EFFECTS OF AN HERBICIDE ON A PLANKTONIC FOOD WEB

by

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(ABSTRACT)

In situ microcosms of a planktonic community were exposed to the triazine herbicide simazine. Randomly-selected sets of microcosms were collected and sampled each week for three weeks (plus Week 0). Samples of ambient water were collected each week for evaluation of enclosure effects. Physical and chemical parameters were measured per microcosm. Simazine was measured at Weeks 0 and 3 only. The following organisms were preserved and quantified: phytoplankton, bacteria, and zooplankton, including ciliates, copepod nauplii, cladocerans and rotifers.

Simazine decreased dissolved oxygen and pH, but increased nitrate and ammonia concentrations compared to control microcosms. A temporary decrease in temperature occurred at Week 1.

Phytoplankton were differentially affected by simazine. Sensitive taxa included *Trachelomonas*, *Glenodinium*, diatoms and several species of relatively minor significance. *Dinobryon* and miscellaneous coccoids were not significantly affected. Phytoplankton $\geq$9 um were more affected by simazine than phytoplankton <9 um. Many

cells <9 um may be facultative or obligate heterotrophs and not susceptible to simazine. Although data were variable, bacteria were also not affected by phytoplankton changes or simazine.

Rotifers were the major zooplankters and the two dominant species, Kellicottia bostoniensis and Keratella cochlearis, were reported to graze exclusively on cells <9 um. Polyarthra vulgaris and Synchaeta pectinata also graze in this size range but are not limited to it. Copepod nauplii/copepodids were present, but adult copepods and cladocerans were rare. The tintinnid ciliate Codonella exhibited a temporary population increase during the study.

Zooplankton were not affected by simazine-induced changes in the phytoplankton. Kellicottia bostoniensis was the only zooplankter affected by simazine: it had lesser mortality in higher concentrations of simazine. Possible reasons for this enhanced survival were discussed. The zooplankton (primarily rotifers) appeared to feed more on heterotrophic cells than on autotrophic cells, largely as a function of food size, and may have been more closely associated with the detrital food chain than the autotrophic food chain.

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1.0 INTRODUCTION

1.1 Background

Food webs consist of numerous interwoven food chains, which are in turn composed of series of trophic levels (Odum 1971). A species may occupy one or several trophic levels, depending on the predator-prey relationships it has with other organisms present. This is especially true in complex and changing natural systems, such as plankton communities. For example, Bird and Kalff (1986) found the "autotrophic" flagellate Dinobryon to be responsible for more consumption of bacteria in a lake than the microcrustacea, rotifers and ciliates combined. Dinobryon could then be considered as an important component of both the grazing and detritus food chains, depending on whether it was considered autotrophic or bactivorous.

Predator-prey relationships are products of various abilities (attack, capture, ingestion, defense, escape, etc.) and limitations (size, speed, etc.). Direct microscopical observation of planktonic organisms can provide valuable information on predator-prey interactions (e.g., Gilbert and Starkweather 1978, Buikema et al. 1978). However, extrapolation of this information alone to complex natural conditions is difficult. Indirect evidence of planktonic predator-prey relationships can be obtained by: comparisons of predator population numbers and reproduction

with prey densities (Edmondson 1965, Pourriot 1977); radioactively labelled food items (Haney 1971, Gilbert and Bogdan 1981); ingestion of similarly-sized particles (Wilson 1973); and predator exclusion (i.e., Neill and Peacock 1980). Taken collectively, information from these direct and indirect approaches probably indicates true predator-prey relationships. Such collective knowledge can then be used to explain events observed in natural plankton communities.

Many studies of plankton trophic interactions consider the crustaceans (copepods and cladocerans) and fully or partially exclude smaller forms like rotifers and ciliate protozoans. This pattern may be related to traditional plankton sampling methods (Likens and Gilbert 1970, Pace and Orcutt 1981), and perhaps to methods of sample preservation, processing and enumeration.

Planktonic rotifers have been acknowledged to form a significant component of the zooplankton and to be the most important soft-bodied invertebrates of the plankton (Wetzel 1983, Hutchinson 1967). Makarewitz and Likens (1975, 1979) demonstrated that rotifers can be of major importance in energy transfer and nutrient cycling in lakes, due to abundance and rapid reproductive capabilities. For example rotifers, cladocera and copepods of three lakes in the USSR were roughly equivalent in production ($g/m^3/day$), but

rotifers had a biomass turnover time about 10X more rapid than the other two groups (Winberg et al. 1970).

Pace and Orcutt (1981) found protozoans (ciliates and amoebae) to be the most abundant zooplankters in Lake Oglethorpe throughout the year. Rotifers were the next most important group, followed by nauplii and macrozooplankton (copepods and cladocerans).

1.2 Microcosms

Microcosms have been defined as "experimental units designed to contain important components and to exhibit important processes occurring in a whole system" (Draggan 1977). Laboratory microcosms have been used to model ecological processes (Cooke 1977, Leffler 1978); to study responses of complex assemblages of organisms to toxicant stress (Taub et al. 1980); and to model environmental fates of chemicals (Cole and Metcalf 1980).

In situ microcosms can provide realistic information on a natural system because they are subject to the conditions of that system (weather, photoperiod, etc.). Marshall and Mellinger (1978, 1980) used 8 L translucent carboys as in situ microcosms to study the effects of cadmium on natural plankton communities. They found results to be very comparable to experiments using 1.5 X 10^5 L enclosures in the Experimental Lakes Area of Canada.

The use of microcosms can permit replication and enhanced manageability in studies of natural systems. Administration of toxicants to entire lakes or ponds can be avoided and multiple dose levels can be used. Small enclosures have potential liabilities for studying plankton, primarily due to high surface-area-to-volume ratios and lack of mixing (Dudzik et al. 1979, Eppley et al. 1978). Periphyton growth in a bottle will have a greater impact on the enclosed plankton than in a lake or pond and will reduce the ability of the enclosed microcosm to model plankton interactions. Lack of mixing may differentially affect the survivability of enclosed organisms.

1.3 Simazine

Simazine is a triazine herbicide, used for selective weed control in various crops and nonselectively for vegetation control of noncropland (WSSA 1974). Simazine is also registered for the control of aquatic algae and macrophytes (VCES 1984) and has been documented in such usage (Walker 1964, Tucker et al. 1983). Application concentrations recommended by the manufacturer range from 0.5 to 2.5 mg/L.

Simazine may also enter aquatic habitats in ug/L quantities via runoff (Waldron 1974). Simazine is not very soluble in water (5 mg/L at 25 C) but is strongly adsorbed

on soil particles (WSSA 1974). Soluble runoff may be less important than runoff associated with erosion and sediment transport. Simazine appears to be persistent in aquatic systems, especially shallow, well mixed lakes and ponds. Schwartz et al. (1981) administered simazine to Ashurst Lake, AZ (mean depth = 4.1 m) at a concentration of 0.45 mg/L. Concentrations in the water column rapidly decreased to 0.3 mg/L after treatment and remained at or near this level for six months. Simazine was still present at 0.14 mg/L in the water after two years.

Mauck et al. (1976) found a rapid decrease in pond water concentrations of simazine after application ($\geq$50%), but levels of about 10% original concentration were present after 346 days. Mixing of sediments and water by wind action was considered to be responsible for concentrations in the water column. Tucker and Boyd (1981) demonstrated that sediments are the major sink for simazine applied to ponds and that pond water alone (without sediments) maintained high dissolved simazine concentrations for 32 days.

Simazine acts by inhibiting the Hill reaction of photosynthesis (Moreland et al. 1959). Specifically, noncyclic photophosphorylation is blocked by stopping electron flow between Q^- and cytochrome b_{559} (Goodwin and Mercer 1983). This inhibition reduces the production of

oxygen, and photosynthetic carbon assimilation as well (Ashton and Crafts 1979). Strictly autotrophic cells survive only as long as stored energy reserves (starches, oils) support metabolic activities. This action would theoretically affect phytoplankton species differently: obligate autotroph cells might be affected more than facultative or heterotrophic cells. Also, species with large energy stores may survive longer than species with small energy stores.

Four mg/L simazine interfered with Daphnia pulex molting, reduced growth, delayed reproductive maturity, and lowered fecundity in chronic toxicity tests (Fitzmayer et al. 1982). Simazine was reported to have a "very low toxicity" to goldfish, rainbow trout and bluegill sunfish (WSSA 1974).

1.4 Purpose and Hypothesis

The purpose of this study was to illustrate the trophic relationships between zooplankton and phytoplankton after perturbation of the phytoplankton community by simazine.

Hypothesis: Simazine indirectly reduces zooplankton numbers and alters zooplankton composition by reducing densities and altering composition of phytoplankton.

2.0. MATERIALS AND METHODS

2.1 Study Site

This study was conducted in Stout's Pond, a small softwater farm pond in Montgomery Co., VA (W 80 22'30" 654.5 km E, N 37 02'30" 4100 km N). The pond is formed by impoundment of two first order streams on its east side; no other major inflow sources are known. The pond is drained by a standpipe near the earthen dam at the west side. The north and east slopes of the surrounding area are pasture, and the south side is steeply sloped and wooded. The pasture is used for grazing cattle and has been previously fertilized. Cattle were prevented access to the pond by a fence. Stout's Pond was estimated to have 0.31 ha surface area, 260 m shoreline, and 1.2 m mean depth (Taylor 1984). Maximum depth in the present study was about 3 m. The pond has a soft mud bottom with minimal macrophytic growth at the margins.

2.2 Experimental Design

Preliminary experiments were conducted to: a) determine an appropriate dose range, b) work out logistical problems (microcosm depths, flotation method, attachment and removal, etc.), and c) obtain experience with subsampling and analytical procedures (organism identifications, water chemistry, etc.).

