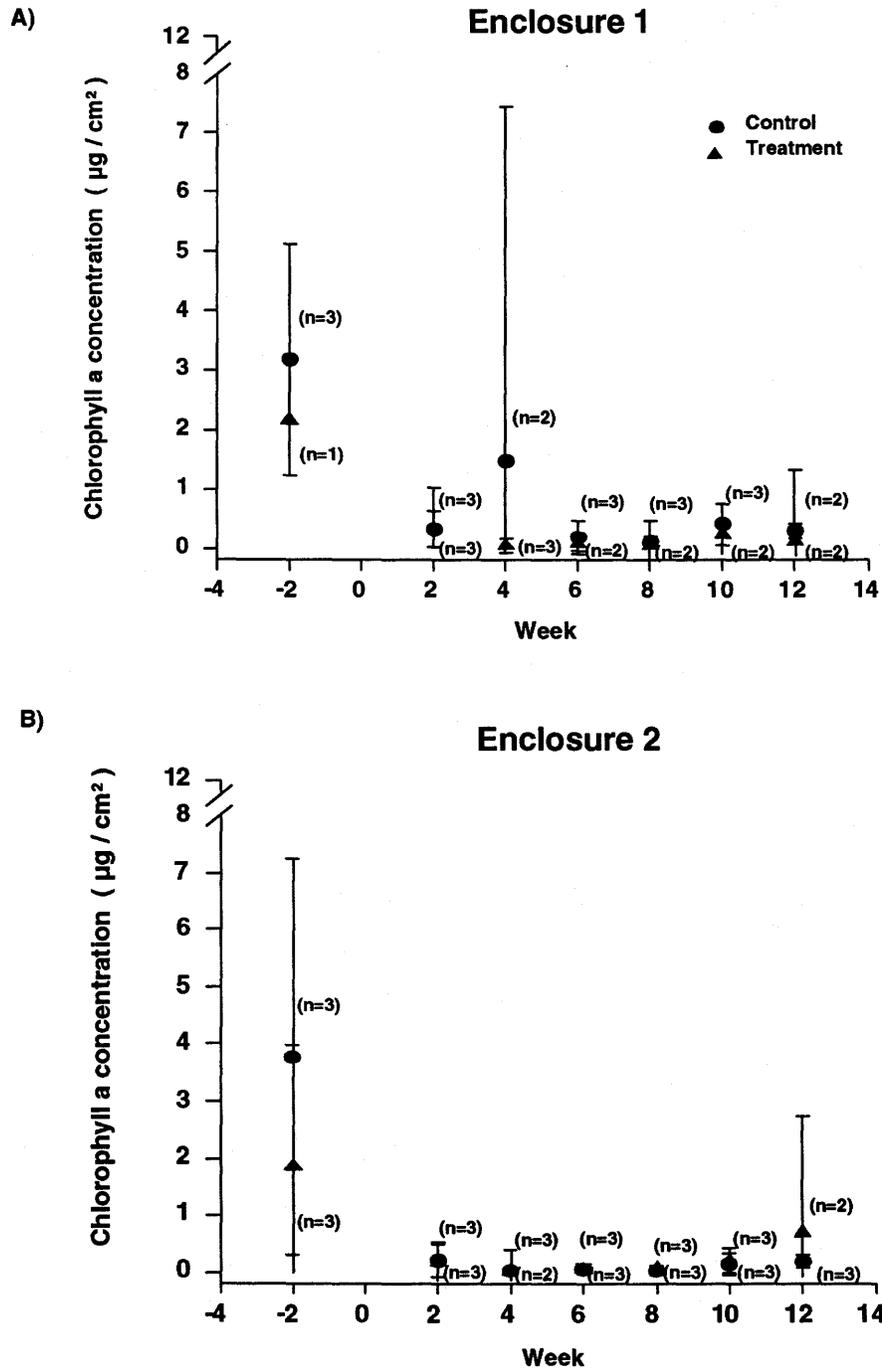


Figure 4.4. Chlorophyll *a* concentration of periphyton in enclosures 1 and 2. Values are means with 95% confidence intervals. Treatment was applied on day 0.

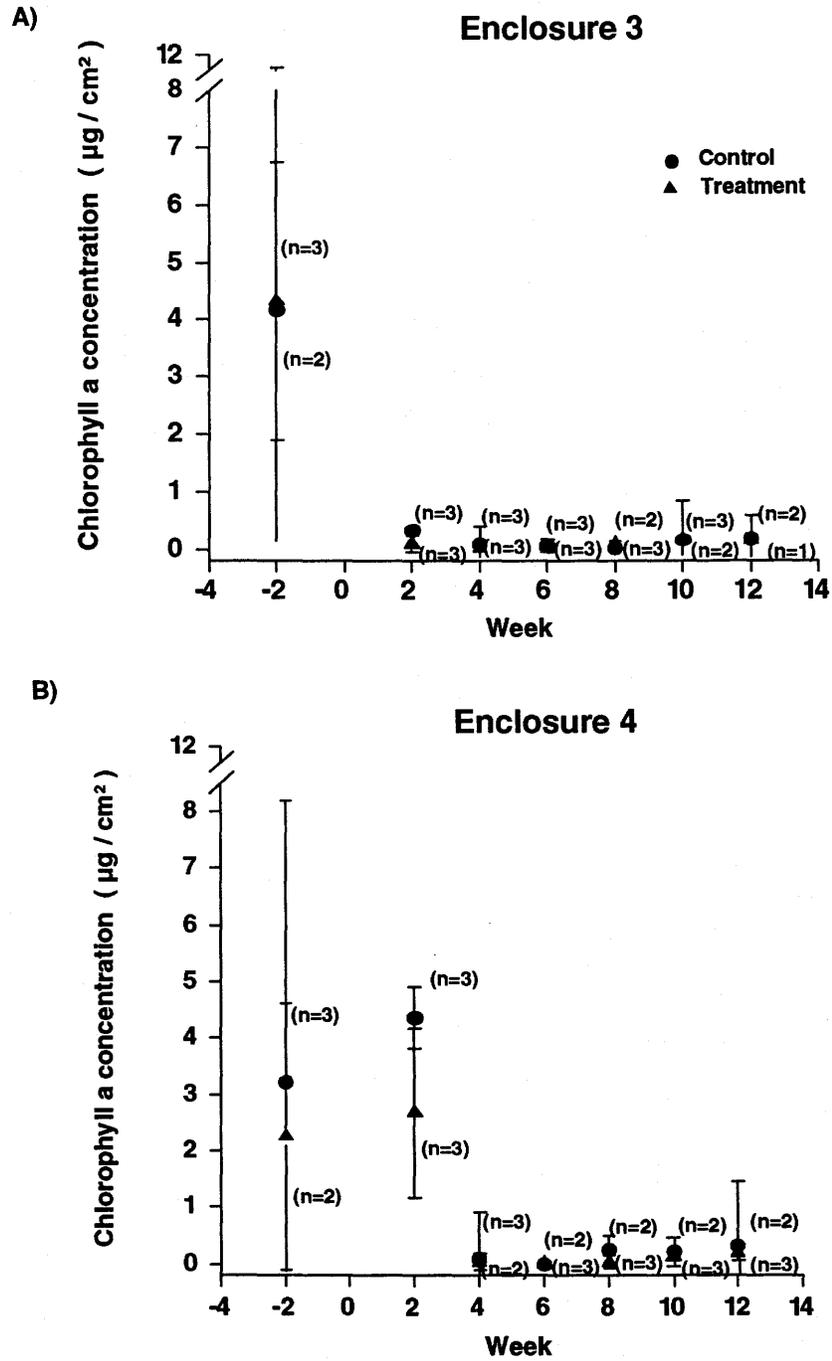


Figure 4.5. Chlorophyll *a* concentration of periphyton in enclosures 3 and 4. Values are means with 95% confidence intervals. Treatment was applied on day 0.

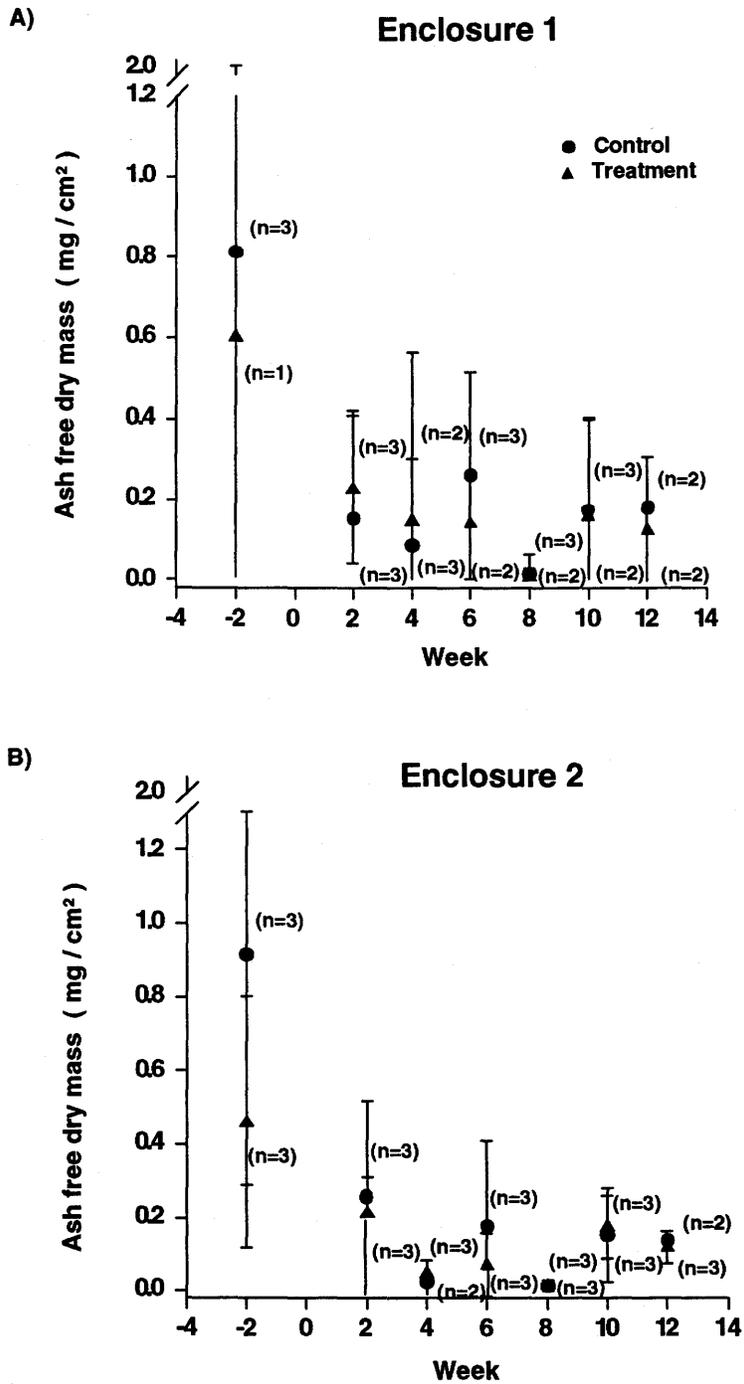


Figure 4.6. Ash free dry mass of periphyton in enclosures 1 and 2. Values are means with 95% confidence intervals. Treatment was applied on day 0.

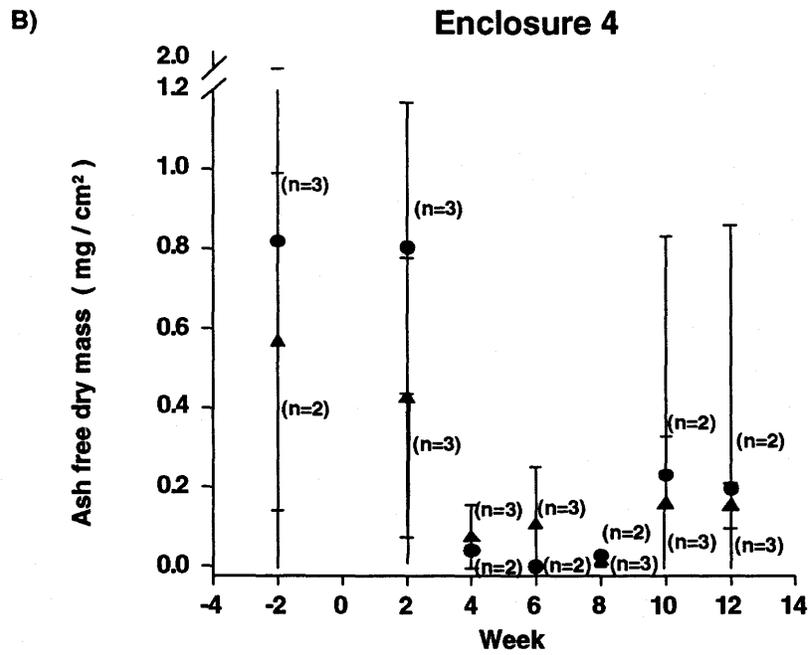
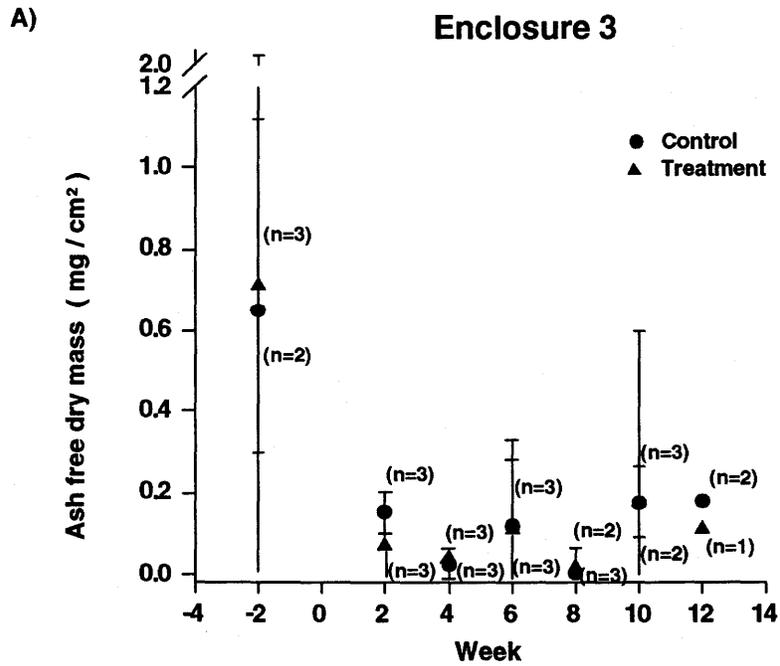


Figure 4.7. Ash free dry mass of periphyton in enclosures 3 and 4. Values are means with 95% confidence intervals. Treatment was applied on day 0.

4.3.4 *Daphnia pulex*

Gravid females were measured for body length, body weight, clutch size, mean egg volume, mean egg lipid volume and MLI. For all variables, ANOVA revealed significant differences associated with either enclosure, interaction between enclosure and treatment, and/or interaction between enclosure and sampling date. Therefore, subsequent analyses were done for each enclosure separately. Data from pre-treatment and day 30 samples were omitted from the statistical analysis and the analysis was performed with post-treatment samples taken 2, 6, 10, and 20 days after triallate application. Post-treatment data from day 30 were excluded because the majority of the *D. pulex* in this sample had never been exposed to triallate. There are 3 reasons to justify this omission: 1) triallate in water decreased sharply within 3 days and was almost 0 after 10 days (chapter 3); 2) maximum lifespan of *D. pulex* is only about 42 days at 18 °C and 3) *D. pulex* reproduce rapidly so that samples taken at day 30 would be composed mainly of animals born after triallate had disappeared from the water. In pre-treatment samples, there was no difference between treatments for all variables measured except in enclosure 3 where body length, body weight, mean egg volume and mean egg lipid volume of controls were significantly greater than treatment animals.

Because of the influence of body size on reproduction, analysis of covariance was used to look at effects of the toxicant on reproductive parameters while controlling body size. Body length was used as a covariable in the ANCOVA. Likewise, the number of eggs produced may influence egg size and egg lipid content, so analysis of covariance was used to evaluate effects of triallate on egg size and lipid content while controlling for effects of clutch size.

Enclosure 1

Treated *D. pulex* had significantly smaller body length, body weight, clutch size and MLI than control *D. pulex* but mean egg volume and mean egg lipid volume were not different (Figures 4.8 to 4.10; Table 4.4). Variation associated with sampling date and the interaction of sampling date and treatment was significant in this analysis. Clutch size, mean egg volume, mean egg lipid volume, and MLI all covaried positively with body length and there was no significant treatment effect. The overall effect of triallate was a reduction in body size. Indirect effects on reproduction manifested by a decrease in clutch size and MLI were directly related to smaller body size in treated *D. pulex* (Table 4.5). Mean egg volume and mean egg lipid volume did not covary with clutch size and no treatment effect was detected with ANCOVA (Table 4.6).

Enclosure 2

There was no difference between treatments for the variables body length, body weight, clutch size, and MLI but mean egg volume and mean egg lipid volume were larger in treated *D. pulex* (Figures 4.11 to 4.13; Table 4.4). Clutch size, mean egg lipid volume and MLI all covaried with body length, but mean egg volume did not. ANCOVA indicated no treatment effects on any of the variables (body weight, clutch size, mean egg volume, mean egg lipid volume, and MLI) corrected for body length (Table 4.5). Mean egg lipid volume covaried with clutch size, while mean egg volume did not, and no treatment effect was observed for either variable (Table 4.6).

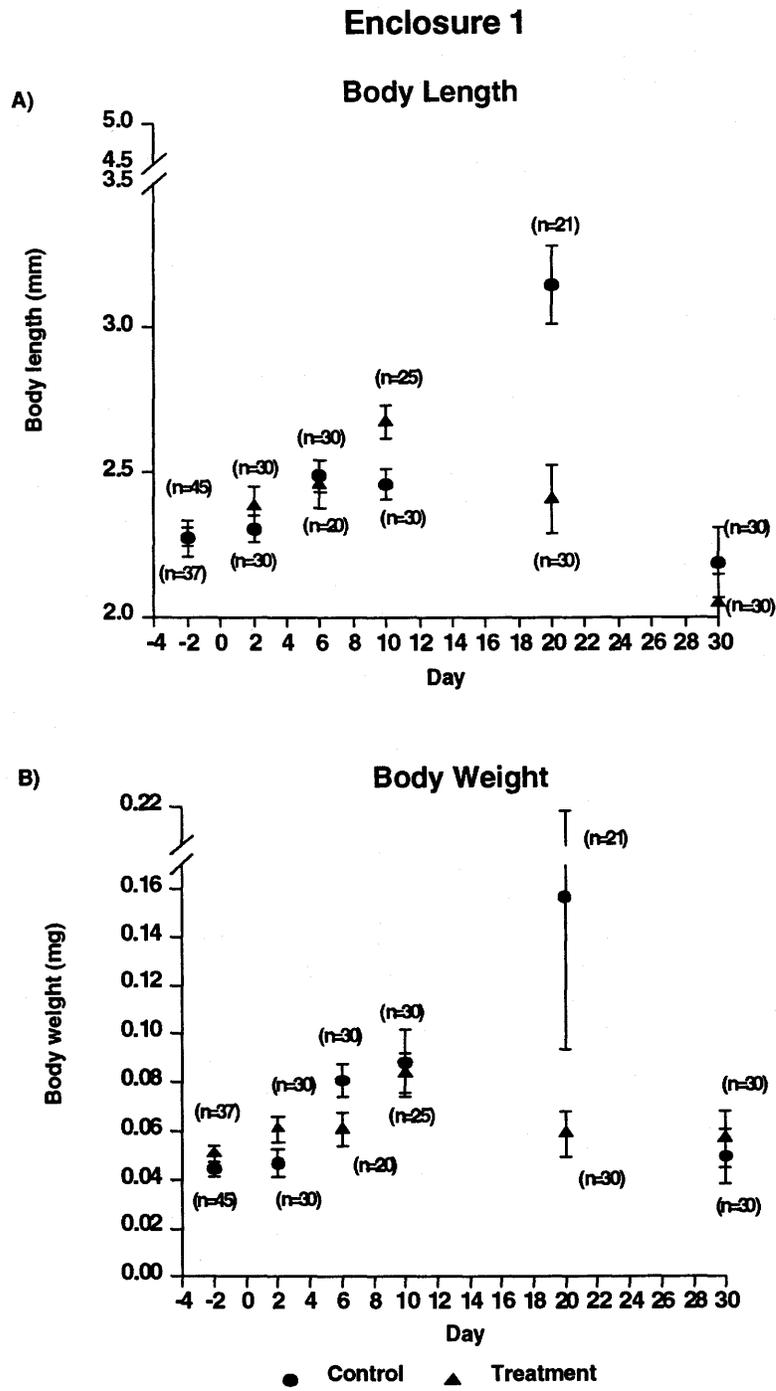


Figure 4.8. Body length (A) and body weight (B) for *Daphnia pulex* in enclosure 1. Values are means with 95% confidence intervals and, number of females is shown in parentheses.

