IMPACTS OF THE PYRETHROID INSECTICIDE CYFLUTHRIN ON AQUATIC INVERTEBRATE POPULATIONS IN OUTDOOR EXPERIMENTAL TANKS

DISSERTATION

Presented to the Graduate Council of the
University of North Texas in Partial
Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

By

Philip C. Johnson, B.S., M.S.
Denton, Texas

May, 1992

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The chemical fate and biological impacts of cyfluthrin in aquatic ecosystems were investigated using microcosms (1.9 m^3 concrete tanks) during 1989. Results were compared to a concurrent pesticide registration study using mesocosms (634.7 m^3 earthen ponds). Ten spray drift and five soil runoff simulations were conducted. Pesticide loadings were scaled by system volume, with the same experimental design in ponds and microcosms. Aqueous cyfluthrin concentrations and sediment residue values were generally higher in microcosms, while aqueous half-life was shorter in the smaller systems.

Biological effects (zooplankton, macroinvertebrate colonization and aquatic insect emergence) showed parallel response patterns in both systems. Larger cladocerans (*Diaphanosoma brachyurum*), mayflies (*Callibaetis* and *Caenis*), and Tanypodinae chironomids (particularly *Labrundinia*) and *Chaoborus* populations were reduced in pesticide treatments, while oligochaetes, rotifers, gastropods, some odonates, Ceratopogonidae, and Chironominae

chironomids were not reduced, or increased in treated systems.

Bluegill sunfish stocked in microcosms were sexually immature, while bluegill stocked in mesocosms were sexually mature resulting in large fish populations in mesocosms. Microcosms also developed larger macrophyte populations and contained artificial refugia, resulting in lower predation pressure. Greater predation in mesocosms resulted in decreased populations of many invertebrates, particularly cladocerans. Some responses were more pronounced in microcosms, while other effects were more apparent in mesocosms. Secondary effects differed among these systems due to fish predation, habitat differences and sampling methodology.

Single species bioassays using *Chironomus tentans*, *Hyalella azteca*, and *Daphnia magna* were conducted using microcosm water and sediments. These tests helped evaluate pyrethroid bioavailability, and corresponded well with population responses of sensitive taxa found within the microcosms.

Sediments contained detectable cyfluthrin residues in November 1989, raising sediment toxicity concerns. Sampling during June 1990 did not detect residual pyrethroid toxicity in either sediment residues, *Hyalella azteca* bioassays or presence of pyrethroid sensitive taxa.

This study suggests that microcosms may prove useful for pesticide hazard assessment in the future. Scaling of chemical fate parameters to smaller systems needs further study, however.

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CHAPTER 1

GENERAL INTRODUCTION

Standardized mesocosm experiments are currently used in the assessment of pesticide impacts, and constitute the final tier of aquatic testing during the FIFRA registration process (Touart 1988). Aquatic mesocosms are partially enclosed outdoor experimental systems, which can be replicated, yet possess a degree of realism not found in laboratory systems (Odum 1984).

Mesocosms allow study of chemical effects on populations of various species under conditions of "real-world" exposure (Crossland and Bennett 1989). Differences in toxicity between lab and field studies are often attributed to actions of the environment on the chemical, therefore coupling of chemical fate and toxicological effects are critical for interpretation of results (Cairns 1989).

Mesocosm tests are not viewed as replacements of single species bioassays (Cairns 1989). Rather, these tests are part of a tiered testing sequence. Single species tests are inadequate when chemical fate is altered significantly under field conditions, when organisms can avoid a toxicant, or when secondary effects occur due to alterations in

competitive or predatory-prey relationships (La Point et al. 1989). It is therefore felt that testing in replicated, semi-natural systems add insight to the assessment of risks to the environment.

Mesocosm research is very expensive, with studies commonly costing in excess of one millon dollars. Recent interest in smaller scale systems has promoted recommendations by the Aquatic Effects Dialogue Group (AEDG 1990) that microcosm testing be investigated. Simultaneous aquatic microcosm (concrete tank) and mesocosm (earthen pond) experiments were conducted in order to explore the utility of using smaller systems in the hazard assessment process, and to define scaling relations between these different systems.

We chose cyfluthrin (a pyrethroid insecticide) as a model compound for this comparison. Synthetic pyrethroids are an important insecticide class, used extensively on various crops in many nations. My dissertation research involved investigation of cyfluthrin impacts in microcosms. Comparison with mesocosm results allowed identification of differences and similarities between the two designs.

Historical Use of Microcosms

The term microcosm generally is reserved for small laboratory systems containing multiple species. Giesy and

Allred (1985) define microcosms as systems < 10 L, mesocosms having volumes > 10 L but < 1000 L, while macrocosms are systems > 1000 L. The term macrocosm has not gained wide recognition, but many would agree that mesocosms would need to be larger than 1000 L. Outdoor experimental tank systems have been referred to as microcosms, mainly to distinguish them from earthen ponds. I have used this terminology for this paper.

Microcosms are less expensive to build, and sampling costs are lower, compared with mesocosm experiments. Reduced cost could then be translated into a larger number of replicates. Enhanced replication increases the statistical power for parametric tests, and may reduce intersystem variation (i.e., lower CV's). Additional replicates would allow one to "discard" tanks from the rest of the experimental units because of extreme divergence prior to application of a stressor compound. Microcosms could allow the use of more complex designs such as two-way factorial designs, testing of chemical interactions, evaluating contaminant exposure to varied fish communities, etc. Further scaling research is needed to advance the state of the science. Microcosms permit visual observations of the entire system, which in turn may promote inclusion of behavioral endpoints into the assessment process. Perhaps the most important advantage of microcosms is the ability to

"customize" the experimental design to address specific concerns about a pesticide, tailoring the test to the toxicological profile of a given compound.

It has long been recognized that microcosms are cost effective models for determining the environmental fate of chemicals (Draggan 1976) and that microcosm size is an important variable. The level of complexity within a given microcosm experiment is another factor, with complex systems providing more environmental realism at the expense of simplification (Draggan 1976). Field microcosms add an additional element of reality and complexity by incorporating native species complexes (La Point and Perry 1989). The reduction in spatial heterogeneity inherent to microcosms is an asset since this decreases the sampling necessary to characterize the system (Dudzik et al. 1979). Expansion to the point that considerations of scale and edge are not problematical would negate the advantages of reduced scale in microcosms (Giesy and Odum 1980). Microcosms and mesocosms are not miniature ecosystems but rather are surrogates for important cause/effect pathways in natural systems (Odum 1984, Cairns 1988b). Cairns (1988b) makes no distinction between microcosms and mesocosms because both encompass higher levels of biological organization and because both have a high degree of environmental realism, relative to single-species bioassays conducted in the lab.

Dudzik et al. (1979) noted that scaling concerns, such as periphyton growth on the sides of microcosms and shallow depth were confounding factors in simulating pelagic systems. These microcosms intentionally modeled a shallow pond environment containing macrophytes. Littoral environments are generally easier to replicate in microcosms, with relatively long-lived organisms lending stability to the system (Giddings 1986).

Studies with atrazine (Larsen et al. 1986) and with chlorpyrifos (Stay et al. 1989) indicated that similar results, such as the same LOEL (Lowest Observed Effects Level), may be determined in small laboratory microcosms and in outdoor ponds or littoral enclosures. Few studies have directly compared the responses to pesticides obtained from outdoor microcosms to larger mesocosm-scale systems (Heimbach in press, and Howick et al. in press). Preliminary indications from these comparative studies suggest similarity among responses from outdoor microcosms and experimental ponds.

Pyrethroid and Cyfluthrin Characteristics

Pyrethroids have become the most significant new class of insecticides in several decades (Coats and Bradbury 1989). The use of pyrethroids has been increasing, representing 30% of all insecticides sold in 1982 (Smith and

Stratton 1986). Pyrethroids are being considered as replacements for organochlorine, organophosphorus and methylcarbamate insecticides, which are more toxic to higher animals and/or are more persistent in the environment (NRCC 1986). With the rise in pyrethroid usage, entrance into aquatic systems is likely and estimation of potential impacts are needed (Coats and Bradbury 1989, Eidt et al. 1989).

Modern pyrethroid insecticides are synthesized molecules based on a class of chemicals (pyrethrins) found in the flowers of *Chrysanthemum cinerariaefolium*. Use of natural pyrethrins (pyrethrum extract) dated to the mid-nineteenth century, and remain in use as domestic insecticides today (Davies 1985). Artificial pyrethroids are more toxic to insects, more stable and more species-specific than natural pyrethrins (Mauck et al. 1976). The first pyrethroid synthesized was allethrin, in 1945 (Davies 1985). Early pyrethroids were similar to natural pyrethrins in chemical structure and biological activity.

Permethrin was among the first light-stabilized pyrethroids, and was more than twice as toxic as earlier pyrethroids (Davies 1985). Pyrethrins degraded in sunlight too rapidly for commercial use, so light-stabilization was crucial. Other photo-stable pyrethroids discovered in the time-period 1968-74 included fenvalerate, cypermethrin and

deltamethrin (Davies 1985). These compounds were more resistant to chemical and biological degradation (Coats et al. 1989).

Cyfluthrin is a "new-generation" pyrethroid insecticide introduced by Bayer Corporation as a cotton insecticide in 1981. BAYTHROID^{R} is the emulsified concentrate of cyfluthrin, with a granular pellet formulation also on the market. Cyfluthrin is structurally identical to cypermethrin, with the addition of a fluorine atom to one of the benzene rings (Figure 1), giving cyfluthrin a spectrum of activity similar to cypermethrin (Davies 1985).

Pyrethroids are currently applied to crops by ground or aerial application, used in greenhouses and in livestock buildings, and used in ear tags for livestock. Forest application has been considered by the Canadian government for spruce budworm control (NRCC 1986, Eidt et al. 1989). Use of pyrethroids for larval mosquito/midge control has also been suggested over the past two decades (Mulla et al. 1975, Ali et al. 1978, Ali and Mulla 1980, Helson and Surgeoner 1986), which would

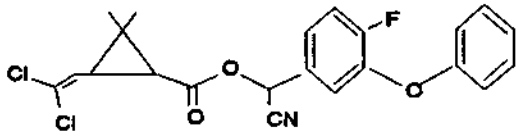

Figure 1. Molecular structure of cyfluthrin.

introduce high concentrations into aquatic habitats. Permethrin is used in Great Britain at concentrations of 10 to 20 μg/L for removal of aquatic invertebrates in drinking water mains (Fielding and Haley 1989).

Pyrethroid Mode of Action

Symptoms of pyrethroid poisoning in invertebrates and vertebrates are similar. Initial symptoms are incoordination and locomotor instability (knockdown) followed by tremors, convulsions and death (Wouters and van den Bercken 1978).

Because of the differential toxicity of enantiomers, molecular shape is probably the most important characteristic of pyrethroid activity (Wouters and van den Bercken 1978). Pyrethroids generally show a negative relationship between temperature and toxicity, as does DDT (Wouters and van den Bercken 1978). This phenomenon is uncommon among most pesticides.

The pyrethroids are thought to act at or near the sodium channel of the nerve, with calcium channels possibly playing a role (Clark and Brooks 1989, Soderlund and Bloomquist 1989). Nerve function is ultimately disrupted via membrane depolarization (Clark and Brooks 1989).

Cyfluthrin is a Type II pyrethroid, as are most "new generation" formulations. This class of pyrethroid appears

to possess a nerve-blocking mode of action, in contrast to Type I pyrethroids that induce repetitive nerve discharge (Matsumura 1985). Repetitive discharge is not observed in Type II pyrethroids. Type II pyrethroids affect sodium channels by impairing closure of the channel at the appropriate time. In the most potent Type II pyrethroids, the open channel state is extended indefinitely (Clark and Brooks 1989). The primary site of action of pyrethroids appears to be the central nervous system (Matsumura 1985, Clark and Brooks 1989).

Cyfluthrin is a highly lipophilic pesticide with an octonol/water partition coefficient (K_{ow}) of 420,000 (Wauchope et al. 1992). Compound liposolubility enhances transport across the insect cuticle, with rapid initial pickup of the toxicant but slower infusion rates from the cuticle to the body (Matsumura 1985). Solvents (i.e., emulsifiable formulations) may enhance toxicity by facilitating transport across membranes (Matusmura 1985).

Pyrethroid Toxicity

Pyrethroids are relatively nontoxic to both mammals and birds. These animals rapidly metabolize pyrethroids, primarily through ester cleavage (Litchfield 1985, Leahey 1985, WHO 1989). Birds are even less sensitive than mammals (Hill 1985). In general, pyrethroids show almost no

mutagenic, carcinogenic or teratogenic effects in mammalian or submammalian assays (Litchfield 1985). Some pyrethroids may cause immunosuppression in mammals (WHO 1989).

Humans exposed dermally to high pyrethroid concentrations may experience a rash or burning sensation (Litchfield 1985). Human effects are thought to be negligible, even for agricultural workers and pesticide applicators (Litchfield 1985, WHO 1989). Due to low application rates and rapid degradation, residues in food are generally low and do not exceed the "Allowable Daily Intake" when "Good Agricultural Practices" are used (WHO 1989).

Fish are more sensitive than mammals, by one to three orders of magnitude (Bradbury and Coats 1989a). This greater toxicity reflects different metabolic pathways in fish, with oxidative degradation predominating and ester hydrolysis playing a minor role (Bradbury and Coats 1989a). Fish apparently lack the enzyme system for hydrolysis (Haya 1989). While biotransformation rates are slower in fish, biotransformation alone cannot explain this differential toxicity to fish (Haya 1989). Toxicity of the major metabolites of cypermethrin are approximately three orders of magnitude lower than the parent pyrethroid (Haya 1989).

Laboratory studies indicate that some important fish food organisms are more susceptible to pyrethroids than even

the most sensitive fish species tested. This may result in secondary effects on fish, particularly larval fish that are more prone to starvation and have a restricted diet (NRCC 1986).

Rainbow trout are approximately three times more susceptible to cypermethrin than common carp (Shires 1983). Sheepshead minnows are > 100 times more tolerant of pyrethroids than mysid shrimp (Hansen et al. 1983). Newly hatched larval fish or small juveniles are most sensitive to pyrethroids (Haya 1989).

Pyrethroids may affect survival, swimming ability and cause deformities in fish larvae (Spehar et al. 1983). Age and food availability modify toxicity of permethrin in larval and juvenile fish (Holdway and Dixon 1988). Morphological changes in gill tissue have been noted at sublethal concentrations of permethrin (Kumaraguru et al. 1982, Drenner et al. In Review). Fish may acclimate to physiological stress at sublethal concentrations, but reduced energy input and utilization can decrease production (Kumaraguru and Beamish 1986).

Standard laboratory bioassays may overestimate impacts on fish since adsorption of pyrethroids to suspended particulate matter can lower aqueous concentrations (Day and Kaushik 1987c), ameliorating toxic effects (Crossland 1982, Coats et al. 1989). Pyrethroids generally are more toxic at

low temperatures (Coats et al. 1989), and show greater toxicity at high salinity and high water hardness (Dyer et al. 1989).

Fish do not usually exhibit acute mortality during field trials when pyrethroids are applied at field rates (Crossland et al. 1982, Shires and Bennett 1985, Muir et al. 1985, Hill, et al. 1988).

Pyrethroid impacts on algae, zooplankton, and macroinvertebrates will be discussed within these separate chapters.

Study Objectives

This research will address several factors for which data are either nonexistent, or that exist in proprietary reports not published in the open literature.
These include:

1. Investigation of the role of physical scale in the assessment of pesticide impacts. Large scale (0.1 acre pond ecosystem) mesocosms are commonly used in pesticide registration studies. These experiments are expensive and logistically difficult. This microcosm study was conducted concurrently with a mesocosm registration experiment, which allowed evaluation of using microcosms as surrogates for required mesocosm tests.

In addition to the applied aspects of this study, the question of physical scale in ecological testing has received considerable attention (Stephenson et al. 1984, Kaushik et al. 1986, Allen and Hoekstra 1987, Frost et al. 1987, Resh and Rosenberg 1989, Ward 1989, Solomon et al. 1989, Menge and Olson 1990). Some have suggested that whole-lake testing was the only valid approach (Carpenter and Kitchell 1988), however lack of replication has hampered the inferential capability of whole-ecosystem manipulations (La Point and Perry 1989). More testing at a range of physical sizes ranging from beaker scale to lakes will help address questions of scale (Stein et al. 1987, La Point and Perry 1989).

2. Evaluation of the impacts of BAYTHROID^{R} (the EC formulation of cyfluthrin) on aquatic systems. Knowledge of cyfluthrin impacts on aquatic biota are limited, with proprietary lab toxicity data (Mobay Corporation) being the main source of data. A cursory investigation of BAYTHROID^{R} was conducted in unreplicated microcosms located in Germany (Heimbach et al. 1992), but an in-depth study was lacking.

3. Field studies of impacts of pyrethroids on benthic invertebrates have received less attention than impacts on zooplankton (Kaushik et al. 1985, Helson and Surgeoner 1986, Day et al. 1987, Day and Kaushik 1987a and 1987b, Yasuno et al. 1988). While several pyrethroid registration studies

have been conducted, much of these data were unpublished. Only recently, with studies of esfenvalerate (Fairchild et al. 1992, Lozano et al. 1992, Webber et al. 1992) and experiments with PP321 in North Carolina (Hill et al. 1988), have replicated experiments documented pyrethroid impacts on macroinvertebrates. These studies were often general in defining impacts to macroinvertebrates, with few details. Some early work involved unreplicated applications of cypermethrin to ponds and ditches (Crossland et al. 1982, Shires and Bennett 1985).

4. Pyrethroid sediment toxicity is a major concern due to their low water solubility and high partition coefficients. In complex ecological systems it is often hard to partition ecological interactions from toxicological effects. To address these questions, *Chironomus tentans* and *Hyalella azteca* bioassays were conducted using sediments taken from microcosms. Larvae of *C. tentans* and *Daphnia magna* neonates were also exposed to treated microcosm water. I will compare bioassay results with impacts on microcosm invertebrate communities. Combining bioassay results with field measurements should provide a more complete picture than either method used in isolation.

5. Finally, the longevity of pesticide impacts on aquatic communities has been little studied. Mesocosm studies to date have only lasted one year, with ponds drained for fish

harvest in November or December. During the microcosm fish harvest (November 1989) a portion of the water was transfered to a holding tank, all fish were removed, and the water was pumped back to the original microcosm. Disruption to the hydrosoil was minimal. Sampling the microcosms in the early Summer of 1990 was conducted to determine any residual effects on sensitive taxa, and to evaluate recolonization of populations impacted by fish and BAYTHROID^{R}.

CHAPTER 2

METHODS AND MATERIALS

Site description and test initiation

Fourteen earthen ponds (mesocosms) were constructed in the cotton growing region of north Texas for use in the cyfluthrin pesticide re-registration study. The bottom of each pond was lined with clay and covered by approximately 15 cm of topsoil. Mesocosm dimensions were 30 m x 16 m at the surface (0.12 acre), maximum depth of 2 m with a 2:1 slope at each end. Cylindrical concrete microcosms (1.5 m x 1.3 m) were used as the smaller test systems. A layer of topsoil (ca. 10 cm) obtained from the same source as mesocosm soil was added to each tank. Water and sediments were screened for pesticide residues prior to use. System metrics and scaling relationships among the two systems are summarized in Table I.

Mesocosms were filled with water from an on-site maintenance pond, and microcosms were filled from an adjacent mesocosm. Filling with natural pond water inoculated these systems with representative zooplankton and phytoplankton populations. Additional biological "inocula" (macroinvertebrates) were collected locally, ensuring the establishment of relatively diverse invertebrate

communities. Natural colonization by insects occurred throughout the study.

Microcosms and mesocosms utilized separate water circulation systems that served to mix water among all tanks or ponds during an equilibration period (Johnson et al., In Press). Mixing was stopped, and microcosms and mesocosms were isolated at test initiation. Evaporative losses for both systems were replaced with ground water from a deep water well.

Mesocosms were each stocked with eighteen male and eighteen female sexually-mature bluegill sunfish, *Lepomis macrochirus* Rafinesque. Adult fish (mean length of 13.2 cm ± 1.58 cm at study initiation) reproduced during the experimental period. A average of 12,961 (± 3,872) juvenile fish, with a modal size class of 2.0-2.9 cm, were harvested

Table I. Physical dimensions of experimental systems and scaling relationships between microcoms and mesocosms.

	Microcosms	Mesocosms	Scaling*
Volume (m^3)	1.95	635	325
Surface Area (m^2)	1.77	480	271
Wall+Bottom Area (m^2)	6.99	516	74
Surface/Volume Ratio	3.58	0.81	0.226

* Scaling = Mesocosm/Microcosm

from mesocosms at study termination. Microcosms were each stocked with eight sexually-immature bluegill sunfish, (mean length of 6.87 cm ± 0.88 cm at study termination; Morris 1991, Morris et al. In Press). Fish loadings are given in Table II. Although the carrying-capacity of the microcosms was unknown, outdoor model ecosystems of moderately large size (> 1000 L) are generally capable of supporting fish (Giddings 1980).

Due to concerns regarding untested fish stocking levels, a single artificial refugium was placed in each microcosm. Refugia were cylinders of 0.25 inch mesh plastic netting (high density polyethylene), and were filled approximately halfway with plastic cylinders (5 cm OD x 5 cm

Table II. Initial bluegill sunfish loadings, and final fish biomass and densities in micocosms and mesocosms. Values are scaled per cubic meter of water.

	Microcosms		Mesocosms	
	Initial	Final	Initial	Final
Fish Density (#/m^3)	4.10	4.07	0.057	21.1
Fish Biomass (g/m^3)	10.73	17.91	2.360	6.08

high) manufactured as surface area enhancers for sewage treatment plants (Actifill '50' units; Norton Chemical Process Products, MA). Each refugium contained approximately 100 Actifill units. Refugia occupied approximately 4.2% of the microcosm surface area and 3.8% of the microcosm total volume. Refugia prevented invertebrates from being eliminated by fish predation. Smith (1985) found that refugia allowed zooplankton populations to coexist with silver carp and channel catfish in 1000 L tanks.

Table III. Nominal cyfluthrin (AI) loadings in microcosms and mesocosms. Spray drift was applied once a week for ten weeks. Runoff applications occured once every two weeks, for a total of five applications.

	Spray Drift		Runoff	
	Percent Drift	Target (µg/L)	Percent Runoff	Target (µg/L)
Dose 0	0.0	0.0000	0.0	0.0000
Dose 1	1.0	0.0356	0.3	0.2143
Dose 2	2.5	0.0911	0.3	0.2143
Dose 3	5.0	0.1780	0.3	0.2143
Dose 4	5.0	0.1780	1.5	1.0714

Pesticide Application

BAYTHROID^{R}, the emulsified concentrate formulation of cyfluthrin, was used in this study. BAYTHROID^{R} is 26.2% cyfluthrin, the rest of the formulation consisting of solvents and/or emulsifiers to enhance dispersal and solubility in water. The pesticide application schedule in this study was determined from maximum loadings allowable under label directions. Ten spray drift (SD) applications were conducted, each a week apart. Simulated pesticide runoff (RO) was modeled using five biweekly stormwater runoff events (Appendix Tables 1 and 2).

Treatments consisted of controls (D0; Dose 0) and four concentrations of cyfluthrin (D1 to D4). Replication in both mesocosms and microcosms was as follows: D0 = 3, D1 = 2, D2 to D4 = 3. One microcosm at D2 was handled differently, resulting in abnormally high turbidities. This tank was subsequently dropped from the design. Microcosm and mesocosm pesticide applications were performed concurrently and concentrations in microcosms were computed to match mesocosm concentrations. Pesticide loadings were scaled down by volume, as opposed to surface area. Pesticide loadings in microcosms would have been slightly higher if concentrations were scaled by surface area (Table I).

Treatments were characterized by differing SD and RO loadings, resulting in four different application levels (Table III). Spray drift D1 through D3 increased in concentration, while D4 spray drift was identical with D3. Treatments D1 through D3 held runoff constant, while D4 had a higher (5x) runoff value (Table III). This allowed evaluation of the relative importance of spray drift (free pesticide) versus runoff (sediment bound) input. Pesticide loadings corresponded to 1.0, 2.5 and 5.0 % drift in the spray drift applications (percentage of field application rate), while runoff simulations were conducted at 0.3 and 1.5 % runoff.

Microcosm drift applications were obtained by preparing a concentrated stock solution in water. Aliquots were pipetted into glass beakers and added to microcosms by pouring evenly across the water's surface. Mesocosm spray drift applications were more complex, utilizing a modified GAMACO^{R} bridge spanner (Johnson et al., In Press). Application stock solutions were added to five gallon stainless steel canisters that were pressurized using CO_2. Pressurized liquid was delivered to the ponds through thirteen TK-552.5 flood jet R spray nozzles attached to the spanner, selected in order to minimize small droplet emission. The pesticide was introduced into mesocosms by

driving the spanner at approximately 0.4 meters per second while spraying 10 to 15 cm above the pond surface.

Runoff application soil slurries for mesocosms were prepared in cement mixers, where they were mixed for one hour prior to application. Microcosm slurries were prepared in glass beakers and allowed to stand (covered with aluminum foil) for one hour, simulating the mesocosm mixing period. Slurries were added to mesocosms using the bridge spanner, modified by the addition of Herd 1-92 K broadcast spreaders. The spanner was driven the length of the pond while broadcasting the soil/water mixture at approximately 38 to 40 cm above the surface. Microcosms were treated by pouring slurries evenly across the tank surface.

Sample Collection and Analysis

Sampling schedules for all biological and residue parameters are given in Appendix Table 1 for microcosms and Appendix Table 2 for mesocosms.

Pyrethroid water-column residue samples were collected in teflon bottles, transferred to 1 L volumetric flasks and extracted with hexane. Water-column samples were collected one hour after spray drift and runoff pesticide application. Water-column half-life residue samples were collected at 1, 8, 24 and 48 hours after spray drift application.

Sediment samples were collected using a coring device that held a sleeve of butyrate tubing. Overlying water was drained from the cores in the field. Sediment cores were frozen and the top 2 cm were sectioned with a saw. Cyfluthrin was extracted from sediment samples with acetone. Acetonitrile was added to remove water from the acetone fraction, and this extract was evaporated to dryness by rotary evaporation. Hexane was added and samples were then sonicated.

Cyfluthrin residues were analyzed using an HP-5890A gas chromatograph with an electron capture detector, using cool on-column injections techniques.

Macroinvertebrate artificial substrate (MAS) samplers were used to estimate epibenthic macroinvertebrate population density. Colonization substrates were constructed using the Actifill surface enhancers described above. Actifill units (14 in mesocosm samplers, 8 in microcosm samplers) were bound together using plastic cable-ties and were weighted with pebble-filled 125 mL Nalgene bottles. Mosquito netting on the bottom of the samplers helped retain macroinvertebrates during retrieval. Mesocosms contained six littoral zone MAS samplers per pond, with a combined surface area of 1.615 m^2. Microcosms had two smaller MAS samplers per tank with a combined surface area of 0.308 m^2. Samplers were introduced into microcosms

and mesocosms for one month, allowing colonization. The MAS samplers were then removed and scrubbed gently to dislodge organisms, which were retained on a 180 μm mesh sieve, and preserved in Kahle's solution (Borror et al. 1989). Samplers were replaced to allow further colonization.

Benthic macroinvertebrates were collected from mesocosms only using an Ekman grab sampler. Samples were preserved in Kahle's solution (Borror et al. 1989) and stained with Rose Bengal.

Insect emergence from mesocosms was measured using pyramid shaped floating emergence traps, similar to LeSage and Harrison (1979). Emergence from microcosms was quantified using two measures: adult emergence and exuviae (cast exoskeleton) production. Emerging adult insects were collected with semi-submerged funnel traps (after Davies 1984). Exuviae were either skimmed from the water's surface using dip nets or collected above the waterline using forceps. Both exuviae and adult insects were preserved in Kahle's solution.

Zooplankton were collected with an integrated water column tube sampler (schedule R-4000 PVC tubing, 2" diameter) in both systems. Five liters per microcosm/mesocosm were concentrated using a 30 μm mesh net and were preserved with Lugol's solution.

Phytoplankton were collected using the tube sampler described above, and a grab sample (250 mL) was preserved in Lugol's. Periphyton were allowed to colonize glass microscope slides for two weeks in floating periphytometers. Periphyton and planktonic chlorophyll analyses involved acetone extraction followed by spectrophotometric measurement (APHA 1985). Algal biomass was determined as ash-free dry weight by scraping slides into crucibles, obtaining dry weights, ignition in a muffle furnace and reweighing (APHA 1985).

Water chemistry parameters such as total suspended solids, total phosphorus, nitrates, nitrites, ammonia, TOC, DOC, POC and turbidity were analyzed using standard methods (APHA 1985). Dissolved oxygen, water temperature (surface and bottom) and surface pH were measured weekly using calibrated meters. Gross photosynthesis and community respiration were determined using the three point diel oxygen pulse method (Lind 1979).

All weeks were plotted when outlining effects in microcosms. Four critical time periods were selected for graphical representation of biological effects when comparing microcosms and mesocosms. These were; 1) prior to pesticide application, weeks -1 or -1a; 2) during the middle of the treatment period, weeks 5 or 6; 3) preceding the last pesticide application period, weeks 9 or 10; and 4) end of

experiment, post application, weeks 18 or 19. Although data were collected more often (Appendix Tables 1 and 2), it was felt that visual trends would be obscured by presenting all weeks, for all taxa, in both mesocosms and microcosms. These same dates were selected for mounting of larval chironomids and MAS richness calculations.

Fish Removal and Recovery Sampling

In November of 1989, water from each microcosm was pumped to a holding tank, fish were harvested, and the same water was then returned to the original microcosm. Care was taken so that surficial sediments were relatively undisturbed. These tanks were not sampled during the winter of 1989-90. Microcosms were sampled for sediment residues, MAS colonization and exuviae during the weeks of 29 May and 12 June, 1990.

Single-Species Bioassays

Daphnia magna Straus bioassays were conducted during the last week of August 1989, using <24 h old neonates from laboratory cultures. Individuals were acclimated to control microcosm water overnight using a drip flow-through system. *D. magna* were therefore 24-48 h old at the time of testing. Individuals were introduced into 250 mL Pyrex beakers containing treated or control microcosm water. Water was

collected at the surface one hour post-application. Beakers were placed in an on-site, semi-covered, ambient temperature water bath. Two replicate beakers, each containing ten individuals, were used for every microcosm. All tests were single-pulse exposures conducted following spray-drift simulations. Survival was determined at 24, 48 and 72 hours.

Water exposure bioassays using laboratory cultured *Chironomus tentans* Fabricius were conducted the last week of pesticide application (week 10, September). Ten day old *C. tentans* larvae (2nd instar) were used in all *Chironomus* testing. These water-column exposures were identical with *D. magna* trials, with the exception that small amounts of paper toweling were provided as substrate to minimize stress.

Sediments for bioassays were collected from microcosms three times during the study; one day after the last pesticide application (week of 5-12 September 1989), at study termination (6-13 November 1989), and during the next summer (31 July - 07 August 1990). *C. tentans* larvae were the test organisms during the first two assays, and *Hyalella azteca* (Saussure) were used in the last test.

Six sediment cores were collected from each microcosm for the chironomid bioassays, two for residue determination and the remaining cores for toxicity testing. Intact cores,

with sediment and overlying water relatively undisturbed, were transported to the lab. Cores were allowed to settle for 2-3 hours and any large predators (such as odonates) that could interfere with the test were removed. Three *Chironomus tentans* larvae (10 day post-hatch, second instar) were introduced into each core and allowed to burrow. It was felt that testing within intact cores would allow a more realistic relationship between overlying water and surficial sediment layers. Water-sediment ratios were approximately 2:1.

Cores were covered with parafilm to retard evaporation and placed in a Precision Illuminated Incubator at 22 °C. Midge larvae were fed a suspension of Tetramin Conditioning Food once midway through the test. After seven days, core sediments were washed through a 180 μm mesh sieve, retaining the chironomids. Larvae were prodded with a probe to distinguish living chironomids. Endpoints for *C. tentans* were mortality (September and November assays), and average dry weight biomass (November test only). Biomass was determined by drying larvae at approximately 40 °C for 24 hours and weighing.

Hyalella azteca bioassays were conducted during the spring of 1990. Amphipods are among the most pyrethroid sensitive taxa (Anderson 1982), and it was felt that they would be a superior assay organism for determining any

residual toxicity. Preliminary tests during the weeks of 7-14 June 1990 were unsatisfactory, with control survival averaging 43%. Subsequent laboratory testing with microcosm control water suggested that high pH values (mean 9.6) were responsible for this phenomenon. Lab culture water was at a pH of 7.9. An attempt was made to acclimate *H. azteca* to higher pH by serial replacement of 20% water volume using microcosm control water, twice a day, with no success. When pH reached approximately 8.6, mortality occurred. It was decided to use microcosm sediments combined with overlying water from laboratory cultures.

Sediments for amphipod testing were collected from microcosms using an Ekman grab. Approximately 10 mL of sediment (obtained from the top 2 cm of sediment) was placed in 50 mL glass beakers, with ten replicate beakers per microcosm. Beakers were transported to the lab and 40 mL culture water was added to each beaker (4:1 water-sediment ratio). Sediments were allowed to settle for 24 hours and a single *H. azteca* was added to each beaker. Amphipods were fed Tetramin Conditioning Food suspension once midway through the test, and sediments were sieved after seven days. Individuals were prodded with a probe to verify survival.

Statistical Analyses

To assess treatment effects, one-way analysis of variance procedures were computed for each week, for all water chemistry parameters and for each taxon using SAS (SAS 1985) software. This was followed by Dunnett's multiple range tests of $\log_{10}$ (x+1) transformed data (α=0.05) to determine which treatments differed significantly from controls. Mesocosms and microcosms were evaluated separately.

Water-column bioassay data (*C. tentans*, *D. magna*) and *H.azteca* data were analyzed using Toxstat 3.0 (Gulley et al. 1989). Statistical tests for bioassays were selected using criteria from Weber, et al. (1989). Percent survival data (all tests except *Hyalella azteca* bioassay) were transformed using the arc sine of the square root transformation and were analyzed using the T-Test with Bonferroni adjustment.

Since *H. azteca* data were binary (0 or 1; live or dead), the Fisher exact test was conducted using SAS software (SAS 1985). Chironomid sediment core bioassay data could not be analyzed by Toxstat, therefore these data were assessed using Dunnett's MRT on ranked data using SAS.

Tabular comparison of pesticide impacts in mesocosms and microcosms were conducted when contrasting these two systems (Tables VI and XVII). Significant impacts, either increases or decreases in treated tanks or ponds relative to

controls, were tallied during the ten week application period (Dunnett's MRT on $\log_{10}$-transformed (x+1) data; α=0.05). For these tables, treatments were determined to differ from controls if Dunnett's values were significantly different for more than one week during the application period. One-time differences were judged to not reflect the general response pattern for the whole experiment.

The cumulative number of significant differences for various biota were determined via Dunnett's MRT (α=0.05), and numbers were tallied for the nineteen week application and post-application period (Figure 101). Unlike graphical representations for each taxon during the comparison chapter (Figures 88-100), all available data were used in these analyses (Tables VI and XVII, Figure 101).

Bray-Curtis cluster analyses were conducted using SIGTREE (Nemec 1991). The lowest taxonomic level was used for all cluster analyses. Bootstrap techniques were used to assess the statistical significance of the clusters, α=0.05, iterations=1000 (Nemec and Brinkhurst, 1988). Bray-Curtis cluster analysis was chosen since it is sensitive to changes in taxonomic composition (Pontasch et al. 1989), and bootstrapping methods were available to assess statistical differences.

Taxonomic Keys

The primary keys used for zooplankton and macroinvertebrate identifications are listed below. A list of microcosm biota is included in Appendix Table 9.

Protozoans were keyed to species using Lee et al. (1985) and Pennak (1989). Rotifers were keyed to species whenever possible, using Edmondson et al. (1959), Stemberger (1979) and Pennak (1989). Copepoda and Cladocera were identified to species (when possible) using Edmondson et al. (1959) and Pennak (1989).

Mayflies were keyed to genus using Edmunds (1984) and Edmunds et al. (1976). Callibaetis were keyed to species using Check (1982). Trichopterans were identified to genus using Wiggins (1984) and Wiggins (1977). Lepidoptera were keyed to genus using (Lange 1984). Odonates were keyed to genus or species (depending on availability of adult material) using Westfall (1984), Needham and Westfall (1955), and Johnson (1972). Naiads of one abundant, difficult Libellulid (*Dythemis fugax*) were reared to confirm identification. Confirmation via rearing was necessary for this species since it had previously been described as a stream dweller (Westfall 1984).

Polhemus (1984) was used for keying hemipterans to family and/or generic levels. Aquatic Coleoptera were identified to genus using White et al. (1984). Non-

chironomid Diptera were identified to genus using Teskey (1984), Merritt and Schlinger (1984), Newson (1984), Carpenter and LaCasse (1955), and Usinger (1956).

Larvae, pupae and adult chironomids were identified to genus (and commonly to subgenric species-groups) primarily using the Wiederholm series (Wiederholm 1983, 1986, 1989) and Oliver (1981). Adults (and some pupal exuviae) were keyed to species whenever possible. Keys used for specific identifications included Townes (1945), Reiss (1974), Grodhaus (1987), Curry (1958), Epler (1987), Epler (1988), Sublette (1964), Maschwitz (1976), Roback (1980), and Roback (1985). Species-level taxonomy is currently confused for some chironomid groups such as the species Tanytarsus. In these instances, sub-generic differences (i.e., pupal exuviae shagreen/setation patterns) were used in distinguishing different groups within a species.

CHAPTER 3

CHEMICAL FATE IN MICROCOSMS AND MESOCOSMS

Introduction

Most pyrethroids are lipophilic, halogenated and exhibit very low water solubilities (Coats and Bradbury 1989), with solubilities in the 1-10 μg/L range (Coats et al. 1989). Thus, they tend to adsorb to soils, sediments and plant cuticles (Rawn et al. 1982, Muir et al. 1985, Cotham and Bidleman 1989).

Pyrethroids show rapid but shallow penetration of sediments, with the majority of the pesticide within the upper 1-2 cm (Sharom and Solomon 1981, Solomon et al. 1985, Heinis and Knuth 1992). Contaminant partitioning is probably a function of sediment characteristics such as grain size and organic content. Uptake from sediments may be due to exposure to interstitial water or ingestion of contaminated sediment (Chapman 1986).

Chemical fate studies indicate that pyrethroids are not highly persistent in the environment, with a typical half-life in water measured in hours or days, while terrestrial soils range from one to four weeks (Smith and Stratton 1986). Residues are persistent longer in estuarine systems than in freshwater (Smith and Stratton 1986).

Volatilization is an important loss mechanism in photostable pyrethroids (Rawn et al. 1982, Maguire et al. 1989). Hydrolysis, which is pH dependant, is also significant (Chapman and Cole 1982). Biodegradation of fenvalerate by microbes may play an important role (Cotham and Bidleman 1989).

Pyrethroids are not expected to biomagnify through the food chain due to low persistence and high metabolism within most organisms (Spehar et al. 1983). Bioaccumulation of pyrethroids directly from water and sediments have been found for certain organisms such as snails, oysters, stoneflies, and fish (McLeese et al. 1980, Anderson 1982, Spehar et al. 1983, Schimmel et al. 1983).

Results

Water column residue samples were collected near the surface of the water and near the bottom, above the sediments, one hour after applying BAYTHROID^{R}. Pesticide residues found in the water column declined rapidly after application. Multiple applications with subsequent dissipation resulted in a "jagged" or "sawtooth" pattern in microcosms (Figure 2). Residue levels generally increased with treatment level (Figure 2). Dissipation of cyfluthrin residues from the water column differed among the two systems, with a half-life of approximately 22 h within

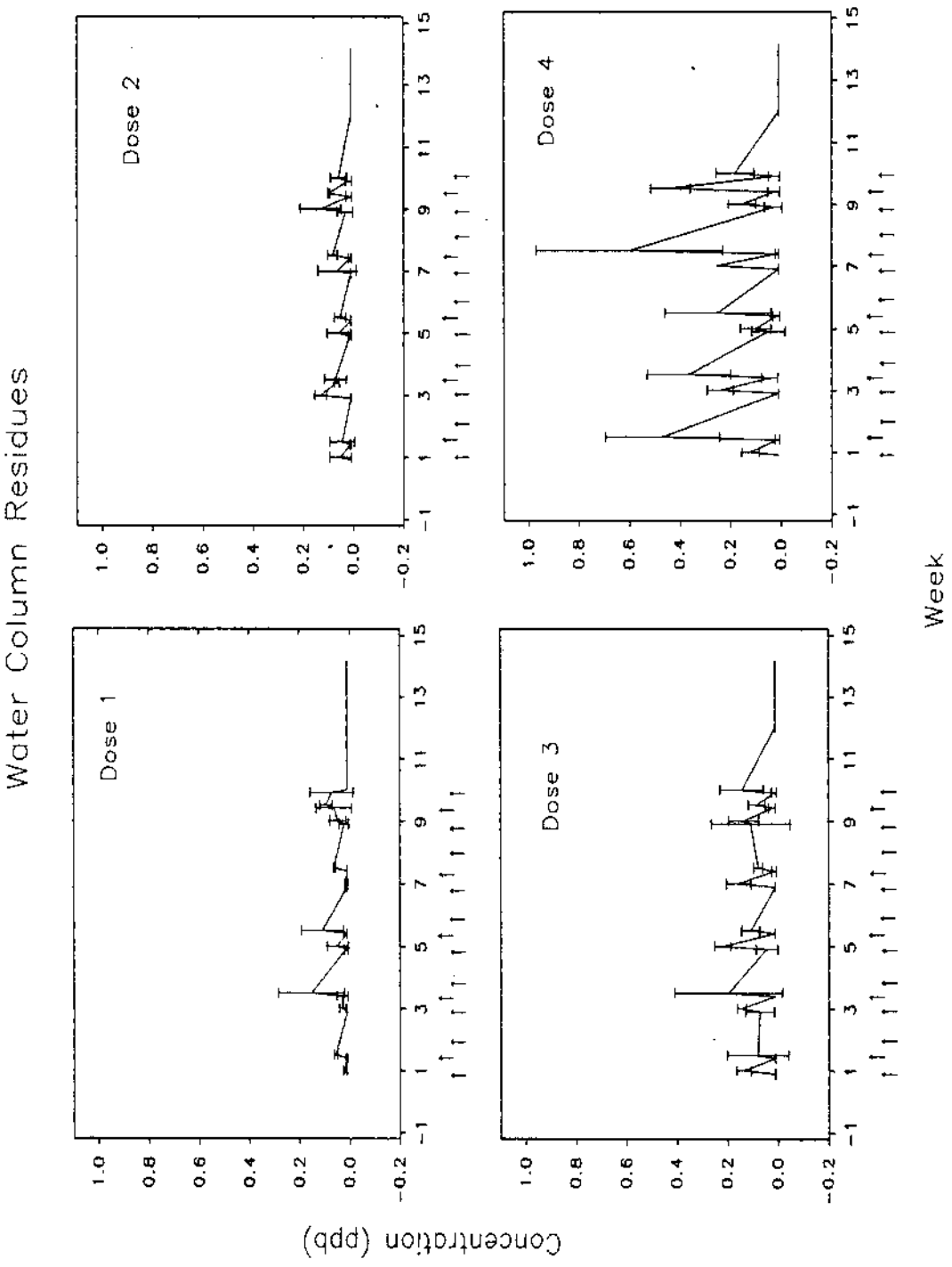

Figure 2. Cyfluthrin residues in treated microcosms through time (mean ± S.D.). Arrows indicate pesticide applications. Pyrethroid was added ten times as simulated drift and five times as a soil runoff simulation.

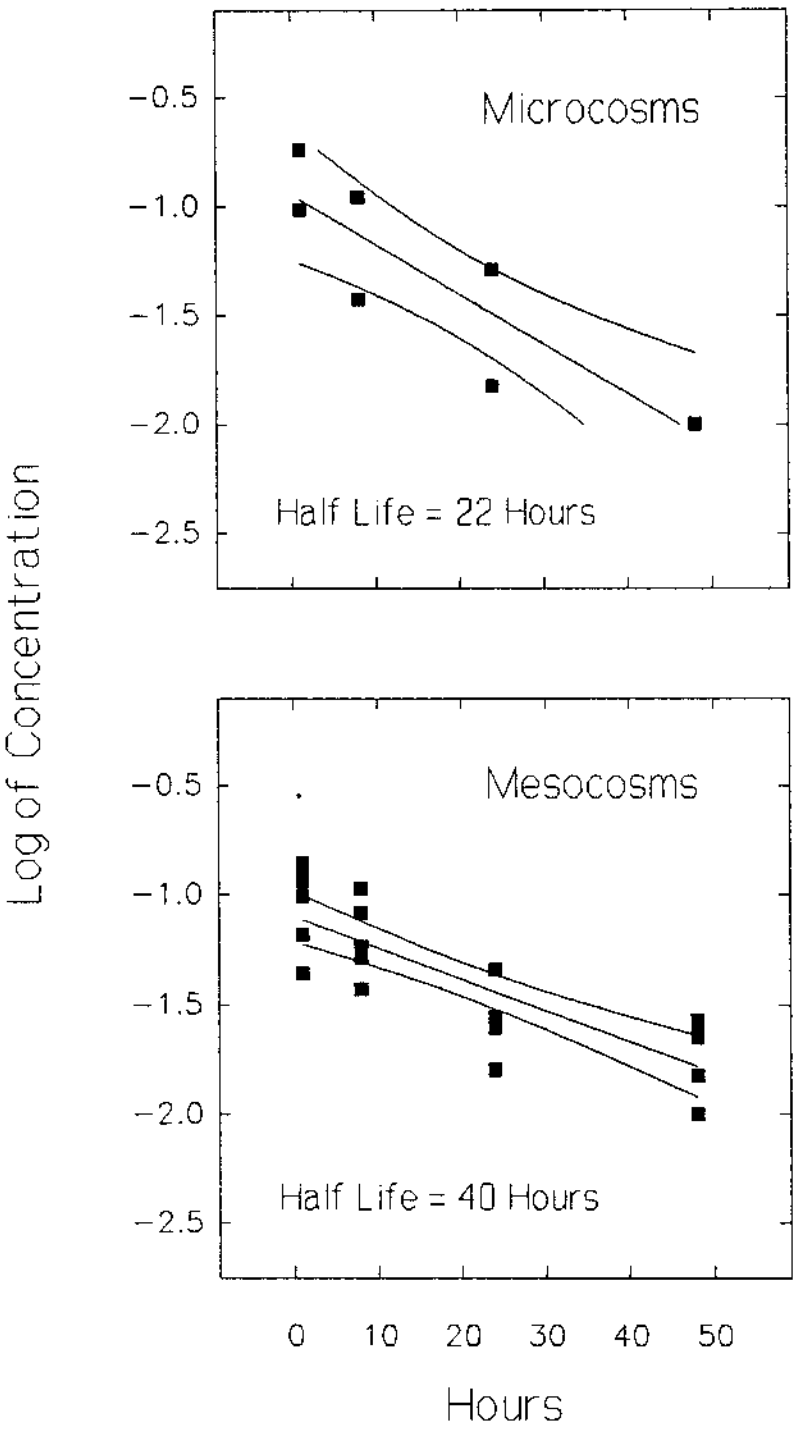

Figure 3. Dissipation of cyfluthrin from the water column in microcosms and mesocosms following spray-drift applications. Lines represent linear regression and 95% confidence intervals.

microcosms and 40 h within mesocosms (Figure 3).

Maximal pyrethroid values are generally obtained one hour after dosing (Heinis and Knuth 1992). Following spray drift treatments, surface values were generally higher than, or equal to, nominal values. Bottom values were generally lower than nominal concentrations. Mean concentrations were calculated across the experimental period, averaging surface and bottom concentrations. Microcosm spray drift values were consistently higher and closer to nominal targets than were mesocosm concentrations (Table IV).

Water column residues collected one hour after applying runoff treatments were similar in both mesocosms and microcosms (Table IV). Percent of nominal values following runoff treatments were consistently lower than after spray drift application.

Sediment residue levels were generally higher in microcosms, compared to mesocosms (Figure 4). Detectable parent compound was present in both systems at study termination (week 18).

Discussion

Chemical fate characteristics of BAYTHROID^{R} shared common patterns among the two systems but were apparently influenced by scaling relationships such as surface/volume ratios. High surface to volume ratios found in microcosms

Table IV. Cyfluthrin (AI) nominal loading rates (ppb) and percent of nominal detected via residue sampling (± S.D). Values are composited surface and bottom water collected one hour after application, averaged over the experiment.

Treatment Level and Parameters		Spray Drift		Run Off	
		Micro	Meso	Micro	Meso
Dose 0	Nominal Conc.	0.0000	0.0000	0.0000	0.0000
	Percent of Nominal	---	---	---	---
	Standard Deviation	---	---	---	---
Dose 1	Nominal Conc.	0.0356	0.0356	0.2143	0.2143
	Percent of Nominal	81.92	75.76	44.51	44.75
	Standard Deviation	65.24	36.05	30.42	16.12
Dose 2	Nominal Conc.	0.0911	0.0911	0.2143	0.2143
	Percent of Nominal	90.16	63.44	32.76	56.53
	Standard Deviation	59.74	30.49	14.73	34.69
Dose 3	Nominal Conc.	0.1780	0.1780	0.2143	0.2143
	Percent of Nominal	86.86	71.89	50.89	49.95
	Standard Deviation	29.24	38.49	49.33	30.14
Dose 4	Nominal Conc.	0.1780	0.1780	1.0714	1.0714
	Percent of Nominal	92.39	82.01	39.56	42.40
	Standard Deviation	39.95	30.68	21.40	21.22

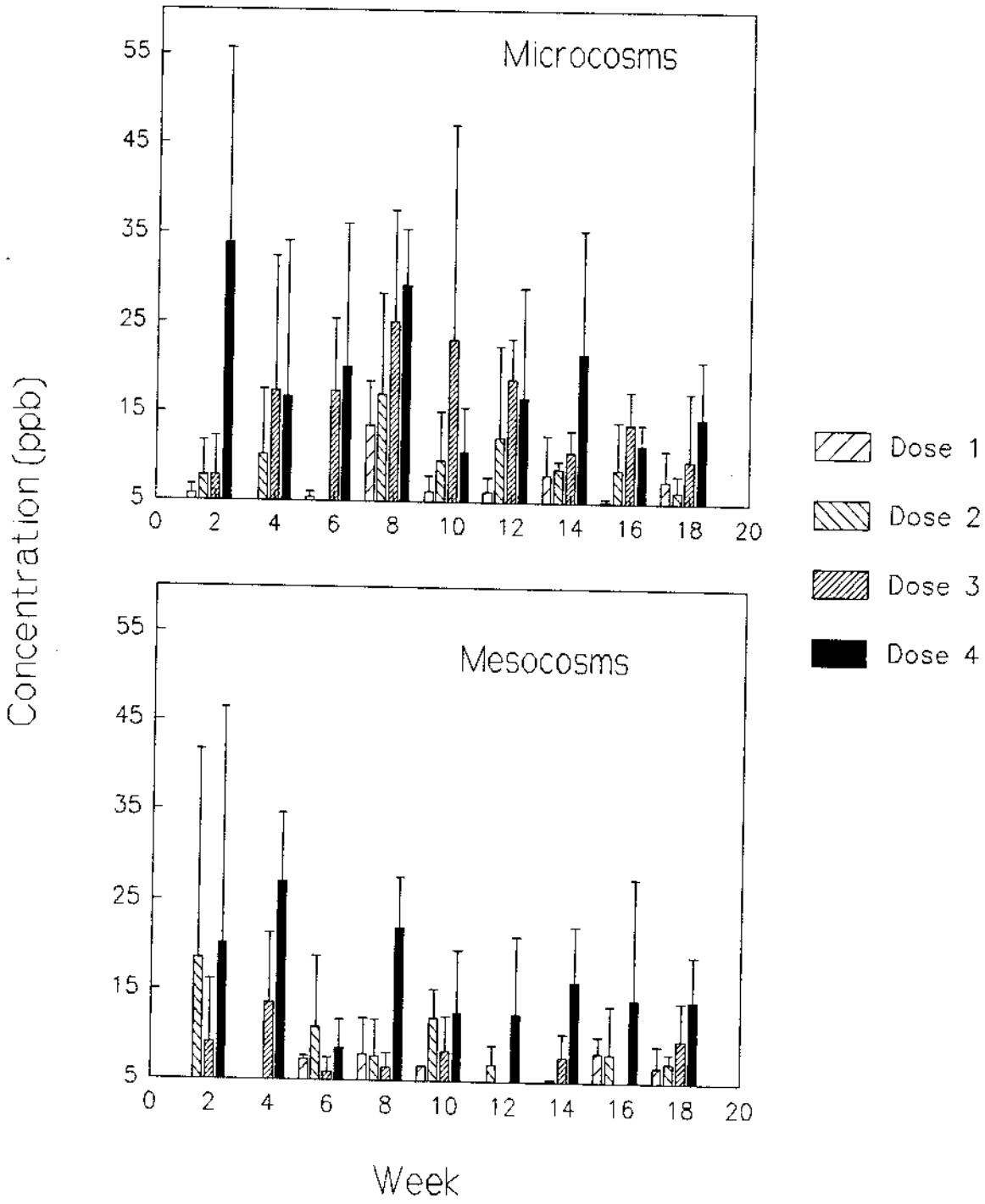

Figure 4. Sediment residues collected from microcosms and mesocosms via core sampling (mean ± S.D.). Samples were obtained from the top 2 cm of sediment.

influence chemical fate (Dudzik et al. 1979). Pyrethroids should sorb rapidly to the walls and bottom of microcosms, effectively reducing the amount of bioavailable pesticide relative to mesocosms. More macrophyte growth occurred in microcosms than in mesocosms, providing additional surface area for sorption. Pyrethroids undergo basic hydrolysis (Heinis and Knuth 1992), thus higher pH values in microcosms (discussed later) also may have contributed to breakdown of the parent compound. A combination of these factors were probably responsible for the shorter pesticide half-life in microcosms. The microcosm's half-life in this study may not be definitive, however, since fewer samples were collected compared to mesocosms, with resulting increases in 95% confidence intervals (Figure 3). It also should be noted that all microcosm values would fit within the observed range of mesocosm concentrations. Other studies suggest that pyrethroids show typical half-lives in water measured in hours or days (Smith and Stratton 1986, Fairchild et al. 1992, Heimbach et al. 1992, Heinis and Knuth 1992, Lozano et al. 1992).

The method of pesticide application may have partially offset these potential sinks, with mesocosms receiving a spray application and microcosms treated with a larger droplet size. Since volatilization of sprayed pesticide may be an important loss mechanism in photostable pyrethroids

(Rawn et al. 1982, Maguire et al. 1989), microcosms actually achieved drift concentrations closer to target values (Table IV). Also, pesticide was delivered to microcosms more rapidly since the application process was simpler. Finally, more complex application gear (bridge spanner with many feet of teflon tubing, spray nozzles and stainless steel spray tank) provided more surface area for pre-application sorption.

Water column residues collected an hour after runoff applications were very similar in both systems, and probably reflect sedimentation of bound pyrethroid during this interval (Table IV). Since soil for runoff applications was obtained from a common stock, similar patterns in both systems were anticipated.

Sediment residue values were somewhat higher in microcosms relative to mesocosms (Figure 4) and probably reflect scaling (surface area/volume) relations. Basin morphology and light penetration also may play a role in chemical partitioning behavior to the sediments. Given the smaller surface area (smaller fetch) of microcosms, one would expect less wind generated mixing. With a sloped bottom and the potential for traveling surface waves in mesocosms, it is likely that water-column mixing is greater in the higher energy environment of mesocosm littoral zones. Lower mixing rates might result in a pronounced surface

micro-layer, resulting in a worst-case scenario. Higher sediment residues found in microcosms may be a result of rapid sedimentation of particles within microcosms due to reduced mixing and little resuspension of bound pyrethroids. Also microcosms in this study had lower turbidity values compared to mesocosms (discussed later). Reduced water-column colloids in microcosms may have resulted in rapid partitioning to the sediments.

Organophosphate insecticide residues were higher in microcosms, compared to mesocosms levels, in a similar scaling study (Howick et al., In Press). This phenomenon should be investigated in other studies to determine if this relationship has wide applicability.

Exposure to sunlight also can influence the chemical fate of photo-labile compounds such as pyrethroids. Attenuation of light in aquatic systems is dependent upon absorption of light by colloidal particulate matter and dissolved organic compounds (e.g., humic acids). These compounds effectively decrease percent transmission and selectively shift absorption by increasing absorption of UV light, particularly in the top 1 m (Wetzel 1983). Increased primary production generally decreases light penetration through absorption and reflection of light energy. In littoral zones of mesocosms and throughout microcosms with extensive productivity, light attenuation in the top meter

of water column would be significant. Finally, biotic factors such as microbial activity will influence pesticide concentrations in sediments. The role of microbial degradation has rarely been investigated in microcosm and mesocosm studies.

Detectable residues were still present in microcosm and mesocosm sediments at the end of sampling in November, 1989 (Figure 4). Heimbach et al. (1992), in a microcosm study of cyfluthrin in Germany, noted that maximal sediment concentrations were measured 2 days after application and remained constant for about one month. Concentrations declined below their limits of detection (0.5-1 μg/kg) within two months after application to low dose tanks. Residues were present in high-rate tanks longer than two months after application. Esfenvalerate was still present in Minnesota limnocorral sediments up to 60 days after application (Lozano et al. 1992). Microcosm sediments were sampled for residues in June 1990 in order to determine long-term pyrethroid persistence. All values were below the 10 ppb limits of quantitation.

Summary

Pesticide residue behavior differed somewhat among the two systems. Initial (hour 1) levels were generally closer to target values in microcosms following drift applications,

but dissipation was apparently more rapid in the smaller systems.

Water-column residue levels following runoff applications were much lower than target levels at one hour after application. This was thought to reflect sinking of sediment-associated pyrethroids. Responses were similar in both mesocosms and microcosms.

Sediment residues were quite variable, but the general pattern was for enhanced levels in microcosms. Supportive results have been noted in a similar scaling study. Cyfluthrin was still present in sediments at fish-harvest in November 1989. Microcosm sediments were resampled in June 1990, with all residue levels below limits of detection at this time.

CHAPTER 4

PHYSICAL AND CHEMICAL PARAMETERS

Introduction

Water chemistry parameters are measured in virtually every field study or mesocosm experiment. Keeping with this tradition, a suite of chemical and physical endpoints were measured during this study. These could potentially serve two purposes; to measure pesticide impacts within microcosm, and secondly to use as comparative tools for contrasting microcosms vs. mesocosms.

Results

Microcosm Response

Turbidity values were both visually (Figure 5) and statistically (Table V) reduced in treated microcosms. This phenomenon was so striking, it was clearly noticeable by visual inspection of tanks during the later part of the experiment. High rate (D4) microcosms were most affected.

Other water chemistry parameters showed few statistically significant responses, and relating these differences to pesticide treatment was difficult (Table V). Most differences were restricted to pre-application periods (alkalinity, ammonia, nitrite, nitrate, and DOC) or

differences were single occurrences without an exposure-response relationship (TSS, TOC, POC).

Total suspended solids were generally higher in treated tanks throughout the application period (Figure 5), however these differences were never significant (Table V). After treatment ceased, controls were similar to treatments.

Alkalinity values were very dissimilar among treatments prior to dosing, but treatments converged by week 1 (Figure 6). Alkalinity rose substantially throughout the study but no treatment-related trends were apparent. Significant differences were limited to pre-application weeks (Table V).

Water hardness declined throughout the study (Figure 6). Values were very similar among treatments and were never statistically different (Table V).

Nitrite and nitrate values (Figure 7) did not exhibit exposure-response relationships. Significant differences were limited to pre-application weeks (Table V). Ammonia samples (Figure 8) did not show clear patterns and the only significant differences were prior to the first application (Table V). Phosphorous values were significantly enhanced twice during the study (Table V), but trends were non-existent (Figure 8).

Dissolved organic carbon values did not show consistent responses (Figure 9), with significance limited to pre-application samples (Table V). Particulate organic carbon

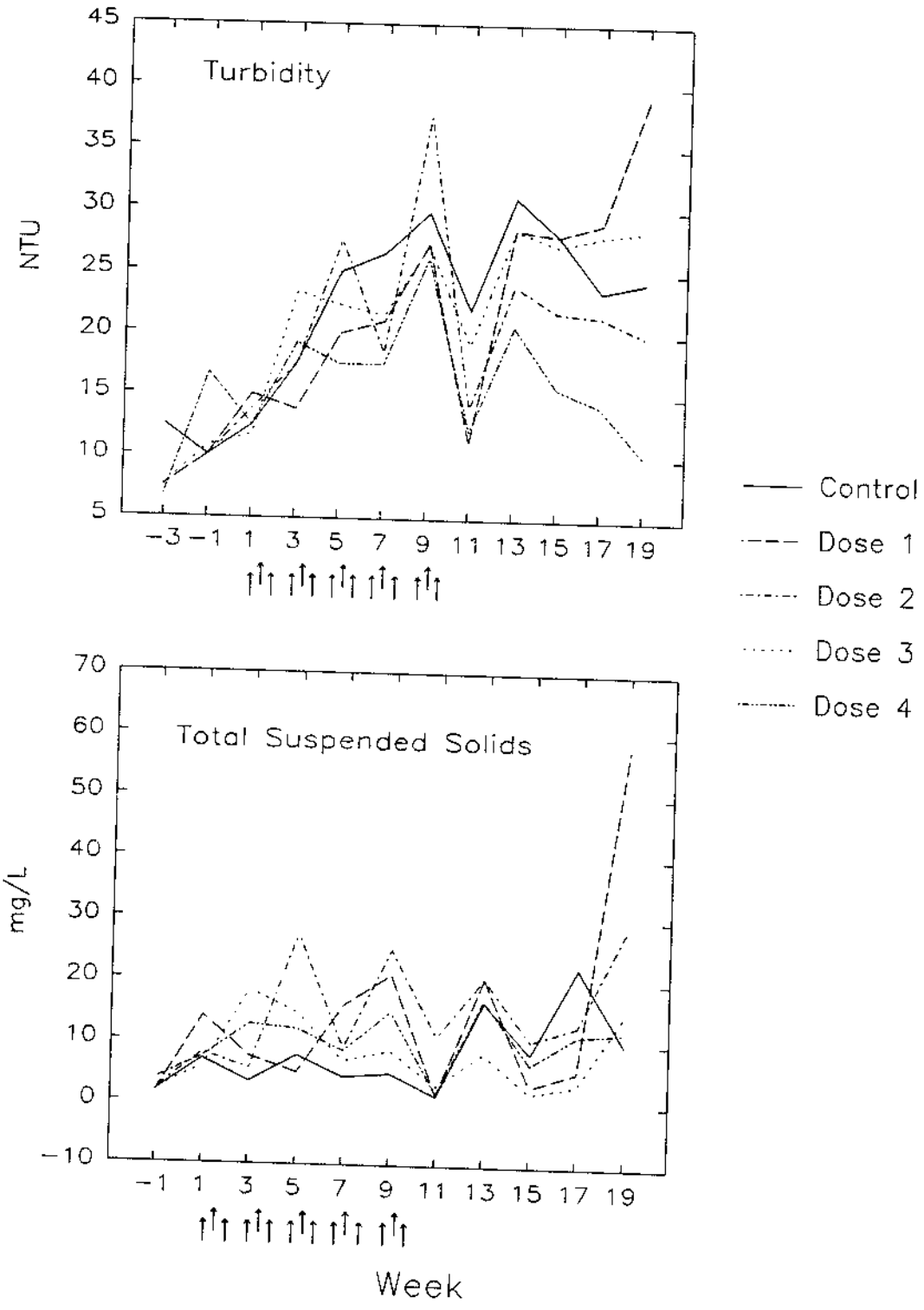

Figure 5. Mean turbidity (NTU) and total suspended solids (mg/L) values from microcosms. Arrows indicate pyrethroid applications.

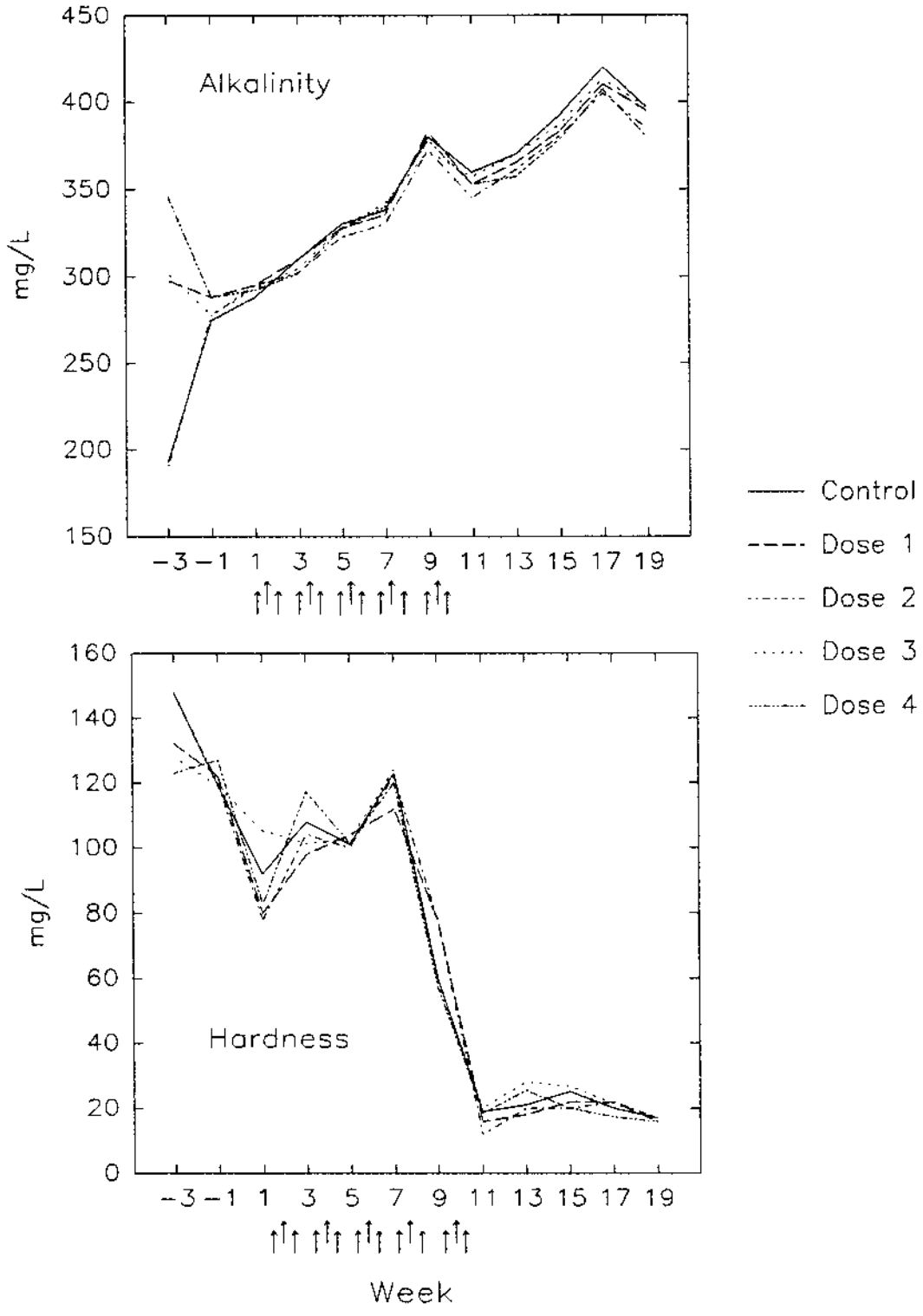

Figure 6. Mean alkalinity (mg/L) and water hardness (mg/L) in microcosms. Arrows indicate pyrethroid applications.

Table V. Statistically significant differences for water quality parameters in microcosms (Dunnett's MRT). D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

Parameter		Week Number											
		-3	-1	1	3	5	7	9	11	13	15	17	19
Alkalinity	D1	↑	↑										
	D2												
	D3	↑											
	D4	↑	↑										
Hardness	D1												
	D2												
	D3												
	D4												
pH	D1												
	D2												
	D3												
	D4												
Temperature	D1												
	D2												
	D3												
	D4												
Dissolved Oxygen	D1												
	D2												
	D3												
	D4												
Turbidity	D1	↓					↓						↑
	D2						↓						
	D3	↓					↓						
	D4	↓				↓	↓						↓
Total Suspended Solids	D1	ND											↓
	D2	ND											
	D3	ND											
	D4	ND											

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.
ND=Data missing (No Data), cannot perform MRT

Continuation of water quality parameter significant differences.

Parameter		Week Number											
		-3	-1	1	3	5	7	9	11	13	15	17	19
Ammonia	D1												
	D2												
	D3												
	D4	↑											
Nitrite	D1	↓	ND										
	D2		ND										
	D3	↓	ND										
	D4	↓	ND										
Nitrate	D1	ND											
	D2	ND											
	D3	ND											
	D4	ND	↓										
Total Phosphorus	D1												↑
	D2												
	D3						↑						
	D4												
Total Organic Carbon	D1												↑
	D2												
	D3												
	D4												
Particulate Organic Carbon	D1												↑
	D2												
	D3												
	D4												
Dissolved Organic Carbon	D1												
	D2												
	D3												
	D4												

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.
ND=Data missing (No Data), cannot perform MRT

values (Figure 9) were frequently higher in treated tanks relative to controls but differences were only significant once, during week 19 (Table V). Total organic carbon values were lower in controls compared to treatments (Figure 9) but variability apparently limited statistical significance to D1 during week 19 (Table V).

Microcosm/Mesocosm Comparison

Mesocosm parameters demonstrated some significant differences between controls and treated ponds, but exposure-response relationships within treated mesocosms were rare. Alkalinity increased in both systems while hardness decreased in both. Microcosm alkalinity values were consistently higher than mesocosm values (Table VI). Phosphate, nitrite and ammonia in treated mesocosms were lower than controls, whereas microcosms showed no patterns related to pesticide input and no patterns through time. Nitrates did not show treatment effects in either system. Total suspended solids showed few trends in mesocosms. Organic carbon values (total, dissolved and particulate fractions) did not show trends with pesticide concentration in mesocosms. Organic carbon values were somewhat higher in microcosms relative to mesocosms (Table VI).

Mean turbidity values were affected in mesocosms, with controls significantly higher (Dunnett's MRT, α=0.05) than

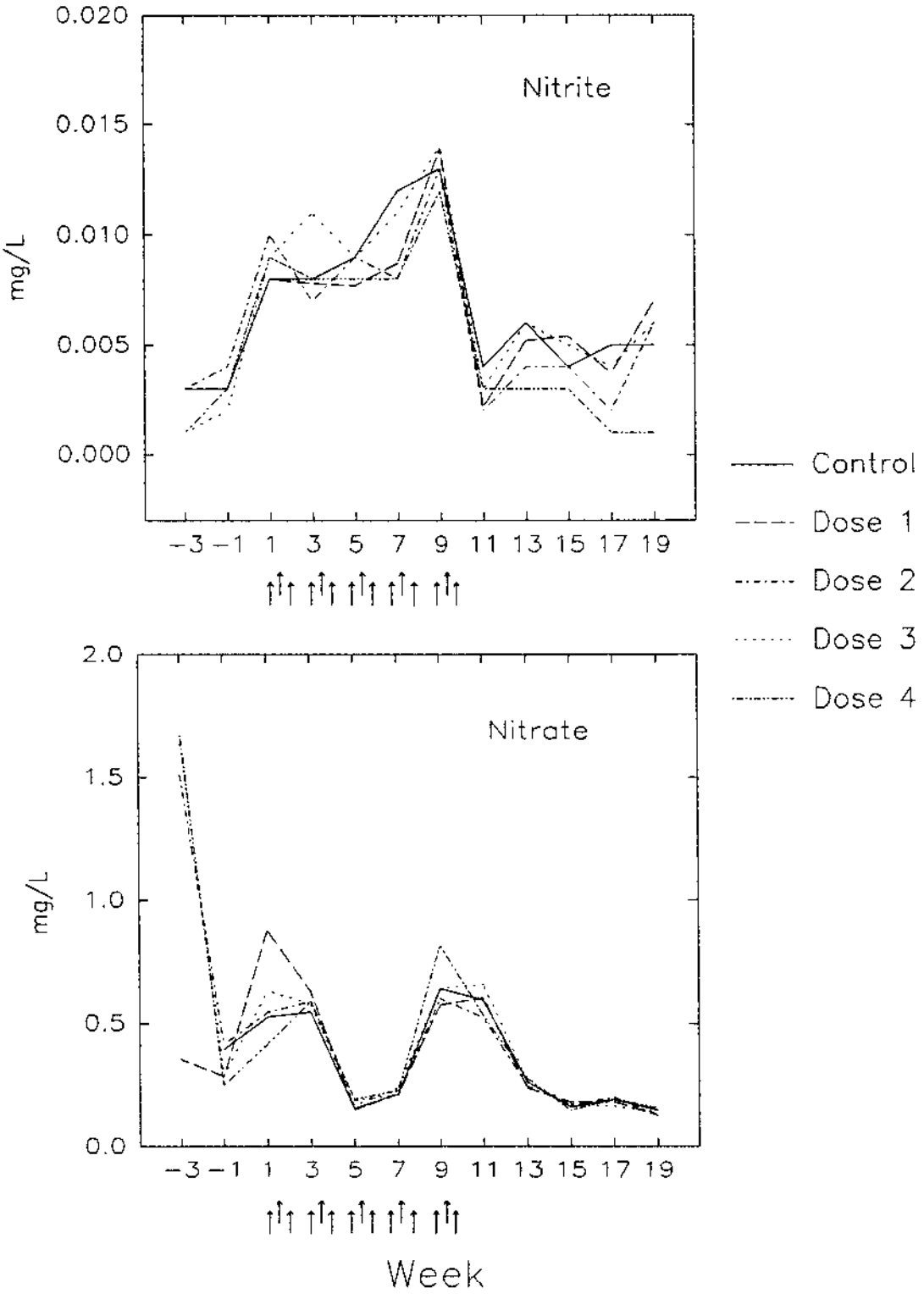

Figure 7. Mean nitrite (mg/L) and nitrate (mg/L) values from microcosms. Arrows indicate pyrethroid applications.

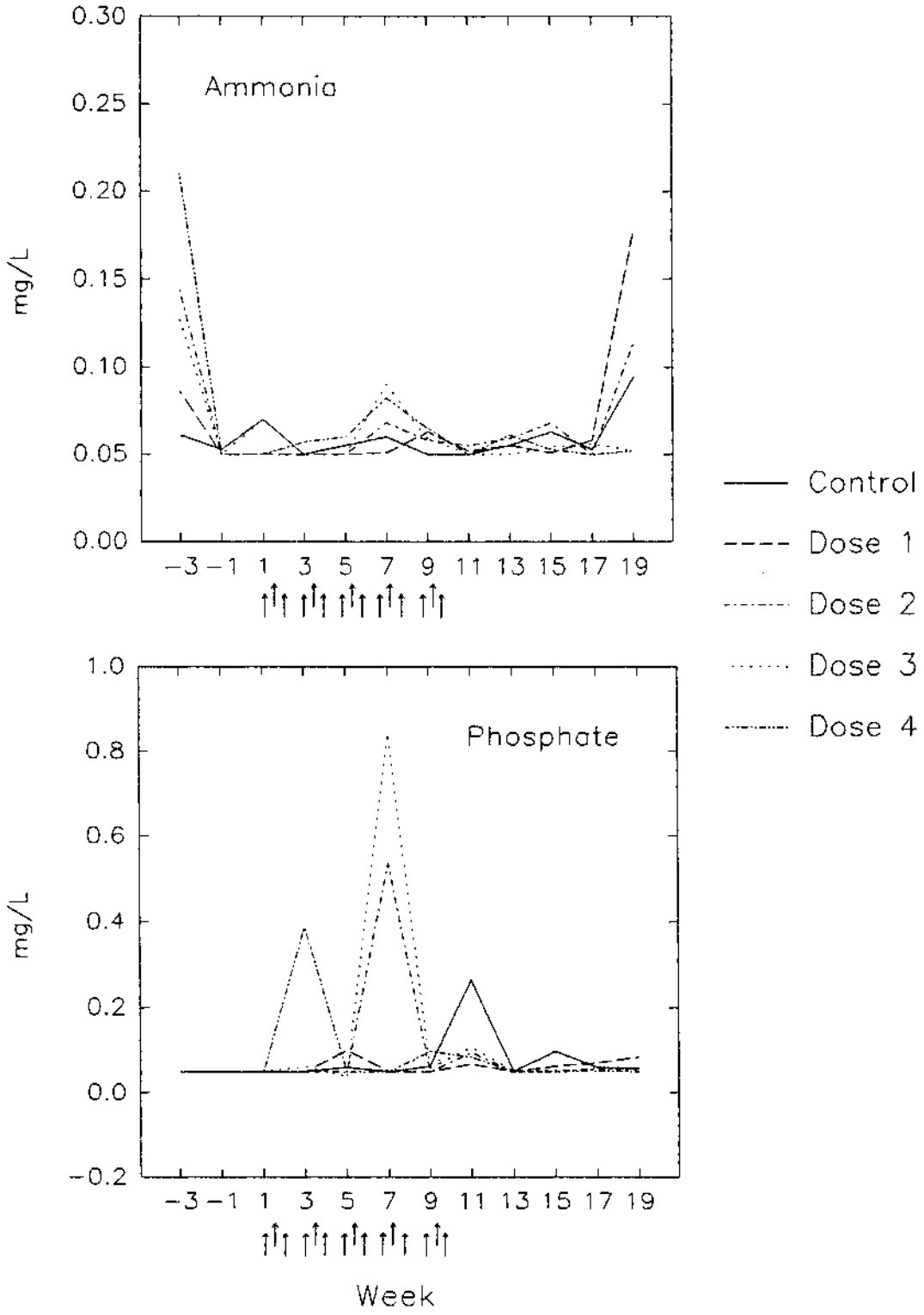

Figure 8. Mean ammonia (mg/L) and phosphorus (mg/L) values from microcosms. Arrows indicate pyrethroid applications.

all treatments from week 3 to study termination (Table VI). Microcosms showed a more complex pattern, with controls higher than dose 4 at weeks 5, 7 and 11 through 19 (Figure 5). Turbidities were consistently higher in mesocosms relative to microcosms (Table VI).