A split plot design was used for the experiment with main effects being simazine dose and time. Four dosages were used: 0.0, 0.1, 0.5, and 1.0 mg/L simazine and the experiment was conducted over a 3 week period (December 1-22, 1984). Forty-eight microcosms (sealed, 4 L clear glass bottles) were placed into the pond. The microcosms were closed systems; they received light but did not exchange nutrients or organisms. Three replicate microcosms per dose were randomly selected and removed at Week 0 and each week thereafter. Seven microcosms could not be used in the experiment because of breakage that presumably occurred during Week 1 when the pond was ice-covered. The loss of replicates at certain dose-time combinations made the experiment unbalanced for statistical analyses, e.g., only one replicate was available for 0.5 mg/L simazine at Week 2. Ambient water was also sampled from the pond each time microcosms were collected to account for enclosure effects.

2.3 Container Preparation and Dosing

The bottles (apple cider jugs) were purchased from Murray Cider Co. and had not been used prior to purchase. All bottles were sequentially washed with soap and water, sulfuric acid washed, rinsed in distilled water, acetone rinsed and air dried prior to use.

Princep (Ciba-Geigy) is a commercially available herbicide containing 4 lbs simazine/gal Princep (1 g

simazine/2.1 mL Princep). A stock solution was prepared containing 1 g simazine/L deionized-distilled water. The bottles received appropriate amounts of stock solution prior to being filled with pond water on Day 0 of the field experiment.

2.4 Microcosm Preparation and Placement

Pond water was obtained with an ITT-Jabsco "Water Puppy" self-priming pump, powered by a 12 V marine battery. Pond water was pumped at a rate of approximately 4 L/min through a 1.5 cm diameter garden hose. The hose intake was connected to a t-joint made from pipe fittings; this served to enlarge the intake area and weighted the end of the hose. This collection method may have introduced bias because organisms that are strong swimmers, such as copepods, can avoid currents at the hose intake (Edmondson and Winberg 1971).

Water was pumped from a depth of 20-30 cm into three 20-gal trash cans while slowly rowing about the middle of the pond. The trash cans were lined with plastic bags that had been preleached with tap water for 2 d to reduce the potential for contamination of the sampled water by leachates (plasticizers, etc.). Water in the trash cans was then continuously stirred with an oar and pumped into the bottles at random. Each bottle was filled completely to eliminate air spaces that may trap zooplankton at the

air-water interface and sealed with aluminum foil-lined twist-on caps. Completely filled bottles contained 3.96 L.

Week 0 microcosms were prepared as above. They were not placed in the pond, but were transported directly to the laboratory for subsampling and analysis. All other microcosms were suspended below the surface to a depth of about 25 cm by attachment to a float (Figure 1). Each crossbar passed through a "sandwich," constructed of round plastic electrical outlet covers held tightly together by bolts. A U-bolt through one side of each "sandwich" was slipped onto the metal pipe and each crossbar received two bottles so that it was balanced on the pipe. The finger hole of each bottle was slipped over the end of a crossbar, so that the bottles hung vertically just below the surface of the water. Catch pins through holes in the crossbars prevented bottles from falling off the apparatus.

2.5 Subsampling and Preservation

Dissolved oxygen (DO), pH and temperature were measured in the field. In the laboratory, the microcosms were gently shaken to stir contents, and subsamples were removed for additional water chemistry and simazine analyses. These subsamples were frozen until they could be analyzed.

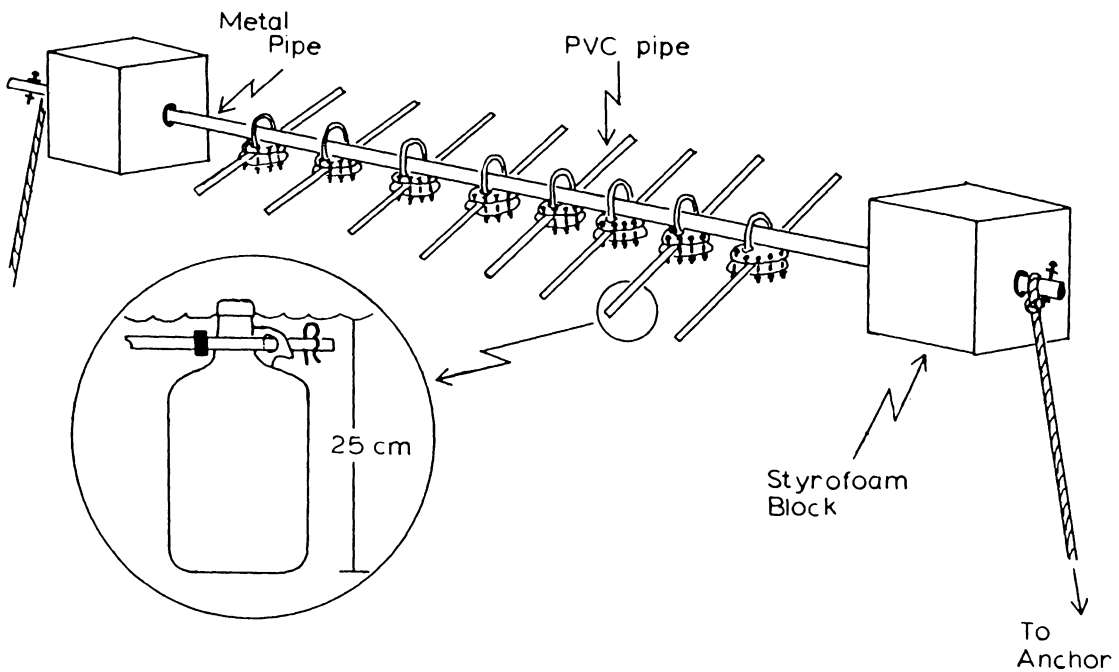

FIGURE 1. Flotation apparatus for microcosms.

Samples of live organisms were examined with a Sedgwick-Rafter counting chamber on a Zeiss binocular microscope at 80 and 200X. This was done to aid in organism identification during quantitative analyses of preserved specimens, and as an attempt to determine relative abundances of organisms, especially those that may rupture, disintegrate or contract upon preservation (e.g., ciliates).

Organisms were then narcotized with club soda and preserved in sodium acetate-buffered 4% formalin (Steedman 1976). The preserved samples (about 3.5 L) were allowed to settle for several days and concentrated by siphoning off the uppermost 2.7 L of water. The remaining 800 mL were then stirred and transferred to 1 L jars. The entire process was repeated with the final transfer of samples to 150 mL jars for storage. Siphoned water was periodically examined; only occasional phytoplankton cells were noted and no zooplankton were lost by this concentration technique.

2.6 Physical & Chemical Measurements

Water Chemistry

The following physical and chemical parameters (excluding simazine) were measured: Secchi depth, temperature, dissolved oxygen (DO), pH, hardness, total alkalinity, conductivity, nitrate, nitrite, ammonia, ortho-

phosphate, and sulfate. The first four parameters were measured in the open water each week; temperature, DO and pH were also measured immediately upon removal of each microcosm from the pond. Temperature and DO were measured with a Yellow Springs Instruments Model 407A oxygen meter (air calibrated) and pH was measured with an Orion Model 54A pH meter (calibrated with pH 7 and 10 standard buffer solutions). Field measurements were made between 10:00 am and 12:00 p.m. each week to minimize diurnal differences in photosynthesis and metabolism and allow for more valid comparisons of data over time.

All other parameters were measured in the laboratory. Because of limited sample volume, titrimetric and colorimetric methods were modified; 25 mL of water sample were used for each analysis of hardness, total alkalinity and conductivity. Five mL were used for analysis of ammonia, nitrite and ortho-phosphate. Calculations and reagent volumes were adjusted accordingly. Hardness and total alkalinity were measured by titration according to Standard Methods (APHA 1984). Conductivity was measured with a Yellow Springs Instruments Model 32 conductance meter and standardized to 25 C (APHA 1984). Nitrate and sulfate were measured by ion exchange chromatography on a Dionex ion chromatograph (Model 14U). Standard solutions were injected after every third sample to determine anion

concentrations. Nitrite, ammonia and ortho-phosphate were measured by colorimetry on a Perkin-Elmer Model 55E spectrophotometer (APHA 1984).

Simazine

Simazine extractions and analyses were conducted in the Pesticide Residue Analysis Laboratory of the Department of Biochemistry and Nutrition, (VPI&SU, Blacksburg, VA). Procedures used by the Pesticide Laboratory for extraction of simazine from soils were modified for use in water samples and are described below. Simazine extractions and analyses were conducted only for samples colllected in the first and last weeks of the study. This was done to verify initial concentrations and to determine the extent of simazine degradation.

Sample containers were wide-mouth glass jars with foil-lined screw caps. Each sample jar was washed with soap and water, rinsed with sulfuric acid, acetone-rinsed and air-dried prior to use. Glassware used in extractions was washed in soap and water, propanol-rinsed, acetone-rinsed and oven-dried.

Two hundred mL water samples for simazine analysis were frozen until they could be analyzed. Frozen samples were thawed, allowed to warm to room temperature, and poured into 500 mL Erlenmeyer flasks. Each original sample jar was rinsed with 25 mL methylene chloride to remove

simazine residue; the rinsate was poured into the water sample. An additional 50 mL methylene chloride was then added to the sample. A small amount of sodium chloride was added to the flask to reduce surface tension of the water, which enhanced mixture of the methylene chloride and water when stirred. The contents of the Erlenmeyer flask were vigorously stirred with Teflon-coated magnetic stir bars on stir plates for 20 min.

Water and methylene chloride were then transferred to a 500 mL separatory funnel. The flask was rinsed with 10 mL methylene chloride, and this rinsate was poured into the separatory funnel. The mixture was allowed to separate and the methylene chloride drawn off into a beaker. An additional 50 mL methylene chloride was added to the separatory funnel and the separatory funnel was vigorously shaken by hand for 5 min. This was again allowed to separate with the methylene chloride drawn off into the same beaker. The water remaining in the separatory funnel was discarded. The methylene chloride extract was then poured through a sodium sulfate column to remove any water. This sodium sulfate column was rinsed with an additional 25 mL methylene chloride to ensure removal of simazine residual from the column.