Enclosure 1

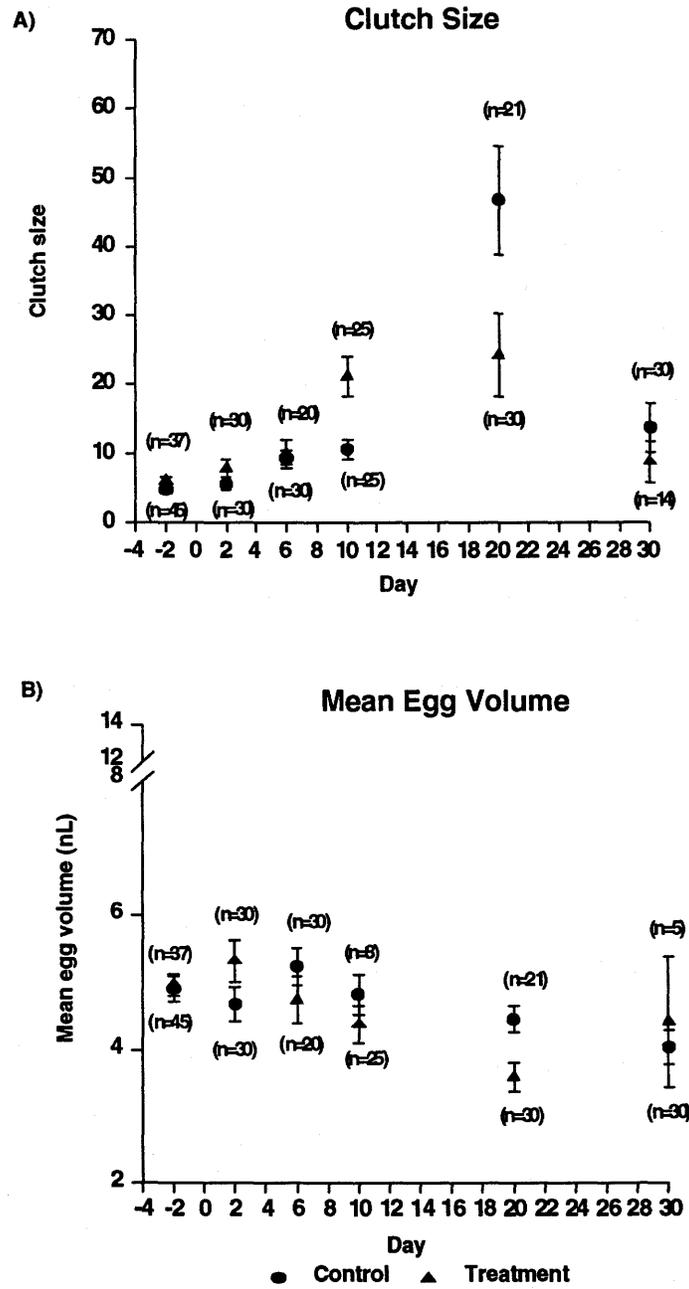


Figure 4.9. Clutch size (A) and mean egg volume (B) for *Daphnia pulex* in enclosure 1. Values are means with 95% confidence intervals and, number of females is shown in parentheses.

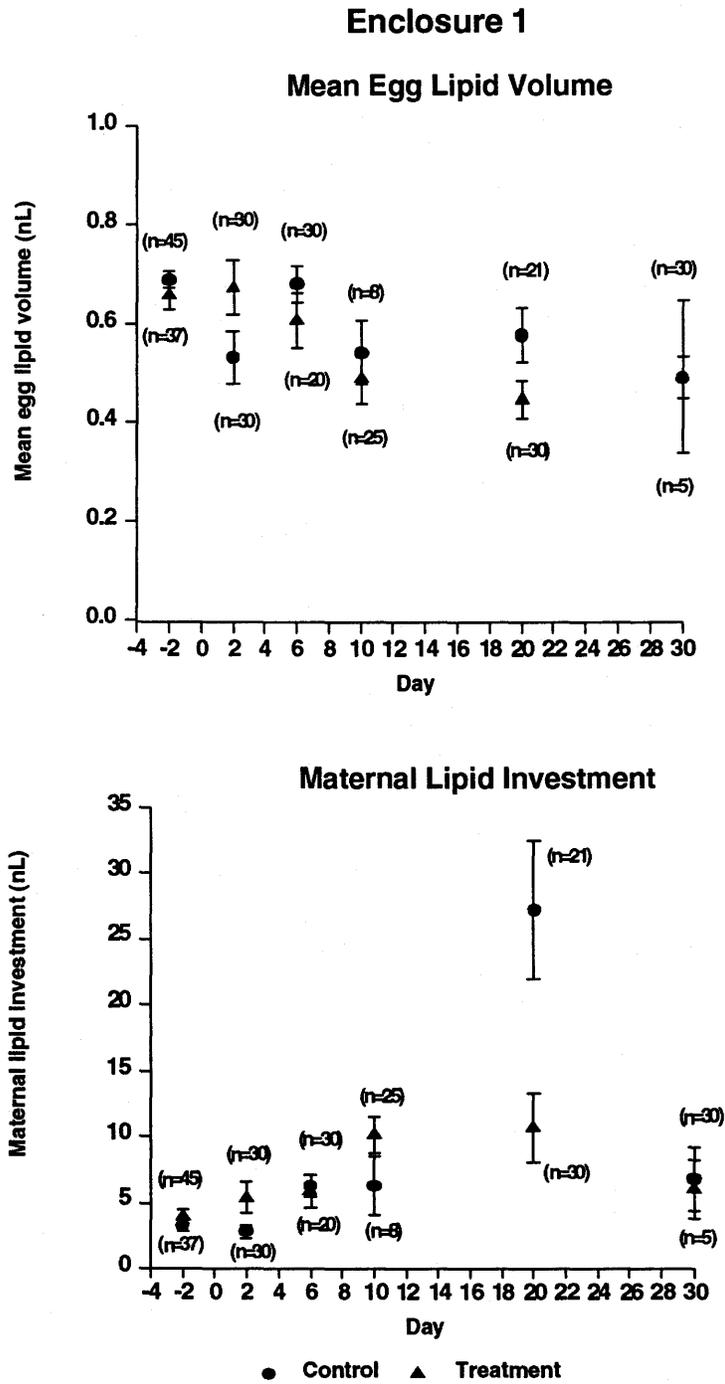


Figure 4.10. Mean egg lipid volume (A) and maternal lipid investment (B) for *Daphnia pulex* in enclosure 1. Values are means with 95% confidence intervals and, number of females is shown in parentheses.

Enclosure 2

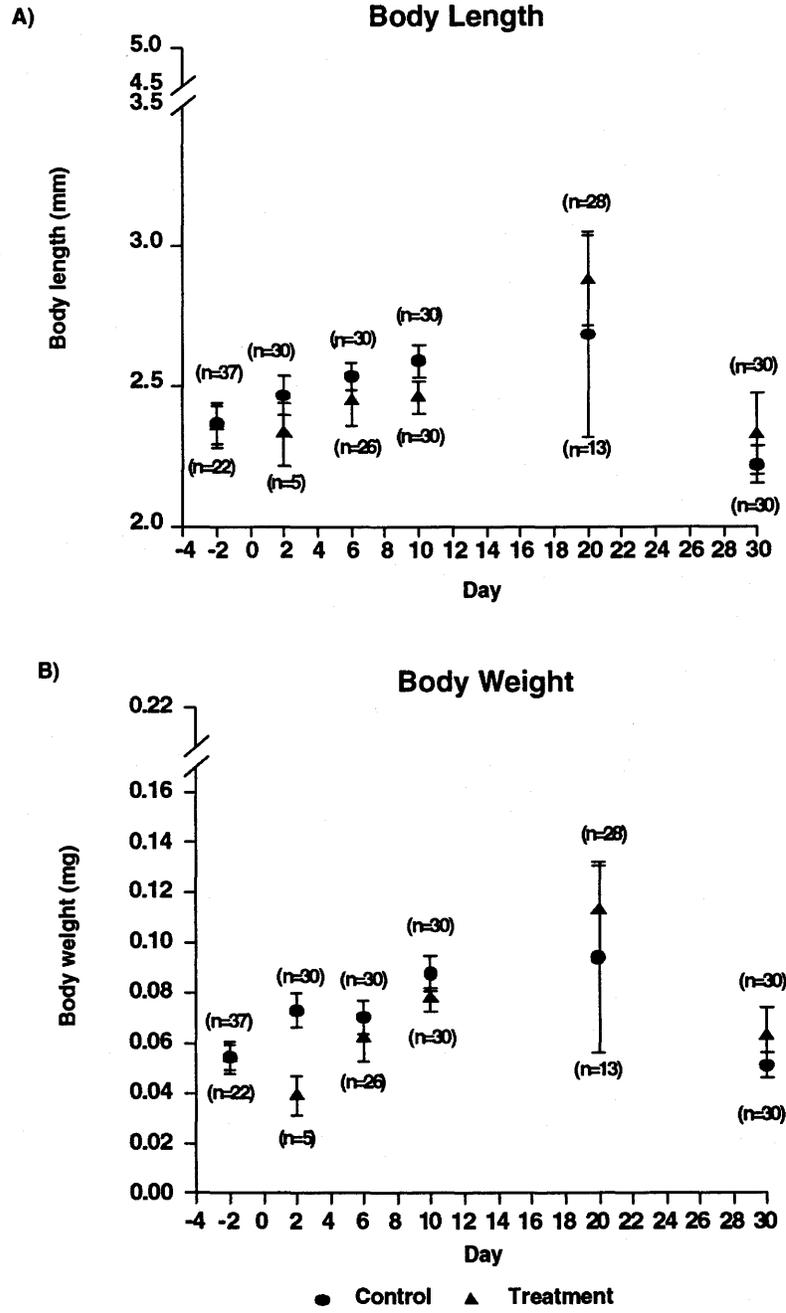


Figure 4.11. Body length (A) and body weight (B) for *Daphnia pulex* in enclosure 2. Values are means with 95% confidence intervals and, number of females is shown in parentheses.

Enclosure 2

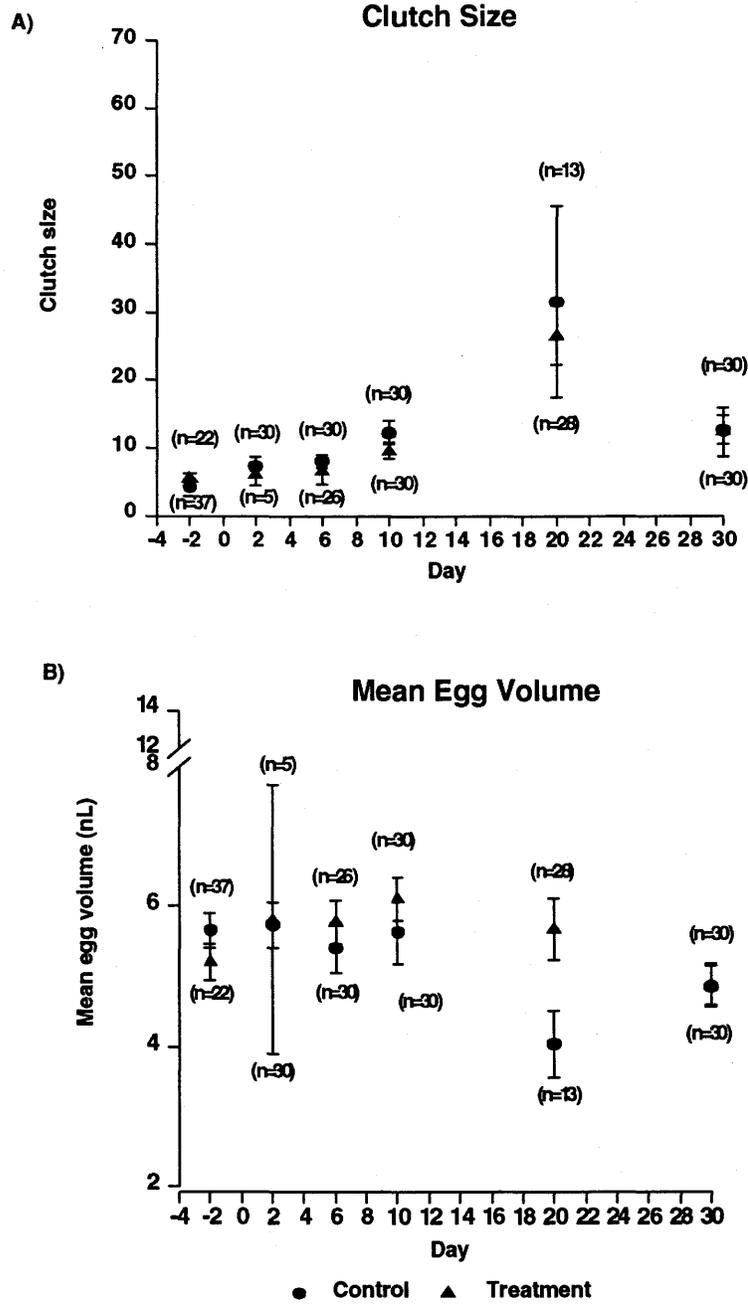


Figure 4.12 Clutch size (A) and mean egg volume (B) for *Daphnia pulex* in enclosure 2. Values are means with 95% confidence intervals and, number of females is shown in parentheses.

Enclosure 2

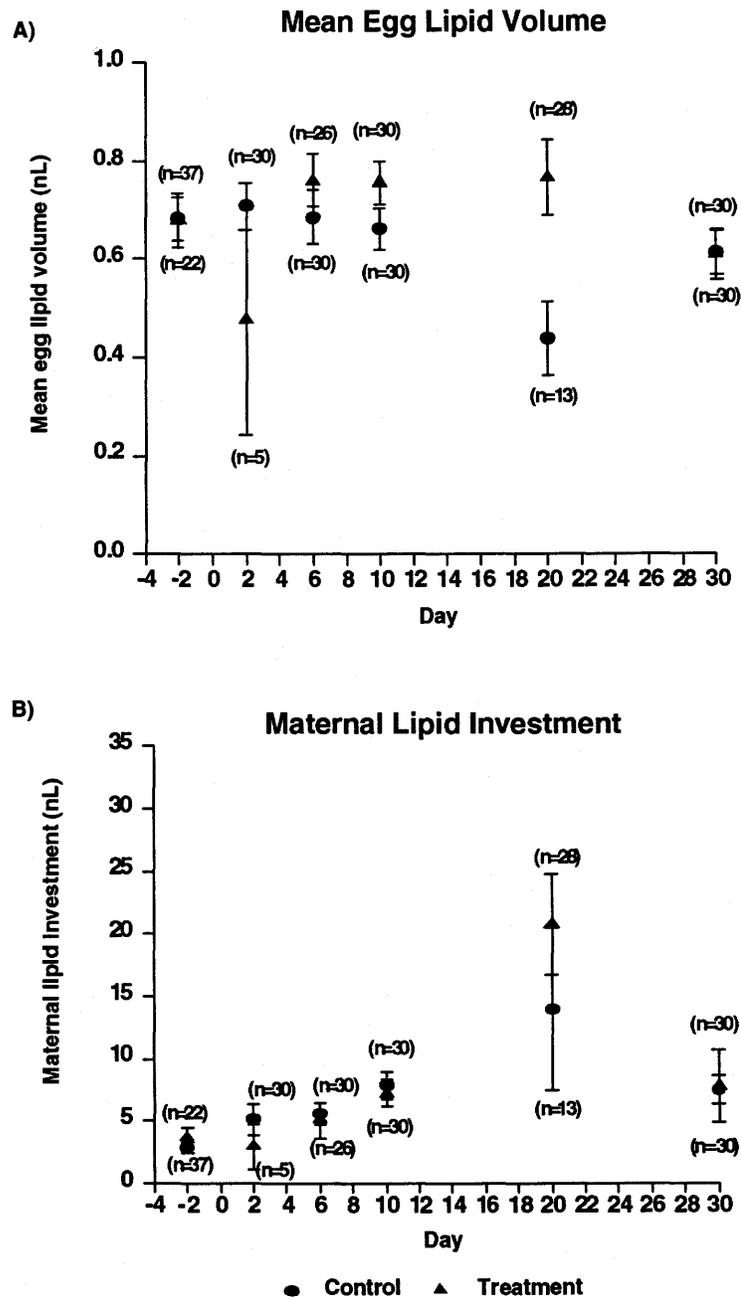


Figure 4.13 Mean egg lipid volume (A) and maternal lipid investment (B) for *Daphnia pulex* in enclosure 2. Values are means with 95% confidence intervals and, number of females is shown in parentheses.

Table 4.4. Body size and reproductive parameters for *Daphnia pulex* in treatment and control cells of each enclosure. Values are means (± 1 SE).

	Enclosure 1		Enclosure 2		Enclosure 3		Enclosure 4	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Body Length (mm)	2.43 \pm 0.02	2.65 \pm 0.02***	2.53 \pm 0.04	2.57 \pm 0.03	2.48 \pm 0.03	2.53 \pm 0.02	2.41 \pm 0.04	2.57 \pm 0.03***
Body Weight (mg)	0.067 \pm 0.002	0.085 \pm 0.002***	0.073 \pm 0.004	0.081 \pm 0.003	0.065 \pm 0.002	0.072 \pm 0.002	0.058 \pm 0.013	0.088 \pm 0.009
Clutch Size	13.12 \pm 0.87	20.69 \pm 0.87***	12.15 \pm 1.13	14.75 \pm 0.86	16.86 \pm 1.05	12.99 \pm 0.90**	8.97 \pm 1.07	14.94 \pm 0.76***
Mean Egg Volume (nL)	4.62 \pm 0.08	4.69 \pm 0.07	5.85 \pm 0.14	5.21 \pm 0.10***	3.77 \pm 0.11	4.83 \pm 0.09***	4.79 \pm 0.16	5.30 \pm 0.11*
Mean Egg Lipid Volume (nL)	0.57 \pm 0.01	0.57 \pm 0.01	0.69 \pm 0.02	0.62 \pm 0.01**	0.43 \pm 0.02	0.61 \pm 0.01***	0.61 \pm 0.02	0.64 \pm 0.02
MLI (nL)	7.09 \pm 0.64	11.65 \pm 0.51***	9.03 \pm 0.76	8.15 \pm 0.58	6.62 \pm 0.48	7.52 \pm 0.41	5.34 \pm 0.75	8.93 \pm 0.53***

* P < 0.05, ** P < 0.01, *** P < 0.001

Table 4.5. Reproductive parameters corrected for the effect of body length for *Daphnia pulex* in treatment and control cells of each enclosure. Values are least square means \pm 1 SE derived from ANCOVA.

	<u>Enclosure 1</u>		<u>Enclosure 2</u>		<u>Enclosure 3</u>		<u>Enclosure 4</u>	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Clutch Size	15.20 \pm 0.54	12.62 \pm 0.92	11.82 \pm 1.53	14.00 \pm 0.46	14.70 \pm 0.63	10.55 \pm 0.63**	9.37 \pm 0.73	11.98 \pm 0.51***
Egg Volume (nL)	4.75 \pm 0.09	4.66 \pm 0.12	5.63 \pm 0.33	5.24 \pm 0.10	3.92 \pm 0.13	4.71 \pm 0.13	4.91 \pm 0.16	5.16 \pm 0.11
Egg Lipid Volume (nL)	0.59 \pm 0.02	0.56 \pm 0.02	0.76 \pm 0.05	0.63 \pm 0.01	0.45 \pm 0.02	0.60 \pm 0.02	0.63 \pm 0.02	0.61 \pm 0.02
MLI (nL)	8.50 \pm 0.39	6.40 \pm 0.52	8.85 \pm 1.00	8.01 \pm 0.30	6.04 \pm 0.34	6.22 \pm 0.34	5.89 \pm 0.51	6.85 \pm 0.36***

* P < 0.05, ** P < 0.01, *** P < 0.001

Table 4.6. Mean egg volume and mean egg lipid volume corrected for the effect of clutch size for *Daphnia pulex* in treatment and control cells of each enclosure. Values are least square means \pm 1SE derived from ANCOVA.

	<u>Enclosure 1</u>		<u>Enclosure 2</u>		<u>Enclosure 3</u>		<u>Enclosure 4</u>	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Mean Egg Volume (nL)	4.69 \pm 0.15	4.51 \pm 0.19	6.36 \pm 0.71	5.13 \pm 0.16	3.85 \pm 0.15	4.60 \pm 0.13**	5.25 \pm 0.38	5.20 \pm 0.17
Mean Egg Lipid Volume (nL)	0.61 \pm 0.03	0.55 \pm 0.03	0.90 \pm 0.10	0.64 \pm 0.02	0.45 \pm 0.02	0.58 \pm 0.02***	0.69 \pm 0.05	0.63 \pm 0.02*

* P < 0.05, ** P < 0.01, *** P < 0.001

Enclosure 3

There were no differences between treatments for the variables body length, body weight and MLI (Figures 4.14 and 4.16; Table 4.4). Clutch size was significantly bigger in treated *D. pulex* compared to control whereas mean egg volume and mean egg lipid volume were significantly smaller (Figures 4.15 and 4.17). However, mean egg volume and mean egg lipid volume were significantly smaller in the treatment cell in pre-treatment samples and the differences remained in all post-treatment samples. Because of the pre-existing differences I conclude that clutch size is the only variable that showed significant effects between treatments in this enclosure. Clutch size and MLI covaried with body length, whereas mean egg volume and mean egg lipid volume did not. ANCOVA indicated a significant treatment effect on clutch size but no effects were observed for body weight, mean egg volume, mean egg lipid volume and MLI (Table 4.5). Mean egg volume and mean egg lipid volume did not covary with clutch size but a significant treatment effect was obtained with ANCOVA (Table 4.6). For the same clutch size, treated *D. pulex* had significantly smaller mean egg volume and mean egg lipid volume.