Mean dissolved oxygen, temperature and pH showed no pesticide effects (Table VI). Mean pH values were consistently higher in microcosms compared to mesocosms (Figure 10). Temperatures in control microcosms and mesocosms were generally similar through time (Figure 10), although the amplitude of fluctuations was somewhat greater in microcosms at the end of the experiment.

Discussion

Microcosm Response

Microcosm turbidity tended to increase through time (Figure 5). This may reflect two phenomena. First, simulated runoff events introduced soil into the tanks. Colloids from these slurries probably increased turbidity. Secondly, phytoplankton chlorophyll-a and biomass also increased through time (Figure 11, Chapter 5). Some of the observed light attenuation may have been due to algal cells. Treatment-related reductions in turbidity were observed in both microcosms and mesocosms. One possibility is that pyrethroids may have caused flocculation of particulates.

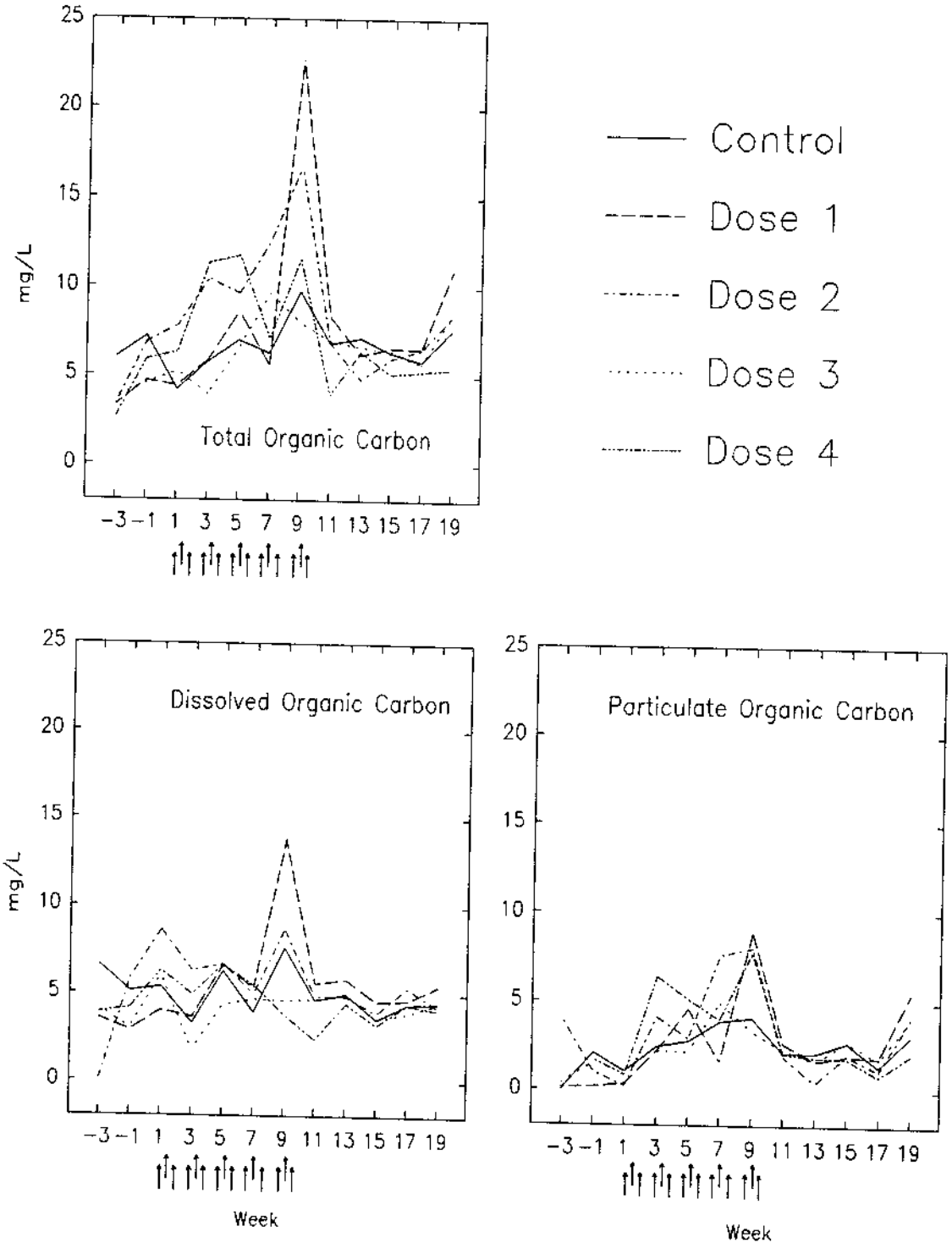

Figure 9. Mean dissolved, particulate and total organic carbon (mg/L) from microcosms. Arrows indicate pyrethroid applications.

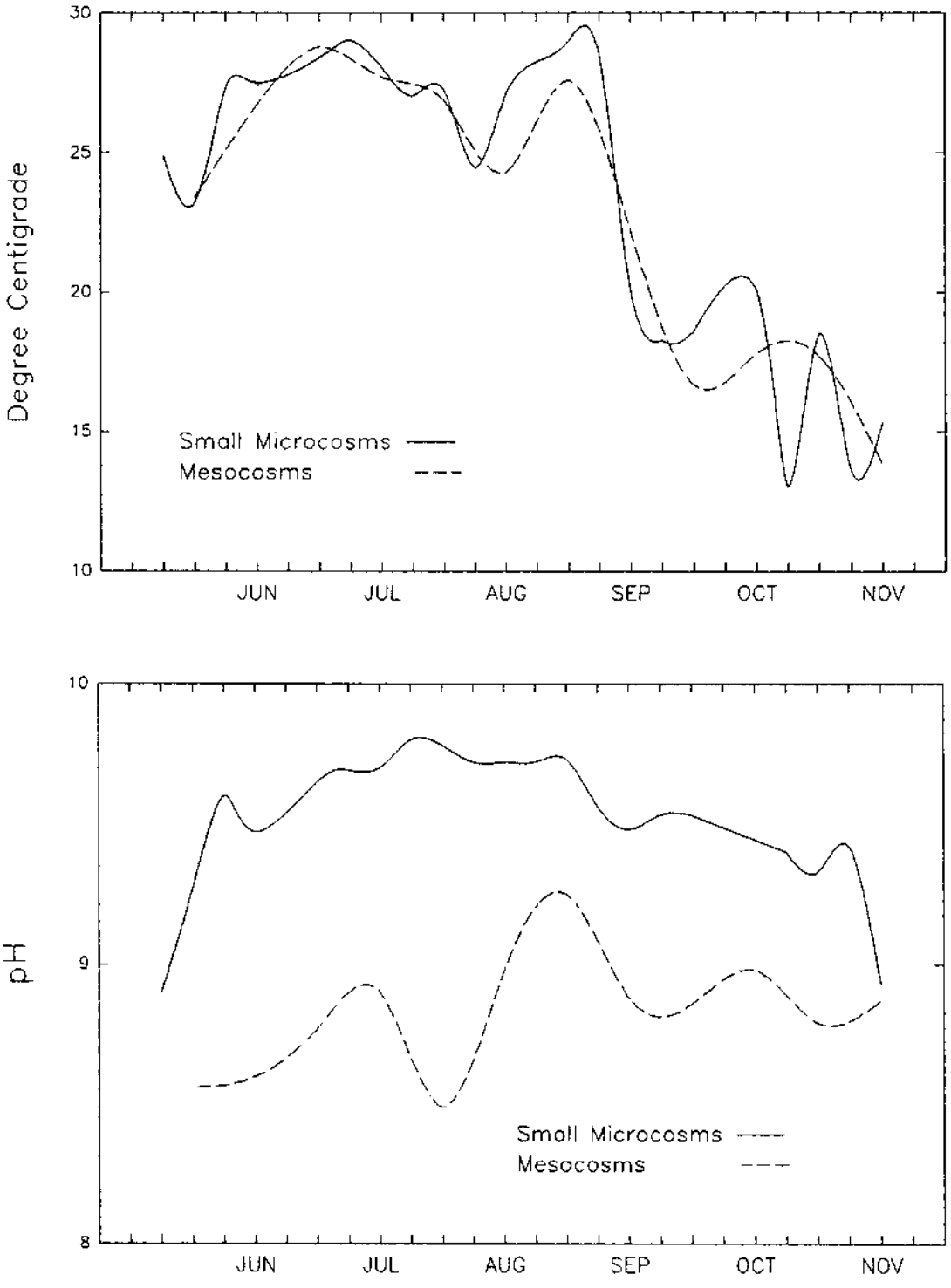

Figure 10. Mean water temperature (C) and water pH from control microcosms and control mesocosms during the experimental period.

Table VI. Summary of Dunnett's analyses during application period only. To be conservative, treatments were noted here only if more than one difference was found during application.

Parameter	Micro Impct	Micro Treat	Meso Impct	Meso Treat	Compare
Residues					
Sediment	NA	NA	NA	NA	Mic>Mes
Half-life	NA	NA	NA	NA	Mic<Mes
% of Nominal	NA	NA	NA	NA	Mic>=Mes
Water Chem.					
Nitrogen	-	-	R	D1-4	Mic<=Mes
Phosphorus	-	-	R	D1-4	Mic<Mes
Alkalinity	-	-	-	-	Mic>Mes
Hardness	-	-	-	-	Mic>=Mes
Organic Carbon	-	-	-	-	Mic<=Mes
TSS	-	-	-	-	Mic<Mes
Turbidity	R	D4	R	D1-4	Mic<Mes
pH	-	-	-	-	Mic>Mes
Temperature	-	-	-	-	Mic=Mes

NA Not Applicable
I Sig. Increase
R Sig. Reduction
\- No Impact

Impct = Impact
Treat = Treatment Level
Compare = System Comparison

Stratton and Corke (1981) found technical grade permethrin to cause adhesion of algae, bacteria and silica powder to *Daphnia magna*. Drenner et al. (In Review) also noted a

relationship between turbidity and pyrethroid loadings. Some pyrethroids do not appear to influence turbidities, however, raising the possibility that constituents of the EC formulation may be responsible. Formulations are proprietary, and differ for each compound. A formulation-control would need to be conducted to evaluate its role in this process.

Alkalinity increased during the study (Figure 6). Alkalinity ranges from < 5-500 mg/L in natural waters, with higher alkalinities generally in areas with clay or loam soils (Boyd 1990). Increasing alkalinities may have resulted from well water additions used to replace evaporation, and/or from runoff applications that used a sandy-loam slurry. Total hardness decreased during the study (Figure 6). Microcosms therefore shifted from moderately hard water (75-150 mg/L) to soft water (0-75 mg/L). Boyd (1990) suggests that when total alkalinity exceeds total hardness, some of the bicarbonate and carbonate is associated with potassium and sodium, rather than only with calcium and magnesium.

Nitrites were generally higher in microcosms during the dosing period (Figure 7), potentially due to soil runoff inputs. Nitrates did not show this pattern. As expected, nitrates greatly exceeded nitrites (Boyd 1990). Ammonia values were often near the 0.05 mg/L detection limit, with

no obvious patterns among treatments (Figure 8). Total phosphate levels were often near the detection limit, and were well within the range of natural lakes, which rarely exceed 1 mg/L (Boyd 1990).

Dissolved organic carbon (DOC) often exceeded particulate organic carbon (POC), however the DOC:POC ratio was never in the 10:1 range described by Wetzel (1983) for unproductive to moderately productive lakes. Deviations from 10:1 often occur during periods of intensive algal and bacteria growth with ratios in the 1:1 range (Wetzel 1983). Total organic carbon (Figure 9) was often higher in treated tanks. Reasons for these increases were unclear.

Many of the observed significant differences occurred during the pre-application period. By week -1 or week 1, treatments often appeared to become more similar. This trend was particularly striking for alkalinity (Figure 6), nitrates (Figure 7), and ammonia (Figure 8). This may have reflected convergence due to pre-treatment circulation of water among microcosms.

Microcosm/Mesocosm Comparison

Mean temperatures were very similar between the mesocosms and the small concrete microcosms buried in the ground, tracking each other through time during the 1989 comparative study (Figure 10). These results suggested that

ground-burial was sufficient for minimization of temperature fluctuations in microcosm-scale systems.

Mean pH values (calculated using hydrogen ion concentrations and reconverted to pH) were consistently higher in microcosms relative to mesocosms (Figure 10). Microcosms had substantially greater macrophyte growth than mesocosms. Microcosms also had a higher surface/volume ratio (Table I), which may have resulted in enhanced periphytic growth in relation to the system volume. Increased primary productivity may result in elevated CO_2 losses from the water column, with subsequent increases in pH (Wetzel 1983). Water chemistry parameters such as alkalinity, hardness, organic carbon, nitrogen series, (etc.), were analyzed to characterize test systems. These variables generally showed no response to pesticide treatment. Mean dissolved oxygen, temperature and pH showed no pesticide effects.

High turbidities in control ponds may have been linked with higher values for other parameters (i.e., phosphate, nitrite and ammonia). Microcosms also exhibited a trend toward higher control turbidities compared to D4 tanks. Turbidity was consistently higher in mesocosms relative to microcosms. Higher turbidities were attributed to intense rains in May and June, 1989. During rainstorm events, mesocosms received soil inputs via runoff while microcosms

did not. Differential turbidities may have influenced macrophyte growth in these systems, with more macrophytes in microcosms. Higher turbidity may have reduced macrophyte development in mesocosms (see Wetzel 1983).

Comparison with Other Mesocosm Studies

Water chemistry parameters have rarely been mentioned in published mesocosm literature. Hill et al. (1988) reported no significant pyrethroid impacts on chemistry parameters. Lozano et al. (1992), and Fairchild et al. (1992) did not discuss chemical parameters. Webber et al. (1992) observed some statistical significance for certain variables, but differences were not consistent among treatments. These results were not unexpected. Species composition and other structural properties are often more sensitive than functional properties due to species replacements and functional redundancy (Odum 1985, Schindler 1987). It should be noted that functional measurements are generally not conducted in mesocosm tests, and functional impacts conceivably could be early warning indicators of ecosystem impacts (Cairns 1986).

Summary

Microcosm water quality parameters showed little response to pyrethroid application, with the notable

exception of turbidity, which was lower in high-rate microcosms. Water circulation among microcosms may have helped to reduce differences in water quality among different tanks.

Mesocosms experienced higher turbidities relative to microcosms, probably due to storm-induced runoff from the berms separating mesocosms, and due to a greater fetch on the larger systems that would help keep particulates in suspension. Turbidity values from treated mesocosms were lower than control levels, suggesting that the pyrethroid (or formulation) affected particulates. Possible reasons for this were discussed. Some water quality parameters responded to pesticide application in mesocosms, and may have been related to differential turbidities with associated particulates. Water temperatures were similar in both systems, whereas pH levels were consistently higher in microcosms. Results of other mesocosm studies suggest that water quality parameters were generally not very sensitive to pyrethroid insecticides.

CHAPTER 5

PHYTOPLANKTON AND PERIPHYTON

Introduction

Pyrethroids have generally not impacted algal populations directly. Cypermethrin and fenvalerate did not reduce terrestrial soil algae populations and even enhanced growth of certain species (Megharaj et al. 1986, 1987).

In aquatic systems permethrin was essentially non-toxic to most algal types, with effects on algal growth present in the low ppm range (Stratton and Corke 1982). Photosynthesis was unaffected at > 100 ppm. Degradation products may be up to 10X more inhibitory to algae than the parent compound (Stratton and Corke 1982).

Results

Microcosm Response

Phytoplankton chlorophyll-*a* was consistently higher in treated microcosms relative to controls (Figure 11), but never significantly (Table VII). Chlorophyll-*a* levels rose during the study, becoming most variable at the end of the experiment.

Phytoplankton biomass increased through time, but treatment-related trends were less obvious (Figure 11).

Phytoplankton biomass was statistically enhanced only once, in D1 at the end of the study (Table VII).

Periphyton chlorophyll-*a* and biomass showed few treatment-related trends (Figure 12). Significant periphyton impacts were limited to the pre-application period (Table VII).

Microcosm/Mesocosm Comparison

Phytoplankton biomass levels (mg/L) were similar in mesocosms and microcosms (Table VII). Statistical significance was rarely observed in either system. Phytoplankton chlorophyll-*a* levels (mg/L) were generally higher in treated microcosms, compared with the controls (Figure 11). This trend was not seen in the mesocosms. Phytoplankton chlorophyll-*a* levels were very similar in both systems and tracked each other well through time (Table VII). Surprisingly, periphyton chlorophyll-*a* levels (mg/mm^2) were lower in microcosms (Table VII). Microcosm periphyton biomass values (mg/mm^2) were either lower than mesocosms or were similar in concentration (Table VII). Periphyton colonization was not influenced by treatment level.

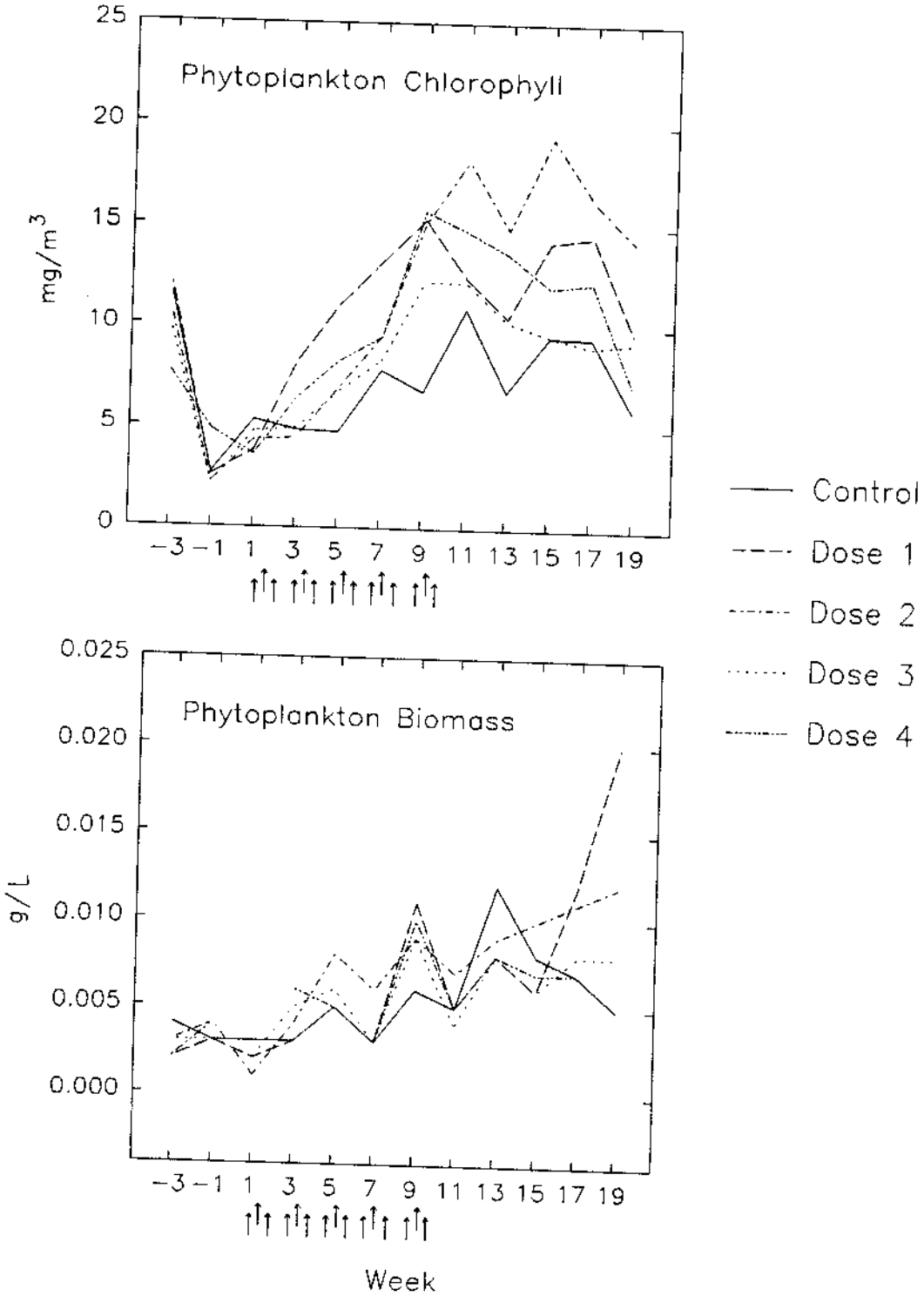

Figure 11. Mean phytoplankton chlorophyll-a (mg/m^3) and phytoplankton biomass (g/L) from microcosms. Arrows indicate pesticide applications.

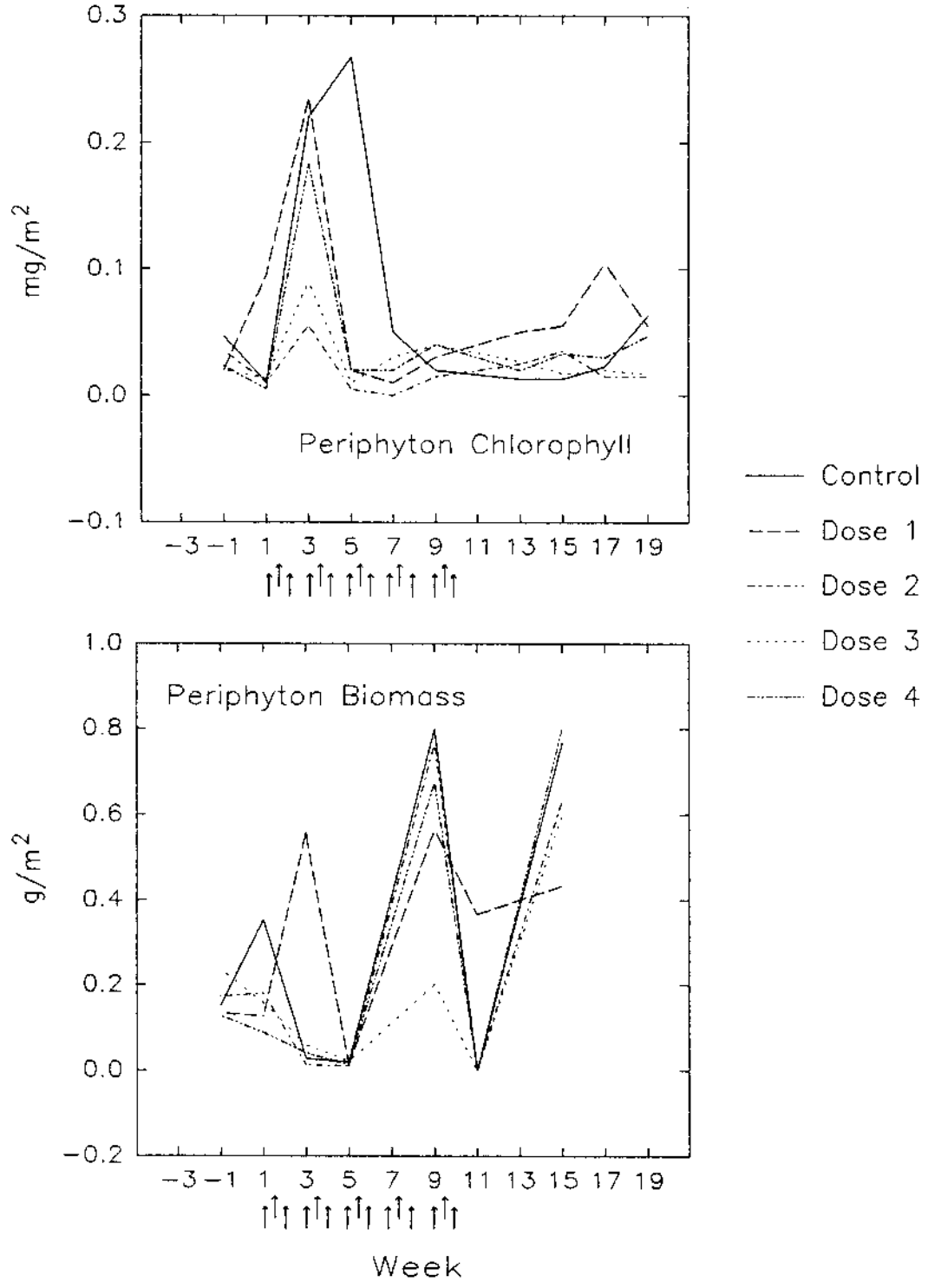

Figure 12. Mean periphyton chlorophyll-a (mg/m^2) and periphyton biomass (g/m^2) values from microcosms. Arrows indicate pyrethroid applications.

Table VII. Statistically significant differences for phytoplankton and periphyton measurements in microcosms (Dunnett's MRT). Bottom table compares microcosms with mesocosms. D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

Parameter		Week Number											
		-3	-1	1	3	5	7	9	11	13	15	17	19
Phytoplankton Chlorphyll-a	D1												
	D2												
	D3												
	D4												
Phytoplankton Biomass	D1												↑
	D2												
	D3												
	D4												
Periphyton Chlorophyll-a	D1	ND	↓						ND				
	D2	ND							ND				
	D3	ND	↓						ND				
	D4	ND	↓						ND				
Periphyton Biomass	D1	ND					ND			ND		ND	ND
	D2	ND					ND			ND		ND	ND
	D3	ND	↑				ND			ND		ND	ND
	D4	ND					ND			ND		ND	ND

Parameter	Microcosm Impact	Mesocosm Impact	Comparison
<u>Phytoplankton</u>:			
Chlorophyll-a	-	-	Mic=Meso
Biomass	-	-	Mic=Meso
<u>Periphyton</u>:			
Chlorophyll-a	-	-	Mic<Meso
Biomass	-	-	Mic<Meso

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.
ND=Data missing (No Data), cannot perform MRT

Discussion

As anticipated, phytotoxicity was not apparent in algal populations. If anything, populations increased, suggesting a secondary effect. Enhanced phytoplankton biomass and chlorophyll-a levels in treated microcosms may have reflected reductions in grazer populations, discussed later in the paper. The location of periphytometers (at the water surface in the middle of the tank) may have limited the likelihood of detecting secondary effects on either pelagic or benthic grazers.

Mesocosm studies investigating pyrethroids have found mixed algal responses. Hill et al. (1988) reported no pyrethroid impacts on phytoplankton or periphyton cell numbers, cell volumes, biomass, or taxonomic diversity in North Carolina. Lozano et al. (1992), in a Minnesota limnocorral study of esfenvalerate, found no impacts on phytoplankton chlorophyll-*a*, but did observe enhancements in phytoplankton biovolumes that they attributed to invertebrate reductions. *Chara* populations also increased in this study.

Webber et al. (1992) noted phytoplankton density, biomass and primary productivity enhancements at higher pyrethroid levels in Alabama mesocosms using esfenvalerate. Fairchild et al. (1992) observed reductions in high-rate phytoplankton chlorophyll-a levels in Missouri mesocosms

treated with esfenvalerate. They attempted to relate this trend to declines in bluegill recruitment with subsequent increases in zooplankton grazing pressure.

Yasuno et al. (1988) observed no effects on chlorophyll-a or primary productivity in a pond enclosure study of permethrin effects. They did note reductions in *Ceratium hirundinella*, a large phytoplankter. Day et al. (1987) observed increases in small, readily digestible Chlorophyta following zooplankton reductions by fenvalerate applied to limnocorrals.

Summary

Phytoplankton chlorophyll-a levels were generally higher in treated microcosms, yet variability precluded statistical significance. Few consistent trends were observed for phytoplankton biomass, periphyton biomass or periphyton chlorophyll-a. Increased algal populations have been attributed to elimination of grazers in other pesticide studies.

No clear responses were observed in mesocosms for any algal measures. Microcosm and mesocosm phytoplankton levels were similar, however mesocosm periphyton levels exceeded microcosm values.

CHAPTER 6

ZOOPLANKTON - GENERAL RESPONSE

Introduction

A number of studies have found cladocerans and chaoborids to be very sensitive to pyrethroids, while copepods and ostracods were less affected and rotifers often increased or were unaffected (Miura and Takahashi 1976, Kaushik et al. 1985, Helson and Surgeoner 1986, Day et al. 1987, Yasuno et al. 1988, Hill et al. 1988). Young Cladocera may be 1.8 to 3 times more sensitive than adults (Day and Kaushik 1987b). *Daphnia magna* neonates (6-24 h old) were more sensitive to deltamethrin than juveniles that were 48 to 72 h old (Xiu et al. 1989). Nauplii were more sensitive to permethrin than adult copepods or copepodid stages (Yasuno et al. 1988).

Chronic, sub-lethal exposure to pyrethroid insecticides resulted in reduced production of young and shortened generation times in zooplankton (McKee and Knowles 1986, Day and Kaushik 1987a). Filtration rates of invertebrates may be reduced, with *Ceriodaphnia lacustris* showing the greatest sensitivity (Day et al. 1987, Day and Kaushik 1987b). Pyrethroid levels < 0.01 µg/L have shown few impacts on zooplankton in the field (Day 1989). Laboratory bioassays

using *D. magna* and *Ceriodaphnia dubia* compared BAYTHROID^{R} to other new-generation pyrethroids (such as bifenthrin, lambdacyhalothrin and tralomethrin). This study suggested that cyfluthrin was among the most toxic of these new formulations (Mokry and Hoagland 1990), thus information about the toxicity of cyfluthrin to zooplankton in the field is necessary for assessment of risks to the aquatic environment.

Laboratory experiments manipulating dissolved organic carbon (DOC) levels have shown reduced toxicity of various pyrethroids to *Daphnia magna* in the presence of elevated DOC (Day 1990). Bioassays with fenvalerate in the laboratory have shown reduced toxicity to *Daphnia galeata mendotae* in the presence of algal cells (Day and Kaushik 1987c). Thus, laboratory "clean-water" bioassays might not be directly translatable to field conditions. Semi-field bioassays with *D. magna* were conducted using microcosm water collected from the tank surface one hour after spray drift application. Results of this test were compared to impacts on microcosm Cladocera, particularly *Diaphanosoma brachyurum* the dominant large cladoceran in the microcosms.

Results

Zooplankton Community Structure

Total zooplankton population densities generally increased at treatments D2-D4 (Figure 13) near the end of the application period. Statistically significant increases were observed during weeks 10, 11, and 13 (Table VIII). Treatment D1 was similar to controls.

Taxa richness gradually declined in all microcosms through time (Figure 14). Richness values from treated microcosms were consistently lower than corresponding controls, but statistical significance was never achieved (Table VIII) due to high variability within treatments.

Total Cladocera numbers were significantly reduced at D4 during weeks 3, 10 and 17 (Table IX). The general pattern for D1-D3 was a reduction in mean Cladocera levels during the application period, with subsequent population increases in D1-D3 after pyrethroid treatment ended (Figure 15).

Within the Cladocera, *Diaphanosoma brachyurum* (Figure 16) and *Chydorus sphaericus* (Figure 17) were both sensitive to cyfluthrin application. *D. brachyurum* populations were significantly reduced at levels as low as D1 (Table IX), while *C. sphaericus* were significantly reduced at D3-D4. Populations of *D. brachyurum* were near zero at all levels (D1-D4) throughout the study. *C. sphaericus* populations

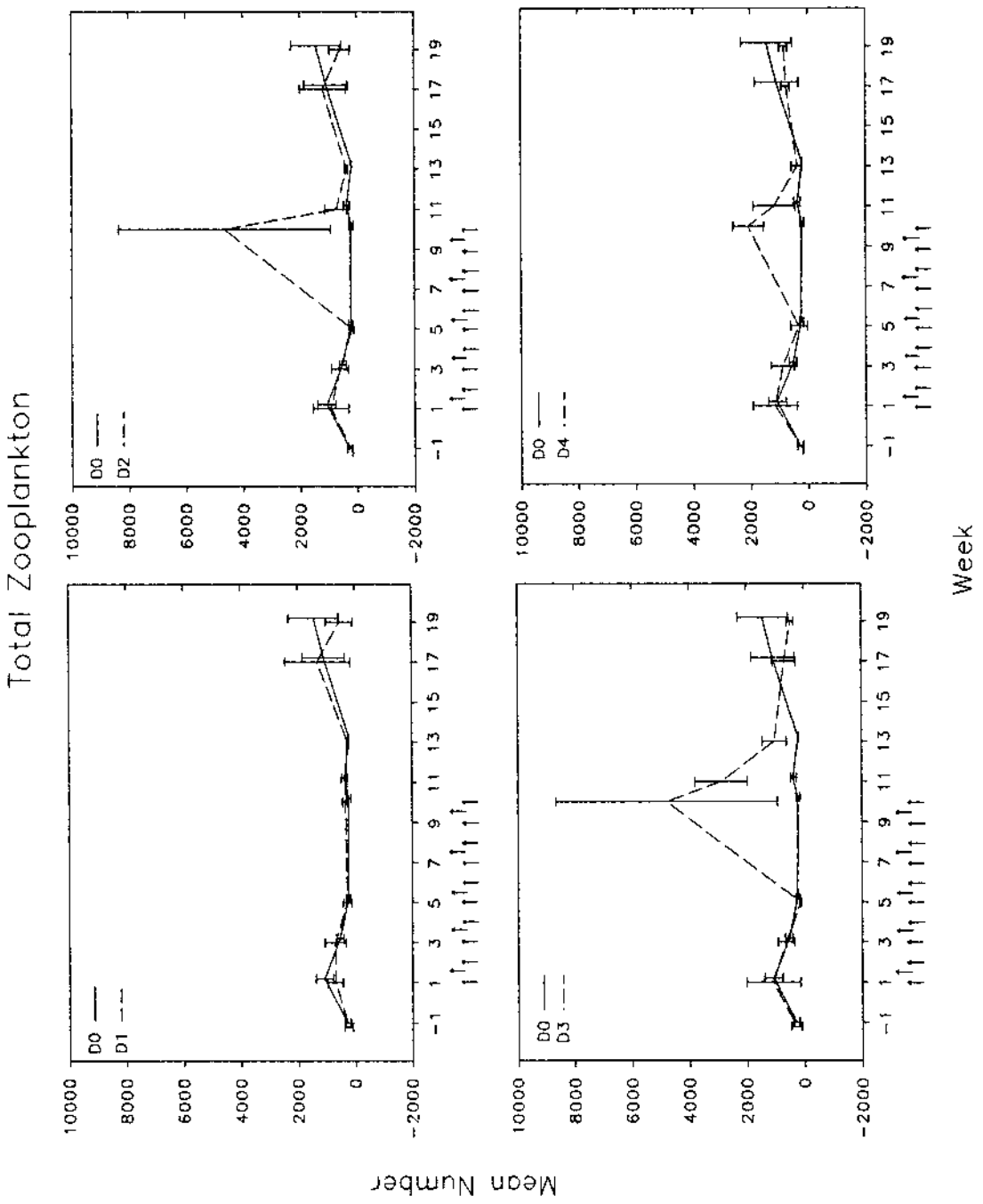

Figure 13. Zooplankton total population numbers (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

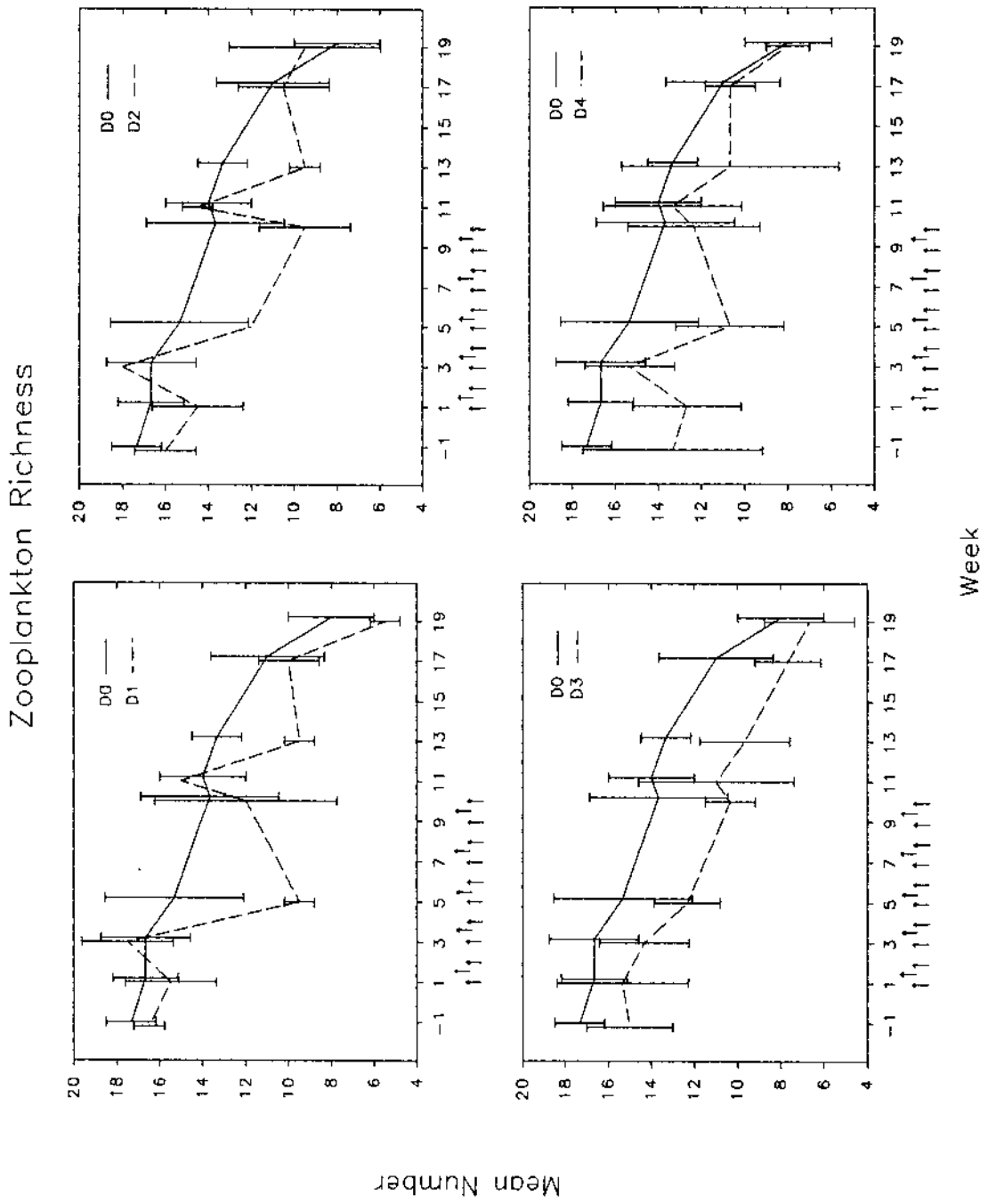

Figure 14. Zooplankton richness (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

increased after cessation of pesticide treatments (Figure 17).

Not all cladocerans were negatively impacted. *Alona rustica* were often higher in treatments, relative to controls (Figure 18). *Macrothrix rosea* populations from treated microcosms were also equal to or greater than controls (Figure 19). These cladocerans demonstrated few statistically significant differences (Table IX).

Some cladocerans (*Bosmina longirostris*, *Ceriodaphnia lacustris*, *Latonopsis occidentalis*, *Pleuroxus denticulatus* and *Scapholeberis kingi*) were rare, precluding evaluation of BAYTHROID^{R} impacts.

Copepod populations were dominated by immature stages (cyclopoid copepodites, cyclopoid and calanoid nauplii). Cyclopoid copepodite numbers were low until the end of the experiment, when populations increased (Figure 20). Increases were generally greater in treated tanks. Nauplii populations showed a similar pattern (Figure 21). Adult *Diaptomus* populations were too low for assessment of impacts. No statistically significant differences were observed in copepods, presumably due to low sample sizes.

Rotifers dominated microcosm zooplankton communities, in terms of both numbers and taxonomic richness. Total Rotifera responses (Figure 22) were similar to the total zooplankton population discussed above (Figure 13) due to

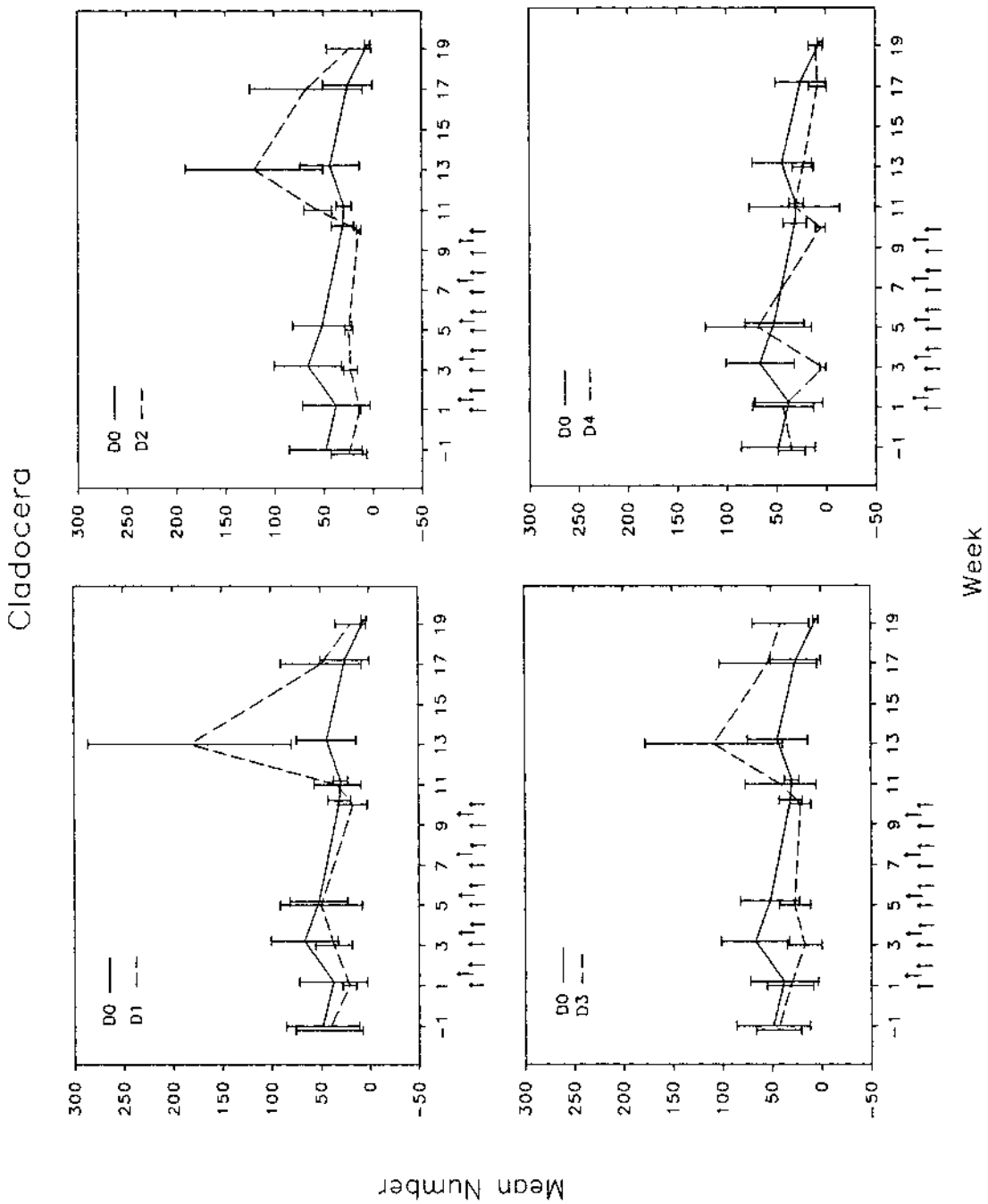

Figure 15. Cladocera populations (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

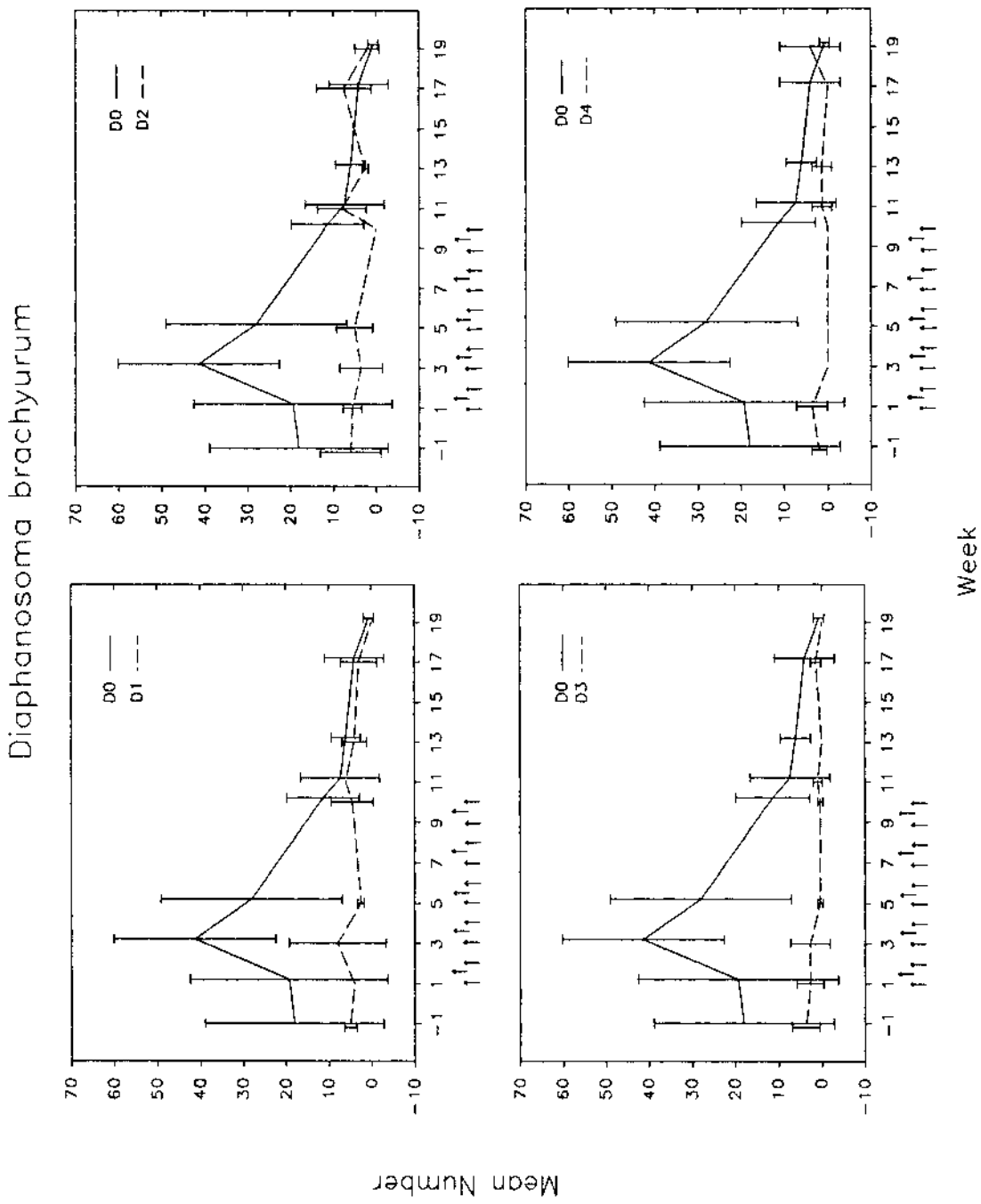

Figure 16. *Diaphanosoma brachyurum* populations (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

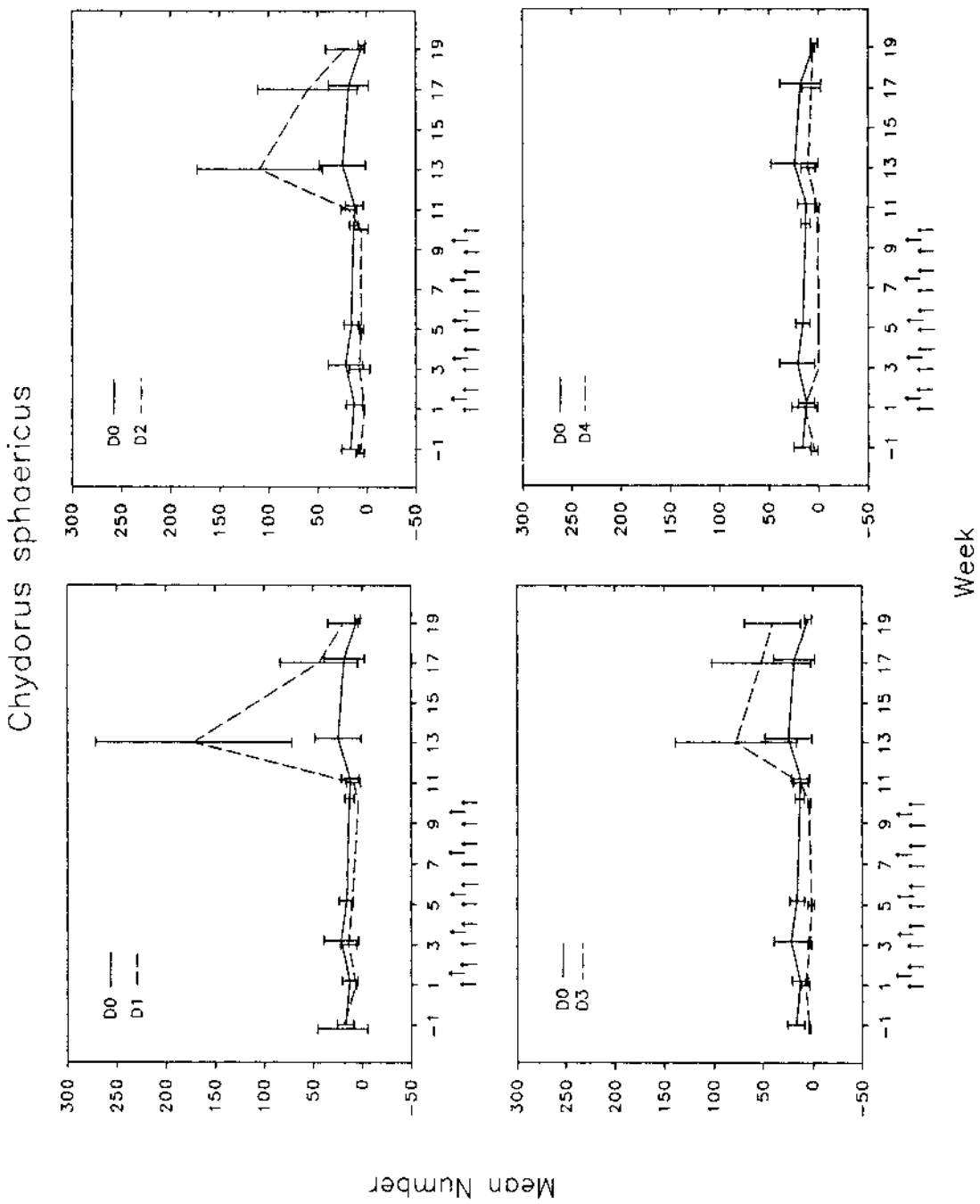

Figure 17. *Chydorus sphaericus* populations (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

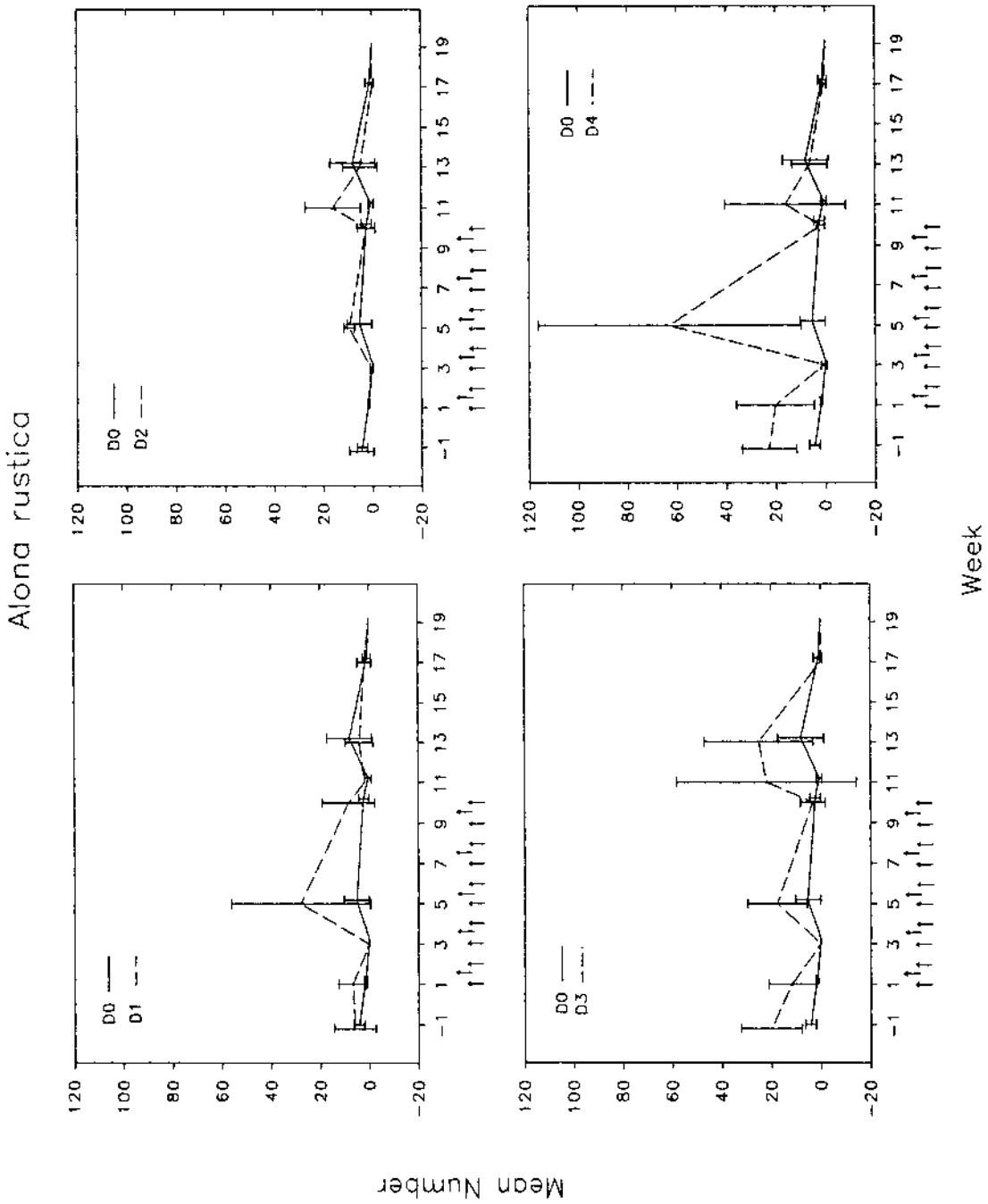

Figure 18. *Alona rustica* populations (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

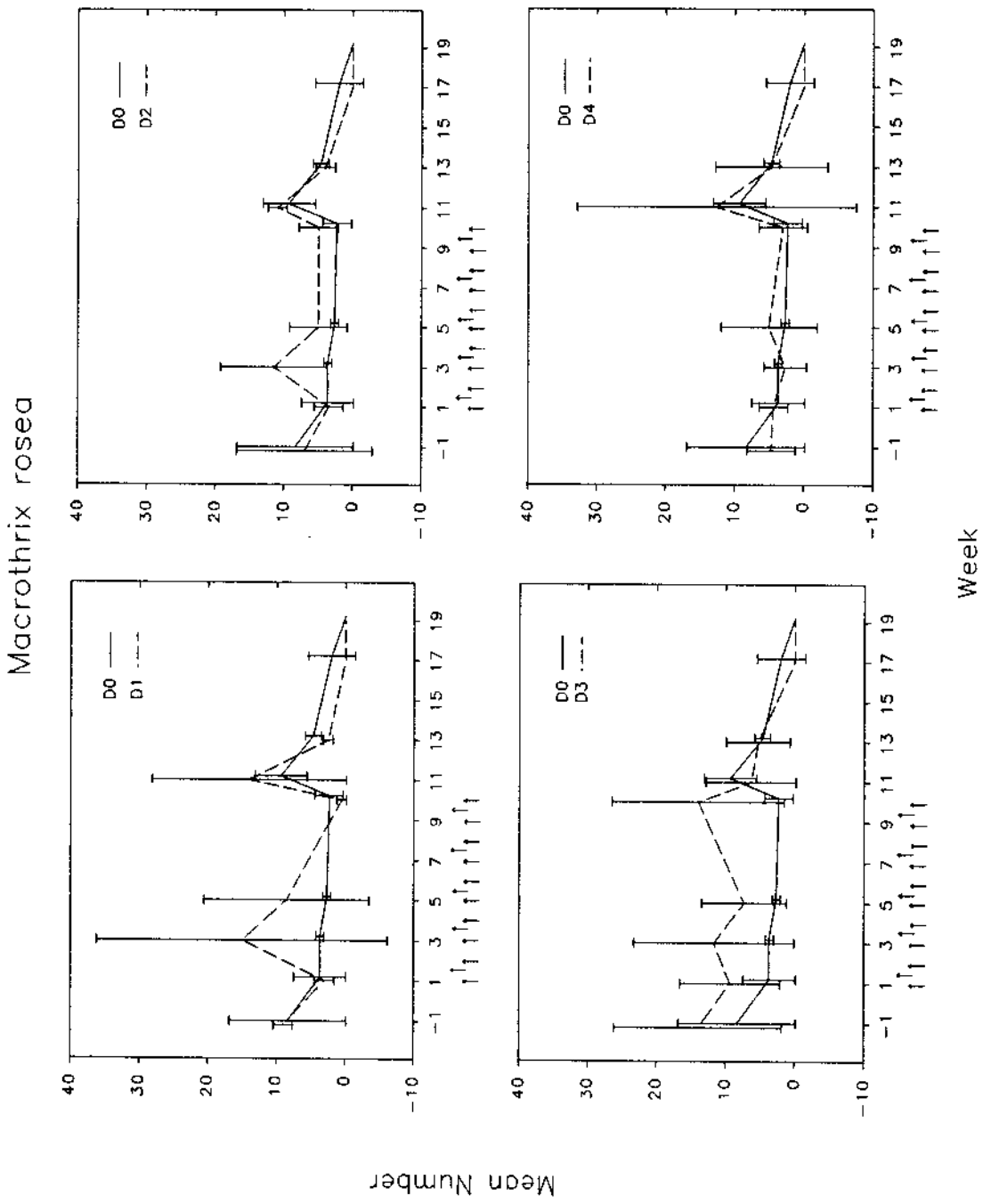

Figure 19. *Macrothrix rosea* population (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

their abundance. Rotifera populations were statistically enhanced at D2-D4 (Table X) during week 10, with rotifer populations reaching high densities within high rate tanks (Figure 22).

Members of the genus *Brachionus* were common in microcosms, particularly in treated tanks. *B. angularis* (Figure 23) populations were statistically increased at D2 and D4 during the experiment (Table X). *B. havanensis* were almost never found in control microcosms, but very high densities were observed at D2-D4 (Figure 24), with significant enhancements at D3 (Table X). *B. quadridentatus* differed from the other two *Brachionus* species, with consistently low numbers at all treatment levels (Figure 25). Populations from treated microcosms never exceeded control levels.

Other rotifer species differed in their responses to cyfluthrin. *Filinia longiseta* showed a mixed response, with occasional increases at high rates (Figure 26). *Polyarthra remata* (Figure 27) were significantly increased at D1-D4 during week 10 and at D3-D4 during week 11 (Table X). *Monostyla bulla* increased dramatically in D4 microcosms (Figure 28), with significant increases during week 5 (Table X). *Monostyla closterocerca*, on the other hand, did not show consistent enhancements in treated microcosms (Figure 29). Both *Lecane luna* and *Lecane leontina* populations

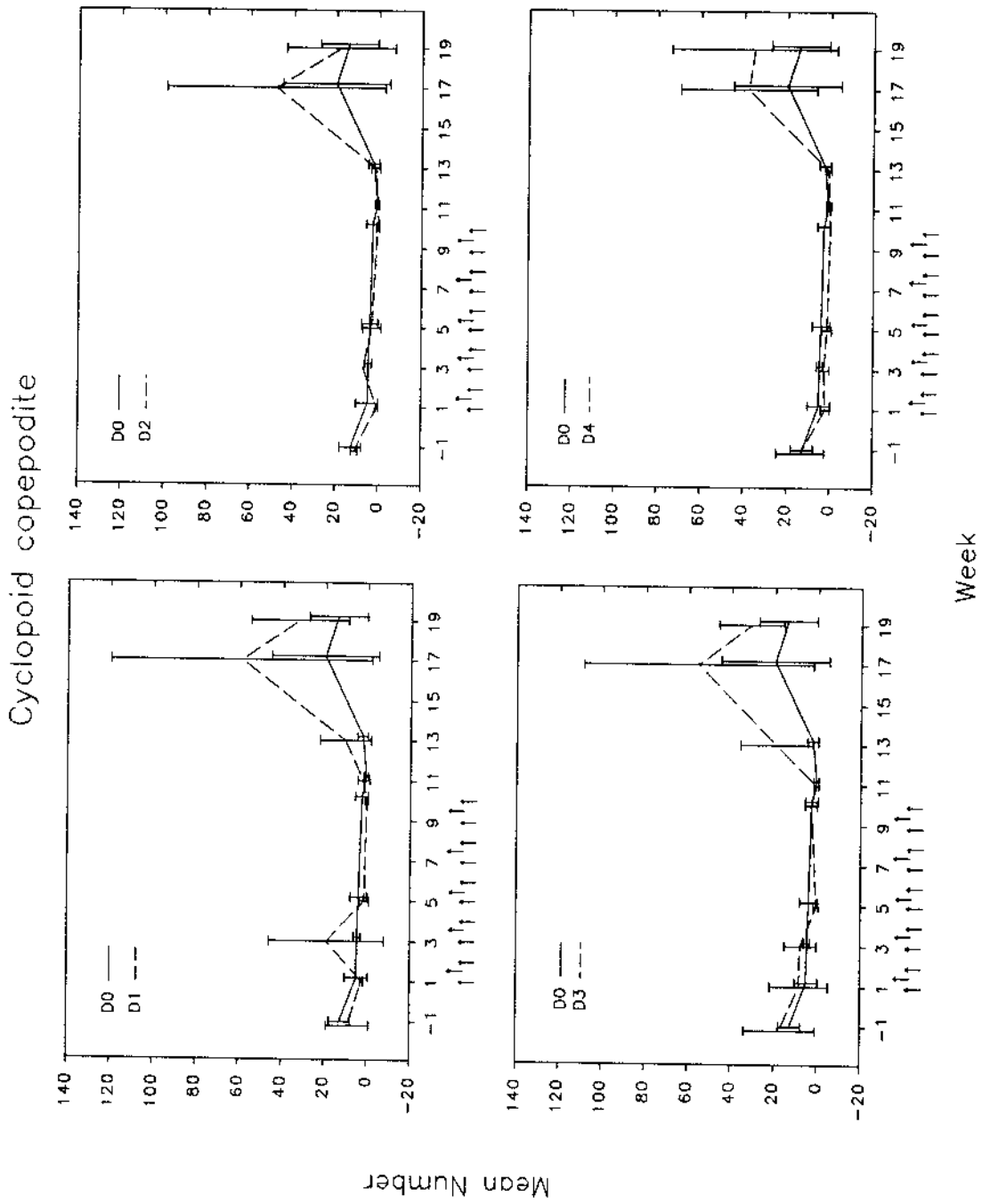

Figure 20. Cyclopoid copepodite population (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

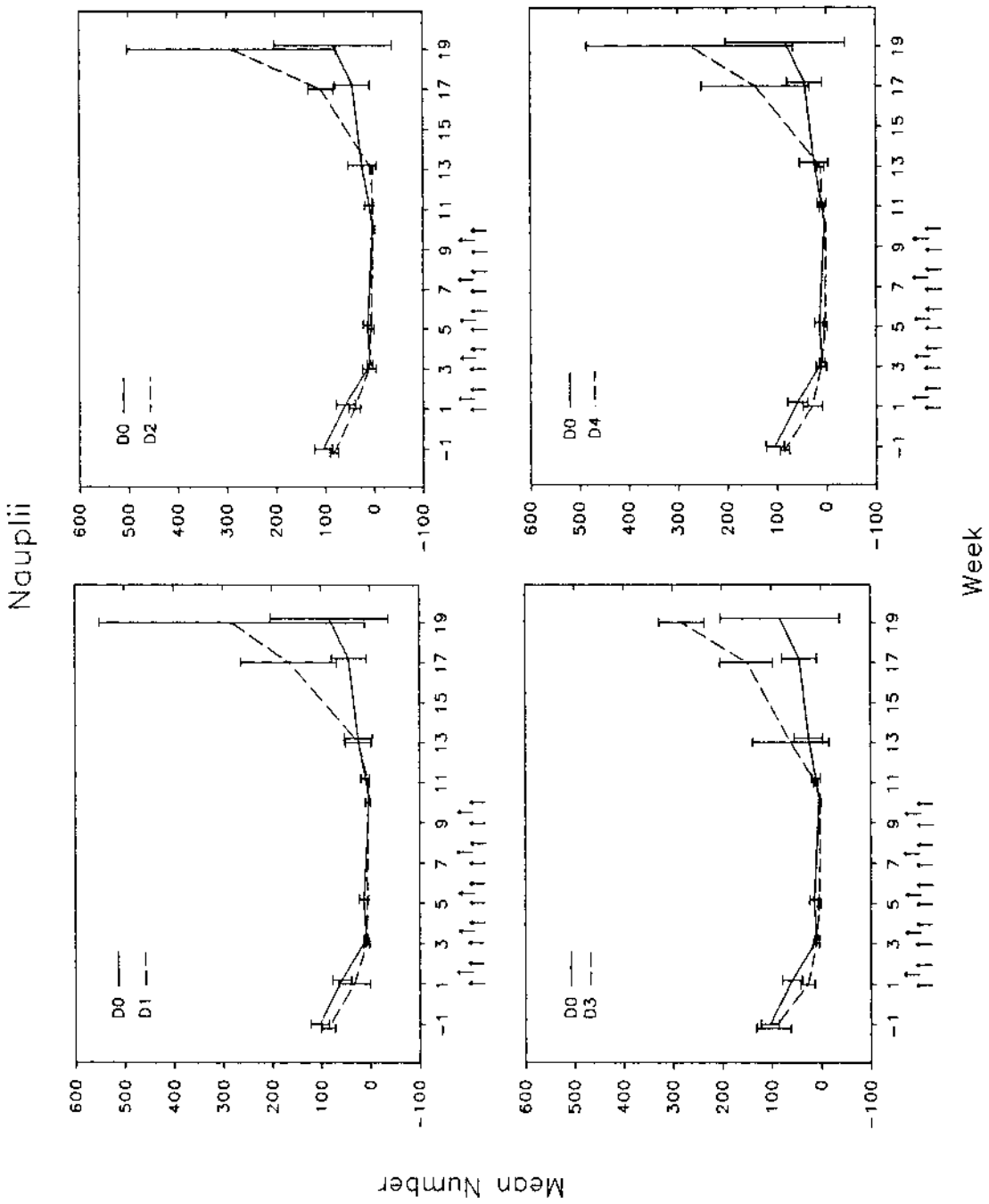

Figure 21. Nauplii population (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

Table VIII. Results of Dunnett's MRT for zooplankton total number and richness. D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

Parameter		Week Number								
		-1	1	3	5	10	11	13	17	19
Total Zooplankton	D1									
	D2					↑				
	D3					↑	↑	↑		
	D4					↑				
Zooplankton Richness	D1									
	D2									
	D3									
	D4									

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

increased in treated microcosms (Figures 30 and 31, respectively).

Bray-Curtis cluster analysis was employed as a method for evaluating the total zooplankton community response in a holistic fashion. All treatments were quite similar in composition and densities of dominant taxa (Figure 32) prior to pesticide application (week -1a). Similarities among treatments (clusters) were high, ranging from 0.72 to 0.79 (Appendix Table 3). Bootstrapping of replicates (Nemec 1991) resulted in probabilities that were not close to statistical significance (Appendix Table 3).

Table IX. Summary of statistically significant differences (Dunnett's MRT) for dominant Crustacea from microcosms; cladocerans and copepods.

Crustacea		Week Number								
		-1	1	3	5	10	11	13	17	19
Total Cladocera	D1									
	D2									
	D3									↑
	D4			↓		↓			↓	
Alona rustica	D1									
	D2									
	D3									
	D4		↑							
Chydorus sphaericus	D1									
	D2									
	D3			↓	↓					↑
	D4				↓	↓	↓			
Diaphanosoma brachyurum	D1				↓					
	D2					↓				
	D3			↓	↓	↓		↓		
	D4			↓	↓	↓				
Macrothrix rosea	D1									
	D2									
	D3									
	D4									
Nauplii	D1									
	D2									
	D3									
	D4									
Cyclopoid copepodite	D1									
	D2									
	D3									
	D4									

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

Table X. Summary of statistically significant differences for dominant rotifers from microcosms (Dunnett's MRT). D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

Rotifera		Week Number								
		-1	1	3	5	10	11	13	17	19
Total Rotifera	D1							↓		
	D2					↑				
	D3					↑	↑	↑		↓
	D4					↑				
Brachionus angularis	D1									↓
	D2					↑				
	D3									
	D4			↑						↓
Brachionus havanensis	D1									
	D2									
	D3						↑			
	D4									
Brachionus quadridentata	D1									
	D2									
	D3									
	D4									
Filinia longiseta	D1									
	D2									
	D3									
	D4									
Hexartha mira	D1									
	D2	↑								
	D3									
	D4									
Lepadella patella	D1									
	D2									
	D3									
	D4									

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

Continuation of Rotifera significant differences.

Rotifera		Week Number								
		-1	1	3	5	10	11	13	17	19
Lecane leontina	D1									
	D2									
	D3									
	D4									
Lecane luna	D1									
	D2									
	D3									
	D4									
Monostyla bulla	D1				↑					
	D2				↑					
	D3									
	D4				↑					
Monostyla closterocerca	D1									
	D2									
	D3									
	D4									
Monostyla lunaris	D1									
	D2									
	D3									
	D4									
Monostyla quadridentatus	D1									
	D2								↑	
	D3									
	D4							↑		
Platyias patulus	D1									
	D2									
	D3									
	D4									

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

Continuation of Rotifera significant differences.

Rotifera		Week Number								
		-1	1	3	5	10	11	13	17	19
Polyarthra remata	D1					↑				
	D2					↑				
	D3					↑	↑			
	D4					↑	↑			
Testudinella patina	D1									
	D2									
	D3									
	D4									
Unknown Rotifer #1	D1									
	D2									
	D3									
	D4									
Unknown Rotifer #2	D1									
	D2									
	D3									
	D4									

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

By week 5, during the middle of the ten week application period, treatments began to diverge (Figure 33). Treatments clustered in an exposure-response relationship, with D4 exhibiting the greatest differences (similarity of 0.33; Appendix Table 3). Control microcosms showed a greater diversity and evenness of taxa (Figure 33), while D4 was dominated by *Monostyla bulla* and *Alona rustica*. Bootstrapping analysis (Appendix Table 3) suggested that D4

was somewhat different from other treatment levels (p=0.118).

Community analysis at week 10 showed increasing divergence of treatments D2-D4, with these treatments clustered together, while D0-D1 clustered together (Figure 34). Similarity between the D0-D1 and D2-D4 clusters (Appendix Table 3) was extremely low (0.102). Bootstrapping generated a statistical significance probability of p=0.123. Large population booms of *B. angularis* and *B. havanensis* were the driving force behind this response.

At the end of sampling in November 1989 (week 19), control and treated microcosms were again dissimilar in community structure. Controls were dominated by the rotifers *Polyarthra remata* and *B. angularis*, while treated tanks had larger nauplii populations (Figure 35). Treatment levels D1-D4 were fairly similar to each other, with similarities ranging from 0.78 to 0.69 (Appendix Table 3), while controls were not similar to any of the treatments (similarity=0.32). Bootstrapping generated a p=0.161 probability level for statistical separation of the D0-D1 linkage (Appendix Table 3).