The methylene chloride extract was evaporated down to approximately 1 mL and 10 mL benzene was added. Benzene

was the preferred final solvent because of its limited interference with sample measurements. The benzene-methylene chloride was then evaporated to approximately 1 mL, and 10 mL benzene again added and evaporated to about 1 mL. The concentrate was transferred into a ground-glass stoppered centrifuge tube. An additional 10-15 mL benzene was used to rinse the beaker and this was added to the centrifuge tube. Upon evaporation to 2 mL, this final concentrate was used in subsequent analyses.

Simazine analyses were conducted on a Camag high performance thin layer chromatograph (HPTLC) with a Spectrophysics 4270 integrator. The TLC plates were prepared with a Camag Nanomat. Machine-integrated peak areas of the chromatograms were used to measure samples with 0.1 mg/L because baseline variability and small peaks made hand-measurement of peak heights inaccurate. Peak heights were used for 0.5 and 1.0 mg/L samples because the larger peaks could be accurately measured against baseline and were more reliable than peak areas. Sample concentrations were calculated against a standard curve.

The standards were generated by pipetting volumes of EPA standard simazine (diluted in benzene) directly into benzene. The accuracy of the sample extraction procedure was assessed by comparison of "spiked" standards against the "pure" standards. "Spiked" standards were prepared by

pipetting Princep into distilled water to make the same target concentrations as used in the study: 0.1, 0.5, and 1.0 mg/L. These solutions were mixed and extracted according to the procedure described above.

2.8 Organism Analyses

Zooplankton

Zooplankton were counted using a Zeiss binocular microscope at 80 and 200X. The preserved sample was thoroughly stirred with a pipette and a one mL aliquot removed with the same pipette. A 5 mm inside-diameter pipette was used to avoid potential clogging with large organisms and differential entry of organisms of different morphologies (Edmondson and Winberg 1971). This aliquot was placed in a Sedgwick-Rafter chamber, the chamber was closed with a coverslip, and the entire contents of the chamber was counted. Only organisms judged to have been alive upon preservation were counted. The basis for this judgment was the presence of intact internal organs, such as the vitellarium, stomach, bladder, etc. Eggs attached to adults and separated eggs were counted. Separated eggs were identified to species by comparison of their size, shape, and morphology with attached eggs.

Three replicate aliquots were counted for each preserved sample, and the mean of these replicates was used in statistical analyses. Total counts of all zooplankton

were used to determine the variability of enumeration. Coefficients of variation (CV) between replicate aliquots of a sample ranged from 2.4 to 13.7%, with a mean of 7.35%. The CV's between replicate microcosms ranged from 0.4 to 25.4%, with a mean of 9.2%.

Densities of each species were calculated and expressed as numbers of organisms, or eggs, per liter. Percent compositions of the dominant rotifer species were calculated from the ratios of each species' densities to total rotifer density. Egg ratios (eggs/female) per species were calculated as the number of eggs/number of adults (females). No male rotifers were observed in any of the samples.

The instantaneous rate of population increase (r), instantaneous birth rate (b), and instantaneous rate of mortality (d) were calculated according to the following equations from Paloheimo (1974):

$$r = \frac{\ln(N_{t1}) - \ln(N_{t2})}{t1 - t2} = b - d$$

$$b = \ln[(C_t/N_t) + 1]/D$$

$$d = b - r$$

where; N_{tx} = population density at time t_x,
C_t = number of eggs at time t,
C_t/N_t = egg ratio (B),
D = egg development time.

The egg development time (D), necessary for calculation of (b), is temperature dependent (Edmondson 1965). Development times per species were calculated using regression data in Edmondson (1965) and the temperature measured at each week of the experiment.

The instantaneous rate of population increase (r) was calculated from the difference between population numbers over a time interval ($t_1 - t_2$) and was reported per time interval. Calculation of (d) for a time interval required an appropriate (b). There are two ways to calculate (b): 1) use of component values (B and D) from the beginning of the interval, with the assumption that the values represent the whole interval, or 2) use of average values, assuming an even change during the interval (Edmondson 1960). Because marked temperature variation occurred during the experiment, the second assumption was considered most valid to calculate (b).

Population parameters related to eggs were not calculated for Synchaeta pectinata. This species rarely carries its eggs (Ruttner-Kolisko 1974), making enumeration of eggs very difficult.

Phytoplankton

Phytoplankton were identified and counted using a Diavert inverted microscope at 400 and 1000X. A preserved sample was thoroughly stirred and a one mL aliquot was

removed with an Eppendorf automatic pipette. The aliquot was placed in a settling chamber and the settling chamber was filled with tap water. A few drops of dilute detergent was added to each chamber upon filling to prevent clumping of cells. Phytoplankton were allowed to settle overnight.

One subsample was counted per sample. A minimum of 30 microscope fields was counted at 400X, and at least 50 fields counted at 1000X for each settled chamber. Only cells judged to have been alive upon preservation (intact cell membrane) were counted. Total numbers of phytoplankton were used to determine variability between replicate microcosms. Coefficients of variation ranged from 2.0 to 39.6%, with a mean of 19.4%. Densities for each taxa were calculated and expressed as number of cells/mL.

Taxa were separated into two size classes: < 9 and ≥ 9 um diameter. The longest axis was used to classify taxa, such as the pennate diatoms, that are not accurately represented by diameter measurements. Nine microns was chosen as the critical size for two reasons. First, various authors (i.e., Naumann 1923, Edmondson 1965, Pourriot 1977) have indicated that the upper size limit of prey items for Kellicottia and Keratella species is about 10 microns and Kellicottia bostoniensis and Keratella cochlearis were the dominant zooplankters in this study.

Second, 9 um was the length of one side of a single Whipple grid square at 1000x on the inverted microscope, as measured by a stage micrometer. The other dominant rotifer species, Polyarthra vulgaris and Synchaeta pectinata are reported to exhibit wider ranges of prey size and type (Pourriot 1977, Edmondson 1965, Hutchinson 1967). It was therefore expected that this size classification might explain any changes in the distribution of available food items for major zooplankters.

Biovolumes of each taxa were estimated using mensuration formulae of solid geometric shapes (Eshbach 1936). Percent biovolumes for each taxa (group) were calculated from the ratios of each taxa (group) biovolume to total algal biovolume. Biovolumes and % biovolumes were also calculated for the size classes by summing the values calculated for each taxa within the size classes.

Bacteria

Bacteria were quantified with a method similar to that of Porter and Feig (1980). The bacteria were stained with 4'6 diamidino-2-phenylindole (DAPI), a DNA-specific stain. After staining, bacteria can be distinguished from detrital material. When excited with light at a wavelength of 365 nm, the DNA-DAPI complex fluoresces bright blue, while DAPI that is not bound to DNA fluoresces yellow.

The sample preparation procedure was as follows. All water used in the procedure was autoclaved, deionized-distilled water. All glassware was acid washed and autoclaved. Nucleopore (NP) filters (pore size = 0.2 um) were soaked for 10 min. or more in a Petri dish containing Irgalan Black (Baker) solution (2 g/L plus 20 mL acetic acid). This provided a dark background for improved visibility of fluorescing cells. The black NP filters were then rinsed in two baths of autoclaved water, placed on a wetted glass fiber filter (for support) and clamped into a small glass filtration unit. A sample was thoroughly stirred with an autoclaved automatic pipette tip and a 1.0 mL subsample was pipetted onto the black NP filter. This was followed by 0.1 mL of a 0.1 ug/mL DAPI (Sigma) solution, for a final concentration of 0.01 ug DAPI/mL sample. The sample-DAPI solution was gently mixed and allowed to stand for 5 min. The solution was then filtered under a 5 psi vacuum. The vacuum was released and 2.0 mL of autoclaved water pipetted onto the NP filter with a another autoclaved pipette tip. This was gently mixed and filtered again at 5 psi vacuum. The filter was removed, air dried, and placed on a thin layer of low-fluorescing immersion oil (Cargille type B) that had been applied to an acetone-rinsed microscope slide. Another drop of immersion oil was placed on top of the filter, followed by a #1

coverslip.

Each prepared slide was examined at 1000X (oil immersion) on an Olympus microscope equipped with a 365 nm wavelength light source. Three replicate slides were prepared for each preserved sample, with 10 randomly selected fields of vision (Whipple grids) counted per slide. The mean of these slide counts was used to represent that sample in analyses. Coefficients of variation between replicate slides (subsamples) of microcosm ranged from 1.3 to 47.1%, with a mean of 22.0%. The CV's between replicate microcosms ranged from 4.3 to 54.6% with a mean of 23.0%.

Bacterial cell densities (cells/mL) were then calculated by relating the volume of sample to the proportion of total filter area counted. No attempt was made to identify bacterial cells, with all types of cells considered equally.

2.9 Statistics

Statistical analyses were conducted using the Statistical Analysis System (SAS) and Sokal and Rohlf (1981). Analyses made with SAS were Analysis of Variance (ANOVA) and Duncan's New Multiple Range Test (Duncan's) (Ray 1982). Comparisons of treatments to controls were made by hand-calculated Dunnett's tests (Steel and Torrie 1960).

3.0 RESULTS

3.1 Physical/Chemical

Eleven physical and chemical parameters were measured in the microcosms, and the Secchi depth was recorded for ambient water each week. Physical/chemical data are presented in Appendix 1. Of the 11 measurements, 6 parameters remained constant with dose and time (Table 1). Five parameters were affected by dosage and/or time: temperature, dissolved oxygen, pH, nitrate and ammonia. Data for these parameters and Secchi depths are presented in Table 2.

Mean water temperature of the microcosms at week 0 was 9.0 C. The temperature of the bottled ambient sample was 2 C higher than ambient. Higher water temperatures in bottles were probably due to exposure to a higher air temperature during microcosms preparation; temperatures were presumed to rapidly return to ambient when placed in the pond.