Enclosure 4

Treated *D. pulex* had significantly smaller body length, clutch size and MLI than controls (Figures 4.17 to 4.19; Table 4.4). There also was a trend ($P=0.051$) for smaller body weight among treated animals (Figure 4.17). Mean egg volume was significantly smaller in treated *D. pulex* but there was no difference between treatments for mean egg lipid volume (Figure 4.18 and 4.19). There was a weak correlation between body weight

and body length ($P=0.054$). ANCOVA indicated that clutch size, mean egg volume, mean egg lipid volume, and MLI were all related to body length. When body length was used as a covariable in ANCOVA, there was a significant treatment effect on the variables clutch size and MLI (Table 4.5). A decrease in body length caused a decrease in clutch size, egg volume and MLI, but there were additional effects of triallate on clutch size and MLI. Mean egg volume and mean egg lipid volume did not covary with clutch size. ANCOVA indicated a significant treatment effect for mean egg lipid volume but not for mean egg volume (Table 4.6). For the same clutch size, treated *D. pulex* had significantly larger mean egg lipid volume than control animals.

Daphnia pulex had a similar response to triallate in 2 of 4 enclosures (enclosures 1 and 4) (Table 4.7). The main effects of triallate were reduced body size and decreased reproduction manifested by smaller clutch size and MLI. However, in the two remaining enclosures, body size was not significantly reduced in treated *D. pulex* compared to control animals, and effects on mean egg volume and mean egg lipid volume were inconsistent. Mean egg volume and mean egg lipid volume did not differ between treatments in enclosures 1 and 3, they were bigger in treated *D. pulex* in enclosure 2 and mean egg volume was smaller in enclosure 4 although mean egg lipid volume was unchanged in that enclosure. Effects of triallate on body size were similar and triallate did not cause additional treatment effects on body weight independent of its effect on size. Effects on reproduction seemed to be mediated through effects on body size in some enclosures but not in others.

Enclosure 3

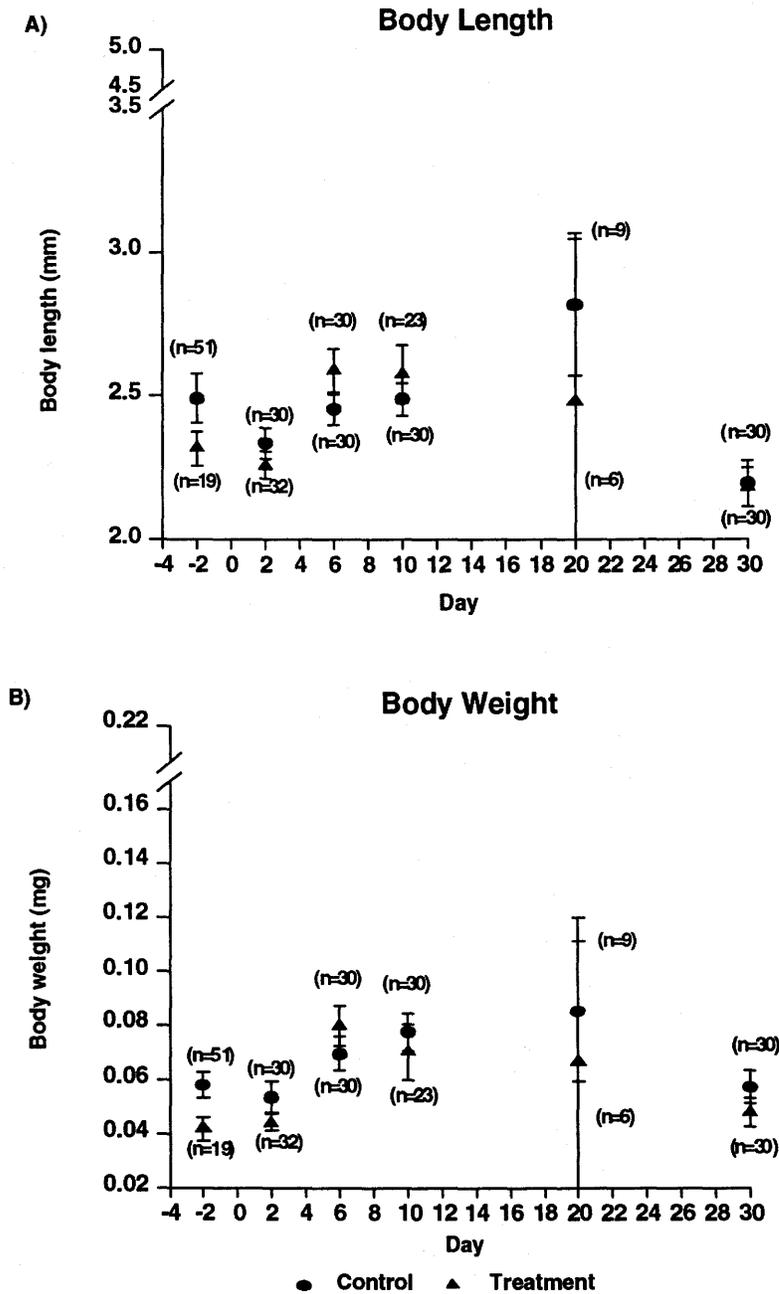


Figure 4.14. Body length (A) and body weight (B) for *Daphnia pulex* in enclosure 3. Values are means with 95% confidence intervals and, number of females is shown in parentheses.

Enclosure 3

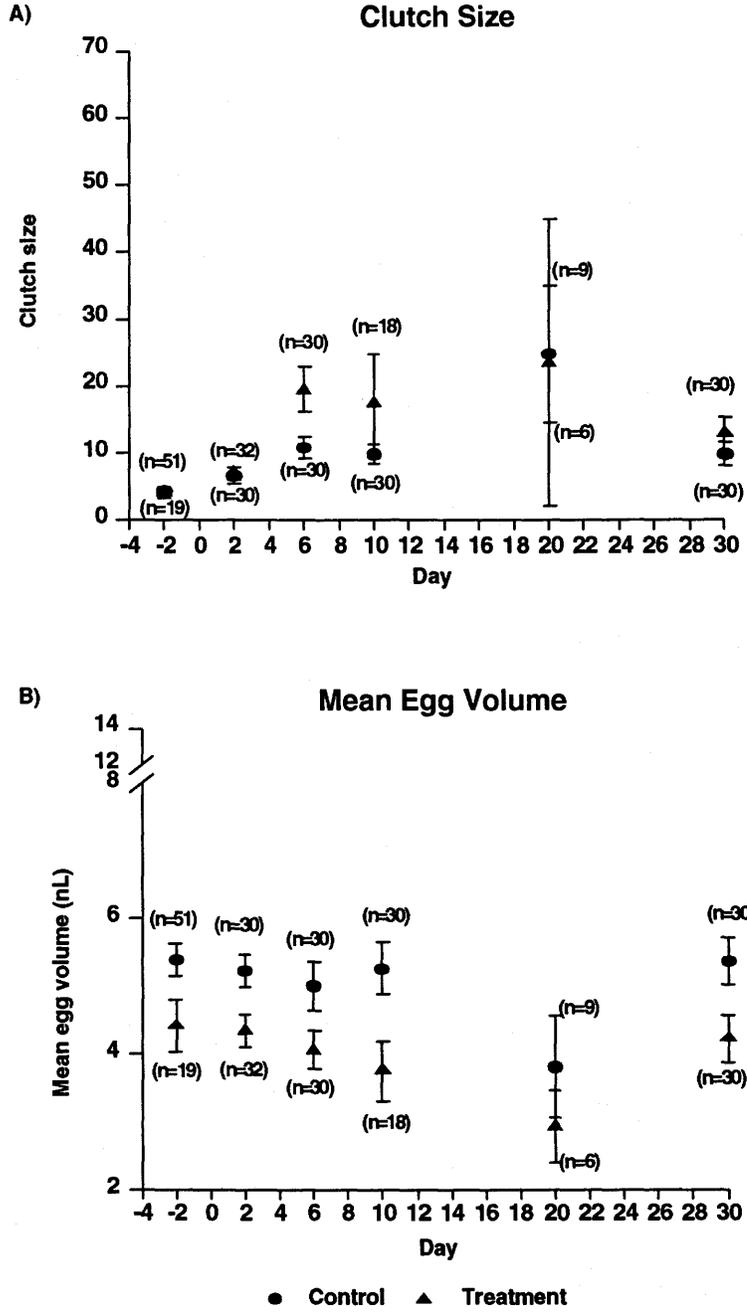


Figure 4.15. Clutch size (A) and mean egg volume (B) for *Daphnia pulex* in enclosure 3. Values are means with 95% confidence intervals and, number of females is shown in parentheses.

Enclosure 3

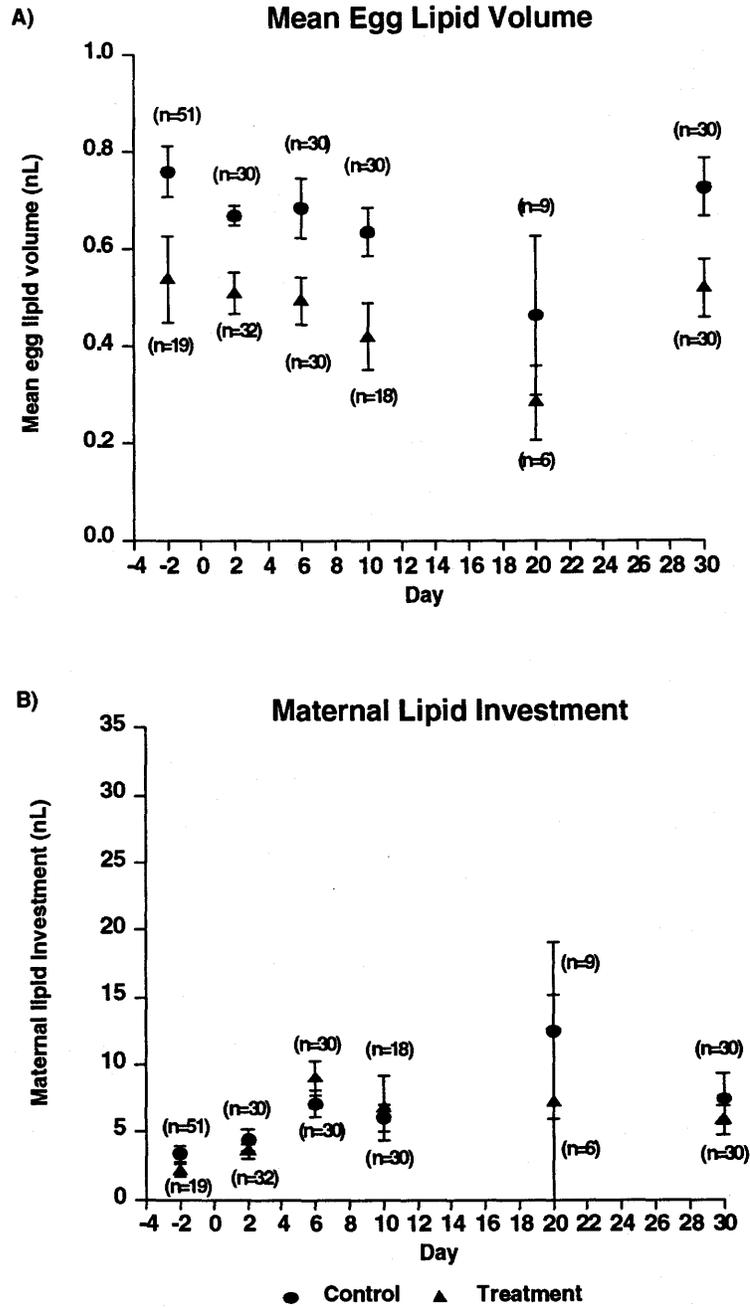


Figure 4.16. Mean egg lipid volume (A) and maternal lipid investment (B) for *Daphnia pulex* in enclosure 3. Values are means with 95% confidence intervals and, number of females is shown in parentheses.

Enclosure 4

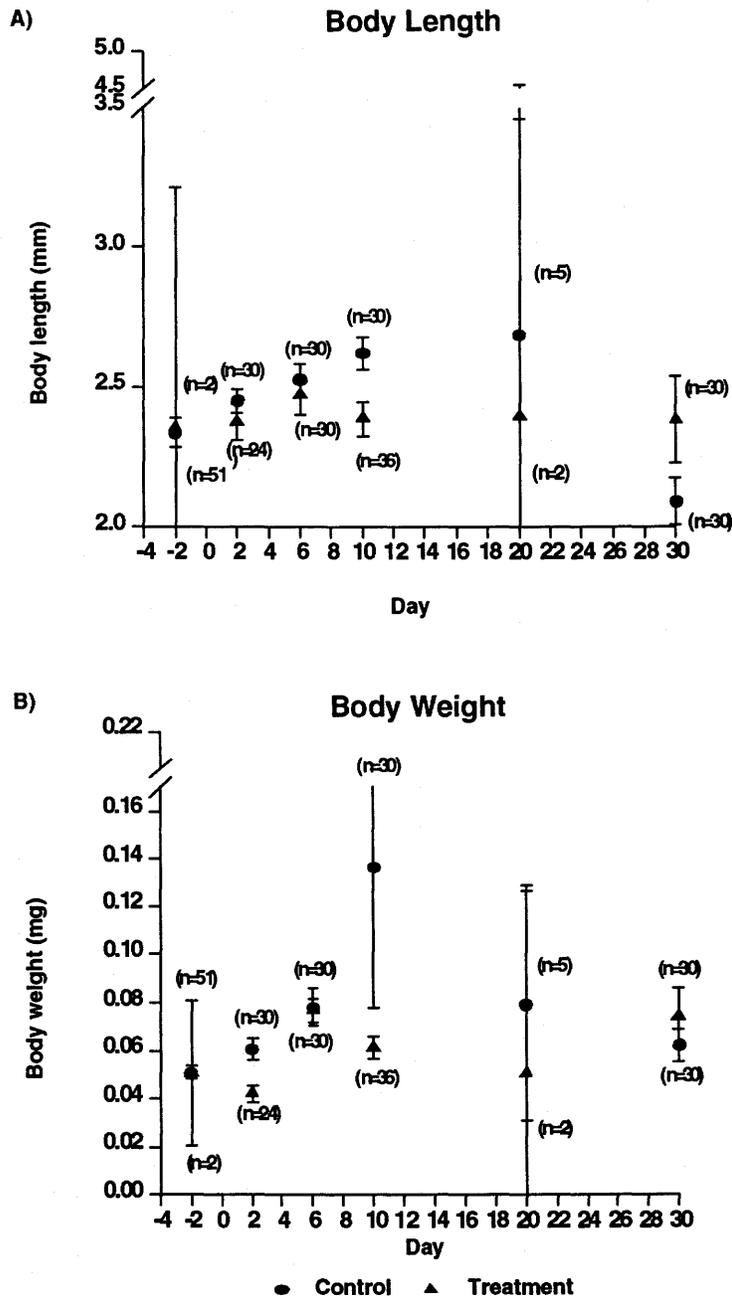


Figure 4.17. Body length (A) and body weight (B) for *Daphnia pulex* in enclosure 4. Values are means with 95% confidence intervals and, number of females is shown in parentheses.

Enclosure 4

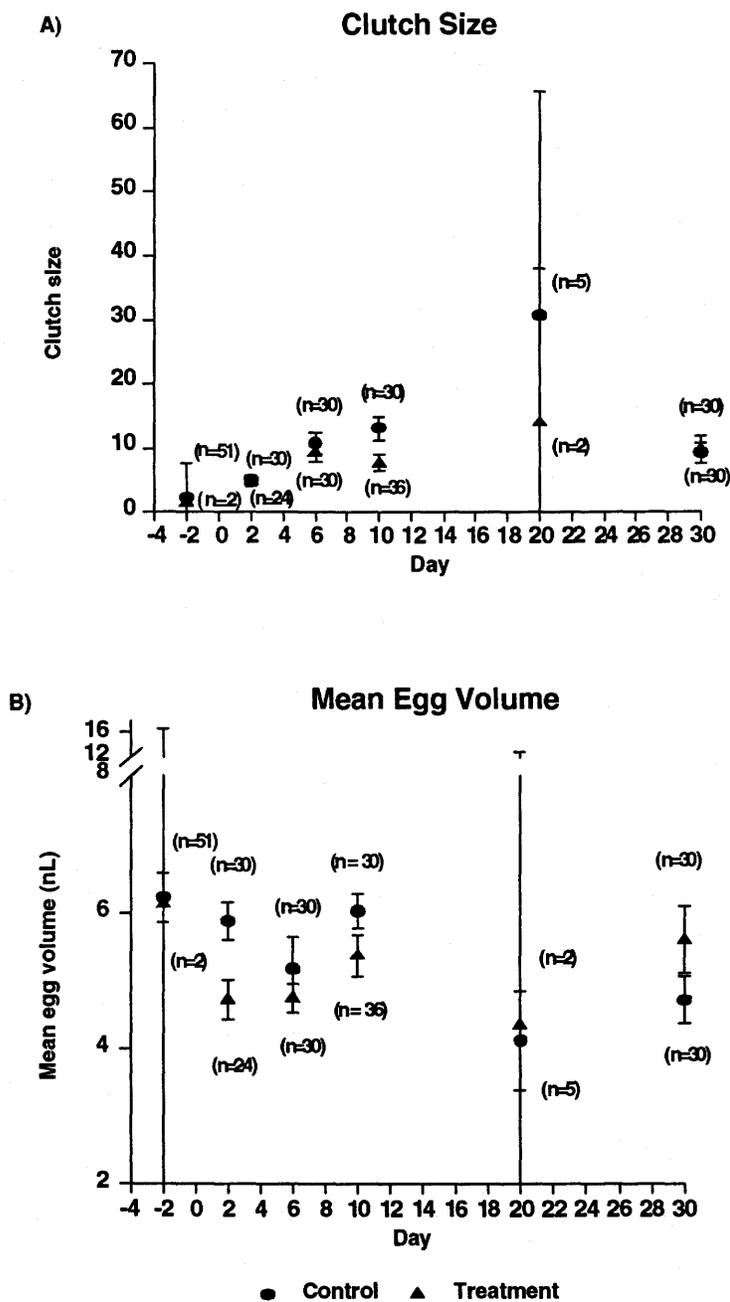


Figure 4.18. Clutch size (A) and mean egg volume (B) for *Daphnia pulex* in enclosure 4. Values are means with 95% confidence intervals and, number of females is shown in parentheses.

Enclosure 4

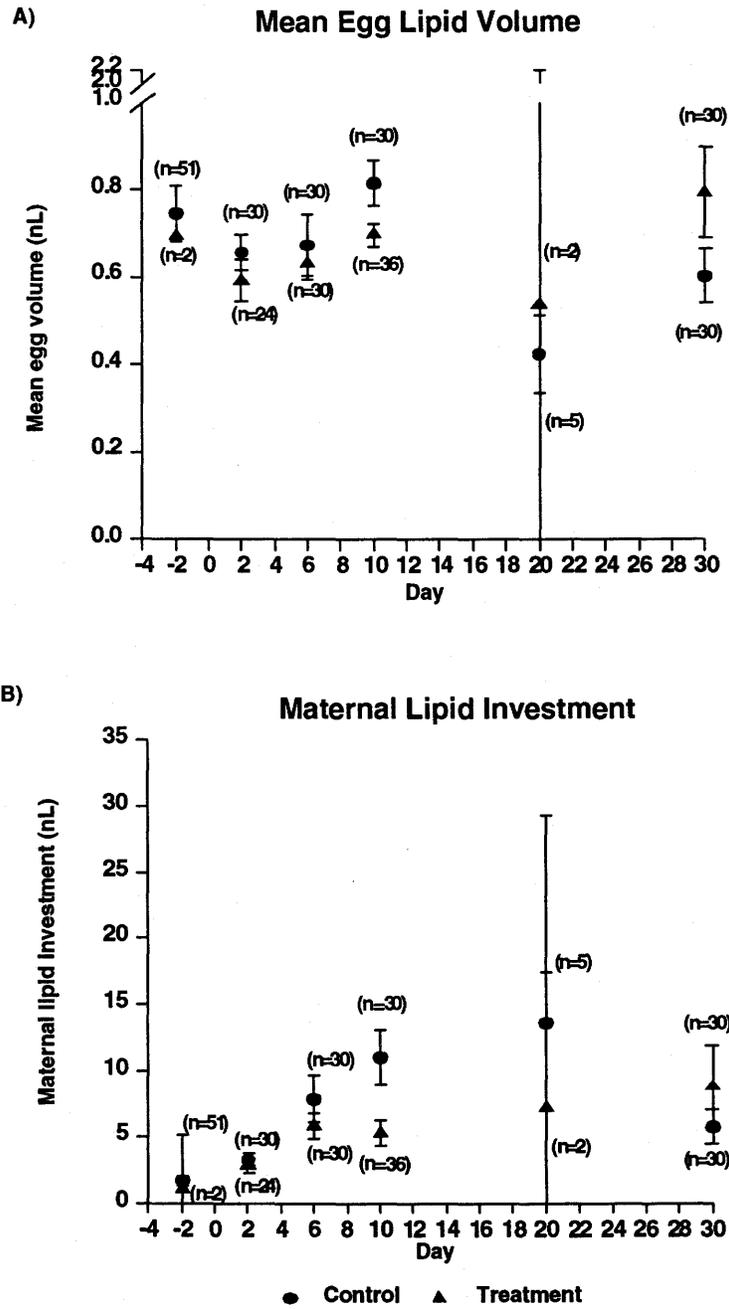


Figure 4.19. Mean egg lipid volume (A) and maternal lipid investment (B) for *Daphnia pulex* in enclosure 4. Values are means with 95% confidence intervals and, number of females is shown in parentheses.