Daphnia Bioassay

Cyfluthrin exposure to 24-48 h old juvenile *D. magna* resulted in little mortality at 24 h (Figure 36), with the

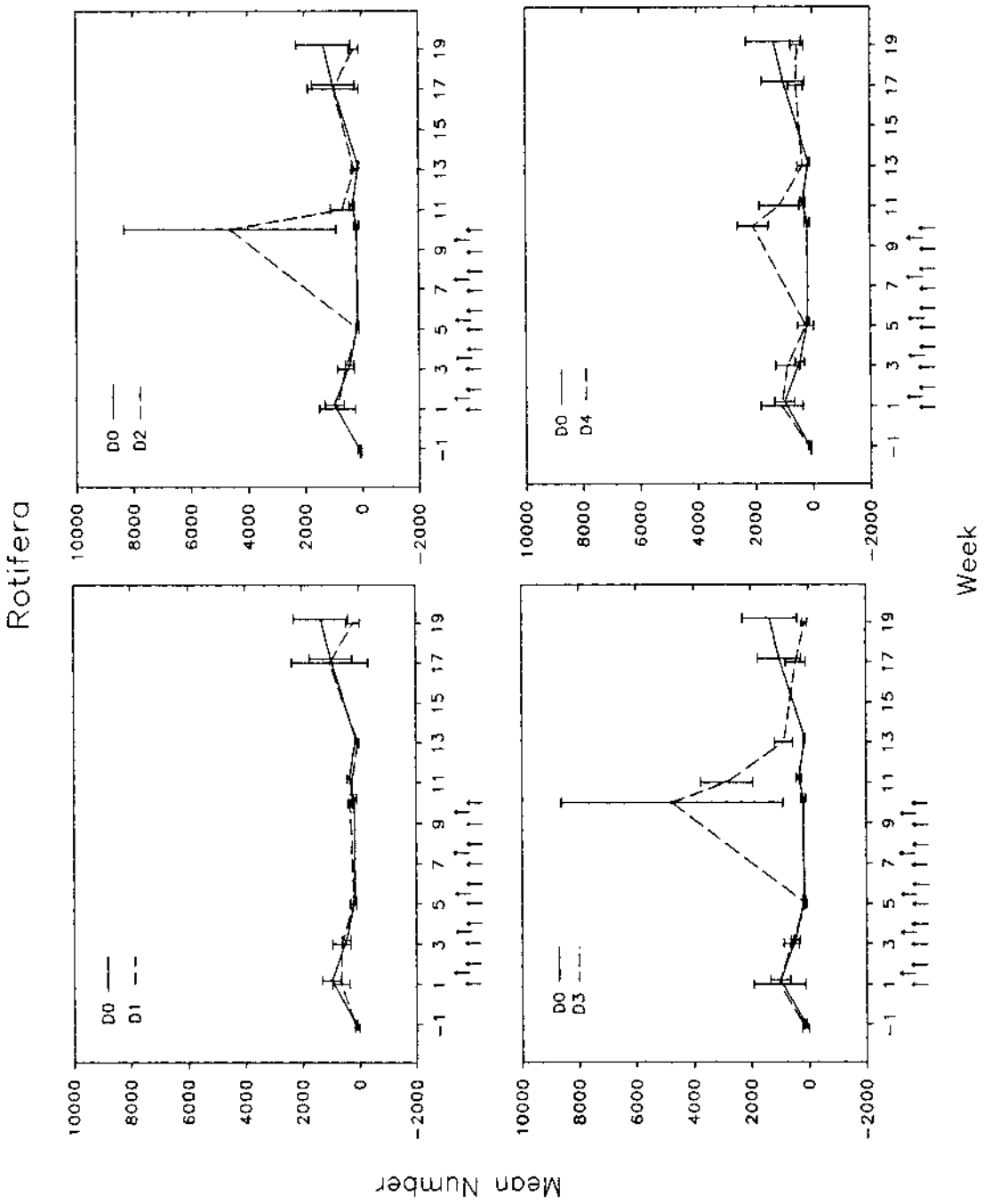

Figure 22. Total Rotifera population (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

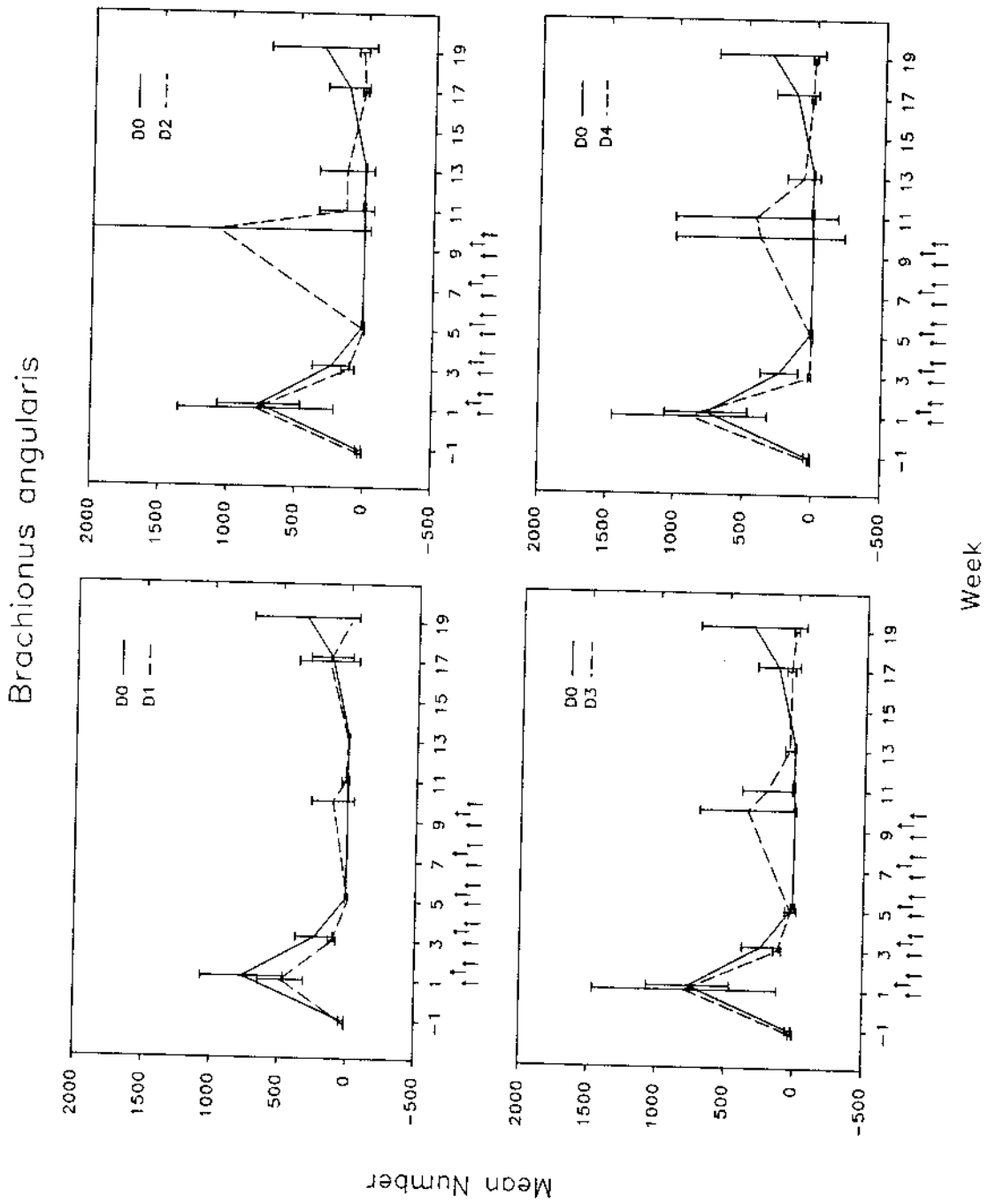

Figure 23. *Brachionus angularis* population (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

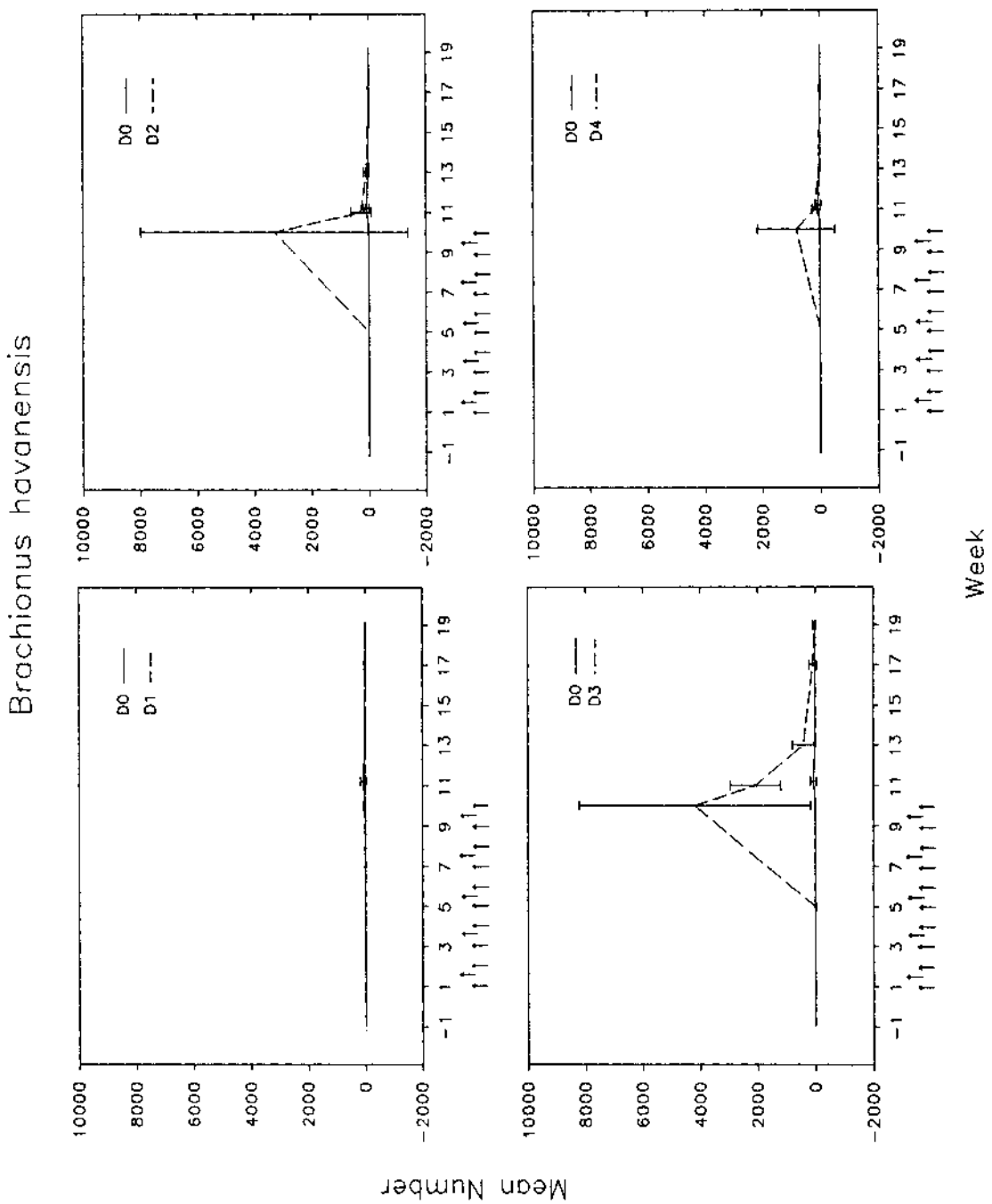

Figure 24. *Brachionus havanensis* population (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

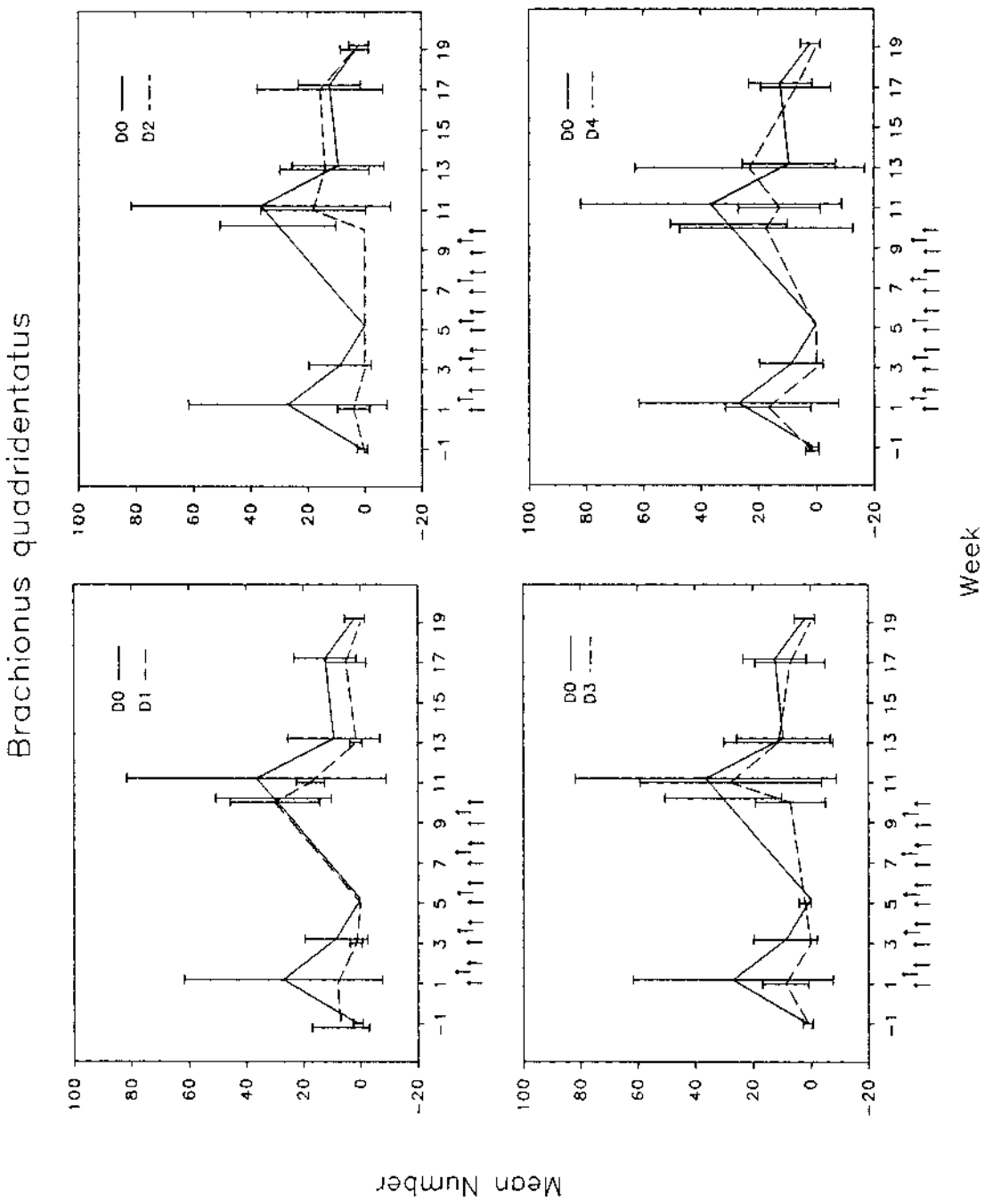

Figure 25. *Brachionus quadridentatus* population (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

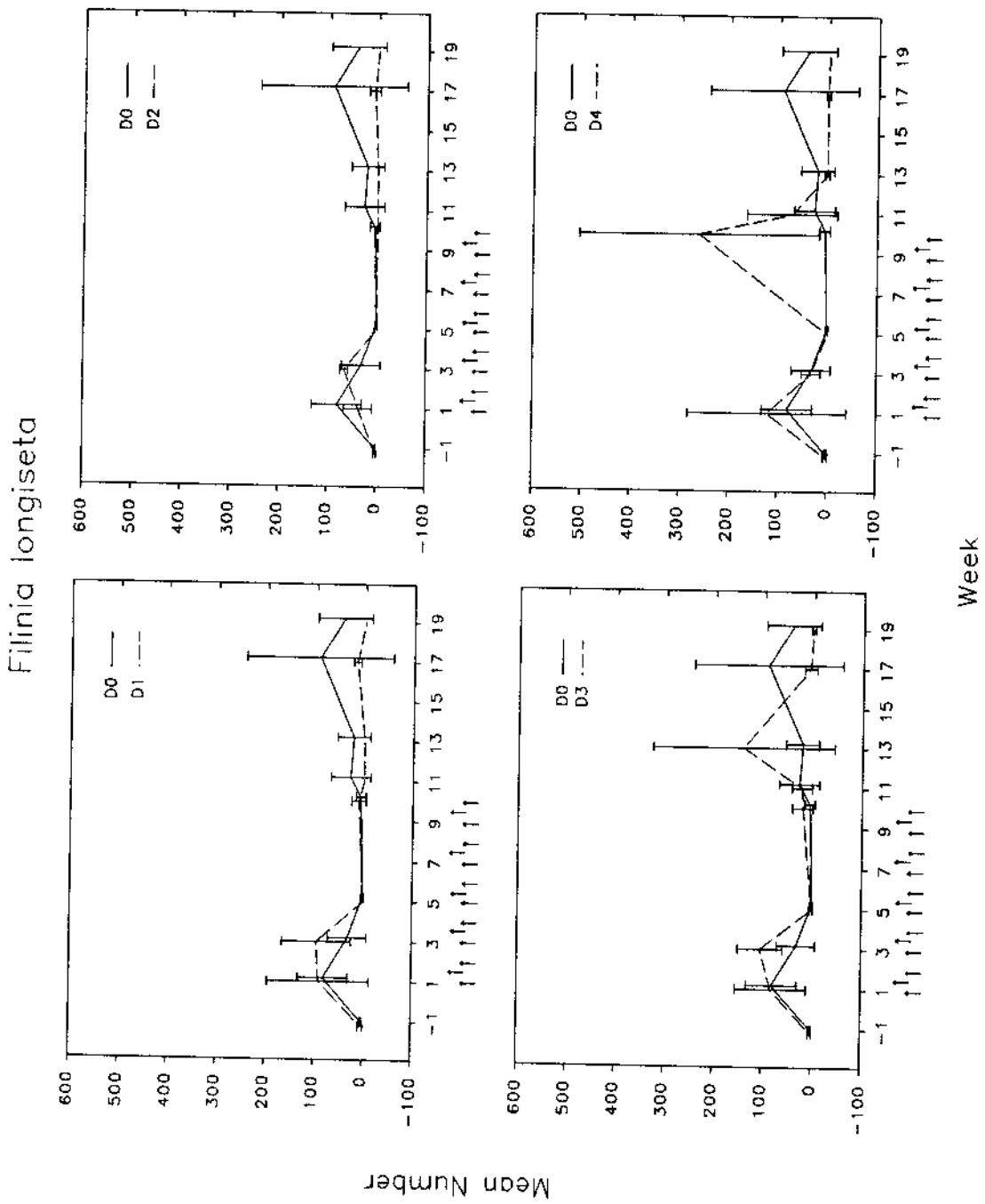

Figure 26. *Filinia longiseta* population (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

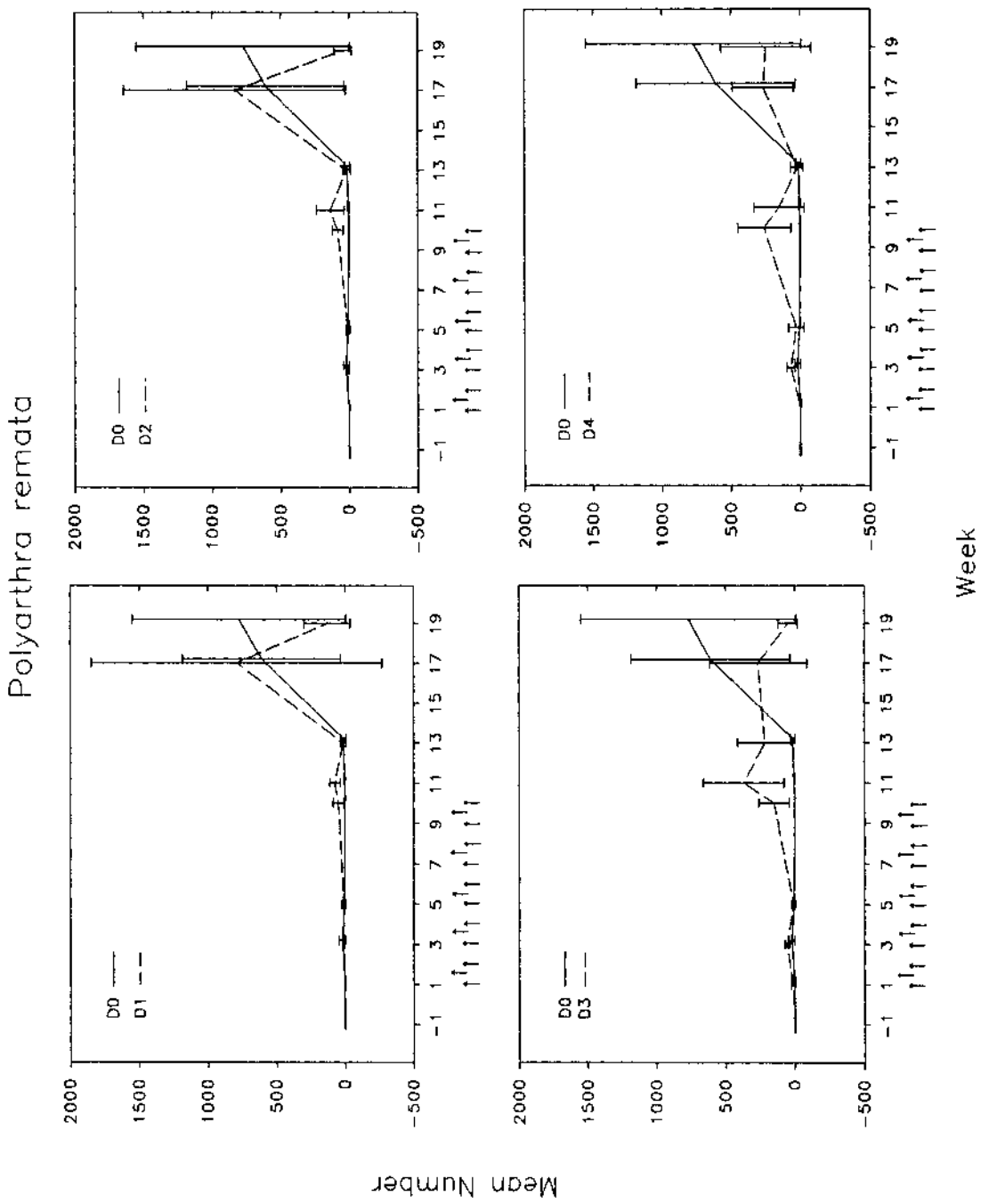

Figure 27. *Polyarthra remata* population (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

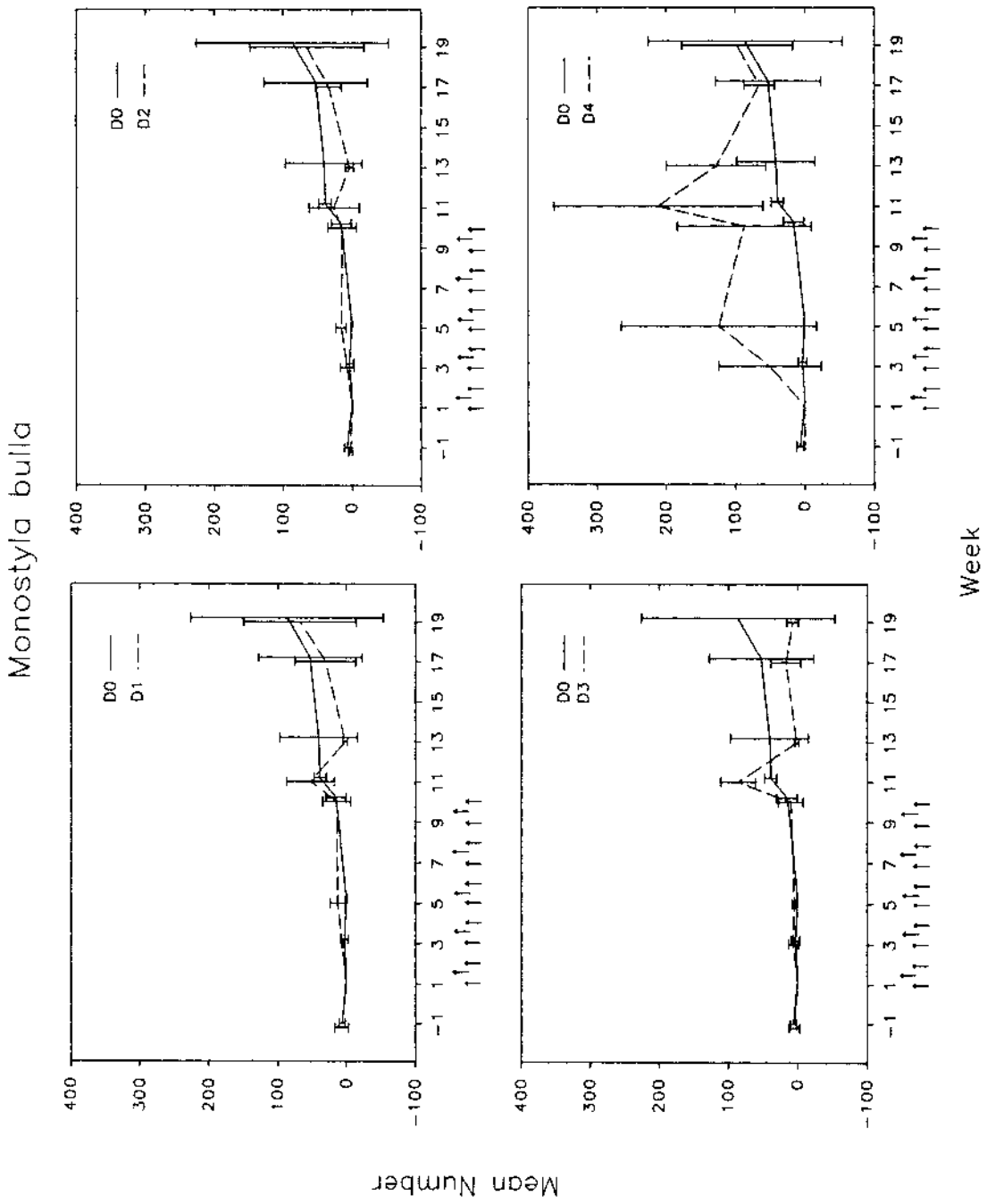

Figure 28. *Monostyla bulla* population (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

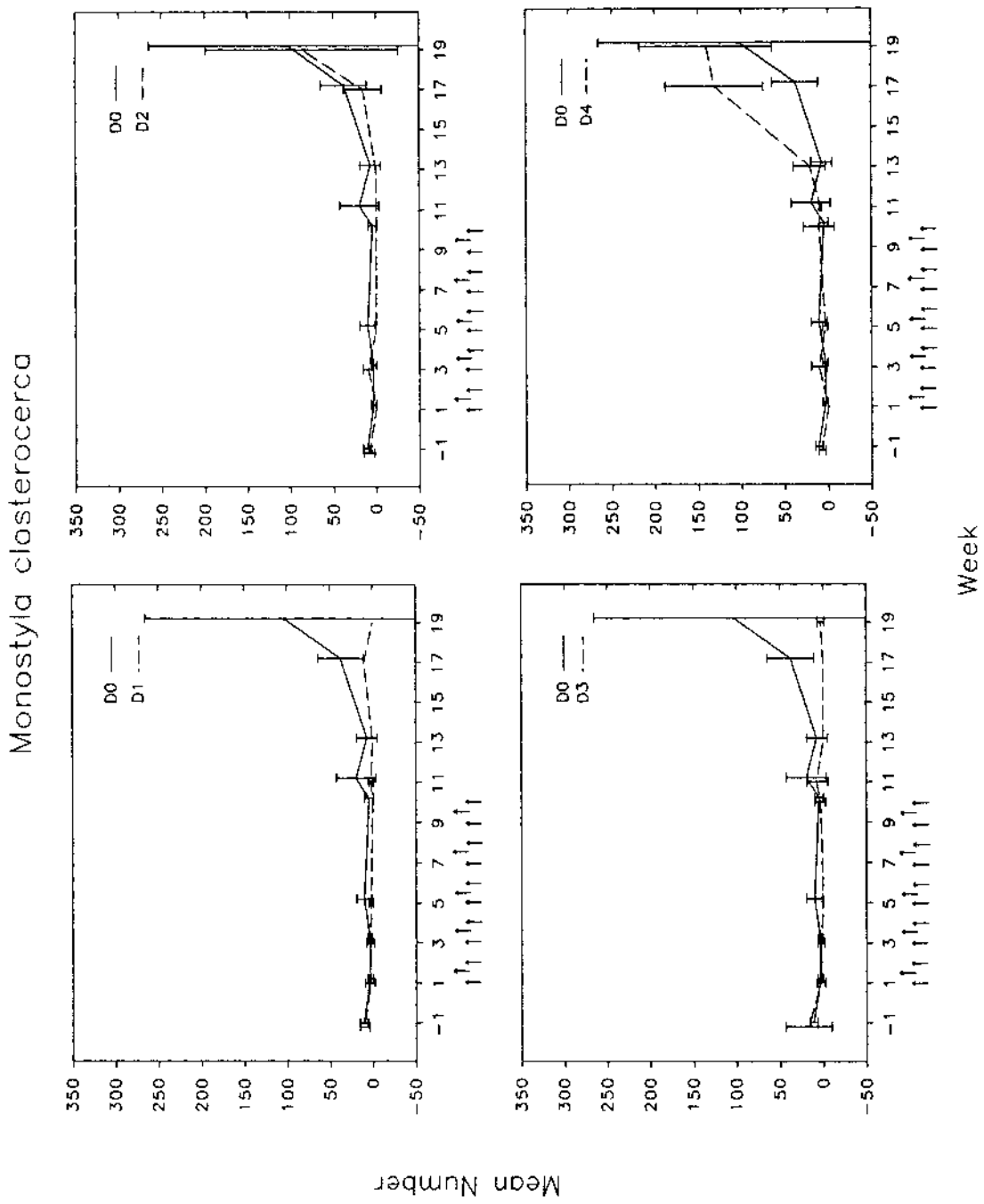

Figure 29. *Monostyla closterocerca* population (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

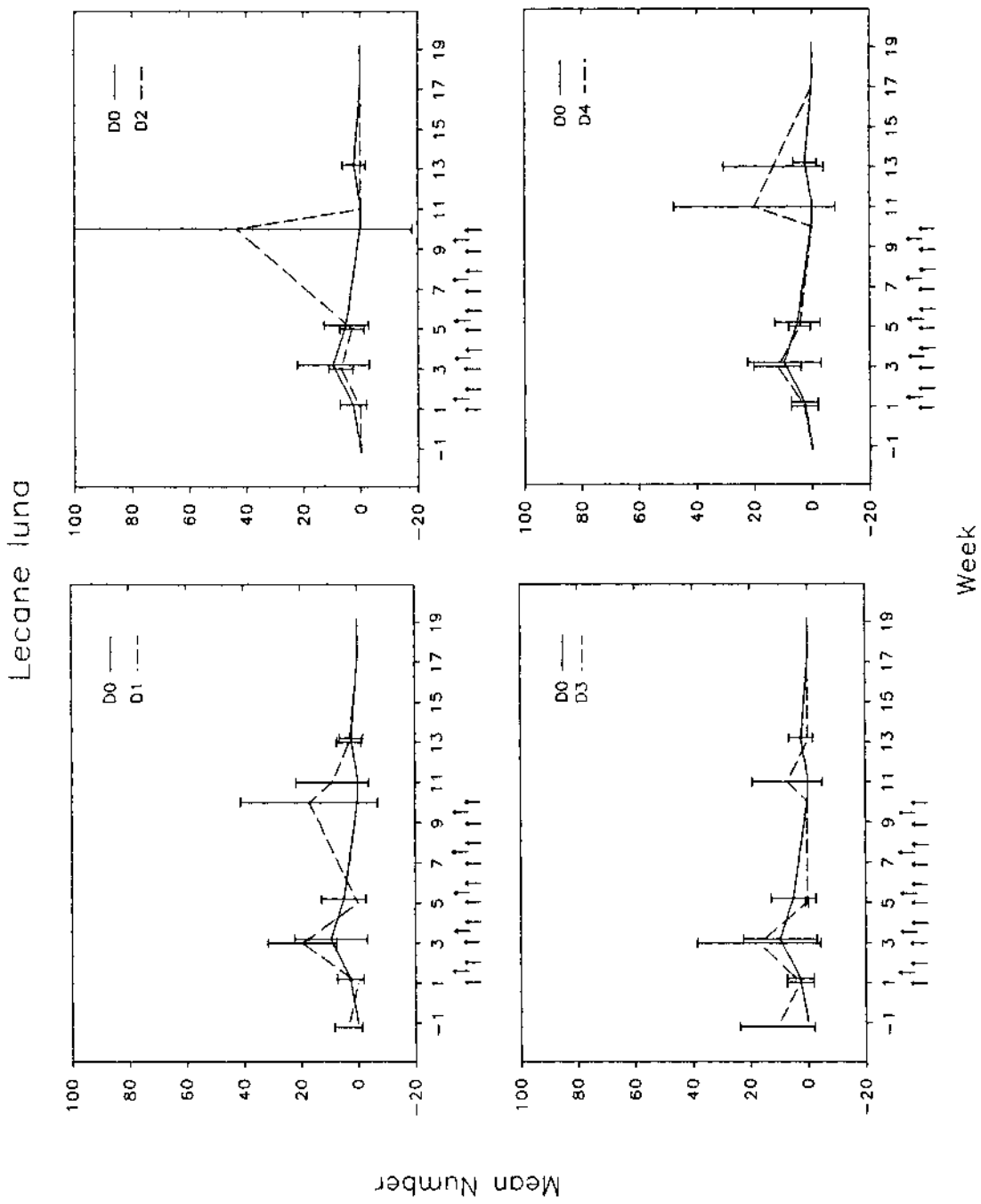

Figure 30. *Lecane luna* population (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

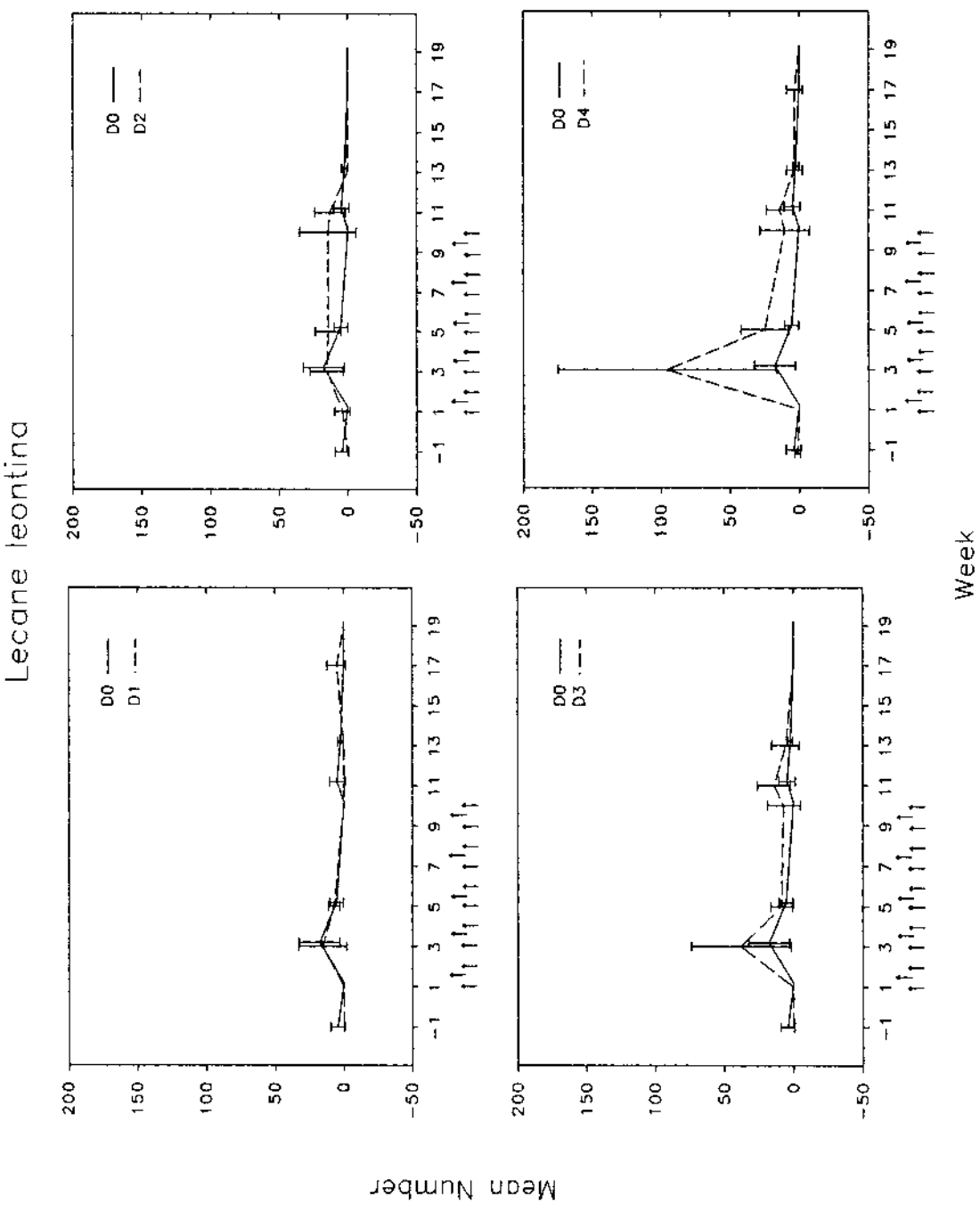

Figure 31. *Lecane leontina* population (mean ± S.D.) through time. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

only significant difference observed at D3 (T-Test with Bonferroni correction, α=0.05). Percent survival was significantly reduced in treatments D1, D3, and D4 at 48 and 72 h.

Discussion

Zooplankton Community Structure

Heimbach et al. (1992) studied impacts of cyfluthrin on zooplankton in small, unreplicated microcosms. They observed reductions in Crustacea, particularly *Daphnia*. *Daphnia* recovered within 2-4 weeks after the single application. Increases in Rotifera populations were also observed.

Yasuno et al. (1988) observed reductions in the rotifers *Hexarthra mira*, but numbers of *Keratella valva* increased after permethrin applications to pond enclosures. The rotifers *Polyarthra trigla*, *Brachionus angularis* and *Keratella cochlearis* did not respond to pyrethroid application. The crustaceans *Daphnia rosea* and *Acanthodiaptomus pacificus* both were reduced by permethrin.

Kaushik et al. (1985) also studied permethrin and found enhancements of *Keratella cochlearis*, *Kellicotia longispina* and *Polyarthra* sp. in limnocorrals. Total rotifer numbers increased during this study while Cladocera and copepods were reduced.

Fenvalerate application to limnocorrals resulted in increased rotifer populations and decreases in Cladocera (Day et al. 1987). Nauplii recovered within one week, while cladocerans recovered in three weeks. Cyclopoid copepods took five weeks to recover, reflecting a longer generation time.

Lozano et al. (1992) found copepods to be sensitive to esfenvalerate applied to mesocosms, but Chydoridae (*Alona*, *Chydorus*, *Pleuroxus*) and Daphnidae (*Ceriodaphnia*, *Simocephalus*) were less affected. This differed from the fairly high sensitivity of *Chydorus sphaericus* in the microcosm study, but the *Alona* results were similar. Eight rotifer genera increased in abundance after application of esfenvalerate, and total rotifer abundance was positively correlated with esfenvalerate concentration (Lozano et al. 1992). A mesocosm study in North Carolina with lambdacyhalothrin (Hill et al. 1988) produced significant reductions in crustaceans, but no rotifer enhancements were noted in this short paper.

Webber et al. (1992) noted reductions in copepod and nauplii populations due to esfenvalerate, while rotifers were enhanced at the high dose rate. Cladoceran populations were eliminated from all mesocosms prior to dosing (due to fish predation), making pesticide impacts impossible to evaluate.

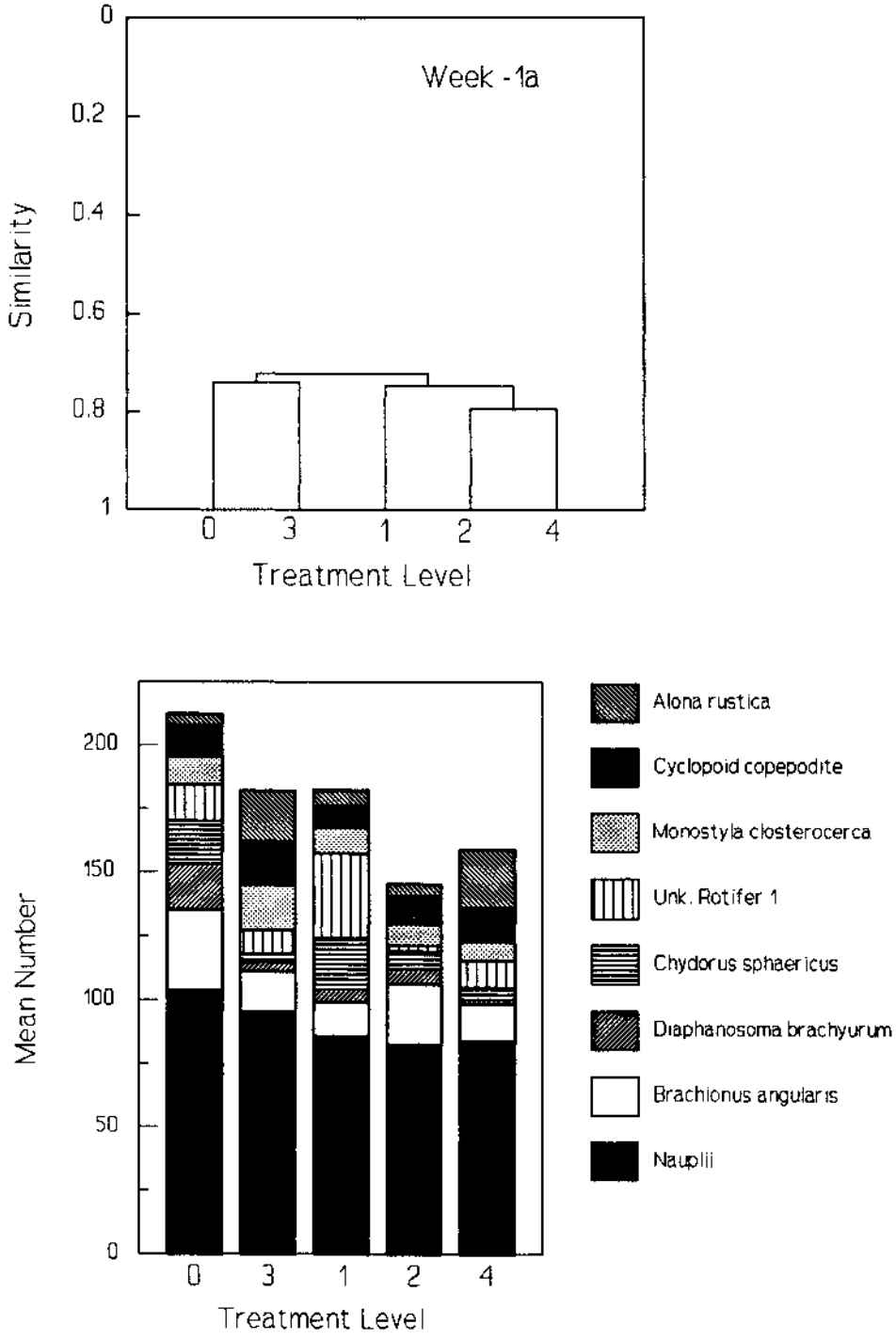

Figure 32. Bray-Curtis cluster analysis of zooplankton populations one week prior to application. Treatment levels range from controls (0) to high rate (4).

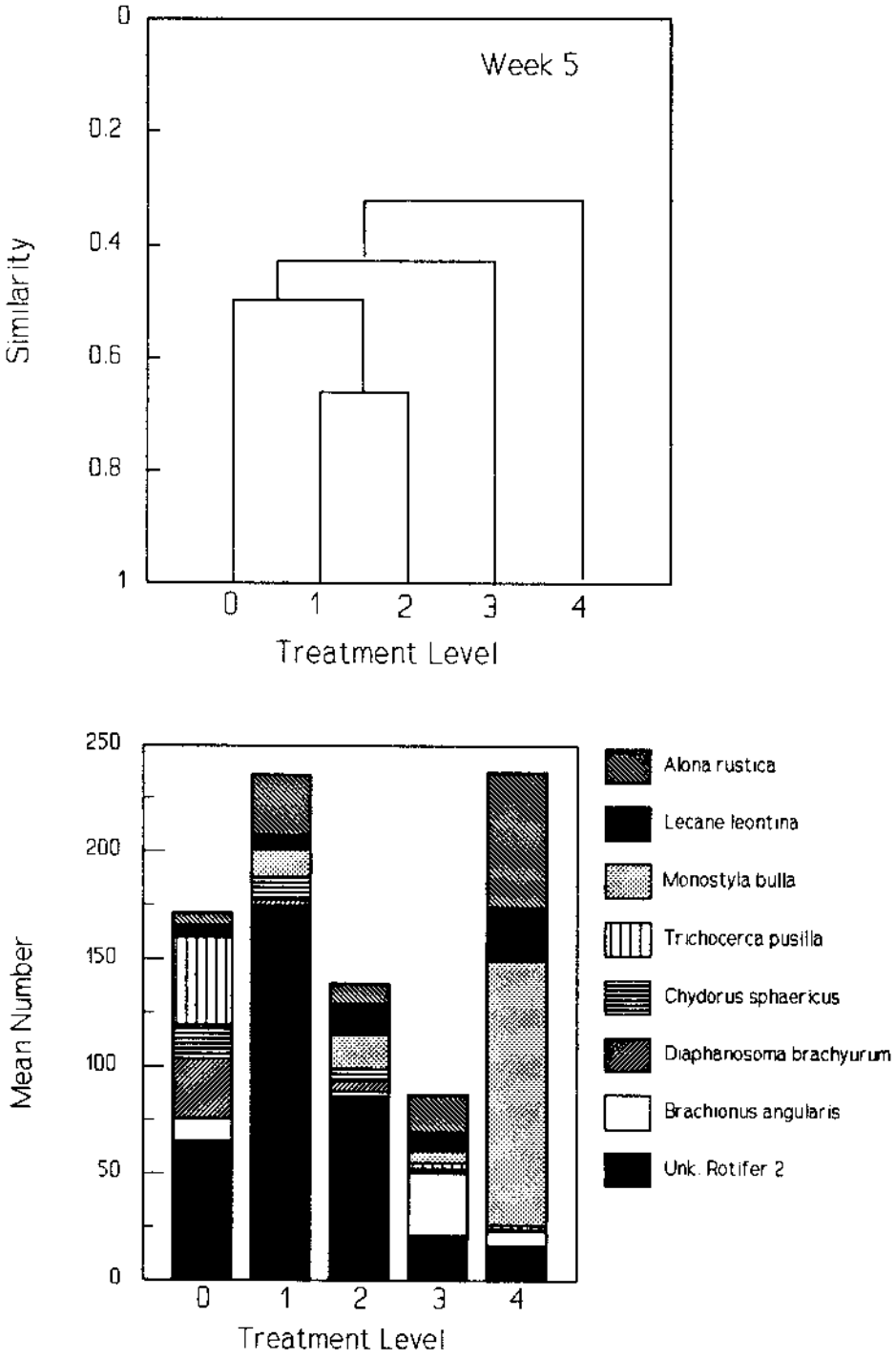

Figure 33. Bray-Curtis cluster analysis of zooplankton during the middle of the application period. Treatment levels range from controls (0) to high rate (4).

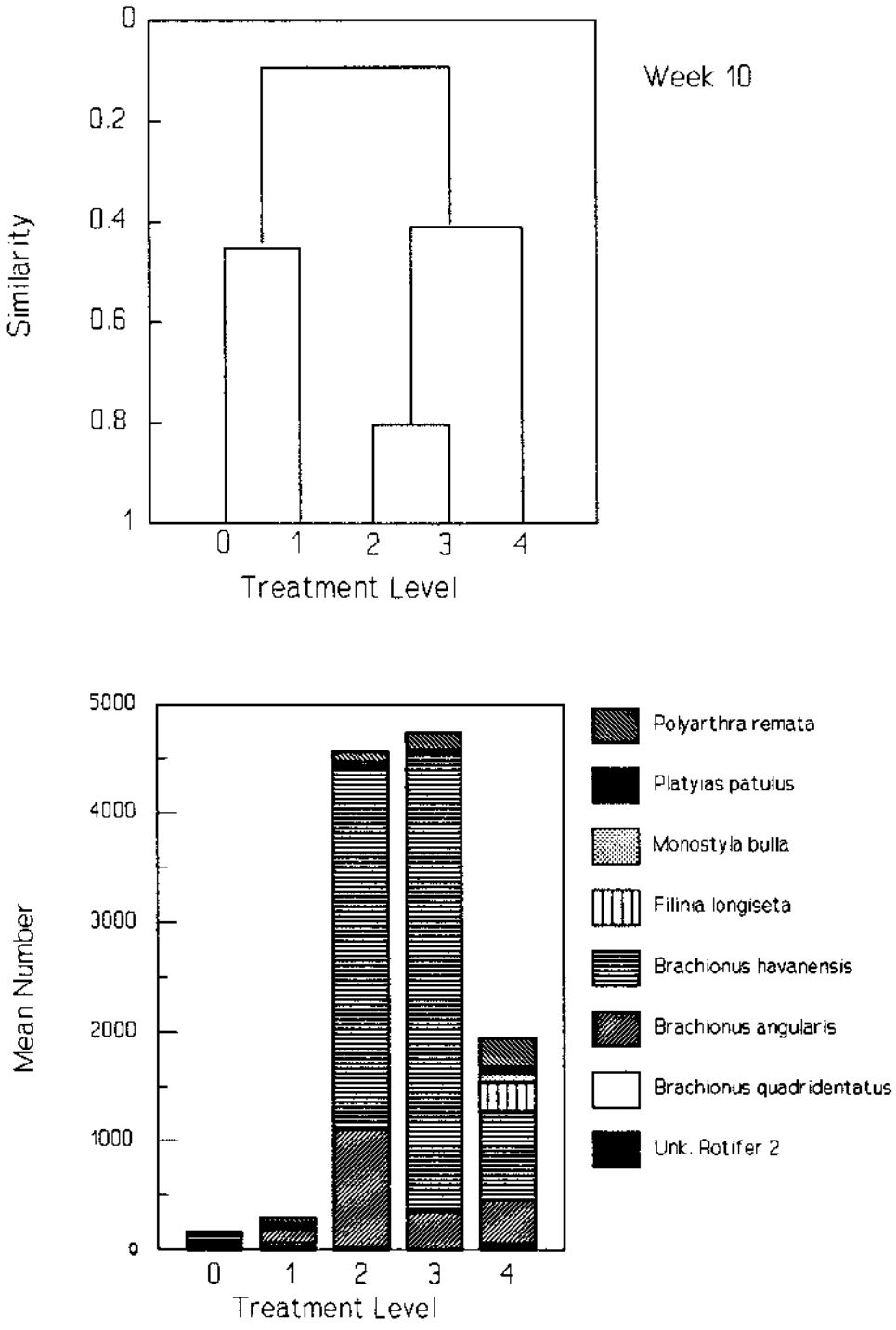

Figure 34. Bray-Curtis cluster analysis of zooplankton at the end of the application period. Treatment levels range from controls (0) to high rate (4).

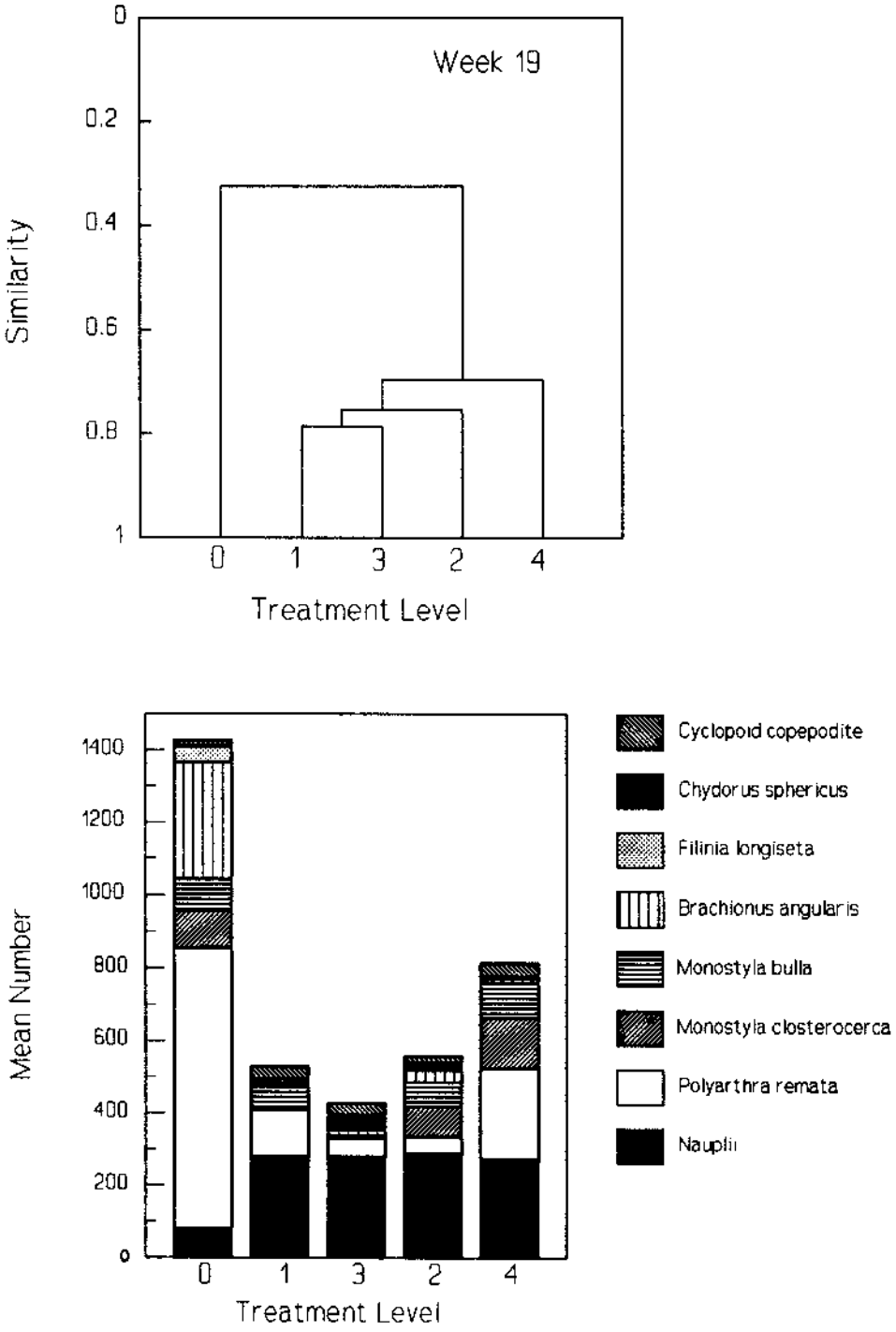

Figure 35. Bray-Curtis cluster analysis of zooplankton at the end of the experiment. Treatments range from control (0) to high rate (4).

Bluegill predation also masked esfenvalerate impacts on Cladocera in a Missouri mesocosm study (Fairchild et al. 1992). Copepods were sensitive to the pyrethroid, with an unusual "spiking" pattern (population pulses) noted in treated mesocosms. Rotifers again increased at high rates.

Experiments with single and repeated applications of carbaryl (a carbamate insecticide) found that single applications resulted in rapid cladoceran recovery, with suppression of rotifer blooms (Hanazato and Yasuno 1990). Repeated applications resulted in large rotifer populations. The later scenario is similar to the microcosm study, where repeated applications of a short-lived pesticide resulted in large rotifer populations.

Knowledge of zooplankton life histories can add perspective to observed responses. For example, the rotifer *Brachionus angularis* are considered r-strategists (Walz 1987), showing higher reproductive, mortality and population growth rates than *Keratella cochlearis*, another rotifer species. In this study, *B. angularis* and *B. havanensis* were two of the dominant species present in pesticide-related rotifer blooms. *B. quadridentatus* (Figure 25) did not exhibit this pattern, indicating the importance of species-level identifications whenever feasible.

In a review paper, Niemi et al. (1990) found that cladocerans and rotifers subjected to pulse stressors such

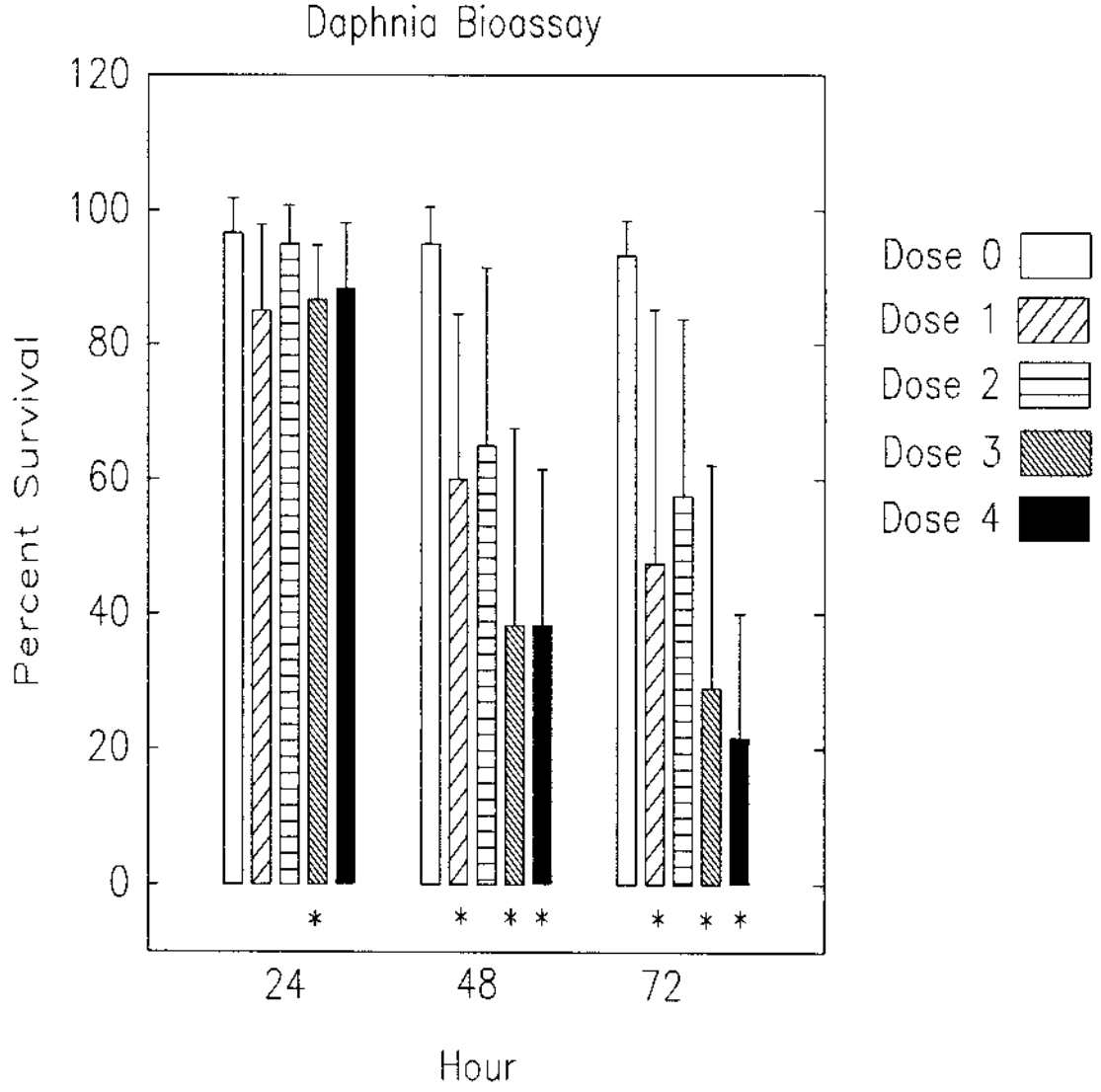

Figure 36. *Daphnia magna* water-column bioassay. Neonates were exposed to a single pulse dose event over a 72 hour period. Water was collected from microcosms surface waters one hour following a spray-drift application.

as insecticides recovered within an average of 0.28 to 0.35 years, respectively. Copepods took longer to recover, with an average of 0.88 years for recovery. Soto (1989) noted that parthenogenetic species, such as cladocerans and rotifers, have greater capabilities for both colonization and persistence since a single individual represents a viable propagule. Copepods, with obligate sexual reproduction, have a reduced likelihood of colonization and an increased probability of low density populations becoming extinct. Furthermore, those sexual taxa with the ability to store sperm (most cyclopoid copepods) may increase the odds of successful colonization compared with taxa that cannot, such as most freshwater calanoids (Soto 1989).

Little research has been done exploring why various cladocerans differ in sensitivity to pesticides. One explanation might be exposure, with *D. brachyurum* being a more planktonic species (potentially exposed to higher surface concentrations), while many Macrotricidae (*Macrothrix*) were associated with benthic deposits (Campbell and Clark 1987). Members of the genus *Alona* were commonly found in periphytic habitat in Texas ponds (Campbell and Clark 1987).

The general zooplankton community response in microcosms was one of reduced taxonomic richness combined with increased numbers of a few species. This general

pattern has been observed in many studies (see Hurlbert 1975), and this response to environmental stress has been called "Thienemann's Principle" (Heckman 1983).

In summary, pyrethroid impacts on zooplankton communities were quite predictable, having been well documented in the past using systems ranging from small microcosms (Heimbach et al. 1992) and small enclosures (Yasuno et al. 1988), to larger limnocorrals (Kaushik et al. 1985, Day et al. 1987), littoral corrals (Lozano et al. 1992) and pond mesocosms (Hill et al. 1988, Webber et al. 1992, Fairchild et al. 1992). The cyfluthrin microcosm experiment identified similar responses, suggesting that smaller scale systems may prove useful for assessing pesticide impacts on zooplankton communities.

Daphnia Bioassay

Xiu et al. (1989) found little acute toxicity of deltamethrin to 48-72 h old juvenile *D. magna* during the first 24 h period, but acute toxicity increased greatly at 48 and 96 h. Neonates (6-24 h old), on the other hand, were impacted heavily by deltamethrin at 24 h. They suggest that this phenomenon may reflect the frequency of molts and metabolic rates of neonates vs. juveniles. Their data paralleled these results (Figure 36), since *D. magna* individuals in the microcsom study were approximately 24-48

h old at the time of testing (individuals were allowed to acclimate to microcosm control water overnight prior to testing).

In standard bioassays with fenvalerate, *Daphnia galeata mendotae*, *Ceriodaphnia lacustris*, and *Diaptomus oregonensis* were all more sensitive than *D. magna* (Day and Kaushik 1987b). Day (1989) suggested that field toxicity results were more accurately predicted in the laboratory using bioassays conducted on field-collected organisms, compared to tests using the standard bioassay organism, *D. magna*. In the cyfluthrin test, no native *Daphnia* were present in microcosms, with *Diaphanosoma brachyurum* being the closest analog. Statistically significant reductions were observed at D1 for both *D. magna* (percent survival) and for *D. brachyurum* (population density). In this sense, both tests were of similar sensitivity. Since the NOEC for both *D. magna* and *D. brachyurum* would lay somewhere below concentrations used for the microcosm test, more detailed comparison of the sensitivity of these two assays cannot be conducted.

Summary

Some cladocerans (notably *D. brachyurum* and *C. sphaericus*) were significantly reduced by BAYTHROID^{R} application. Other cladocerans were unaffected or increased

in density (*A.rustica* and *M. rosea*). Total cladoceran numbers increased beyond control levels once pesticide stress was removed.

Population densities of many rotifer species increased following cyfluthrin application. These included *B. angularis*, *B. havanensis*, *M. bulla* and *P. remata*. Some of these taxa (i.e., *B. angularis*) were r-selected strategists that could exploit conditions when competitors or predators were reduced.

These two general responses (reductions in cladocerans and subsequent rotifer blooms) have been noted during other field and enclosure experiments.

Bioassays with *D. magna* using treated microcosm surface water were generally as sensitive as toxicological responses in the most pyrethroid-sensitive cladoceran inhabiting microcosms (*D. brachyurum*). Lowest observed effect levels (LOEL) were at the lowest treatment level (D1) for both approaches.

CHAPTER 7

MACROINVERTEBRATES - GENERAL RESPONSE

Introduction

Pyrethroids affect almost all invertebrates when introduced into aquatic systems, either directly through toxicity or indirectly through species interactions and reduced prey base (Smith and Stratton 1986). Arthropods are approximately one order of magnitude more sensitive than fish (Haya 1989).

Aquatic invertebrates differ in sensitivity to pyrethroid exposure. Anderson (1982) found decreasing sensitivity from amphipods > mayflies > stoneflies and caddisflies > snails. The duration of exposure is critical (Anderson and Shubat 1984), thus pyrethroid impacts in streams should be less than lakes or ponds. "Pulse-dose" exposures (rapid declines in water concentration due to sorption and degradation) are typical of the pyrethroids, and bioassays with continuous replacement of fresh pesticide may not reflect true exposures (Clark et al. 1987, Clark et al. 1988, Jarvinen et al. 1988, Clark et al. 1989, Baughman et al. 1989).

Water mites, amphipods and mayflies were more sensitive to cypermethrin than were *D. magna*, with dipteran larva and

corixids being the least sensitive (Stephenson 1982). In marine systems, mysid shrimp and grass shrimp were very sensitive (Clark et al. 1987, Clark et al. 1989).

The acute LC_{50} of permethrin for *Daphnia magna* (0.2 - 0.6 µg/L) was approximately ten times lower than the LC_{50} for mosquito larva (2.5 µg/L), thus larvicidal applications of pyrethroids for mosquito control may not be recommended (Stratton and Corke 1981).

Incoordination and lack of feeding has been observed in benthic macroinvertebrates (Anderson 1982). Grass shrimp development (completion of metamorphosis) may be affected at low fenvalerate concentrations (McKenney and Hamaker 1984). Biochemical parameters such as RNA, DNA, ADP and glycogen levels responded to sublethal concentrations of fenvalerate (McKee and Knowles 1986).

Mollusks (such as snails) were tolerant of pyrethroids, with no acute effects at water solubility (Anderson 1982, Spehar et al. 1983, Coats et al. 1989). In marine systems, oysters were insensitive (Clark et al. 1989).

Laboratory systems containing both water and sediment showed reduced (5 fold) toxicity to invertebrates compared to water alone (Hill 1985). Thus, investigation of toxic effects in natural systems is necessary for informed hazard assessment.

Muirhead-Thompson (1978) noted increased drift (releasing from substrate and drifting down-stream) in invertebrates after permethrin application. Mayflies, amphipods, and blackfly larva increased drift at both lethal and sublethal concentrations. The Trichopteran *Hydropsyche* showed delayed drift, with no drift during the 30 minute experimental period but substantial drift within an hour. The caddisfly *Brachycentrus* showed little propensity to drift even at lethal concentrations. Cypermethrin also may increase sublethal drift (Crossland et al. 1982).

Application of permethrin and deltamethrin to riverine forest habitat in Africa for tsetsefly control resulted in near elimination of mayfly larva and small shrimp (Everts et al. 1983). Caddis and blackflies were affected but showed quick recovery. Surface dwelling insects such as water-striders showed high mortality. The application of pyrethroids did not have a sufficient effect on the tsetse population (Everts et al. 1983).

Mayflies, amphipods, water mites and surface-dwelling groups such as Notonectidae, Corixidae, Gerridae and Velidae were often strongly affected by pyrethroids (Miura and Takahashi 1976, Crossland 1982, Hill 1985, Shires and Bennett 1985, Helson and Surgeoner 1986, Hill et al. 1988). Oligochaetes were often unaffected (Hill 1985, Shires and Bennett 1985). Fenvalerate, however, reduced the number of

annelid species colonizing estuarine colonization chambers (Tagatz et al. 1987).

Chironomids can be either target or nontarget species, depending on their numbers and location (Anderson 1989). Chironomid responses will be detailed in the next chapter.

Results

Total Numbers and Taxonomic Richness

The total number of organisms colonizing artificial substrate samplers (MAS) in treated microcosms were either equal to controls or were higher (Figure 37). Statistically significant increases were found for D3 during week 9 only (Table XI). Total numbers of exuviae collected from treated microcosms were generally reduced by pesticide application (Figure 38). Total exuviae were significantly reduced during weeks 2-8, at levels as low as D1 (Table XII). The total number of emerging insects collected in funnel traps was also reduced (Figure 39), particularly in D3 and D4. Significant differences in emergence trap populations were found during the treatment period (Table XIII) for D3 and D4.

Taxonomic richness (total number of taxa collected for a given sampling period) in MAS was calculated at four time periods. These included pre-application (week -1), middle of application period (week 5), end of application period

(week 9), and end of study sampling (week 19). These were chosen since larval chironomids (the most diverse group in the microcosms) were mounted on slides and identified to genus (or species-group when possible) on these dates. At other times the Chironomidae were only identified to subfamily, making richness values questionable. MAS richness was reduced with treatment (Figure 40), with significant declines at D4 during weeks 5, 9 and 19 (Table XI). All treatments were significantly impacted during week 19. Reductions in exuviae taxa richness demonstrated increasing impact with treatment level (Figure 41), with significant reductions at treatments D3 and D4 (Table XII). Emergence trap richness showed few significant declines (Table XIII) due to high control and treatment variability (Figure 42).

Responses of Abundant Taxa

Various macroinvertebrate taxa differed in sensitivity to cyfluthrin. One of the most heavily impacted groups was the phantom midge *Chaoborus*. While MAS samples showed no significant impacts (Table XI) due to low sample size (Figure 43), exuviae samples demonstrated dramatic, significant reductions in this group across all treatment levels for most of the experiment (Figure 44, Table XII). Emergence trap sampling collected few *Chaoborus* (Figure 45),

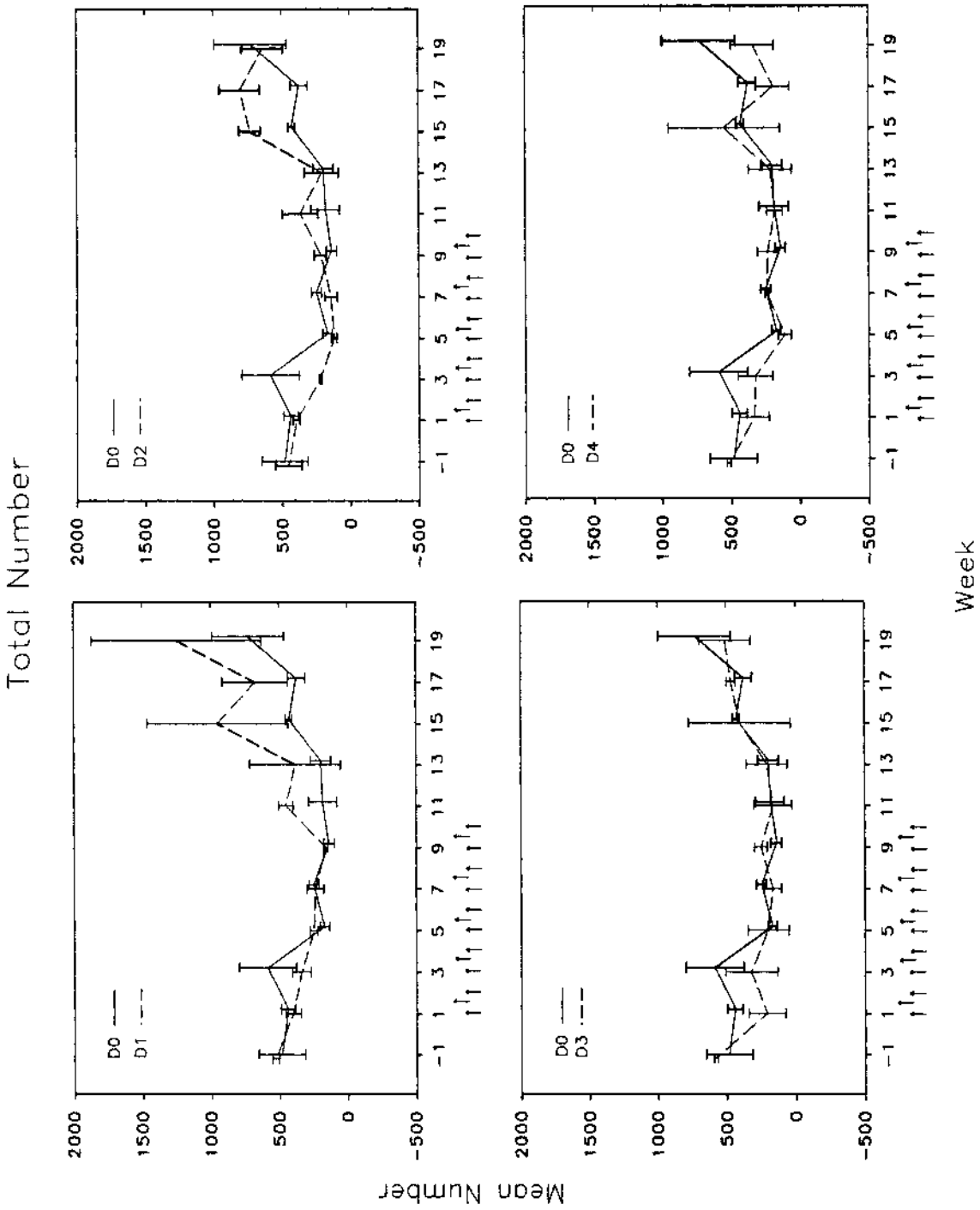

Figure 37. Total number of organisms collected from MAS samplers in microcosms. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4. Arrows indicate pyrethroid applications.

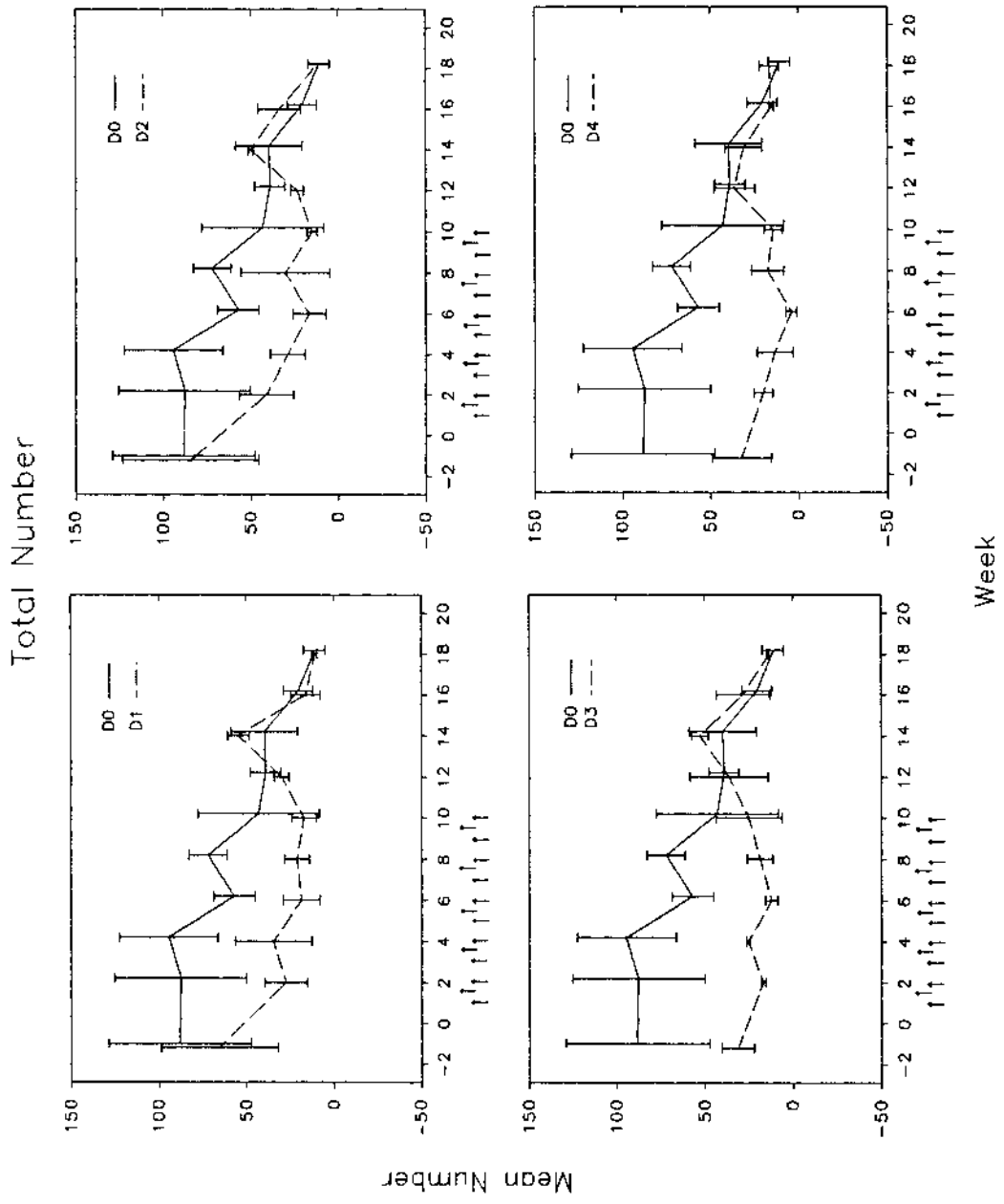

Figure 38. Total number of exuviae collected from microcosms. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4. Arrows indicate pyrethroid applications.

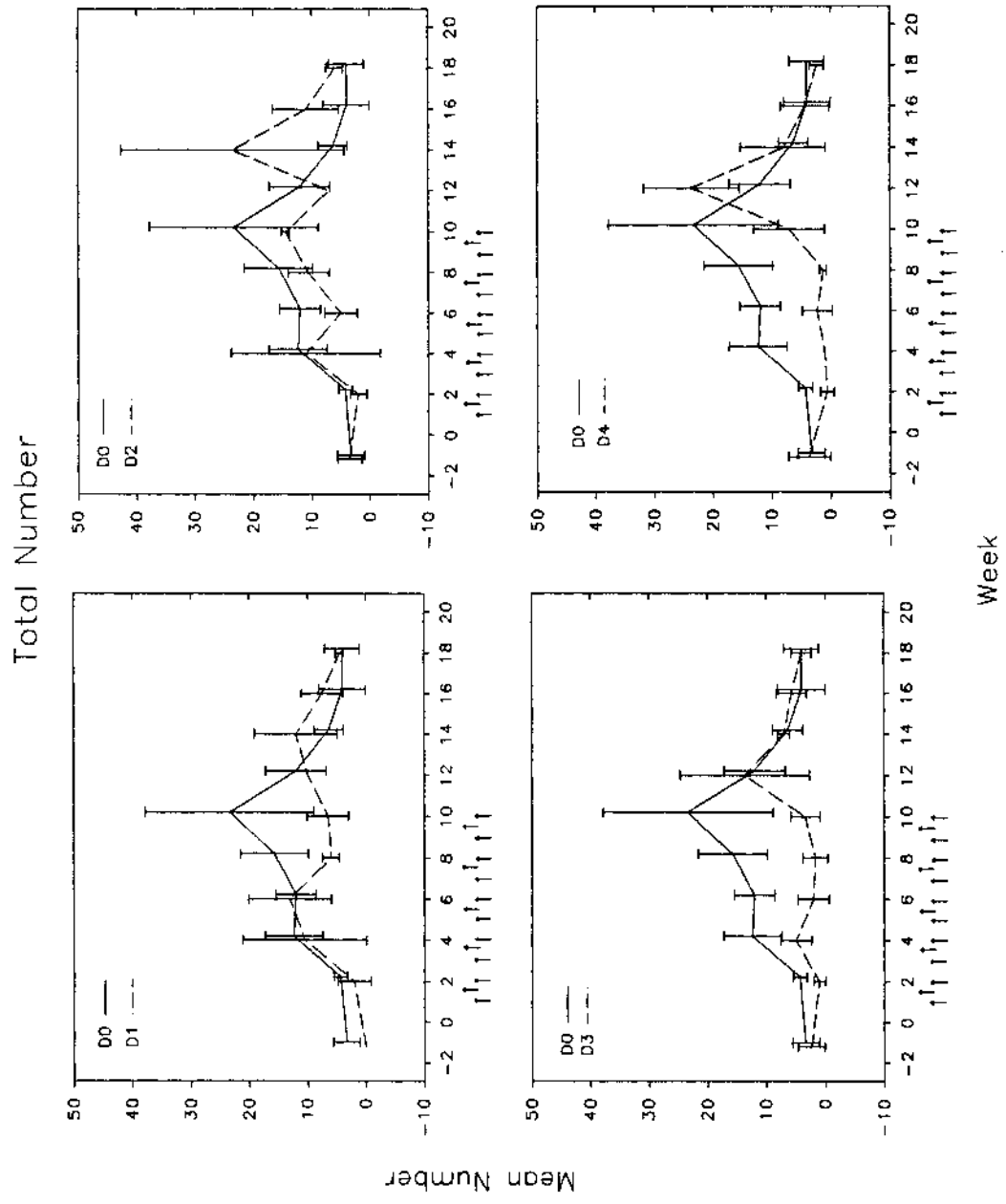

Figure 39. Total number of individuals collected from microcosm emergence traps. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4= Dose 4. Arrows indicate pyrethroid applications.