Temperature differed over time. A sharp drop in temperature occurred at Week 1, to a mean value of 1.5 C (Figure 2). Approximately 10-15 cm of ice was present at this time, with no snow cover. Water temperatures returned to 7.6 and 10.0 C at Weeks 2 and 3, respectively.

The freeze of Week 1 was temporary, but had considerable effect on other parameters. Changes in water

TABLE 1. Physical and Chemical Parameters of Stout's Pond Not Affected by Simazine Dose or Time[a].

Parameters	Mean Value (std. dev.)
Water Hardness (mg/L as CaCO3)	25.1 (0.75)
Total Alkalinity (mg/L as CaCO3)	27.9 (2.45)
Conductivity (micromhos)	68.6 (1.92)
Nitrite (mg/L)	<0.05
ortho-Phosphate (mg/L)	<0.05
Sulfate (mg/L)	1.60 (0.05)

(a) Values <0.05 were below detection limits.

TABLE 2. Physical/Chemical Parameters Affected by Simazine Dosage and/or Time(a).

Week	Dose(b)	Water Temp. (C)	pH	D.O. % Saturation	Nitrate(c)	Ammonia(c)	Secchi Depth (cm)
0	amb	8.2 (---)	7.6 (---)	87.0 (---)	0.24 (----)	<0.05 (----)	46
0	0.0	9.1 (0.2)	7.6 (0.0)	85.7 (0.5)	0.24 (0.01)	<0.05 (----)	
0	0.1	9.3 (0.0)	7.6 (0.0)	87.0 (0.0)	0.25 (0.00)	<0.05 (----)	
0	0.5	9.0 (0.5)	7.5 (0.1)	84.7 (0.5)	0.25 (0.00)	<0.05 (----)	
0	1.0	9.1 (0.2)	7.5 (0.1)	85.3 (0.5)	0.27 (0.00)	<0.05 (----)	
1	amb	2.1 (---)	7.2 (---)	90.0 (---)	0.18 (----)	<0.05 (----)	74
1	0.0	2.2 (0.1)	7.2 (0.2)	95.5 (0.5)	0.06 (----)	<0.05 (----)	
1	0.1	1.4 (0.1)	7.6 (0.1)	93.7 (1.7)	0.07 (0.02)	<0.05 (----)	
1	0.5	1.4 (0.3)	7.3 (0.3)	90.0 (5.7)	0.18 (0.02)	<0.05 (----)	
1	1.0	1.7 (0.3)	7.4 (0.2)	79.5 (0.5)	0.23 (0.01)	<0.05 (----)	

(a) Numbers in parentheses = std. dev. of replicate microcosms per dose per week. (---) indicates no std. dev. was calculated (single sample or below detection limit).
(b) Simazine concentration (mg/L); "amb" = ambient surface water sample.
(c) units = mg/L.

continued-

TABLE 2 (Cont'd). Physical/Chemical Parameters Affected by Simazine Dosage and/or Time(a).

Week	Dose(b)	Water Temp. (C)	pH	D.O. % Saturation	Nitrate(c)	Ammonia(c)	Secchi Depth (cm)
2	amb	8.0 (---)	7.5 (---)	95.0 (---)	0.18 (----)	<0.05 (----)	92
2	0.0	7.7 (0.9)	7.7 (0.1)	87.3 (3.3)	<0.05 (----)	<0.05 (----)	
2	0.1	7.5 (0.5)	7.7 (0.0)	89.0 (9.0)	<0.05 (----)	<0.05 (----)	
2	0.5	7.0 (---)	7.5 (---)	68.0 (---)	0.15 (----)	0.06 (----)	
2	1.0	7.5 (0.5)	7.4 (0.1)	69.0 (1.0)	0.23 (----)	0.06 (0.00)	
3	amb	10.3 (---)	7.3 (---)	88.0 (---)	0.36 (----)	<0.05 (----)	68
3	0.0	9.9 (0.1)	8.4 (0.0)	87.3 (0.9)	0.55 (0.03)	<0.05 (----)	
3	0.1	10.0 (0.0)	8.3 (0.0)	84.0 (1.4)	0.59 (0.24)	<0.05 (----)	
3	0.5	9.8 (0.2)	7.7 (0.0)	68.7 (1.9)	0.70 (0.01)	0.07 (0.01)	
3	1.0	10.0 (0.1)	7.3 (0.0)	54.5 (2.5)	0.97 (0.01)	0.10 (0.01)	

(a) Numbers in parentheses = std. dev. of replicate microcosms per dose per week. (---) indicates no std. dev. was calculated (single sample or below detection limit).
(b) Simazine concentration (mg/L); "amb" = ambient surface water sample.
(c) units = mg/L.

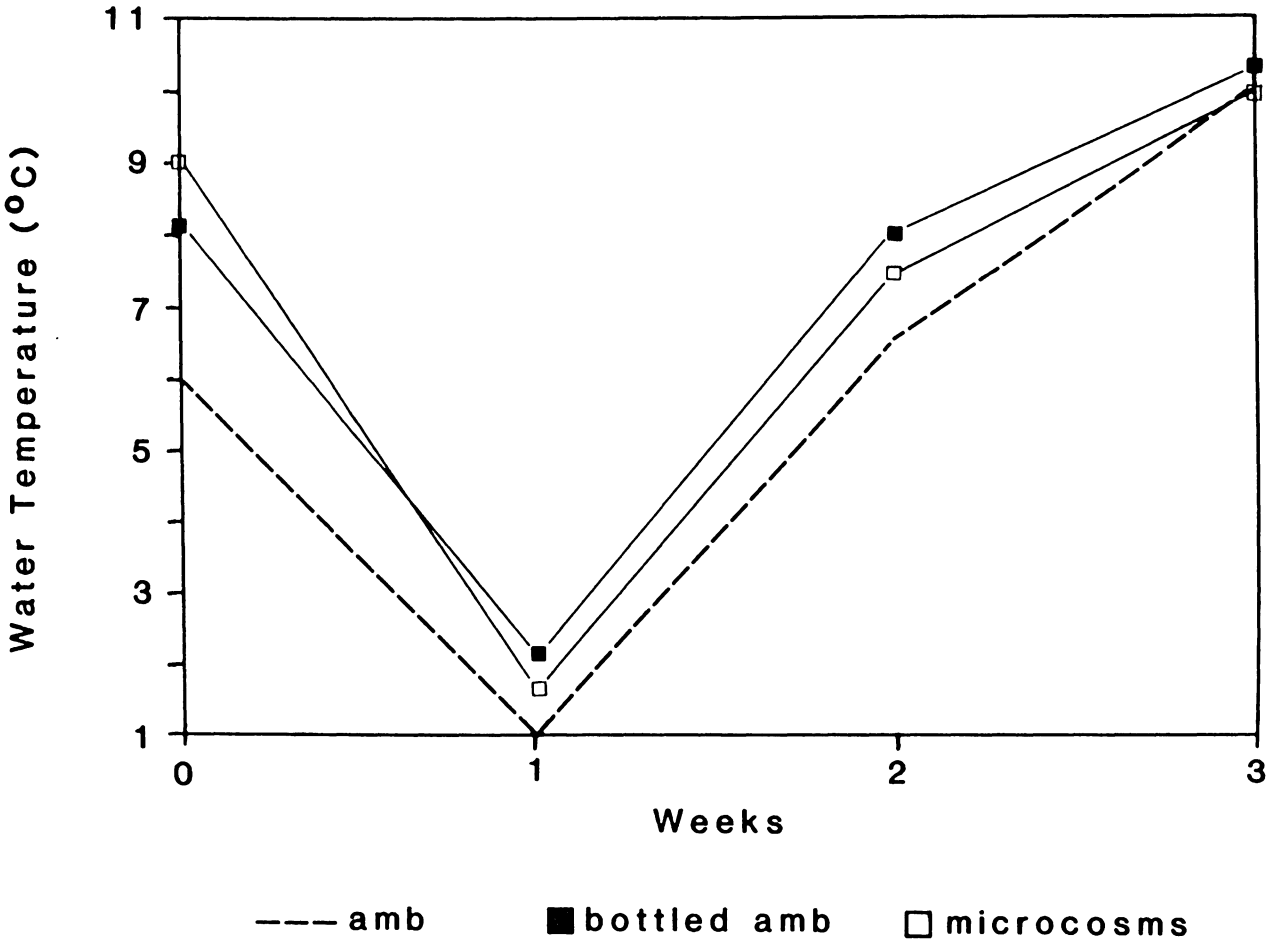

FIGURE 2. Ambient and microcosm mean temperatures

temperature affected the solubility of dissolved oxygen. Dissolved oxygen data were converted to percent saturation to correct for temperature differences between measurements. Control microcosms maintained a $\geq$85% saturation and were similar to ambient conditions (Figure 3). As simazine dose increased, % oxygen saturation decreased over time; the lowest was 55% saturation (6.0 mg/L) for the highest dose at week 3.

A split plot ANOVA indicated that simazine dose affected DO ($p=0.0001$); a Duncan's test indicated that DO was significantly reduced in doses of 0.5 and 1.0 mg/L simazine ($p=0.05$). Comparisons of treated and control microcosms were made with Dunnett's test (Figure 3). These results indicated that there was no enclosure effect on DO but dose affected DO concentration.

It should be noted that 6.0 mg/L DO (70% saturation at 22 C) was measured in Stout's Pond in August, 1984 during a preliminary experiment. The dominant zooplankters in the winter experiment were also present in August, 1984, although in different relative proportions. This indicated that the lowest measured DO in the winter experiment was within the natural tolerance range of the zooplankton and the pond.