Table 4.7. Summary of direct and indirect effects of triallate on *Daphnia pulex*.

	Enclosure 1	Enclosure 2	Enclosure 3	Enclosure 4
Direct	Reduced body size	Larger mean egg volume and mean egg lipid volume	Larger clutch size	Reduced body size Reduced clutch size and MLI
Indirect	Smaller clutches overall, lower lipid investment in clutch	No significant effects	No significant effects	Smaller clutches; overall; lower lipid investment in clutch

In enclosure 1, reduction in clutch size and MLI was associated with smaller body size of treated *D. pulex*. Additional effects on clutch size and MLI were observed in enclosure 4. Mean egg volume and mean egg lipid volume covaried with body length, except in enclosure 3, and for mean egg lipid volume in enclosure 2. Effects on mean egg volume and mean egg lipid volume seemed to be mediated through effects on body size except mean egg lipid volume in enclosure 2 and mean egg volume in enclosure 4.

Egg size and the amount of lipid they contain was highly variable across all clutch sizes. Mean egg volume and mean egg lipid volume did not covary with clutch size except mean egg lipid volume in enclosure 2. In all enclosures, the amount of egg lipid was proportional to the size of the egg and was not influenced by treatment.

4.3.5 *Hyalella azteca*

Analysis of variance revealed significant differences among enclosures for the variables body length, body weight and clutch size. Consequently, separate statistical analyses were performed for each enclosure. There were few *H. azteca* in pre-treatment samples of enclosures 1, 2 and 3. Those samples were lost, so amphipods present in pre-treatment samples of zooplankton were used for measurements. For these enclosures, due to low sample size, it was not possible to evaluate differences between treatment and control cells prior to triallate application. In enclosure 4, where sufficient animals were taken, there were no differences between treatment and control enclosures for body length, body weight or clutch size.

There is a positive relationship between body size and number of eggs produced by *H. azteca* (Pennak 1985). Thus, analysis of covariance was used to control the effect

of body size when assessing the impact of triallate on reproduction. Although length and weight are expected to be closely related, stressed animals may be lighter for their size, and this effect was also tested with ANCOVA.

Enclosure 1

The variables body length, body weight and clutch size were not significantly different between treatments. There was, however, an interaction between sampling day and treatment for the variable body length. Body length of control *H. azteca* was larger than treated animals 6 days post-treatment but was smaller 20 days after application (Figure 4.20; Table 4.8). ANCOVA showed that the variables body weight and clutch size covaried with body length. Clutch size was significantly smaller in treated *H. azteca* when body length was controlled in ANCOVA (Table 4.9).

Enclosure 2

The variables body length, body weight and clutch size were not significantly different between treatments (Figure 4.21; Table 4.8). Much variation was associated with sampling date. The variables body weight and clutch size covaried with body length, but ANCOVA indicated no effects of treatment on these variables when body length was controlled statistically (Table 4.9).

Enclosure 3

Body length was significantly bigger in treated *H. azteca* compared to control animals and this difference was apparent 20 days post-treatment (Figure 4.22; Table 4.8). Body weight and clutch size were not significantly different although there was a trend for larger body weight ($P = 0.074$) and smaller clutch size ($P = 0.065$) in treated *H.*

azteca compared to controls. ANCOVA showed that body weight and clutch size were significantly greater in control *H. azteca* when body length was controlled statistically (Table 4.9).

Enclosure 4

Body weight and clutch size were significantly bigger in control than in treated animals whereas body length was not different (Figure 4.23; Table 4.8). ANCOVA indicated that body weight and clutch size were significantly bigger in control *H. azteca* when body length was controlled in the analysis (Table 4.9).

Triallate affected body size and reproduction of *H. azteca* but the response varied among enclosures (Table 4.10). Body weight was significantly reduced in enclosure 4. In enclosure 3, body length was larger in treated *H. azteca* although they had reduced weight for their body length. Effects varied with sampling date in enclosure 1; treated *H. azteca* had reduced body length after 6 days but larger after 20 days and overall effect was not significant.

Clutch size covaried with length and weight of *H. azteca* in all enclosures. Clutch size was lower in enclosure 4 and was nearly significantly reduced ($P = 0.065$) in enclosure 3. Clutch size was also reduced in enclosure 1 when clutch size was corrected for body length.

Enclosure 1

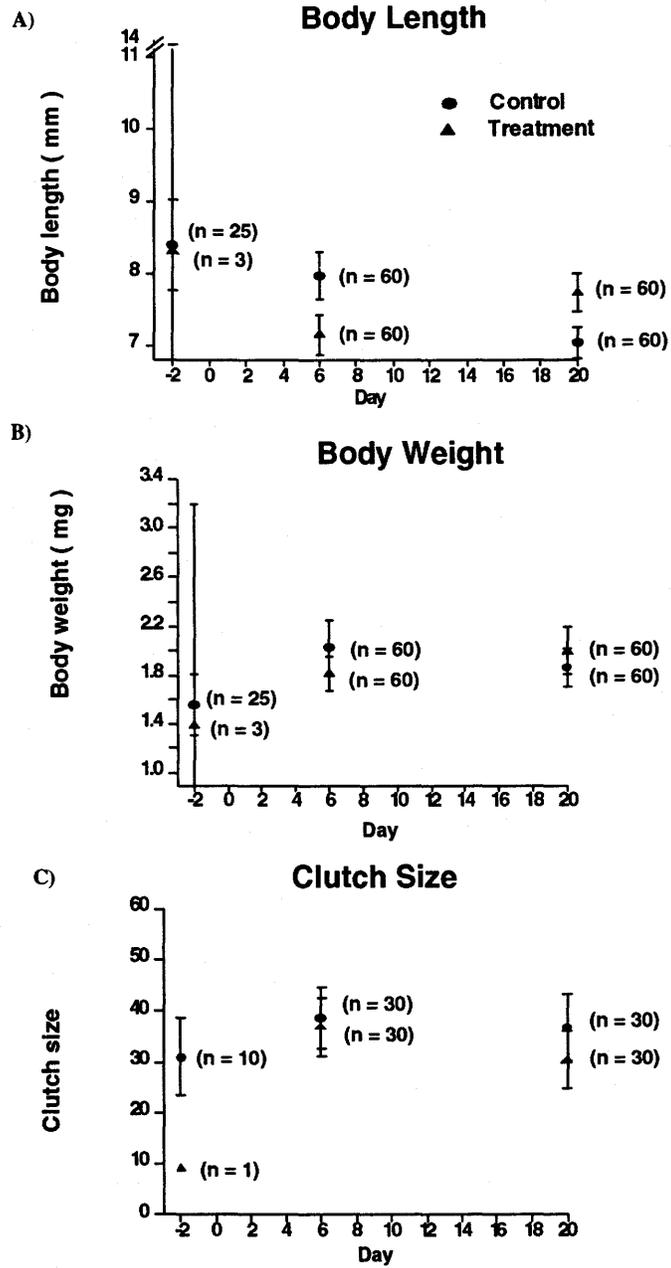


Figure 4.20. Body length (A), body weight (B) and clutch size (C) for *Hyalella azteca* in enclosure 1. Values are means with 95% confidence intervals. Number of animals is shown in parentheses.

Enclosure 2

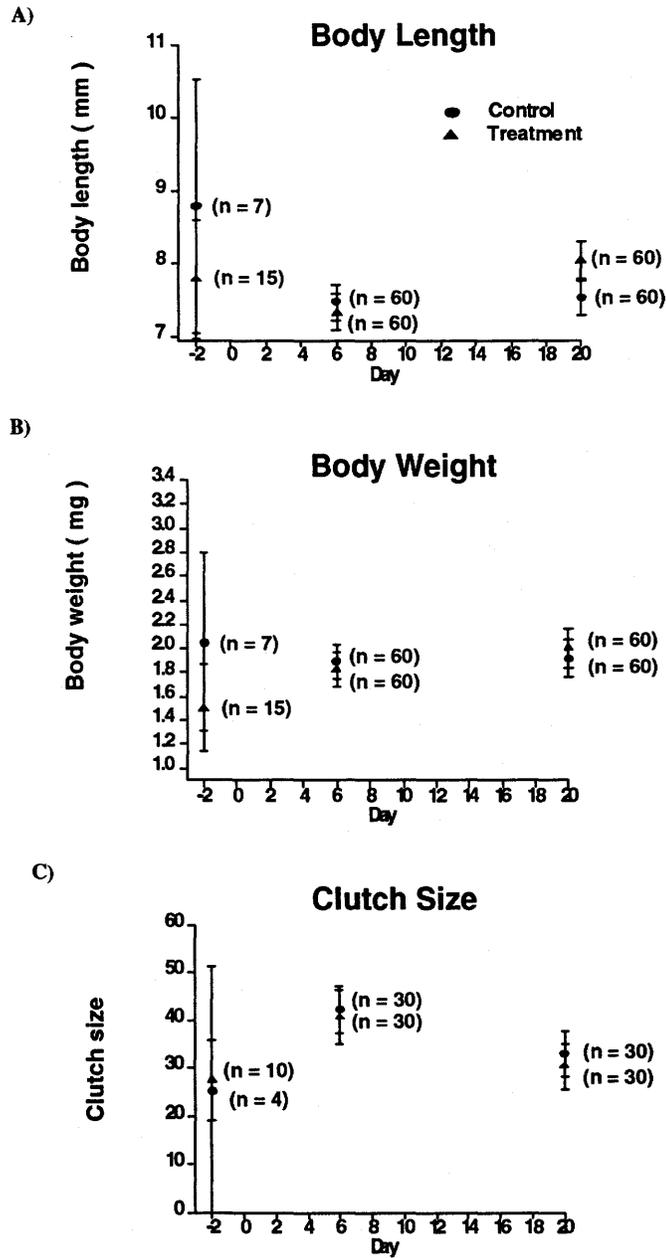


Figure 4.21. Body length (A), body weight (B) and clutch size (C) for *Hyaella azteca* in enclosure 2. Values are means with 95% confidence intervals. Number of animals is shown in parentheses.

Enclosure 3

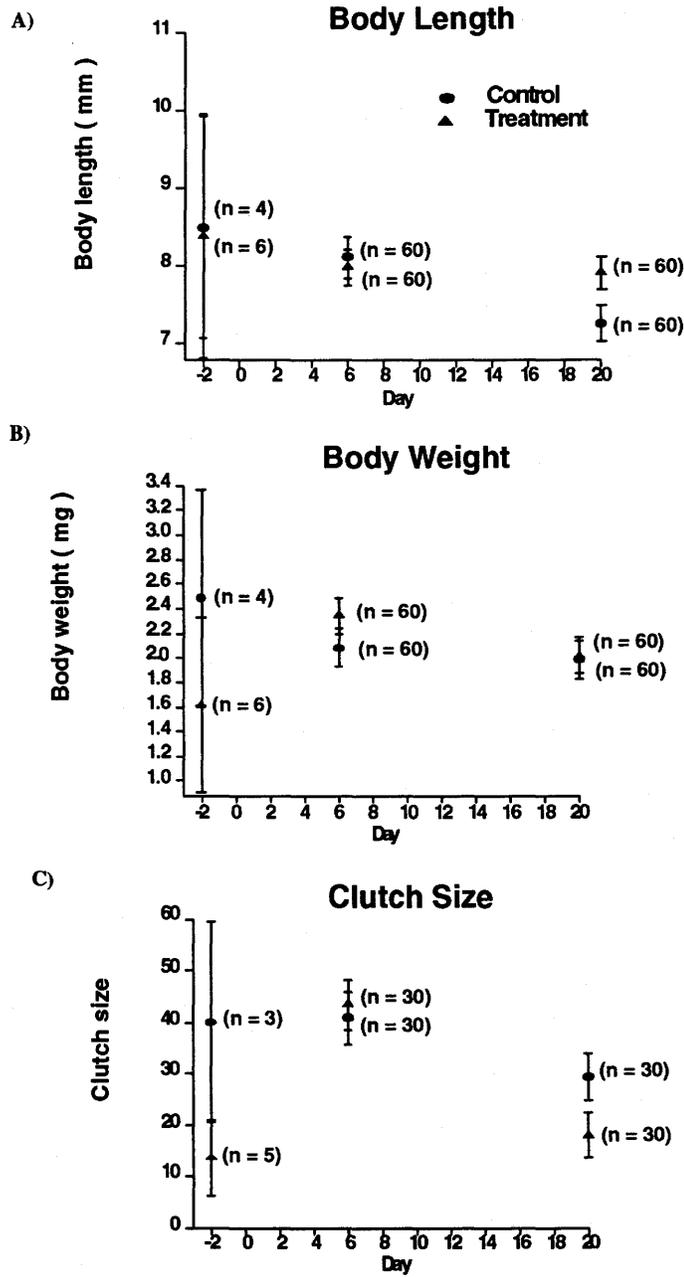


Figure 4.22. Body length (A), body weight (B) and clutch size (C) for *Hyalella azteca* in enclosure 3. Values are means with 95% confidence intervals. Number of animals is shown in parentheses.

Enclosure 4

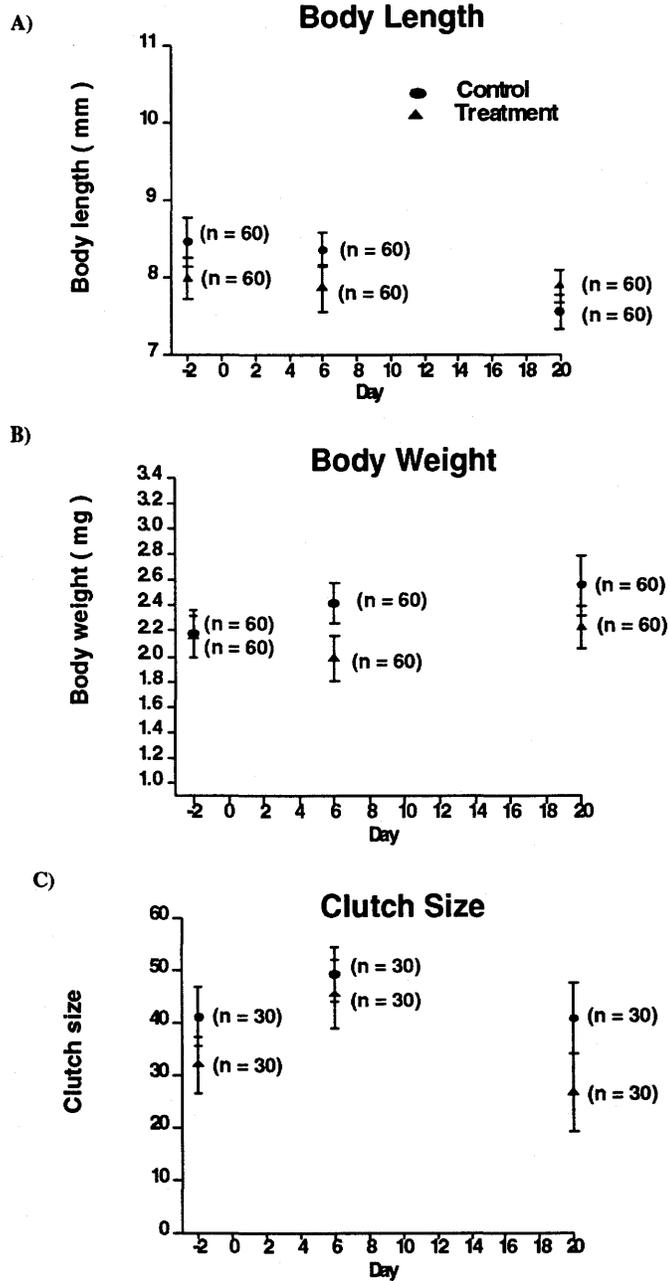


Figure 4.23. Body length (A), body weight (B) and clutch size (C) for *Hyaella azteca* in enclosure 4. Values are means with 95% confidence intervals. Number of animals is shown in parentheses.

Table 4.8. Body size and reproductive parameters for *Hyalella azteca* in treatment and control cells of each enclosure. Values are means (± 1 SE).

	<u>Enclosure 1</u>		<u>Enclosure 2</u>		<u>Enclosure 3</u>		<u>Enclosure 4</u>	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Body Length	7.44 \pm 0.10	7.51 \pm 0.10	7.69 \pm 0.09	7.50 \pm 0.09	7.95 \pm 0.08	7.69 \pm 0.08*	7.87 \pm 0.09	7.95 \pm 0.09
Body Weight	1.91 \pm 0.06	1.95 \pm 0.06	1.92 \pm 0.05	1.91 \pm 0.05	2.18 \pm 0.05	2.04 \pm 0.05	2.11 \pm 0.06	2.49 \pm 0.06***
Clutch Size	33.68 \pm 2.06	37.73 \pm 2.06	35.58 \pm 1.73	37.72 \pm 1.73	30.77 \pm 1.65	35.10 \pm 1.65	36.07 \pm 2.55	45.07 \pm 2.08**

* P < 0.05, ** P < 0.01, *** P < 0.001

Table 4.9. Body weight and clutch size corrected for the effect of body length for *Hyalella azteca* in treatment and control cells of each enclosure. Values are least square means \pm 1 SE derived from ANCOVA.

	<u>Enclosure 1</u>		<u>Enclosure 2</u>		<u>Enclosure 3</u>		<u>Enclosure 4</u>	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Body Weight	1.89 \pm 0.03	1.95 \pm 0.04	1.85 \pm 0.03	1.95 \pm 0.03	2.13 \pm 0.03	2.15 \pm 0.04	2.13 \pm 0.04	2.53 \pm 0.04*
Clutch Size	33.79 \pm 1.74	39.14 \pm 1.78*	37.20 \pm 1.44	37.34 \pm 1.33	29.02 \pm 1.41	37.68 \pm 1.55*	36.43 \pm 2.05	47.09 \pm 1.88*

* P < 0.05, ** P < 0.01, *** P < 0.001

Table 4.10. Summary of effects of triallate on *Hyaella azteca*.

Enclosure 1	Enclosure 2	Enclosure 3	Enclosure 4
Reduced clutch size (clutch size adjusted for body length) Effects on body length varied with sampling day	No significant effects	Larger body length. Smaller body weight and clutch size adjusted for body length	Reduced body weight Reduced clutch size

4.4 Discussion

4.4.1 Physicochemical Characteristics

Triallate did not change the physicochemical characteristics of the water although my ability to detect changes was limited by low sampling effort. In microcosm experiments, Johnson (1986) also found no effect of triallate on pH and conductivity, but he observed an increase in dissolved oxygen level. This increase was attributed to a stimulation of photosynthesis by the herbicide. This phenomenon was not observed in this study. Community metabolism can be assessed using either dissolved oxygen or a combination of pH, alkalinity and conductivity (Brock *et al.* 1993). Moreover, it has been repeatedly shown that DO, pH, alkalinity and conductivity are sensitive indicators of metabolic effects of toxicants in freshwater ecosystems (Brock *et al.* 1993). Since these parameters often are highly correlated, treatment effects on these endpoints can be regarded as a syndrome of stress. This was not the case in my mesocosm experiment, suggesting that photosynthesis and respiration of the community were not adversely affected by triallate. Alternatively, a much more detailed investigation may be required, involving greater sampling intensity to detect treatment effects.

4.4.2 Phytoplankton

Phytoplankton biomass, as estimated by chlorophyll *a* concentration, was not affected by triallate for any of the size fractions: nanoplankton, edible algae, inedible algae, and for total algae. However, my ability to discern effects was limited by a lack of replication within enclosures and high variability between enclosures. An alternative

hypothesis that I could not adequately evaluate is that lowering the number of grazers (e.g. *Daphnia*) with triallate compensates for lowered production.

Toxicity of triallate to algae has been studied by other workers in laboratory bioassays and microcosm experiments. Turbak *et al.* (1986) conducted an algal bioassay with *Selenastrum capricornutum* for 2-3 weeks duration using the commercial triallate formulation Fargo (10% ai). They obtained an EC50 of 6.20 µg/L in water and 11.2 µg/L in standard algal growth medium based on algal cell number. Johnson (1986) also used *S. capricornutum* in a short term growth bioassay and found reduced algal growth (cell counts) by more than 40% at triallate (as Fargo) concentrations of 100 µg/L and 1000 µg/L. Kratky and Warren (1971) used the alga *Chlorella pyrenoidosa* in a short term bioassay and found less than 50% inhibition of chlorophyll production when tested with 1000 µg/l and 10000 µg/L triallate concentrations. Finally, Waiser (1995) studied a natural phytoplankton assemblage in a microcosm and found that triallate reduced chlorophyll *a* concentration at 1000 µg/L of triallate but not at 100 µg/L concentration. Variability in findings of these authors may be related to different sensitivities of algal species to triallate. Aquatic algae exhibit considerable variability in their reactions to herbicides (Heckman 1995). The experimental conditions as well as the different formulations of triallate tested, for instance, technical grade versus commercial formulations (Fargo or Avadex BW), may explain some of the variation. Buhl and Faerber (1989) found that Fargo was twice as toxic to *Chironomus riparius* than technical grade triallate, although toxicity to *D. magna* was similar for the emulsifiable concentrate and technical material (Mayer and Ellersieck 1986). Differences in test

conditions as well as source population used in the bioassay likely influenced the response of algae (Heckman 1995).