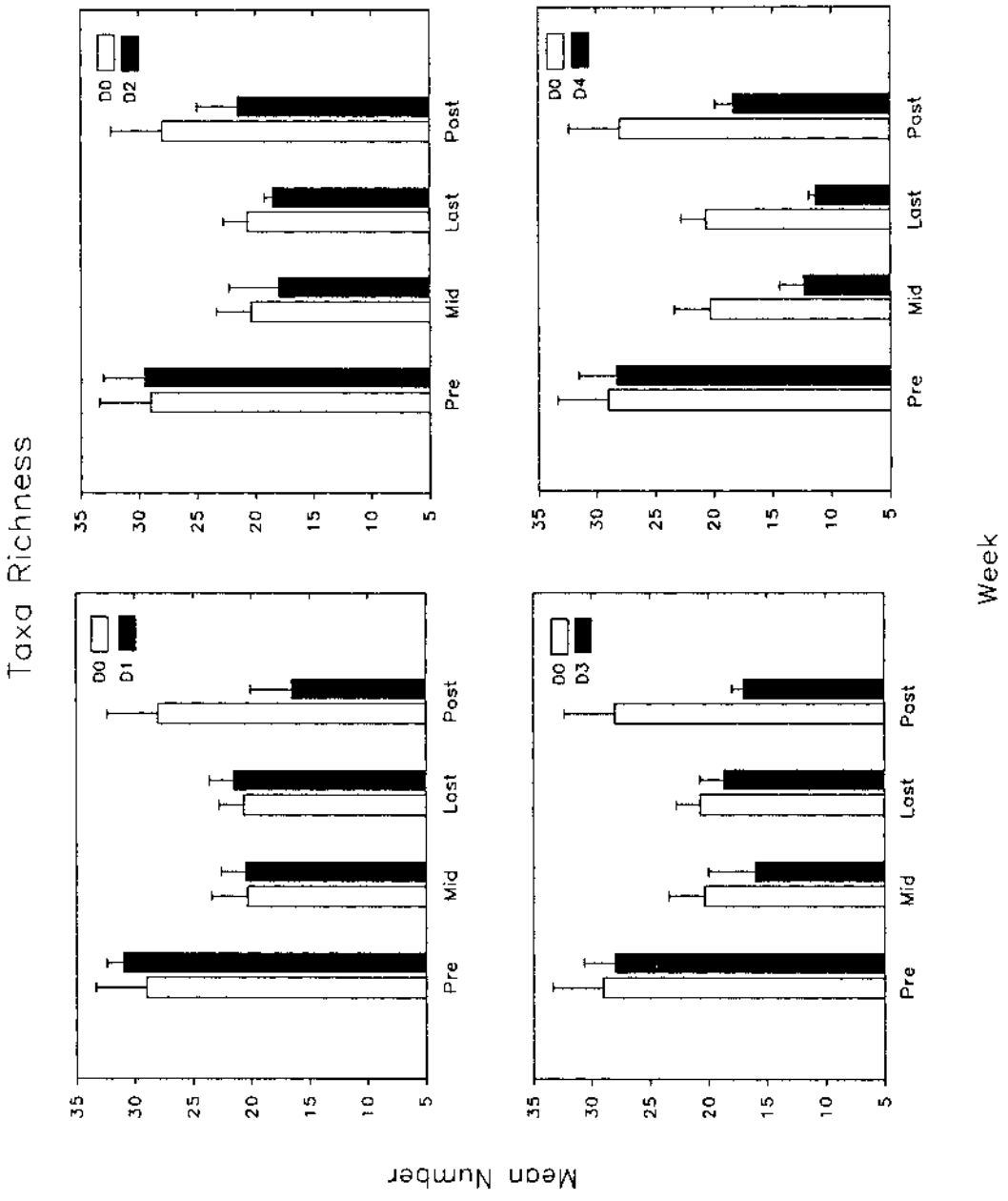

Figure 40. Taxa richness (number of taxa) collected from microcosm MAS samplers. Pre=Before first application, Mid=Middle of application period, Last=Final application, Post=Study termination.

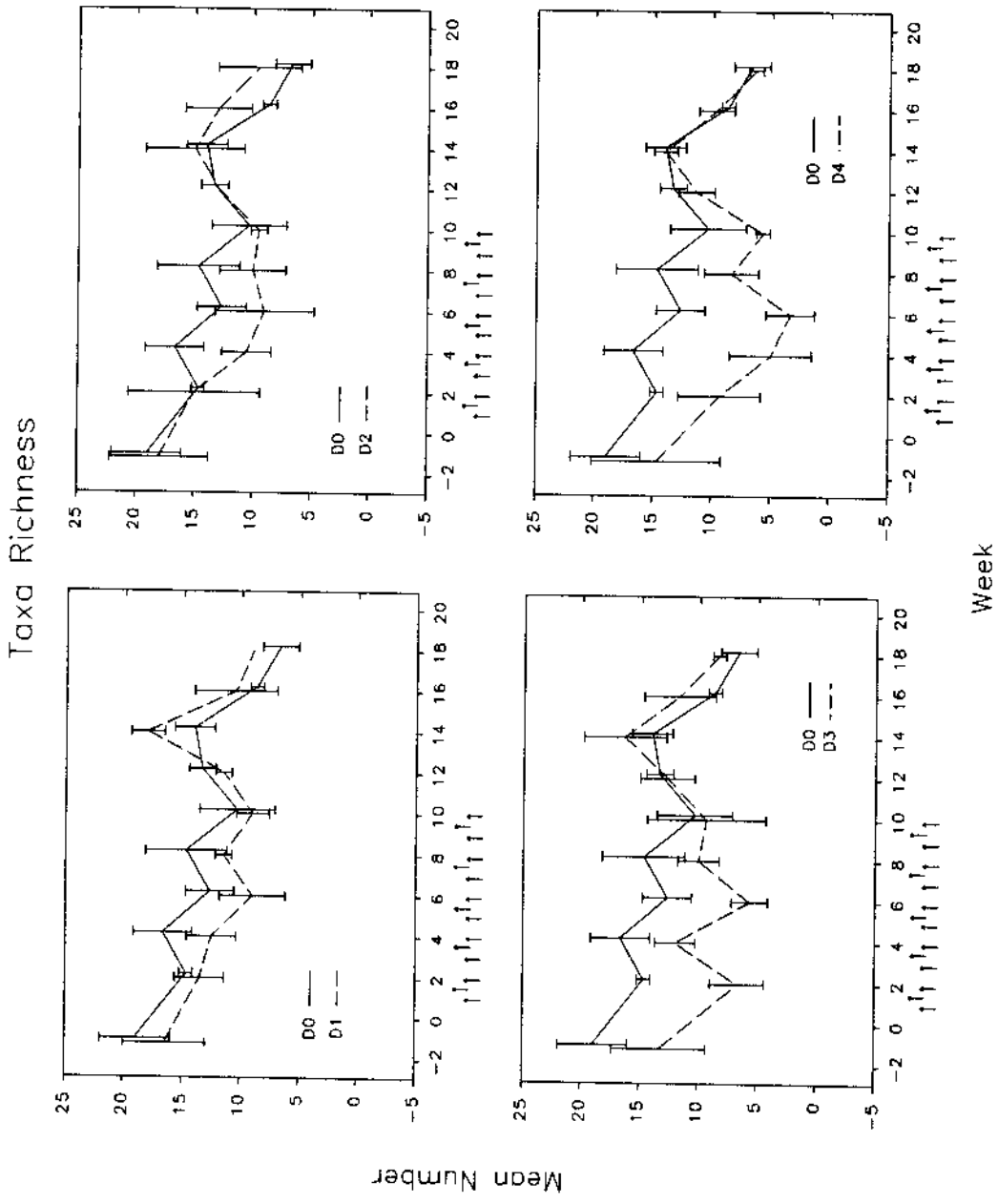

Figure 41. Taxa richness (number of taxa) of exuviae collected from microcosms. D0=Controls, D1=Dose Level 1, D2=Dose Level 2, D3=Dose Level 3, D4=Dose Level 4. Arrows indicate pyrethroid applications.

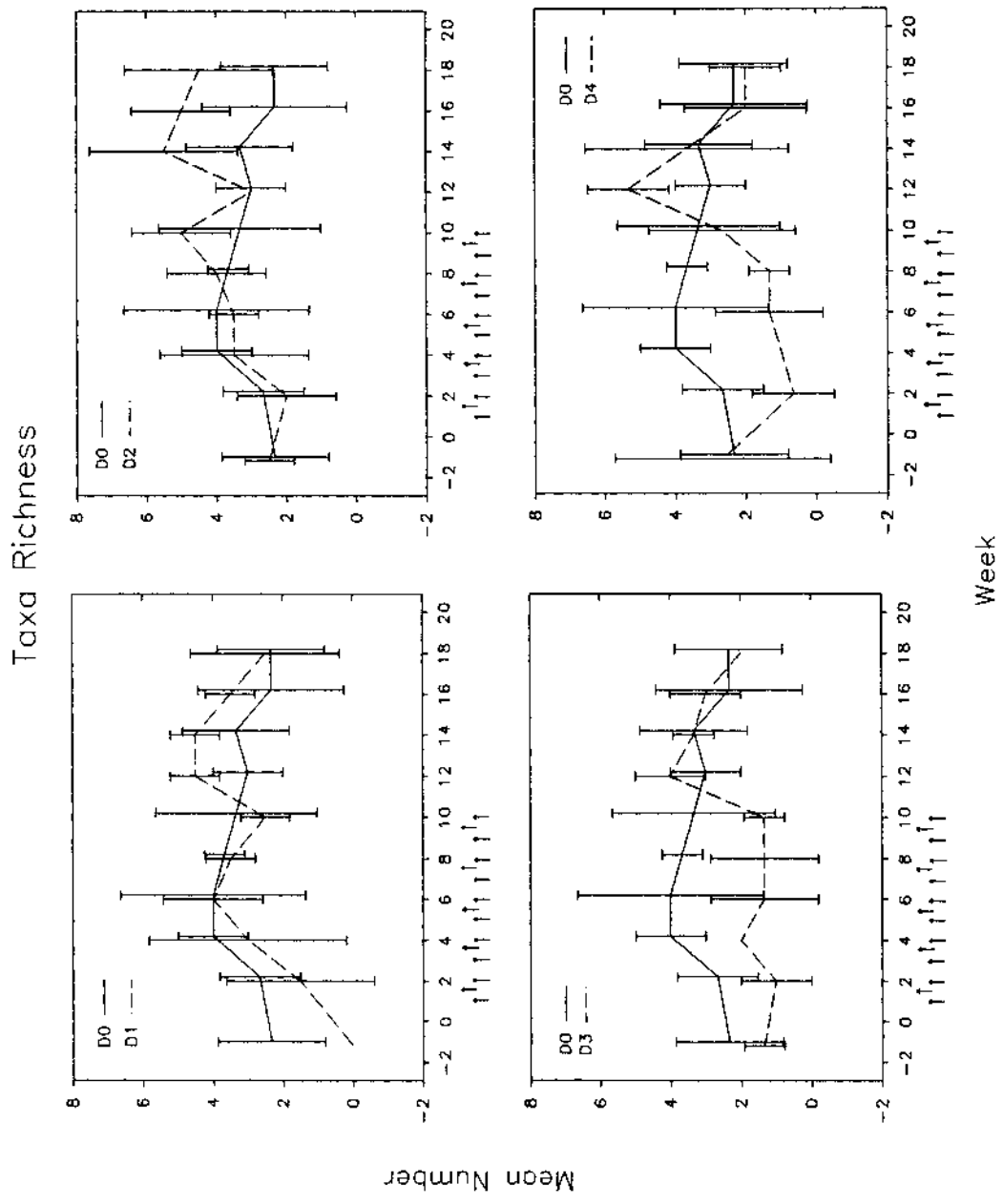

Figure 42. Taxa richness (number of taxa) collected with emergence traps in microcosms. D0=Controls, D1=Dose Level 1, D2=Dose Level 2, D3=Dose Level 3, D4=Dose Level 4. Arrows indicate pyrethroid applications.

with significant impacts observed during week 4 only (Table XIII).

Four genera of caddisflies were commonly collected from microcosm MAS samplers; *Oecetis* (Leptoceridae), *Orthotrichia* (Hyroptilidae), *Oxyethria* (Hydroptilidae) and *Cyrnellus* (Polycentopodidae). All the *Oecetis* keyed to species were *inconspicua*. *Triaenodes* (Leptoceridae) was represented by a single larvae from one micrcosm. Due to low sample sizes, total Trichoptera were graphed (Figure 46), however generic level analyses were performed for calculation of statistical significance (Table XI). Trichopterans were significantly reduced at all treatment levels (D1-D4), primarily during week 3 when control densities were maximal. All trichopterans were essentially reduced to zero by repeated cyfluthrin applications (Figure 46). Caddisfly densities were too low in exuviae or emergence traps to allow estimation of impacts on emergence.

Three mayfly genera were collected from microcosms; *Callibaetis* (Baetidae), *Caenis* (Caenidae) and *Hexagenia* (Ephemeridae). *Hexagenia* were rare, precluding analysis of impacts for this group. *Callibaetis* were keyed to the species *floridanus* (Check 1982), and all nymphs appeared to belong to a single species. This group was reduced in cyfluthrin treated microcosms (Figure 47 and 48), with significant differences detected in MAS and exuviae samples

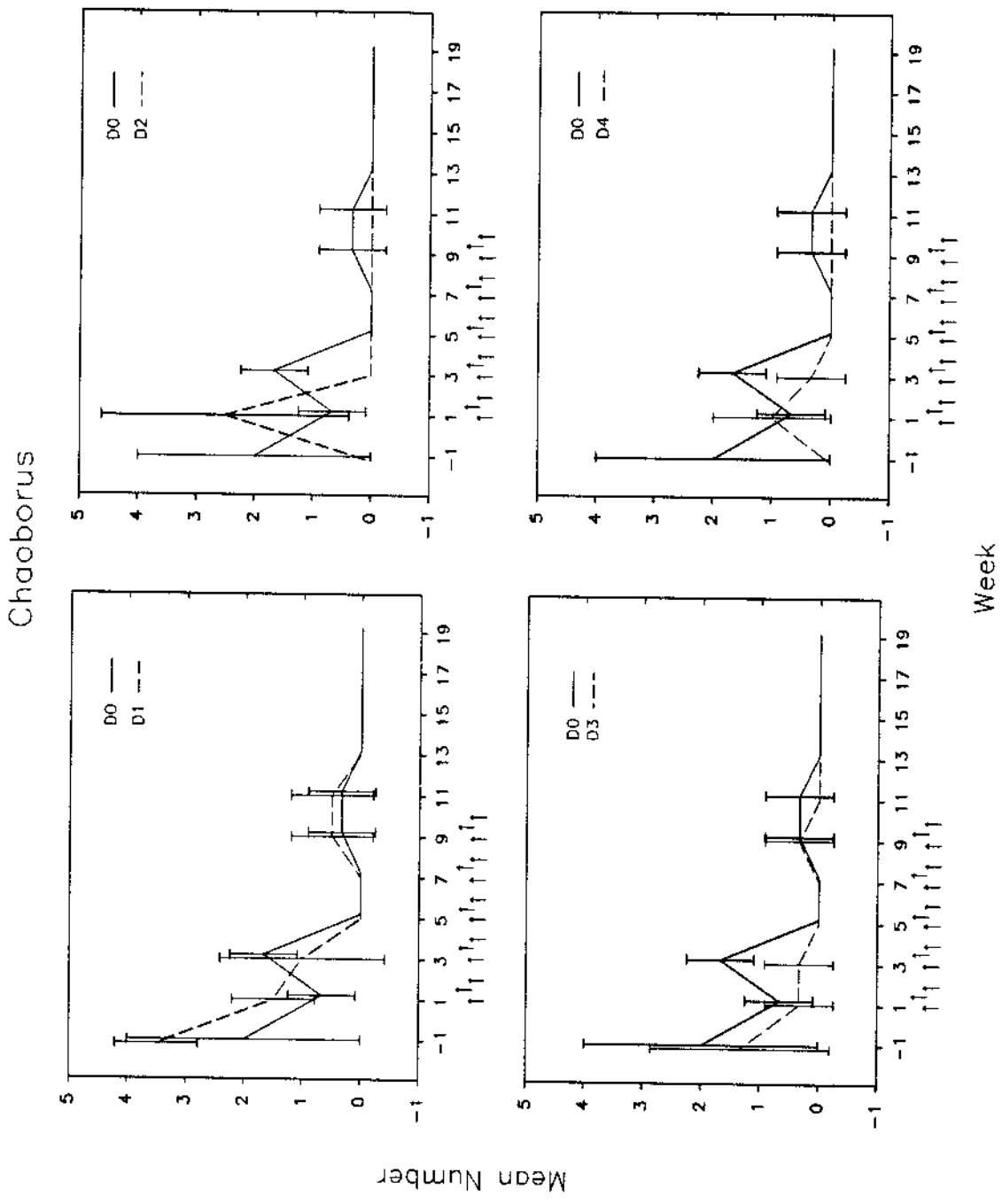

Figure 43. Mean number of *Chaoborus* larvae (± S.D.) colonizing artificial substrates during the experiment. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

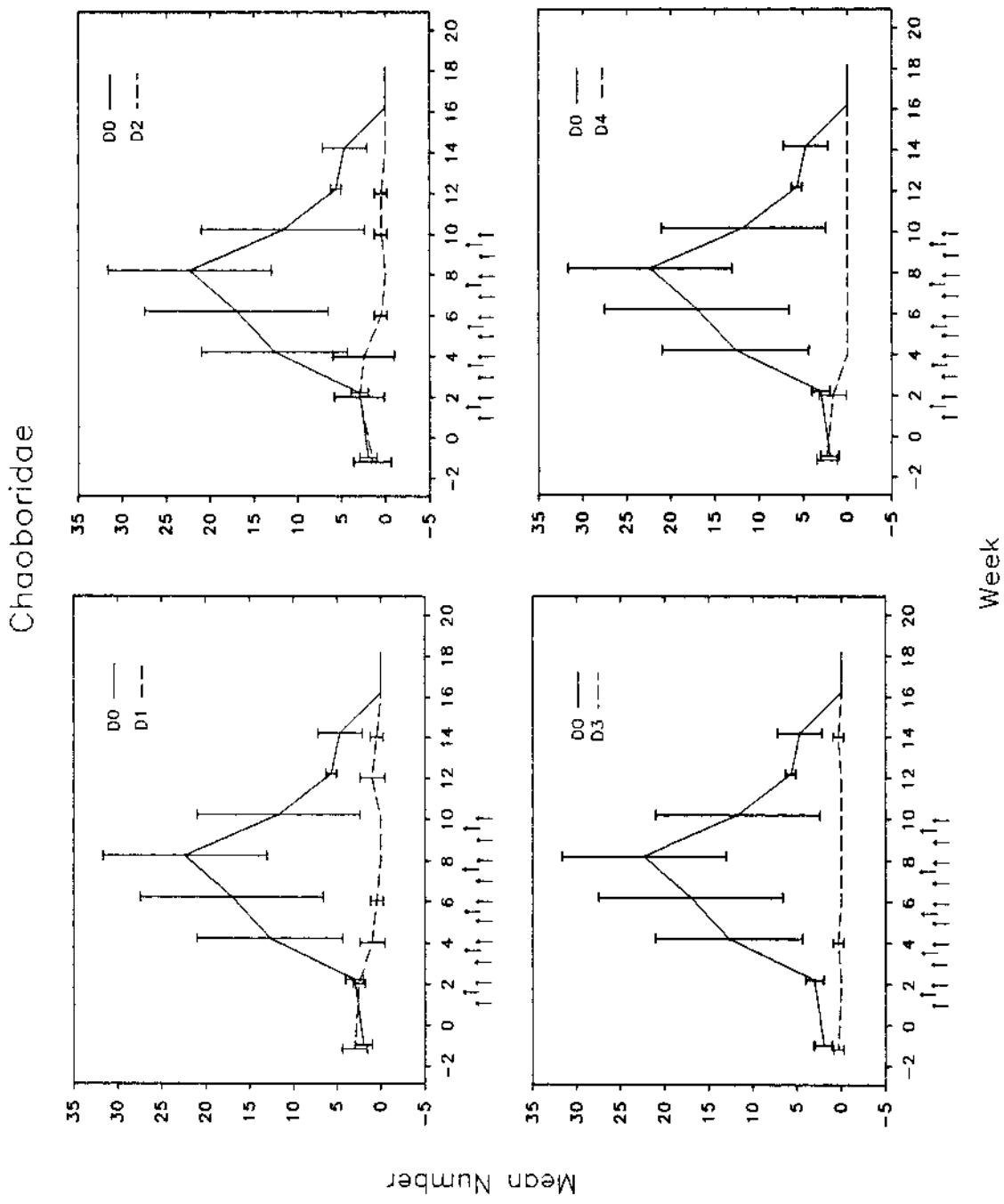

Figure 44. Mean number of *Chaoborus* exuviae (± S.D.) collected from microcosms. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

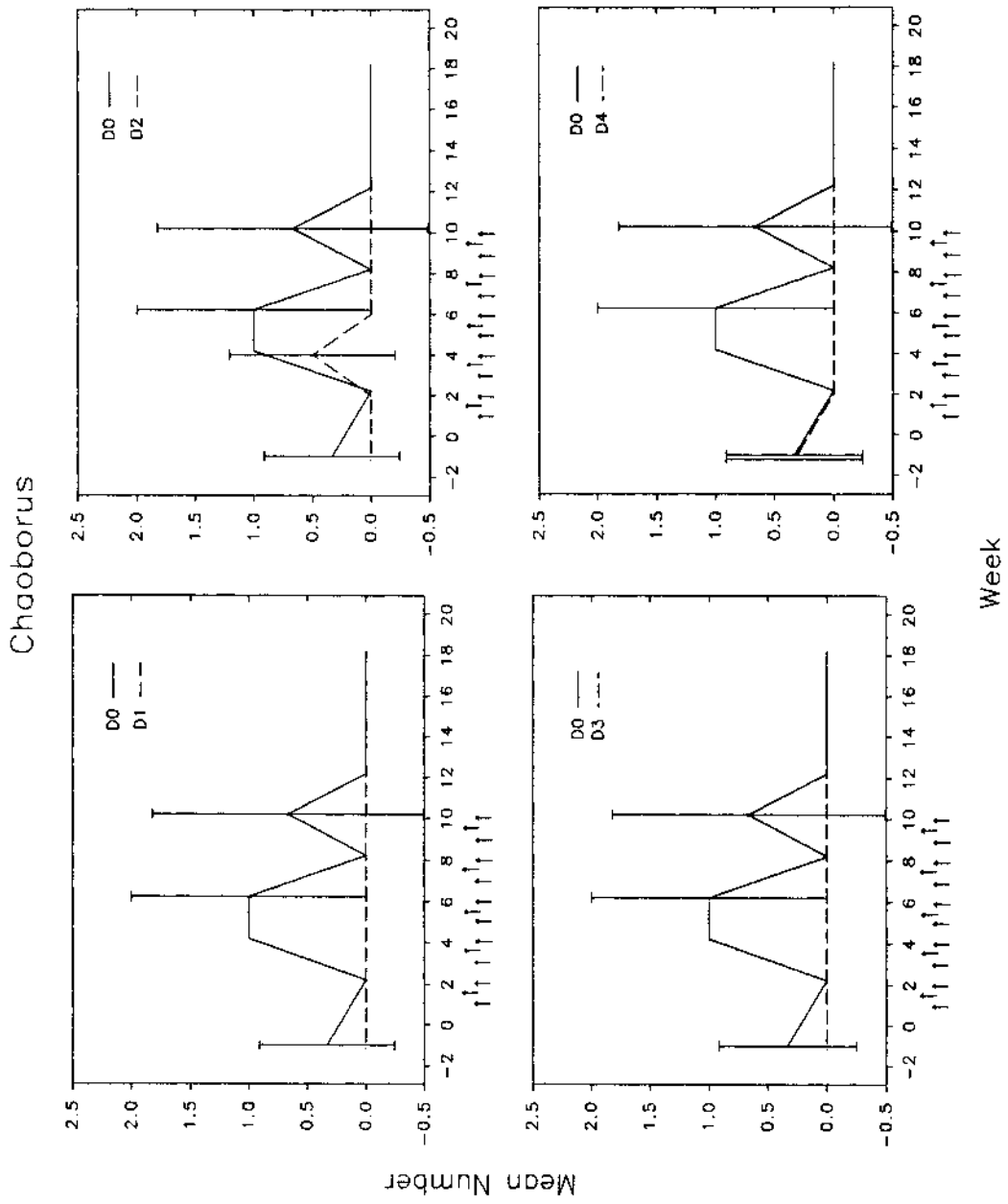

Figure 45. Mean number of *Chaoborus* adults collected in emgergence traps (mean ± S.D.). Arrows represent pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

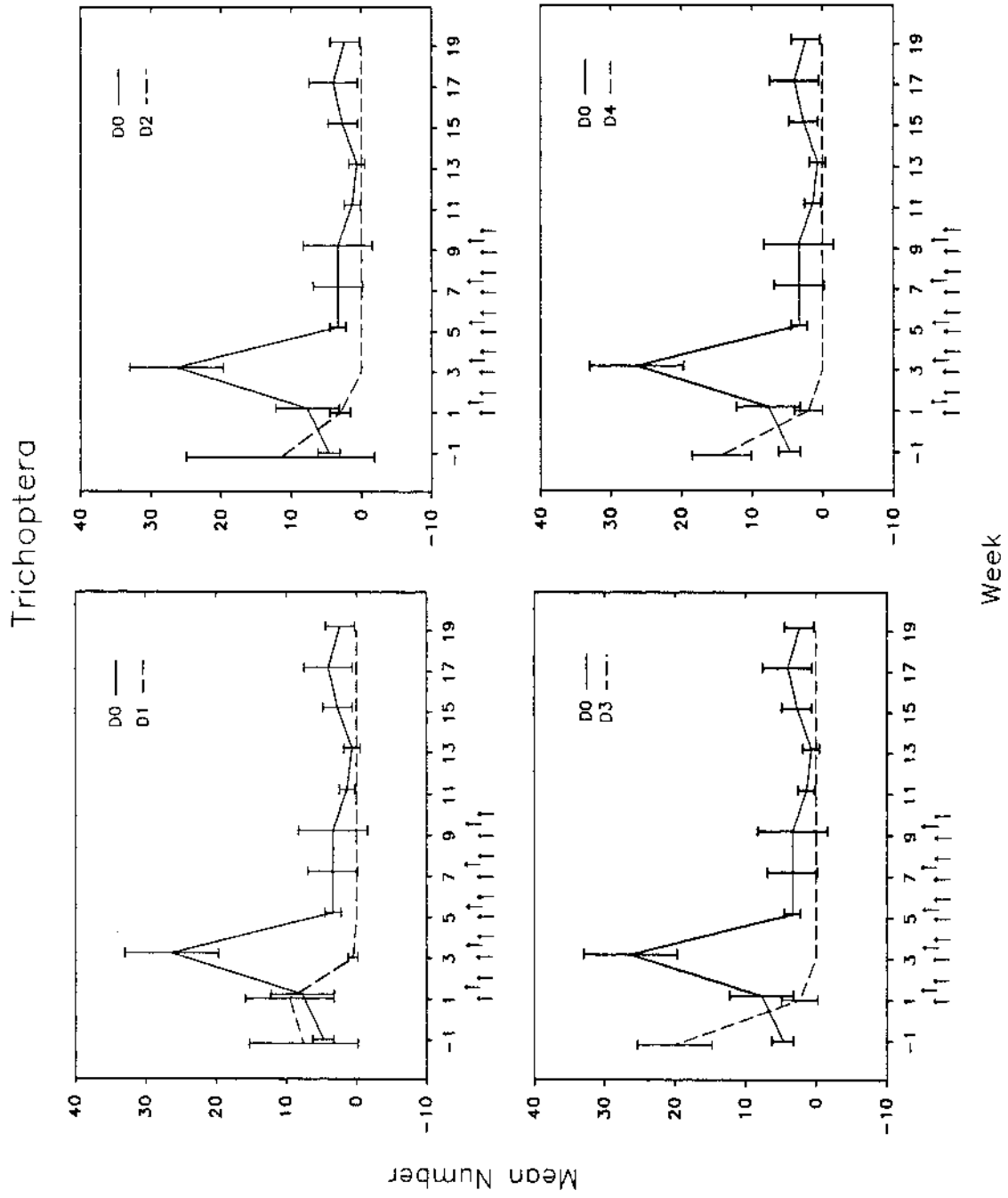

Figure 46. Mean number of Trichoptera larvae (± S.D.) colonizing artificial substrates during the experiment. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

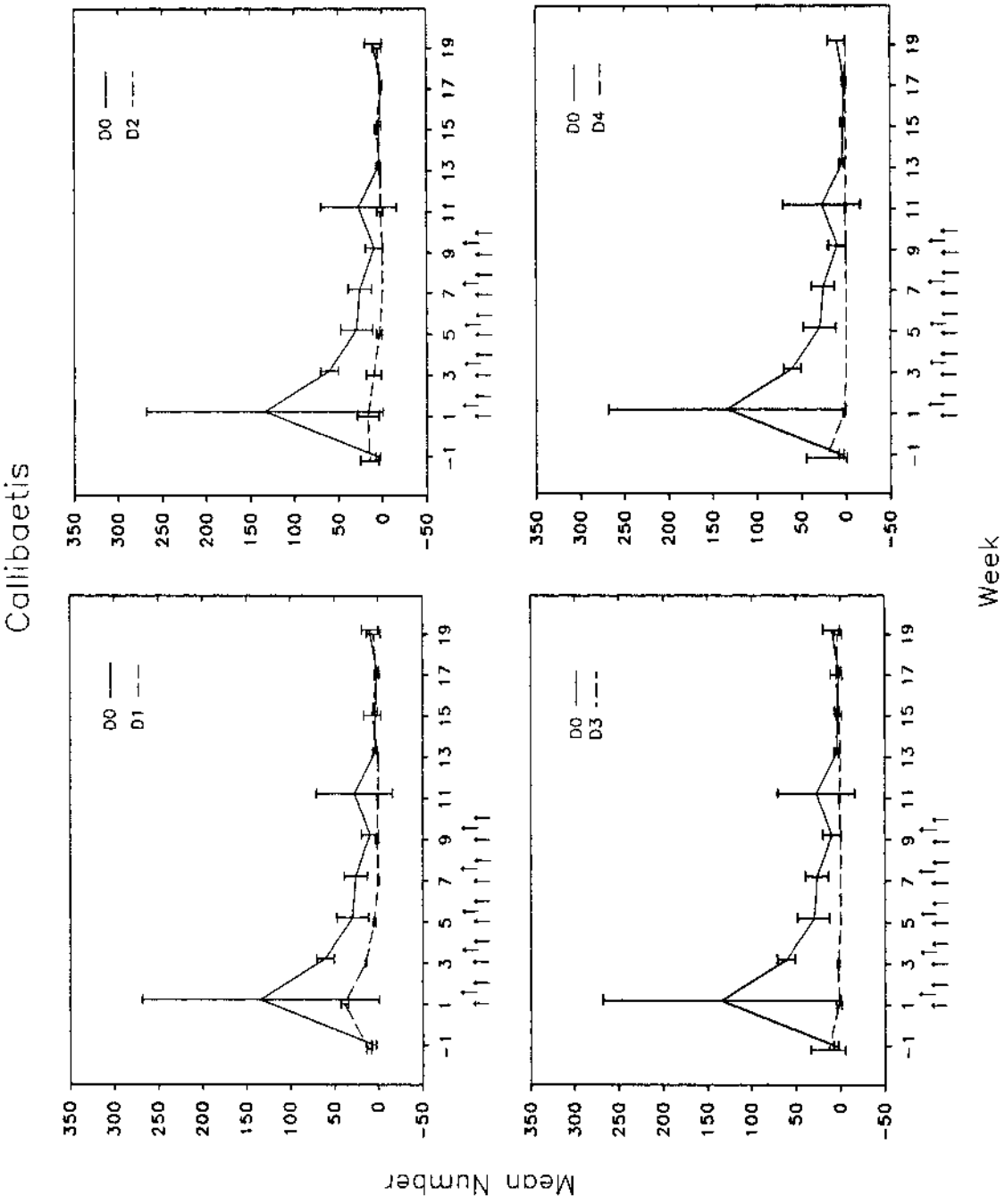

Figure 47. Mean number of *Callibaetis* larvae (± S.D.) colonizing artificial substrates during the experiment. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

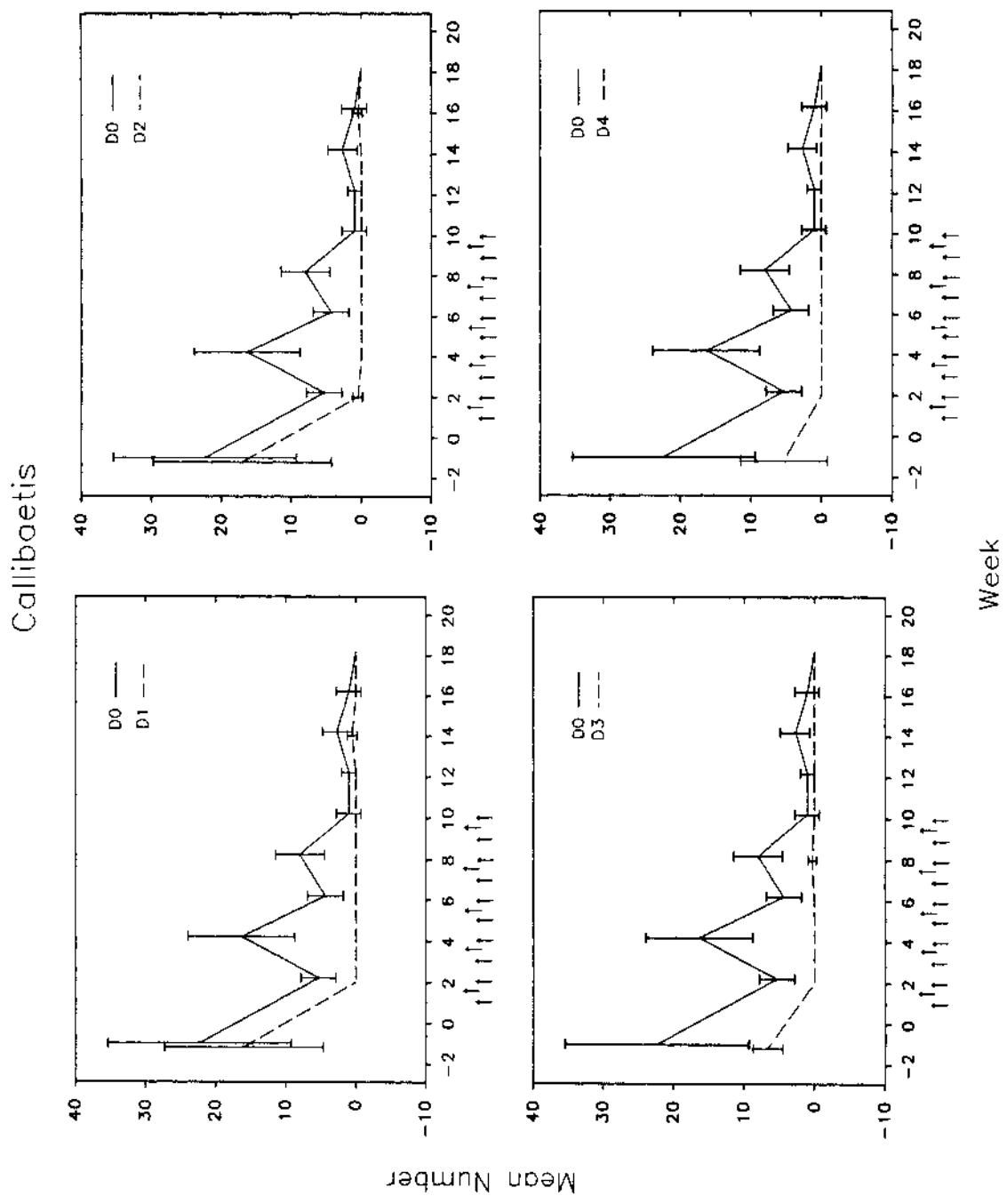

Figure 48. Mean number of *Callibaetis* exuviae (± S.D.) collected from microcosms. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

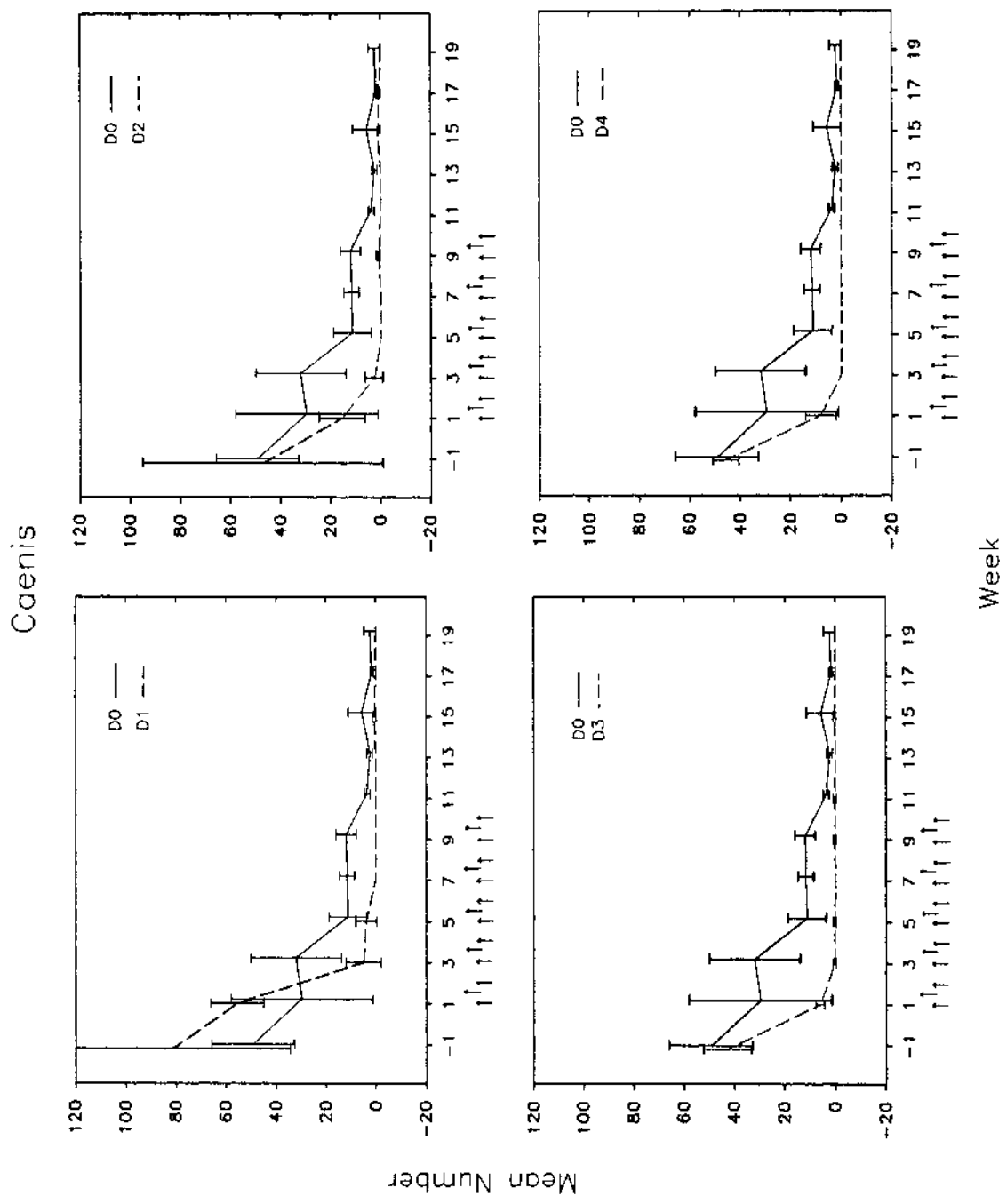

Figure 49. Mean number of *Caenis* larvae (± S.D.) colonizing artificial substrates during the experiment. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

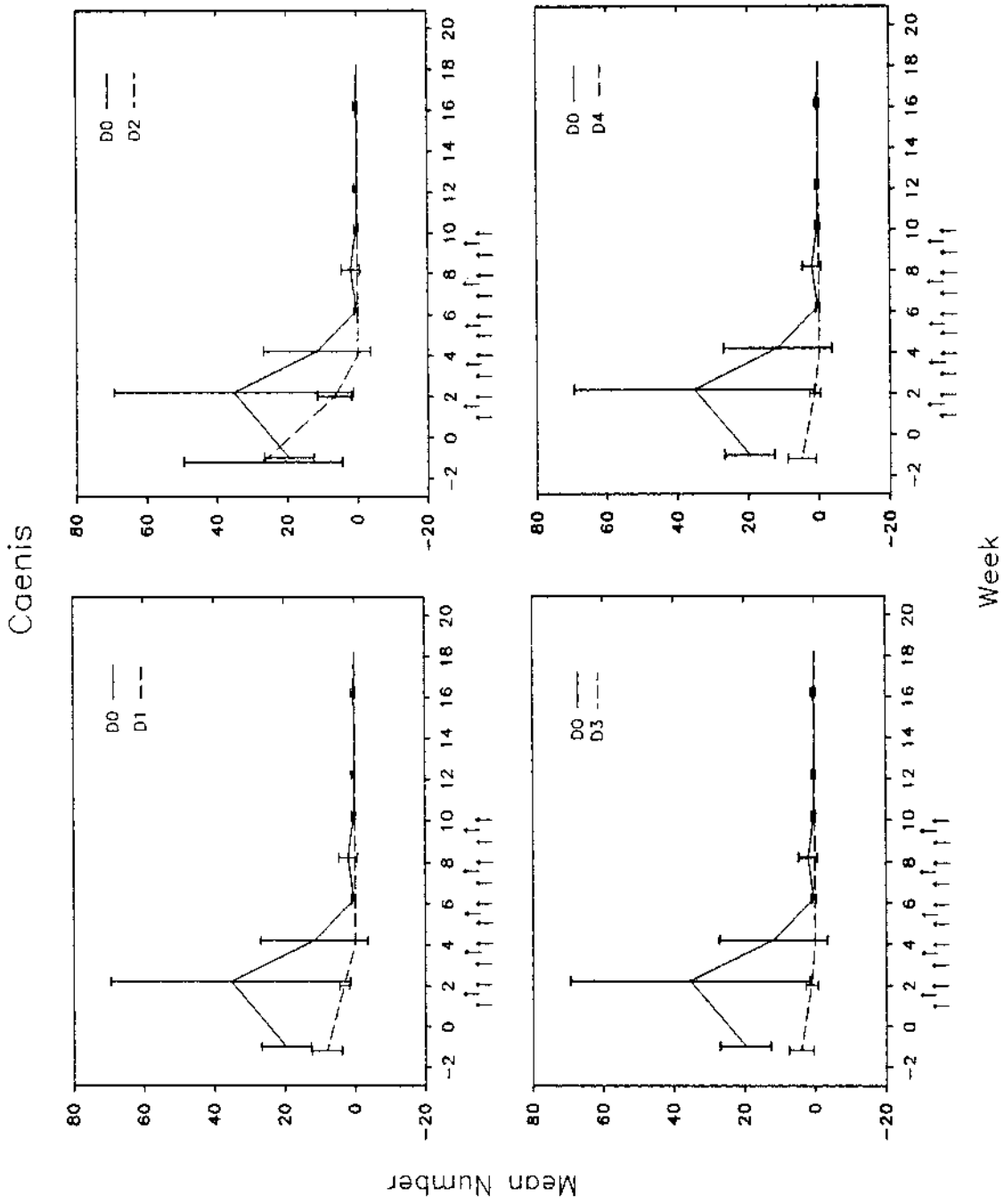

Figure 50. Mean number of *Caenis* exuviae (± S.D.) collected from microcosms. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

Table XI. Statistically significant differences in mean MAS sampler density in microcosms (Dunnett's MRT). D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

MASS Taxa		Week Number										
		-1	1	3	5	7	9	11	13	15	17	19
Callibaetis	D1			↓	↓	↓			↓			
	D2			↓	↓	↓	↓					
	D3		↓	↓	↓	↓	↓		↓			
	D4		↓	↓	↓	↓	↓		↓			
Caenis	D1					↓	↓	↓	↓	↓	↓	↓
	D2			↓	↓	↓	↓	↓	↓		↓	↓
	D3			↓	↓	↓	↓	↓	↓	↓	↓	↓
	D4			↓	↓	↓	↓	↓	↓	↓	↓	↓
Amphipoda	D1		↓	↓								
	D2		↓	↓								
	D3		↓	↓								
	D4		↓	↓								
Chaoborus	D1											
	D2											
	D3											
	D4											
Coenagrionidae	D1											
	D2											
	D3											
	D4										↓	↓
Libellulidae	D1											
	D2											
	D3											
	D4											

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

Continuation of MAS sampler significant differences.

MASS Taxa		Week Number										
		-1	1	3	5	7	9	11	13	15	17	19
Trichoptera (Total)	D1			↓	↓					↓		
	D2			↓	↓					↓		
	D3			↓	↓					↓		
	D4			↓	↓					↓		
Oecetis (Trichoptera)	D1			↓								
	D2			↓								
	D3	↑		↓								
	D4			↓								
Orthotrichia (Trichoptera)	D1			↓								
	D2			↓								
	D3			↓								
	D4			↓								
Oxyethria (Trichoptera)	D1			↓	↓							
	D2			↓	↓							
	D3		↓	↓	↓							
	D4		↓	↓	↓							
Cyrnellus (Trichoptera)	D1											
	D2											
	D3											
	D4											
Hydrophilidae	D1					↓	↓	↓				
	D2											
	D3					↓	↓	↓				
	D4			↓	↓	↓	↓	↓				
Chironomidae	D1											
	D2											
	D3											
	D4											

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

Continuation of MAS sampler significant differences.

MASS Taxa		Week Number										
		-1	1	3	5	7	9	11	13	15	17	19
Physidae	D1											
	D2											
	D3											
	D4											
Planorbidae	D1											
	D2											
	D3											
	D4											
Hydracarina	D1											
	D2		↑		↓							
	D3				↓							
	D4										↓	↓
Naididae	D1										↑	
	D2										↑	
	D3											
	D4						↑					

Parameter		Week Number										
		-1	1	3	5	7	9	11	13	15	17	19
Total Number	D1											
	D2											
	D3						↑					
	D4											

Parameter		Week Number			
		-1	5	9	19
Taxa Richness	D1				↓
	D2				↓
	D3				↓
	D4		↓	↓	↓

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

at all exposure levels (Table XI and XII). Adult emergence of *Callibaetis* was not adequately sampled by submerged funnel traps.

Caenis mayflies were reduced to near zero in treated microcosms (Figures 49 and 50), with significant differences detected in MAS samples at D1-D4 (Table XI). *Caenis* exuviae production in control tanks was quite variable (Figure 50), resulting in no significant differences.

Amphipods were fairly rare in MAS samplers, and densities declined rapidly with time (Figure 51). Treatments declined much more rapidly than controls, however, with significant differences found at all cyfluthrin levels (Table XI).

Chironomids, when analyzed at the family level (Chironomidae), were not impacted by cyfluthrin in MAS samples (Figure 52, Table XI). Chironomidae were significantly reduced by the pesticide in exuviae samples (D2-D4; Figure 53 and Table XII) and in emergence trap samples (D3-D4; Figure 54 and Table XIII). Family level trends will be contrasted with subfamily and generic level analyses in the next chapter.

Beetle (Coleoptera) larvae and adults from microcosms were dominated by members of the family Hydrophilidae, primarily the genera *Berosus* and *Helophorus*. Other types (fairly rare) included *Tropisternus* (Hydrophilidae),

Table XII. Statistically significant differences in mean exuviae density in microcosms (Dunnett's MRT). D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

Exuviae Taxa		Week Number									
		-1	2	4	6	8	10	12	14	16	18
Callibaetis	D1		↓	↓	↓	↓					
	D2		↓	↓	↓	↓			↓		
	D3		↓	↓	↓	↓			↓		
	D4		↓	↓	↓	↓			↓		
Caenis	D1										
	D2										
	D3										
	D4										
Chaoborus	D1			↓	↓	↓	↓	↓	↓		
	D2				↓	↓		↓	↓		
	D3		↓	↓	↓	↓	↓	↓	↓		
	D4			↓	↓	↓	↓	↓	↓		
Chironomidae	D1										
	D2				↓						
	D3		↓		↓						
	D4		↓	↓	↓	↓					
Coenagrionidae	D1										
	D2										
	D3										
	D4										
Libellulidae	D1										
	D2										
	D3										
	D4	↓									

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

Continuation of exuviae significant differences.

Exuviae Parameter		Week Number									
		-1	2	4	6	8	10	12	14	16	18
Taxa Richness	D1										
	D2										
	D3		↓								
	D4			↓	↓	↓					
Total Number	D1		↓								
	D2		↓								
	D3				↓	↓					
	D4		↓	↓	↓	↓					

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

Paracymus (Hydrophilidae), *Laccophilus* (Dytiscidae), and *Peltodytes* (Haliplidae). Hydrophilidae were the only taxa numerous enough for statistical treatment. Hydrophilidae were significantly reduced at doses D1, D3 and D4 (Figure 55 and Table XI). Adult beetles (various genera) were observed immobilized/dead on the water surface in microcosms following BAYTHROID^{R} application. This phenomenon was observed at all treatment levels.

Damselflies were restricted to the family Coenagrionidae. All naiads keyed belonged to the genus *Enallagma*. The few adults identified to species were

Enallagma civile. Routine counts were taken to the family level since most individuals were too small for reliable generic identification. Coenagrionidae naiads in MAS were similar to controls at D1, were somewhat higher that controls at D2 and D3, and were lower than controls at D4 (Figure 56). The only significant differences detected were at D4 near the end of the study (Table XI). Exuviae samples showed few distinctive trends (Figure 57) and never demonstrated statistical significance (Table XII). Damselflies were almost never collected in funnel traps.

Dragonflies were represented by members of four families; Aeshnidae, Gomphidae, Corduliidae and Libellulidae. Only the last group was sufficiently abundant for statistical treatment. Libellulidae were dominated by small instars during the summer (when pesticide impacts were occurring), thus they were analyzed at the family level for graphical and Dunnett's analysis. Mature naiads (from MAS or exuviae) were keyed to genus whenever possible. Libellulidae were apparently not affected by cyfluthrin in artificial substrate or exuviae samples (Figures 58 and 59; Tables XI and XII). Few dragonflies were collected in emergence traps.

Water mites (Hydracarina) were rare at first, but reached high densities by the end of the study (Figure 60). Treatments D1 and D2 were fairly similar to controls. A

Table XIII. Statistically significant differences in number of individual from emergence traps in microcosms (Dunnett's MRT). D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

Emergence Trap Taxa		Week Number									
		-1	2	4	6	8	10	12	14	16	18
Callibaetis	D1										
	D2										
	D3										
	D4										
Caenis	D1										
	D2										
	D3										
	D4										
Chaoborus	D1			↓							
	D2										
	D3			↓							
	D4			↓							
Chironomidae	D1										
	D2										
	D3		↓		↓	↓					
	D4		↓	↓	↓	↓					

Parameter		Week Number									
		-1	2	4	6	8	10	12	14	16	18
Total Number	D1										
	D2										
	D3					↓	↓				
	D4			↓		↓					
Taxa Richness	D1										
	D2										
	D3										
	D4			↓							

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

trend toward reduced densities was observed at D3, but differences were never significant (Table XI). Water mites in D4 microcosms were significantly lower that controls at the end of the study (Figure 60, Table XI).

Snails (Gastropoda) belonged to two families, Physidae and Planorbidae. Population densities in experimental tanks were highest in early Summer or late Fall, and lowest during mid-Summer (Figures 61 and 62). No significant differences were observed (Table XI).

Oligochaetes were dominated by the family Naididae. Genera observed included *Dero*, *Chaetogaster*, and *Stylaria*. Counts were conducted at the family level due to high densities, difficulty of routine identification at the lower taxonomic levels, and the anticipation of few pesticide impacts on this group. Naidid populations in treated tanks were generally greater than control populations, particularly at D1 and D2 (Figure 63). Significant increases were noted during weeks 9 and 17 (Table XI).

Bray-Curtis Cluster Analyses

Artificial substrate and exuviae samples were analyzed by Bray-Curtis cluster analysis combined with a bootstrap procedure (Nemec 1991) for determination of statistical separation of clusters (treatment levels). Multivariate analyses such as cluster analysis hold the promise of

holistically evaluating the whole community at once, rather than the taxa-by-taxa approach used above. Analyses were conducted four times during the study (see MAS richness discussion above), and the lowest possible level of taxonomic identification was used for each individual.

The macroinvertebrates colonizing MAS samplers prior to the initial application were fairly similar among all treatments (Figure 64). Dominant taxa included naidid oligochaetes, physid and planorbid snails, and mayflies (*Caenis* and *Callibaetis*). Similarity coefficients equaled or exceeded 0.68, with no significant separation of clusters (Appendix Table 4).

Dominant MAS macroinvertebrates during week 5 were naidid oligochaetes, *Callibaetis* mayflies (in controls), *Labrundinia* chironomids (in controls), and *Goeldichironomus* chironomids (in treated tanks). Treatment levels 1 and 3 were the most similar, while controls were least similar to other treatments (Figure 65). Clusters were not statistically separated from each other (Appendix Table 4).

Near the end of the application period (week 9), abundant macroinvertebrates from MAS were Naididae, Coenagrionidae, *Caenis* mayflies, and several chironomids (*Labrundinia*, *Apedilum*, *Goeldichironomus* and *Parachironomus*). Cluster analysis organized treatments in increasing order (Figure 66), with D0 and D1 being quite

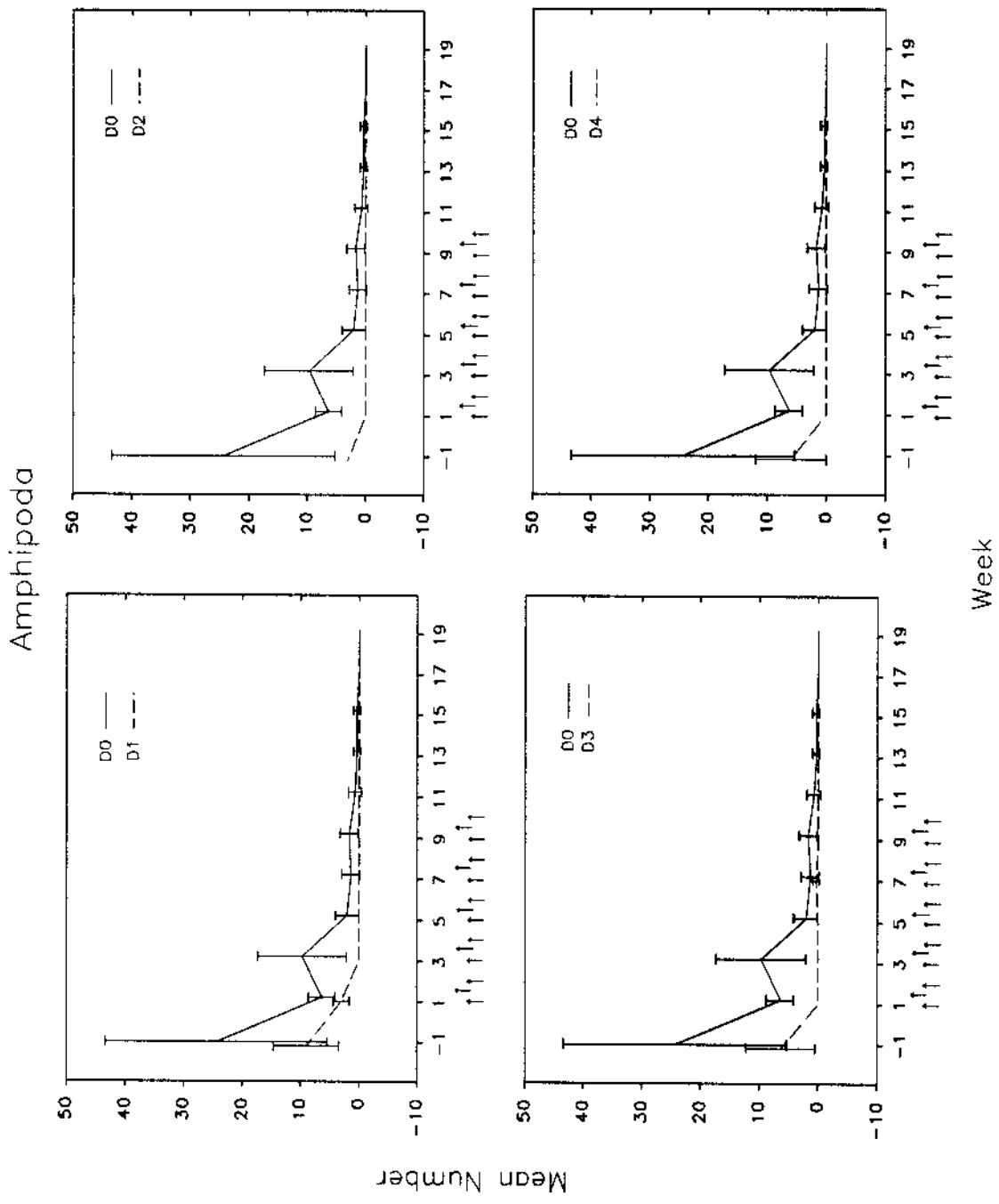

Figure 51. Mean number of Amphipoda (± S.D.) colonizing artificial substrates during the experiment. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

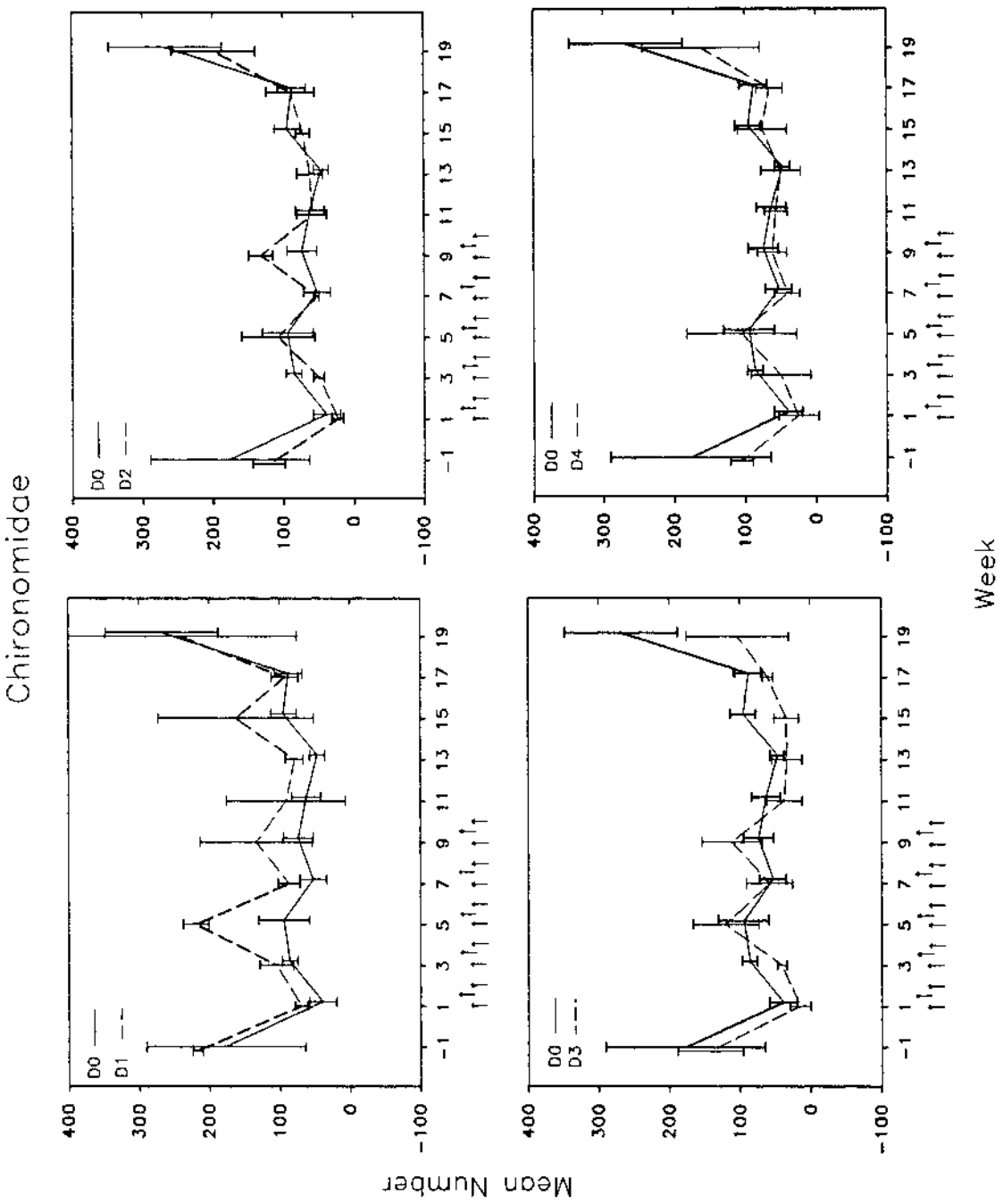

Figure 52. Mean number of Chironomidae larvae (± S.D.) colonizing artificial substrates during the experiment. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

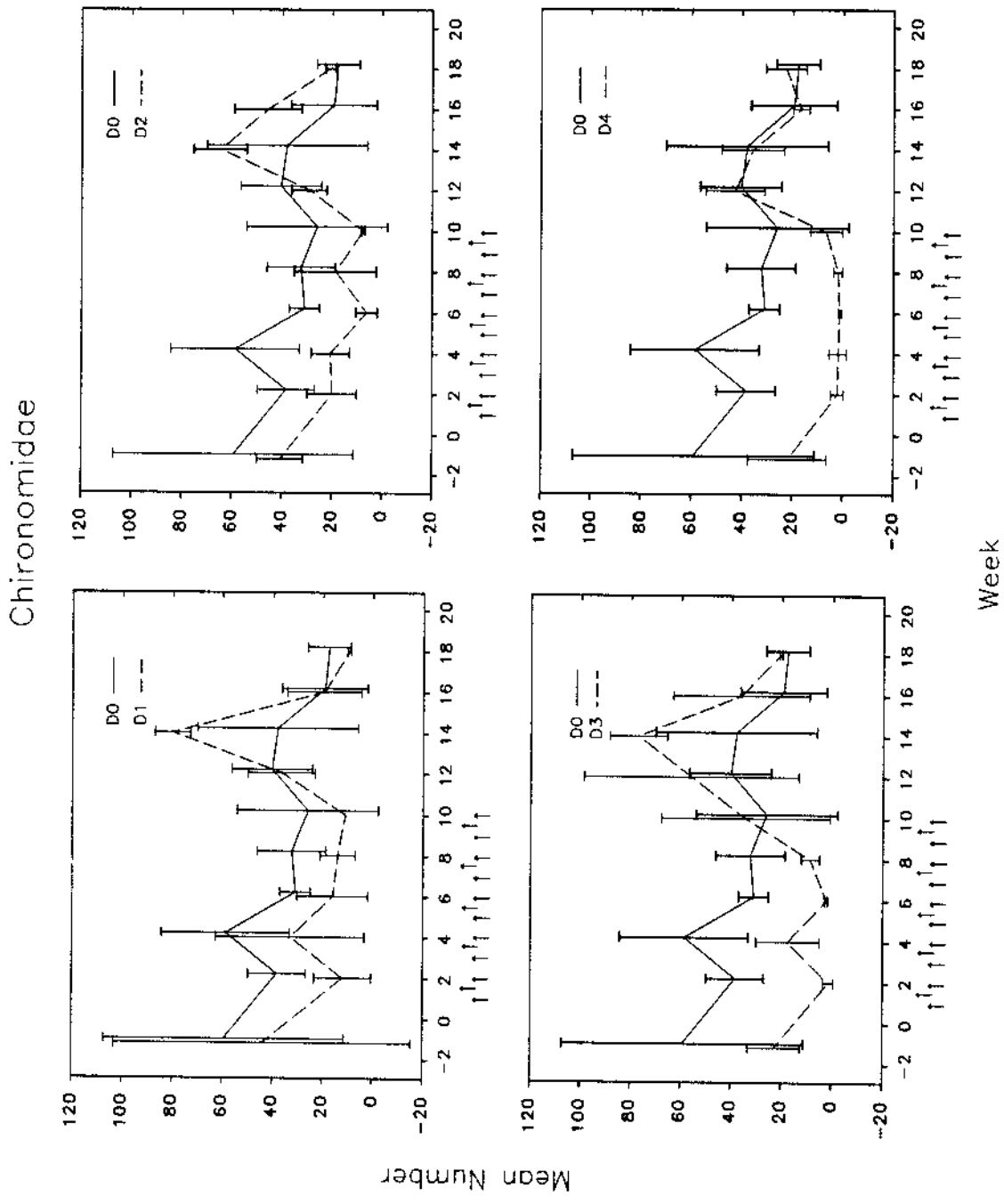

Figure 53. Mean number of Chironomidae exuviae (± S.D.) collected from microcosms. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

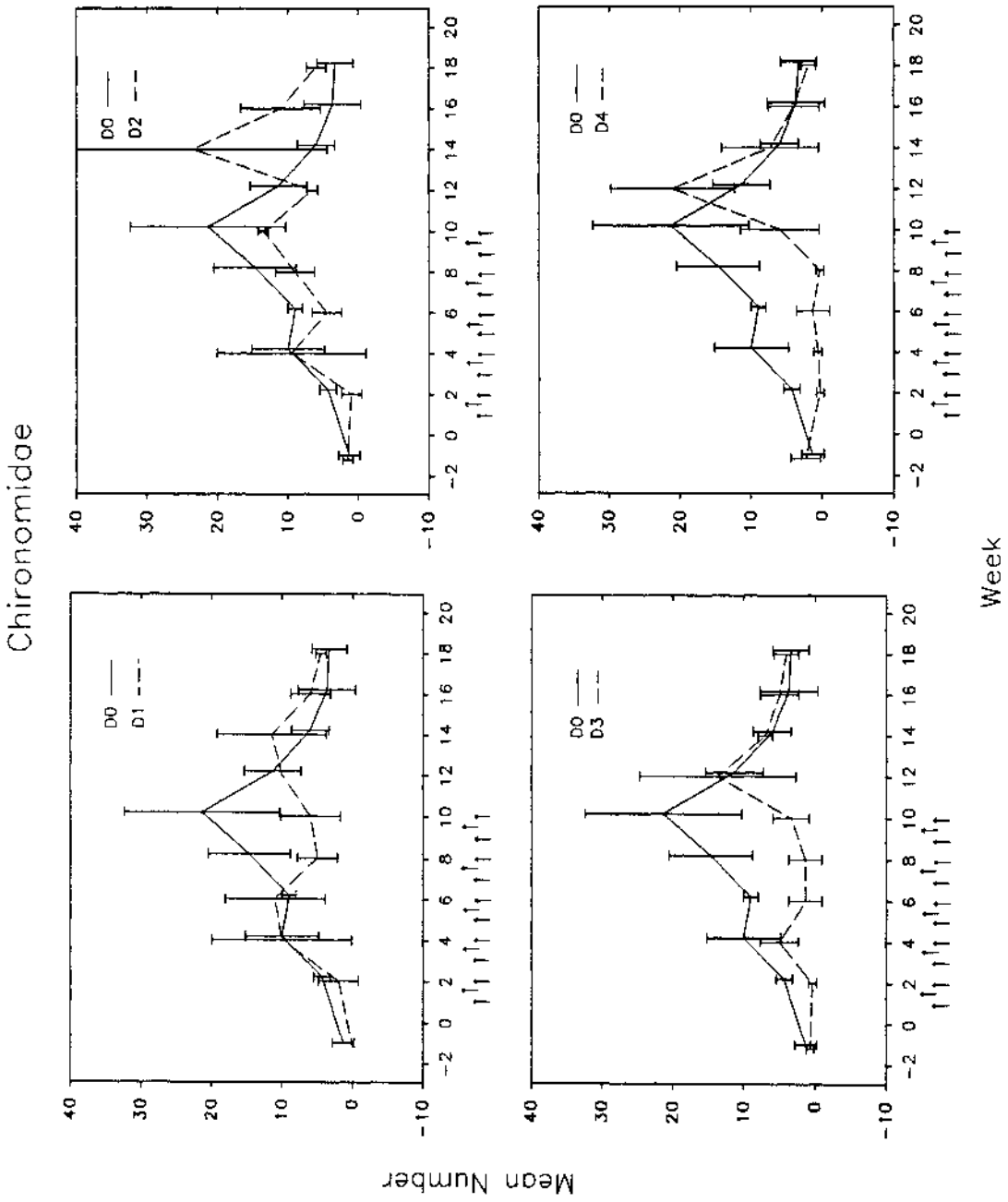

Figure 54. Mean number of Chironomidae adults (± S.D.) collected with emergence traps from microcosms. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

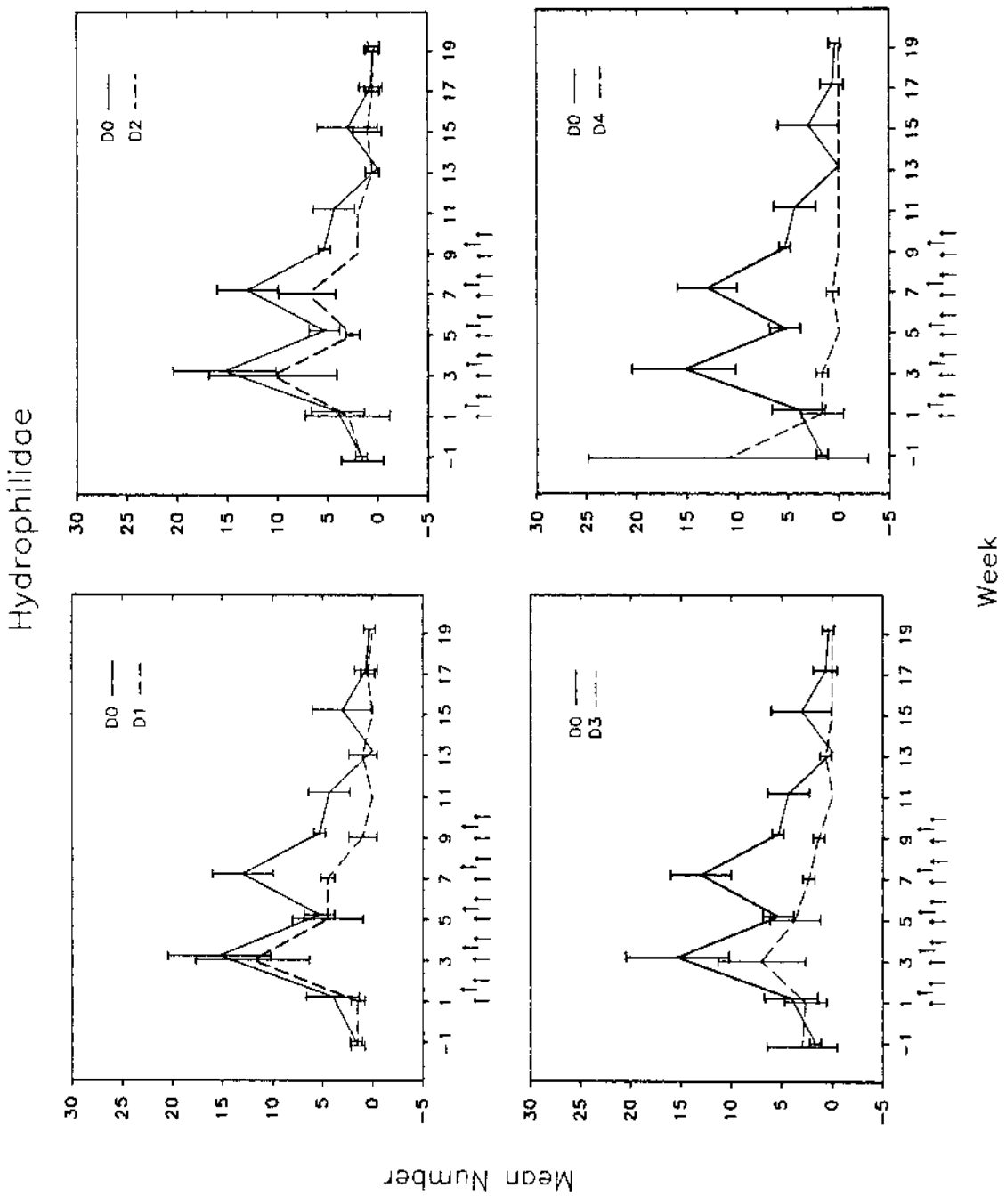

Figure 55. Mean number of Hydrophilidae larvae (± S.D.) colonizing artificial substrates during the experiment. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

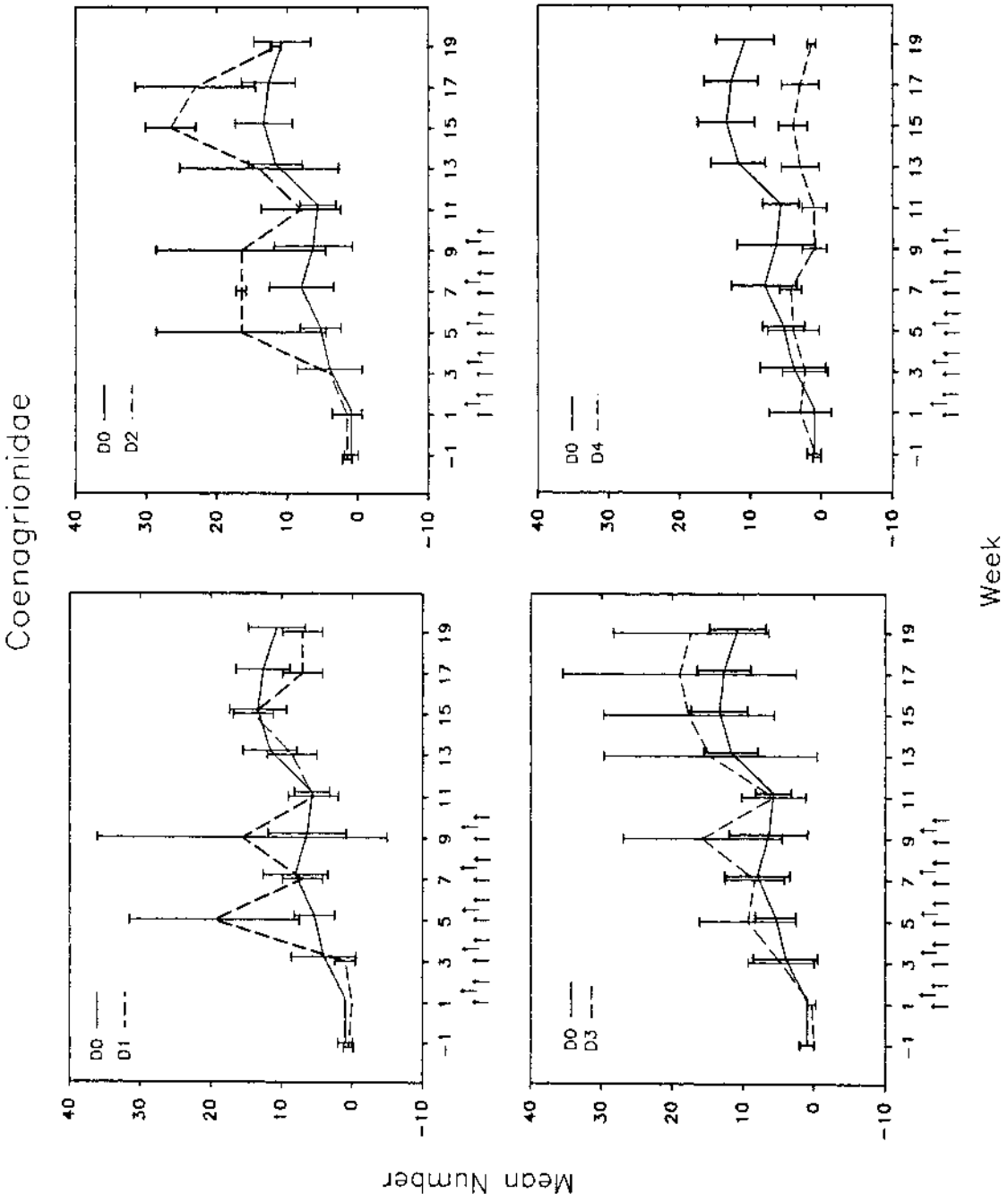

Figure 56. Mean number of Coenagrionidae naiads (± S.D.) colonizing artificial substrates during the experiment. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

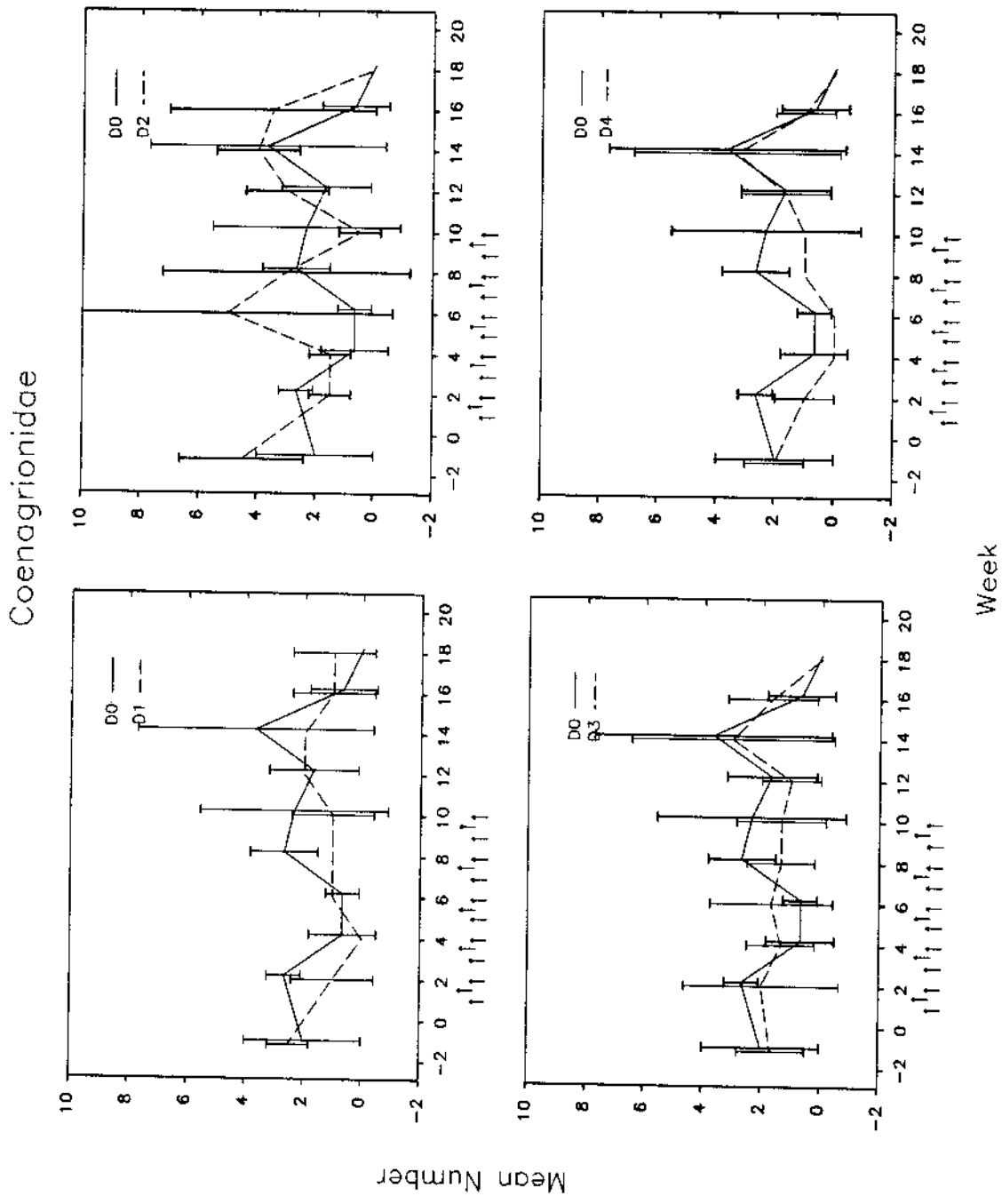

Figure 57. Mean number of Coenagrionidae exuviae (± S.D.) collected from microcosms. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

different from D4. No clusters were separated statistically by bootstrap analysis, with the lowest probability at the D0-D2 linkage (p=0.142; Appendix Table 4).