The pH of the microcosms was negatively affected by herbicide dose (Figure 4). No significant differences

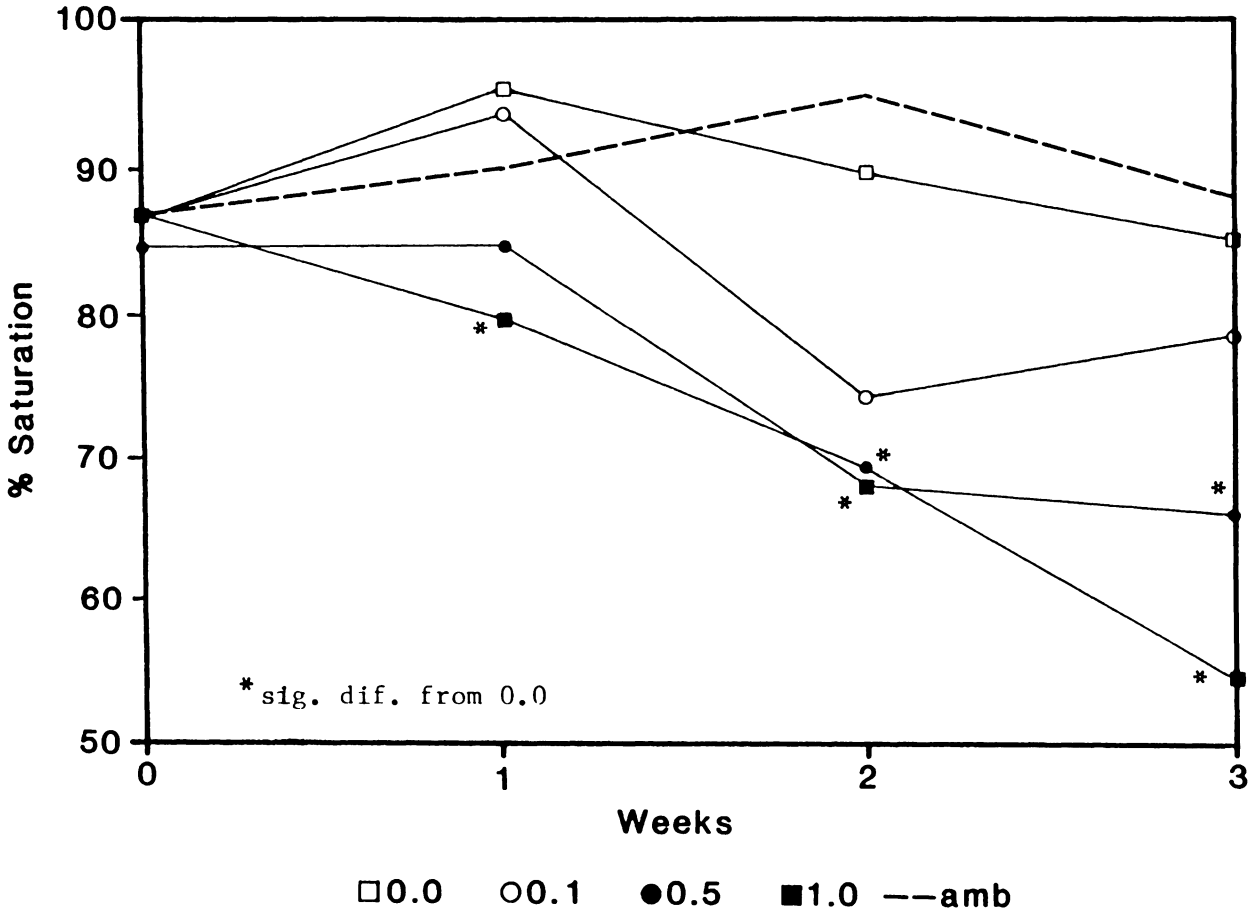

FIGURE 3. Mean dissolved oxygen % saturation per dose

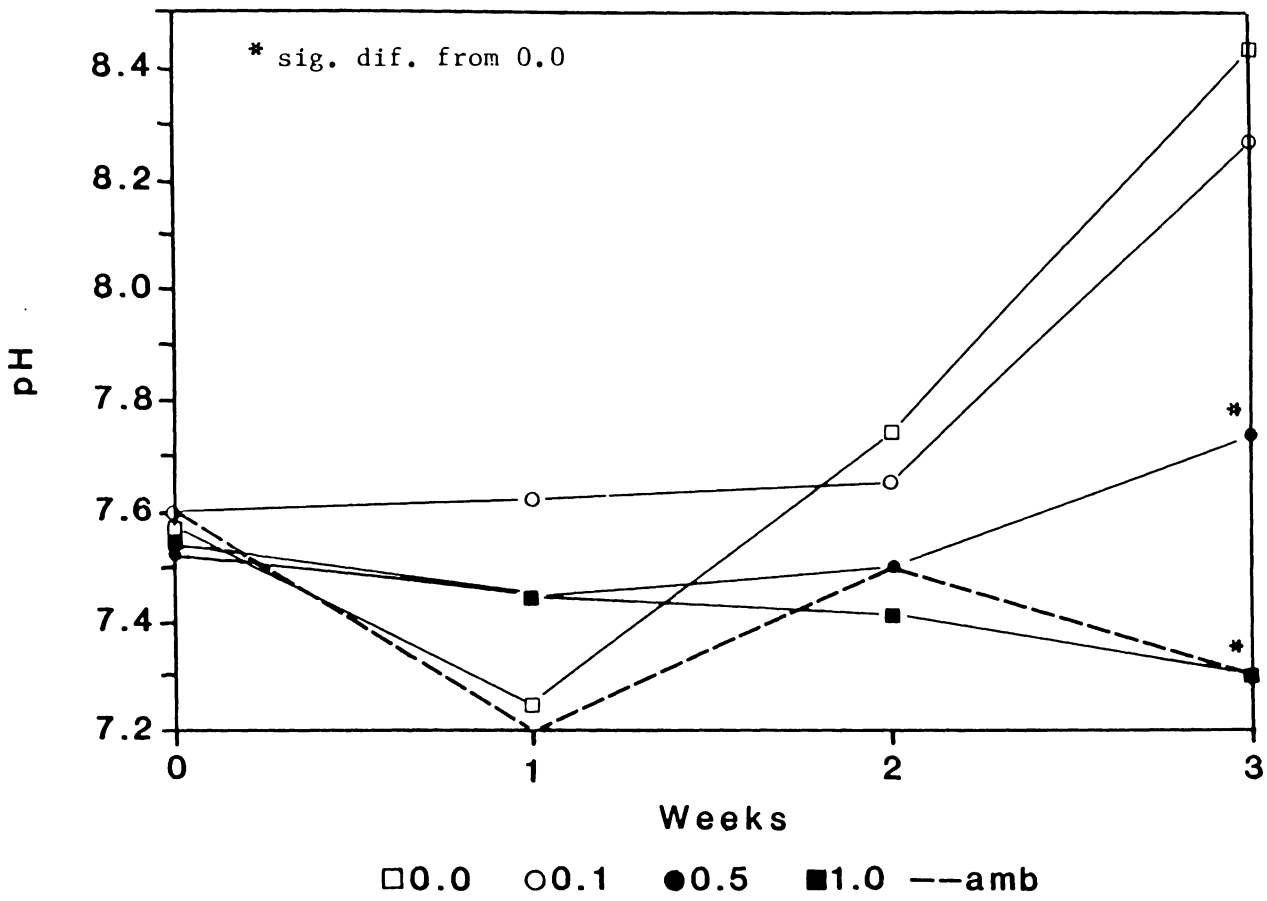

FIGURE 4. Mean pH per dose

occurred between treatments (p=0.05) during Weeks 0-2. At Week 3, pH values were greatly separated in order of dosage, ranging from 8.4 for controls to 7.3 for 1.0 mg/L simazine. Both 0.5 and 1.0 mg/L were significantly different from control pH, and 0.1 mg/L was not significantly different from control (p=0.05).

Ammonia and nitrate were positively affected by simazine dose. Ammonia increased significantly at weeks 2 and 3 for the two highest doses of simazine (Figure 5a). Nitrate levels increased for all dosages at Week 3 (Figure 5b) with 1.0 mg/L the only dosage significantly different from control (p=0.05).

3.2 Simazine

Simazine concentrations in the microcosms did not significantly decrease during the field experiment. Week 0 measured concentrations were not significantly different from Week 3 measured values (p=0.05). Data are presented in Appendix 2. Target simazine concentrations were 0.1, 0.5 and 1.0 mg/L. Corresponding average measured concentrations were 0.096, 0.565 and 1.100 mg/L simazine. Percent recoveries of 0.1, 0.5 and 1.0 mg/L "spiked" standards were 129, 140 and 118%, respectively. These recoveries >100% may have been due to variability in simazine content of Princep or experimental error. No simazine was detected in ambient water samples or control