Although the biomass of phytoplankton was not changed by triallate contamination, it is very possible that some of the algae may have been affected while others were not. Rapid shifts in algal species are not evident in general indicators of resource quality such as chlorophyll *a* concentration (Kerfoot *et al.* 1988). Therefore, although I found no differences in phytoplankton standing crop, it is possible that the community structure of algae may have been altered by the herbicide. In addition to shifts in algal composition, phytoplankton communities can show changes in genetic composition after herbicide exposure where tolerant strains of algal species replace sensitive strains (Kasai and Hanazato 1995). Unfortunately, taxonomic enumeration of algae to assess structural change in the community was not performed in this study. I made an effort, however to evaluate the effects of triallate on the biomass of different size fractions (nanoplankton, edible algae, inedible algae). This approach is useful to observe shifts in the dominant size fractions representing algae of different quality as food for zooplankton (Stevenson and Lowe 1986).

4.4.3 Periphyton

Results of this study indicate a similar response between phytoplankton and periphyton communities. Sensitivity should be similar if both communities are composed of the same algal groups. This feature was observed in productive waters where a qualitative interrelationship existed between the phytoplanktonic and periphytic algae (van Dijk 1993). Changes in species composition in the periphyton community cannot be

verified without species enumeration. I found no reports concerning effects of triallate on periphyton communities. However, other herbicides affect periphyton communities. For example, periphyton communities in limnocorrals were adversely affected by the herbicide atrazine at a concentration of 100 µg/L (Herman *et al.* 1986). Chlorophyll, organic matter and total algal biomass were reduced and there were changes in the community composition. Cyanophyta were eliminated while most Bacillariophyceae and Chlorophyta species remained viable although stressed. Impact of atrazine on periphyton depends on the composition of the periphyton community at the time of exposure (Herman *et al.* 1986). Other triazine herbicides, simazine and terbutryn, caused substantial reductions in chlorophyll *a* content and carbon assimilation following a single dosing in *in situ* enclosures (Goldsborough and Robinson 1983). They found a rapid (1 week) recovery of periphyton communities and suggested that long-term impact on periphyton of a single dosage of these herbicides may be minimal. However, if these reductions occur at a critical time for grazers, then effects may be extensive further up the food chain.

4.4.4 *Daphnia pulex*

Effects of triallate on *D. pulex* were much less severe than expected based on previous laboratory bioassays (Johnson 1986, Mayer and Ellersieck 1986). The concentration applied in this study (250 µg/L) was 4 times higher than the 48 h LC50 value (57 µg/L) derived for *D. magna* by Mayer and Ellersieck (1986) but I did not detect mortality of zooplankton. In contrast, others have reported sharp declines of certain zooplankton groups or species after exposure to pyrethroid insecticides (Kaushik

et al. 1985, Yasuno *et al.* 1988, Lozano *et al.* 1992, Tidou *et al.* 1992, Webber *et al.* 1992). Rapid dissipation of triallate from the water within 48 h to levels below the 48 h LC50 value (57 µg/L) may have limited the exposure of *D. pulex* to triallate. Some mortality may have occurred following the herbicide application but it likely was minimal or else there was a rapid recovery and the *D. pulex* population was largely unaffected.

Effects observed on *D. pulex* are principally sublethal: reduced body size or lower reproductive output, but results varied among enclosures. Reduced body size and reproduction in treated *D. pulex* were observed in two of the four enclosures. The other two enclosures showed no effect on body size but an increase in some reproductive parameters when exposed to triallate. Existing conditions before treatment will influence the response of the system to pesticide exposure (Hanazato and Yasuno 1990). Pairing of the treatment and control cells was effective in obtaining similar conditions in corresponding treatment and control enclosures. Only enclosure 3 was different for a few parameters of *D. pulex* in the pre-treatment samples. The 4 enclosures were located in different parts of the pond with considerable distance separating them and, therefore, heterogeneity within the aquatic community may have resulted in spatial variation in predation pressure and competition. In addition, clonal variation of *D. pulex* may have occurred in different parts of the pond. Fish were absent from the pond but *Chaoborus* larvae, an important dipteran predator of *D. pulex*, were present. Spatial heterogeneity and patchiness in physical properties of the sediment, or macrophyte beds providing refuge to zooplankton may also be a factor. Sampling date was also an important source of variation. Although a normal pattern of increasing body size and clutch size with time

in the spring was observed. There was, however, some interaction between sampling date and treatment that made patterns more difficult to interpret.

Effects of triallate on body size and reproduction may be in part attributed to the direct toxicity of this herbicide to *D. pulex*. Previous studies in the laboratory (Mayer and Ellersieck 1986, Johnson 1986) and in microcosms (Johnson 1986) revealed effects of triallate on the survival ability of *D. magna* at concentrations below 100 µg/L. Life history traits are well described in laboratory studies, however, and a decrease in growth and reproduction seems a common response to sublethal concentration of toxicants (see chapter 6).

Comparison of my findings with other field studies is difficult since in other zooplankton studies, community structure is typically evaluated rather than life history traits. One exception is the study by Schartau (1994) that looked at effects of low cadmium (Cd) contamination on freshwater populations and communities in limnocorrals. The cladoceran *Bosmina longispina* was affected by both reduced densities, reduced growth and reduced reproduction when exposed to water concentrations of 3 and 5 ppb Cd. Reproduction was also affected when *B. longispina* were exposed to 1 ppb Cd although results were more ambiguous at this low level.

Food chain effects may still play a role in the reduction of growth and reproduction of *D. pulex* but it is not possible to quantify to what degree. Although I was unable to detect a reduced biomass of algae (phytoplankton and periphyton), a shift in the community structure in favor of algal species of poor nutritional quality to *D. pulex* may have occurred. Kerfoot *et al.* (1988) observed that *Daphnia* fluctuations in a

mesotrophic lake corresponded to shifts in resource quality (algal species of different quality) and the demographics of coexisting grazers. Gelatinous greens, small, thick-walled green algae and other small protected species were all available in the same size fraction but were resistant to gut passage and so provided little nutrition to the zooplankton. Others (Arts *et al.* 1992, Vanni and Lampert 1992) have also reported differential food quality of some algal species, and effects on life history traits and fitness in *Daphnia* spp. Nutrient limitation and toxicant exposure can also reduce the quality of algae. Sterner (1993) found reduced growth of *Daphnia* spp. being fed algae low in phosphorus content. Gorbi and Corradi (1993) found that growth and fecundity were dramatically reduced in daphnids fed algae exposed to chromium (VI), and the effect was attributed to alterations in the nutritive value of the algae due to chromium treatment rather than to a toxic effect of the metal.

4.4.5 *Hyaletta azteca*

The impact of triallate on *H. azteca* resembles the effects observed on *Daphnia*, with distinct responses in size and reproduction in different enclosures. Reductions in body size and reproduction of both species were observed in enclosures 1 and 4. Effects were also recorded in enclosure 3 for *H. azteca*, but not for *D. pulex*.

In preliminary acute range-finding tests in the laboratory, I found that the sensitivity of *H. azteca* and *D. pulex* to triallate was in the same concentration range. Nebeker *et al.* (1984) also found similar sensitivity of both species to a number of toxicants. However, exposure to triallate is probably greater for *H. azteca* than for *D. pulex* and effects on amphipods should be more pronounced. Amphipods are exposed to

triallylate present in both water and sediment. The levels measured in sediments were high and although the bioavailability of triallylate associated with sediment was not quantified, it probably contributed to the total exposure of amphipods to triallylate. Because they are exposed to triallylate from sediment for an extended period of time, their exposure and therefore their body burden is expected to be greater than planktonic organisms. High levels of triallylate were measured in amphipods (chapter 3). Nevertheless, effects on *H. azteca* were similar to those observed with *D. pulex* but are expected to last for a longer time period.

Implications of reduced body size for *H. azteca* are principally a reduction in the number of offspring produced. Reduced reproduction through smaller body size or directly due to the effect of a toxicant affects population growth. Impacts on populations of *H. azteca* could potentially influence foods available to large invertebrates or higher organisms which prey on this amphipod. However, potential food web interactions have not been evaluated.

5.0 EFFECT OF TRIALLATE ON PHYTOPLANKTON, PERIPHYTON AND THE CLADOCERAN *DAPHNIA PULEX* IN MICROCOSM EXPERIMENTS

5.1 Introduction

An experiment to study effects of triallate on selected organisms in littoral enclosures was presented in chapter 4 of this thesis. Due to the variability of natural systems, and insufficient replication for some of the parameters tested, the impact of triallate on algae and the zooplankton *Daphnia pulex* was not clearly evaluated. Therefore, microcosm experiments were conducted in a simplified system to resolve some of these uncertainties. The microcosm included the water phase only and focused on phytoplankton, periphyton and *D. pulex*. Two experiments were conducted and each had a different objective. In the first experiment, the goal was to evaluate effects of triallate on phytoplankton and periphyton, while the second experiment focused on the effects of triallate on *D. pulex*.

5.2 Materials and Methods

5.2.1 Microcosms

Microcosm experiments took place in the summer of 1994 in a permanent pond located near St. Denis, Saskatchewan (chapter 2); the same pond where the mesocosm experiment (chapter 3 and 4) was performed in 1993. Two experiments were conducted: one in May and one in July. Microcosms consisted of 18-L clear plastic bottles filled with pond water containing a natural assemblage of phytoplankton. The zooplankton *D.*

pulex was added to the microcosms in the second experiment (July). Bottles were submerged in shallow water in the pond to use natural temperature and light conditions.

To obtain a uniform source of water for use in all microcosms, pond water was collected in a large (600 L) plastic tank. Water was screened through a 50 μm mesh to remove zooplankton and was mixed by hand with a wooden blade. Ten 1 L water samples were taken from the tank at the start of the experiment to provide an estimate of initial phytoplankton biomass. A strip of ceramic tile (2 cm x 20 cm), attached to a string for easy recovery, was placed inside each bottle and laid on the bottom. Bottles were filled with filtered water and triallate (as Avadex BW) was applied before the bottles were capped. Bottles were secured horizontally, at a same depth, on individual supports about 20 cm above the sediment with the upper side of the bottle being approximately 50 cm below the water surface. There were 3 concentrations of triallate tested: 0 (control), 150 $\mu\text{g/L}$ and 250 $\mu\text{g/L}$, with 5 bottles per treatment. Sampling was performed after 5 and 20 days when bottles were removed. Thus, a total of 30 bottles was used in each experiment.

The same procedure was followed in a second experiment except for the addition of *D. pulex*. Pre-adult *D. pulex* were collected from the pond with a plankton net and 100 individuals of similar size were added to each bottle at the start of the experiment.

5.2.2 Phytoplankton

Phytoplankton biomass was estimated using size-fractionated chlorophyll analysis as in chapter 4. A 1 L sample was taken from each bottle and care was taken to keep the samples cool in the dark during travel. A 250 ml aliquot of water was filtered in the

laboratory within 6 h of collection. The filters were kept in the dark at -40 °C until analyzed. Chlorophyll analysis was done on each fraction separately and total chlorophyll *a* was obtained by summing the 3 fractions. Chlorophyll *a* was extracted with 90% ethanol and quantified with a fluorometer following Nusch (1980).

5.2.3 Periphyton

Ceramic strips were removed from each bottle and periphyton was scraped off the tile on an area of 36 cm² with a razor blade and collected in a plastic vial. Periphyton samples were frozen at -40 °C and kept in the dark until analyzed. Periphyton growth was estimated by ash free dry mass and chlorophyll *a* content. Periphyton was resuspended in 20 ml of deionized water and homogenized with a Polytron. Two aliquots were taken and filtered on separate GFC filters: one for chlorophyll analysis, the other for ash free dry mass determination. Chlorophyll analysis was described by Nusch (1980). Ash free dry mass determination followed the method of Aloi (1990).

5.2.4 *Daphnia pulex*

Water was filtered through a fine mesh (50 µm) to collect *D. pulex*. Samples were counted under a dissecting microscope in the laboratory and *D. pulex* were separated by age (adults and young).

5.2.5 Statistical Analysis

Multivariate analysis of variance was used to evaluate simultaneously the effect of triallate on 3 size fractions of phytoplankton and total chlorophyll *a*. ANOVA was used to analyze periphyton and *D. pulex* data. A Tukey's multiple comparison test was used when a significant treatment effect was detected with ANOVA or MANOVA. Data were

transformed (log or square root) prior to conducting tests when variances were heteroscedastic. Transformations and statistical analyses are described by Zar (1984). Analyses were executed on the Statistical Analysis System (SAS Institute 1990).

5.3 Results

5.3.1 Experiment 1 (May)

5.3.1.1 Phytoplankton

There was a significant effect associated with sampling date, or the interaction of sampling date and treatment, so MANOVA was performed separately per sampling day. Analyses were performed on untransformed data. Significant treatment effects on phytoplankton biomass were observed (Table 5.1). There was a slight stimulation in phytoplankton biomass in the lower (150 $\mu\text{g/L}$) triallate treatment after 5 days when chlorophyll *a* concentration in the 1.2 μm fraction and total chlorophyll *a* was significantly higher than controls (Table 5.2). Phytoplankton biomass in the higher (250 $\mu\text{g/L}$) treatment group was not different from the 150 $\mu\text{g/L}$ or control groups. After 20 days, however, chlorophyll *a* concentrations in the 30 μm fraction, and total chlorophyll *a*, were significantly lower in the 150 $\mu\text{g/L}$ and 250 $\mu\text{g/L}$ groups compared to controls.

5.3.1.2 Periphyton

Sufficient periphyton growth on ceramic tiles was present only after 20 days. ANOVA was performed on untransformed data. There was a significant treatment effect on periphyton biomass, measured by ash free dry mass and chlorophyll *a* content (Table 5.3). Both measures were significantly higher in the 250 $\mu\text{g/L}$ treatment than in control samples (Table 5.4).

Table 5.1. Results of MANOVA for the effect of treatment on chlorophyll *a* (Chl *a*) concentration for 3 size fractions of phytoplankton and for total chlorophyll *a* exposed for 5 and 20 days in May 1994. Shown are F-values.

Size fractions	Source	DF	5 Days	20 Days
1.2 μm	Treatment	2	5.34*	1.09
20 μm	Treatment	2	2.46	0.28
30 μm	Treatment	2	1.39	10.72**
Total Chl <i>a</i>	Treatment	2	5.64*	8.34**

* P < 0.05, ** P < 0.01, *** P < 0.001; DF is degrees of freedom.

Table 5.2. Chlorophyll *a* concentration (mg/L) for 3 size fractions of phytoplankton and for total chlorophyll *a* exposed to 3 concentrations of triallate for 5 and 20 days in May 1994.

Size fractions (Initial)	Treatment	Time ^a	
		5 Days	20 Days
1.2 μm (49.21 \pm 2.08)	Control	14.13 \pm 1.06 a	15.22 \pm 1.80 a
	150 $\mu\text{g/L}$	20.37 \pm 1.46 b	13.04 \pm 0.61 a
	250 $\mu\text{g/L}$	18.81 \pm 1.63 a, b	17.92 \pm 3.58 a
20 μm (0.44 \pm 0.05)	Control	0.42 \pm 0.11 a	3.11 \pm 0.95 a
	150 $\mu\text{g/L}$	0.99 \pm 0.24 a	2.32 \pm 0.61 a
	250 $\mu\text{g/L}$	0.57 \pm 0.20 a	2.71 \pm 0.64 a
30 μm (1.66 \pm 0.19)	Control	2.59 \pm 0.32 a	70.07 \pm 16.13 a
	150 $\mu\text{g/L}$	4.85 \pm 1.28 a	17.03 \pm 2.60 b
	250 $\mu\text{g/L}$	3.21 \pm 1.10 a	15.42 \pm 1.94 b
Total Chl <i>a</i> (51.31 \pm 2.08)	Control	17.14 \pm 1.33 a	88.39 \pm 17.98 a
	150 $\mu\text{g/L}$	26.22 \pm 1.50 b	32.38 \pm 2.98 b
	250 $\mu\text{g/L}$	22.59 \pm 2.66 a, b	36.06 \pm 4.59 b

^a Shown are means \pm SE ($n = 5$). Means with the same letter are not significantly different.

Table 5.3. Results of ANOVA for the effect of treatment on chlorophyll *a* (Chl *a*) concentration and ash free dry mass (AFDM) of periphyton exposed for 20 days in May 1994. Shown are F-values.

	Source	DF	20 Days
Chl <i>a</i>	Treatment	2	5.49*
AFDM	Treatment	2	4.99*

* P < 0.05, ** P < 0.01, *** P < 0.001; DF is degrees of freedom

Table 5.4. Chlorophyll *a* concentration (Chl *a*) and ash free dry mass (AFDM) of periphyton exposed to 3 concentrations of triallate for 20 days in May 1994.

	Treatment	20 Days ^a
Chl <i>a</i> (mg/cm ²)	Control	0.094 ± 0.055 a
	150 µg/L	0.503 ± 0.059 a, b
	250 µg/L	0.580 ± 0.155 b
AFDM (mg/cm ²)	Control	0.125 ± 0.083 a
	150 µg/L	0.271 ± 0.032 a, b
	250 µg/L	0.453 ± 0.095 b

a Shown are means ± SE (n = 5). Means with same letter are not significantly different.

5.3.2 Experiment 2 (July)

MANOVA performed on phytoplankton data as well as ANOVAs performed on periphyton and *D. pulex* data revealed significant effects associated with sampling date and the interaction between sampling date and treatment. Therefore, phytoplankton, periphyton and *D. pulex* data were analyzed separately per sampling day.

5.3.2.1 Phytoplankton

MANOVA was performed on untransformed data and significant treatment effects on phytoplankton biomass were observed on both sampling days (Table 5.5). After 5 days, significantly higher phytoplankton biomass was present in the 1.2 μm fraction of the 250 $\mu\text{g/L}$ treatment than in the control group (Table 5.6). Treatment effects were observed for the 1.2 μm and 20 μm fractions, and for total chlorophyll *a* after 20 days. For these fractions, significantly higher phytoplankton biomass was present in the 150 $\mu\text{g/L}$ and 250 $\mu\text{g/L}$ treatments than in control groups.

5.3.2.2 Periphyton

ANOVA was performed on untreated data. There was a significant treatment effect on periphyton biomass observed on both sampling days (Table 5.7). After 5 days, chlorophyll *a* content was lower in control and 150 $\mu\text{g/L}$ samples compared to the 250 $\mu\text{g/L}$ treatment (Table 5.8). Ash free dry mass differed significantly only between the 150 $\mu\text{g/L}$ and 250 $\mu\text{g/L}$ treatments only. After 20 days, chlorophyll *a* concentration in the 150 $\mu\text{g/L}$ treatment was significantly higher than controls or the 250 $\mu\text{g/L}$ treatment. Ash free dry mass was significantly different only between the 150 $\mu\text{g/L}$ and control groups.

Table 5.5. Results of MANOVA for the effect of treatment on chlorophyll *a* (Chl *a*) concentration for 3 size fractions of phytoplankton and for total chlorophyll *a* exposed for 5 and 20 days in July 1994. Shown are F-values.

Size fractions	Source	DF	5 Days	20 Days
1.2 μm	Treatment	2	4.15*	85.57***
20 μm	Treatment	2	0.94	6.19*
30 μm	Treatment	2	2.79	0.39
Total Chl a	Treatment	2	3.26	74.53***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; DF is degrees of freedom.

Table 5.6. Chlorophyll *a* concentration (mg/L) for 3 size fractions of phytoplankton and for total chlorophyll *a* exposed to 3 concentrations of triallate for 5 and 20 days in July 1994.

Size fractions (Initial)	Treatment	Time ^a	
		5 Days	20 Days
1.2 μm (56.73 \pm 3.92)	Control	7.93 \pm 0.39 a	4.66 \pm 0.37 a
	150 $\mu\text{g/L}$	10.30 \pm 0.72 a, b	13.68 \pm 0.57 b
	250 $\mu\text{g/L}$	13.05 \pm 2.02 b	15.96 \pm 0.89 b
20 μm (4.60 \pm 0.47)	Control	0.49 \pm 0.19 a	0.36 \pm 0.16 a
	150 $\mu\text{g/L}$	0.18 \pm 0.09 a	1.92 \pm 0.47 b
	250 $\mu\text{g/L}$	0.34 \pm 0.18 a	2.07 \pm 0.43 b
30 μm (9.17 \pm 1.00)	Control	0.65 \pm 0.06 a	1.85 \pm 0.51 a
	150 $\mu\text{g/L}$	0.41 \pm 0.21 a	1.62 \pm 0.26 a
	250 $\mu\text{g/L}$	1.95 \pm 0.83 a	2.08 \pm 0.27 a
Total Chl <i>a</i> (61.32 \pm 4.26)	Control	9.08 \pm 0.57 a	6.87 \pm 0.72 a
	150 $\mu\text{g/L}$	10.89 \pm 0.97 a	17.24 \pm 0.93 b
	250 $\mu\text{g/L}$	15.33 \pm 2.88 a	20.11 \pm 0.76 b

a Shown are means \pm SE ($n = 5$). Means with the same letter are not significantly different.

Table 5.7. Results of ANOVA for the effect of treatment on chlorophyll *a* (Chl *a*) concentration and ash free dry mass (AFDM) of periphyton exposed for 5 and 20 days in July 1994. Shown are F-values.

	Source	DF	5 Days	20 Days
Chl <i>a</i>	Treatment	2	10.67**	18.93***
AFDM	Treatment	2	5.80*	5.07*

* P < 0.05, ** P < 0.01, *** P < 0.001; DF is degrees of freedom

Table 5.8. Chlorophyll *a* concentration (Chl *a*) and ash free dry mass (AFDM) of periphyton exposed to 3 concentrations of triallate for 5 and 20 days in July 1994.