At the end of the experiment (week 19), large populations of naidid oligochaetes and water mites (Hydracarina) dominated MAS collections (Figure 67). A relationship between dose level and cluster order was less apparent at this time due to divergence of D1 from the other treatments. Again, statistical significance of clustering was not found, with the lowest probability at p=0.18 (Appendix Table 4).

Exuviae samples were also evaluated using cluster analysis. Pre-treatment sampling (Figure 68) revealed high numbers of mayflies (*Caenis*, *Callibaetis*) and chironomids (*Tanypus*, *Procladius*, *Cladotanytarsus*). No significant differences among clusters were detected by bootstrapping (Appendix Table 5).

During the middle of the application period (week 6), controls appeared to be very different from treated microcosms (Figure 69). Taxa common in controls (*Chaoborus*, *Labrundinia* and *Callibaetis*), were rare in other treatments. Exuviae similarities were low (the control-treated microcosm linkage similarity was only 0.26), but statistical significance was not found via bootstrapping of samples (p=0.13; Appendix Table 5).

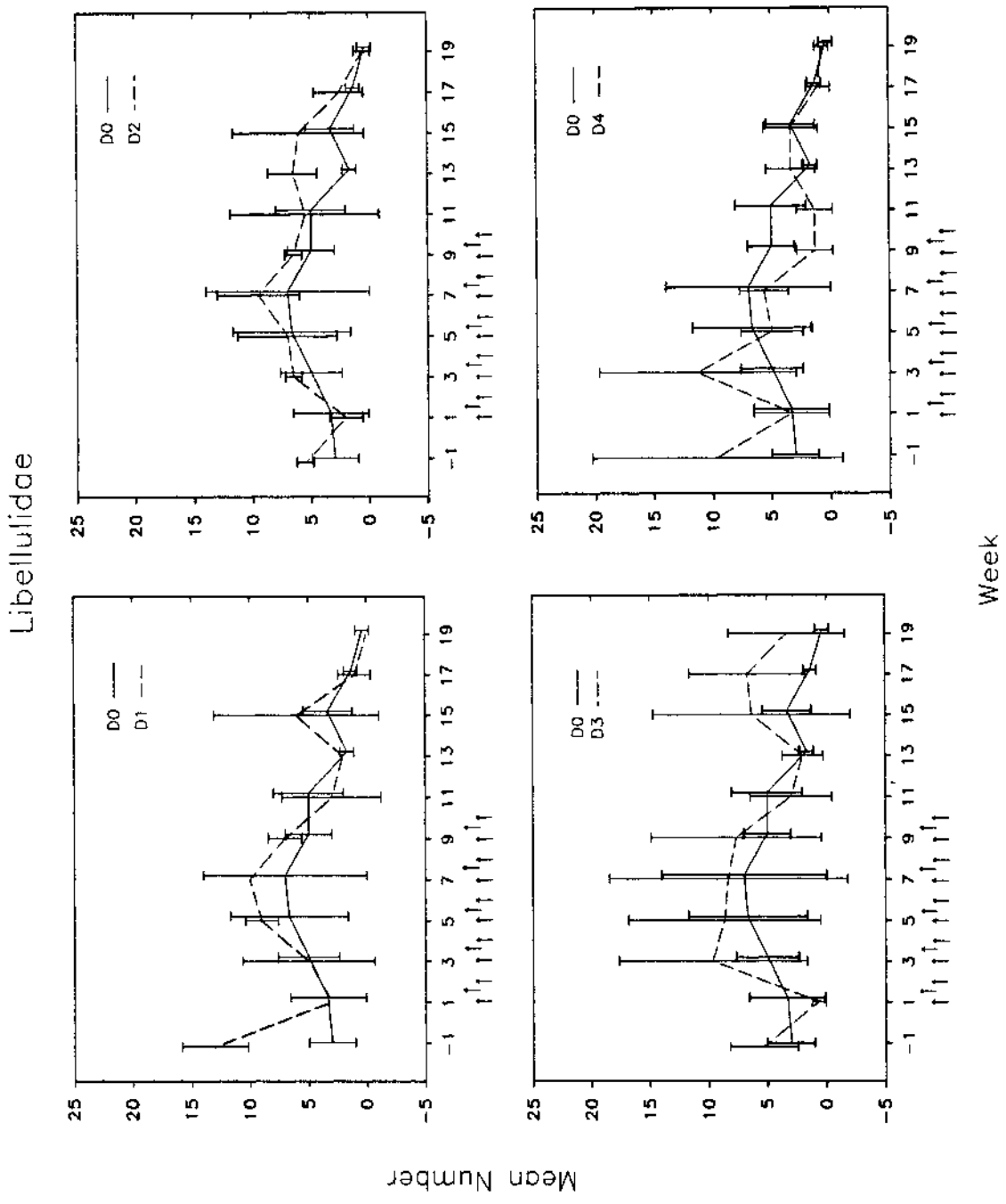

Figure 58. Mean number of Libellulidae naiads (± S.D.) colonizing artificial substrates during the experiment. Arrows represent pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

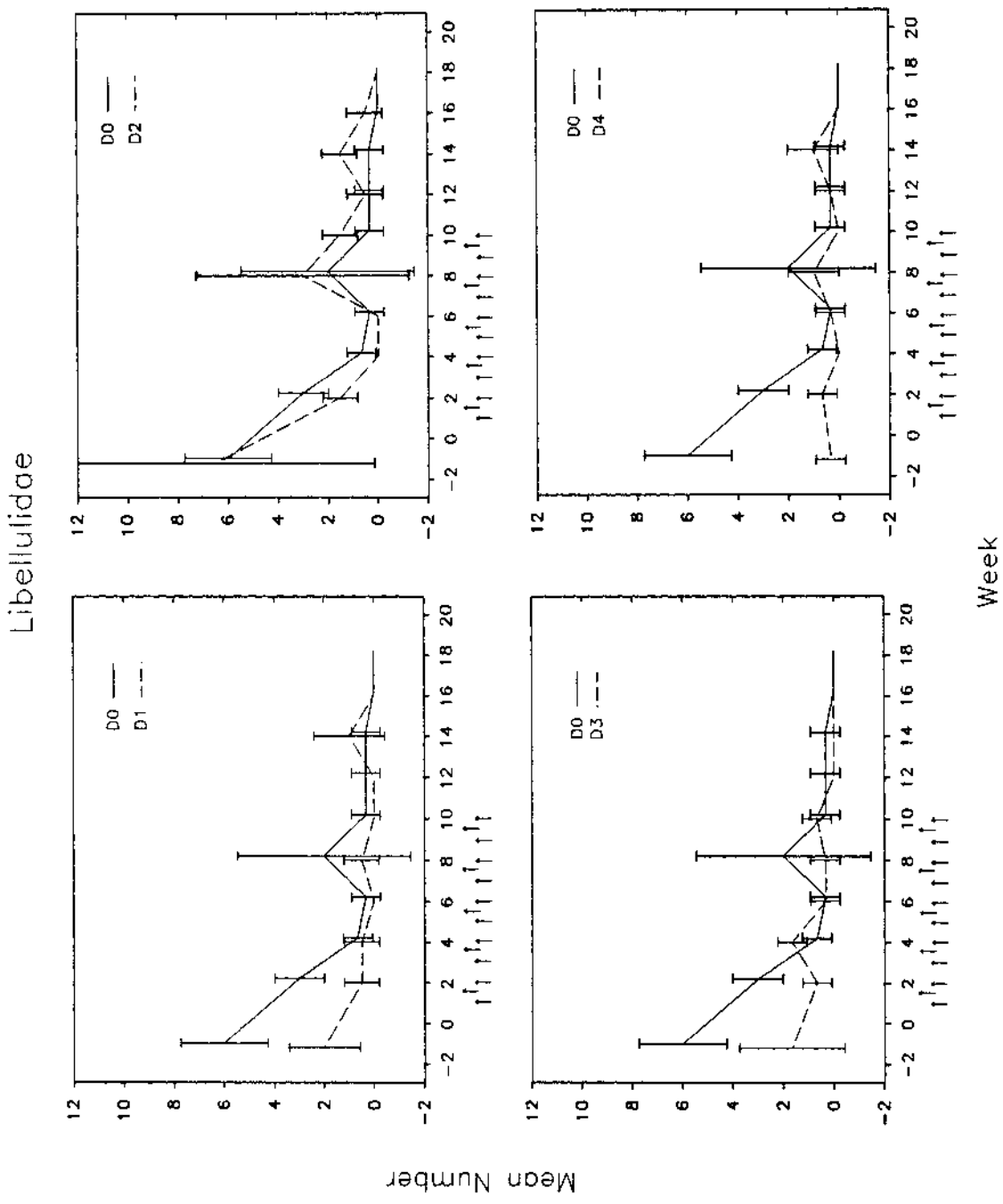

Figure 59. Mean number of Libellulidae exuviae (± S.D.) collected from microcosms. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

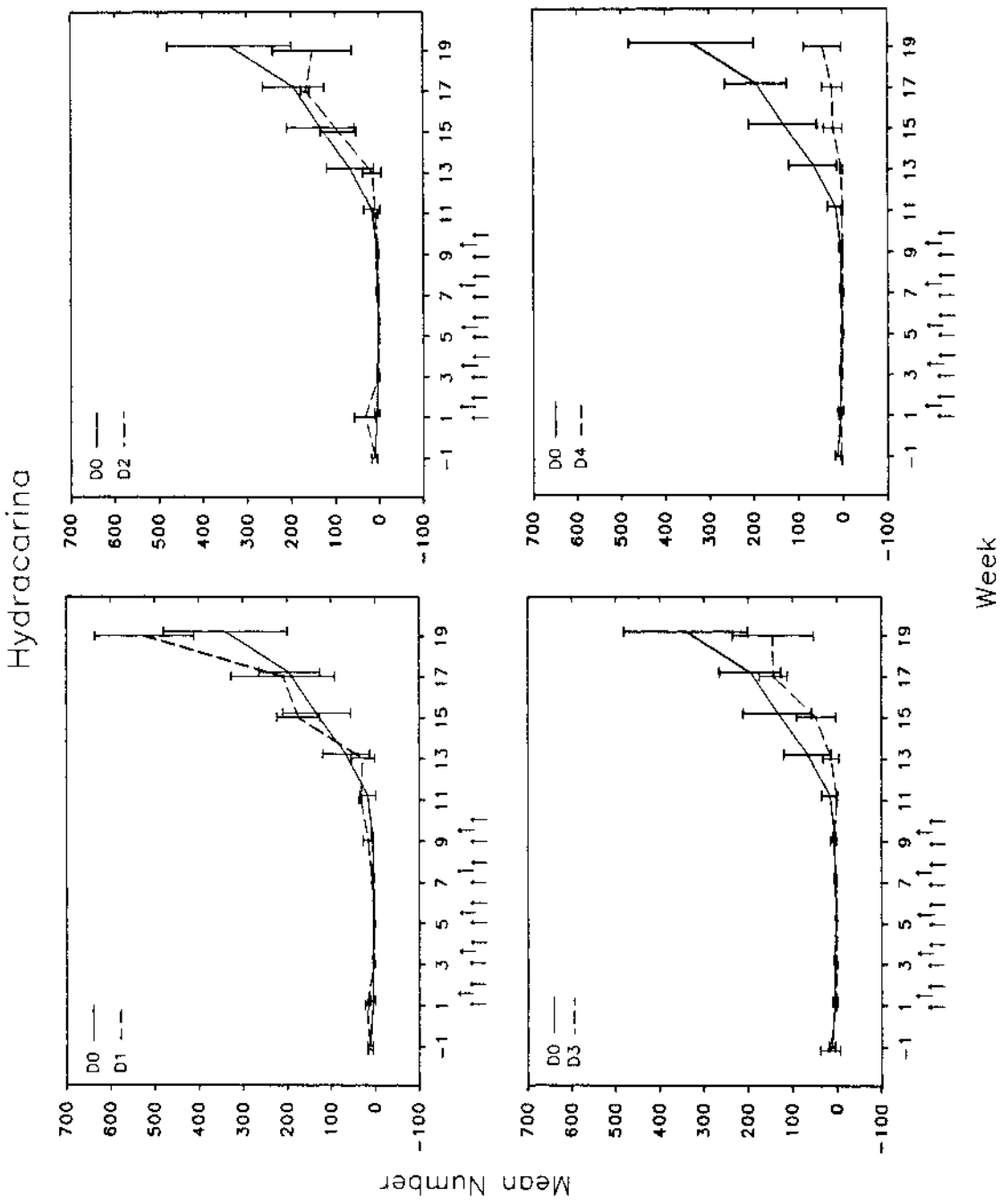

Figure 60. Mean number of Hydracarina (± S.D.) colonizing artificial substrates during the experiment. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

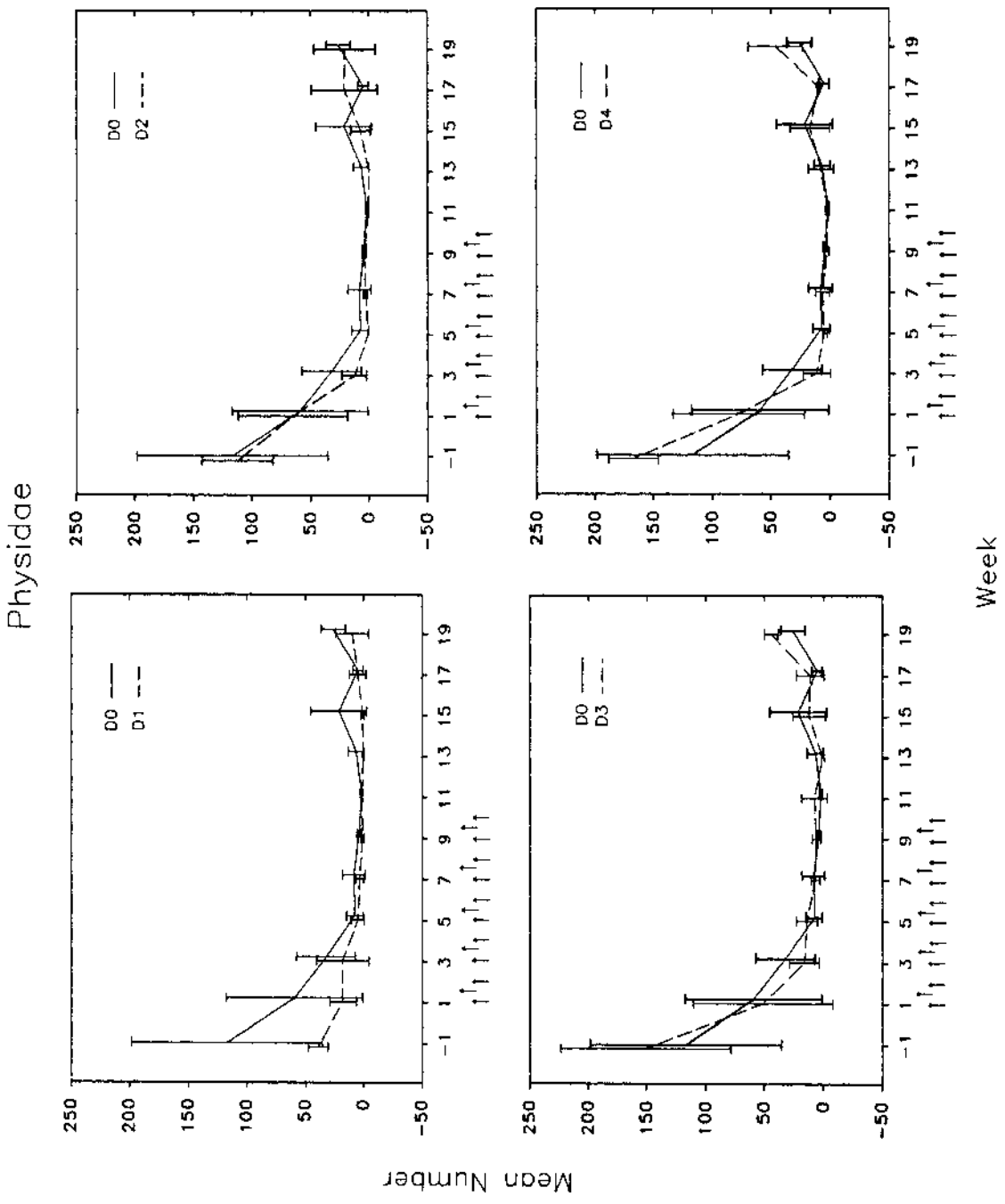

Figure 61. Mean number of Physidae (± S.D.) colonizing artificial substrates during the experiment. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

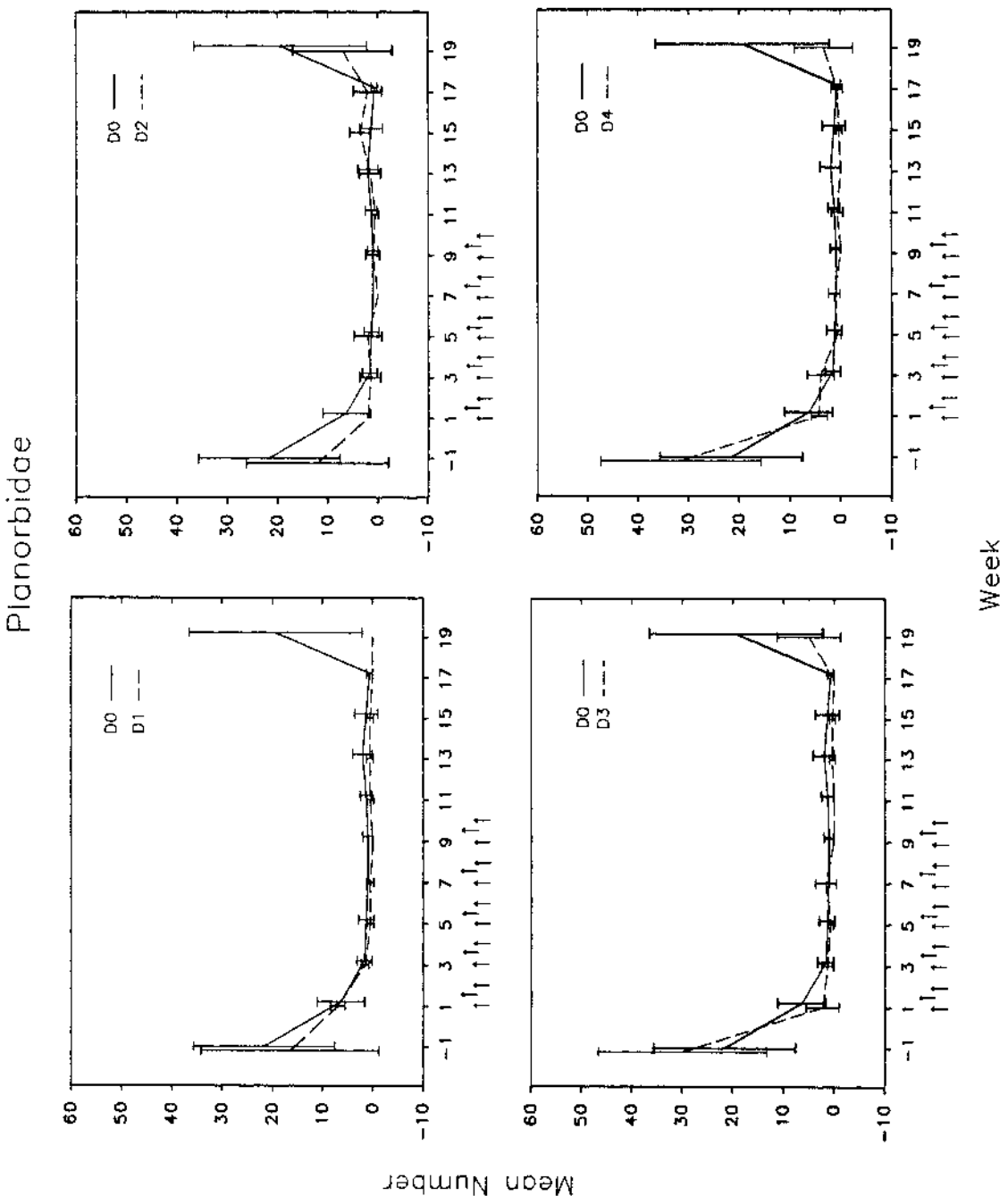

Figure 62. Mean number of Planorbidae (± S.D.) colonizing artificial substrates during the experiment. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

Exuviae sampling during the last week of application (week 10) again indicated low similarity between controls and treatments (Figure 70), but results were not statistically significant ($p=0.177$; Appendix Table 5).

By the end of the experiment, exuviae numbers were lower than at other sampling periods (probably due to lower temperatures, see Figure 10), and each treatment was dominated by differing taxa (Figure 71). *Labrundinia* exuviae were most common in control tanks, *Apedilum* reached high densities in D2 and D4, while *Endochironomus* were most abundant in D3 microcosms. No significant differences in community structure were determined via bootstrapping (Appendix Table 5; lowest probability at D2-D3 linkage with $p=0.13$).

Discussion

Microcosm Responses and Comparison with Other Field Studies

Sensitive and insensitive macroinvertebrates were identified from microcosms. Groups reduced by cyfluthrin included *Chaoborus*, *Caenis*, *Callibaetis*, various caddisfly genera, hydrophilid beetles, amphipods, and Chironomidae. Water mites and coenagrionids were only reduced at the highest treatment. Snails and libellulid dragonflies were apparently unaffected while naidid oligochaetes increased in number. These results agreed with some recently published

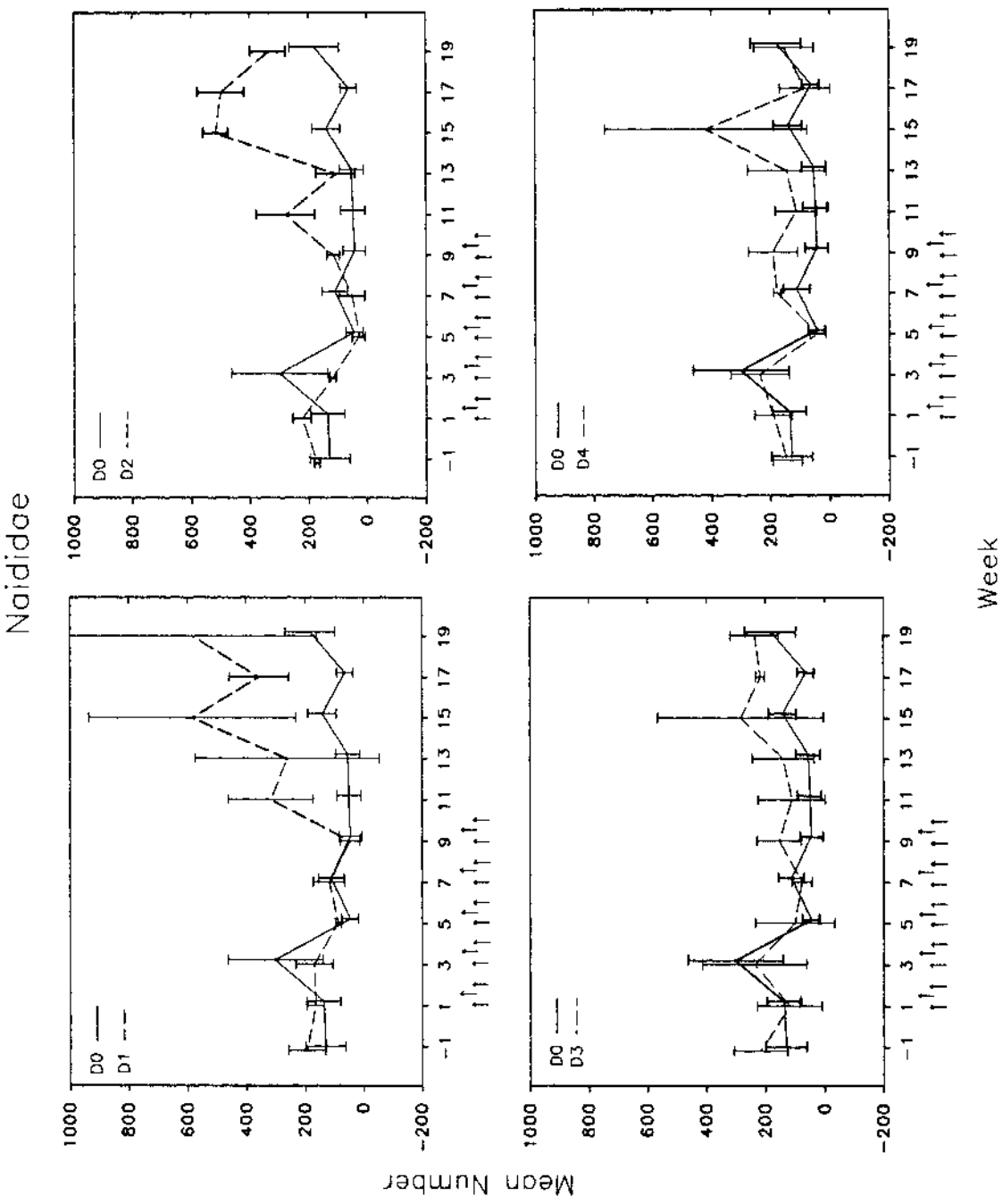

Figure 63. Mean number of Naididae (± S.D.) colonizing artificial substrates during the experiment. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

field studies of pyrethroids applied to mesocosms, but were dissimilar in other respects. Comparison of concurrent microcosm and mesocosm BAYTHROID^{R} studies will be detailed in Chapter 9.

Lozano et al. (1992) found that *Hyalella azteca*, larval dipterans (i.e., chironomids) and odonates (Coenagrionidae) were among the most sensitive taxa in limnocorrals treated with esfenvalerate in Minnesota. Oligochaetes and water mites were not sensitive and *Caenis* were somewhat sensitive to esfenvalerate. Oligochaetes increased in abundance 25 days after each application, not unlike increases found during the microcosm study. These researchers only evaluated artificial substrate colonization.

Webber et al. (1992) also evaluated esfenvalerate impacts, using mesocosms in Alabama. This study used artificial substrates, dredge samples and floating pyramid emergence traps for monitoring macroinvertebrates. Several dipterans (various chironomids and *Chaoborus*), and the trichopteran *Orthotrichia* were impacted by the pyrethroid. Total emergence (mostly chironomids) was reduced in treated ponds. This study was very sketchy, with few details.

Fairchild et al. (1992) sampled macroinvertebrates using a benthic uplift sampler. They tested esfenvalerate using mesocosms at Columbia, Missouri. This group found Ephemeroptera, Diptera and Gastropoda to be the most

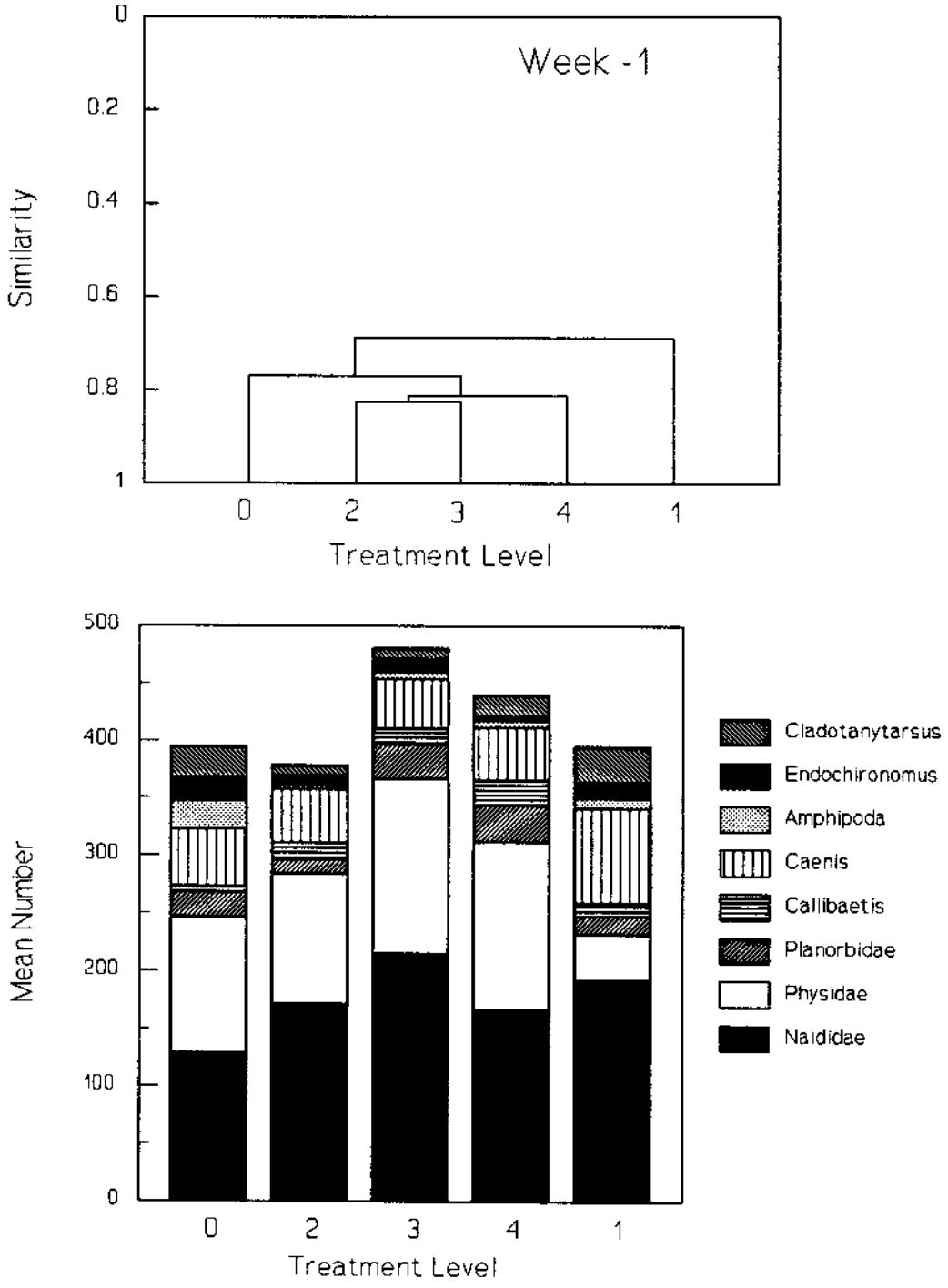

Figure 64. Bray-Curtis cluster analysis of macroinvertebrates colonizing artificial substrates prior to the initial application. Treatments range from controls (0) to high rate (4).

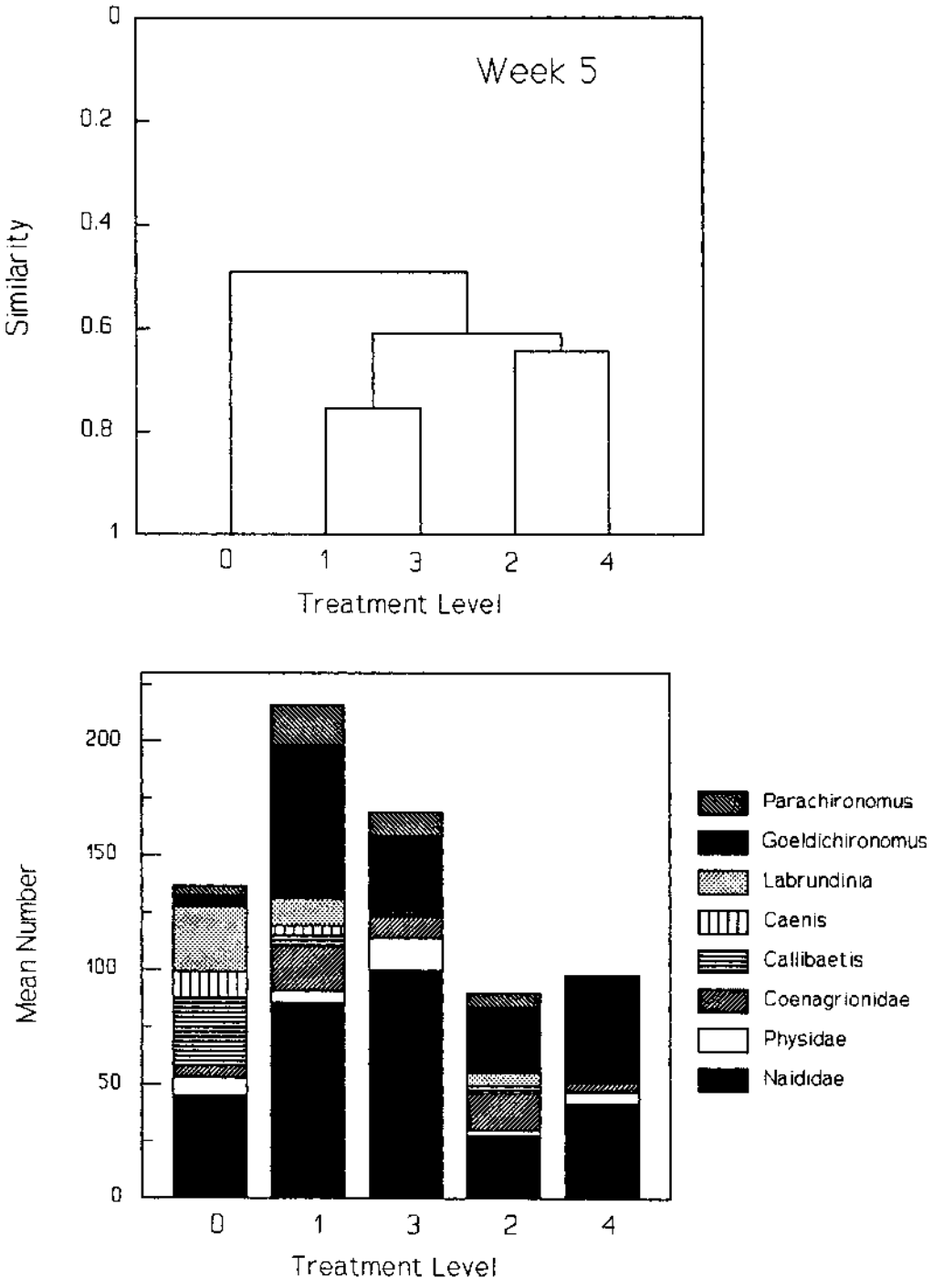

Figure 65. Bray-Curtis cluster analysis of macroinvertebrates colonizing artificial substrates during the middle of the application period. Treatments range from contols (0) to high rate (4).

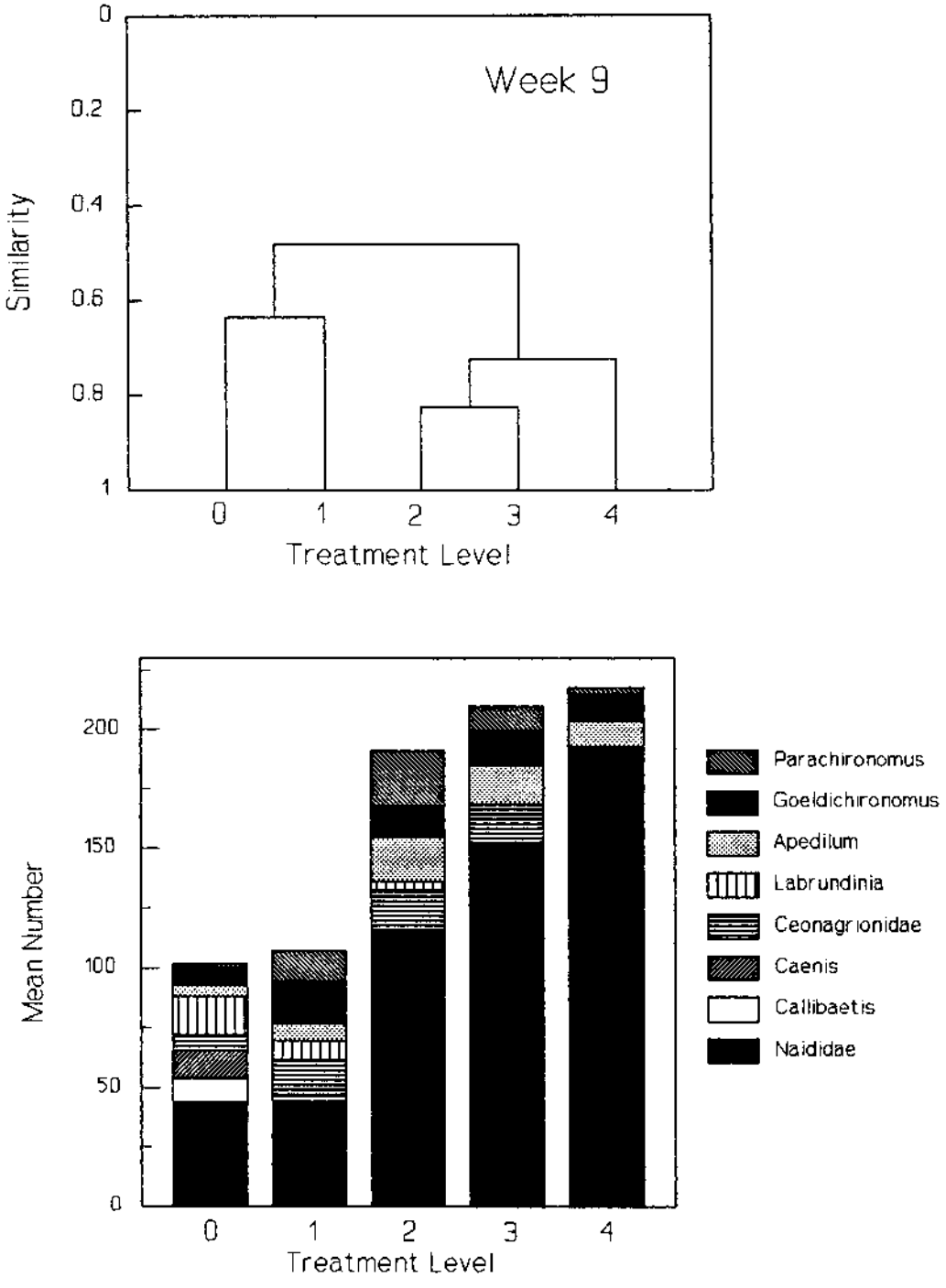

Figure 66. Bray-Curtis cluster analysis of macroinvertebrates colonizing artificial substrates one week prior to the final pesticide application. Treatments range from controls (0) to high rate (4).

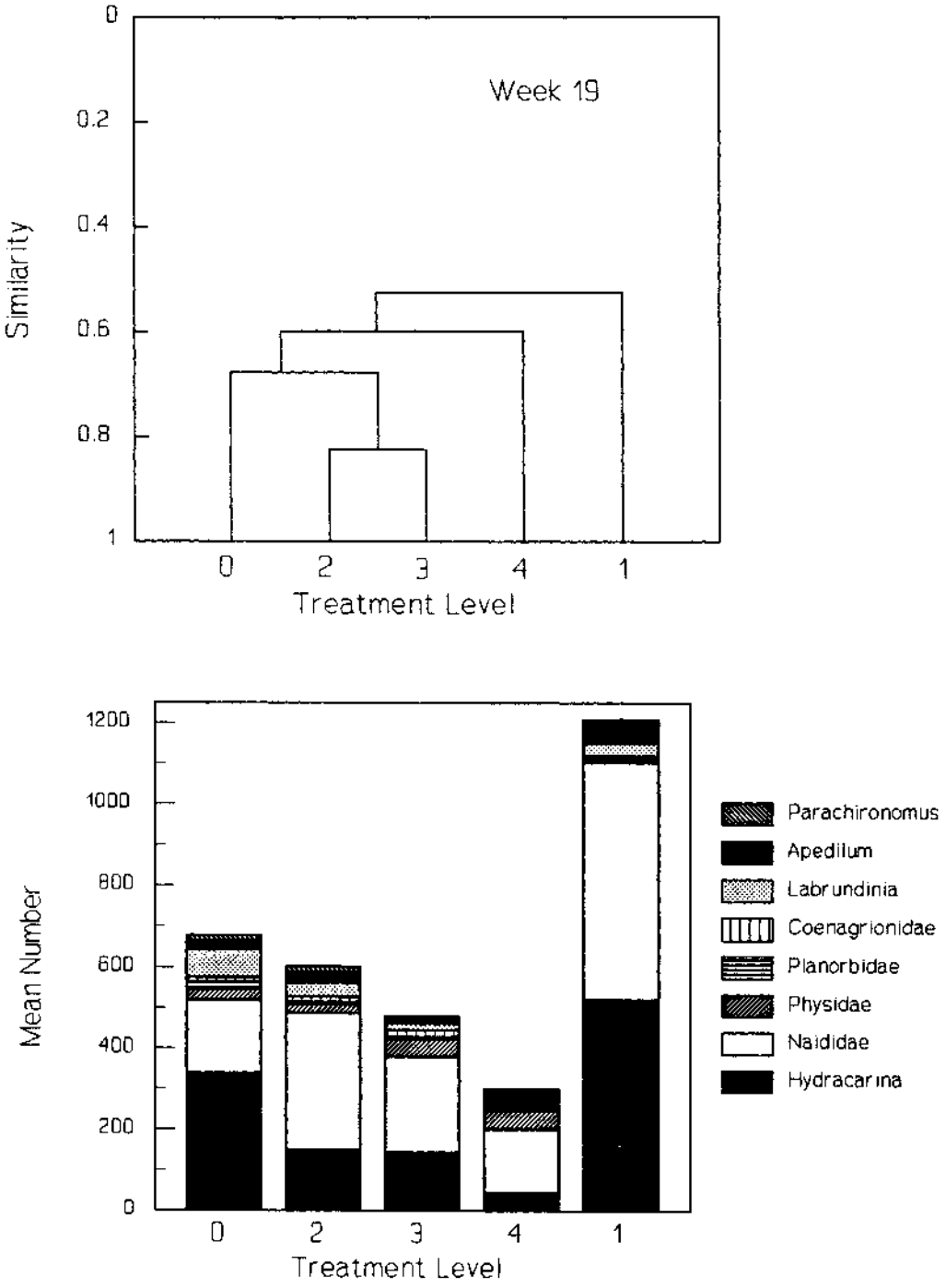

Figure 67. Bray-Curtis cluster analysis of macroinvertebrates colonizing artificial substrates during the final sampling week. Treatments range from controls (0) to high rate (4).

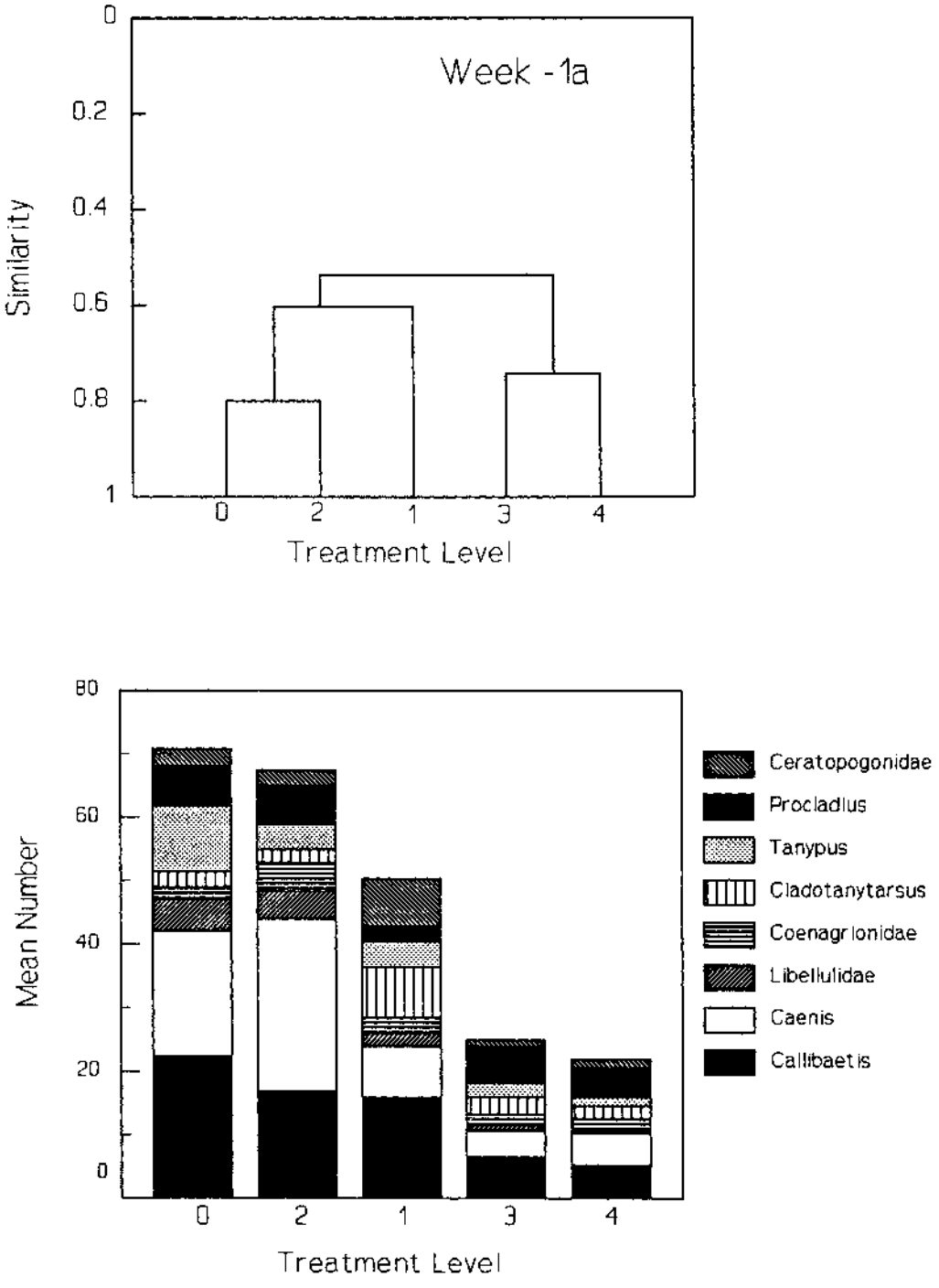

Figure 68. Bray-curtis cluster analysis of exuviae collected prior to the first pesticide application. Treatments range from controls (0) to high rate (4).

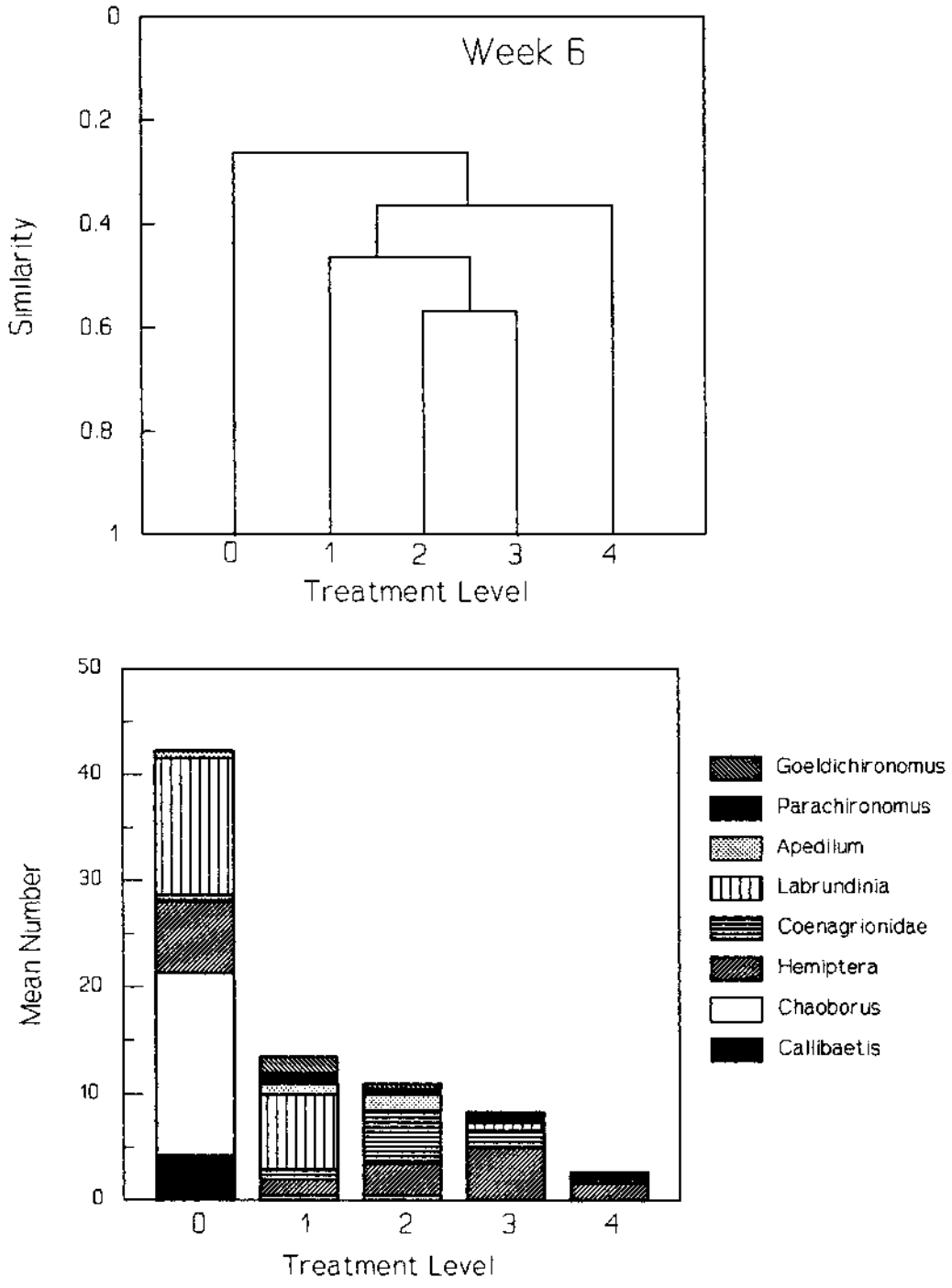

Figure 69. Bray-Curtis cluster analysis of exuviae collected from microcosms during the middle of the application period. Treatments ranged from controls (0) to high rate (4).

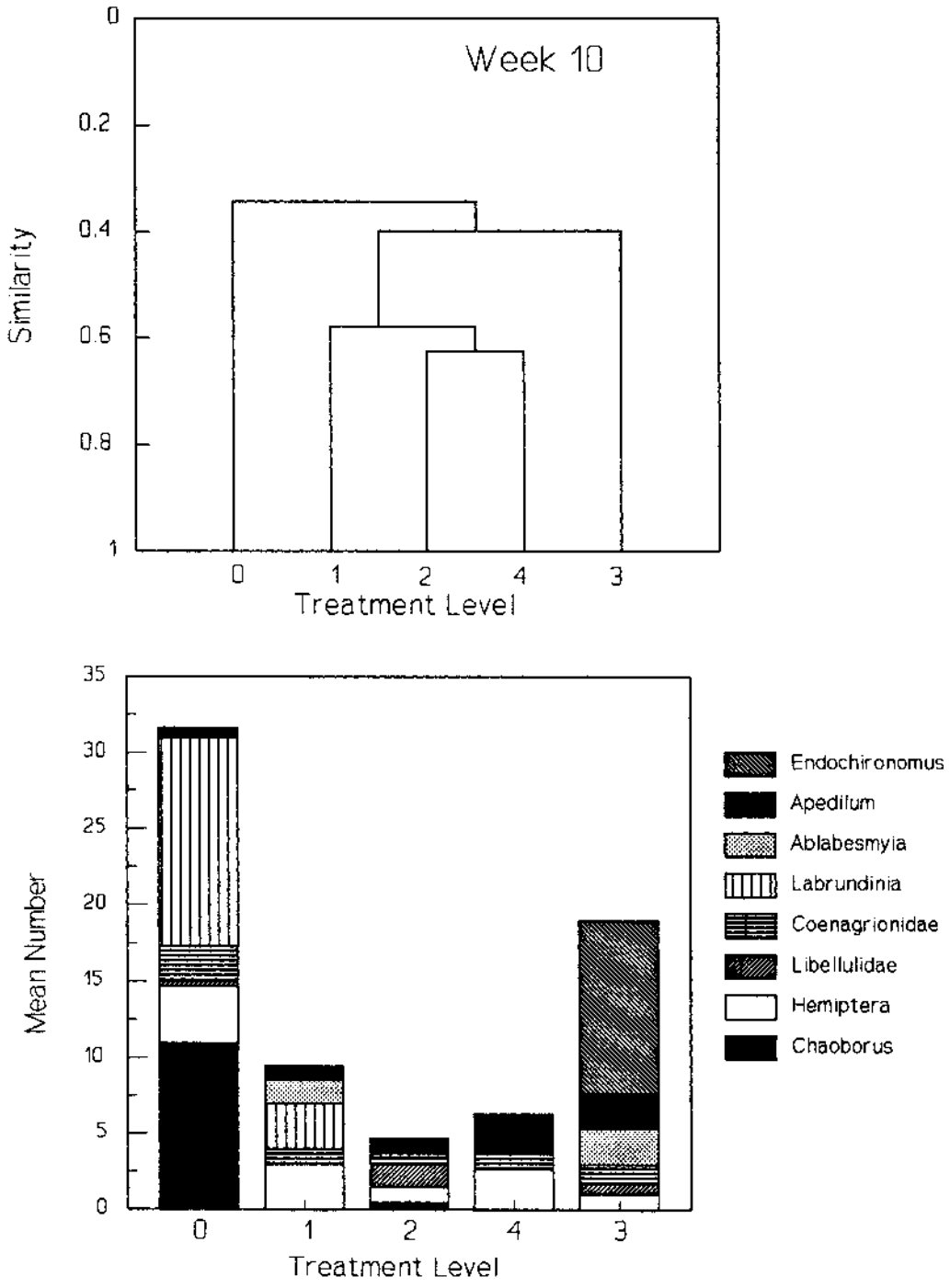

Figure 70. Bray-Curtis cluster analysis of exuviae collected from microcosms during the final application period. Treatments range from controls (0) to high rate (4).

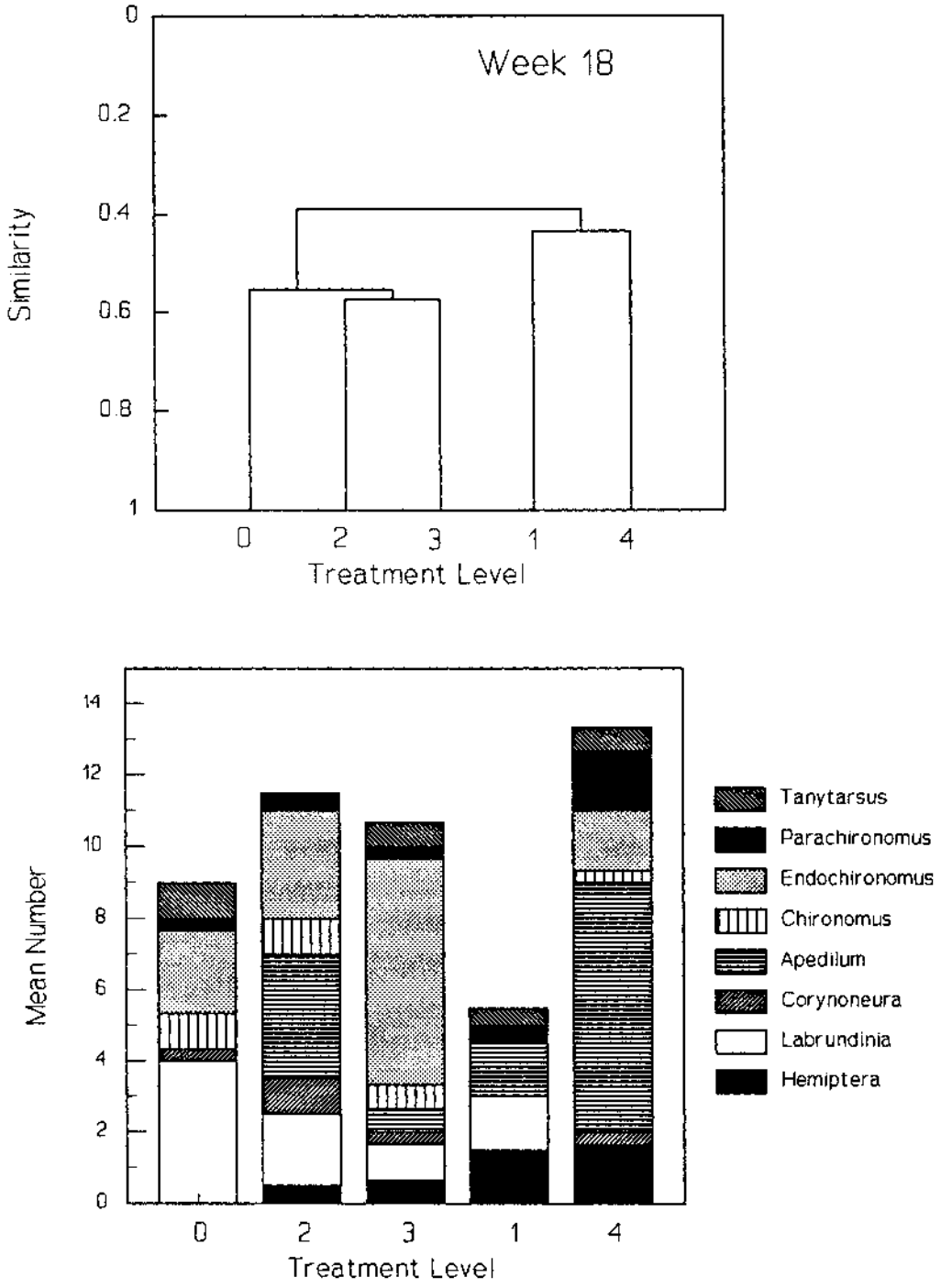

Figure 71. Bray-Curtis cluster analysis of exuviae collected from microcosms one week prior to study termination. Treatments range from controls (0) to high rate (4).

sensitive taxa. They felt that odonate toxicity was not well addressed by their sampling methods. Looking at the data, the gastropod response was variable and toxic effects may have been spurious. Based on the range of responses found in the cyfluthrin microcosm study, it would appear that classification to the order level (as in this mesocosm study) was inadequate.

Hill et al. (1988) studied lambdacyhalothrin (PP321) in mesocosms in North Carolina. Rankings of impacts listed baetid and caenid mayflies, water mites, Gerridae, Veliidae, Trichopterans (family Leptoceridae), and Tanypodinae chironomids as very sensitive or fairly sensitive (impacted in low or medium rate ponds). Taxa impacted at high rates only included zygopterans (damselflies), Belostomatidae, Notonectidae, Ceratopogonidae (Dipterans) and Haliplidae (Coleopterans). Turbellaria, Mollusca, Oligochaeta, Hydrophilidae beetles and Chironominae chironomids were all listed as unaffected.

It is difficult to extrapolate results across different compounds (pyrethroids differ in toxicity), and location/sampling methods appear to play a role, judging from the three esfenvalerate studies. Even so, many widespread taxa appear to be sensitive to various pyrethroids used in different parts of the country. Indeed, microcosm studies might be useful in the future as a way of

extrapolating toxicological results to differing conditions or locales.

Sampling and Analytical Methodologies

Some taxa were sampled most effectively by MAS samplers (i.e., Trichoptera). Trichopterans apparently avoided emergence traps, or emergence success was low for this group in microcosms. While speculative, bluegill predation on emerging caddisflies may have been important since they would be fairly large, obvious prey.

Caenis were dramatically reduced in MAS but no statistical differences were observed for exuviae due to lower densities. Baetid mayfly impacts were identified well using both exuviae and MAS, but all mayflies avoided funnel traps. This agrees with Dewey (1986) who found that Odonata, dytiscid beetles, baetid mayflies and ceratopogonid flies were not sampled effectively by submerged funnel emergence traps in mesocosms.

Other groups were more effectively sampled by exuviae collection. The most obvious example is *Chaoborus*, which were poorly sampled in MAS and emergence traps. This result is not surprising, since *Chaoborus* are semi-pelagic insects that rest near the sediments during daylight and migrate through the water-column at night. Sampling efficiency has important ramifications for ecological testing. In this

instance, the number of significant differences identified for *Chaoborus* were apparently related to sample sizes within control microcosms, since densities in treated microcosms neared zero for all sampling methods. Chironomidae numbers were fairly high in both MAS and exuviae, but very different conclusions would be reached if only emergence or artificial substrate sampling was employed. MAS suggested that no impacts occurred, whereas emergence (both traps and exuviae) identified significant reductions. Chironomid responses are detailed in Chapter 8.

Statistical differences did not always agree with visual trends. For instance, Dunnett's MRT differences (Table XI) observed in Hyracarina populations during week 1 (D2) and week 5 (D2, D3) might or might not be ecologically important, since densities were very low. Alternatively, obvious differences in Hydracarina populations were observed during weeks 13-19 for D4 microcosms (Figure 60) while statistical significance was limited to weeks 17 and 19 (Table XI). These contrasts between statistical significance and "graphical significance" can be observed in almost any ecological study and underscore the danger in relying totally on statistical determinations.

Bray-Curtis/bootstrapping analyses were ineffective for identifying statistical significance from these data. No significant differences were identified for either MAS or

exuviae samples during the application period, though controls were often quite different from treatments. Intra-treatment variance was apparently high enough to preclude significance. Also, insensitive taxa were "lumped" with impacted types, in contrast to taxa-specific analyses such as Dunnett's MRT where impacted groups were considered in isolation. As with other metrics such as diversity, richness or evenness that attempt to consolidate community-wide responses into a single number, the sensitivity of this whole-community approach appears to be low. Use of Bray-Curtis cluster analysis will be discussed further in the next chapter, dealing with impacts on chironomid populations.

Summary

Total numbers in MAS generally increased, while taxa richness decreased. Both numbers and richness of emergence (exuviae and funnel trap) samples decreased in response to cyfluthrin.

Many groups of macroinvertebrates responded to pyrethroid application. Mayflies, chironomids, amphipods, caddisflies, chaoborids, damselflies, and water mites were significantly reduced at differing treatment levels. Many were impacted at the lowest level (D1). Naidid oligochaetes

were enhanced, particularly at lower treatment levels, while snails and dragonfly naiads were apparently unaffected.

These results were broadly similar to those of published mesocosm studies, still some differences in sensitivity were found for specific taxa.

Dunnett's MRT analyses detected differences not found using Bray-Curtis cluster analysis. This particular clustering technique may be too conservative for analysis of impacted communities.

CHAPTER 8

CYFLUTHRIN IMPACTS ON CHIRONOMIDS

Introduction

The family Chironomidae is the most widely distributed and frequently most abundant group of insects in freshwater environments (Morris and Brooker 1979, Pinder 1986). Chironomids were numerically important and quite diverse during this experiment. It was felt that focusing on this group would result in a better understanding of pesticide impacts on the macroinvertebrate community.

Three subfamilies of chironomids are commonly found in northern Texas; the Tanypodinae, Chironominae and Orthocladiinae. Chironomids differ greatly in feeding strategies, and few chironomids appear to be limited to a single mode of feeding (Pinder 1986). Larval Tanypodinae are generally classified as predators, but few are obligate carnivores (Oliver 1971). Gut analyses of Tanypodinae from streams in the Coweeta area of North Carolina found 81-91% of the diet consisting of animal material (Lugthart et al. 1990). Oligochaetes (Loden 1974, Soster and McCall 1989) and Chironominae larvae (Kajak and Dusoge 1970, Baker and McLachlan 1979) are common prey of Tanypodinae. Other subfamilies such as the Orthocladiinae and Chironominae have

been broadly classified as collector-gatherers (Wallace et al. 1989). Some normally detritivorous species may be facultative predators, however (Pinder 1986).

Chironomids demonstrate a range of sensitivity to pyrethroids, with LC50 values ranging from 6.5 μg/L to 0.12 μg/L for various compounds and different chironomid species (Anderson 1989). Most experiments have identified chironomid LC50's in the < 1.0 μg/L range.

Few studies have evaluated insecticide impacts on chironomids in detail, with analyses at the generic level being quite rare. No studies in the open literature document pesticide impacts on larval, pupal and adult chironomids. No other study has combined bioassays with community responses for evaluating pesticide impacts on midges.

Results

Subfamily Level Analyses

Orthocladiinae were fairly rare in microcosm samples, with maximal values in spring and fall. Low numbers resulted in an absence of significant differences throughout the experiment. The two dominant chironomid subfamilies (Tanypodinae and Chironominae) differed substantially in their responses to cyfluthrin.

Tanypodinae were significantly reduced at levels D2-D4 consistently in MAS, exuviae and emergence trap samples (Table XIV, XV, and XVI respectively). Tanypodinae were significantly reduced at D1 in emergence trap and MAS samples at least once during the sampling period. Larval Tanypodinae colonizing MAS showed a clear exposure-response relationship, with the severity and duration of impact directly related to dose level (Figure 72). Tanypodinae exuviae (Figure 73) and adult emergence (Figure 73) were also reduced by cyfluthrin, particularly at high treatment levels, with no recovery at D4.

Chironominae larvae (Table XIV), exuviae (Table XV) and adult emergence (Table XVI) from treated microcosms showed a pattern of significant enhancement over control levels. Chironominae larvae collected with MAS were generally higher than controls at all treatments, with the highest densities in D1 microcosms (Figure 75). Chironominae exuviae production was generally similar to control levels throughout the application period, with dramatic increases in emergence during weeks 12-16 after pesticide stress was removed (Figure 76). Adult emergence measured with funnel traps also showed substantial increases at D3 and D4 following cessation of pyrethroid treatments (Figure 77).

Generic Level Analysis and Bootstrapping

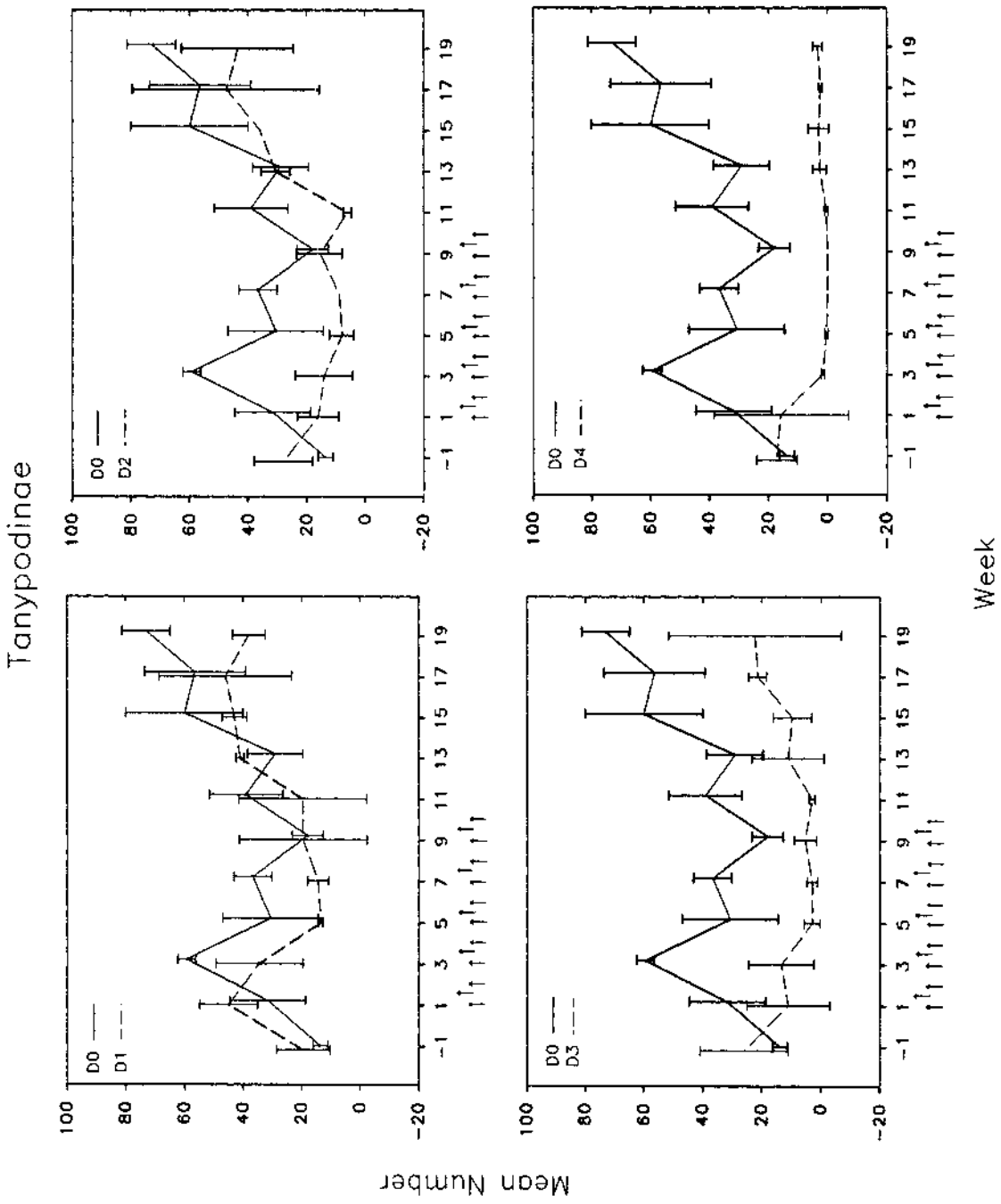

Figure 72. Mean number of Tanypodinae larvae (± S.D.) colonizing artificial substrates. Arrows represent pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

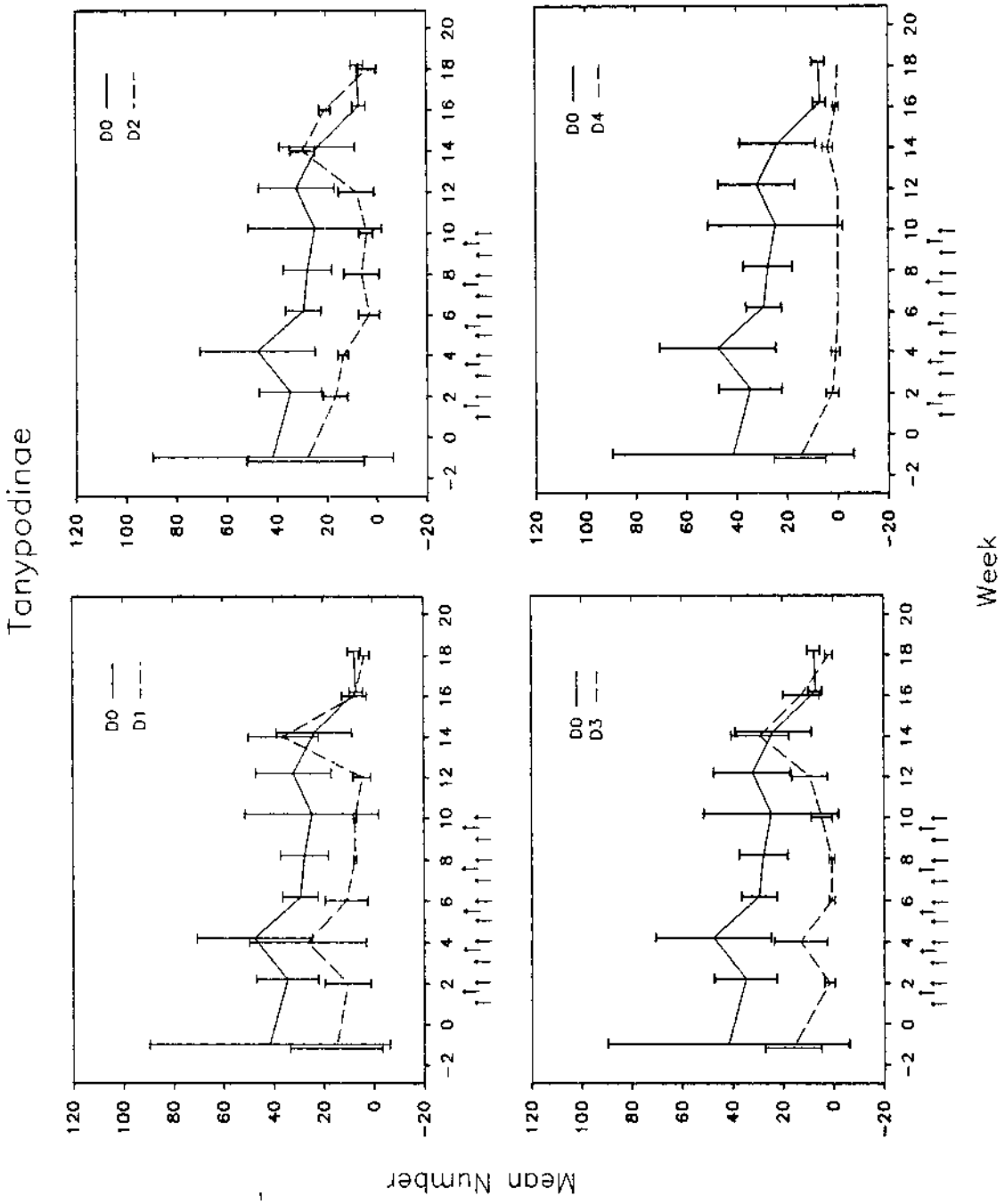

Figure 73. Mean number of Tanypodinae exuviae (± S.D.) collected from microcosms. Arrows represent pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose Level 4.

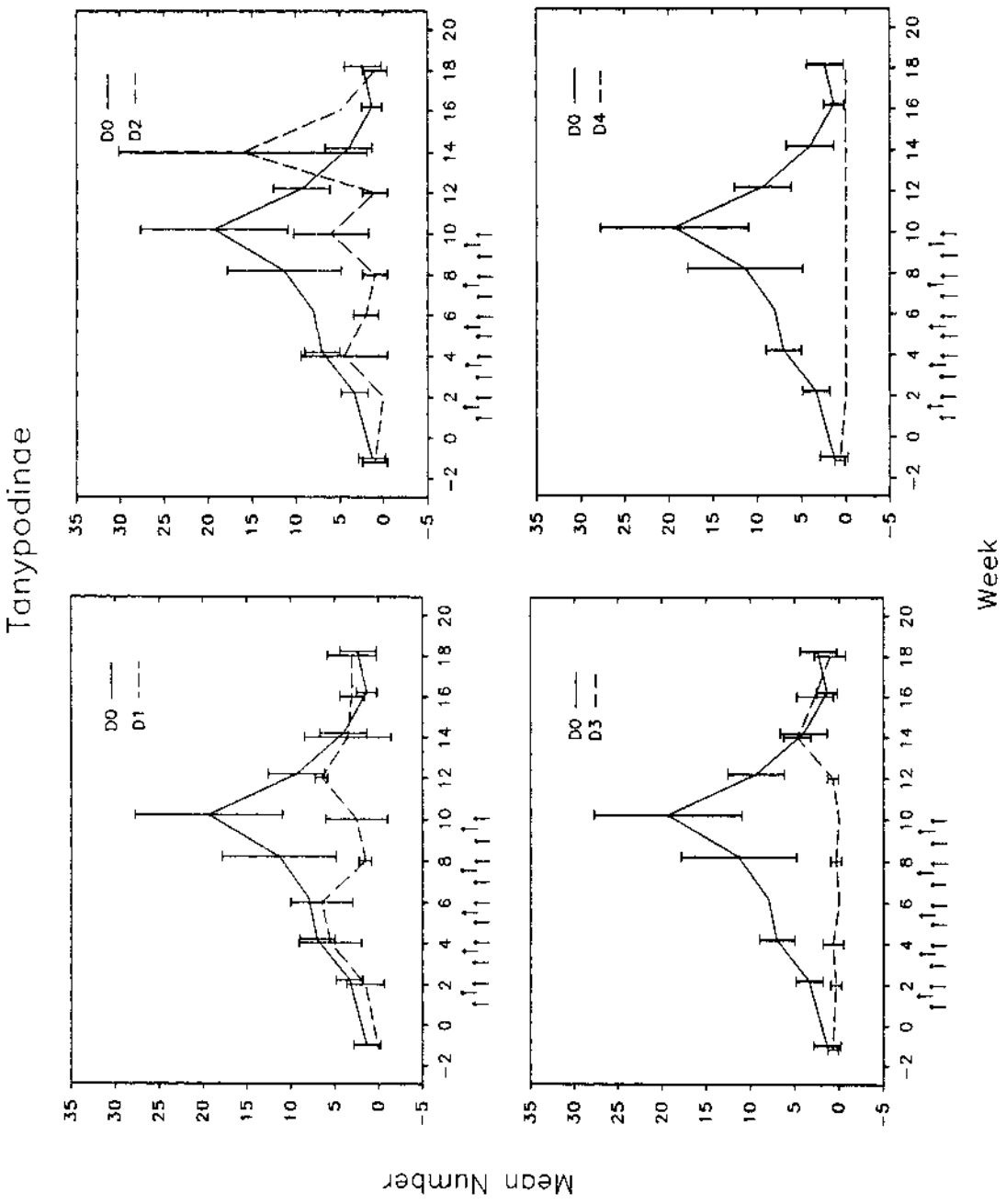

Figure 74. Mean number of Tanypodinae adults (± S.D.) collected with emergence traps from microcosms. Arrows indicate pesticide applications. D0=Control, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

Identification of chironomids at the generic and species-group levels revealed changes in dominant taxa through time relative to pesticide loadings. Due to the labor involved in mounting specimens of larvae and pupal exuviae, four key sampling periods were chosen for analysis. Generic analyses of MAS samples and exuviae were conducted prior to pesticide application (weeks -1 and -1a), during the middle of the dosing period (weeks 5 and 6), at the end of the application period (weeks 9 and 10), and near study termination (weeks 18 and 19). Emergence trap specimens were routinely identified to genus or species bimonthly.