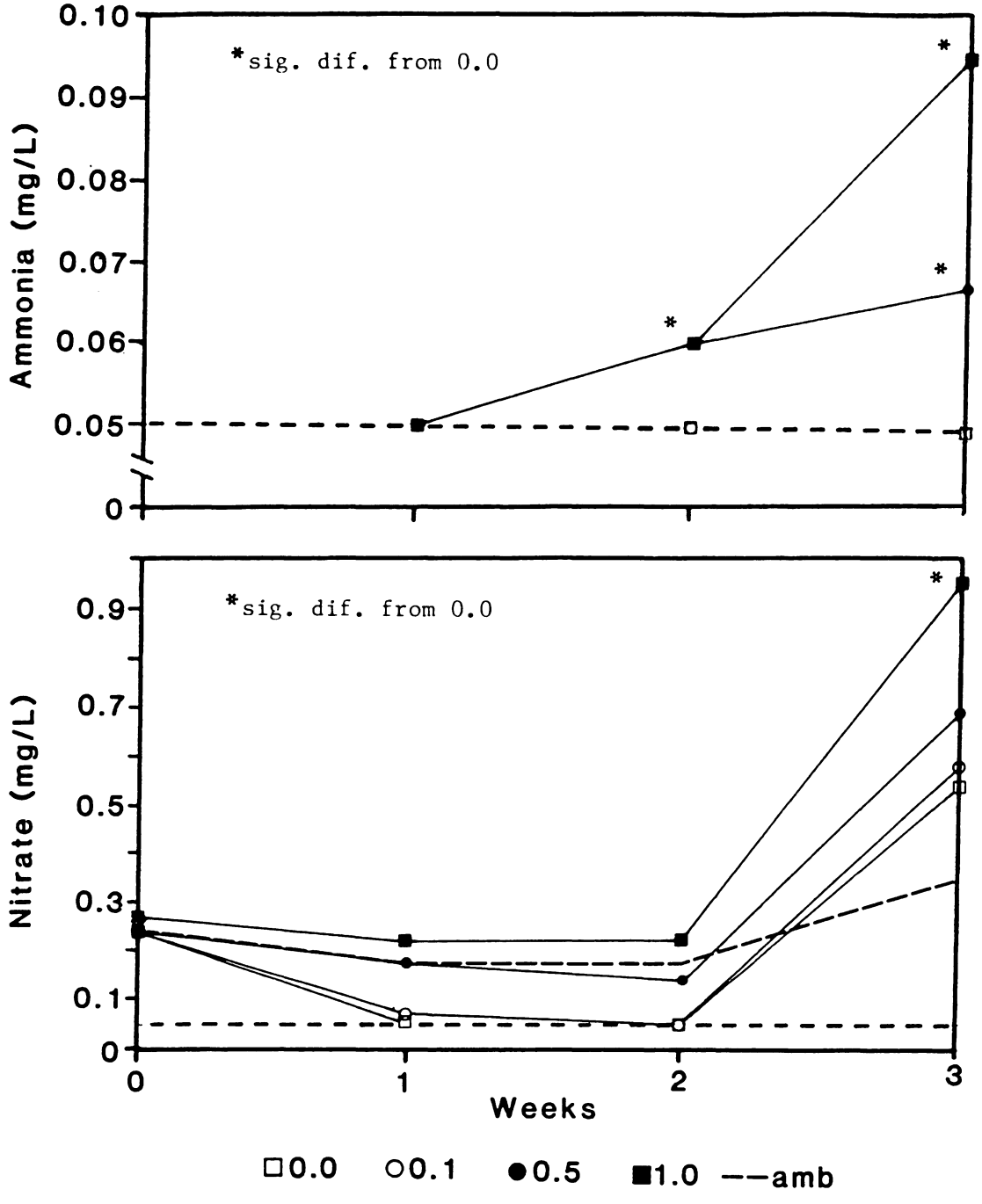

FIGURE 5. Nitrogenous compounds: a) ammonia and b) nitrate mean concentrations per dose

microcosms.

3.3 Phytoplankton

Twenty-five different algal taxa or groups were quantified. The most common taxa/groups, their typical dimensions and size class assignments are shown in Table 3. Rare taxa ($\leq$ 5% of total density or biovolume) were not considered in analyses other than total biovolume. Densities and % biovolumes are presented in Appendix 3.

The group labelled as miscellaneous coccoids included various flagellate and non-flagellate cells that could not be confidently identified to genus. This group was divided into two size classes and counted. All other taxa were also classified by size.

Total algal densities were variable but remained at about the same level through Week 2 (Figure 6a). The only dose that exhibited a net decrease in densities by Week 2 was 1.0 mg/L. Densities in control and 0.1 mg/L microcosms increased at Week 3, while 0.5 and 1.0 mg/L microcosms did not. Time and dose-time interaction were statistically significant (p=0.03, 0.05; respectively), but significant variability between replicate microcosms (p=0.02) for this pooled data made analyses inconclusive.

Total algal biovolume exhibited a variable but general decrease over the course of the experiment (Figure 6b). Week 0 total biovolumes ranged from 9.5 to 15 x 10^6 um^3/mL

TABLE 3. Major Phytoplankton Dimensions, Calculated Volumes (um^3) and Size Class Assignments.

Taxa/Group	Dimensions (WxLxD, um)	Approx. Solid Geometric Shape	Calculated Cell Volume[a]
	<9 um Size Class		
Micractinium	4x4x4	sphere	33
Trachelomonas volvocina	4x4x4	sphere	33
Golenkinia	6x6x6	sphere	113
Misc. coccoid (3-8 um)	6x6x6	sphere	113
Selenastrum minutum	4x4x12	cylinder	150
	>9 um Size Class		
Scenedesmus	3x10x3	ellipsoid	47
Ankistrodesmus falcatus var. mirabilis	1x45x1	cylinder	140
Small Diatoms	1x60x1	cylinder	200
Misc. coccoids (9-15 um)	12x12x12	sphere	905
Sm. Trachelomonas spp.	12x16x12	ellipsoid	1200
Ankistrodesmus falcatus	4x140x4	cylinder	1750
Synedra (diatom)	4x150x4	cylinder	1900
Dinobryon spp.	14x40x1	cylinder	2050
Trachelomonas spp.	24x30x24	ellipsoid	9000
Glenodinium	30x30x32	sphere	15000

(a) Cell volumes calculated using mensuration formulae of approx. solid geometric shapes (Eshbach 1936).

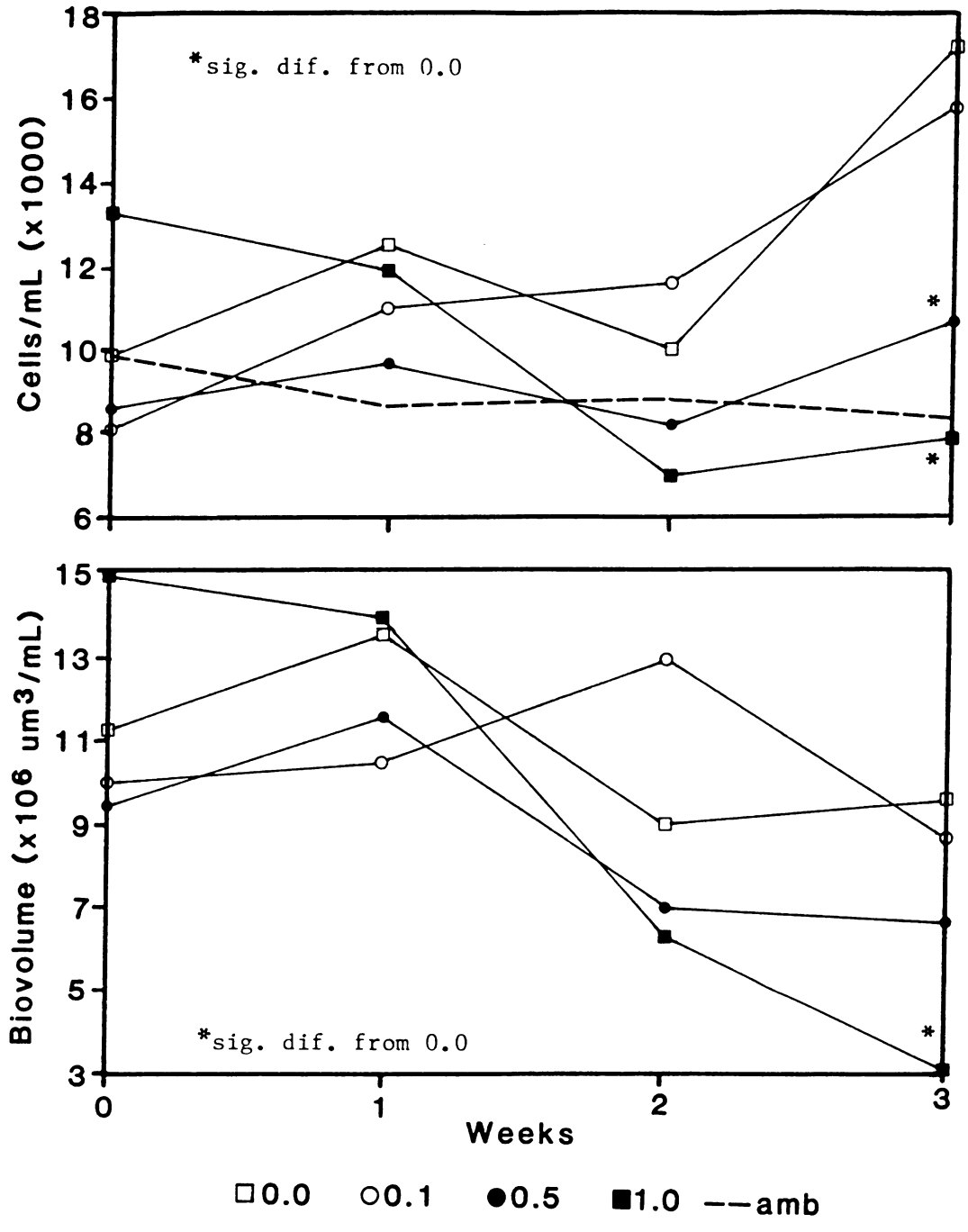

FIGURE 6. Total phytoplankton a) mean densities and b) mean biovolumes per dose

and week 3 values ranged from 3 to 9.5 x 10^6 um^3/mL, depending on dose. Time and dose-time interaction were highly significant (p=0.0004 and 0.02, respectively). The only mean value significantly different from control was 1.0 mg/L simazine at Week 3 (p=0.05). The amount of decrease appeared to be dose-related. Control and 0.1 mg/L microcosms exhibited little or no decline in total algal biovolume, 0.5 mg/L microcosms were intermediate, and 1.0 gm/L microcosms showed a five-fold decrease from Week 0 to Week 3.

Dinobryon spp. was the dominant member of the Week 0 phytoplankton assemblage (approx. 3,000/mL and 55% of total algal biovolume). Dinobryon mean densities and mean % biovolumes for 1.0 mg/L simazine at Weeks 0 and 1 appeared to be higher than other values but were not significantly different from controls, nor were dose or dose-time interaction significant (Figure 7a).