	Treatment	Time ^a	
		5 Days	20 Days
Chl <i>a</i> (mg/cm ²)	Control	0.020 ± 0.004 a	1.721 ± 0.277 a
	150 µg/L	0.008 ± 0.002 a	5.568 ± 0.521 b
	250 µg/L	0.100 ± 0.025 b	3.247 ± 0.497 a
AFDM (mg/cm ²)	Control	0.018 ± 0.002 a, b	0.392 ± 0.020 a
	150 µg/L	0.009 ± 0.002 a	0.681 ± 0.105 b
	250 µg/L	0.043 ± 0.012 b	0.481 ± 0.038 a, b

a Shown are means ± SE ($n = 5$). Means with same letter are not significantly different.

5.3.2.3 *Daphnia pulex*

Triallate greatly reduced the number of individuals present in microcosms after 5 and 20 days (Tables 5.9 and 5.10). *Daphnia pulex* was eliminated at a triallate concentration of 250 µg/L whereas *D. pulex* exposed to 150 µg/L suffered some mortality but were not eliminated, and some individuals were able to reproduce. After 5 days, few adults and no offspring were found in the 250 µg/L group, and surviving adults in the 150 µg/L group reproduced poorly compared with control *D. pulex*. These differences were magnified further after 20 days, when no *D. pulex* were found in the 250 µg/L group and relatively few persisted in the 150 µg/L group.

5.4 Discussion

5.4.1 Experiment 1 (May)

In the first experiment, triallate produced a slight stimulatory effect on the 1.2 µm fraction of phytoplankton, also reflected in total chlorophyll *a*, after 5 days. However, toxicity was apparent after 20 days when phytoplankton biomass in the 30 µm fraction (and total chlorophyll *a*) increased significantly in control samples while treated samples remained near the same level. After 20 days, periphyton biomass was significantly higher in the 250 µg/L treatment group compared with the control group.

Table 5.9. Results of ANOVA for the effect of treatment on the number of surviving young and adult *Daphnia pulex* exposed to 3 concentrations of triallate for 5 and 20 days in July 1994. Shown are F-values for ANOVA models. Data were log transformed prior to analysis in all cases except with adults on day 5 when square root transformation was used.

Age group	Source	DF	5 Days	20 Days
Young	Treatment	2	410.11***	3.67***
Adult	Treatment	2	332.20***	10.14**

* P < 0.05, ** P < 0.01, *** P < 0.001; DF is degrees of freedom.

Table 5.10. Number of surviving young and adult *Daphnia pulex* after 5 and 20 days of exposure to 3 concentrations of triallate in July 1994. See Table 5.9 for description of transformations used prior to analyses.

Age group	Treatment	Time ^a	
		5 Days	20 Days
Young	Control	743.60 ± 36.39 a	357.60 ± 184.16 a
	150 µg/L	12.60 ± 1.21 b	9.80 ± 4.04 b
	250 µg/L	0.00 ± 0.00 c	0.00 ± 0.00 c
Adult	Control	97.20 ± 0.58 a	803.75 ± 281.98 a
	150 µg/L	71.00 ± 4.54 b	35.40 ± 15.80 b
	250 µg/L	2.00 ± 0.95 c	0.00 ± 0.00 c

a Shown are means ± SE (n = 5). Means with the same letter are not significantly different.

This stimulatory effect observed on periphyton may be related to the changes in phytoplankton biomass in the different treatments because periphytic algae are competitively depressed by phytoplankton in productive lakes (e.g. Hosseini and van der Valk 1989, Hansson 1992). Light and nutrient availability are the main factors regulating periphytic biomass in lakes (Hansson 1992). High phytoplankton biomass reduces light penetration limiting growth of benthic periphyton. In this experiment, smaller periphyton biomass in control microcosms is associated with an elevated chlorophyll *a* concentration in the 30 µm fraction and total chlorophyll *a*. Although I did not measure light penetration, reduced light reaching the bottom of the bottle could explain the reduction in periphyton growth observed in the control microcosms.

5.4.2 Experiment 2 (July)

The goal of this experiment was to evaluate the toxicity of triallate to *D. pulex* and particularly to verify sublethal effects observed in the mesocosm experiment (chapter 4). However, measurement of sublethal effects was precluded by mortality in treatment microcosms. *Daphnia pulex* was eliminated from the 250 µg/L treatment and were significantly reduced in the 150 µg/L treatment (after 20 days). At the same time, control *Daphnia* increased to a high density and experienced a high reproductive rate.

Phytoplankton and periphyton were probably influenced by triallate and grazing pressure from *D. pulex*. The effect of grazing was particularly evident after 20 days. On that day, edible fractions of algae (1.2-20 µm and 20-30 µm) were significantly reduced in control samples compared to other treatments while the biomass of inedible algae (> 30 µm) was the same. Reduction or alteration of the grazer population by toxicants

indirectly changes phytoplankton assemblages (e.g. Tidou *et al.* 1992, Webber *et al.* 1992). Size-fractionated biomass measurement was essential to evaluate changes to a specific size fraction of phytoplankton. Munawar *et al.* (1989) recommend the use of size-fractionated measurements on phytoplankton because effects of various contaminants are often either missed or masked when total phytoplankton community assays are used.

This experiment demonstrated direct and indirect effects of triallate. Toxic effects on algae and *D. pulex* were identified together with indirect effects produced by interactions between organisms present in the microcosms. Triallate exposure was different in this experiment compared to the mesocosm study (chapter 4). The microcosm formed a closed system including only a water phase and the distribution pattern of triallate was expected to be very different from the pattern experienced in natural systems. Loss of triallate by binding to plastic and to microbial degradation are 2 processes that could decrease triallate concentration in water in microcosms, but volatilization and partitioning to the sediment were not possible in this system. Partitioning to sediment and possibly volatilization were the main processes responsible for the majority of dissipation of triallate in natural system (chapter 3).

5.5 Conclusion

Microcosm experiments were conducted on a natural assemblage of phytoplankton with and without *D. pulex*. These experiments were designed to complement the mesocosms and resolve some of the uncertainties regarding the impact of triallate on phytoplankton, periphyton and *D. pulex*. Two experiments were performed

and each had a different goal. The first experiment evaluated effects of triallate on phytoplankton and periphyton. Triallate produced a slight stimulatory effect on phytoplankton after 5 days but toxicity was apparent after 20 days and phytoplankton biomass in the 30 μm fraction was significantly greater (twofold) in control microcosms. Periphyton biomass, however, was higher in treated microcosms compared with controls, and this response may have been linked to the influence of shading by the phytoplankton community (particularly 30 μm fraction) in control samples. The second experiment focused on the effect of triallate on *D. pulex*. Mortality was important; the number of individuals was reduced significantly at the 150 $\mu\text{g/L}$ treatment and *D. pulex* were completely eliminated at the 250 $\mu\text{g/L}$ concentration. In this experiment, the effect of triallate on phytoplankton and periphyton cannot be dissociated from the effect of grazing by *D. pulex*. Grazing by *D. pulex* was probably responsible for a significant reduction in biomass of edible algae (1.2 and 20 μm fractions) in control microcosms containing a high density of *Daphnia* whereas biomass of inedible algae (>30 μm) was unaffected. Periphyton was probably affected by triallate and grazing by *D. pulex*. Differences in the exposure level of triallate in microcosms compared to the mesocosms likely explains increased toxicity to phytoplankton and *D. pulex* in these experiments.

6.0 LABORATORY EXPERIMENTS WITH *DAPHNIA PULEX*

6.1 Introduction

Pesticides and other chemicals can produce adverse impacts on aquatic organisms (reviewed in chapters 3-5). *Daphnia pulex* is one of several species of zooplankton that are important components of wetland food webs by forming a major link between primary producers and predators. Toxicity of triallate has been evaluated in the laboratory for a number of aquatic organisms including, algae, *Daphnia*, chironomids and fish (Mayer and Ellersieck 1986, Buhl and Faerber 1989, Kent *et al.* 1992). Mayer and Ellersieck (1986) measured a 48 h median effective concentration (EC50) of 80 µg/L for *D. magna*. In a field experiment (chapter 4), *D. pulex* exposed to an initial triallate concentration of 250 µg/L suffered reduced body size and reproduction. Results of the field study were not always consistent, however, and there were differences in responses among enclosures. Laboratory tests described in this chapter were designed to complement the field experiments (chapters 4 and 5), by evaluating direct toxic effects of triallate to *D. pulex* and sublethal effects of triallate, principally growth and reproduction, under more controlled conditions.

Daphnia pulex was the dominant cladoceran zooplankton in Gursky's pond where the field experiment was conducted (chapter 2). The main characteristics of *D. pulex* were described in chapter 4, but some features that make the study of reproduction

relatively easy in *Daphnia* spp. are summarized below. *Daphnia* reproduce parthenogenetically and have a short reproductive cycle (approximately 3 days at 21 °C). The eggs are carried in a transparent brood chamber, and mature through five developmental stages (Threlkeld 1979). The first stage is characterized by a spherical lipid droplet. This lipid reserve is composed of triacylglycerol and provides energy to the embryo throughout early development (Goulden and Henry 1984). Eggs and lipid droplets are easily counted and measured.

Laboratory experiments include acute and chronic tests. The goal of the acute test was to provide an estimate of triallate toxicity while the chronic test exposed *D. pulex* to low concentrations of triallate over a longer period of time to study sublethal effects, principally on growth and reproduction. Reproductive effort was evaluated on the basis of numbers of eggs produced, their size and the amount of lipid they contain. Maternal lipid investment (MLI) (Arts and Sprules 1988) provided an estimate of the total amount of energy a female allocated to reproduction because it considers not only the number of eggs produced but the amount of lipid energy reserve they contain.

Sublethal effects of triallate on *Daphnia* remain undefined but have the potential to adversely affect *Daphnia* populations. These laboratory tests in combination with the field experiments (chapter 4 and 5) provide a more complete understanding of the impact of triallate on these organisms.

6.2 Materials and Methods

6.2.1 Test Chemical

A commercial formulation of triallate, Avadex BW (Monsanto Co.), was used for toxicity testing. Test solutions were prepared by diluting Avadex BW in filtered pond water. Concentrations were based on the active ingredient, triallate (400 g triallate/L of Avadex BW). Test concentrations were made by nominal dilution of a stock solution of 200 mg/L. Concentrations were 0 (control), 125, 200, 315 and 500 µg/L for the acute test, and 0 (control), 80 and 125 µg/L for the chronic test. These concentrations were chosen based on the results of preliminary acute range-finding tests. Concentrations in the chronic test were selected to assure good survival until the end of the experiment. All water was treated at the start of the experiment to obtain a decreasing concentration of triallate with time, a pattern similar to the gradual dissipation of triallate from water after a contamination event. Treated water was kept in closed glass bottles (2.5 L) at the same temperature and light regime used in toxicity testing. All glassware used in culturing, testing or preparation of solutions was thoroughly washed with soap and milli-Q water, soaked in a hydrochloric acid solution and rinsed with solvents (acetone and hexane).

6.2.2 Culture and Experimental Conditions

Toxicity testing and culturing of *D. pulex* was done under static renewal conditions. *Daphnia pulex* were kept in environmental chambers at a temperature of 19-21 °C under a 16:8 h light:dark cycle. Water was collected from Gursky's pond near St. Denis, Saskatchewan (chapter 2), filtered (0.45 µm pore size) and aerated before use. Sediment used in the tests was also collected from this pond. Water was changed every

other day when water quality was monitored. Temperature, pH, dissolved oxygen and conductivity were measured in 2 day-old test solutions and new solutions. Temperature was measured with a thermometer, pH measurements were taken using an Accumet pH meter (model 915, Fisher Scientific), dissolved oxygen was measured with a dissolved oxygen meter (HI 8543, Hanna Instruments) and conductivity was measured with a conductivity meter (1481-60, Cole-Parmer Instrument Co.).

6.2.3 Test Organism

Daphnia pulex used in the experiments were also collected from Gursky's pond. Gravid females were collected from the pond with a plankton net and returned to the laboratory. Females were kept individually in 125 ml glass beakers with 100 ml of filtered pond water. They were fed a daily ration of *Chlamydomonas reinherdii* at a concentration of 4.5×10^4 cells/ml/*Daphnia*. Algae were centrifuged and supernatant was removed. Algae were resuspended in filtered pond water and the concentrated algal cells were counted with an haemocytometer to determine the amount of concentrated food needed. Rations deviated slightly from the target (4.5×10^4 cells/ml), but, on average, the ration was identical among all treatments and replicates.

Culture vessels were observed every 12 h for neonates which were removed as soon as they were observed. Day-old *Daphnia* (less than 12 h old) from the third clutch were used in experiments. Offspring from the first and second clutches are generally more variable in size (Glazier 1992) and were not used. Female offspring produced by 10 mothers of similar size with a mean brood size of 23 neonates (range: 16 to 30 neonates) were randomly assigned to treatments.

6.2.4 Acute Toxicity Testing

Acute tests were conducted in 250-ml teflon containers with 2 cm of sediment and 200 ml of test solution. Six concentrations were tested. There were 10 *D. pulex* per test container and 3 replicates per concentration (30 animals per concentration).

Daphnia pulex were observed daily for motility and mortality. The criterion used for immobility was lack of movement (except small movements of the appendages) when probed with a gentle flow of water for 10 s. Animals were considered to be dead when no heart beat was visible when viewed under a dissecting microscope. Dead animals were removed but immobile *D. pulex* were kept in the experiment. The acute test lasted 96 h and *D. pulex* were fed daily as described above (see 6.2.3).

6.2.5 Chronic Toxicity Testing

Chronic tests were run in 250-ml teflon containers with a 2 cm sediment layer and 100 ml of overlying test solution. *Daphnia pulex* were kept individually and were fed daily throughout the experiment. Three concentrations were tested with 20 replicates per concentration. Daily observations were made for survival and evidence of reproduction. When reproduction began, observations were made every 12 h to count and remove offspring. *Daphnia pulex* were removed from the experiment after releasing their second brood and when the third clutch of eggs was freshly deposited in the brood chamber (stage 1 eggs). The exposure ranged from 12 to 15 days. Females were anesthetized with carbonated water and preserved in a saturated sugar formaldehyde solution (4% formaldehyde).

Gravid females with stage 1 eggs were measured for body length, body weight, clutch size, mean egg volume (per female), mean egg lipid volume (per female) and maternal lipid investment (MLI) as described in chapter 4 (see 4.2.5).

6.2.6 Toxicity Data and Statistical Analyses

In the acute test, both median lethal concentration and median effective concentration were estimated with the Spearman-Kärber method (U.S. EPA Toxicity Data Analysis Software, 1994) after 48 h and 96 h of exposure. Median lethal concentration is the concentration at which 50% of the test animals die. Median effective concentration is the concentration at which 50% of the tested animals are affected; immobility was the endpoint used.

Analysis of variance was used to analyze data from the chronic experiment. Tukey's multiple comparison test was used when a significant treatment effect was obtained in the ANOVA. Analysis of covariance was also performed on the data. Analyses were executed on the Statistical Analysis System (SAS Institute 1990).

6.3 Results

6.3.1 Chemical Characteristics of Water

Physical conditions (means \pm 1 SE) were pH, 8.75 ± 0.02 ; conductivity, 0.847 ± 0.0002 ; and dissolved oxygen, 8.4 ± 0.03 mg/L, although dissolved oxygen decreased to 7.2 ± 0.03 mg/L in 2 day-old solutions.

6.3.1 Acute Toxicity Testing

Neonate *D. pulex* were not adversely affected by triallate at levels below 125 μ g/L when exposed for 48 or 96 h, but mortality rose sharply with both exposure periods

at levels above this concentration (Figure 6.1). Lethal concentrations were estimated to be about 30% and 10% greater than effective concentrations at 48 and 96 h, respectively (Table 6.1).

6.3.2 Chronic Toxicity Testing

Survival and reproduction were good in all groups. There was 90%, 80% and 90% survival in control, 80 µg/L and 125 µg/L groups, respectively. Ehippial eggs were produced in the second clutch by a few (20%) *D. pulex* from both treatment groups but none appeared in control *D. pulex*. Ehippial eggs are produced in response to unfavourable conditions (Pennak 1989) indicating that exposure to triallate produced at least some level of stress to *D. pulex*. The third clutch, however, was composed of parthenogenetic eggs in all *D. pulex*.

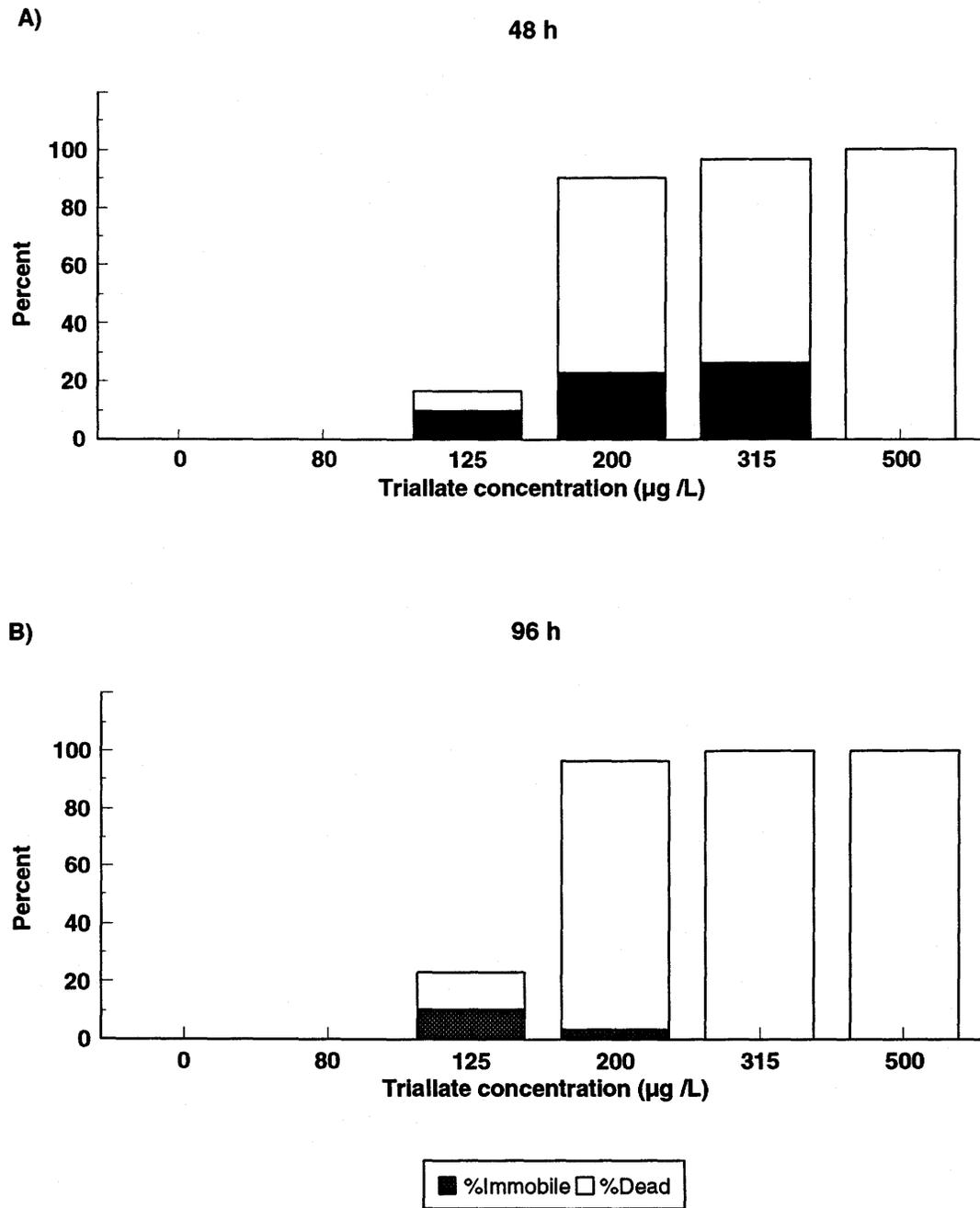


Figure 6.1. A) Static acute toxicity for *Daphnia pulex* after 48 h (A) and 96 h (B) of exposure.

Table 6.1. Acute toxicity for *Daphnia pulex* based on 48 and 96 h of exposure (Figure 6.1). Shown are mean effective (EC50) and lethal (LC50) concentrations, with 95% confidence intervals (CI). EC50 refers to concentrations of triallate required to affect 50% of treated animals, and LC50 is the concentration required to kill 50% of treated animals.

Exposure time	EC50 (µg/L)		LC50 (µg/L)	
	Mean	95% CI	Mean	95% CI
48 h	156	143 - 170	205	182 - 231
96 h	144	134 - 156	153	143 - 165

Chronic exposure to triallate reduced growth and reproduction of *D. pulex*. Body length and body weight were significantly reduced at an exposure concentration of 125 µg/L compared with control and 80 µg/L groups (Figure 6.2, Table 6.2). Likewise, reproductive parameters such as clutch size, mean egg volume (per female), mean egg lipid volume (per female) and MLI were also reduced at 125 µg/L (Figure 6.3, Table 6.2).