Prior to pesticide application (week -1), *Cladotanytarsus*, *Endochironomus* and *Ablabesmyia* were the dominant taxa found in MAS samplers (Figure 78). No significant differences were found via Dunnett's analysis (Table XIV). Similarity coefficients ranged from 0.57 to 0.71 with no significant differences between clusters (Appendix Table 6).

Dominant chironomid pupal exuviae prior to pesticide application (week -1a) included *Tanypus*, *Procladius*, *Ablabesmyia* (all Tanypodinae), and the Chironominae *Cladotanytarsus* and *Chironomus* (Figure 79). No significant differences were found with Dunnett's analysis (Table XV) prior to dosing. No significant differences were found between clusters (Appendix Table 7). Clustering showed no

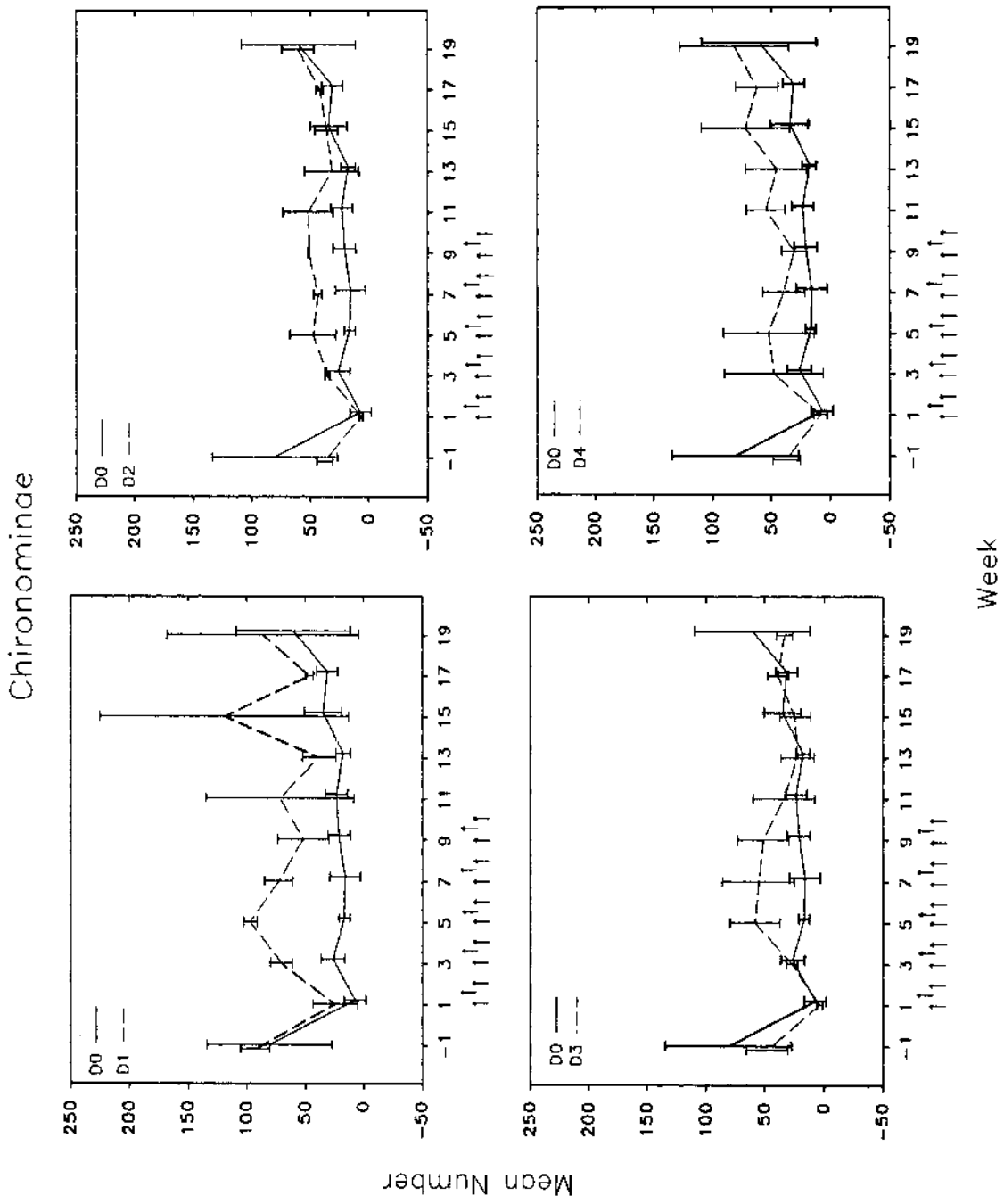

Figure 75. Mean number of Chironominae larvae (± S.D.) colonizing artificial substrates during the experiment. Arrows represent pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose Level 4.

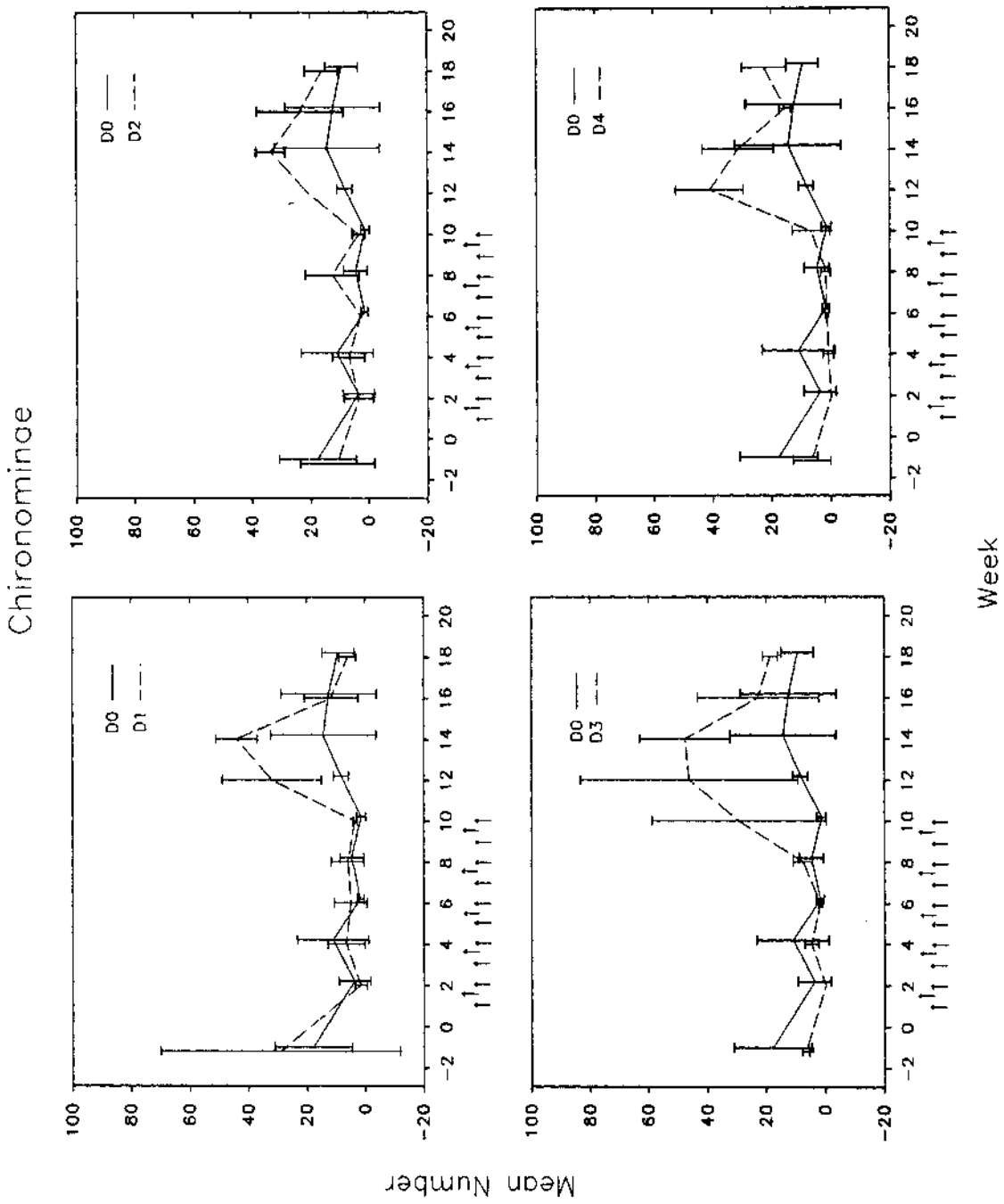

Figure 76. Mean number of Chironominae exuviae (± S.D.) collected from microcosms. Arrows represent pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

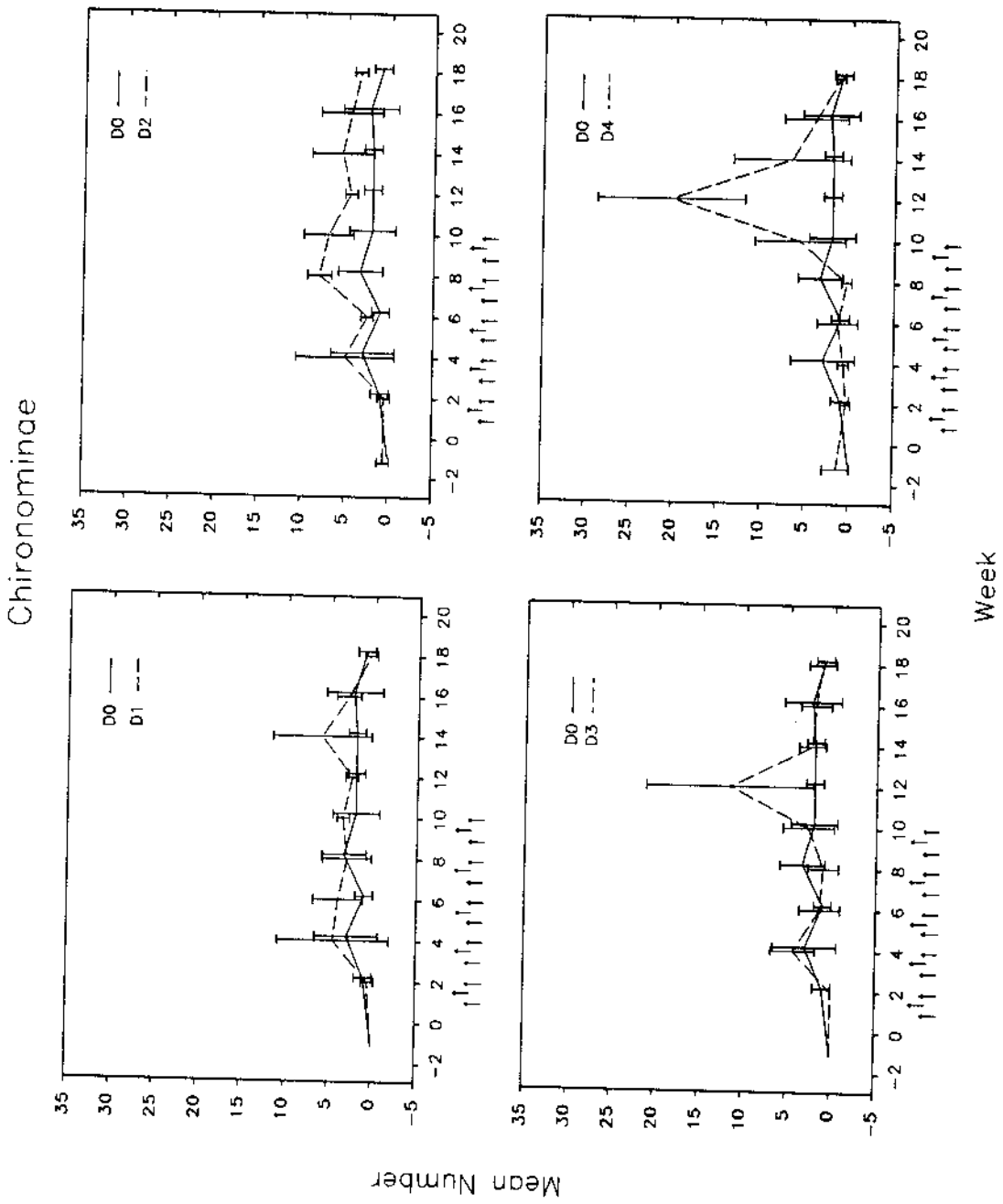

Figure 77. Mean number of Chironominae adults (± S.D.) collected with emergence traps from microcosms. Arrows indicate pyrethroid applications. D0=Controls, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

relationship to treatment level (Figure 79).

Artificial substrate samples during the middle of the application period (week 5) showed reductions in *Labrundinia* (Tanypodinae) larvae (Figure 80). *Labrundinia* were significantly lower in D2-D4 relative to controls (Table XIV). Chironominae genera (particularly *Goeldichironomus*) were dramatically enhanced in treated microcosms (Figure 80). Significant increases were observed for *Dicrotendipes*, *Glypotendipes*, *Goeldichironomus* and *Parachironomus*, with most increases at D1-D3 (Table XIV). Similarity coefficients ranged from 0.32 to 0.79 (Appendix Table 6), with clusters arranged in order of increasing dose (Figure 80). Probability analysis using SIGTREE bootstrapping partitioned D0 from D1-D4 ($p=0.023$; Appendix Table 6).

Emergence (exuviae) at week 6 was dominated by the Tanypodinae *Labrundinia*, *Ablabesmyia* and *Procladius* (*Psilotanypus* and *Holotanypus*) in controls and D1. Total numbers of emerging chironomids showed a clear exposure-response relationship, with few exuviae collected at D4 (Figure 81). *Labrundinia* were significantly decreased at treatments D2-D4 (Table XV). *Procladius* exuviae were significantly decreased at D3-D4 (Table XV). Cluster analysis grouped D0-D1 and D2-D4, with little similarity among these clusters (similarity=0.17). Bootstrapping analysis did not statistically separate D0 from D1

Table XIV. Statistically significant differences for Chironomidae from MAS samplers in microcosms (Dunnett's MRT). D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

Tanypodinae Genera - MASS		Week Number -1	5	9	19
Ablabesmyia	D1				
	D2				
	D3				
	D4				
Clinotanypus	D1				
	D2				
	D3				
	D4				
Labrundinia	D1				
	D2	↑	↓	↓	
	D3		↓	↓	↓
	D4		↓	↓	↓
Larsia	D1				
	D2				
	D3				
	D4				
Procladius	D1				
	D2				
	D3				
	D4				
Tanypus	D1				
	D2				
	D3				
	D4				

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

Continuation of Chironomidae significant differences from MAS samples.

Chironominae Genera - MASS		Week Number -1	5	9	19
Apedilum elachista	D1				
	D2				
	D3				
	D4				
Chironomus	D1				
	D2				
	D3				
	D4				
Cladotanytarsus	D1				
	D2				
	D3				
	D4				
Dicrotendipes	D1		↑		
	D2		↑		
	D3		↑		
	D4				
Endochironomus nigricans	D1				
	D2				
	D3				
	D4				
Glypotendipes	D1		↑		
	D2				
	D3				
	D4				↑

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

Continuation of statistical differences for Chironomidae from MAS samplers.

Chironominae Genera - MASS		Week Number -1	5	9	19
Goeldichironomus holoprasinus	D1		↑		
	D2				
	D3		↑		
	D4		↑		
Parachironomus	D1		↑	↑	
	D2			↑	
	D3			↑	
	D4				
Tanytarsus	D1				
	D2				
	D3				
	D4				
Zavreliella varipennis	D1				
	D2				
	D3				
	D4				

Subfamily MASS		Week Number -1	1	3	5	7	9	11	13	15	17	19
Tanypodinae (Total)	D1					↓						
	D2			↓		↓		↓				
	D3			↓	↓	↓		↓		↓	↓	
	D4			↓	↓	↓	↓	↓	↓	↓	↓	↓
Chironominae (Total)	D1				↑	↑						
	D2											
	D3				↑							
	D4										↑	

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

treatments (p=0.127) if α=0.05 is used.

Labrundinia in MAS samples during week 9 showed an exposure-response pattern (Figure 82) with significant reductions at D2-D4 (Table XIV). *Goeldichironomus, Parachironomus* and *Apedilum* increased in treated microcosms, particularly D2 (Figure 82). *Parachironomus* were significantly enhanced in D1-D3 relative to D0 (Table XIV). Bray-Curtis clustering ranked treatments in increasing dose-order. Bootstrap analysis indicated that D0 differed significantly from D1-D4 (p=0.05; Appendix Table 6).

Week 10 emergence (exuviae) was dominated by *Labrundinia, Ablabesmyia, Apedilum* and *Endochironomus* (Figure 83). *Labrundinia* were significantly reduced at D3-D4 (Table XV). No significant enhancements in Chironominae were observed for this week. Bray-Curtis similarity coefficients were as low as 0.18 (Figure 83). No significant differences were identified via probability analysis (Appendix Table 7).

Labrundinia in MAS continued to be significantly reduced at D3-D4 near the end of the experiment (Table XIV, Figure 84). Dominant taxa during week 19 were *Labrundinia, Apedilum* and *Parachironomus. Glypotendipes* were significantly enhanced at D4 (Table XIV). Clustering did not respond in an exposure-response manner, with D0 being most similar to D2 (Figure 84). Bootstrapping analysis

Table XV. Statistically significant differences in Chironomidae exuviae from microcosms (Dunnett's MRT). D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

Tanypodinae Genera		Week Number -1	6	10	18
Ablabesmyia	D1				
	D2				
	D3				
	D4				
Clinotanypus	D1				
	D2				
	D3				
	D4				
Labrundinia	D1				
	D2		↓		
	D3		↓	↓	
	D4		↓	↓	↓
Larsia	D1				
	D2				
	D3				
	D4				
Procladius	D1				
	D2				
	D3		↓		
	D4		↓		
Tanypus	D1				
	D2				
	D3				
	D4				

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

Continuation of Chironomidae exuviae significant differences from microcosms.

Chironominae Genera		Week Number			
		-1	6	10	18
Apedilum elachista	D1				
	D2				
	D3				
	D4				↑
Chironomus	D1				
	D2				
	D3				
	D4				
Cladotanytarsus	D1				
	D2				
	D3				
	D4				
Dicrotendipes modestus	D1				
	D2				
	D3				
	D4				
Endochironomus nigricans	D1				
	D2				
	D3				
	D4				
Goeldichironomus holoprasinus	D1				
	D2				
	D3				
	D4				

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

Continuation of Chironomidae exuviae significant differences from microcosms.

Chironominae Genera		Week Number -1	6	10	18
Parachironomus	D1				
	D2				
	D3				
	D4				
Polypedilum	D1				
	D2				
	D3				
	D4				
Tanytarsus	D1				
	D2				
	D3				
	D4				
Zavreliella varipennis	D1				
	D2				
	D3				
	D4				

Subfamily		Week Number -1	2	4	6	8	10	12	14	16	18
Tanypodinae (Total)	D1										
	D2				↓	↓					
	D3		↓		↓	↓					
	D4		↓	↓	↓	↓	↓	↓	↓	↓	↓
Chironominae (Total)	D1										
	D2										
	D3						↑	↑			
	D4							↑			

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

showed no significant differences among clusters (Appendix Table 6).

Exuviae during week 18 were dominated by *Labrundinia*, *Chironomus* and *Endochironomus* (Figure 85). *Labrundinia* were significantly reduced at D4, and *Apedilum* were significantly increased at D4 (Table XV). Treatment D2 was most similar to controls, while D1 was least similar (Figure 85). No significant differences between clusters were determined via bootstrapping, with the lowest observed probability of p=0.14 (Appendix Table 7).

Emergence trap samples were analyzed at the generic level on a bimonthly basis. No clustering was conducted since sample sizes were lower than exuviae and results were anticipated to be similar. *Labrundinia* were significantly reduced throughout much of the experimental period. Most reductions occurred at D2-D4, but D1 was also impacted during weeks 10 and 12 (Table XVI). *Parachironomus* were significantly increased during weeks 6, 12 and 16, while *Apedilum* were enhanced during week 12 (Table XVI).

Chironomus tentans Bioassays

Pyrethroid toxicity in the water column was examined using *C. tentans* assays (week 10). No significant toxicity was observed at 24 hours (Figure 86). Significantly lower survival at 48 and 72 hours was exhibited in treatment D4

Table XVI. Statistically significant differences in emergent Chironomidae collected with emergence traps (Dunnett's MRT). D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

Tanypodinae		Week Number									
		-1	2	4	6	8	10	12	14	16	18
Ablabesmyia	D1										
	D2										
	D3										
	D4										
Labrundinia	D1					↓	↓				
	D2		↓		↓	↓		↓			
	D3			↓	↓	↓	↓	↓			
	D4		↓	↓	↓	↓	↓	↓			
Pentaneurini	D1										
	D2										
	D3										
	D4										
Procladius	D1										
	D2										
	D3										
	D4										
Tanypus	D1										
	D2										
	D3										
	D4										

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

Continuation of Chironomidae collected in emergence traps.

Chironominae		Week Number -1	2	4	6	8	10	12	14	16	18
Apedilum elachista	D1										
	D2										
	D3										
	D4							↑			
Chironomus decorus	D1										
	D2										
	D3										
	D4										
Cladotanytarsus	D1										
	D2										
	D3										
	D4										
Dicrotendipes modestus	D1										
	D2										
	D3										
	D4										
Endochironomus nigricans	D1										
	D2										
	D3										
	D4										
Goeldichironomus holoprasinus	D1										
	D2										
	D3										
	D4										

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

Continuation of Chironomidae collected with emergence traps.

Chironominae and Orthocladiinae		Week Number									
		-1	2	4	6	8	10	12	14	16	18
Parachironomus	D1										
	D2				↑						
	D3							↑			
	D4							↑	↑		
Tanytarsini	D1										
	D2										
	D3										
	D4										
Corynoneura (Orthocladiinae)	D1										
	D2										
	D3										
	D4										

Subfamily		Week Number									
		-1	2	4	6	8	10	12	14	16	18
Tanypodinae (Total)	D1					↓	↓				
	D2		↓		↓	↓		↓			
	D3		↓	↓	↓	↓	↓	↓			
	D4		↓	↓	↓	↓	↓	↓			
Chironominae (Total)	D1										
	D2										
	D3										
	D4							↑			

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

compared with controls (T-Test with Bonferroni correction, α=0.05). Control survival exceeded 90% throughout the test period.

Sediment toxicity bioassays (Figure 87) conducted in September (week 10) showed significant reductions in survival in D1-D4 relative to control sediment cores (Dunnett's MRT on ranked data, α=0.05). Avoidance of treated sediments was observed within hours of adding midge larvae to cores. Control survival exceeded 80%.

Sediment bioassays in November (week 19) showed no significant differences in survival between treatments and controls (Dunnett's MRT on ranked data, α=0.05). Control survival exceeded 90% (Figure 87). No significant differences in dry weight were observed among treatments (Dunnett's MRT, α=0.05).

Discussion

Comparison with Other Studies

Significant pyrethroid-related effects were found in Tanypodinae populations at the subfamily level. Hill et al. (1988) found that Tanypodinae were very sensitive to the pyrethroid lambdacyhalothrin in North Carolina mesocosms. Lozano et al. (1992) found Tanypodinae to be as sensitive or more sensitive than Chironominae to esfenvalerate in littoral corrals in Minnesota, while Orthocladiinae were the

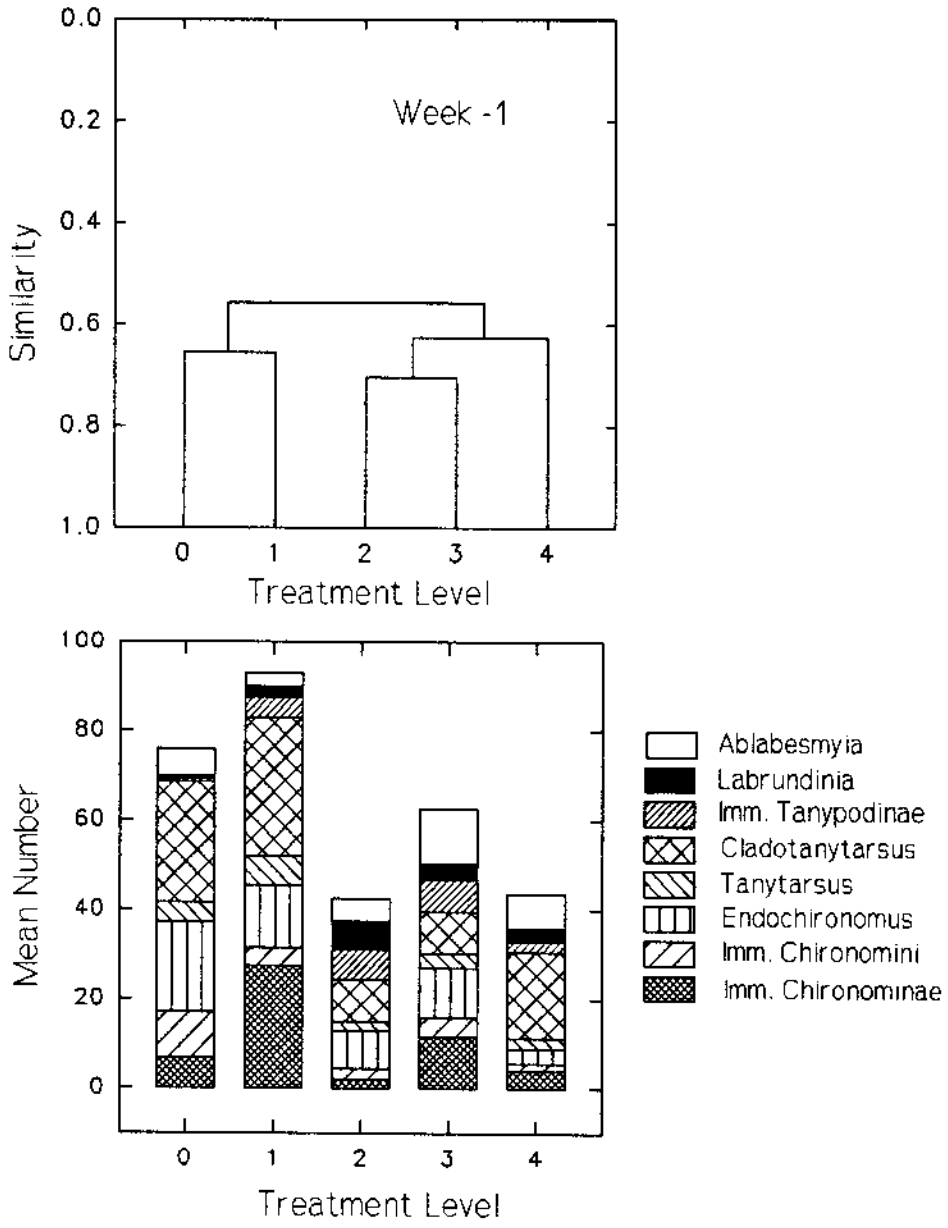

Figure 78. Bray-Curtis cluster analysis of Chironomidae colonizing artificial substrates in microcosms during the pre-treatment period. Treatments range from controls (0) to high rate (4).

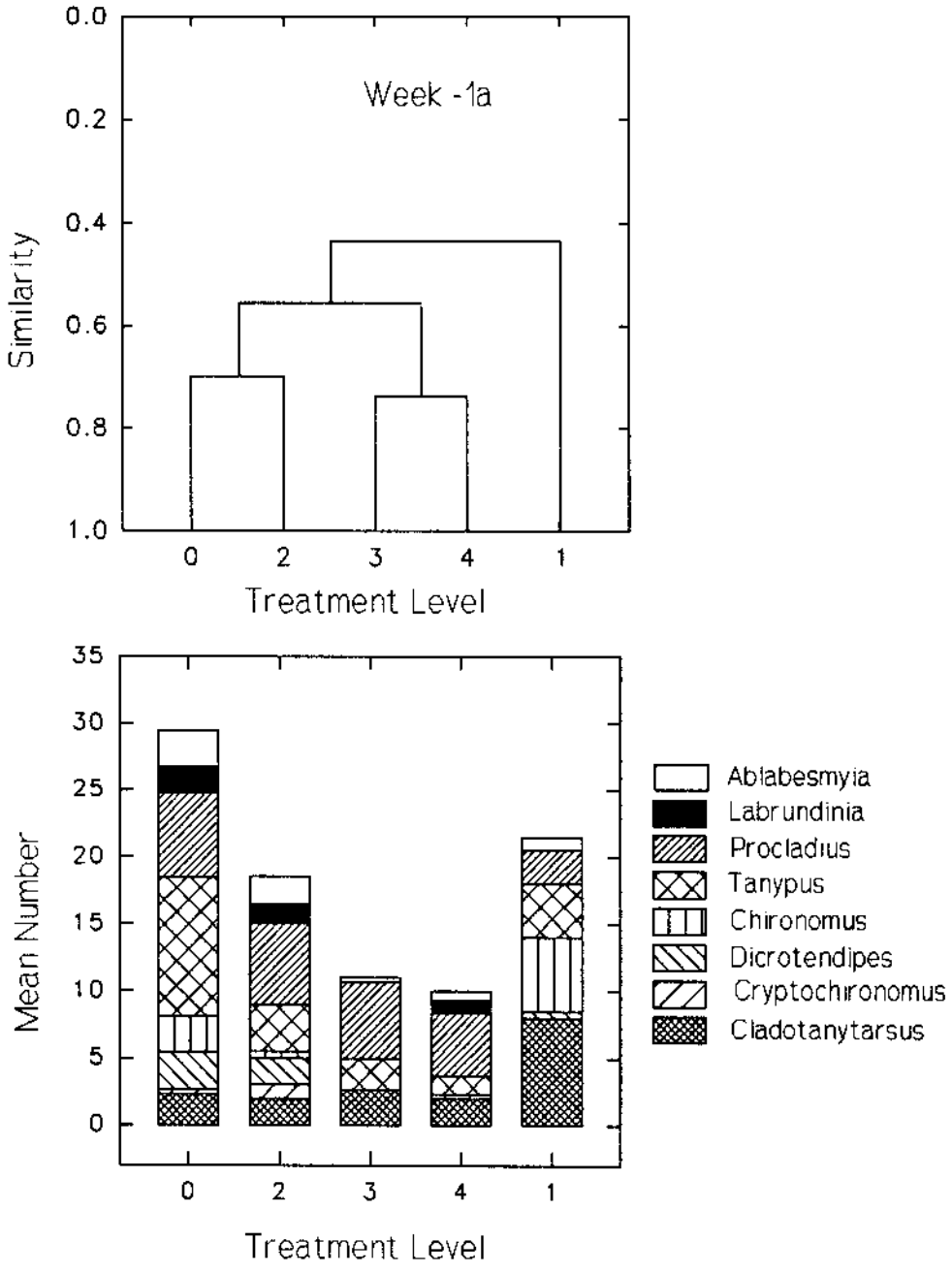

Figure 79. Bray-curtis cluster analysis of Chironomidae exuviae collected from microcosms during the pre-treatment period. Treatments range from controls (0) to high rate (4).

most sensitive subfamily.

Analysis of generic level data indicated that *Labrundinia* was one of the most sensitive chironomids in this microcosm study, being significantly reduced at all exposure levels. Emergence of *Labrundinia pilosella* was reduced by the herbicide atrazine in pond mesocosms (Dewey 1986).

Chironominae populations were enhanced at all exposure levels, often significantly. *Goeldichironomus*, *Parachironomus*, *Dicrotendipes* and *Apedilum* were the dominant genera affected. Increases in these midges may represent indirect effects due to pesticide-related reductions in predator or competitor populations.

Webber et al. (1992) observed reductions of the Tanypodinae *Clinotanypus* and *Procladius*, and the Chironominae *Einfeldia*, *Cladotanytarsus*, *Tanytarsus*, and *Glypotendipes* in high rate treatments of esfenvalerate (a pyrethroid) in mesocosms at Auburn, Mississippi.

Webber et al. (1989) in a study of Wheeler Reservoir found that Tanypodinae were reduced in areas of high DDT and DDD/DDE metabolite residues, while Chironominae became abundant. They felt that organic enrichment, not DDT residues, was responsible for this relationship. Still, these reductions also could be explained by differential toxicity at the subfamily level.

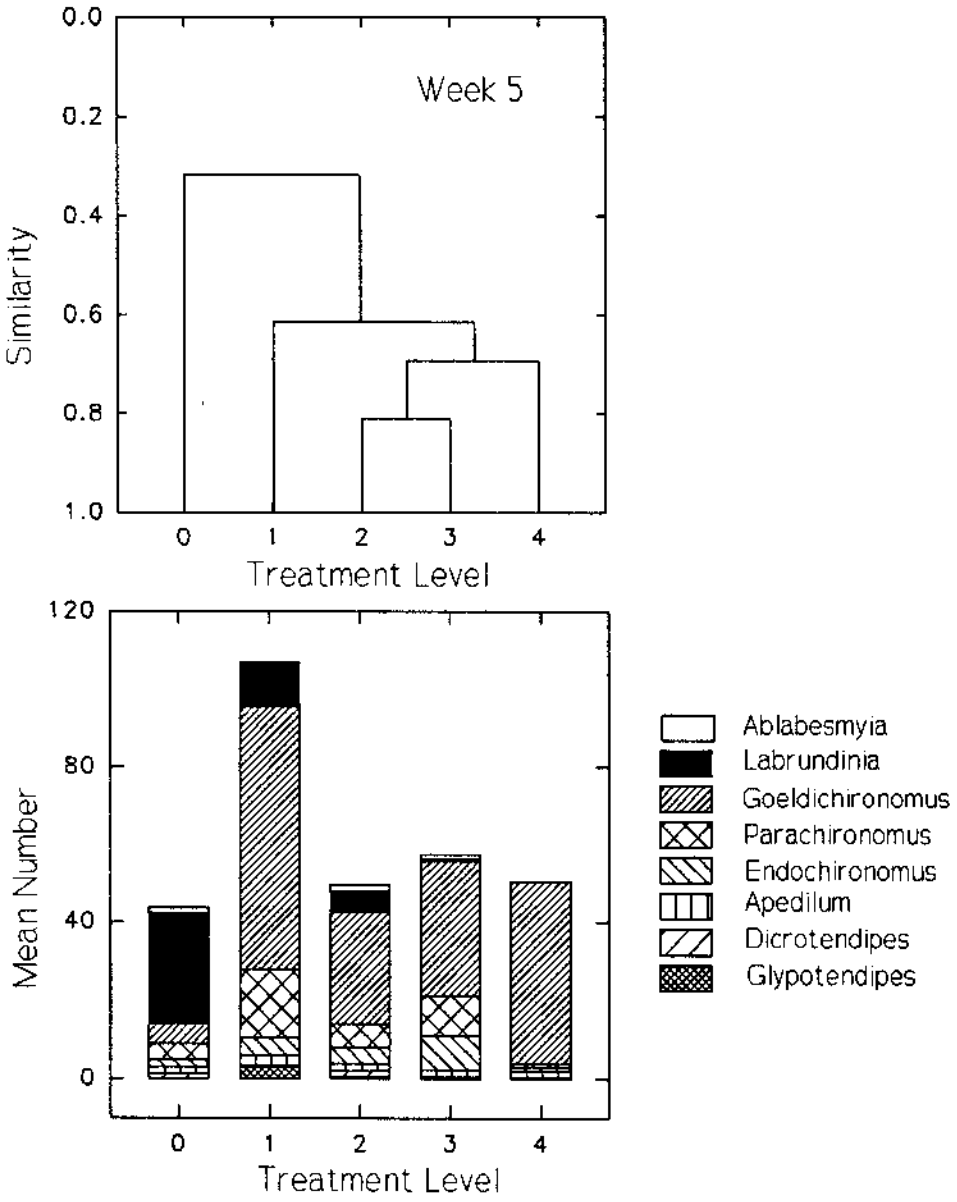

Figure 80. Bray-Curtis cluster analysis of Chironomidae colonizing artificial substrates during the middle of the application period. Treatments range from controls (0) to high rate (4).

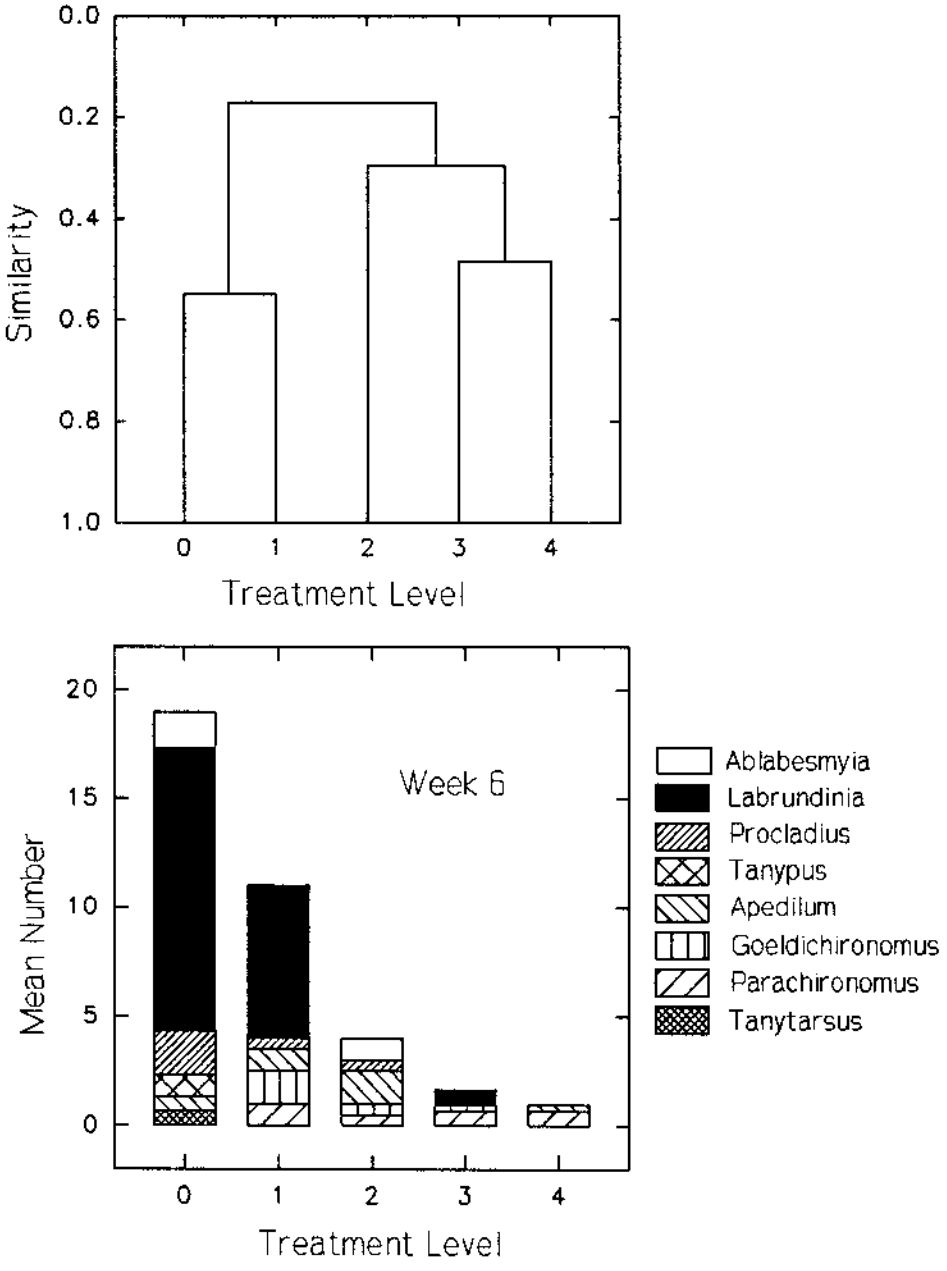

Figure 81. Bray-Curtis cluster analysis of Chironimidae exuviae collected from microcosms during the middle of the application period. Treatments range from controls (0) to high rate (4).

Application of methoxychlor, an organophosphorus pesticide, to streams in the Coweeta study area in North Carolina resulted in dramatic reductions in chironomid densities, biomass and production (Lugthart et al. 1990). This study found both Tanypodinae and non-Tanypodinae chironomids to be very sensitive to methoxychlor application.

Differences in toxicological response at the subfamily level may be related to exposure. Tanypodinae are generally classified as sprawlers and swimmers, and are active predators, not tube-builders (Coffman 1984). Most Chironominae and Orthocladiinae are tube builders (Coffman 1984). Feeding tubes might confer a degree of protection to Chironominae larvae. Tanypodinae larvae do not build tubes and may be more exposed to the pyrethroid. Hershey (1987) has related the amount of time spent outside feeding tubes with susceptibility to damselfly predation. It would be interesting to explore similar relationships between the amount of time outside larval tubes and pesticide toxicity. Differences in exposure might help explain why some chironomids are more susceptible than others.

Sampling Effectiveness and Level of Taxonomic Identification

Some taxa were sampled more effectively by exuviae collection (*Procladius*) while others were sampled more

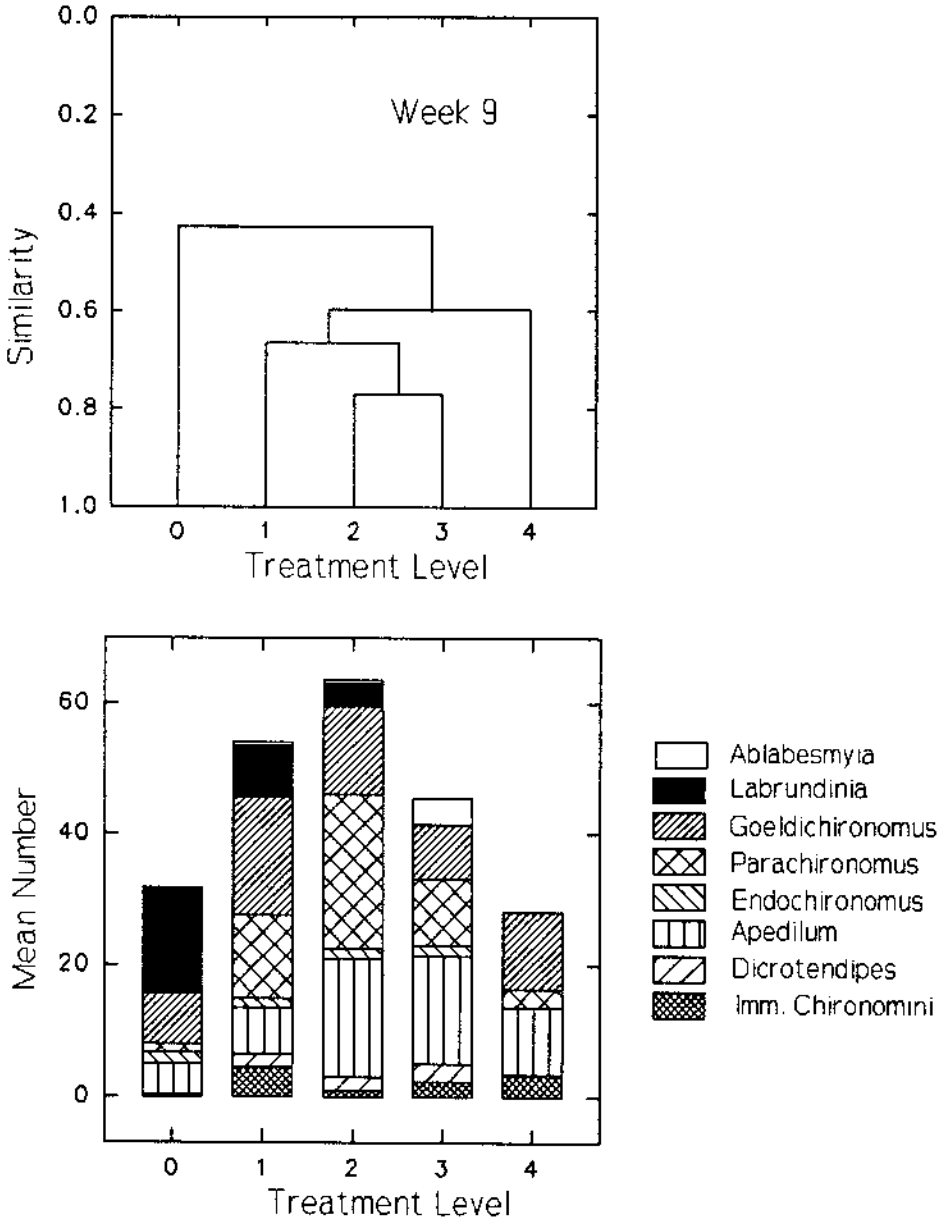

Figure 82. Bray-Curtis cluster analysis of Chironomidae colonizing artificial substrates in microcosms one week before the final application period. Treatments range from controls (0) to high rate (4).

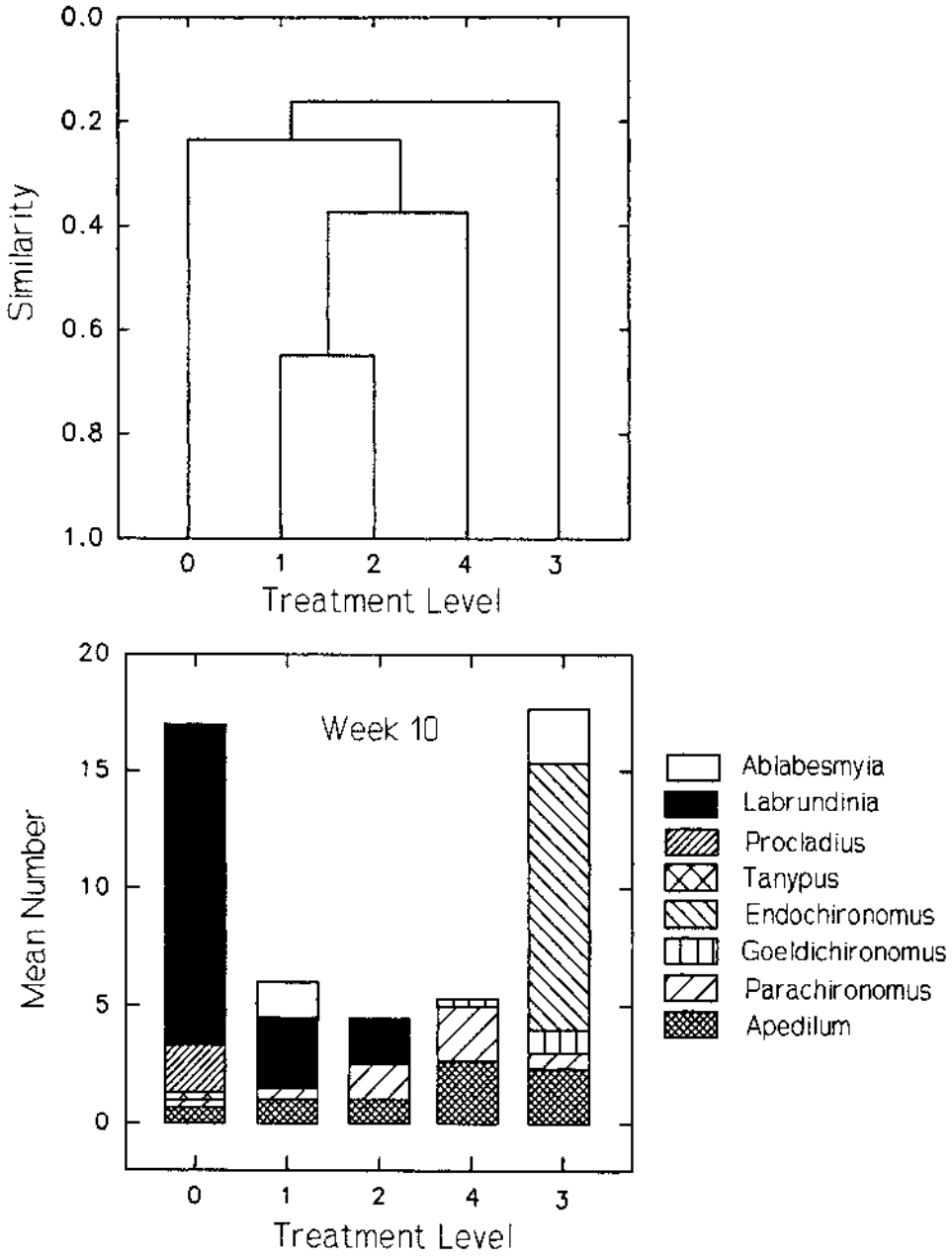

Figure 83. Bray-Curtis cluster analysis of Chironomidae exuviae collected from microcosms during the final application period. Treatments ranged from controls (0) to high rate (4).

effectively in MAS samples (*Glypotendipes*). This may represent habitat preference and/or sampling bias.

Tanypodinae reductions and recovery patterns were broadly similar among exuviae, emergence traps and substrate colonization (Figures 72, 73, and 74; Tables XIV, XV, and XVI). In contrast, Chironominae responses measured by emergence and MAS were very dissimilar (Figures 75, 76, and 77). Larvae in MAS were enhance in pyrethroid-exposed microcosms throughout the treatment period, while emergence increased only toward the end of the experiment. This phenomenon could be caused by a combination of factors. First, larval production may be uncoupled from emergence. Larval populations require a certain amount of time for completion of their life-cycle, resulting in later emergence peaks. A second possibility is that while larval Chironominae were protected from pyrethroids by burrowing in the sediment or constructing feeing tubes (Mulla et al. 1975, Hill et al. 1988), emerging pupae were exposed to higher pesticide levels when swimming through the water-column. These two possibilities are not mutually exclusive.

Higher numbers and greater taxonomic diversity of emergent chironomids were sampled with exuviae, compared to funnel emergence traps. This phenomenon was apparent for both dominant subfamilies, probably reflecting trap avoidance. Some very small Orthocladiinae (i.e.,

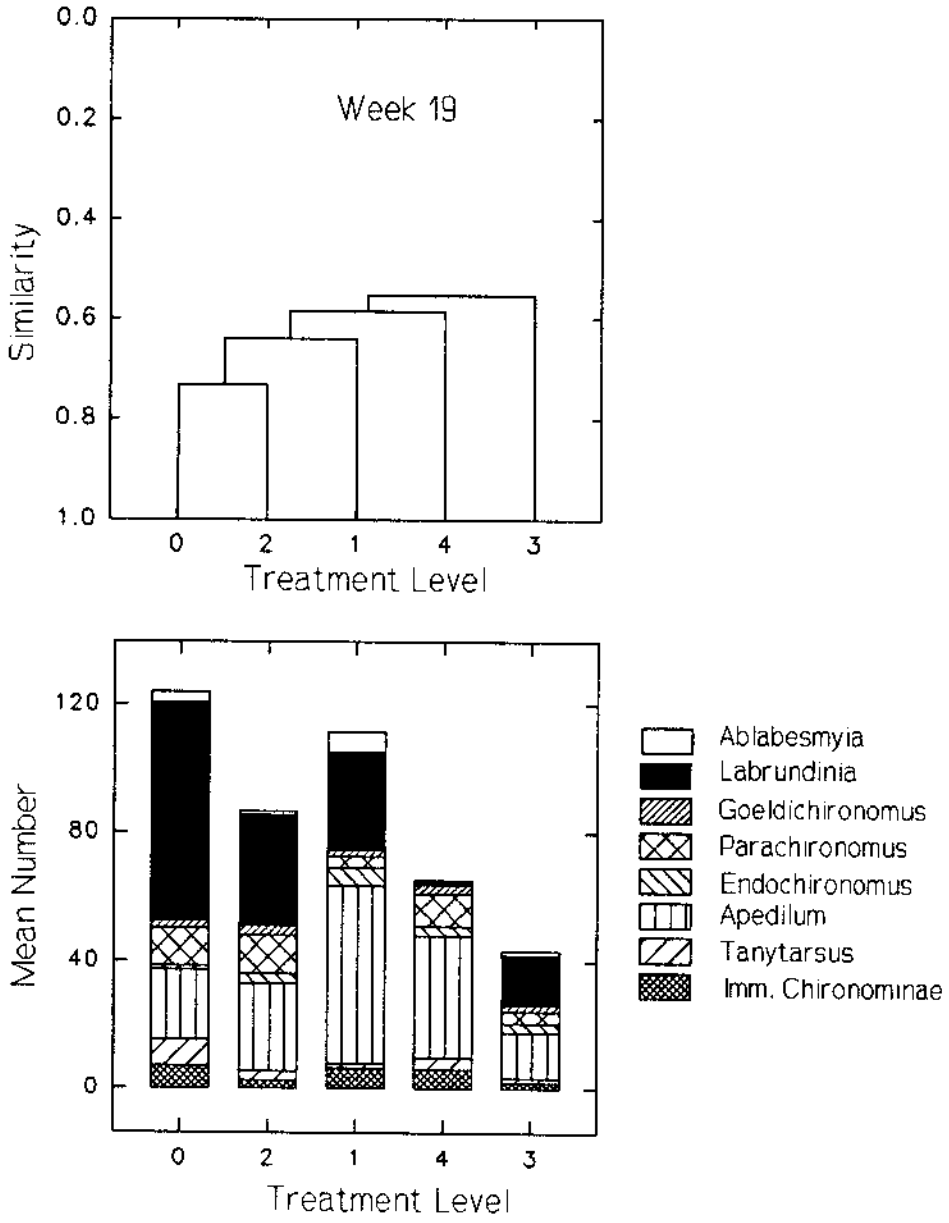

Figure 84. Bray-Curtis cluster analysis of Chironomidae colonizing artificial substrates in microcosms during the final week of sampling. Treatments range from controls (0) to high rate (4).

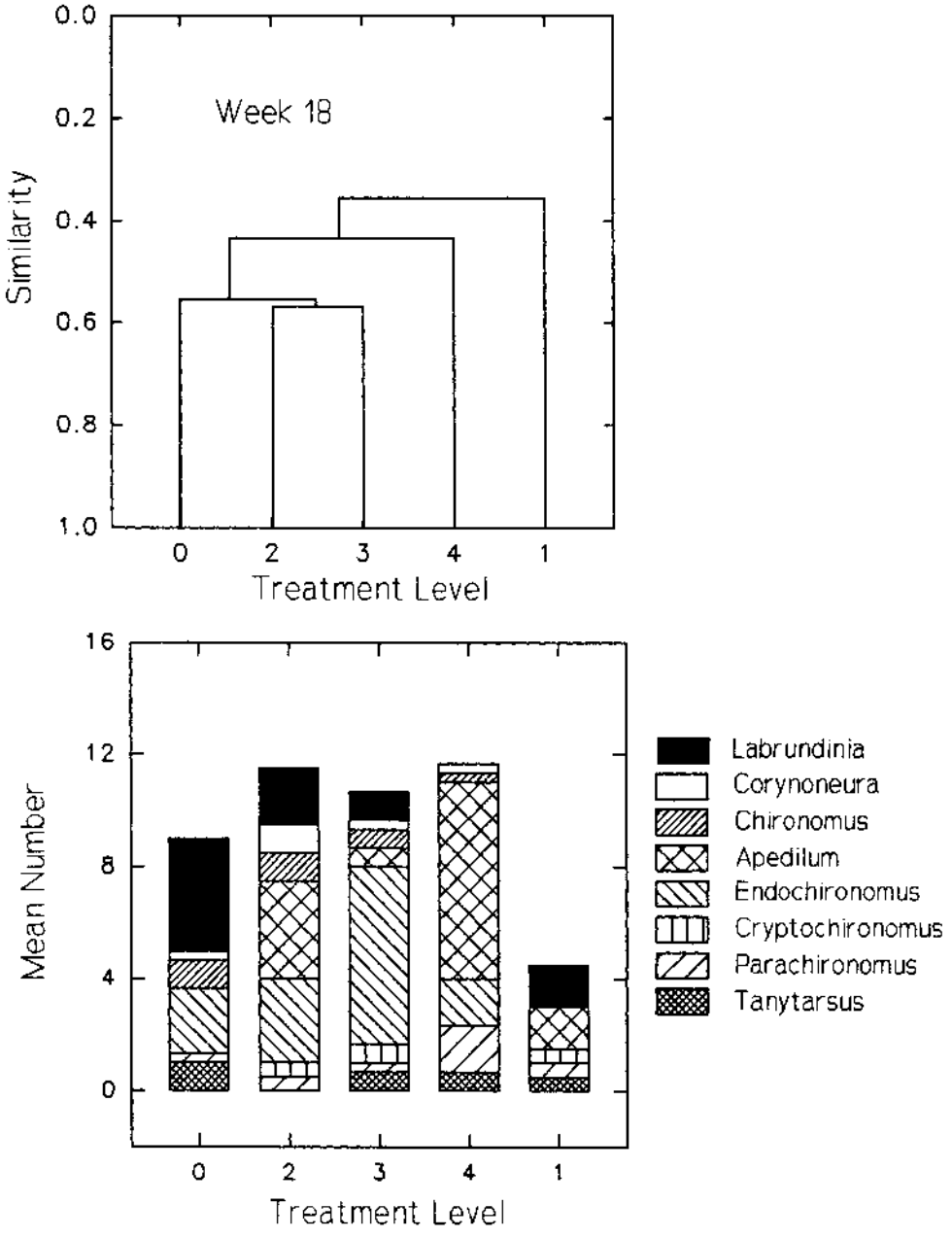

Figure 85. Bray-Curtis cluster analysis of Chironomidae exuviae collected from microcosms one week prior to the final sampling period. Treatments range from controls (0) to high rate (4).

Corynoneura) were more abundant in emergence traps, presumably due to the mesh size used in exuviae nets (110 x 112 μm). Reduced mesh size would be required for effective sampling of these small chironomids. Effective sampling of early instar chironomids often requires small mesh sizes in the 50-70 μm range (Storey and Pinder 1985).

Cluster analysis with subsequent bootstrapping showed mixed success as a method for analyzing chironomid community responses. The whole assemblage was evaluated at once, with significant separation of controls from treatments during weeks 5 and 9 for MAS samples. Significance was established for all treatments (D1-D4) in these cases, thus this method was as sensitive as Dunnett's analysis.

No significant separation of clusters was established for week 6 and 10 exuviae, even though clear pyrethroid impacts existed. Lack of significance in exuviae samples may have reflected low sample sizes. MAS densities always exceeded exuviae numbers. Clustering was not as sensitive as Dunnett's analysis of susceptible taxa (i.e., *Labrundinia*) for exuviae sampling. As discussed in previous chapters, no statistical separation of clusters was achieved for zooplankton, total MAS or total exuviae samples. Significant reductions of pyrethroid-sensitive taxa and significant increases of pyrethroid-tolerant groups were identified via Dunnett's analyses. Thus, the inferential

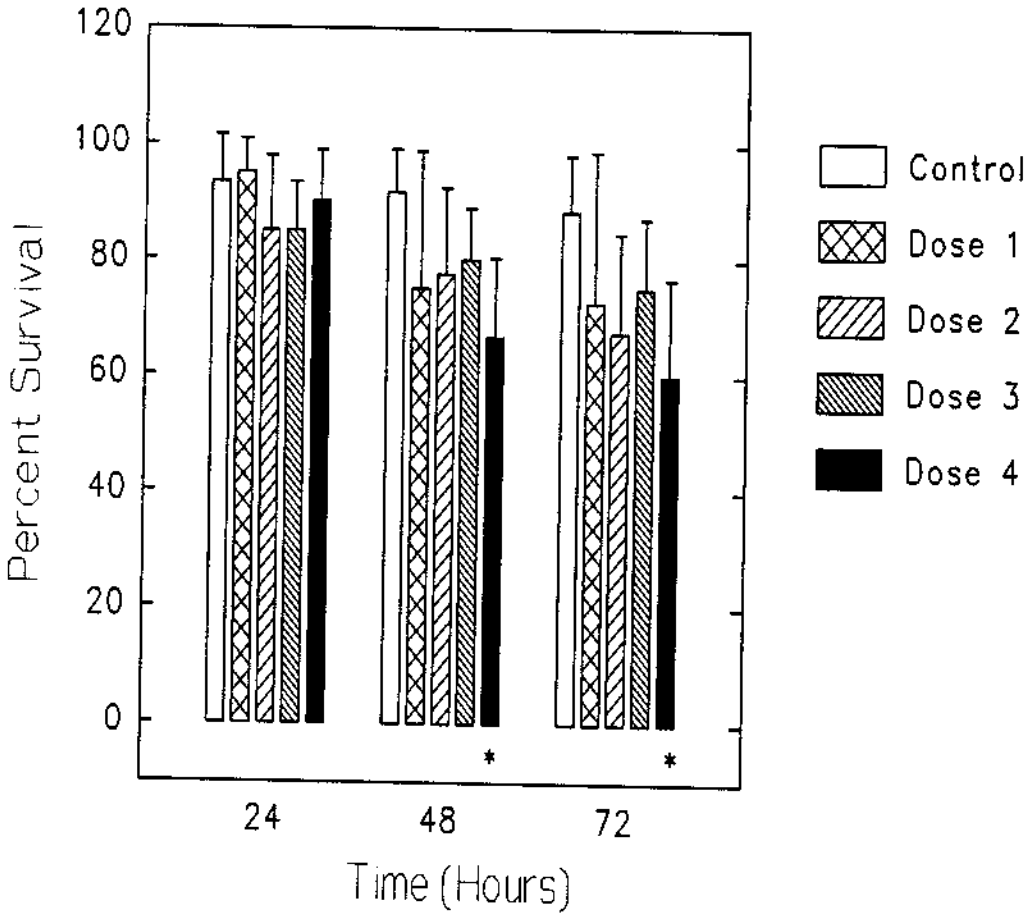

Figure 86. Percent survival (± S.D.) of Chironomus tentans exposed to microcosm water collected one hour after dosing. Trials were 72 hour static exposures. * = Sig. different from controls.

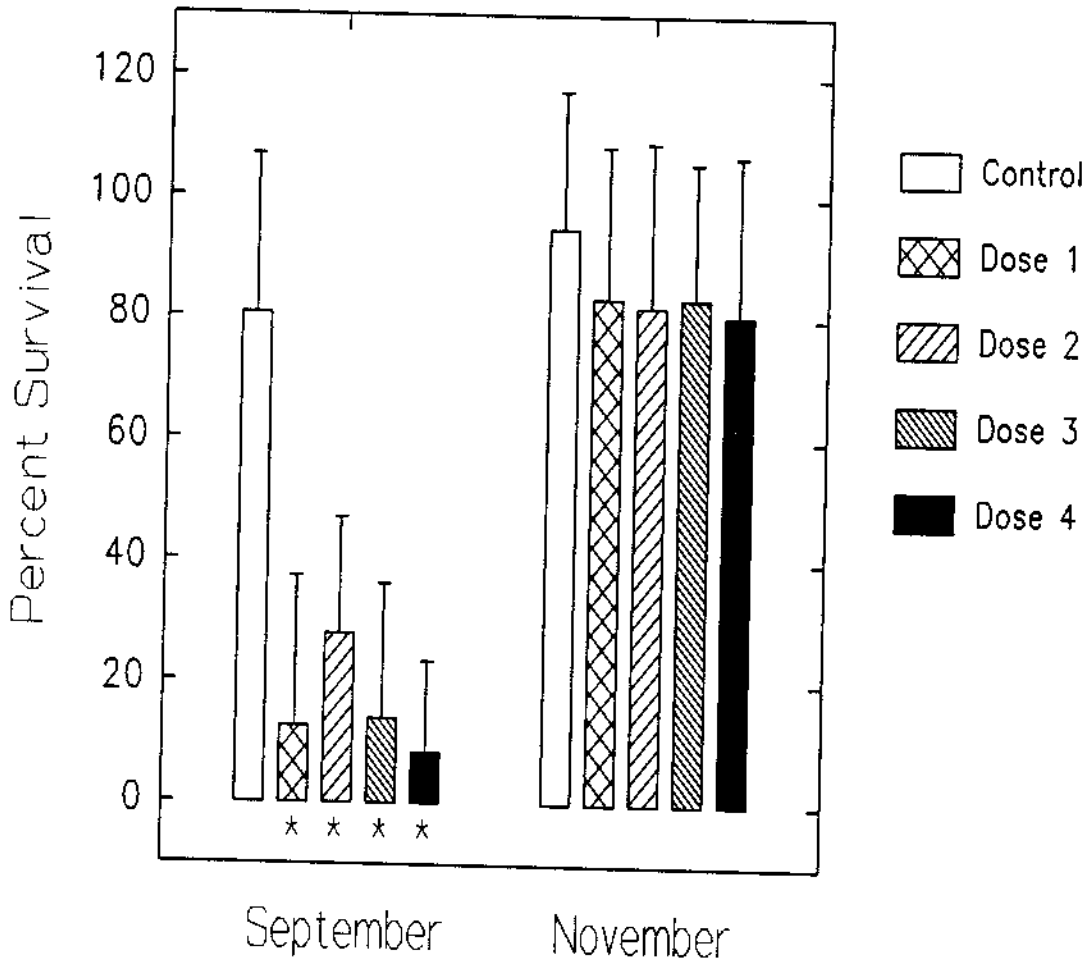

Figure 87. Percent survival of C. tentans exposed to microcosm sediments in cores (7 day test) collected the last week of dosing (September) and final sampling week (November). * = Sig. different from controls.

power of this test was very limited. If one were to use a higher alpha level (α=0.15 for instance), rather than the traditional α=0.05, then many "obvious" differences among treatments would be statistically significant.

Chironomus Bioassays

Chironomus tentans exhibited less toxicity in water column exposure than in sediment assays. This may have reflected lower water column pesticide concentrations (Figures 2 and 4) and shorter exposure times (72 hours vs. seven days). *C. tentans* were less sensitive to cyfluthrin compared with similar *D. magna* bioassays (Figure 36).

Responses of *C. tentans* to contaminated sediments in the September bioassay (week 10) showed higher sensitivity than native Chironominae populations from MAS samplers. One interpretation is that MAS samplers, which rested on top of the sediments, measure epibenthic rather than sediment populations. Exposure to high sediment residues thus would be maximized in bioassays.

At study conclusion, sediment bioassays exhibited no toxicity. This was consistent with recovery of native chironomids and other macroinvertebrates. These results differed from residue analyses, which showed persistent levels of pyrethroid at all treatments (Figure 4). Lower toxicity in November, at a time of only slightly lower

cyfluthrin sediment residues, appears to demonstrate a reduction in cyfluthrin bioavailability through time. It has been noted that pesticides in sediments often become less bioavailable through time. Although pesticide adsorption to sediments should be reversible, studies have shown that desorption is almost invariably slower. The difficulty of desorption or extraction increases with time (Calderbank 1989). A portion of the chemical becomes more firmly bound than average, resulting in "bound residue". With longer residence time in soil, bound pesticide residues tend to lose biological activity and become more resistant to degradation and extraction. This phenomenon has been referred to as "aging" of residues (Calderbank 1989). This pattern was consistent with the microcosm results: although toxicity decreased dramatically from September to November (1989), hexane extractable cyfluthrin residues had not declined considerably during this interval.

Summary

Differences in cyfluthrin toxicity were clearly observed at the subfamily level. Tanypodinae were impacted in MAS, pupal exuviae and adult emergence samples. Chironominae populations were greater in treated MAS compared to controls. Chironominae emergence was low in both control and treated microcosms throughout the

application period, but a large increase in emergence was noted after pesticide stress was removed in D1-D4 tanks.

Community analyses (Bray-Curtis clustering) of MAS data were more successful when just the chironomid taxa were considered, compared with total macroinvertebrate response (Chapter 7). Most chironomids were either increased or decreased by cyfluthrin, so this is not surprising. Exuviae samples did not show statistically significant differences between clusters. This was probably related to small exuviae sample sizes.

Sediment bioassays using *C. tentans* were a very sensitive measure at the end of the application period (September assay). Lack of toxicity in November sampling, combined with persistent cyfluthrin residues, suggest that single-species bioassays may be a powerful tool for determining the bioavailability of pesticides collected in the field. Residue analyses alone would have over-estimated toxicity in November.

CHAPTER 9

COMPARISON OF MICROCOSM AND MESOCOSM IMPACTS

Introduction

As discussed in the introductory chapter, large scale mesocosms (0.1 acre ponds) are the standardized simulated field test used for evaluation of fate and effects of pesticides during the FIFRA registration process. Use of microcosms might provide some distinct advantages (Chapter 1), but undoubtably some compromises will be made when scaling-down. This chapter will review biological endpoints obtained from the microcosm-mesocosm comparison, allowing evaluation of the validity of using microcosms as surrogates for required mesocosm tests. Testing at different scales allowed identification of biological responses that were either similar or dissimilar across test systems. This comparison between microcosms and mesocosms does not address larger scaling questions such as extrapolation to the "real world" (i.e., natural lakes, reservoirs and streams). These extrapolations are probably just as important or more important than comparisons between these model ecosystems.

Zooplankton Results

Total Cladocera were considerably more abundant in microcosms relative to mesocosms (Figure 88). Both pyrethroid-sensitive and insensitive taxa were represented in microcosm samples, but this distinction could not be made in mesocosms due to low numbers (Table XVII). *Diaphanosoma brachyurum* (Figure 89) and *Chydorus sphaericus* (not shown) exhibited population declines consistent with pesticide loadings. *Diaphanosoma brachyurum* were the dominant large-bodied Cladocera in microcosms. *Macrothrix rosea* did not show treatment related responses (Figure 89), while *Alona rustica* (not shown) were enhanced in the highest loadings relative to controls during week five, but were unaffected in any treatments at week nine.

Mature copepods were not abundant in either system. Nauplii were more abundant in microcosms compared to mesocosms (Figure 90). Nauplii in mesocosms exhibited treatment related reductions at low-rate exposure levels, but microcosms did not (Table XVII).

Total rotifer populations were much higher in microcosms compared with mesocosms (Figure 91). Total rotifers were significantly enhanced in microcosm treatments D2 through D4 during the last application week (Table XVII). A similar but non-significant trend was observed in

mesocosms for treatments D3 to D4 during the same period (Figure 91).

Similar rotifer taxa were found in mesocosms and microcosms, but the dominant species differed in these systems. *Brachionus* (*B. angularis* and *B. havanensis*) were most abundant in microcosms and were the driving force behind treatment enhancements during the last application. *Brachionus* were uncommon in mesocosms. Some taxa in mesocosms were rarely seen in microcosms. *Hexarthra mira*, for instance, were common during week five in mesocosms and *Keratella cochlearis* were abundant prior to treatment initiation, but both were rare in microcosms.

Other rotifers were common to both mesocosms and microcosms. *Filinia longiseta* showed similar enhancements with higher treatments in both systems (Figure 92). *Polyarthra remata* showed disparate trends in the two systems. Microcosms experienced enhancements with increasing treatment levels (Figure 93), but mesocosms showed either reductions (week five; "Mid") or slight enhancement (week nine; "Last").

Macroinvertebrate Results

Macroinvertebrates identified as pyrethroid-sensitive or insensitive were similar in both systems. Artificial substrate colonization was effective in evaluating pesticide

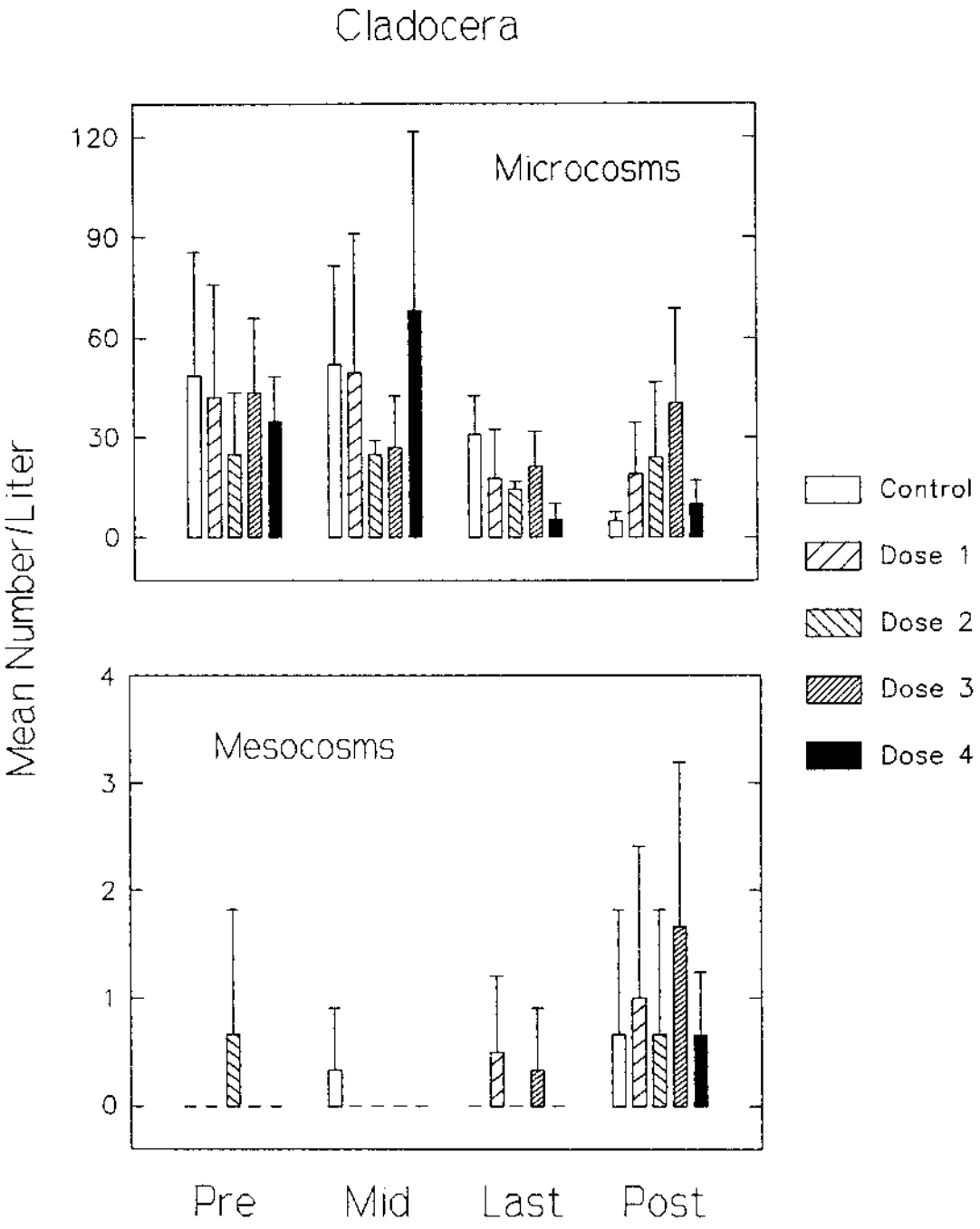

Figure 88. Mean number (± S.D.) of Cladocera collected from microcosms and mesocosms. Pre=Prior to initial application, Mid=Middle of application period, Last=Last application, Post=Study termination.