Because no dose-related differences were identified in Dinobryon densities, the statistical significance of dose for Dinobryon % biovolume (p=0.003) must be attributed to a simazine effect on other taxa (Figure 7b). The apparent insensitivity of Dinobryon to these simazine concentrations permitted it to comprise a greater proportion of total algal biovolume when other taxa were negatively affected.

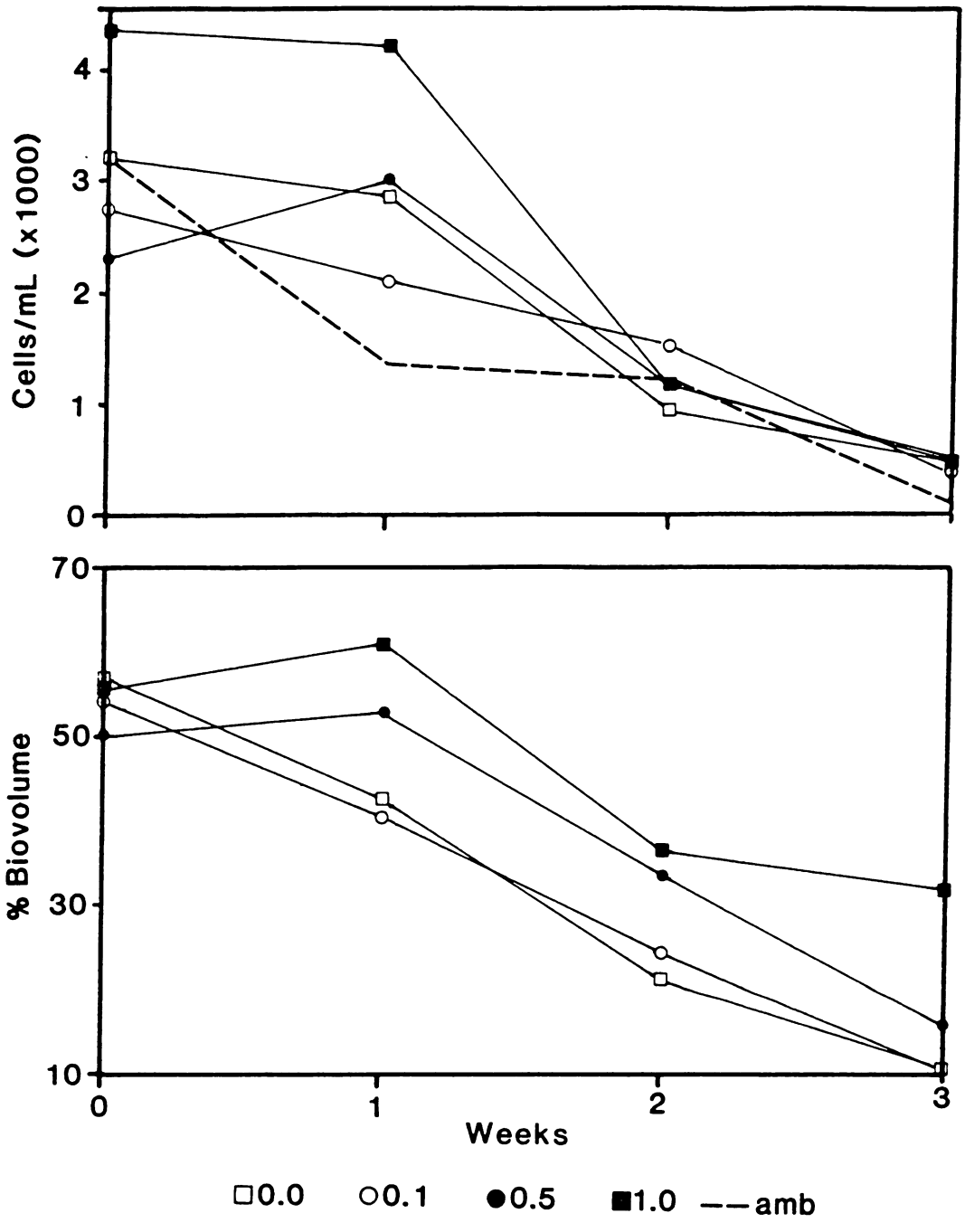

FIGURE 7. Dinobryon a) mean densities and b) mean % biovolumes per dose

Densities of the dinoflagellate Glenodinium were negatively affected by dose and had a significant dose-time interaction (p=0.03 and 0.02, respectively). Glenodinium densities increased in ambient samples and 0.0 and 0.1 mg/L microcosms over time, but decreased in 0.5 and 1.0 mg/L microcosms (Figure 8a). The values of 1.0 mg/L densities for Weeks 2 and 3 were significantly different from controls (p=0.05). The same basic trends were found in % biovolumes (Figure 8b); dose and dose-time interaction were highly significant (p=0.0009, p=0.0002, respectively). Glenodinium was sensitive to the higher concentrations of simazine and the dose-related changes in Glenodinium densities were the primary determinants of its percent biovolume changes. In addition, enclosure may have limited the natural increase in Glenodinium densities at the end of the study.

Trachelomonas densities were not significantly affected by simazine dose or dose-time interaction, but were affected by time (p=0.05). The only mean density value that was significantly different from control was that of 1.0 mg/L at Week 3 (p=0.05); this density was similar to the ambient density at Week 3 (Figure 9a). Trachelomonas may have been affected by 1.0 mg/L simazine at the end of the study. The percent biovolume of Trachelomonas was not related to dose, but was dependent on