Two measures of body size were used in this study: length and weight. Although these two characters are highly correlated, body weight is a volumetric measure and reflects condition of *D. pulex*. Thus, analysis of covariance was performed to see whether treated *D. pulex* had reduced body weight compared to control *D. pulex* of the same body length. ANCOVA indicated that treatment had no effect on body weight when body length was used as a covariate in the ANCOVA (Table 6.3). Triallate did not produce an additional decrease in body weight, so reductions in body size can be equally well described by length or weight measurements. Body weight, however, is more variable (CV = 29.0%) than body length (CV = 7.7%); a common observation in *Daphnia* since weight varies continuously with age whereas body length only changes at the end of an instar. Variation was reduced by measuring weight of somatic tissues: *D. pulex* were taken shortly after they had deposited their clutch in the brood chamber and eggs (stage 1) were removed before weighing.

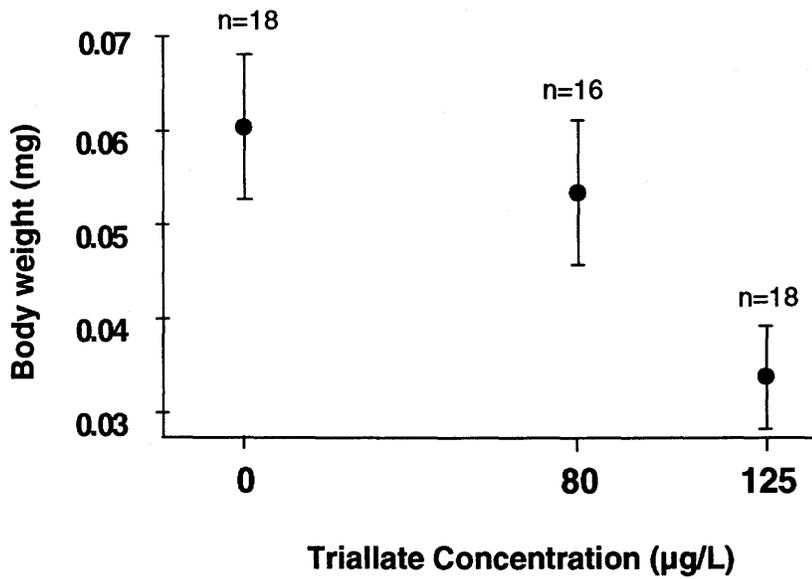
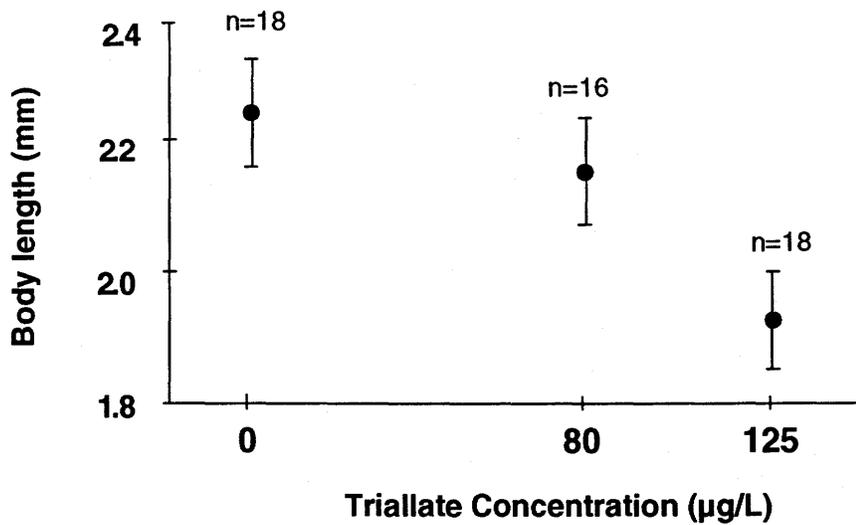


Figure 6.2. Body length and body weight for *Daphnia pulex* exposed to 3 concentrations of triallate (0, 80 and 125 µg/L) for 12 to 15 days in a chronic toxicity experiment. Shown are means (closed circle) with 95% confidence interval (vertical line) and number of females (n).

Table 6.2. Summary of body size and reproductive parameters for *Daphnia pulex* exposed to 3 concentrations of triallate (0, 80 and 125 µg/L) for 12 to 15 days in chronic toxicity experiment. Results of ANOVA as well as mean values (± 1 SE) are presented ($n = 18$ for control, $n = 16$ for 80 µg/L and $n = 18$ for 125 µg/L). Tukey's multiple comparison test was performed when significant treatment effects were obtained in the ANOVA. Means with the same letter are not significantly different.

	ANOVA			MEANS ± 1 SE		
	Source	DF	F Value	Control	80 µg/L	125 µg/L
Body Length (mm)	Treat	2	17.45***	2.238 \pm 0.038 a	2.152 \pm 0.041 a	1.927 \pm 0.038 b
Body Weight (mg)	Treat	2	15.44***	0.059 \pm 0.003 a	0.053 \pm 0.004 a	0.034 \pm 0.003 b
Clutch Size	Treat	2	5.43**	13.111 \pm 0.956 a	12.875 \pm 1.013 a	9.111 \pm 0.955 b
Mean Egg Volume (nL)	Treat	2	4.64*	4.056 \pm 0.127 a	3.902 \pm 0.135 a	3.522 \pm 0.127 b
Mean Egg Lipid Volume (nL)	Treat	2	5.82**	0.501 \pm 0.022 a	0.484 \pm 0.023 a	0.403 \pm 0.022 b
MLI (nL)	Treat	2	9.23**	6.718 \pm 0.551 a	6.248 \pm 0.585 a	3.594 \pm 0.551 b

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, DF is degrees of freedom

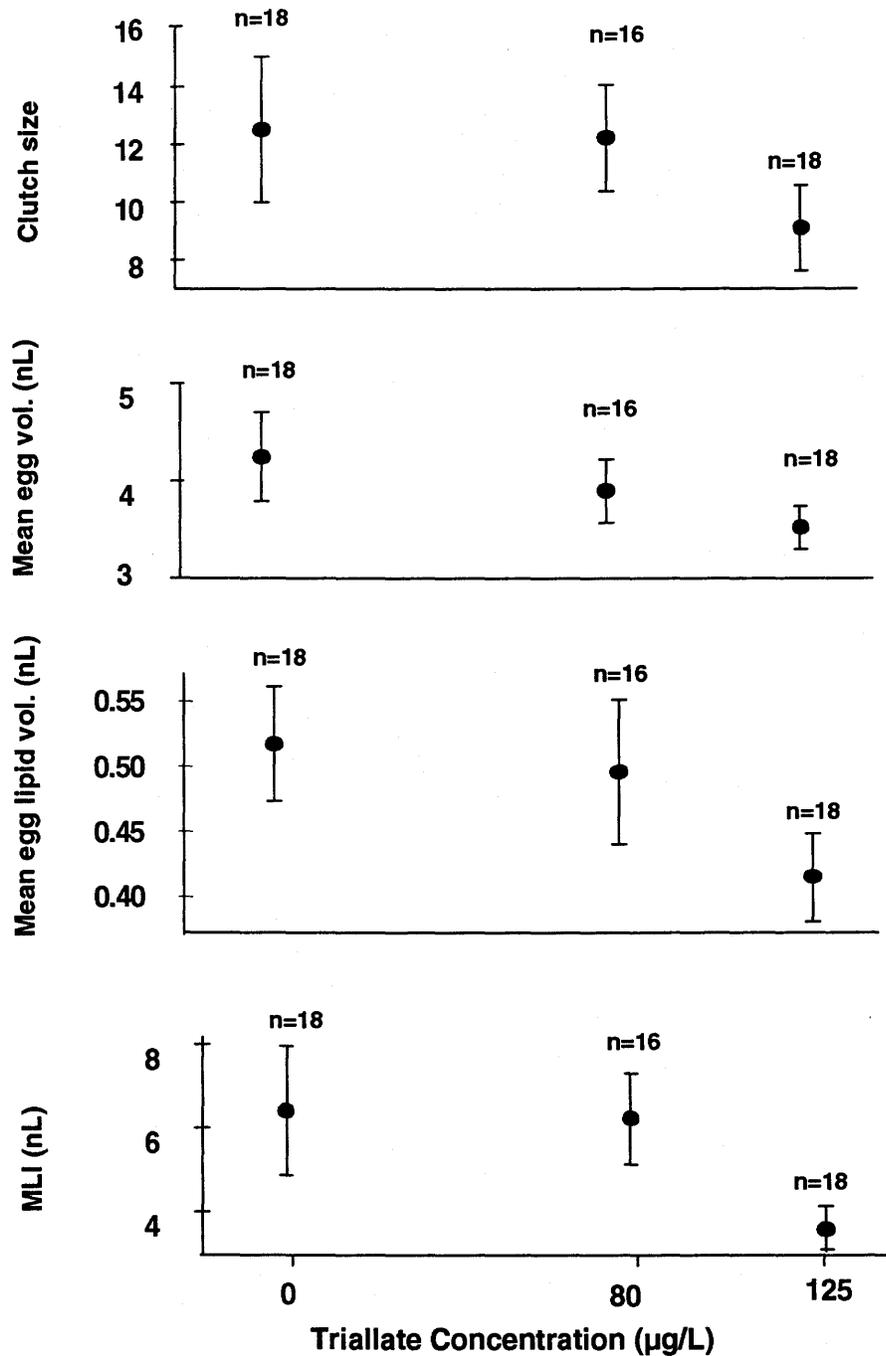


Figure 6.3. Clutch size, mean egg volume, mean egg lipid volume and maternal lipid investment (MLI) of *Daphnia pulex* exposed to 3 concentrations of triallate (0, 80 and 125 µg/L) for 12 to 15 days in a chronic toxicity experiment. Shown are means (closed circle) with 95% confidence interval (vertical line) and number of females (n).

Table 6.3. Results of ANCOVA for the effect of treatment on body weight, clutch size, mean egg volume, mean egg lipid volume and MLI for *Daphnia pulex*. Treatment is main effect and body length is a covariate. Shown are partial F-values.

Source	DF	Body Weight	Clutch Size	Mean Egg Volume	Mean Egg Lipid Volume	MLI
Treatment	2	0.51	3.85*	4.03*	0.34	3.27*
Length	1	107.26***	28.76***	3.92	3.91	23.99***
Treat x Length	2	0.62	3.84*	3.92*	0.38	3.43*
Error MS	46	0	8.15	0.26	0.01	3.23
R-square		0.83	0.61	0.31	0.26	0.59

* P < 0.05, ** P < 0.01, *** P < 0.001; DF is degrees of freedom

Table 6.4. Reproductive parameters corrected for the effect of body length for *Daphnia pulex* exposed to 3 concentrations of triallate (0, 80 and 125 µg/L) for 12 to 15 days in chronic toxicity experiment. Values are least square means ± 1 SE (\bar{n} = 18 for control, \bar{n} = 16 for 80 µg/L and \bar{n} = 18 for 125 µg/L).

	Control	80 µg/L	125 µg/L
Clutch Size	10.7 ± 0.8	12.3 ± 0.8	10.3 ± 1.1
Mean Egg Volume (nL)	4.13 ± 0.15	3.78 ± 0.13	3.64 ± 0.19
Mean Egg Lipid Volume (nL)	0.49 ± 0.03	0.47 ± 0.02	0.42 ± 0.03
MLI (nL)	5.00 ± 0.53	5.87 ± 0.47	4.12 ± 0.67

ANCOVA was used to separate the effects of body size (i.e., body length) and treatment on reproduction. ANCOVA indicated that clutch size and MLI covaried positively with body length (Table 6.3). The same trend was observed for mean egg volume ($P=0.054$) and mean egg lipid volume ($P=0.054$) but neither was statistically significant. After adjusting for effects of body length on the reproductive parameters, additional treatment effects were observed for the variables clutch size, mean egg volume and MLI. Clutch size and MLI were bigger in *D. pulex* from the 80 $\mu\text{g/L}$ group compared with control and 125 $\mu\text{g/L}$ groups (Table 6.4). Mean egg volume, however, was smaller in the 125 $\mu\text{g/L}$ group.

A positive correlation was found between clutch size and body size (Table 6.3) but the relationship between the number of eggs produced and their size in *D. pulex* exposed to triallate remains unexplored. Clutch size was used as a covariable in an analysis to look at the effect of triallate on egg size and egg lipid content. ANCOVA indicated that neither mean egg volume nor mean egg lipid volume covaried with clutch size and there was no significant treatment effect (Tables 6.5 and 6.6). No pattern is apparent in the relationship between clutch size and egg size or egg lipid content (Figure 6.4). Because variation in mean egg lipid volume follows a similar pattern as mean egg volume, ANCOVA was used to factor out the effect of egg size on egg lipid content. After adjusting effects of mean egg volume, no treatment effects were observed on mean egg lipid volume (Table 6.7).

Table 6.5. Results of ANCOVA for the effect of treatment on mean egg volume and mean egg lipid volume for *Daphnia pulex* exposed to 3 concentrations of triallate (0, 80 and 125 µg/L for 12 to 15 days in chronic toxicity experiment. Treatment is main effect and clutch size is a covariate. Shown are F-values.

Source	DF	Mean Egg Volume	Mean Egg Lipid Volume
Treatment	2	0.04	0.25
Clutch size	1	1.86	0.02
Error MS	46	0.29	0.01
R-square		0.19	0.23

* P < 0.05, ** P < 0.01, *** P < 0.001; DF is degrees of freedom

Table 6.6. Reproductive parameters, corrected for effects of clutch size, for *Daphnia pulex* exposed to 3 concentrations of triallate (0, 80 and 125 µg/L) for 12 to 15 days in chronic toxicity experiment. Values are least square means ± 1 SE ($n = 18$ for control, $n = 16$ for 80 µg/L and $n = 18$ for 125 µg/L).

	Control	80 µg/L	125 µg/L
Mean Egg Volume (nL)	4.04 ± 0.15	3.91 ± 0.14	3.33 ± 0.18
Mean Egg Lipid Volume (nL)	0.48 ± 0.03	0.48 ± 0.02	0.38 ± 0.03

Table 6.7. Results of ANCOVA for the effect of treatment on mean egg lipid volume for *Daphnia pulex* exposed to 3 concentrations of triallate (0, 80 and 125 µg/L) for 12 to 15 days in chronic toxicity experiment. Treatment is the main effect and mean egg volume is the covariate. F-values and least square means ± 1 SE are presented (\bar{n} = 18 for control, \bar{n} = 16 for 80 µg/L and \bar{n} = 18 for 125 µg/L). The interaction of treatment and mean egg volume was not significant.

Source	ANCOVA		LEAST SQUARE MEANS ± 1 SE		
	DF	Egg lipid volume	Control	80 µg/L	125 µg/L
Treatment	2	0.84	0.48 ± 0.02	0.48 ± 0.02	0.44 ± 0.02
Mean Egg Volume	1	21.70***			
Error MS	46	0.01			
R-square		0.46			

* P < 0.05, ** P < 0.01, *** P < 0.001; DF is degrees of freedom

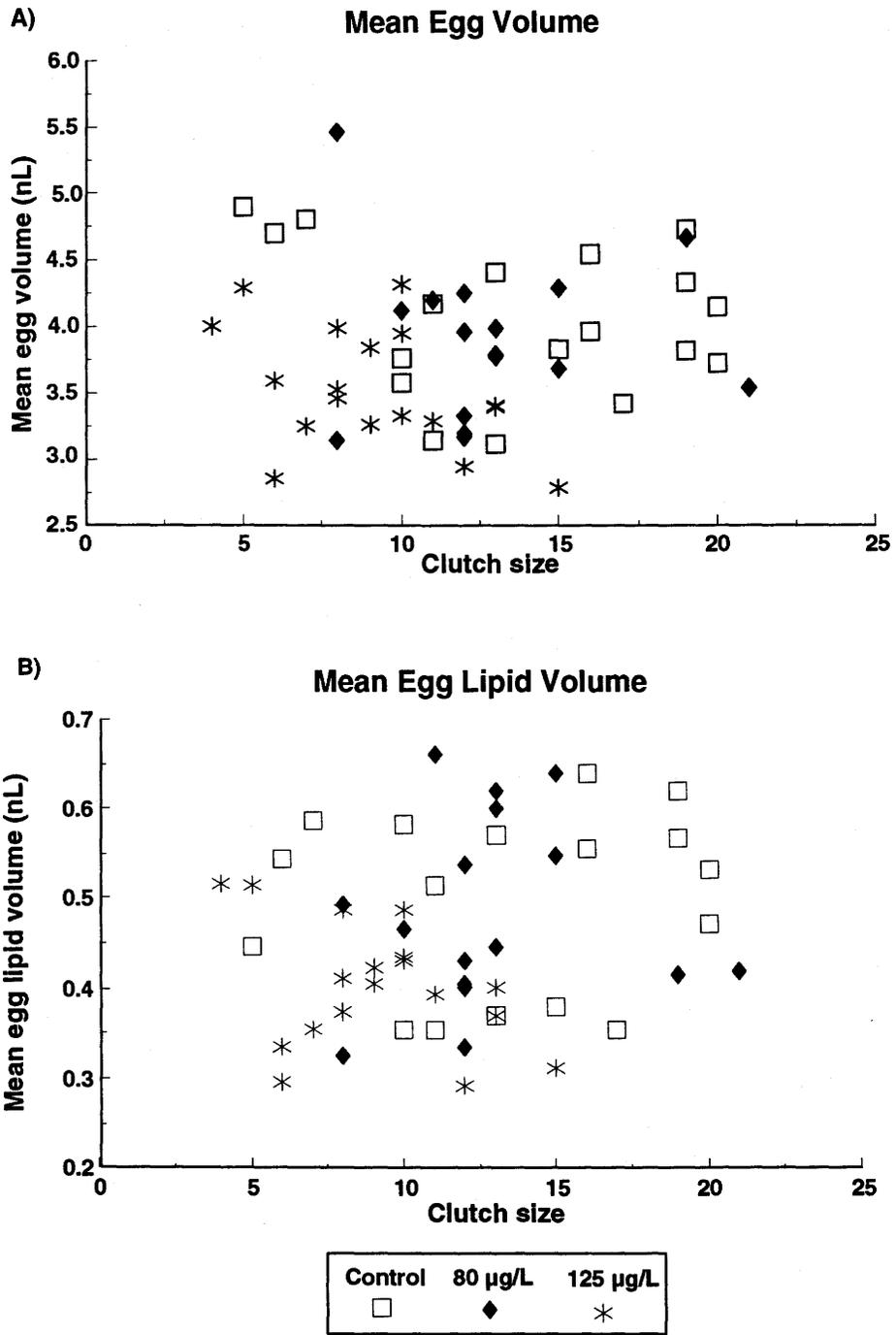


Figure 6.4. Relationships between A) mean egg volume and clutch size and B) mean egg lipid volume and clutch size, for *Daphnia pulex* exposed to 3 concentrations of triallate (0, 80 and 125 $\mu\text{g/L}$) for 12 to 15 days.

6.4 Discussion

6.4.1 Acute Toxicity Experiments

Results from acute tests indicated lower toxicity of triallate to *Daphnia* than previous studies. I obtained a 48 h EC50 estimate of 156 µg/L, about twice the value of 80 µg/L reported by Mayer and Ellersieck (1986). Both tests were done under static conditions, but different species (*D. pulex* vs *D. magna*) were tested and test conditions were different. A two phase system, water and sediment, was used in this study and feeding was continued throughout the experiment. Triallate is lipophilic ($\log K_{ow} = 4.6$) and will bind to sediment and food particles, decreasing the amount present in solution. Nominal concentrations only were used in this study and it is possible that concentrations decreased rapidly because of volatilization, as well as binding to sediment, algae and to test containers.

6.4.2 Chronic Toxicity Experiments

Chronic toxicity of triallate was manifested by reduced growth and reproduction in *D. pulex* exposed to 125 µg/L of triallate. Reproductive parameters were correlated with body length; a strong correlation existed with clutch size and MLI, whereas a weak correlation was observed with mean egg volume and mean egg lipid volume. Reduced clutch size and MLI in *D. pulex* exposed to 125 µg/L of triallate is directly related to their smaller body length. However, *D. pulex* exposed to 80 µg/L of triallate produced significantly more eggs for their size than the control or 125 µg/L groups. The same pattern was observed for the variable MLI adjusted for effects of body length. Smaller mean egg volume and mean egg lipid volume in *D. pulex* exposed to 125 µg/L of triallate is related in part to reduced body size in treated *D. pulex* but reduction in mean egg

volume is also due to additional effects of triallate. However, the decrease in mean egg lipid volume observed in treated *D. pulex* is proportional to the reduction in mean egg volume and therefore relative allocation of lipid to the egg was not affected by triallate.

Exposure to triallate did not change the allocation of resources into individual eggs: treated *D. pulex* laid as many eggs as the control *D. pulex* relative to their size but eggs were smaller with the amount of egg lipid remaining proportional to the size of the egg. They did not adopt a strategy of producing fewer larger eggs, a strategy often observed under conditions of stress such as reduced food supply (e.g. Cowgill *et al.* 1985, Enserink *et al.* 1990, Gliwicz and Guisande 1992, Cox *et al.* 1992).

6.4.3 Reduced Growth and Reproduction in Relation to Energetics

Chronic toxicity of triallate to *D. pulex* was manifested by reduced growth and reproduction. The same response has been observed repeatedly in cladocerans exposed to toxicants (e.g. Schober and Lampert 1977, Van Leeuwen *et al.* 1985, Winner 1988, Chandini 1989, Baird *et al.* 1990, Koivisto *et al.* 1992, Vigano 1993). Baird *et al.* (1990) indicated that *Daphnia* exhibit specific responses under acute exposure to toxic chemicals and that general mechanisms are involved in chronic exposure. Acute responses, which are generally hormonally controlled, are considered to be the initial response to a stressor and often involve the mobilization of energy-related substrates (Giesy and Graney 1989). Chronic stress will initiate compensatory physiological adjustments such that changes in energy metabolism may be required to maintain homeostasis (Giesy and Graney 1989). Chronic exposure of different clones of *D. magna* to cadmium and 3,4-dichloroaniline caused reduced growth and reproduction that were

attributed to reduced food consumption and increased maintenance costs (Baird et al. 1990). Increased protein turnover contributed to elevated maintenance costs (Barber *et al.* 1990). They argued that chronic exposure to toxicants caused a reduction in growth and reproduction and that this was indicative of a general decrease in energy availability.