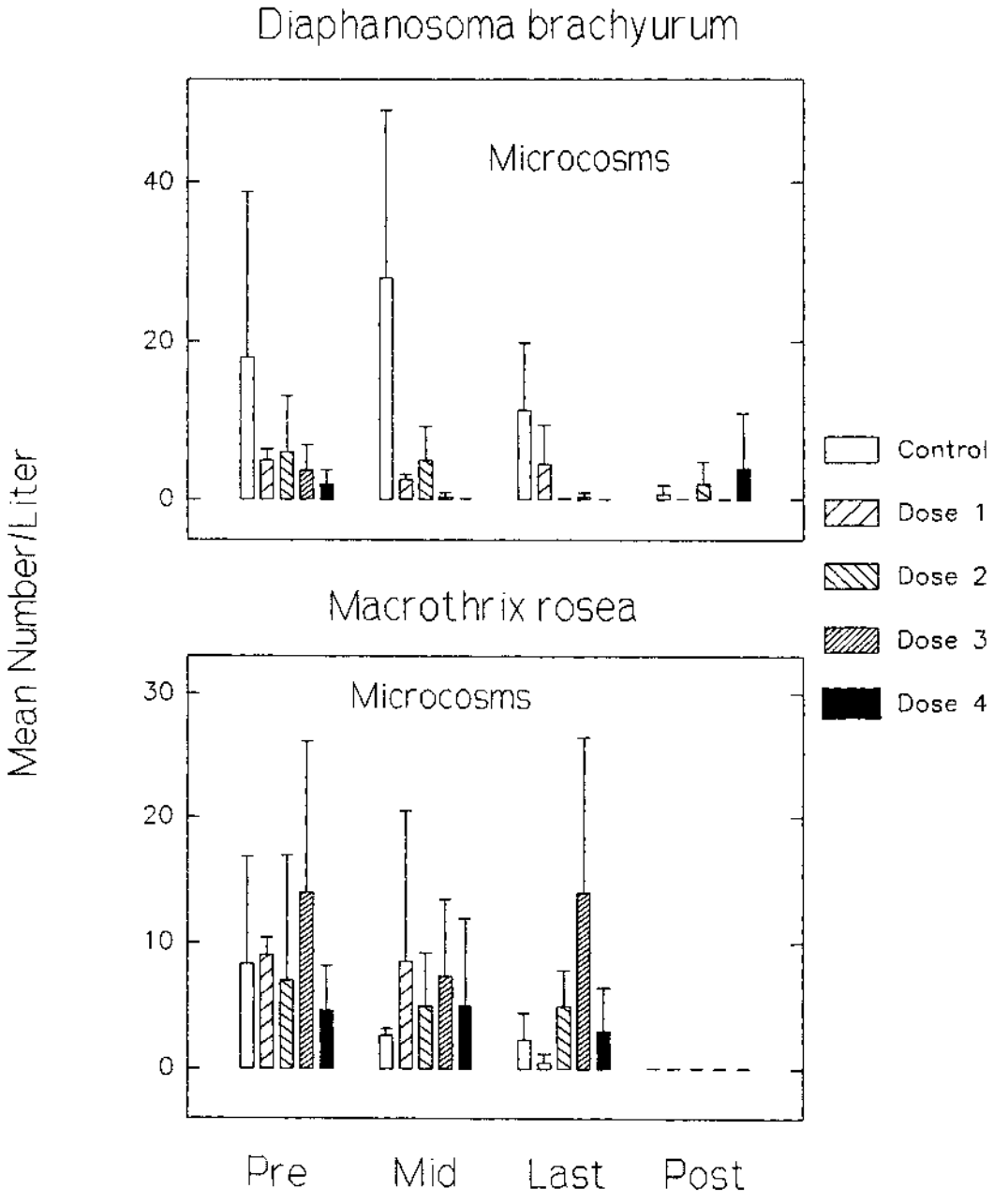

Figure 89. Two common cladocerans (mean ± S.D.) collected from microcosms. Pre=Prior to initial application, Mid=Middle of application period, Last=Last application, Post=Study termination.

impacts in some taxa. The mayfly *Callibaetis floridanus* was significantly reduced at all treatments (D1-D4) in microcosms and reduced at levels D2-D4 in mesocosms (Table XVII). *Callibaetis* nymphs found in control microcosms were more abundant than those in mesocosms, and an exposure/response relationship was evident in the smaller systems (Figure 94). The mayfly *Caenis* experienced higher populations in control mesocosms relative to microcosm controls (Figure 95). *Caenis* nymphs were significantly reduced at all treatments in both systems, as were trichopterans and amphipods (Table XVII).

Chaoboridae MAS colonization was significantly decreased and Ceratopogonidae populations were significantly enhanced with treatment in mesocosms (Table XVII). Parallel responses were not found in concrete tanks due to low numbers of these taxa in microcosm MAS samplers. Significant reductions of Tanypodinae chironomids were detected at lower levels in microcosms (D1-D4) compared to mesocosms (D4 only; Table XVII). Other taxa colonizing artificial substrates were identified as insensitive. Gastropods (not shown) were not impacted by the pyrethroid. Oligochaetes (mostly family Naididae) sampled by MAS demonstrated very similar colonization patterns though the absolute numbers differed greatly (Figure 96). Naididae showed a small increase in microcosms during the last

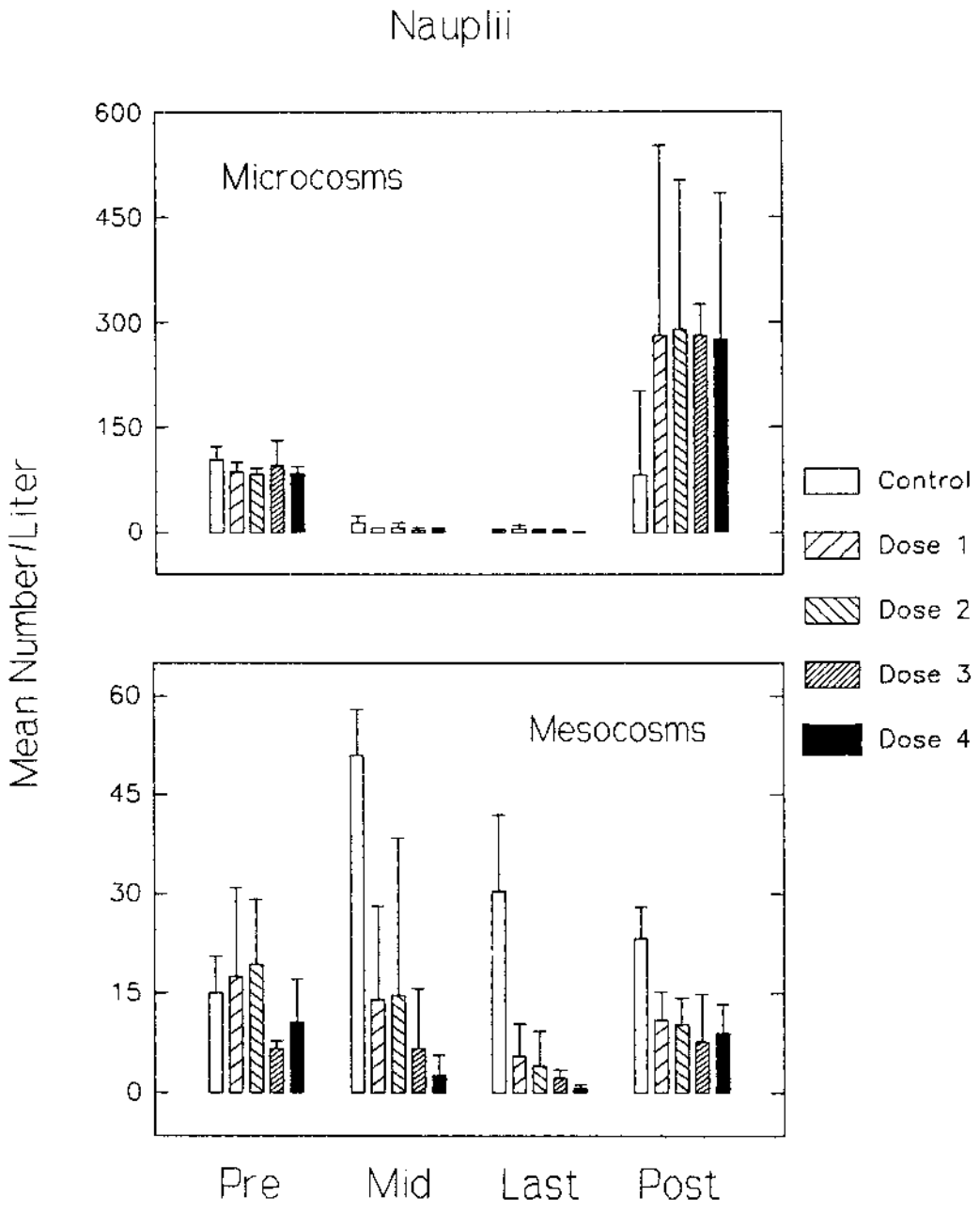

Figure 90. Mean number (± S.D.) of copepod nauplii collected from microcosms and mesocosms. Pre=Prior to initial application, Mid=Middle of application period, Last=Last application, Post=Study termination.

treatment week. Substrate colonization may not be the most effective method for sampling oligochaetes, since Ekman grab samples from mesocosms revealed significant population increases in treated ponds not observed in MAS colonization (Table XVII). Ekman grab samples from mesocosms were dominated by Tubificidae, with some Naididae present.

Large odonate predators were more abundant in microcosms compared with mesocosms (Table XVII). Libellulidae (dragonfly larvae) numbers did not show consistent treatment-related patterns, except for D4 in microcosms (Figure 97). Coenagrionidae (damselfly larvae) also lacked clear treatment-related patterns, being reduced in D4 microcosms and not affected in mesocosms (not shown).

Insect emergence appeared to be an excellent method of detecting pesticide/biotic relations. Both sensitive and insensitive taxa were identified, with fairly good agreement between mesocosms and microcosms. Exuviae collection in microcosms yielded larger sample sizes and larger species diversity compared with funnel traps in microcosms. Thus, all comparisons were of microcosm exuviae and mesocosm pyramid traps.

Chaoborus emergence (Figure 98) was among the most sensitive parameters measured, with similar patterns in both systems. Chironomids in the subfamily Tanypodinae were also identified to be sensitive to pesticide additions (Figure

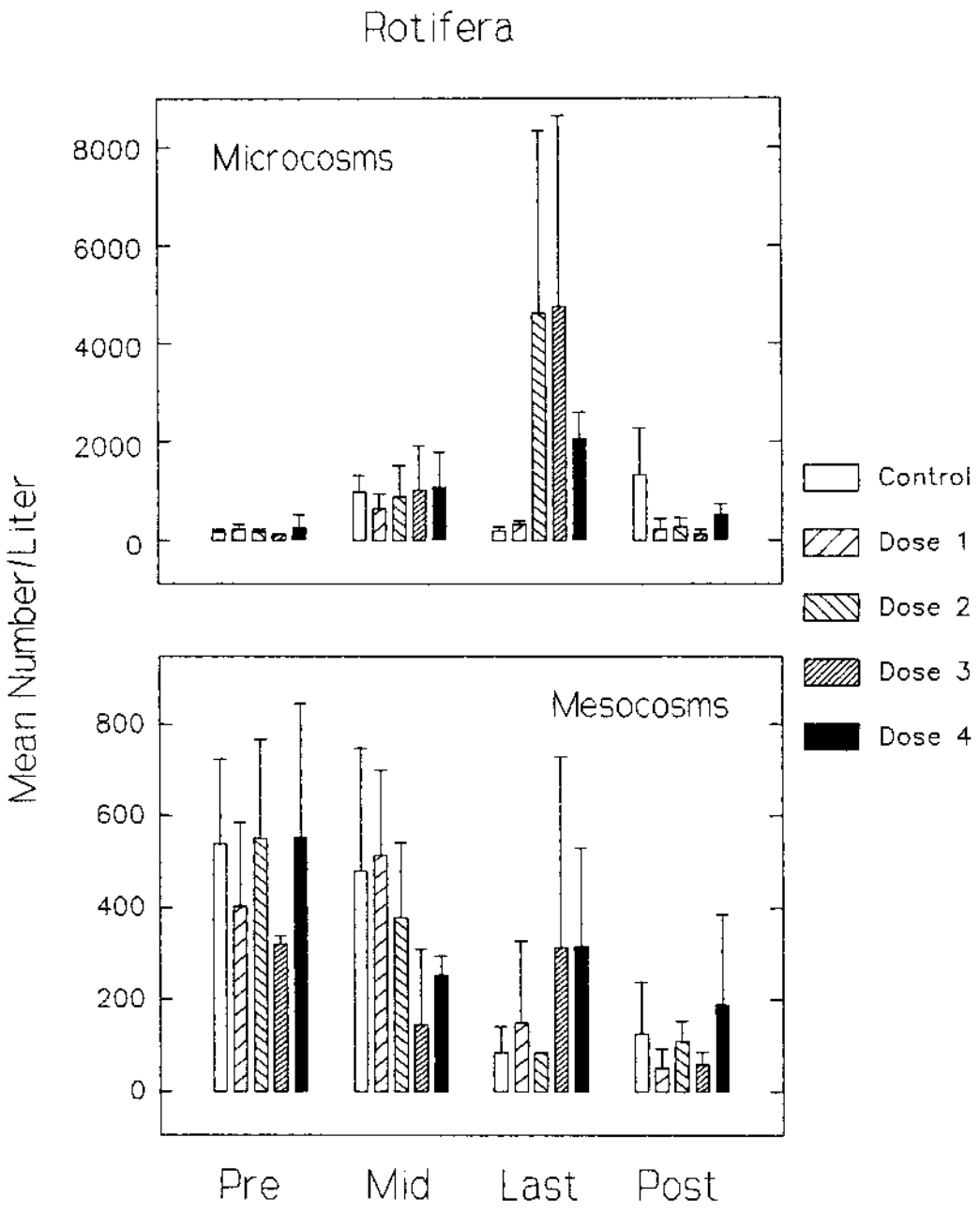

Figure 91. Mean number (± S.D.) of Rotifera collected from microcosms and mesocosms. Pre=Prior to initial application, Mid=Middle of application period, Last=Last application, Post=Study termination.

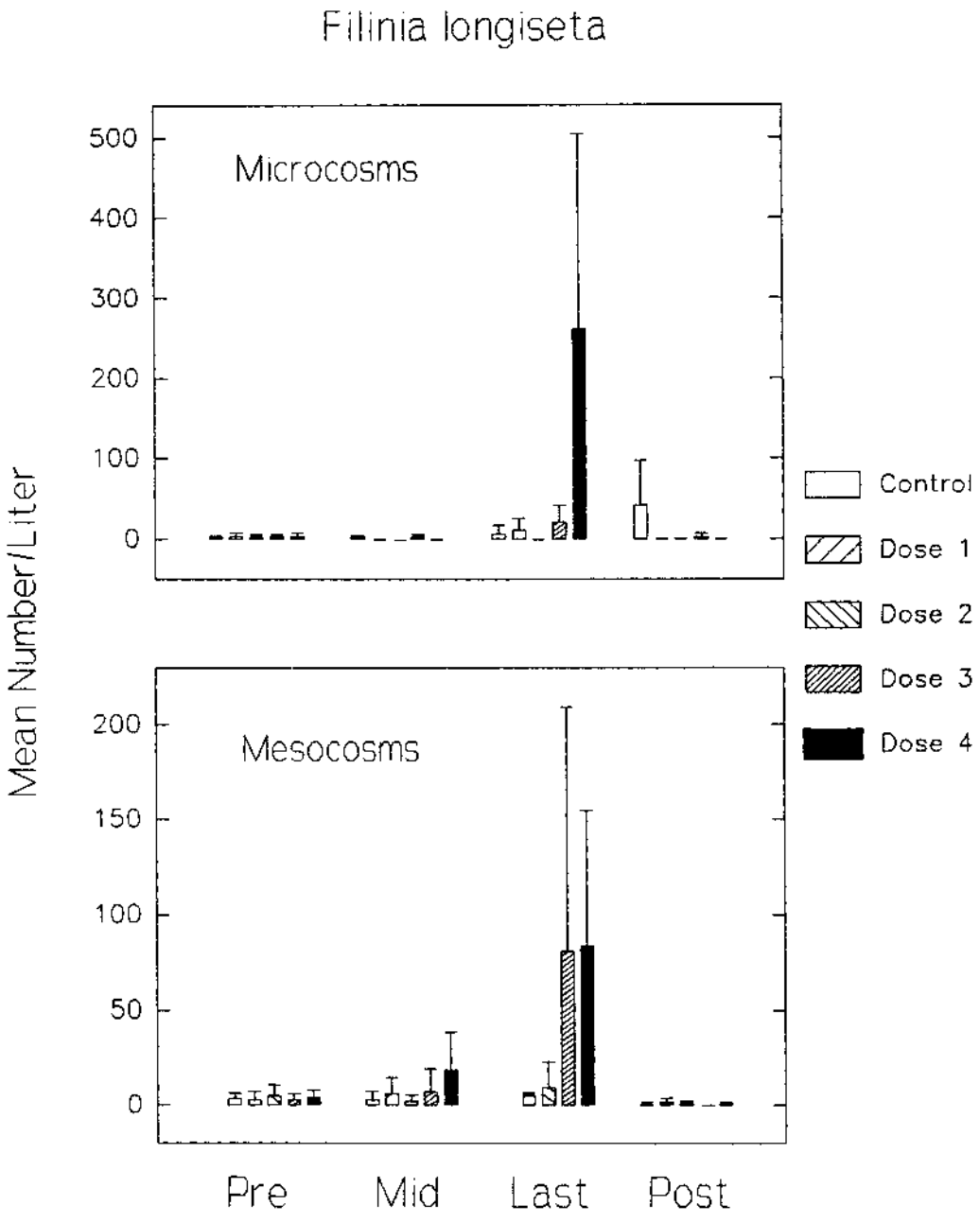

Figure 92. Mean number (± S.D.) of *Filinia longiseta* collected from microcosms and mesocosms. Pre=Prior to initial application, Mid=Middle of application period, Last=Last application, Post=Study termination.

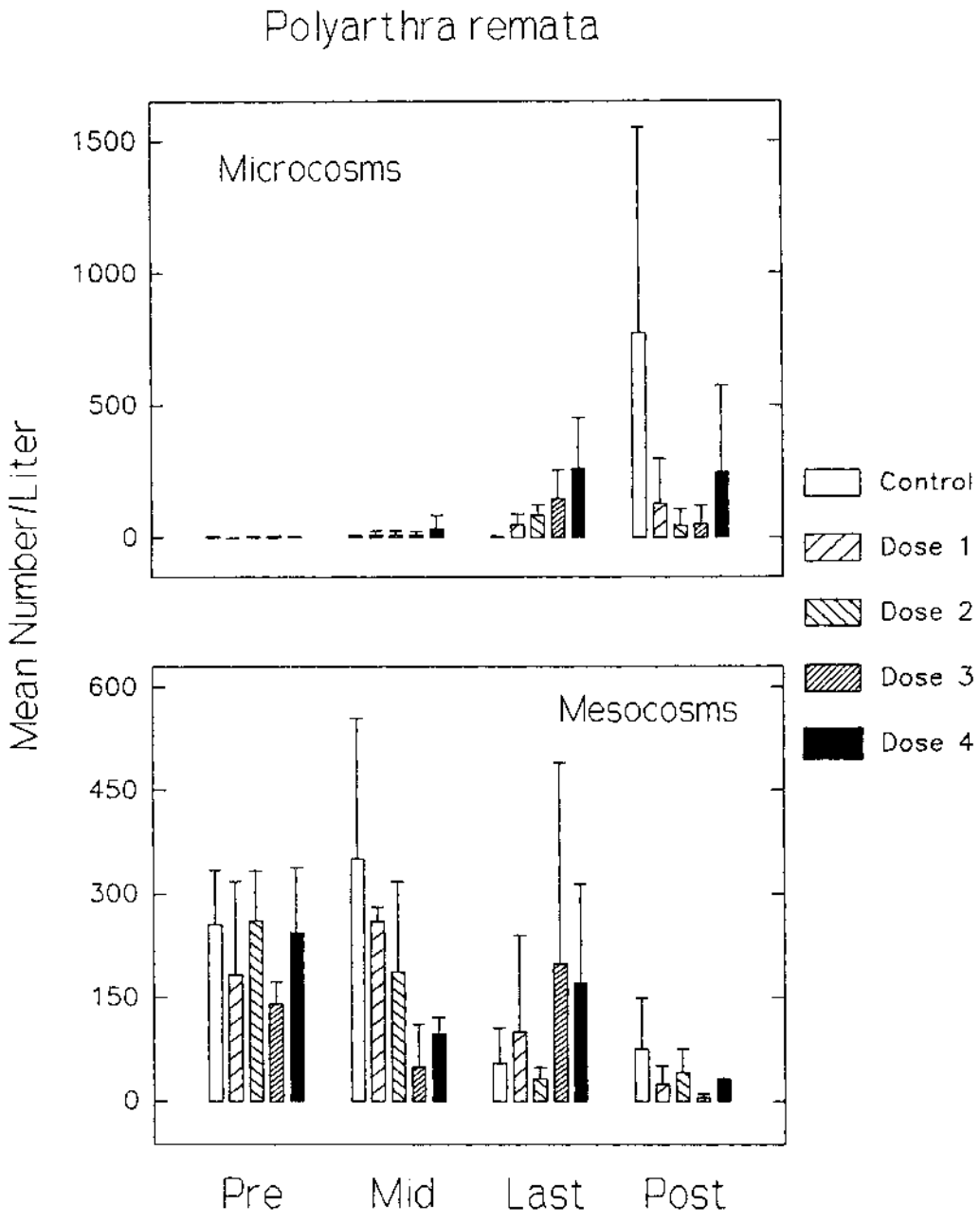

Figure 93. Mean number (± S.D.) of *Polyarthra remata* collected from microcosms and mesocosms. Pre=Prior to initial application, Mid=Middle of application period, Last=Last application, Post=Study termination.

99) while members of the subfamily Chironominae were not (Figure 100). One major difference in the emergence patterns among these systems was the greater number Ceratopogonidae (Diptera) emerging from mesocosms. Ceratopogonid emergence in mesocosms was significantly enhanced at all pesticide loadings (Table XVII).

The total number of significant differences detected over the course of the experiment increased with increasing treatment level (Figure 101). The total number of differences detected were generally higher in mesocosms, primarily due to MAS samples. In microcosms, exuviae collection identified more differences than did emergence traps. More differences in zooplankton densities were found in microcosms.

Discussion

Biological Effects

Population level endpoints were emphasized in this study design and were correspondingly found to be more sensitive than functional parameters. Ecosystem function (nutrient cycling, etc.) may show few long term effects of perturbation, since natural systems have feedback mechanisms that serve as buffers. Structural variables such as species composition are often affected more rapidly than functional variables in ecotoxicological studies (Giddings 1980, Odum

Table XVII. Summary of significant differences during application period only. To be conservative, differences were reported only if more than one difference was observed. Mth=Sampling Method, Imt=Impact, Trt=Treatment Level.

	Microcosms			Mesocosms			Density Compare
	Mth	Imt	Trt	Mth	Imt	Trt	
Zooplankton (#/L)							
Rotifera	TU	I	D2-4	Tu	-	-	Mic>=Mes
Cladocera	TU	R	D4	Tu	*	*	Mic>Mes
Copepoda Nauplii	TU	-	-	Tu	R	D1-4	Mic>Mes
Macroinvert. ($\#/m^2$)							
Gastropoda	MAS	-	-	MAS	I	D1-4	Mic<=Mes
Oligochaeta	MAS	-	-	MAS	-	-	Mic<Mes
				EK	I	D1-4	
Amphipoda	MAS	R	D1-4	MAS	R	D1-4	Mic<Mes
Nematoda	MAS	*	*	MAS	-	-	Mic<Mes
Ephemeroptera							
Caenidae	MAS	R	D1-4	MAS	R	D1-4	Mic<Mes
	ET	*	*	ET	*	*	
	EX	-	-				
Baetidae	MAS	R	D1-4	MAS	R	D2-4	Mic>Mes
	ET	*	*	ET	*	*	
	EX	R	D1-4				
Trichoptera	MAS	R	D1-4	MAS	R	D2-4	Mic=Mes
	ET	*	*	ET	*		
	EX	*	*				

NA Not Applicable
I Sig. Increase
R Sig. Reduction
- No Impact
* Density Too Low

TU Tube Sampler
MAS MAS Sampler
EK Ekman Grab
ET Emergence Trap
EX Exuviae

Continuation of significant impacts on macroinvertebrates in microcosms and mesocosms.

	Microcosms			Mesocosms			Density Compare
	Mth	Imt	Trt	Mth	Imt	Trt	
Macroinv. (Continued)							
Diptera							
Chironomidae							
Chironominae	MAS	I	D1,3	MAS	I	D1-4	Mic<Mes
	ET	-	-	ET	-	-	
	EX	-	-	EK	-	-	
Tanypodinae	MAS	R	D2-4	MAS	R	D4	Mic=Mes
	ET	R	D1-4	ET	R	D1-4	
	EX	R	D2-4	EK	I	D1	
Ceratopogonid	MAS	*	*	MAS	I	D1-4	Mic<Mes
	ET	*	*	ET	I	D1-4	
	EX	*	*	EK	I	D1, 2,4	
Chaoboridae	MAS	*	*	MAS	R	D2-4	Mic<Mes
	ET	*	*	ET	R	D1-4	
	EX	R	D1-4	EK	R	D1-4	
Odonata							
Libellulidae	MAS	-	-	MAS	-	-	Mic>Mes
	EX	-	-				
Coenagrionid	MAS	-	-	MAS	-	-	Mic>Mes
	EX	-	-				
Coleoptera	MAS	R	D1-4	MAS	-	-	Mic=<MAS
Hydrophilid *Berosus*	MAS	R	D1, 3,4	MAS	R	D3-4	

NA Not Applicable
I Sig. Increase
R Sig. Reduction
- No Impact
* Density Too Low for Evaluation

MAS MAS Sampler
ET Emergence Trap
EX Exuviae
EK Ekman Grab

1985, Schindler 1987).

Zooplankton

Studies using other pyrethroids have found cladocerans and chaoborids to be very sensitive, while copepods and ostracods were less affected, and rotifers often increased or were unaffected (Kaushik et al. 1985, Helson and Surgeoner 1986, Day et al. 1987, Yasuno et al. 1988, Hill et al. 1988). These results agree with effects observed in this study, particularly the microcosms (Table XVII). Significant impacts on copepods were detected at lower levels in mesocosms compared with microcosms (Table XVII).

Within the rotifers, differing response patterns were noted when comparing mesocosms with microcosms. *Filinia longiseta* showed general agreement, but *Polyarthra remata* showed variable responses. *Hexarthra mira*, *Keratella cochlearis* and *Brachionus* spp. differed in their importance within the two systems. Increased rotifer densities following reductions in large Cladocera were often attributed to interference and exploitative competition (MacIssac and Gilbert 1989).

Large reductions in copepod nauplii were observed in microcosms during the summer ("Mid" and "Last" periods; Figure 90). This trend was not observed in mesocosms. Summer minima in microcosms brought nauplii densities within

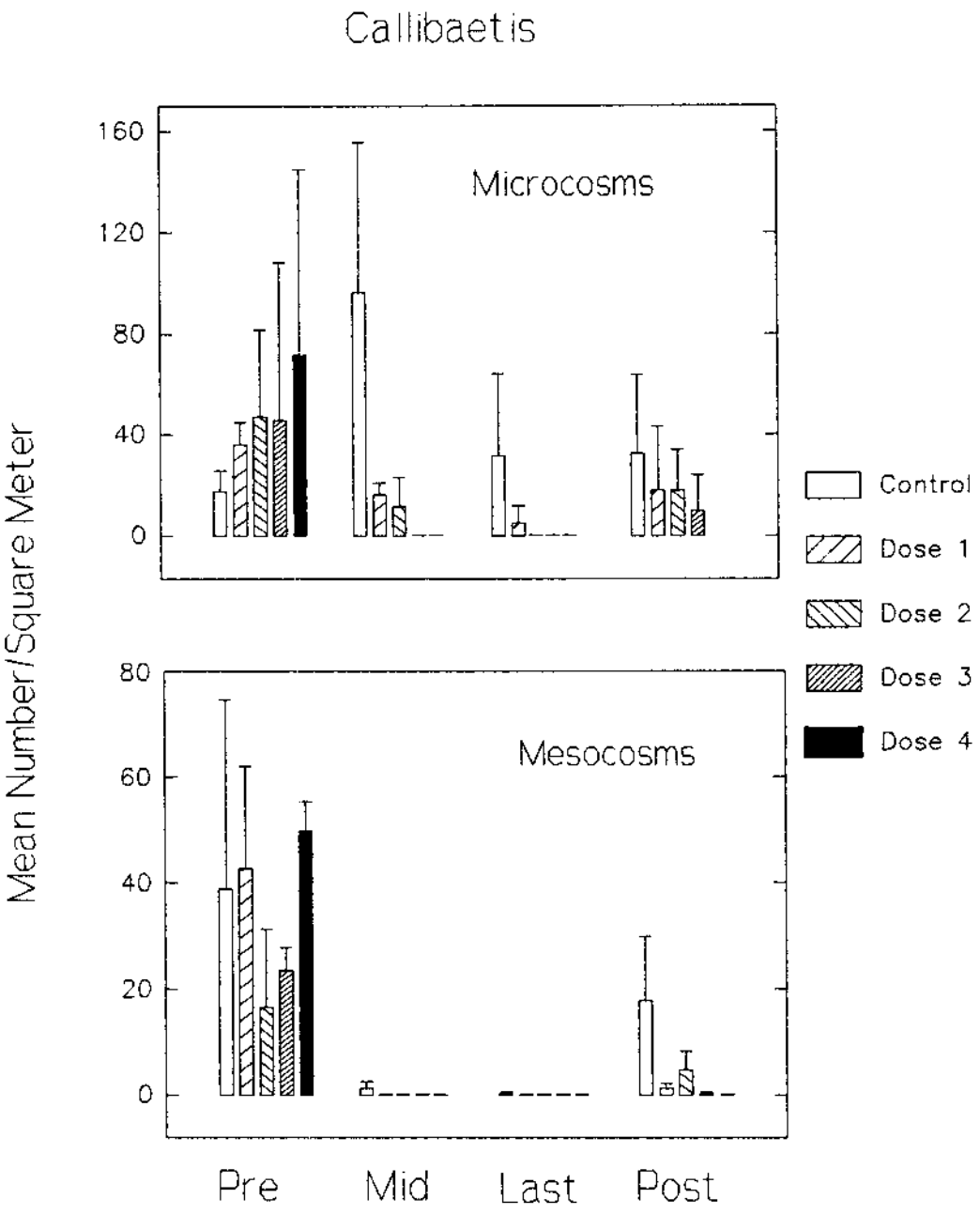

Figure 94 Mean number (± S.D.) of ***Callibaetis floridanus*** collected from microcosms and mesocosms. Pre=Prior to initial application, Mid=Middle of application period, Last=Last application, Post=Study termination.

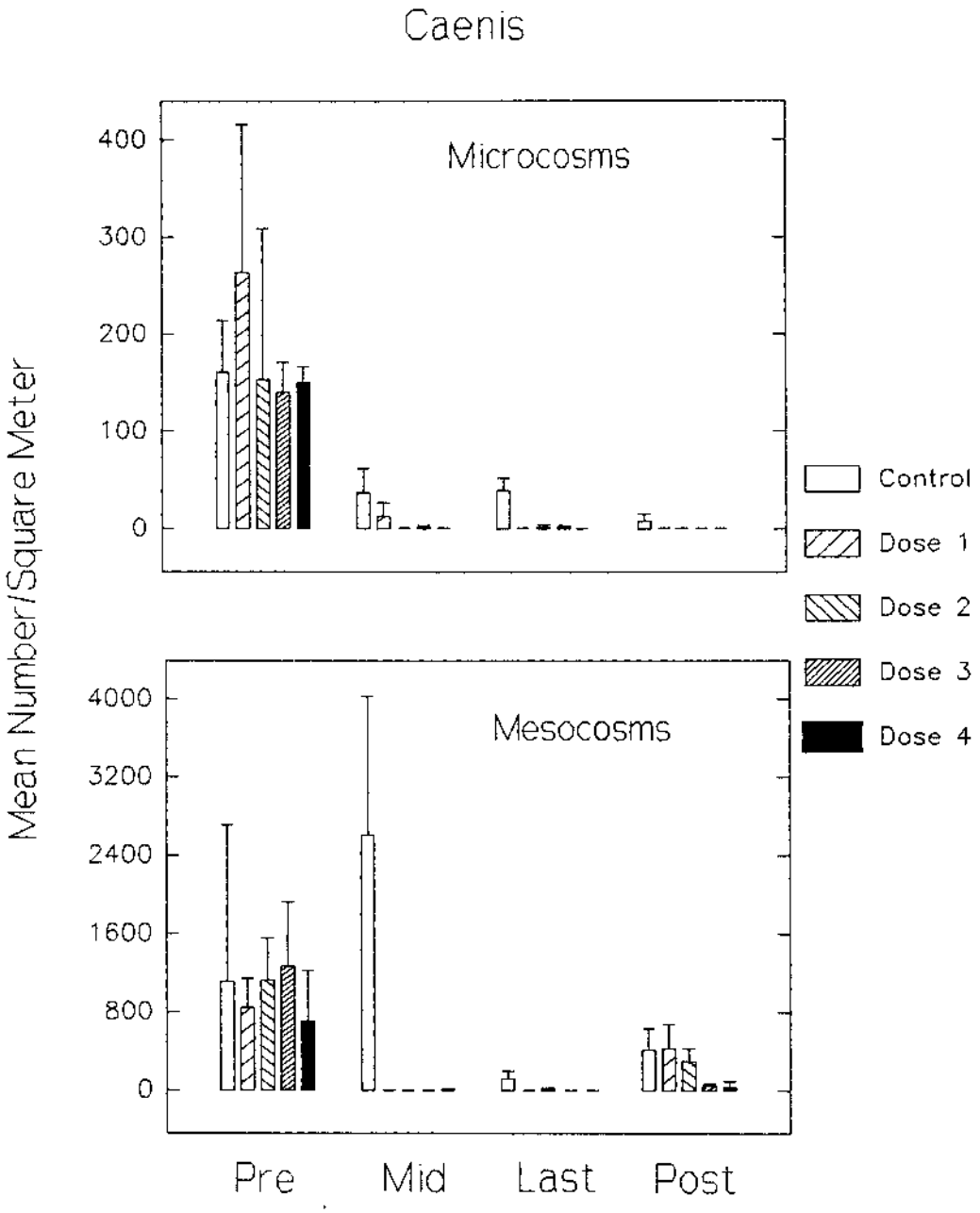

Figure 95 Mean number (± S.D.) of *Caenis* collected from microcosms and mesocosms. Pre=Prior to initial application, Mid=Middle of application period, Last=Last application, Post=Study termination.

the range observed in mesocosms. These population fluctuations were not linked to treatment levels, suggesting competitive or predatory impacts. Thus, secondary interactions showed more diversity compared with pesticide-induced effects.

Macroinvertebrates

Macroinvertebrates differed in sensitivity to pyrethroid exposure. Anderson (1982) found decreasing sensitivity from amphipods > mayflies > stoneflies and caddisflies > snails. Mayflies, amphipods, water mites and surface-dwelling groups (many hemipterans) were often strongly affected by pyrethroids (Crossland 1982, Shires and Bennett 1985, Hill 1985, Helson and Surgeoner 1986, Hill et al. 1988). Oligochaetes are often unaffected (Shires and Bennett 1985, Hill 1985). These trends agreed with sensitivities found during the cyfluthrin experiment.

Midges commonly show differing susceptibility to pyrethroids, with the subfamily Tanypodinae generally more sensitive than the subfamily Chironominae. This difference may reflect differences in exposure since many Chironominae burrow within the sediment or construct feeding tubes (Mulla et al. 1975, Hill et al. 1988). These observations agree with the emergence and epibenthic colonization data in both systems.

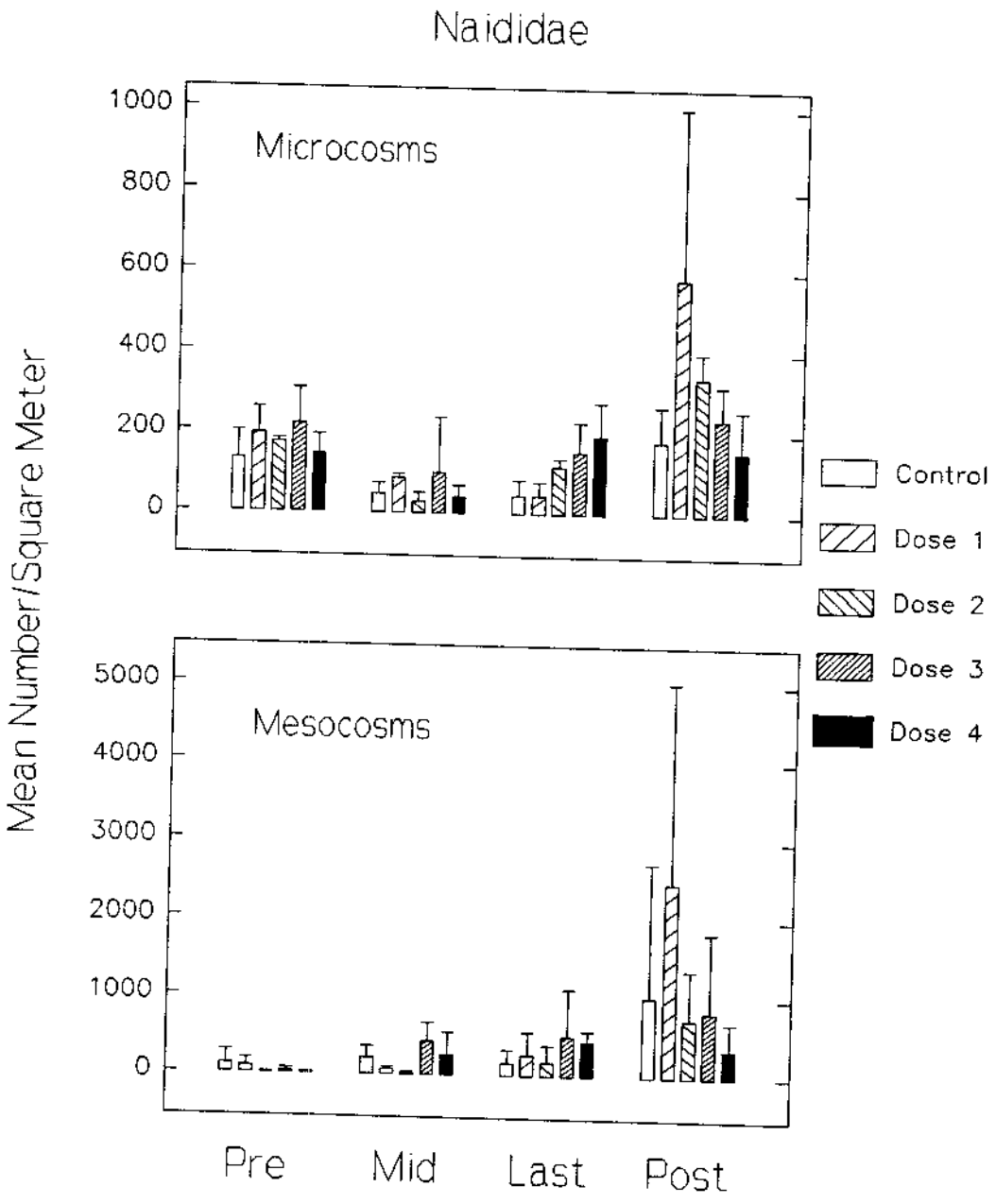

Figure 96 Mean number (± S.D.) of Naididae collected from microcosms and mesocosms. Pre=Prior to initial application, Mid=Middle of application period, Last=Last application, Post=Study termination.

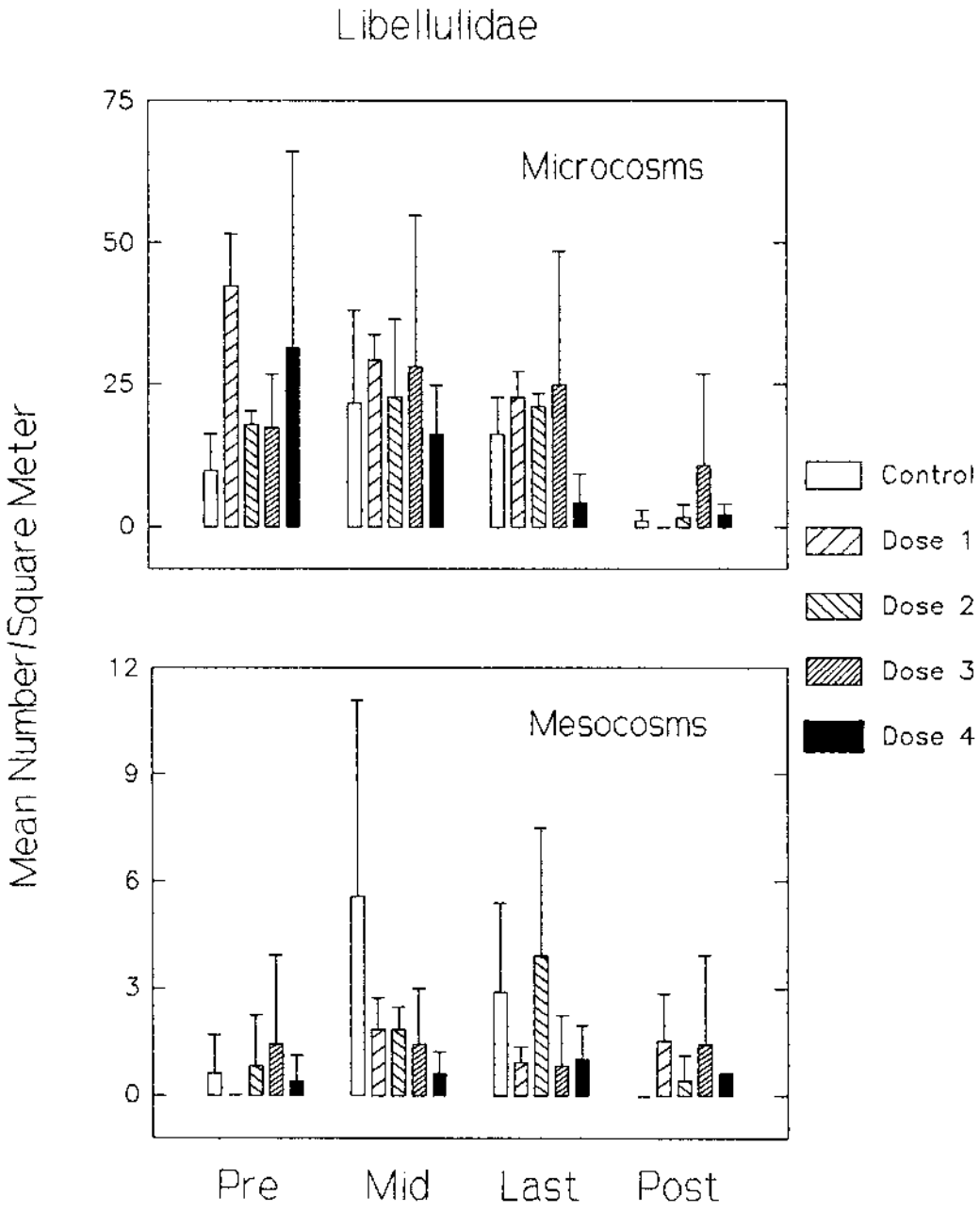

Figure 97 Mean number (± S.D.) of Libellulidae collected from microcosms and mesocosms. Pre=Prior to initial application, Mid=Middle of application period, Last=Last application, Post=Study termination.

More significant differences, both reductions and enhancements, were detected in mesocosms compared to microcosms but similar trends were evident (Figure 101). This may reflect the generally larger sample sizes found in mesocosms, particularly for many macroinvertebrates. Zooplankton were as numerous, or more numerous, in microcosms (Table XVII) and this is reflected in the number of differences identified. In general, a clear treatment-response relationship was seen for cumulative statistical differences over the experimental period in both systems.

Impacts of Fish

Besides direct pesticide impacts, secondary (indirect) impacts are common in ecotoxicological manipulations due to reductions in predators and/or competitors (Hurlbert 1975). In these types of experimental systems, fish obviously have a large impact and determine the abundance of some taxa. Mesocosms were stocked with adult fish (mean length, at study initiation, of 13.197 cm ± 1.58 cm), which reproduced during the experimental period. An average of 12,961 (± 3,872) juvenile fish, with a modal size class of 2.0-2.9 cm, were harvested from mesocosms at study termination. Microcosms were each stocked with eight sexually-immature bluegill sunfish (mean length, at study termination, of 6.87 cm ± 0.88 cm), which did not reproduce. These two systems

thus experienced different predation pressures, with mesocosm bluegills ranging in size from small to very large.

Comparison of microcosms and mesocosms showed higher microcosm densities for cladocerans, copepods, rotifers, libellulid dragonflies, coenagrionid damselflies and baetid mayflies (Table XVII). Caenid mayflies, Chironominae midges, naidid oligochaetes, chaoborids, gastropods and ceratopogonids were more abundant in mesocosms. Some taxa were equally represented in both systems, such as Tanypodinae midges and trichopterans (Table XVII).

Visual planktivores generally select prey based on prey size (Brooks and Dodson 1965, Werner and Hall 1974) and escape ability (Confer and Blades 1975, Drenner and McComas 1980). Thus, large and slow prey like *Daphnia* are subject to intense predation (Stein et al. 1987, Hambright et al. 1986, Vanni 1987). Larval bluegill sunfish feed on small rotifers such as *Polyarthra* and copepod nauplii initially, switching to other rotifers and cyclopoid copepods when fish reach approximately 7 mm in length (Siefert 1972). Fish larger than 8 mm feed primarily on small cladocerans such as *Bosmina*, *Chydorus*, *Diaphanosoma brachyurum* and *Alona* (Siefert 1972). Selective predation of larval and small bluegill on rotifers and nauplii may explain observed reductions of these taxa within mesocosms relative to microcosms, since these small fish size classes were not

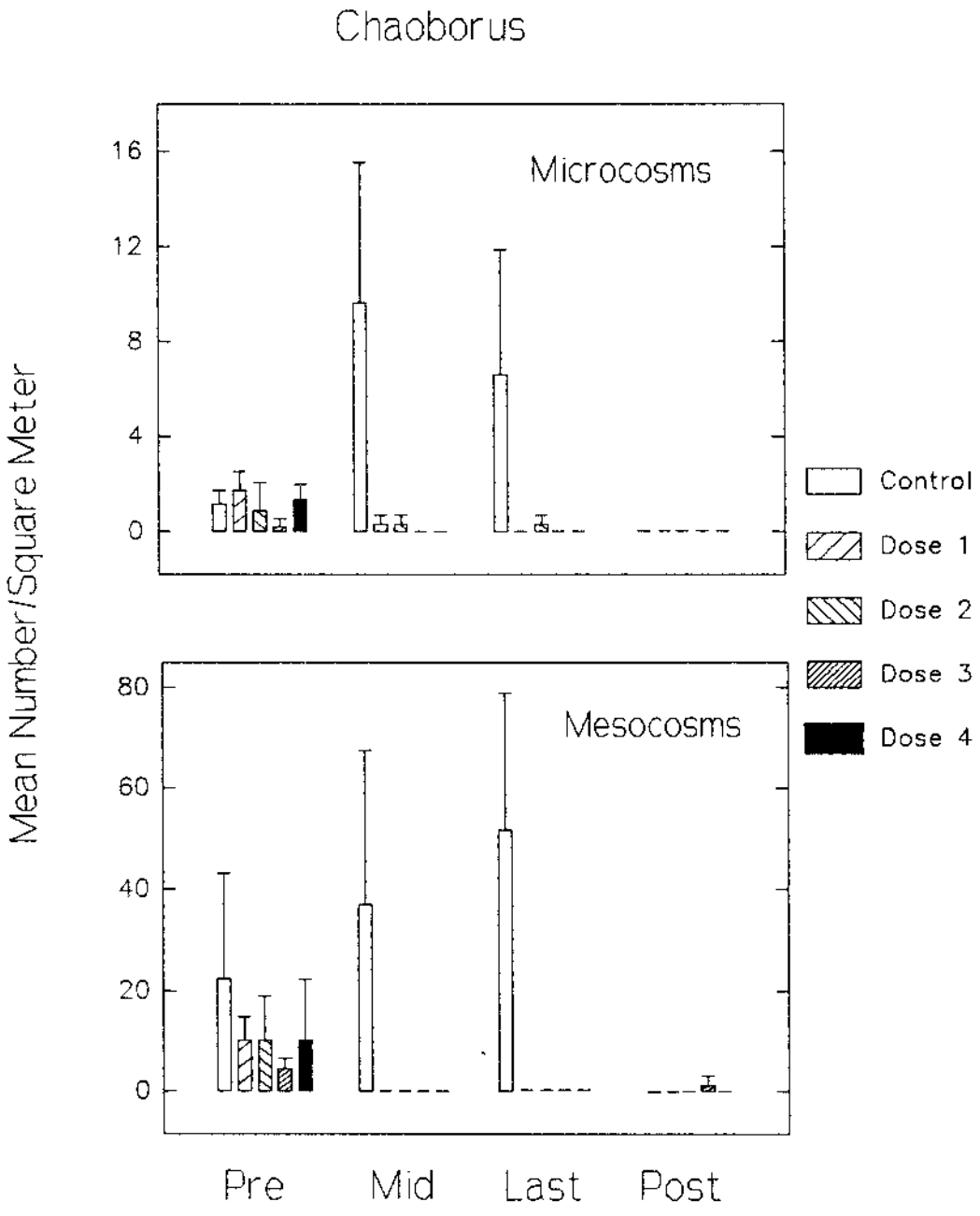

Figure 98 Mean number (± S.D.) of *Chaoborus* collected from microcosms and mesocosms. Pre=Prior to initial application, Mid=Middle of application period, Last=Last application, Post=Study termination.

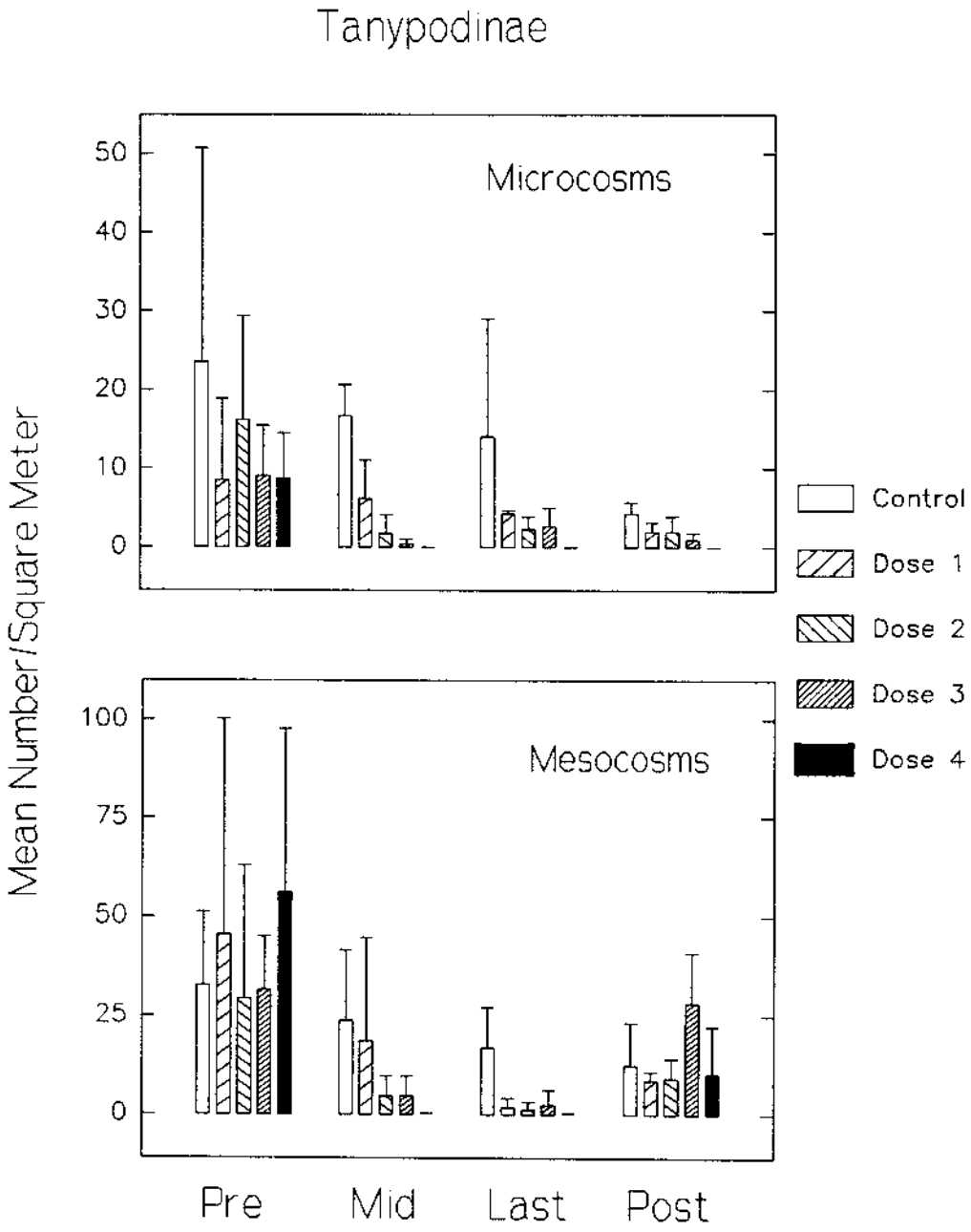

Figure 99 Mean number (± S.D.) of Tanypodinae collected from microcosms and mesocosms. Pre=Prior to initial application, Mid=Middle of application period, Last=Last application, Post=Study termination.

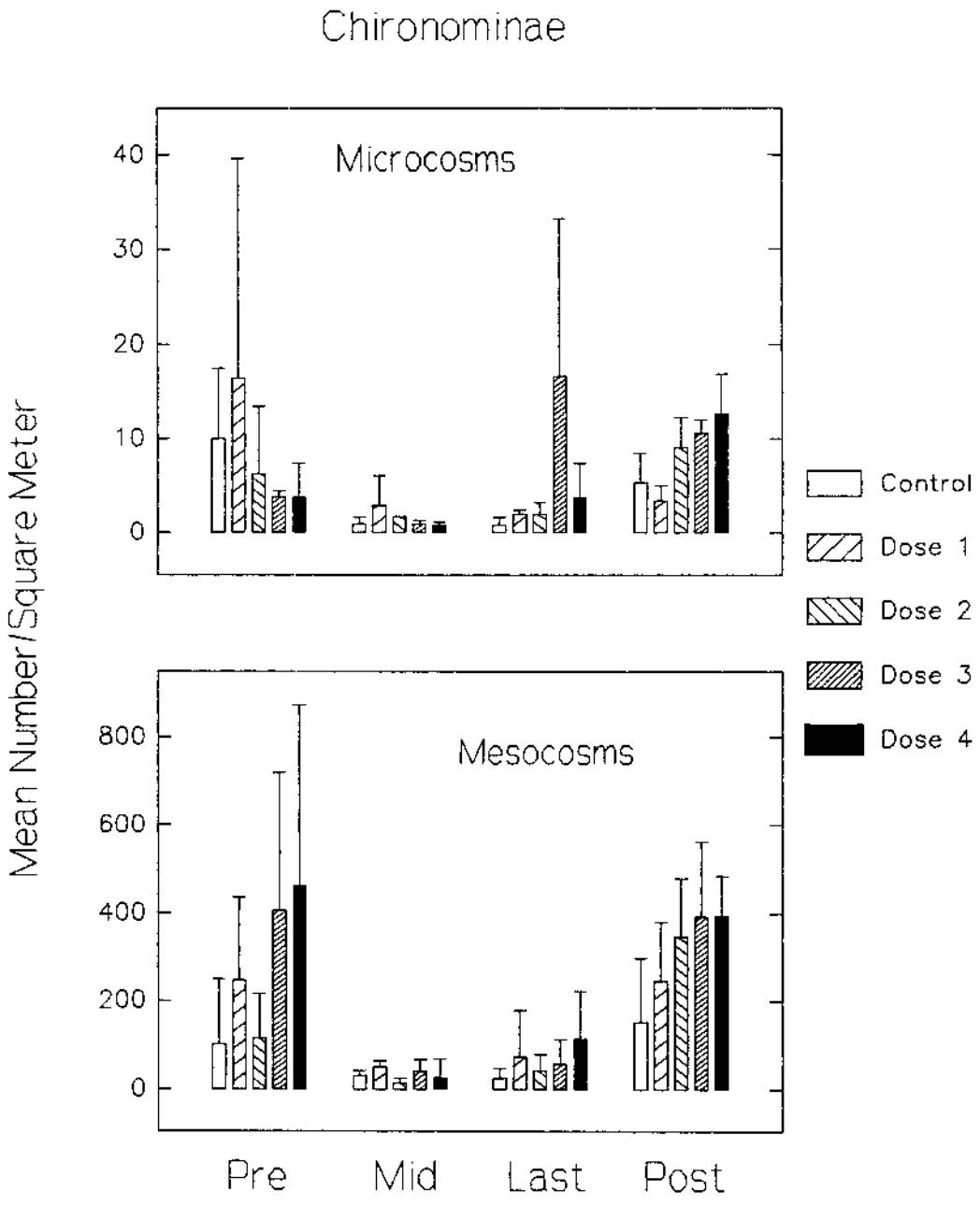

Figure 100 Mean number (± S.D.) of Chironominae collected from microcosms and mesocosms. Pre=Prior to initial application, Mid=Middle of application period, Last=Last application, Post=Study termination.

present in microcosms.

Open water systems and planktivory have received more attention than fish impacts in littoral systems. Bluegill predation on benthic communities is also size selective, with bluegills considered to be "keystone predators" (Butler 1989). Large or active prey are selected, increasing the densities of smaller, more sedentary prey (Morin 1984a, Mittlebach 1988, Butler 1989).

Many differences in macroinvertebrate population levels between mesocosms and microcosms can be explained when prey life-history and bluegill predation are considered. *Callibaetis* nymphs are "swimmers and climbers" (Merritt and Cummins 1978), usually associated with aquatic macrophytes, suggesting increased exposure to fish predation.

Caenis mayfly immatures are classified as "sprawlers", inhabiting depositional areas and sediments and would be less vulnerable to fish predation (Merritt and Cummins 1978). Production of *Caenis simulans* (now *C. amica*) was enhanced in replicated ponds containing bluegills and lower in fishless treatments (Hall et al. 1970). This paralleled the higher *Caenis* population densities in mesocosms relative to microcosms during this study.

Odonates were usually reduced in enclosures or ponds containing bluegills (Hambright et al. 1986, Hall et al. 1970, Morin 1984b). This trend may reflect both direct

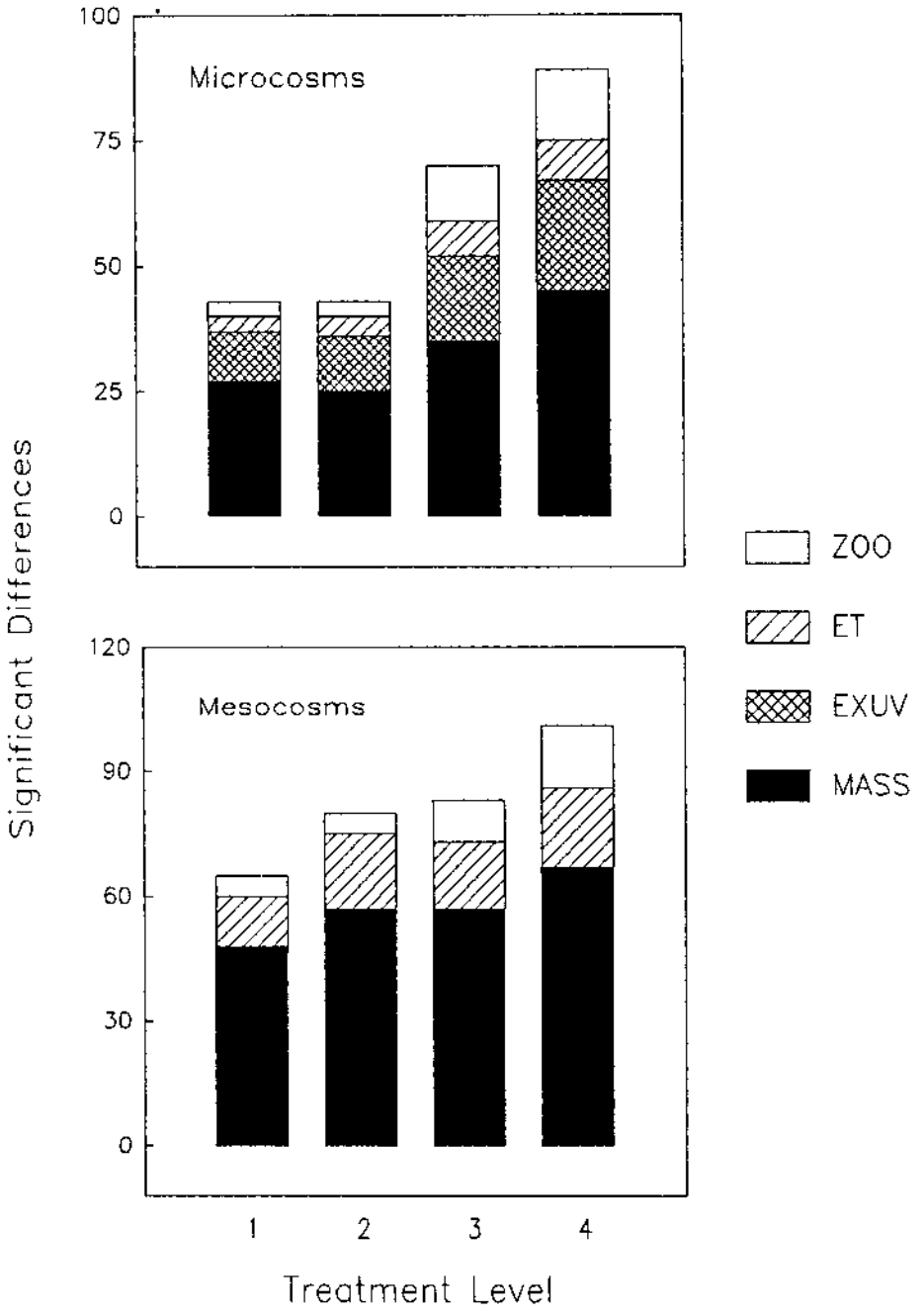

Figure 101. Cumulative number of significant differences (Dunnett's MRT) from microcoms and mesocosms during the application period. Treatments range from low (1) to high (4). ET=Emerg. Trap, EXUV=Exuviae, ZOO=Zooplank., MASS=MAS Samples.

predation of fish on odonates and competition between fish and surviving naiads for common prey items (Morin 1984b). Size-selective bluegill predation resulted in high predation rates on large odonate naiads (Morin 1984a). Odonates colonizing MAS samplers, primarily Libellulidae and Coenagrionidae, were more abundant in microcosms and reduced in mesocosms. Some taxa that did not readily colonize epibenthic substrates (Gomphidae, Aeshnidae) were not sampled effectively, thus population impacts within these groups are currently unknown.

The reduced numbers of odonates in mesocosms relative to microcosms may reflect a combination of factors. First, a broader range of bluegill sizes were represented in mesocosms that might allow predation on several odonate size classes simultaneously. Microcosm bluegills were juvenile fish and may have experienced gape limitations when handling the largest odonate naiads. Alternatively, many Libellulidae and Coenagrionidae are associated with aquatic macrophytes, which were more abundant in microcosms. Higher microcosm odonate populations may have reduced populations of other macroinvertebrates (Naididae, Tanypodinae, and Chironominae, for example).

At a general level chironomid larvae are considered to be preferred bluegill prey (Schramm et al. 1989). Close examination of chironomid responses to fish predation reveal

more complex relationships. Gilinsky (1984) found that chironomids responded variably to fish predation depending upon ecological niche, habitat sampled and season of the year. Somewhat higher Tanypodinae and much higher Chironominae populations were observed in mesocosms relative to microcosms.

Macrophytes may provide a refuge for macroinvertebrates by reducing the searching efficiency of bluegill (Gilinsky 1984, Loucks 1985). Macrophyte density has also been correlated with survival of *Daphnia* populations (Wright and Shapiro 1990). Though macrophytes theoretically reduce search efficiency, macrophytes also provide habitat for epiphytic invertebrates. Bluegill utilization of epiphytic prey may be much greater than predation upon benthic organisms (Schramm et al. 1989). Thus, invertebrate production and fish growth may be maximized at some intermediate macrophyte density (Crowder and Cooper 1982). Higher densities of potential bluegill prey in the microcosms compared to mesocosms probably reflected higher macrophyte densities and the artificial refugia present, besides the obvious differences in fish loadings.

Fishless microcosms were used to help partition fish effects from pesticide stress. The greatest predation impacts were observed on epibenthic/epiphytic

macroinvertebrates and emergent insects (Morris 1991, Morris et al., In Press). Impacts on emerging insects were more pronounced than impacts on macroinvertebrates colonizing artificial substrates (Morris 1991, Morris et al., In Press).

Other Factors

Differences in community structure between the mesocosms and microcosms may also have reflected colonization phenology. Odonate community composition, for instance, can be influenced by which species first colonize a pond (Morin 1984a, Benke 1978). Also, the surface area of water bodies may influence colonization rates (Friday 1987) and the presence of suitable oviposition sites may help determine insect community composition.

Finally, microcosms lacked a depth gradient (vertical walls were present), potentially reducing insect emergence success. Artificial refugia may have helped offset this disadvantage, since refugia were used extensively by emerging odonates. Increased odonate mortality upon emergence was qualitatively noted in some microcosms that were not in the study design and lacked refugia. This suggested that some sort of emergence route linking the microcosm sediments to the surface may be useful and should be incorporated in future microcosm designs.

Summary

Characterization of sensitive and insensitive taxa was similar among the two systems. Responses of sensitive populations showed corresponding patterns, but differences in absolute numbers, among the two systems. These differences in absolute numbers and dominant taxa may be attributable to differences in bluegill predation pressures, habitat (macrophytes) and also may reflect taxa-specific colonization of these systems.

CHAPTER 10

RESIDUAL TOXICITY AND MACROINVERTEBRATE RECOVERY

Introduction

Impacted ecosystems subjected to chemical stress undergo characteristic exposure-response-recovery relationships, which can vary with the duration, intensity and scale of the stressor (Kelly and Harwell 1990). Responses of aquatic invertebrate communities to the application of a pyrethroid insecticide were studied in outdoor microcosms, and subsequent recovery processes were documented.

Study of recovery in freshwater habitats has primarily centered on lotic systems, with studies of lakes and ponds less common (Niemi et al. 1990). While recovery of zooplankters has been studied in lentic systems, macroinvertebrate recovery has mainly been studied in low (first to third) order streams (Niemi et al. 1990).

In general, stressors that physically altered habitat, and were characterized by longer-term chronic impacts, demonstrated longer recovery times. Examples include mining activity, clear-cut logging and channelization, with recovery times measured in decades (Yount and Niemi 1990).

When evaluating the response of a natural community to a particular stressor, it is often difficult to distinguish between ecologically "significant" responses, and those that may occur but do not alter the ecosystem in a meaningful way. For instance, eliminating one species from the ecosystem may or may not constitute a change of importance to the ecosystem; loss of all species performing a common ecosystem function certainly would (Kelly and Harwell 1990). When considering the recovery of a system, it is equally difficult to determine when it has reached a normal or nominal state. Recovery to a pre-impacted state might not even be achievable, since a precise sequence of events, including climatic occurrences, might rarely or never be repeated (Cairns 1990).

Recovery involves the recolonization of organisms from outside sources or internal refugia (Yount and Niemi 1990). Recovery times following pulse disturbances are influenced by the presence of refugia, distance from refugia, time of year of disturbance and life history characteristics of impacted taxa (Niemi et al. 1990).

Aquatic mesocosms and microcosms are experimental systems that are replicated, yet possess a degree of realism not possible in laboratory systems (Odum 1984). Mesocosms and microcosms are not miniature ecosystems but rather are surrogates for important cause/effect pathways in natural

systems (Odum 1984, Cairns 1988b). These simulated field studies should offer unique opportunities for studying exposure-response-recovery relationships.

Mesocosm studies used within the FIFRA pesticide registration process have traditionally lasted less than one calendar year, with pesticide application beginning in spring or summer, monitoring continuing throughout the summer and fall, and study termination in the late fall or early winter. Pesticide impacts and recovery processes in outdoor microcosms were evaluated as part of a comparative study contrasting microcosm (1.9 m^3) and mesocosm (635 m^3) responses to the pyrethroid insecticide cyfluthrin during 1989 (discussed in previous chapters).

Microcosms still had detectable pyrethroid concentrations in the sediments at the termination of monitoring in November 1989. The presence of residual pesticide in aquatic sediments could potentially result in accumulation of residues over time if the same compound was used again the following year. In November of 1989, water from each microcosm was pumped to a holding tank, fish were harvested, and the same water was then returned to the original microcosm. Microcosms were resampled in June 1990 to determine residual pesticide effects and to evaluate the role of recolonization in the recovery of stressed systems.

A *Hyalella azteca* sediment bioassay and sediment residue sampling conducted at this time provided independent lines of evidence for evaluating potential sediment toxicity.

Results

Pyrethroid Residues

Microcosm sediments were sampled in June, 1990. No detectable pyrethroid residues were found in sediments from any microcosms (minimum detectable limit = 10.0 µg/kg).

Macroinvertebrate Community

Macroinvertebrate populations in concrete microcosms were sampled in June 1989, at the initiation of the microcosm study. Dominant taxa colonizing MAS at this time were mayflies (*Caenis* and *Callibaetis*), naidid oligochaetes, Hydracarina, gastropods and chironomids (Figure 102). Exuviae were dominated by Ephemeroptera (mostly *Callibaetis*), Chironomidae and Odonata (Figure 103). *Chaoborus* were important in tanks without fish (NFC).

Artificial substrates (MAS) sampled in 1990 were dominated by Naididae, Hydracarina. Gastropoda and Chironomidae were also abundant in some tanks. Total numbers of organisms colonizing MAS samplers increased over 1989 levels, and very different compositional patterns were

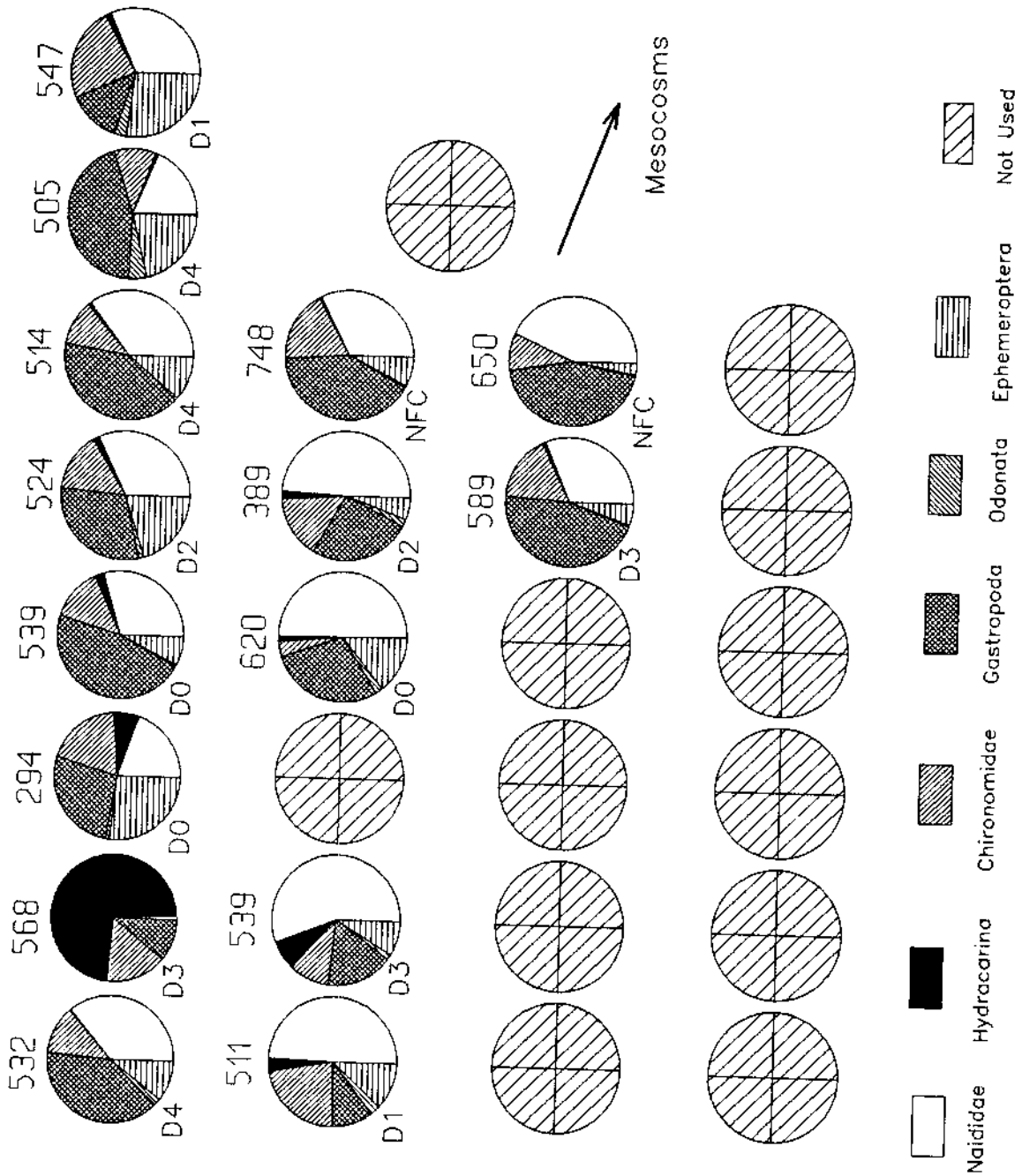

Figure 102. Dominant macroinvertebrates colonizing MAS prior to first application; June 1989. Each microcosm is represented by a pie-chart (percent composition). D0=Control, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4, NFC=Fishless Control.

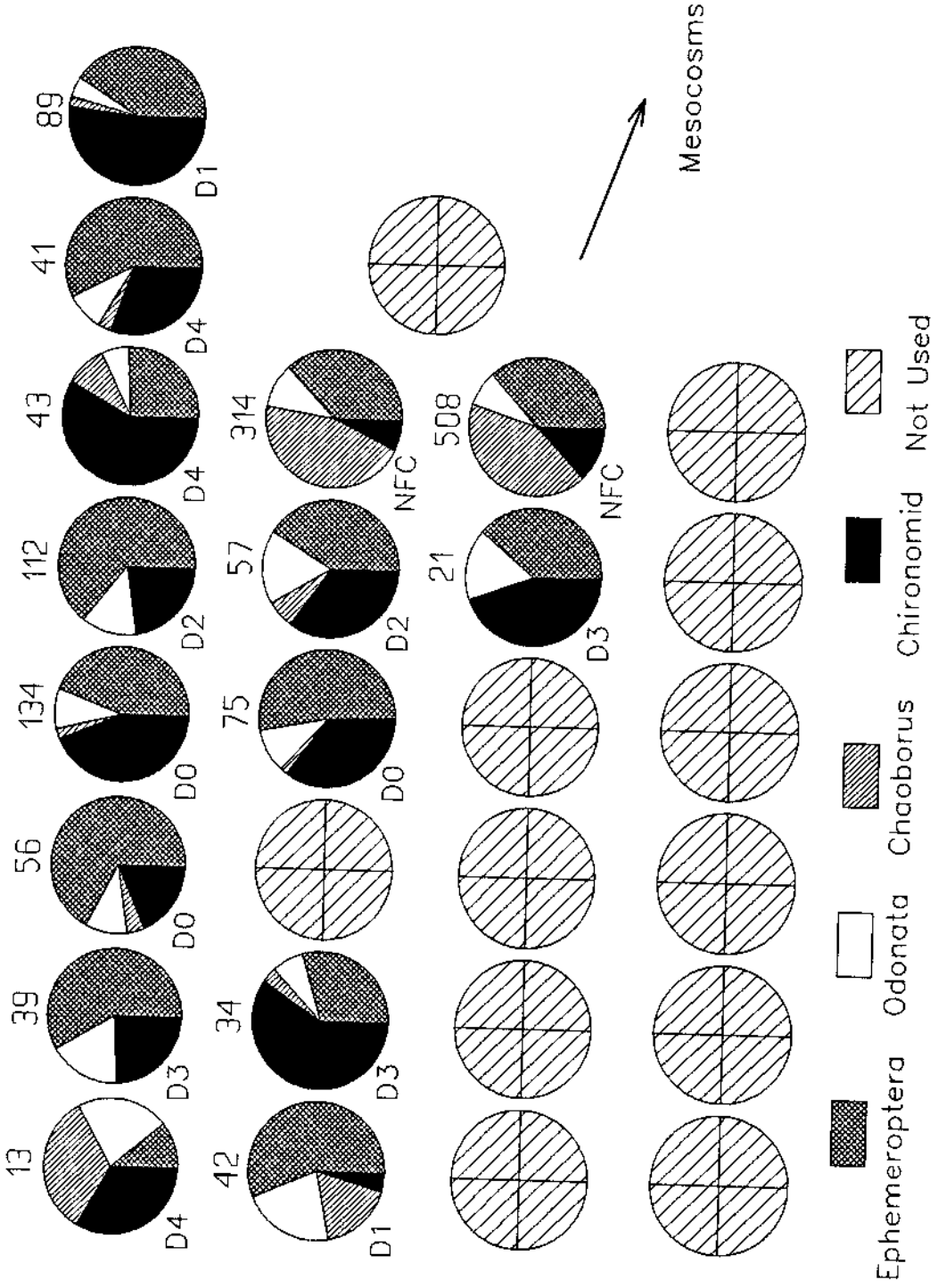

Figure 103. Dominant groups of macroinvertebrate exuviae collected during June 1989, prior to the first application. Each microcosm is represented by a pie-chart (percent composition) of abundant taxa.