Under conditions of limited food resources, *Daphnia* preferentially allocate energy to maintenance (carapace formation and respiration) and decrease the relative allocation of energy to growth and reproduction (Glazier and Calow 1992). The highest priority is given to carapace formation whereas the relative priority of respiration, growth and reproduction varied with species, clone, age and pattern of resource limitation (acute vs chronic). Therefore, a reduction in the supply of energy and increasing costs of maintenance would limit energy available for growth and reproduction.

Although food level was kept constant in this study, triallate may possibly decrease the rate of ingestion and assimilation of algae by *D. pulex*, producing a decrease in energy supply in treated animals. Several pesticides (Day and Kaushik 1987, Day 1989, Fernandez-Caselderrey *et al.* 1994) and metals (Flickinger *et al.* 1982, Bodar *et al.* 1988) affected feeding and/or assimilation of food in *Daphnia*.

In addition, exposure to chemical toxicants can in some cases result in protein damage. For example, protein damage may occur because of structural changes and synthesis errors (Barber *et al.* 1990). In this condition, increased protein synthesis can compensate for degradation of protein. However, an increase in protein turnover could cause a proportional rise in energy required for maintenance.

Although the mode of action of triallate on invertebrates has not been documented, the general mechanism affecting the energy budget of *Daphnia* described by Baird *et al.* (1990) and Barber *et al.* (1990) seems a reasonable explanation for the reduced growth and reproduction observed in this study. Measurement of ingestion and assimilation rate as well as protein turnover rate and energy substrates such as lipid and carbohydrates in *Daphnia* exposed to triallate would further clarify the exact mechanism of toxicity.

6.4.4 Implications of Reduced Body Size

Body size is an important trait in animals, including *Daphnia*, and it directly influences reproduction. Clutch size, egg size and neonate size increase with increasing maternal size (e.g., Kerfoot 1974, Cowgill *et al.* 1985, Lynch 1989, Lynch 1992, Glazier 1992, Lampert 1993). The same relationship between clutch size and maternal size was observed in this study; egg size was also correlated with maternal size but the relationship was weak.

Maternal size is an important determinant of offspring size and cladoceran neonate size is also influenced by environmental conditions. Neonate size is affected by temperature (Brambilla 1982, Arts and Sprules 1988), food conditions (Cowgill *et al.* 1985, Enserink *et al.* 1990, Gliwicz and Guisande 1992) and chemical cues released by predators (Stibor 1992, Weider and Pijanowska 1993, Stibor and Lüning 1994, Lüning 1994). However, in my study, these variables were controlled.

The advantages of large body size in *Daphnia* are numerous. In addition to increased production of offspring, large females produce eggs and neonates of larger size

which confer important survival advantages. Large offspring that contain more lipid have increased survivorship under low food conditions (Tessier *et al.* 1983, Goulden *et al.* 1987). Tessier and Consolatti (1989) observed that large size at birth was always advantageous for general performance of juveniles. Overall, larger *Daphnia* have increased fitness because they produce more offspring with higher survival ability.

Another advantage of large body size is reduced predation by invertebrate predators such as larvae from the phantom midge *Chaoborus* (Hebert 1978). In natural populations, the consequences of reduced juvenile growth and delayed age at maturity may be severe and result in significant mortality (Vanni and Lampert 1992). However, smaller body size are advantageous in the presence of visually-oriented vertebrate predators such as fish and some invertebrates (*Notonecta*, *Corixa*) that selectively prey on large individuals. *Daphnia* have adapted to changing predation pressures, responding to chemical cues from predators as well as showing some degree of phenotypic plasticity through morphological, behavioural and/or life-history adaptation to increase survival and maximize fitness under predation pressure (Weider and Pijanowska 1993). Fish predators are not likely to be the main predators in ponds and sloughs where the highest probability of triallate contamination is likely to occur. In these cases, invertebrate predators (particularly *Chaoborus*) may be more important, favouring large size in *Daphnia*.

6.4.5 Implications of Reduced Reproduction

Reduced production of offspring has an obvious effect on population growth. When recruitment is reduced and mortality remains constant, population growth is reduced and populations may even decline if environmental conditions become unfavourable. Because of its importance in freshwater food webs, effects on *Daphnia* population will be reflected in primary producers, as well as in vertebrate and invertebrate organisms that prey on *Daphnia*. Although we can predict secondary effects resulting from a decrease in *Daphnia* populations, our ability to quantify the response is limited (La Point and Fairchild 1994).

6.5 Conclusion

Laboratory experiments included both acute and chronic tests. In the acute test, a 48-h EC50 (immobility) value of 156 µg/L was obtained, about twice the value reported in other studies. Different test conditions likely were responsible for this difference. In the chronic test, significant effects were observed at a triallate concentration of 125 µg/L and reduced energy allocation in treated *D. pulex* may explain effects on growth and reproduction. The strongest manifestation of triallate toxicity on *D. pulex* was a reduction in body size. Reproduction was reduced as a consequence of smaller body size; fewer offspring were produced and the size of the eggs was smaller with a proportional decrease in egg lipid. Decrease in the size of eggs was due in part to smaller body size but was also directly affected by triallate. Maternal lipid investment, a measure of total reproductive allocation, was also reduced in response to smaller body size. Reduced fitness will result from exposure to triallate as this herbicide influences

reproduction and, possibly, survival. Ultimately, it may have the capacity to lower *Daphnia* populations.

7.0 GENERAL DISCUSSION

7.1 Introduction

Extensive use of pesticides in modern agricultural practices increases the potential for contamination of wetlands. Triallate is one widely used herbicide for the control of wild oats on a variety of crops on the prairies. Triallate is acutely toxic to invertebrates and contamination of wetlands may pose a risk to these organisms that are a vital part of this ecosystem. Environmental contamination by triallate is widespread in areas where the herbicide is extensively used. Presence of triallate in air, water, sediment and biota have been reported (Grover *et al.* 1988a, Kent *et al.* 1992, Waite *et al.* 1992, Waite *et al.* 1995, Donald and Syrgiannis 1995).

In addition to potential for contamination of the environment, and possible persistence in sediments, toxicity of triallate to invertebrates and lack of sufficient data to establish an environmental guideline for the protection of aquatic life motivated this research.

7.2 Fate Study

Information on fate of triallate in mesocosms is an important contribution to the existing data concerning triallate. Information on the distribution of triallate in aquatic systems was needed to supplement available toxicological data since both types of information are required for sound ecological risk assessment. Although the potency of a

compound is the same in laboratory or field situations, the toxicity can be changed by a number of factors affecting the distribution of the toxicant and hence, the exposure of aquatic organisms. For instance, exposure to triallate, a volatile hydrophobic organic compound that strongly binds to organic matter, can be very different in natural systems compared to constant exposure experienced in laboratory bioassays. The amount of dissolved and particulate organic matter in the water and the organic matter content of sediments both influence the distribution of triallate. Wind, temperature, photolysis, volatilization and microbial degradation also play a role.

The study of triallate distribution in mesocosms (chapter 2) clearly shows the rapid dissipation of triallate from water, concomitant with partitioning to sediment organic matter. Consequently, exposure of planktonic organisms to triallate is of short duration. Disappearance of triallate from the water is characterized by a sharp decline in the first 24 h to 48 h followed by a more gradual decrease. Triallate level decreased 100-fold within 10 days (from an initial concentration of about 250 $\mu\text{g/L}$ to a level of 2.5 $\mu\text{g/L}$). Binding to particulate and dissolved organic material in the water column also is probably occurring and reducing the bioavailability of triallate. A significant quantity of data indicates that chemicals bound to dissolved organic carbon (DOC) are not bioavailable (Di Toro *et al.* 1991) and therefore the presence of dissolved organic material in water influences the toxicity of xenobiotics. Day (1991) found reduced toxicity of pyrethroid insecticides to *Daphnia magna* in the presence of dissolved organic carbon (DOC). She indicated that the DOC-pesticide complex may be too large or too polar to penetrate biological membranes. However, the influence of dissolved organic

material on the toxicity of organic compounds is not completely understood. Leek *et al.* (1993), found that the presence of dissolved humic acid reduced the acute toxicity to *D. magna* of certain organic compounds (e.g., diazinon, 4-chloroanilin) but not others (e.g., tetrabromobisphenol-A, pentachlorophenol).

The rising concentrations of triallate that were measured in the sediment 2 days after application coincided with a sharp decrease in water concentration. Levels remained high 30 days post-treatment, and exposure to triallate associated with sediment potentially was important for a much longer period than water exposure. Concentrations measured in sediment cannot be compared or related directly to toxicity for aquatic organisms since I have found no toxicological data for triallate in sediment. Moreover, effects observed in this study are due to water exposure or a combination of water and sediment exposure; sediment exposure alone was not evaluated.

Donald and Syrgiannis (1995) postulated that triallate may persist in sediment for more than 1 year. Information on persistence was not obtained in this study because the mesocosms were not built to withstand winter, and the study was terminated in the fall. Degradation of triallate in the aquatic environment and in sediment, in particular, is not well known. Persistence of triallate in sediment requires further study.

It is difficult to assess toxicity of sediments based solely on the measured concentration of toxicants because toxicity varies widely depending on sediment type and organic matter content (Di Toro *et al.* 1991). It is generally believed that bound residues are not bioavailable and that exposure to toxicants in sediment comes from the dissolved fraction present in pore water (Nebeker *et al.* 1984, Di Toro *et al.* 1991). Toxicity can

therefore be related to the concentration of xenobiotics in interstitial water and is dependent on adsorption/desorption processes in the sediment. Alternatively, toxicity of hydrophobic organic compounds can also be related to the amount of toxicant in relation to organic content of sediment, i.e., concentration expressed on an organic matter content basis (Di Toro *et al.* 1991).

Among the organisms studied in this research, the amphipods *Hyalella azteca* and *Gammarus lacustris* were most exposed to triallate because of their close contact with water and sediment. In the field experiment, they contained high concentrations of triallate shortly after application and, triallate was still present in samples taken 30 days after treatment. The primary route of exposure to triallate for *H. azteca* and *G. lacustris* is unknown. Adams *et al.* (1985) reported uptake and exposure of *Chironomus tentans* to Kepone from overlying water, but interstitial water was the key source of Kepone for uptake and toxicity while Kepone sorbed to food elicited no observable toxic response. Despite differences in physiology and behaviour of amphipods and chironomids, overlying water and interstitial water can be expected to represent important routes of exposure for both species. Amphipods ingest sediments and consume organic material present in sediment. Because triallate is associated with organic material, it may be absorbed from ingested organic material in sediment. Arts *et al.* (1995) suggested that triallate was absorbed by *H. azteca* through ingested food material and demonstrated that triallate was associated with storage lipid adjacent to the gut, lipid-rich tissue such as the nervous system and the triacylglycerol rich eggs of gravid females. Landrum and Scavia (1983) measured uptake of anthracene by *H. azteca* and found that ingested sediment

contributed only 5% to total uptake of this toxicant while interstitial water contributed 72%. Principal routes of uptake of triallate by amphipods have not been elucidated.

7.3 Evaluation of Biological Effects

Biological effects of triallate were evaluated in 3 different test systems: a mesocosm experiment complemented by microcosm and laboratory experiments. All 3 test systems provided evidence of adverse effects to at least some of the organisms. Effects were less pronounced in mesocosms because of their inherent natural variability. Reduced toxicity of triallate to aquatic organisms in natural systems in comparison to microcosm and laboratory studies is probably related to differences in exposure to triallate. Triallate concentration decreased rapidly in water due to partitioning to the sediment and, possibly, volatilization and resulted in a short exposure period to planktonic organisms. Moreover, binding to particulate and dissolved organic carbon present in the water probably reduced the bioavailability of triallate. Considering the concentration of triallate applied to the mesocosms (250 µg/L) and previous laboratory and microcosm studies (Johnson 1986, Mayer and Ellersieck 1986, Buhl and Faerber 1989), more severe effects were expected. The main effects observed in the mesocosm study were reductions in body size and reproduction in *D. pulex* and *H. azteca* but there were not uniform effects in the different replicates. Effects of triallate on *H. azteca* were very similar to effects observed on *D. pulex* despite exposure of *H. azteca* to triallate present in both water and sediment. Depending on persistence and bioavailability of triallate associated with sediment, chronic effects may be important for amphipods and other benthic organisms.

Impacts of a toxicant are dependent on pre-existing conditions in the system (Hanazato and Yasuno 1990). The pond in this study was very productive and *D. pulex* had adequate food resources (e.g., large clutch size was indicative of their well-being). A different scenario can develop, however, if triallate enters a system where the aquatic organisms are stressed by insufficient food resources or extreme environmental conditions (temperature, pH, salinity, hardness etc.). In this case, the impact could be more severe.

Microcosm and laboratory studies with *D. pulex* were done to verify some effects observed in the field experiment under controlled conditions. Toxic effects were more pronounced in these systems. In microcosm experiments, algae and *D. pulex* were affected by triallate at concentrations of 250 µg/L and 150 µg/L, respectively. There was also evidence of interaction between algae and *D. pulex*. Grazing by *D. pulex* reduced significantly the edible fraction (1.2-20 µm and 20-30 µm) of phytoplankton. Significant mortality of *D. pulex* in the 150 µg/L treatment was observed and they were completely eliminated in the 250 µg/L treatment. The nature of the microcosms as a closed system with water phase only eliminated the role of 2 key processes in triallate distribution in the pond: volatilization and partitioning to the sediment. Binding to the bottle and degradation by microorganisms were the only processes likely to reduce triallate level in the microcosms and therefore the exposure may have been higher and lasted for a longer period resulting in increased toxicity.

Laboratory experiments with *D. pulex* included both acute and chronic tests. The main focus of the chronic tests was to evaluate the sublethal effects observed in the field

experiment (reduced body size and reproduction) when exposed to low concentration of triallate. The laboratory experiments confirmed the sublethal effects observed in the field study and indicated that these effects result from direct toxicity of triallate to *D. pulex*. The sensitivity of *D. pulex* to triallate in laboratory experiments was similar to the microcosm experiments but was less than other laboratory experiments conducted by Johnson (1986) and Mayer and Ellersieck (1986).

7.3.1 Test Systems

Mesocosms are useful models of natural systems which avoid unnecessary contamination of an entire body of water. Kaushik *et al.* (1986) consider limnocorrals to be a valuable tool for studying the impact of pesticides. Direct effects as well as indirect effects can be evaluated concurrently with the degradation and dissipation of the pesticide. Recovery of populations affected by a pesticide can also be assessed. Mesocosm studies require many resources, are expensive and variability of the results can be a drawback. Despite differences in responses, laboratory bioassays can provide guidelines that are often protective of the environment at a much lower cost. Because they use sensitive species and maximum exposure conditions, laboratory tests are often more sensitive. However, indirect effects cannot be evaluated. Although knowledge of the biology and ecology of the organisms involved enables one to predict some of the indirect effects, our present knowledge does not permit to quantify these responses (La Point and Fairchild 1994). Mesocosm studies are useful in risk assessment, contributing to ecological knowledge of species interactions and functioning of the system under

study. Mesocosms are also very useful for fate studies because this test system allows natural processes to occur and therefore offers more realism than smaller test systems.

Microcosms are a smaller multispecies version of the mesocosm, offering many of the same advantages. Microcosms can be operated with greater ease and perhaps greater replicability (deNoyelles *et al.* 1994). Microcosm experiments described in chapter 5 would have been more useful if they included sediment and allowed for the natural distribution of triallate.

Despite the limitations of single-species laboratory tests, they are a logical first step in evaluating the potential for toxicity of a compound. Laboratory tests were useful to characterize direct effects of triallate on *D. pulex* (chapter 6). They are also most useful to elucidate mechanisms of toxicity of a contaminant.

7.3.2 Endpoints Tested

Lethal and sublethal effects of triallate were evaluated in this study. Measures of algal biomass as well as density measurements of zooplankton were used in the mesocosm experiment. Direct mortality was recorded in microcosm and laboratory tests. Sublethal effects such as effects on body size and reproduction were evaluated in *D. pulex* and *H. azteca*. Sublethal effects causing reduced survivorship and fecundity are important as they greatly influence the population and community. Thus, effects on size, reproduction and energetics are useful measures of sublethal effects (Giesy and Graney 1989).

Measures of growth and reproduction are widely used in toxicological evaluations. Body size is an important trait in animals. Generally, body size can

influence predation, competition, access to resources and reproduction (Peters 1983).

Because of interactions between organisms in the ecosystem, impacts on one population may affect other populations and alter the balance within the community and ecosystem.

There is growing interest in the study of energetics in ecotoxicology. The basis for using energetics is that energy is required to resist the effects of toxicants and to maintain homeostasis (Giesy and Graney 1989). Eventually, an organism utilizes all of its energy reserves and dies. Energetic state of an organism can be measured by weight gain, kinetic growth rate or energy stores, more specifically the amount of glycogen and lipids and their distributions among tissues (Giesy and Graney 1989). A number of factors must be considered in any study of energetics since nutrition, reproductive conditions and molt status of organisms, among other things, greatly influence the energetic state of an organism (Elendt 1989, Giesy and Graney 1989).

Lipids are an important energy reserve for aquatic invertebrates and vertebrates, effectively buffering them against extreme fluctuations and patchiness of food abundance (Goulden and Place 1990). On a dry mass basis, *Daphnia* spp. generally are composed of 20 to 30% of lipids (Cowgill *et al.* 1984). Furthermore, most lipid is utilized for reproduction every 2 to 4 days (Tessier and Goulden 1982). Lipid deposition in eggs represents a major energy source for the developing embryo and any interference with lipid metabolism in the adult can directly influence the survival of the next generation. Available energy is primarily allocated to maintenance, and excess energy is used for growth and reproduction (Glazier and Calow 1992). *Daphnia* spp. allocates a maximum amount of energy to reproduction and the amount of energy that a female allocates to

reproduction is a good indicator of their energetic state. Clutch size as well as the size and the lipid content of eggs were a sensitive measure of triallate toxicity.

7.4 Implications

Based on this research, the interim guideline for the protection of aquatic life (0.024 µg/L) appears to be protective of the aquatic system. Effects observed in the field experiments were mostly sublethal and were produced by a high concentration of triallate (250 µg/L). Chronic exposure experiments with *D. pulex* in the laboratory resulted in reduced growth and reproduction at a triallate concentration of 125 µg/L but not at 80 µg/L.

The concentration tested in the mesocosm experiment (250 µg/L) represents a worst-case scenario of contamination by runoff. Only direct overspray of a wetland would produce higher concentrations. Environmental levels of triallate are well below this concentration and other concentrations tested in the laboratory. Waite *et al.* (1992) reported a maximum concentration of triallate in spring runoff of 0.98 µg/L. Other concentrations reported in surface water are even lower (Kent *et al.* 1992). Occurrence of triallate in surface water is of short duration since it partitions quickly to the sediment. Often triallate is not detected in surface water although it is present in sediment and biota (Therrien-Richards and Williamson 1987, Donald and Syrgiannis 1995). Although sediment represents the primary sink for triallate in the aquatic system, there is no environmental guideline for triallate in sediment.

Environmental levels in water have been reported occasionally to exceed the interim guideline concentration. The margin of safety (factor of 10) implies that the

likelihood of harmful effects is low since triallate has not been reported at concentrations exceeding 2.4 µg/L. Nonetheless, no severe impacts are expected to occur at this concentration in natural systems since exposure is greatly reduced in the field due to partitioning to organic material. This guideline was established based on laboratory tests using a sensitive species *Ceriodaphnia dubia*.

7.5 Recommendations for Future Research and Conclusion

In the aquatic system, triallate is associated primarily with sediment. Although this phenomenon was suggested by the physicochemical characteristics of triallate, it was confirmed in the fate study (chapter 2) and environmental monitoring studies (Donald and Syrgiannis 1995). Research on the toxicity of triallate in sediment is needed to assess potential for impact on benthic organisms. Chronic effects, in particular, need to be evaluated since benthic organisms are exposed to low concentrations of triallate for extended periods of time. Sorption-desorption processes of triallate in sediment should be studied to evaluate the bioavailability of triallate associated with sediment. Principal routes of uptake by invertebrates should be evaluated along with biotransformation and excretion. Vertebrates can excrete triallate but no information exists for invertebrates.

One important problem is the possibility that triallate persists in sediment. Degradation of triallate in the aquatic environment and particularly in sediment must be studied to determine how long the herbicide remains in the system. This information along with a better knowledge of toxicity of triallate associated with sediment will strengthen our ability to evaluate the risks of triallate contamination in aquatic systems.

The presence of triallate at concentrations routinely measured in surface water is not expected to adversely affect wetlands. Concentrations are too low and triallate disappears quickly from the water. Only rare contamination events produce high water concentrations approaching levels studied in this research (250 µg/L). The major risk of wetland contamination by triallate may come from triallate associated with sediment but this risk has not yet been evaluated. Toxicity associated with environmental levels of triallate regularly measured in sediments has not been studied.

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