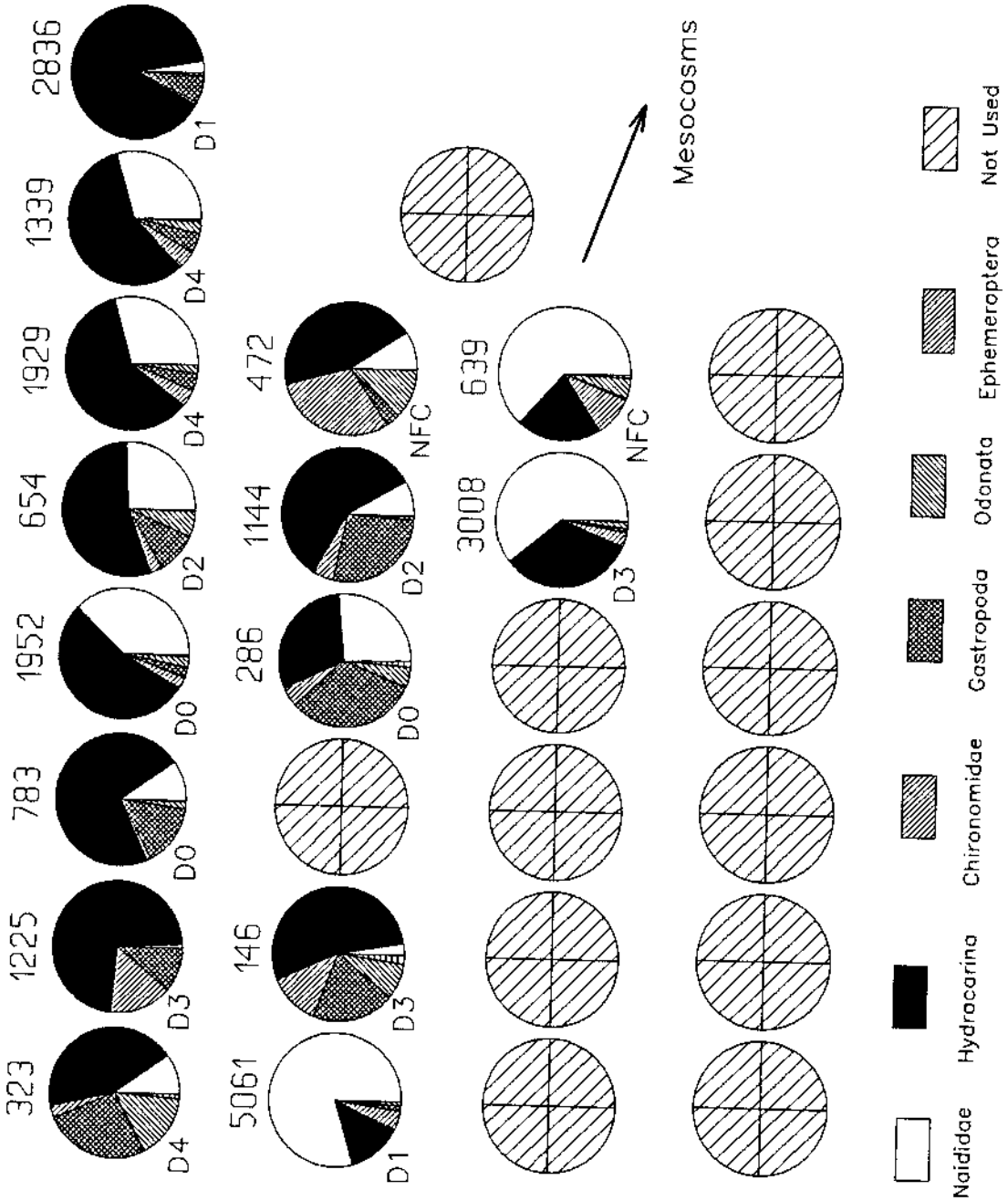

Figure 104. Dominant macroinvertebrates from MAS one year after first application; June 1990. Each microcosm is represented by a pie-chart (percent composition). D0=Control, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4, NFC=Fishless Control.

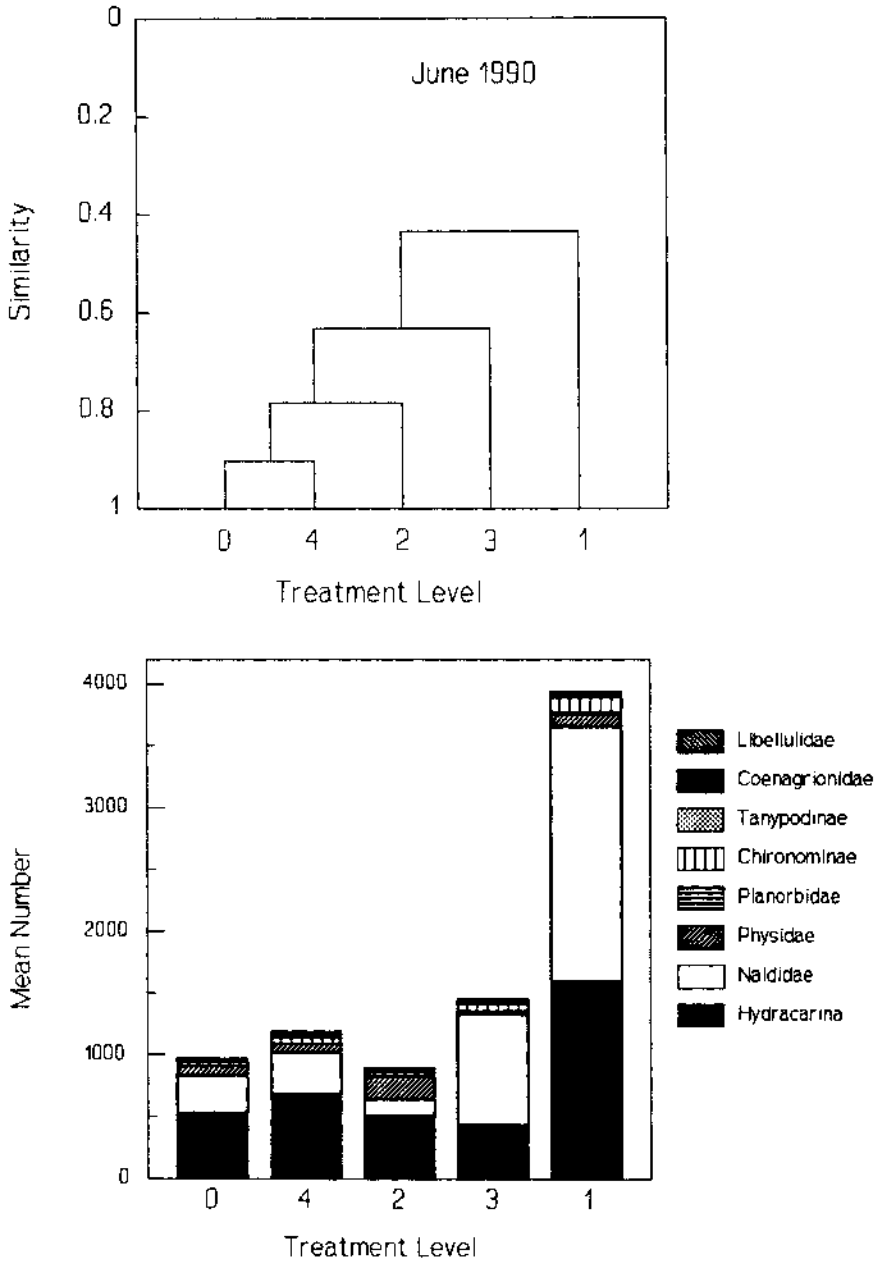

Figure 105. Bray-Curtis cluster analysis for macroinvertebrates colonizing artificial substrates during June, 1990; one year after first application. Treatments range from control (0) to high rate (4).

observed (Figure 104). Clear trends related to treatment level were not evident, with extreme variation occurring even within treatment levels (i.e., D1 microcosms, Figure 104). Cluster analysis of these data (Figure 105, Appendix Table 8) indicated that Controls and D4 were most similar (similarity=0.90), while D1 was most different from other treatments (similarity=0.43). No significant differences were identified via bootstrapping ($p>0.4$, Appendix Table 8).

Exuviae collections in 1990 were dominated by *Callibaetis* mayflies, Coenagrionidae naiads, Tanypodinae chironomids and *Chaoborus* pupae (Figures 106 and 107). Numbers of exuviae collected increased greatly over 1989 levels (Figure 106). Cluster analysis of exuviae samples indicated that D0 and D3 were most similar (similarities=0.74), while D2 and D1 were less similar to controls and clustered separately (Figure 107). Bootstrapping again failed to discern any statistically significant separation of clusters (Appendix Table 8).

Comparisons of treatments to controls using Dunnett's MRT on a taxa-by-taxa basis identified few significant differences. The only differences found for exuviae collections were significantly higher levels of *Oecetis* pupae (a pyrethroid-sensitive trichopteran) at D2 and significant increases in *Orthocladius* (*Orthocladius*) pupae, an orthoclad chironomid at D2 and D4 (Tables XVII and XIX).

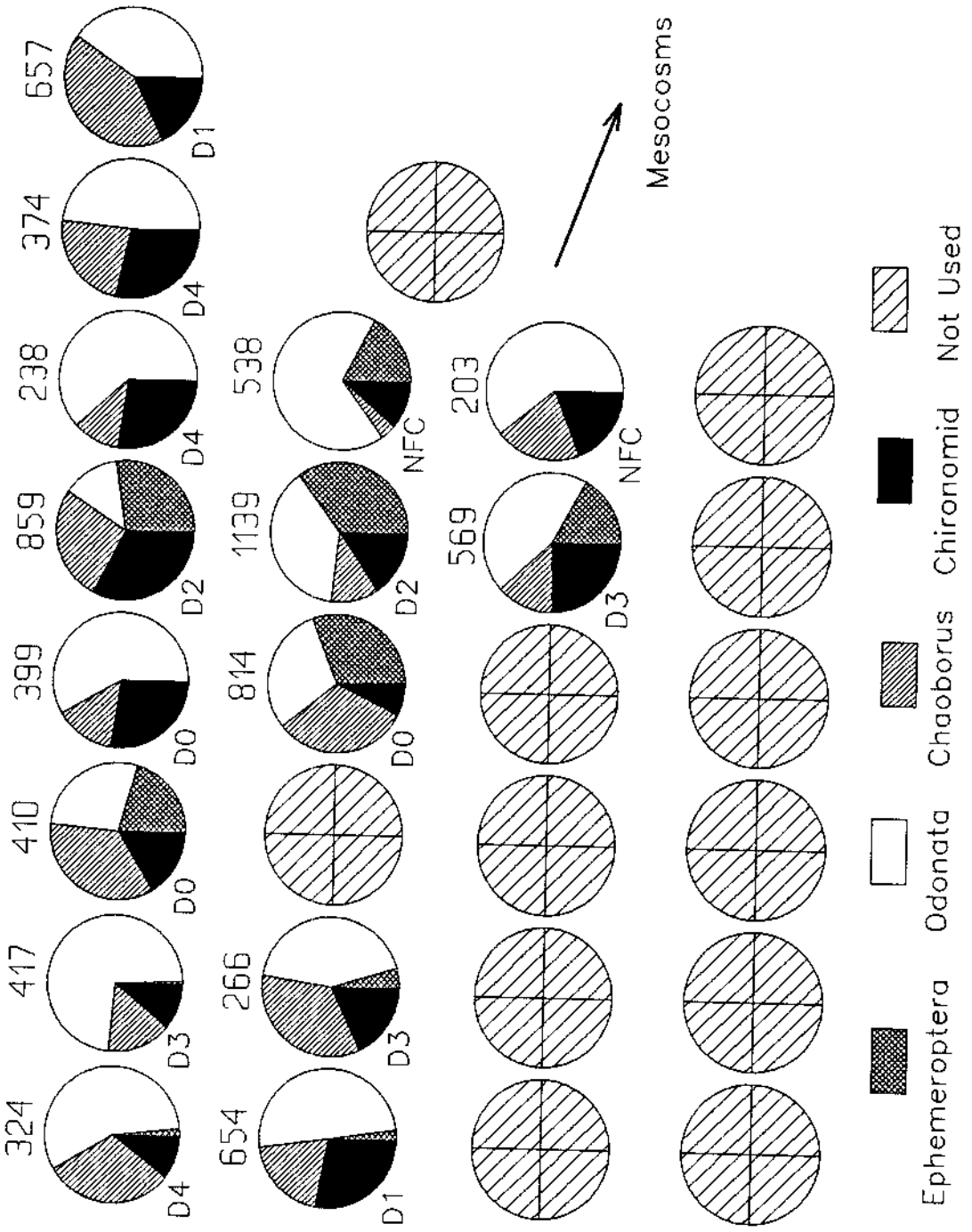

Figure 106. Dominant macroinvertebrate exuviae one year after first application; June 1990. Each microcosm is represented by a pie-chart (percent composition). D0=Control, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4, NFC=Fishless Contol.

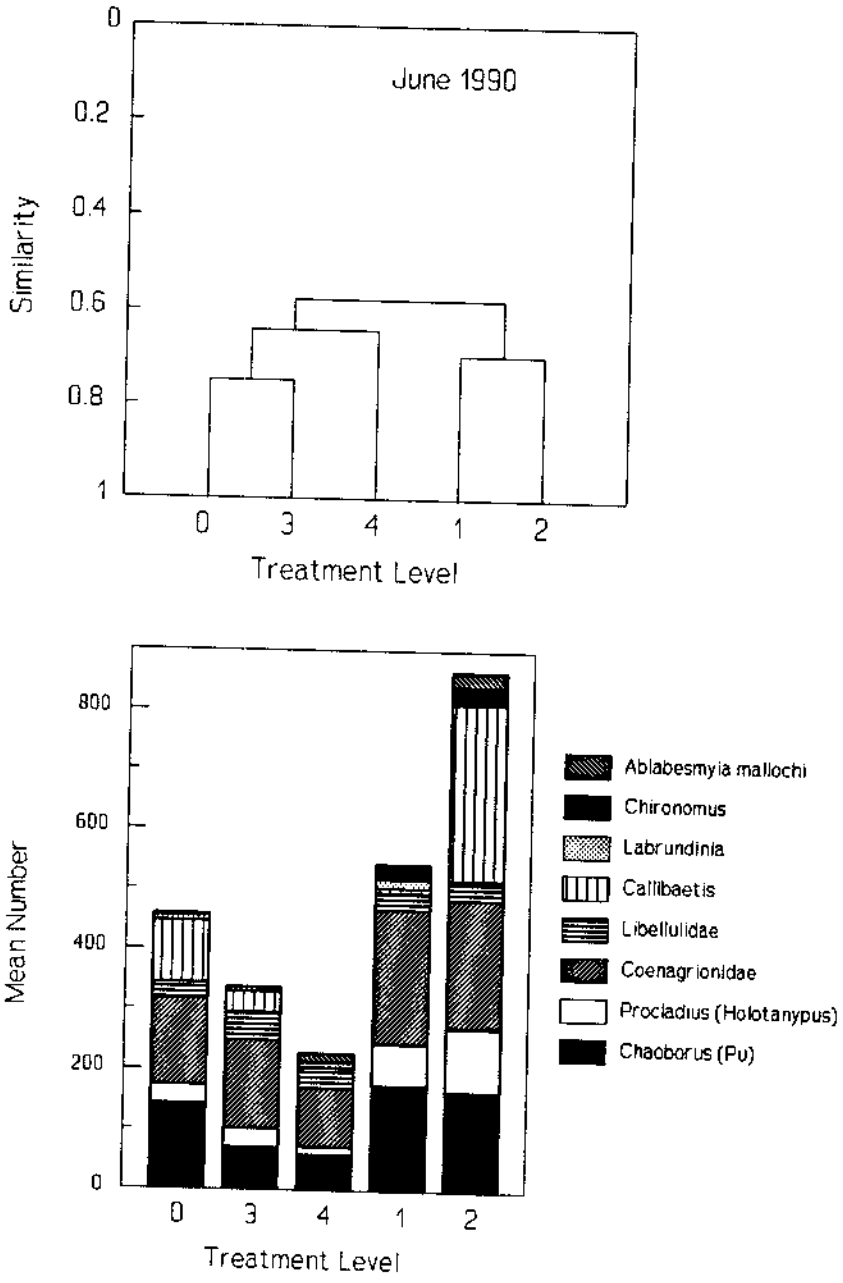

Figure 107. Bray-Curtis cluster analysis of exuviae collected from microcosms during June, 1990; one year after first application. D0=Control, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4, NFC=Fishless Control.

Oecetis and *Orthocladius* (*Orthocladius*) sample sizes were both very low. The only significant differences in MAS were found for *Berosus* (a hydrophilid beetle). *Berosus* larvae were significantly increased at D1 and significantly reduced at D3 (Table XX).

Since the general macroinvertebrate community structure revealed few differences attributable to historical pesticide exposure, the next step might be to focus on the most pyrethroid-sensitive organisms identified the previous year. These macroinvertebrates include mayflies, chaoborids (phantom midges), and Tanypodinae chironomids (see Chapters 7 and 8).

Callibaetis exuviae were extremely variable, with highest values at D2 (Figure 108). Analysis of the spatial distribution of *Callibaetis* exuviae suggests a very patchy distribution for this mayfly (Figure 109). Tanks near the center of the site contained more *Callibaetis* exuviae relative to microcosms on the periphery. *Chaoborus*, among the most pyrethroid sensitive taxa during 1989, were found at every treatment level. Graphically, mean numbers of *Chaoborus* pupal exuviae collected at D3 and D4 were somewhat reduced compared with controls (Figure 108). *Chaoborus* were more widely distributed (Figure 110), and did not exhibit the patchiness observed for *Callibaetis*. *Chaoborus* from MAS samplers (not shown) demonstrated a different pattern, with

Table XVIII. Summary of statistically significant diffences (Dunnett's MRT) for Chironomidae exuviae collected from microcosms during June, 1990.

Chironomid Taxa: Exuviae	D1	D2	D3	D4
Tanypodinae: *Ablabesmyia idei*				
Ablabesmyia mallochi				
Ablabesmyia peleensis				
Clinotanypus				
Labrundinia				
Larsia				
Procladius (Holotanypus)				
Procladius (Psilotanypus)				
Tanypus				
Orthocladiinae: *Corynoneura*				
Orythocladius (Orthocladius)		↑		↑
Chironominae: *Apedilum*				
Chironomus				
Cladotanytarsus				
Cryptochironomus				
Dicrotendipes incurvus				
Dicrotendipes modestus				
Endochironomus				
Parachironomus				
Paratanytarsus				
Tanytarsus				

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

fewer larvae in controls and higher numbers from treated tanks.

Tanypodinae were very abundant in all microcosms. *Labrundinia*, the most common and most heavily impacted Tanypodinae genera during 1989, showed no pesticide-related trends during 1990 (Figure 111). *Labrundinia* populations were low and fairly even in distribution (Figure 112).

Table XIX. Summary of statistically significant differences for non-chironomid exuviae collected from microcosms during June, 1990. D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

Non-Chironomid Taxa: Exuviae	D1	D2	D3	D4
Libellulidae: *Libellula*				
Pachydiplax longipennis				
Pantala flavescens				
Tramea				
Dythemis fugax				
Coenagrionidae				
Ephemeroptera: *Callibaetis*				
Caenis				
Trichoptera: *Oecetis* (pupae)		↑		
Diptera: *Chaoborus*				
Ceratopogonidae				
Hemiptera: Gerridae				
Coleoptera: *Berosus*				

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

Table XX. Summary of statistically significant differences (Dunnett's MRT) for taxa colonizing MAS samplers from microcosms during June, 1990. D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4.

All Taxa: MAS	D1	D2	D3	D4
Ephemeroptera: *Callibaetis*				
Caenis				
Libellulidae				
Coenagrionidae				
Trichoptera: *Oecetis*				
Orthotrichia				
Oxyethria				
Coleoptera: *Berosus* (larvae)	↑		↓	
Diptera: *Chaoborus*				
Ceratopogonidae				
Chironominae				
Tanypodinae				
Physidae				
Planorbidae				
Hydracarina				
Naididae				

↑=Treatment significantly greater than control.
↓=Treatment significantly less than control.

Procladius (*Holotanypus*), another Tanypodinae, reached its highest densities at D2 (Figure 111). This response was primarily due to very high populations in a single tank (D2; 175 pupal exuviae collected, Figure 113). Tanypodinae larvae from MAS samples (not shown) were more common in treated tanks compared to controls.

A perceptible shift within the odonate community occurred during the 1989 - 1990 interval. MAS and exuviae samples from 1989 were either dominated by dragonfly naiads (Libellulide) or demonstrated mixed dominance of Libellulidae and Coenagrionidae (Figure 114). In contrast, coenagrionid naiads were proportionally more abundant in 1990 samples (Figure 115). Total odonate numbers collected by MAS samplers increased from 1989 to 1990. This trend was even more evident from exuviae sampling.

Hyalella bioassay

Hyalella azteca exposed to microcosm sediments exhibited excellent survival (all treatments over 90%; Figure 116), with no significant differences among treatments (Fisher's exact test, α=0.05).

Discussion

Analyses of the macroinvertebrate community within microcosms less than one year after pyrethroid application

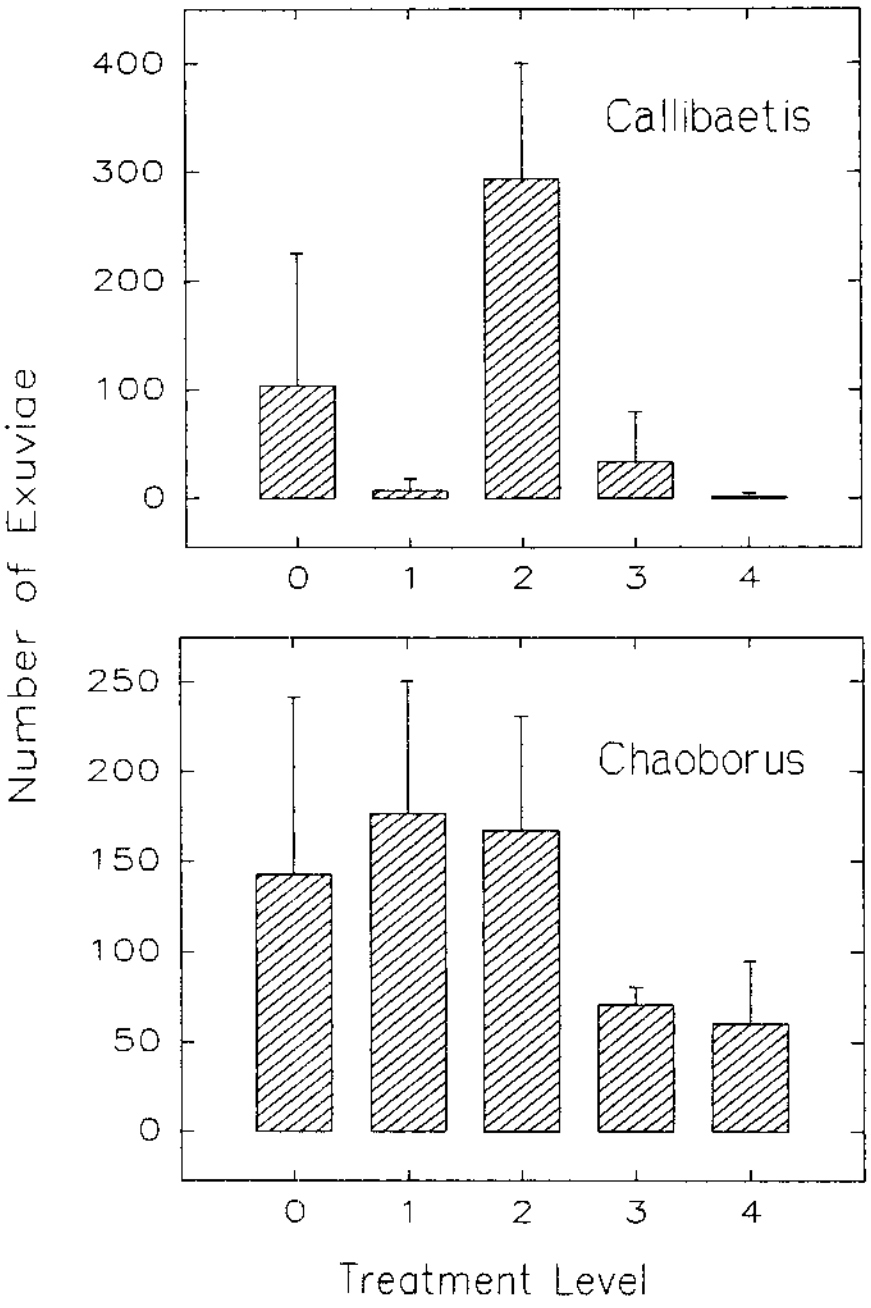

Figure 108. Number of *Callibaetis* and *Chaoborus* exuviae collected during June 1990. Bars represent the mean number of exuviae (± S.D.). Treatments range from control (0) to high rate (4).

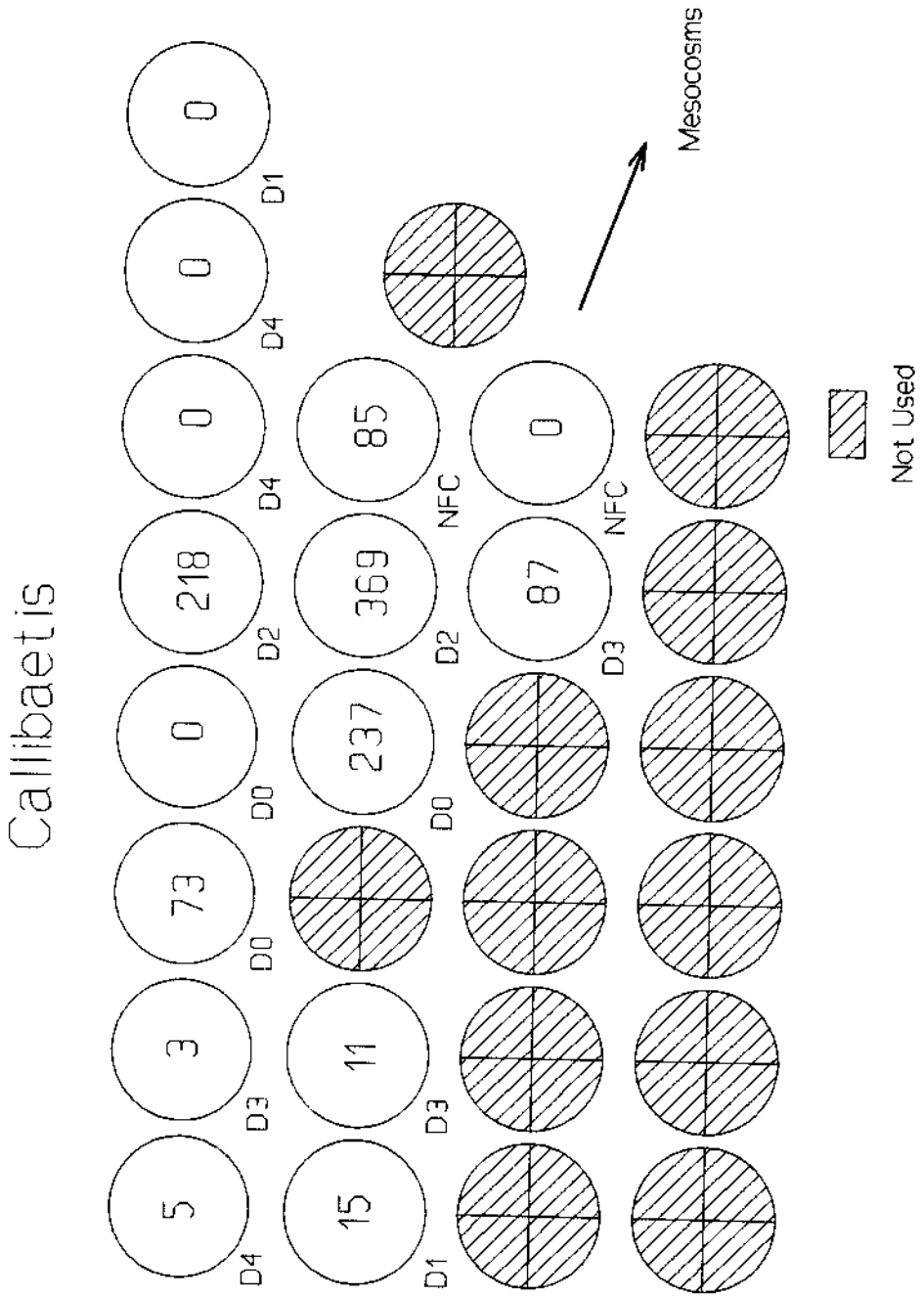

Figure 109. Number of *Callibaetis* exuviae collected during June 1990. Each microcosm shows the number of exuviae collected over a one week period. D0=Contol, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4, NFC=Fishless Control.

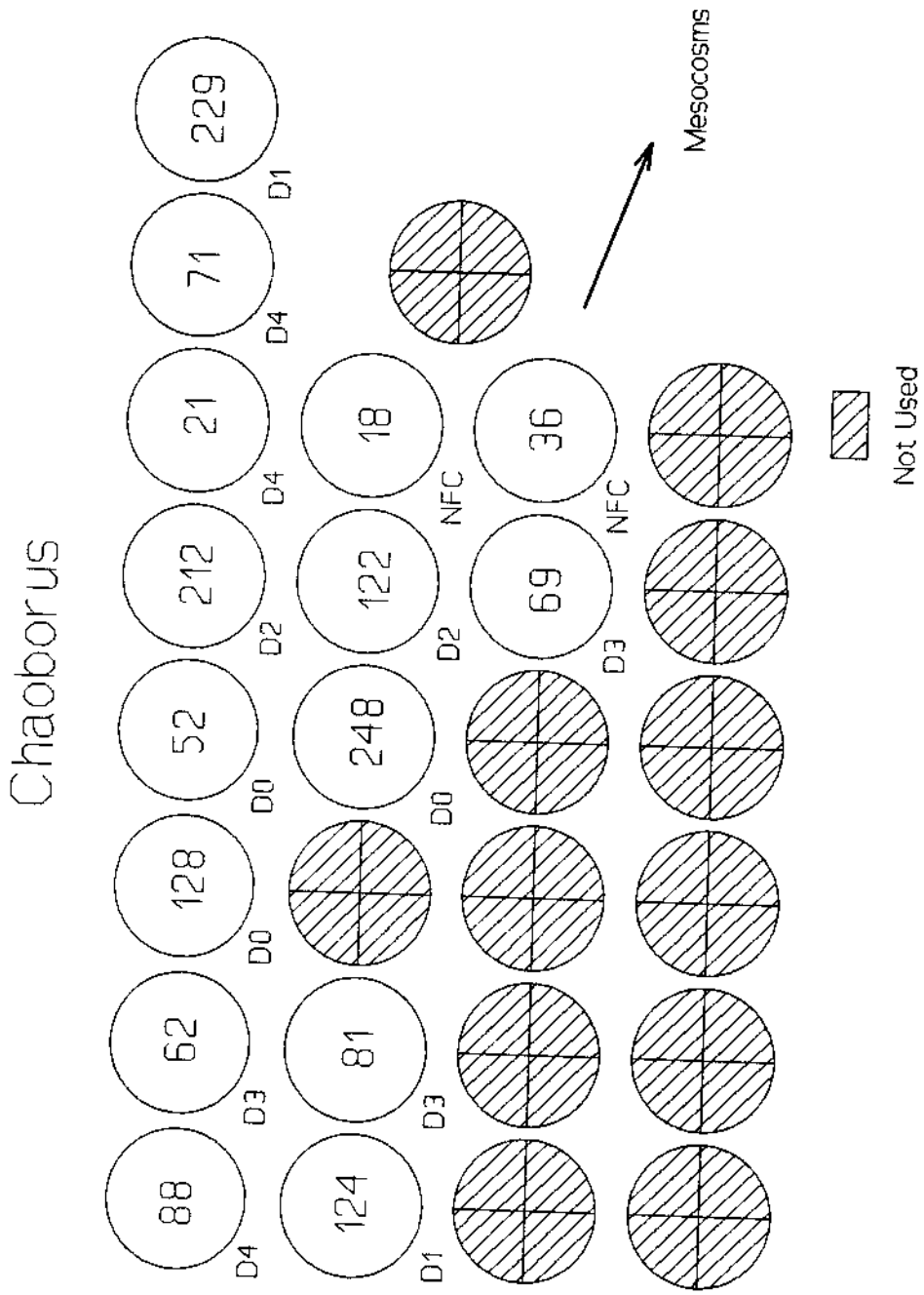

Figure 110. Number of *Chaoborus* exuviae collected during June 1990. Each microcosm shows the number of exuviae collected over a one week period. D0=Control, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4, NFC=Fishless Control.

(applications ended in September 1989) failed to show any definitive residual pesticide effects. Sensitive taxa such as Tanypodinae chironomids, *Callibaetis* mayflies and *Chaoborus* midges either showed no impacts or exhibited contradictory patterns. Sediment bioassays using *Hyalella azteca*, among the most pyrethroid-sensitive taxa known, supports these field observations. In addition, cyfluthrin residues were below detectable levels, adding another line of evidence for lack of long-term impacts.

Total numbers of macroinvertebrates sampled by MAS and exuviae increased from 1989 to 1990. This undoubtably reflected removal of fish from the microcosms. A major difference between 1989 and 1990 samples was the presence of *Chaoborus* in all tanks, which were rare when fish were present, again suggesting that fish-effects were a controlling force during 1989. Coenagrionid numbers also increased dramatically (Figures 114 and 115), suggesting that these insects benefited from fish removal.

Water mites and naidid oligochaetes showed dramatic increases over 1989 population levels. These taxa show "r-selected" characteristics such as good colonization ability (i.e., *Chaoborus* exploited fishless conditions over a short period) and rapid growth rates (naidids may show rapid growth due to asexual reproduction; McElhone 1978, Loden 1981, Smith 1986). Chironomids, particularly those with

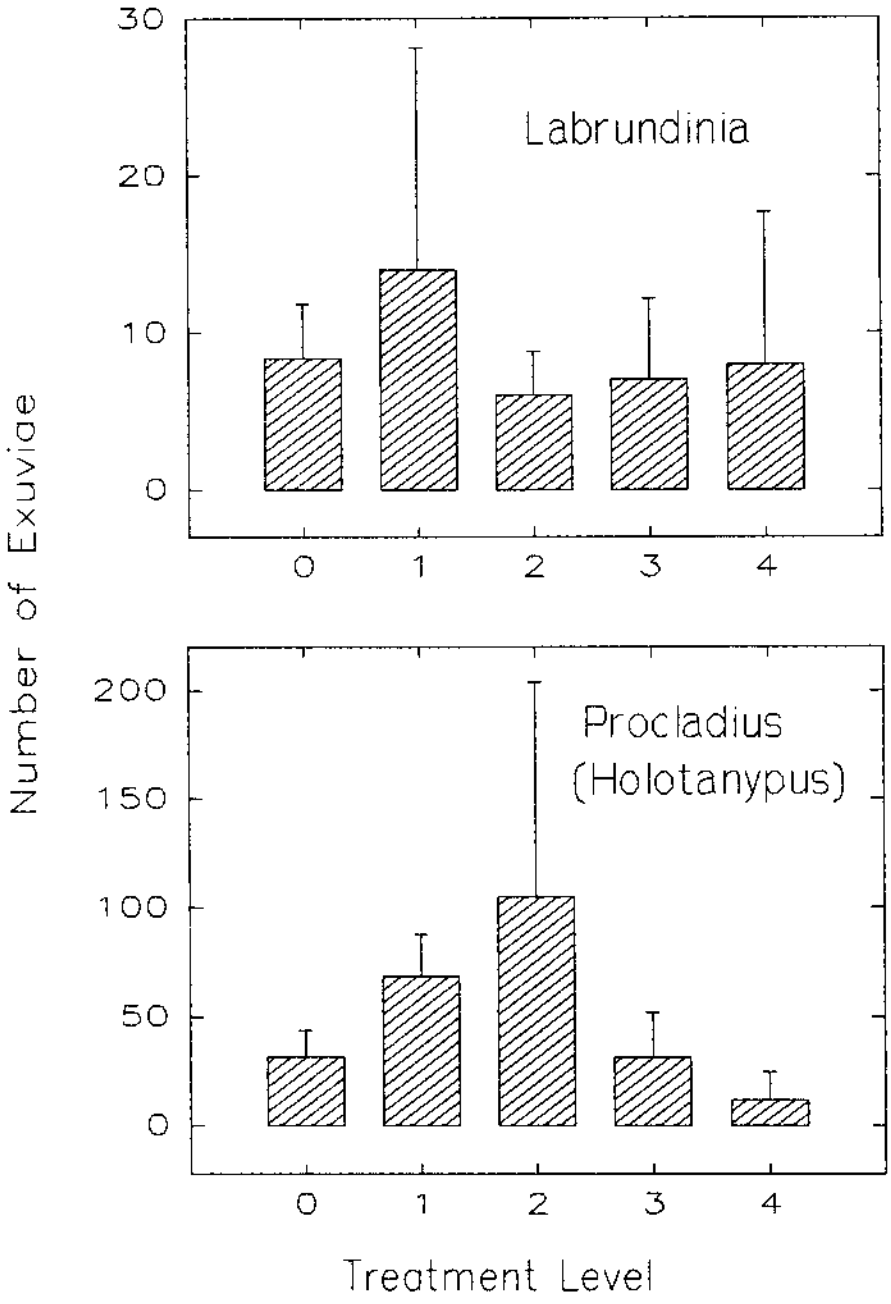

Figure 111. Number of *Labrundinia* and *Procladius* (*Holotanypus*) exuviae collected during June 1990. Bars represent the mean number of exuviae (± S.D.). Treatments range from control (0) to high rate (4).

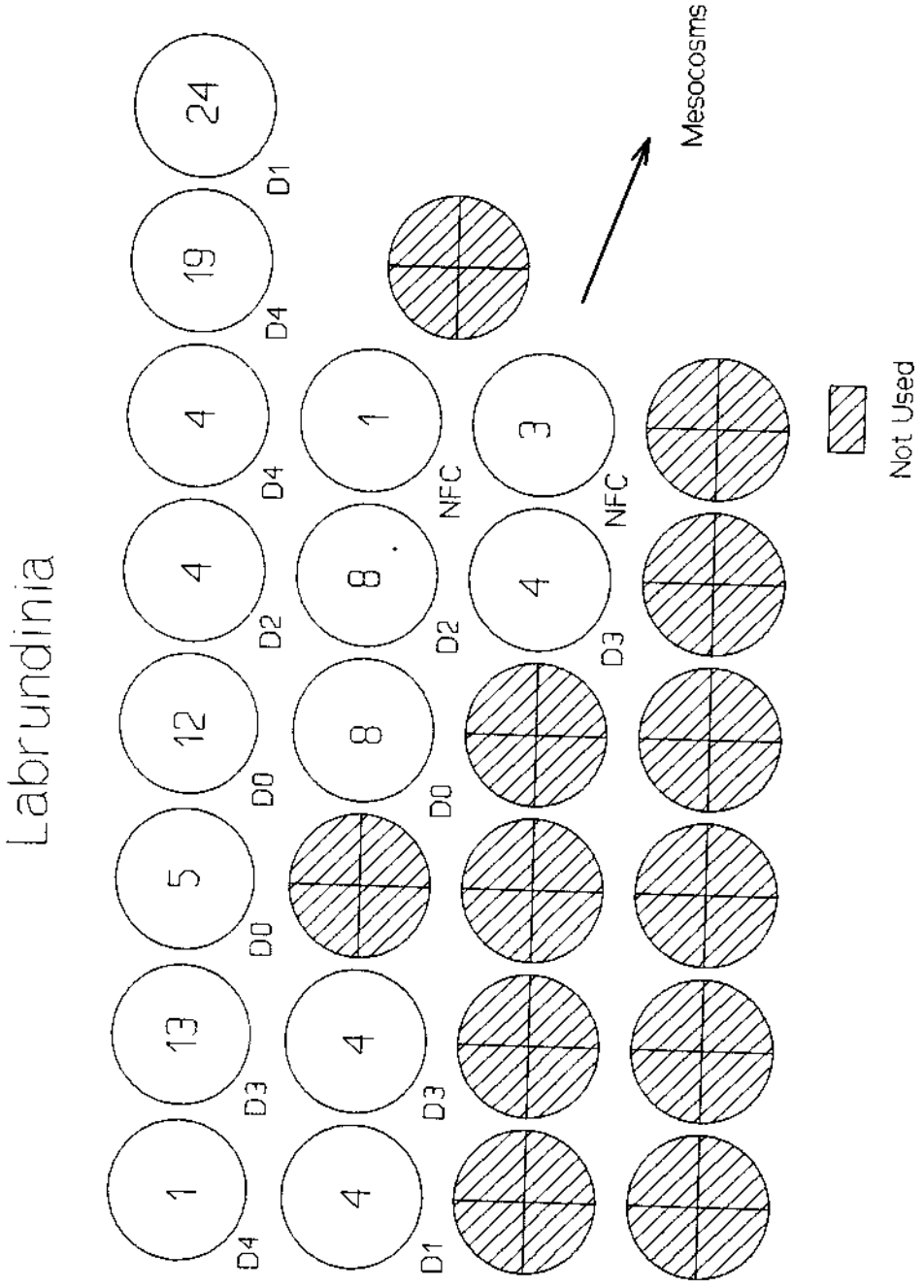

Figure 112. Number of *Labrundinia* exuviae collected during June 1990. Each microcosm shows the number of exuviae collected over a one week period. Treatments range from control (0) to high rate (4).

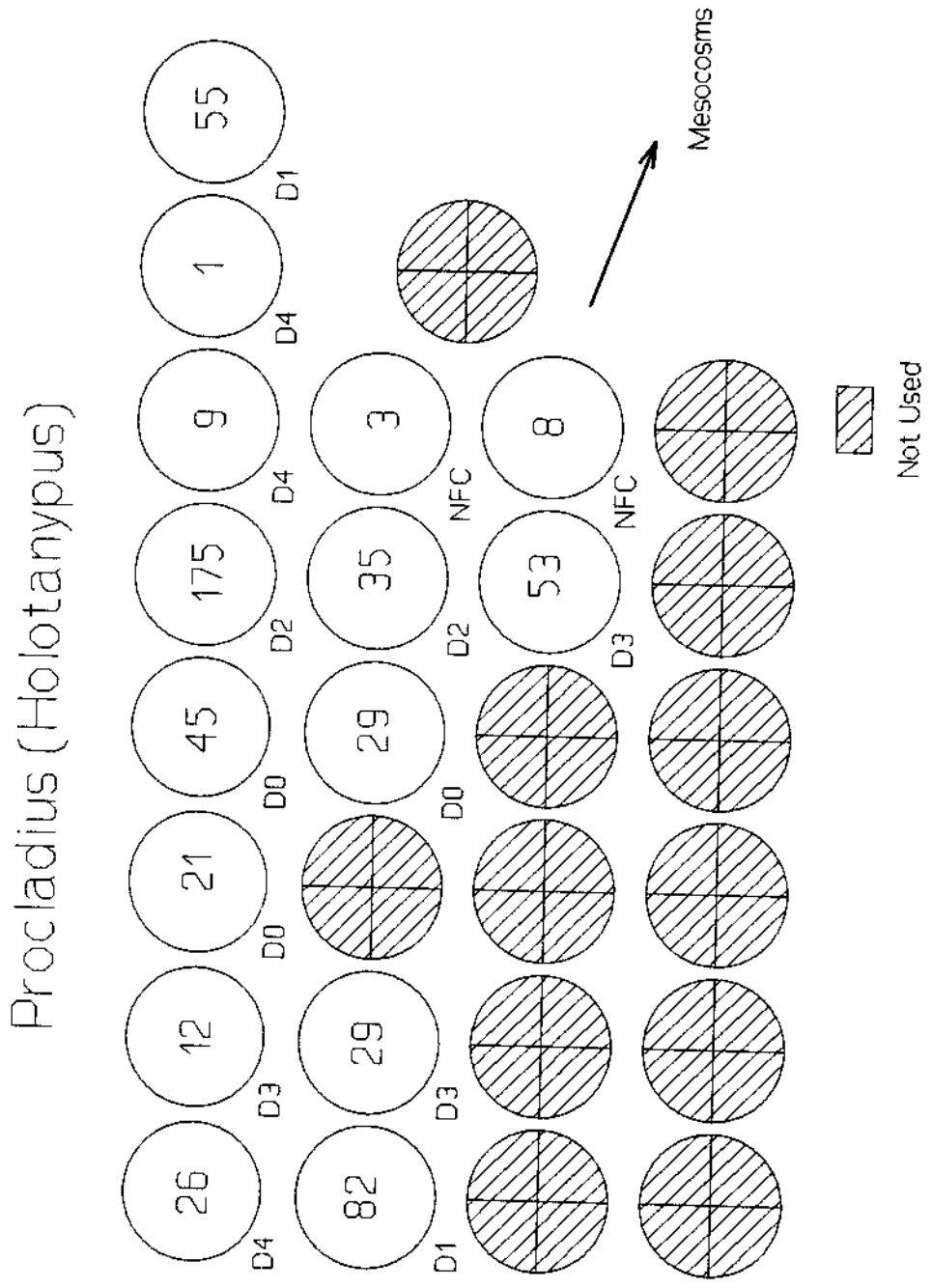

Figure 113. Number of *Procladius* (*Holotanypus*) exuviae collected during June 1990. Each microcosm shows the number of exuviae collected over a one week period. Treatments range from control (0) to high rate (4).

short life-cycles, can recover from perturbations very rapidly and Chironomidae are often among the first insect taxa to recolonize streams following disturbance (Wallace 1990).

High numbers of *Callibaetis* were observed in selected tanks (Figure 109). Microcosms with high mayfly populations were generally in the center of the study site, with fewer *Callibaetis* on the periphery. This spatial distribution shows the stochastic nature of colonization events, and/or responses to unknown factors during oviposition. Extreme variability (i.e., *Callibaetis* and *Procladius* (*Holotanypus*)) potentially hampered detection of significant differences among treatments.

Population structure changed from 1989 to 1990, favoring r-selected taxa. Many colonizers were predators. While differing from 1989 populations, the implications of this divergent community structure were unclear. These results suggested that small microcosms may not be sufficient to support stable macroinvertebrate populations over such a long period (into a second year). This was not surprising. According to island biogeography theory, extinction events would exert a strong influence on community structure in smaller systems due to lower population sizes (Friday 1987), and smaller "islands" may receive fewer immigrants since they represent a smaller

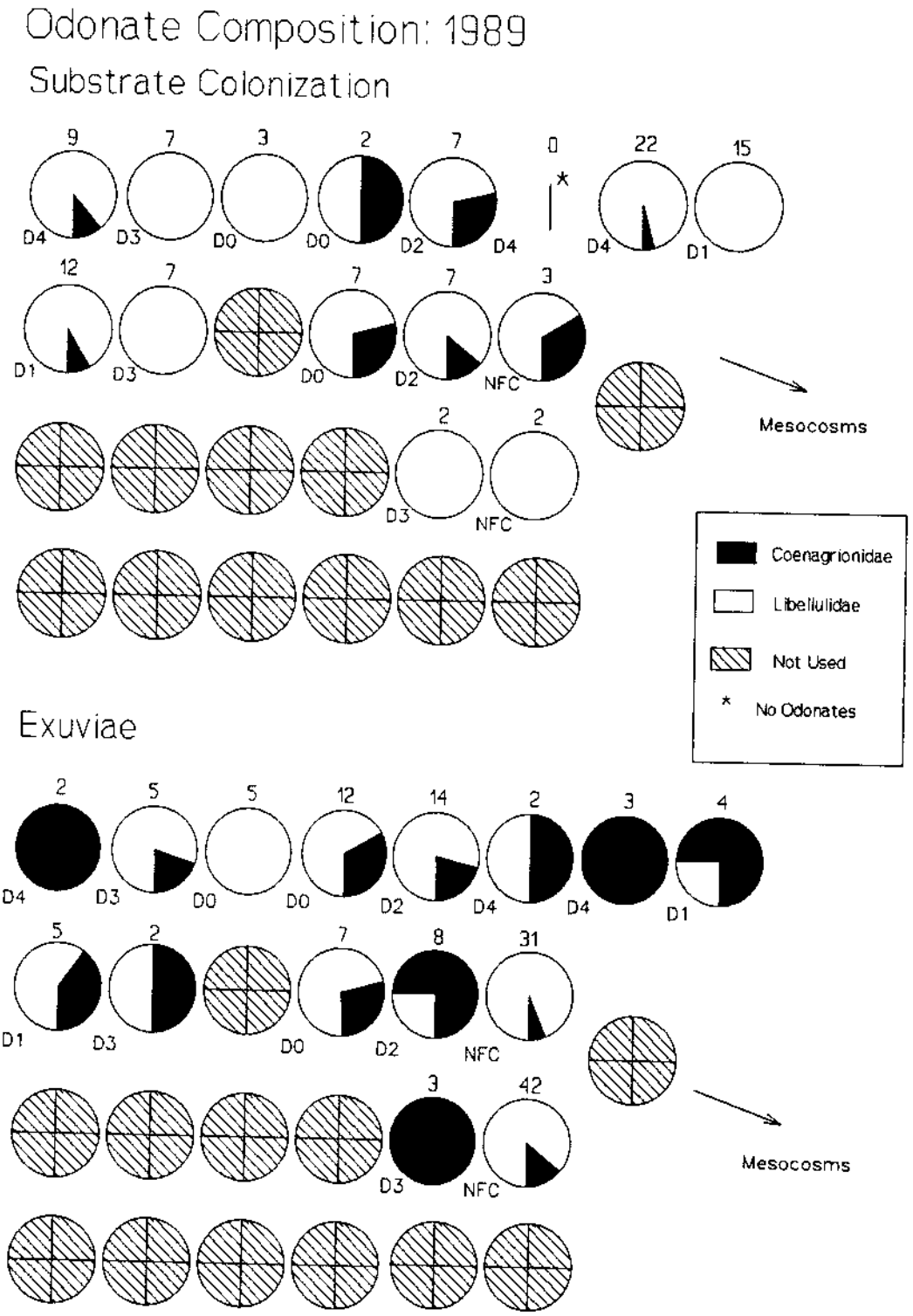

Figure 114. Odonate composition of MASS and exuviae samples from June, 1989. Each microcosm is represented by a pie-chart (percent composition). D0=Control, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4, NFC=Fishless Control.

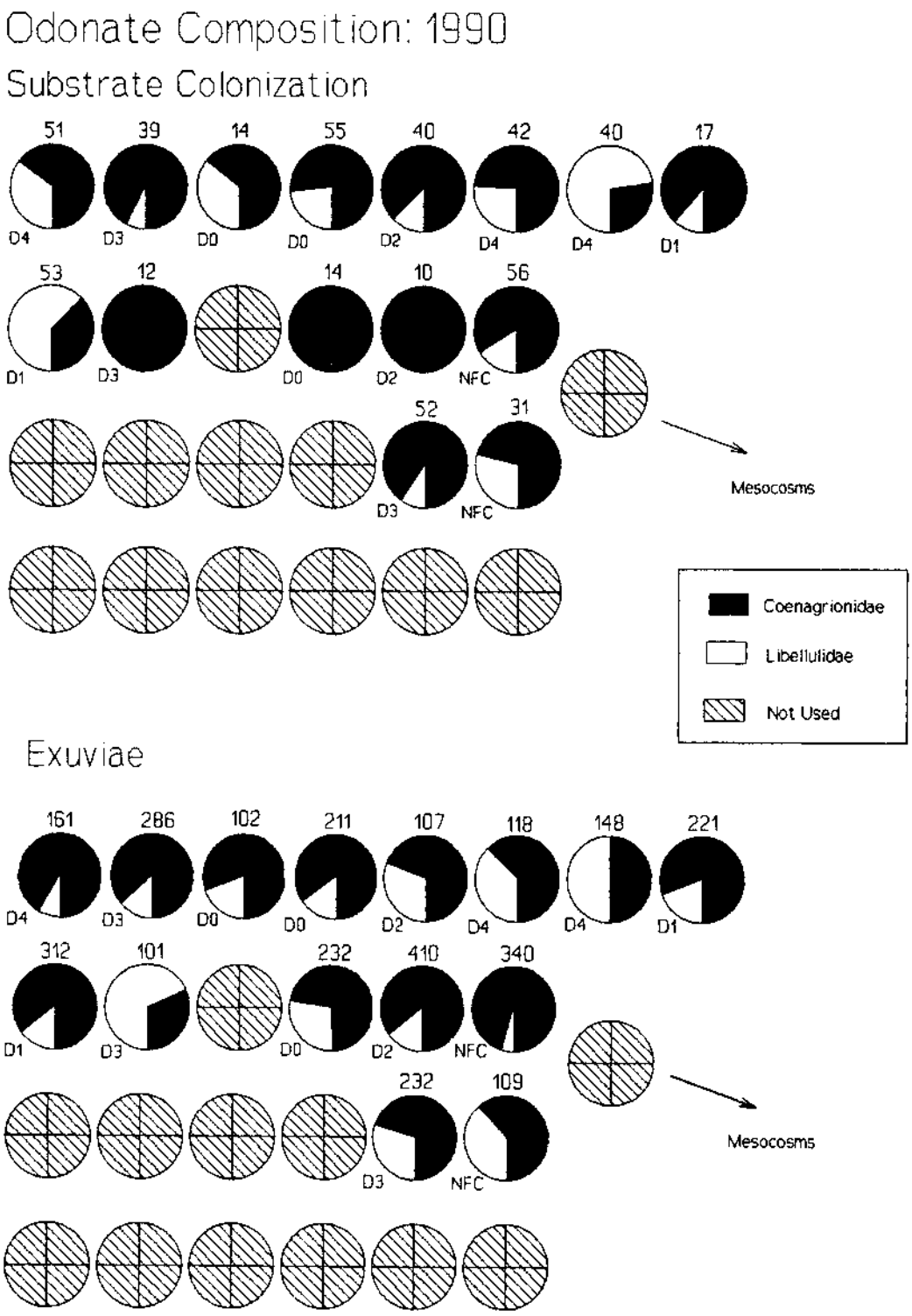

Figure 115. Odonate composition of MASS and exuviae samples from June, 1990. Each microcosms is represented by a pie-chart (percent composition). D0=Control, D1=Dose 1, D2=Dose 2, D3=Dose 3, D4=Dose 4, NFC=Fishless Control.

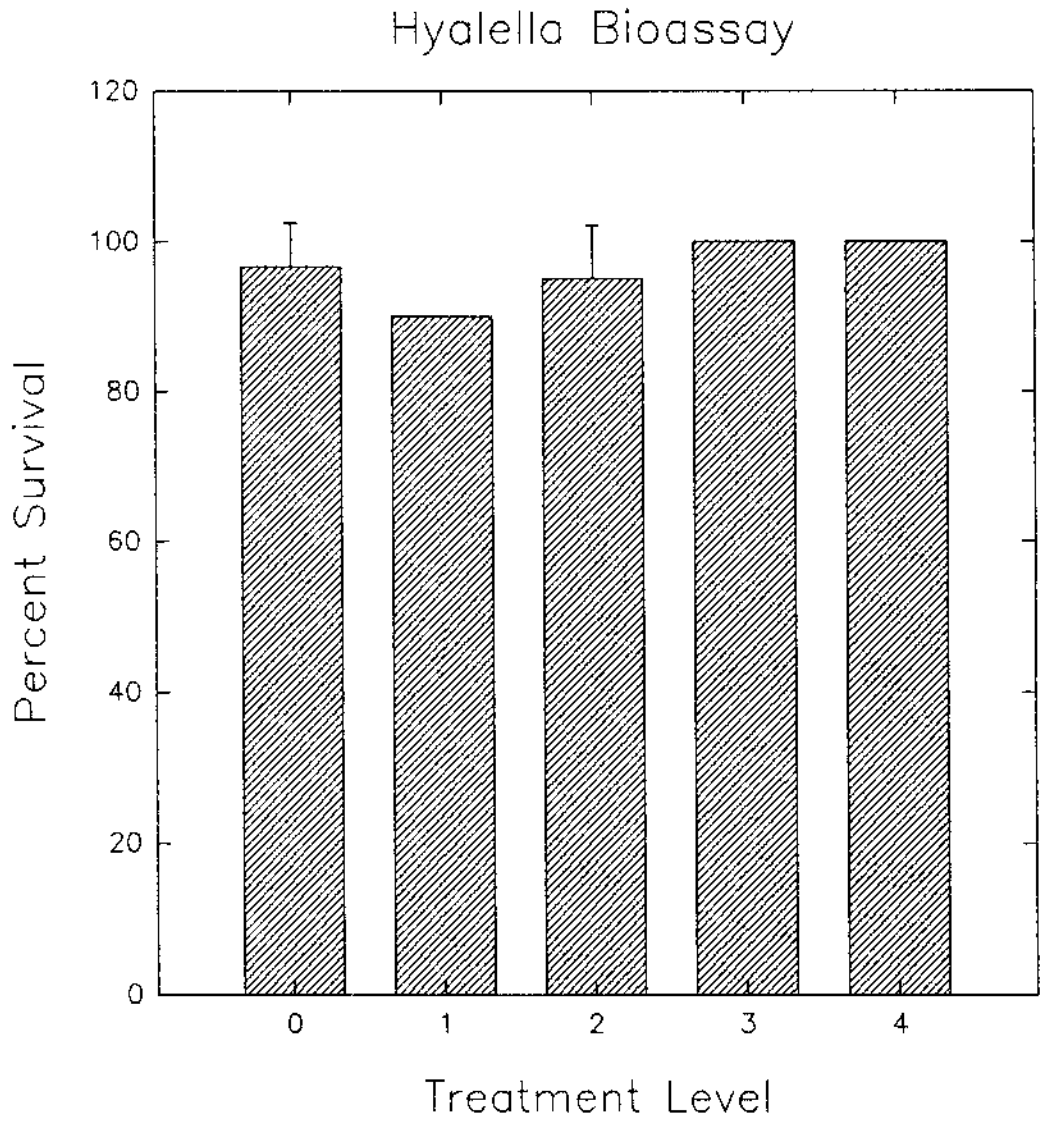

Figure 116. Percent survival (mean ± S.D) of *Hyalella azteca* exposed to microcosm sediments, June 1990. Each *H. azteca* was exposed for seven days in individual beakers containing sediment and lab culture water.

catchment for migrant species (Gore and Milner 1990). Even large ponds within a restricted geographical area may show extreme diverge. Jeffries (1989) noted that a large "element of chance" exists in colonization events. This would be especially true in small microcosms.

Summary

Exuviae and MAS collection did not identify clear residual toxicity of cyfluthrin in microcosms sampled the next summer. *H. azteca* bioassays, combined with sediment residue analysis, suggested that no significant amounts of bioavailable pyrethroid were present in the sediments.

Comparison of microcosm macroinvertebrates in 1989 and 1990 revealed changes in community structure. The 1990 samples were dominated by good colonizers (r-strategists) and fish-intolerant taxa (such as *Chaoborus*). These dramatic shifts could be explained in part by fish removal, but other factors may have been important.

Extreme variability of some taxa (i.e., *Callibaetis* and *Procladius*) suggested that microcosms may hve become quite dissimilar over long time periods, reducing their inferential capability.

CHAPTER 11

CONCLUSIONS

Chemical fate was influenced by system size and application methodology. Sediment residues were higher, and water column half-life was shorter in microcosms. Since at least one other microcosm study has observed the same relationship (higher sediment residues in the microcosms), future testing should further define these scaling relationships.

Characterization of sensitive and insensitive taxa was similar among the two systems. Responses of sensitive populations showed corresponding patterns but differences in absolute numbers among the two systems. These differences in absolute numbers and dominant taxa may be attributable to differences in bluegill predation pressures, habitat (macrophytes) and also may reflect taxa-specific colonization of these systems.

Single-species bioassays were useful in evaluating bioavailability of cyfluthrin in water and sediments, without confounding variables such as predator-prey or competitive interactions. Combining bioassays with field monitoring addresses ecological questions, but also allows

investigation of specific issues such as sediment toxicity or chemical persistence.

It has long been recognized that microcosms would be cost effective models for determining environmental fate of chemicals (Draggan 1976) and that microcosm size is an important variable (Giesy and Allred 1985, Neuhold 1986). This study should contribute to the growing information regarding scaling relationships within ecological systems. These results suggest that microcosms hold considerable promise as a supplement to current methods used for evaluation of pesticide impacts in aquatic systems. While some differences were observed among the two systems, the same sensitive taxa were identified at similar exposure levels in microcosms and mesocosms. The relation of chemical fate to system size deserves greater evaluation in future studies. Smaller scale systems might eventually be used as an intermediate tier in the risk assessment process.

This study suggests that microcosms and mesocosms provide similar effects data, at least for a pyrethroid insecticide. In some instances, microcosm responses were clearer since fish predation impacts were more controlled. Scaling research will undoubtably continue. The real challange will be in "scaling-up" to natural ecosystems.

APPENDIX

Appendix Table 1. Application and sampling schedule for microcosms.

Pre Treatment				Treatment Period										Post Treatment								
Week #	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Microcosm Application Schedule				SD	SD	SD	SD	SD	SD	SD	SD	SD	SD									
				RO		RO		RO		RO		RO										
Res. Water			X	X		X		X		X		X		X		X		X				
Sed. Cores			X		X		X		X		X		X		X		X		X		X	X
Water Chem.		X		X		X		X		X		X		X		X		X		X		X
Zooplankton	X		X	X		X		X		X			X	X		X		X		X		X
Emergence			X		X		X		X		X		X		X		X		X		X	
MAS Samples		X		X		X		X		X		X		X		X		X		X		X
DO/Temp/pH	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Algal Meas.				X		X		X		X		X		X		X		X		X		X

SD = Spray drift pesticide application
RO = Soil runoff pesticide application
Res. Water = Residue samples from water
Sed. Cores = Sediment residues
MAS = Artficial substrates
Emergence = Exuviae and funnel traps
Algal Meas. = Phytoplankton/periphyton

Appendix Table 2. Application and sampling schedule for mesocosms.

Pre Treatment				Treatment Period										Post Treatment								
Week #	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Mesocosm Application Schedule				SD	SD	SD	SD	SD	SD	SD	SD	SD	SD									
				RO		RO		RO		RO		RO										
Res. Water			X	X	X	X	X	X	X	X	X	X	X	X		X		X				
Sed. Cores			X		X		X		X		X		X		X		X		X		X	X
Water Chem.		X		X		X		X		X		X		X		X		X		X		X
Zooplankton	X		X	X		X		X		X		X		X		X		X		X		X
Emergence			X		X		X		X		X		X		X		X		X		X	
MAS Samples		X		X		X		X		X		X		X		X		X		X		X
DO/Temp/pH	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Algal Meas.		X		X		X		X		X		X		X		X		X		X		X

SD = Spray drift pesticide application
RO = Soil runoff pesticide application
Res. Water = Residue samples from water
Sed. Cores = Sediment residues

MAS = Artficial substrates
Emergence = Pyramid emergence traps
Algal Meas. = Phytoplankton/periphyton

Appendix Table 3. Bray-Curtis cluster similarity matrices and associated probabilities generated via bootstrapping. Probabilities are for zooplankton data.

Zooplankton: Week -1 (Figure 32).

Linkage	Clusters	Linked	Similarity	Prob
1	Dose_2	Dose_4	.79376	.88800
2	Dose_1	Dose_2	.74746	.94300
3	Control	Dose_3	.73974	.37900
4	Control	Dose_1	.72221	.95600

Zooplankton: Week 5 (Figure 33).

Linkage	Clusters	Linked	Similarity	Prob
1	Dose_1	Dose_2	.66089	.36700
2	Control	Dose_1	.49626	.30600
3	Control	Dose_3	.42775	.27800
4	Control	Dose_4	.32934	.11800

Zooplankton: Week 10 (Figure 34).

Linkage	Clusters	Linked	Similarity	Prob
1	Dose_2	Dose_3	.80344	.76500
2	Control	Dose_1	.45220	.23600
3	Dose_2	Dose_4	.41278	.42100
4	Control	Dose_2	.10201	.12300

Zooplankton: Week 19 (Figure 35).

Linkage	Clusters	Linked	Similarity	Prob
1	Dose_1	Dose_3	.78617	.76800
2	Dose_1	Dose_2	.75370	.93400
3	Dose_1	Dose_4	.69554	.93300
4	Control	Dose_1	.32449	.16100

Appendix Table 4. Bray-Curtis cluster similarity matrices and associated probabilities generated via bootstrapping. Probabilities are for MASS data (all taxa).

MASS (All): Week -1 (Figure 64).

Linkage	Clusters	Linked	Similarity	Prob
1	Dose_2	Dose_3	.82575	.88100
2	Dose_2	Dose_4	.81343	.96900
3	Control	Dose_2	.77241	.85200
4	Control	Dose_1	.68745	.36500

MASS (All): Week 5 (Figure 65).

Linkage	Clusters	Linked	Similarity	Prob
1	Dose_1	Dose_3	.75601	.70100
2	Dose_2	Dose_4	.64382	.37000
3	Dose_1	Dose_2	.60892	.63900
4	Control	Dose_1	.49284	.20000

MASS (All): Week 9 (Figure 66).

Linkage	Clusters	Linked	Similarity	Prob
1	Dose_2	Dose_3	.82529	.83800
2	Dose_2	Dose_4	.72331	.52300
3	Control	Dose_1	.63509	.37500
4	Control	Dose_2	.48191	.14200

MASS (All): Week 19 (Figure 67).

Linkage	Clusters	Linked	Similarity	Prob
1	Dose_2	Dose_3	.82384	.71300
2	Control	Dose_2	.67561	.28200
3	Control	Dose_4	.59840	.19000
4	Control	Dose_1	.52536	.18200

Appendix Table 5. Bray-Curtis cluster similarity matrices and associated probabilities generated via bootstrapping. Probabilities are for exuviae data (all taxa).

Exuviae (All): Week -1a (Figure 68).

Linkage	Clusters	Linked	Similarity	Prob
1	Control	Dose_2	.80169	.81200
2	Dose_3	Dose_4	.74380	.43800
3	Control	Dose_1	.60467	.35300
4	Control	Dose_3	.53887	.39300

Exuviae (All): Week 6 (Figure 69).

Linkage	Clusters	Linked	Similarity	Prob
1	Dose_2	Dose_3	.56818	.26700
2	Dose_1	Dose_2	.46483	.19100
3	Dose_1	Dose_4	.36646	.16000
4	Control	Dose_1	.26394	.13200

Exuviae (All): Week 10 (Figure 70).

Linkage	Clusters	Linked	Similarity	Prob
1	Dose_2	Dose_4	.62428	.32300
2	Dose_1	Dose_2	.57827	.31800
3	Dose_1	Dose_3	.39770	.17700
4	Control	Dose_1	.34346	.18800

Exuviae (All): Week 18 (Figure 71).

Linkage	Clusters	Linked	Similarity	Prob
1	Dose_2	Dose_3	.57831	.13100
2	Control	Dose_2	.55776	.39300
3	Dose_1	Dose_4	.43902	.14300
4	Control	Dose_1	.39392	.19500

Appendix Table 6. Bray-Curtis cluster similarity matrices and associated probabilities generated via bootstrapping. Probabilities are for MASS data (chironomids only).

MASS (Chironomids): Week -1 (Figure 78).

Linkage	Clusters	Linked	Similarity	Prob	
1	Dose_2	Dose_3	0.71003	0.61900	
2	Control	Dose_1	0.67320	0.50300	
3	Dose_2	Dose_4	0.62755	0.46900	
4	Control	Dose_2	0.56077	0.40600	

MASS (Chironomids): Week 5 (Figure 80).

Linkage	Clusters	Linked	Similarity	Prob	
1	Dose_2	Dose_3	0.79274	0.71700	
2	Dose_2	Dose_4	0.67748	0.59600	
3	Dose_1	Dose_2	0.62528	0.50900	
4	Control	Dose_1	0.32234	0.02300	**

MASS (Chironomids): Week 9 (Figure 82).

Linkage	Clusters	Linked	Similarity	Prob	
1	Dose_2	Dose_3	0.76602	0.71100	
2	Dose_1	Dose_2	0.68292	0.68800	
3	Dose_1	Dose_4	0.59278	0.48200	
4	Control	Dose_1	0.42955	0.05000	**

MASS (Chironomids): Week 19 (Figure 84).

Linkage	Clusters	Linked	Similarity	Prob
1	Control	Dose_2	0.72222	0.37200
2	Control	Dose_1	0.64125	0.42300
3	Control	Dose_4	0.56513	0.42000
4	Control	Dose_3	0.53632	0.41600

** Significant at the 0.05 level of significance.

Appendix Table 7. Bray-Curtis cluster similarity matrices and associated probabilities generated via bootstrapping. Probabilities are for exuviae data (chironomids only).

Exuviae (Chironomids): Week -1a (Figure 79).

Linkage	Clusters	Linked	Similarity	Prob
1	Dose_3	Dose_4	0.73973	0.47800
2	Control	Dose_2	0.70164	0.73300
3	Control	Dose_3	0.56847	0.78400
4	Control	Dose_1	0.46109	0.58700

Exuviae (Chironomids): Week 6 (Figure 81).

Linkage	Clusters	Linked	Similarity	Prob
1	Control	Dose_1	0.54444	0.12700
2	Dose_3	Dose_4	0.50000	0.58300
3	Dose_2	Dose_3	0.31373	0.44300
4	Control	Dose_2	0.17490	0.31800

Exuviae (Chironomids): Week 10 (Figure 83).

Linkage	Clusters	Linked	Similarity	Prob
1	Dose_1	Dose_2	0.63636	0.18600
2	Dose_1	Dose_4	0.38100	0.23100
3	Control	Dose_1	0.23635	0.16300
4	Control	Dose_3	0.18398	0.21000

Exuviae (Chironomids): Week 18 (Figure 85).

Linkage	Clusters	Linked	Similarity	Prob
1	Dose_2	Dose_3	0.55319	0.14100
2	Control	Dose_2	0.53710	0.32400
3	Control	Dose_4	0.40211	0.20300
4	Control	Dose_1	0.36296	0.20800

Appendix Table 8. Bray-Curtis cluster similarity matrices and associated probabilities generated via bootstrapping. Data were collected approximately one year after the initial pyrethroid application.

MASS (All): June, 1990 Recovery Data (Figure 105).

Linkage	Clusters	Linked	Similarity	Prob
1	Control	Dose_4	.90260	.82000
2	Control	Dose_2	.78466	.79800
3	Control	Dose_3	.63333	.63800
4	Control	Dose_1	.43377	.40700

Exuviae (All): June, 1990 Recovery Data (Figure 107).

Linkage	Clusters	Linked	Similarity	Prob
1	Control	Dose_3	.74902	.42200
2	Dose_1	Dose_2	.69478	.36600
3	Control	Dose_4	.64296	.23600
4	Control	Dose_1	.57642	.45400

Appendix Table 9. List of taxa collected from microcosms 1989-1990.

Phylum: Protozoa
Class: Sarcodina
Order: Testacidae
Difflugidae
Difflugia limnetica (Levander, 1902)

Phylum: Platyhelminthes
Class: Turbellaria

Phylum: Rotifera
Class: Monogononta
Order: Ploima
Family: Brachionidae
Anuraeopsis fissa (Gosse, 1851)
Brachionus angularis Gosse, 1851
Brachionus havanaensis Rousselet, 1911
Brachionus quadridentatus Hermann, 1783
Euchlanis Ehrbg., 1832
Platyias patulus (Muller, 1786)
Colurella obtusa (Gosse, 1886)
Lepadella patella (Muller, 1773)
Family: Lecanidae
Lecane flexilis (Gosse, 1886)
Lecane luna (Muller, 1776)
Lecane leontina (Turner, 1892)
Monostyla bulla Gosse, 1886
Monostyla closterocerca Schmarda, 1895
Monostyla hamata Stokes, 1896
Monostyla lunaris (Ehrbg., 1832)
Monostyla quadridentata Ehrbg., 1832
Family: Notommatidae
Cephalodella Bory De St. Vincent, 1826
Family: Trichocercidae
Trichocerca pusilla (Jennings, 1903)
Trichocerca rousseleti (Voigt, 1901)
Family: Synchaetidae
Polyarthra remata Skorikov, 1896

Order: Floscullariacae
Family: Testudinellidae
Filinia longiseta (Ehrbg., 1834)
Testudinella patina (Herman, 1783)
Family: Hexarthridae
Hexarthra mira (Hudson, 1871)
Family: Conochilidae
Conochiloides dossuarius (Hudson, 1885)

Order: Collothecaceae
Family: Collothecidae
Collotheca mutabilis (Hudson, 1885)

Phylum: Nematoda

Phylum: Annelida
Class: Clitella
Subclass: Oligochaeta
Order: Haplotaxida
Family: Naididae
Chaetogaster von Baer, 1827
Dero Oken, 1815
Stylaria Lamarck, 1816

Subclass: Hirudinea

Phylum: Mollusca
Class: Gastropoda
Family: Physidae
Family: Planorbidae

Class: Pelecypoda
Order: Heterodonta
Family: Sphaeriidae

Phylum: Arthropoda
Class: Crustacea
Order: Cladocera
Family: Bosminidae
Bosmina longirostris (Muller, 1785)
Family: Chydoridae
Alona rustica Scott, 1895
Chydorus sphaericus (Muller, 1785)
Pleuroxus denticulatus Birge, 1879
Family: Daphniidae
Ceriodaphnia lacustris Birge, 1883
Scapholebris kingi Sars, 1903
Family: Macrothricidae
Macrothrix rosea (Jurine, 1820)
Family: Sididae
Diaphanosoma brachyurum (Lieven, 1848)
Latonopsis occidentalis Birge, 1891

Order: Copepoda
Suborder: Cyclopoidia
Suborder: Calanoidia
Diaptomus Westwood
Order: Ostracoda

Order: Isopoda
Order: Amphipoda

Class: Arachnoidea
Order: Acarina
Hydracarina

Class: Insecta
Order: Collembola

Order: Ephemeroptera
Family: Baetidae
Callibaetis floridanus Banks, 1900
Family: Caenidae
Caenis Stephens, 1835
Family: Ephemeridae
Hexagenia Walsh, 1863

Order: Odonata
Suborder: Anisoptera
Family: Aeshnidae
Anax Leach, 1815
Family: Gomphidae
Gomphus Leach, 1815
Family: Libellulidae
Celithemis Hagen, 1861
Dythemis fugax Hagen, 1861
Libellula Linnaeus, 1785
Miathyria Kirby, 1889
Pachydiplax longipennis (Burmeister, 1839)
Pantala flavescens (Fabricius, 1798)
Perithemis tenera Say, 1839
Sympetrum Newman, 1833
Tramea Hagen, 1861
Family: Corduliidae
Epicordulia Selys, 1871
Tetragoneuria Hagen, 1861

Suborder: Zygoptera
Family: Coenagrionidae
Enallagma civile (Hagen, 1861)

Order: Hemiptera
Family: Belostomatidae
Family: Corixidae
Family: Gerridae
Family: Hebridae
Family: Macrovelidae
Macrovelia Uhler, 1872
Family: Notonectidae

Family: Veliidae

Order: Coleoptera
Family: Dytiscidae
Copelatus Erichson, 1832
Deronectes Sharp, 1882
Liodessus Guignot, 1939
Laccophilus Leach, 1817
Family: Gyrinidae
Family: Hydrophilidae
Berosus Leach, 1817
Helophorus Fabricius, 1775
Paracymus Thomson, 1867
Tropisternus Solier, 1834
Family: Haliplidae
Haliplus Latreille, 1802
Peltodytes Regimbart, 1878

Order: Trichoptera
Family: Polycentopodidae
Cyrnellus Banks, 1913
Family: Hydroptilidae
Oxyethria Eaton, 1873
Orthotrichia Eaton, 1873
Family: Leptoceridae
Oecetis inconspicua (Walker, 1852)
Triaenodes McLachlan, 1865

Order: Lepidoptera

Order: Hymenoptera
Family: Pomilidae

Order: Diptera
Family: Culicidae
Anopheles Meigen, 1818
Family: Chaoboridae
Chaoborus Lichtenstein, 1800
Family: Chironomidae
Subfamily: Tanypodinae
Tribe: Pentaneurini
Ablabesmyia (*Karelia*) *idei* (Walley, 1925)
Ablabesmyia (*Ablabesmyia*) *mallochi* (Walley, 1925)
Ablabesmyia (*Karelia*) *peleensis* (Walley, 1926)
Labrundinia Fittkau, 1962
Larsia Fittkau, 1962
Tribe: Coelotanypodini
Clinotanypus Kieffer, 1913

Tribe: Macropelopiini
Procladius (*Holotanypus*) Skuse, 1889
Procladius (*Psilotanypus*) *bellus* (Loew, 1866)
Tribe: Tanypodini
Tanypus Meigen, 1803
Subfamily: Chironominae
Tribe: Chironomini
Apedilum elachista Townes, 1945
Chironomus decorus (Johannsen, 1905)
Cladopelma Kieffer, 1921
Cryptochironomus sorex Townes, 1945
Cryptotendipes Lenz, 1941
Dicrotendipes modestus (Say, 1823)
Dicrotendipes incurvus (Sublette, 1964)
Endochironomus nigricans (Johannsen, 1905)
Goeldichironomus holoprasinus (Goeldi, 1905)
Glypotendipes Kieffer, 1913
Lauterborniella Bause, 1913
Microchironomus nigrovittata (Malloch, 1915)
Nimbocerca Reiss, 1972
Parachironomus Lenz, 1921
Parachironomus (*arcuratus* group)
Parachironomus (*varus* group)
Parachironomus (species group *C*)
Polypedilum (*Tripodura*) *digitifer* Townes, 1945
Polypedilum (*Tripodura*) *simulans*
Polypedilum s. str.
Pseudochironomus Malloch, 1915
Zavreliella varipennis (Coquillett, 1902)
Tribe: Tanytarsini
Cladotanytarsus Kieffer, 1921
Paratanytarsus Thienemann & Bause, 1913
Tanytarsus van der Wulp, 1874
Tanytarsus (*elminulus* group)
Tanytarsus (*verralli*, *excavatus*, *recruvatus* groups)
Tanytarsus (*mendax* group)
Tanytarsus allicis Sublette, 1964
Subfamily: Orthocladiinae
Corynoneura Winnertz, 1846
Orthocladius (*Orthocladius*) van der Wulp, 1874
Nanocladius Kieffer, 1913
Parakiefferiella Thienemann, 1936
Family: Ceratopogonidae
Family: Tipulidae
Family: Ephydridae

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