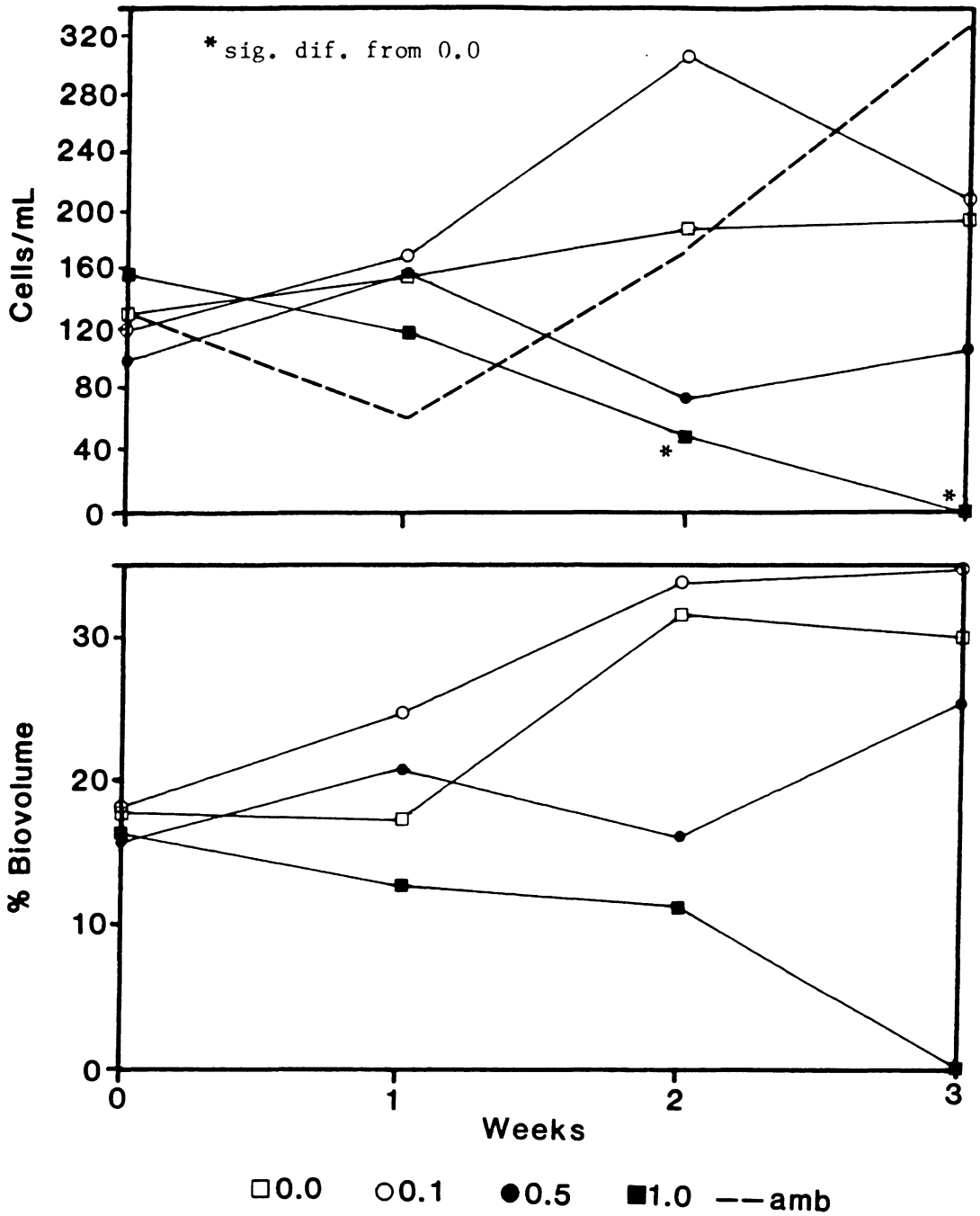

FIGURE 8. Glenodinium a) mean densities and b) mean % biovolumes per dose

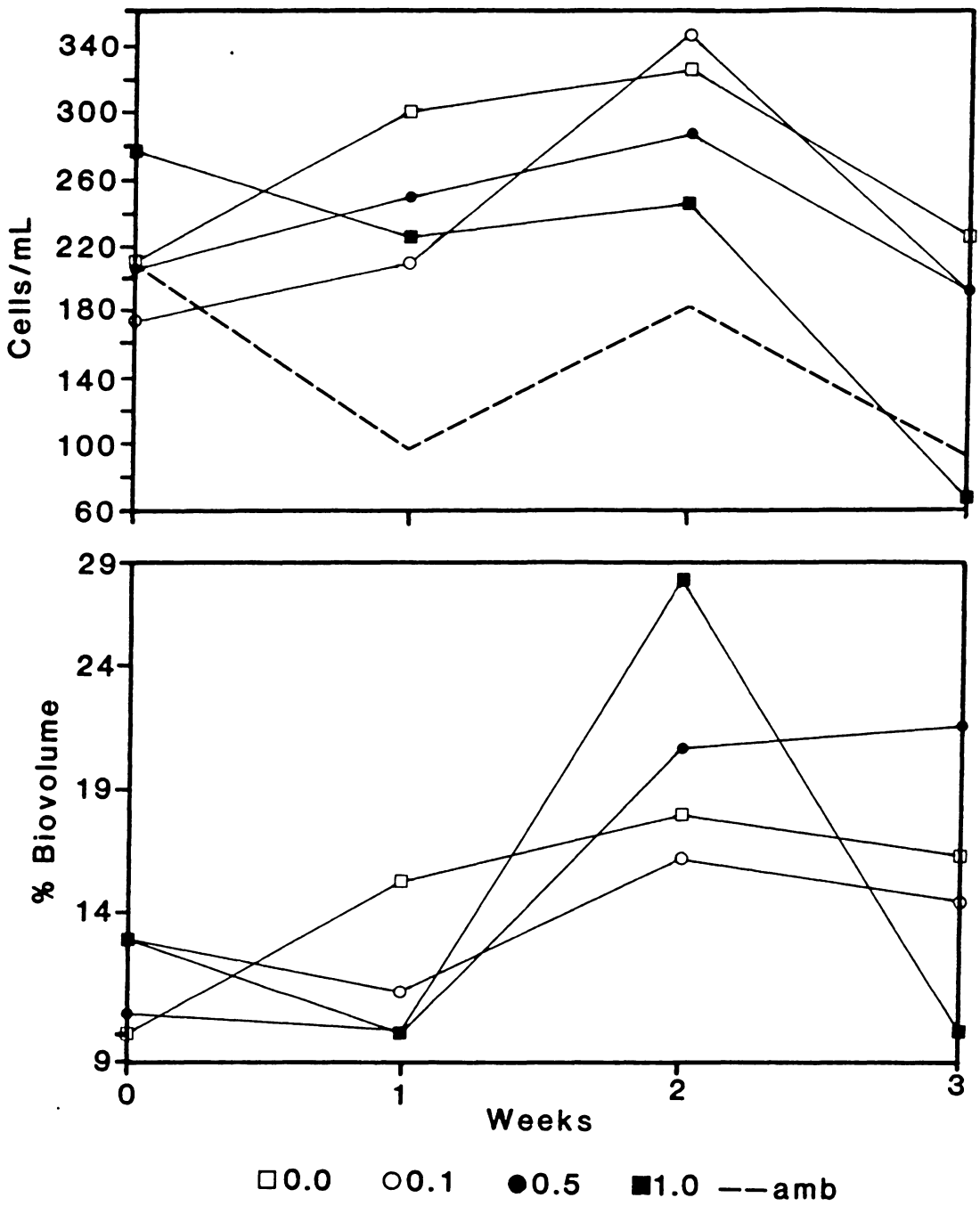

FIGURE 9. Trachelomonas a) mean densities and b) mean % biovolumes per dose

time (p=0.02) and dose-time interaction (p=0.04). Although trends were variable and difficult to interpret (Figure 9b), the peak % biovolume for 1.0 mg/L at Week 2 was probably related to the coinciding decrease in other taxa with the increase in Trachelomonas.

Densities of miscellaneous coccoids (9-15 um) were probably affected by time (p=0.06), with a slight net decrease in densities between Weeks 0 and 3 (Figure 10a). Although not statistically significant, 0.5 and 1.0 mg/L microcosms appeared to have depressed densities relative to 0.0 and 0.1 mg/L microcosms at Weeks 1 and 2. Percent biovolumes of 9-15 um miscellaneous coccoids were not statistically related to any effects and were not statistically different from controls at any week.

Densities of miscellaneous coccoids (3-8 um) had a marginally significant dose-time interaction (p=0.09), but no mean values were different from controls (p=0.05). The dose-time interaction is evident in Figure 10b. Mean densities in 0.5 and 1.0 mg/L microcosms were lower than 0.0 and 0.1 mg/L densities by Weeks 2 and 3. These small miscellaneous coccoids may have been negatively affected by herbicide dose, especially after 1-2 weeks. The data for % biovolume of the 3-8 um cells exhibited a very strong relationship with time (p=0.0002), but not with dose or dose-time interaction. Mean values showed a generally

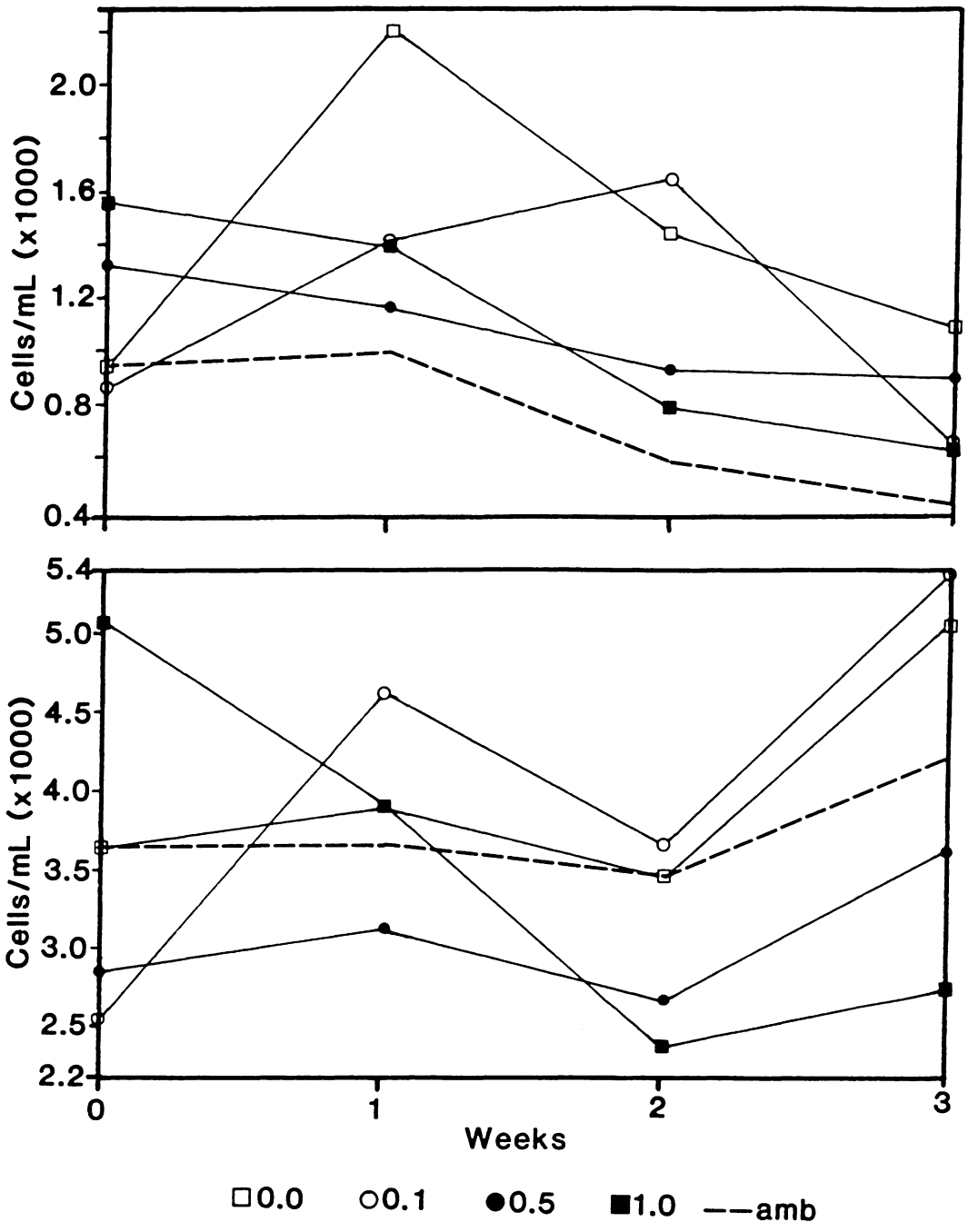

FIGURE 10. Miscellaneous coccoids mean densities a) cells 9-15 um and b) cells 3-8 um

positive trend with time, increasing from 3-4% to 6-10% biovolume. This trend was apparently related to changes in other taxa (e.g., Dinobryon) rather than direct effects.

Several genera of diatoms were present and divided into two size ranges (50-80 and about 150 um length). The smaller group consisted of a smaller Synedra species and a Nitzchia species. Naviculoid, Asterionella and Gomphonema cells were occasionally observed, but were not quantified separately. These genera may have been included in the 50-80 um group when encountered. The larger size group was composed solely of a second Synedra species.

Figure 11a shows mean densities over time per dosage for the smaller diatoms. Dose was not a significant factor ($p=0.11$) but time and dose-time interaction were significant ($p= 0.0001$ and 0.06, respectively). Densities of small diatoms in control and low dose microcosms increased sharply between Weeks 2 and 3. The two highest herbicide doses significantly impaired this bloom of small diatoms at Week 3 ($p=0.05$), so that the density for 1.0 mg/L microcosms closely resembled ambient density.

The dose effect did not carry over to effects on % biovolumes of the small diatoms (Figure 11b). This was probably because other taxa (e.g., Glenodinium) were more severely affected by the simazine, while the diatoms in 0.5 and 1.0 mg/L simazine maintained about the same densities,

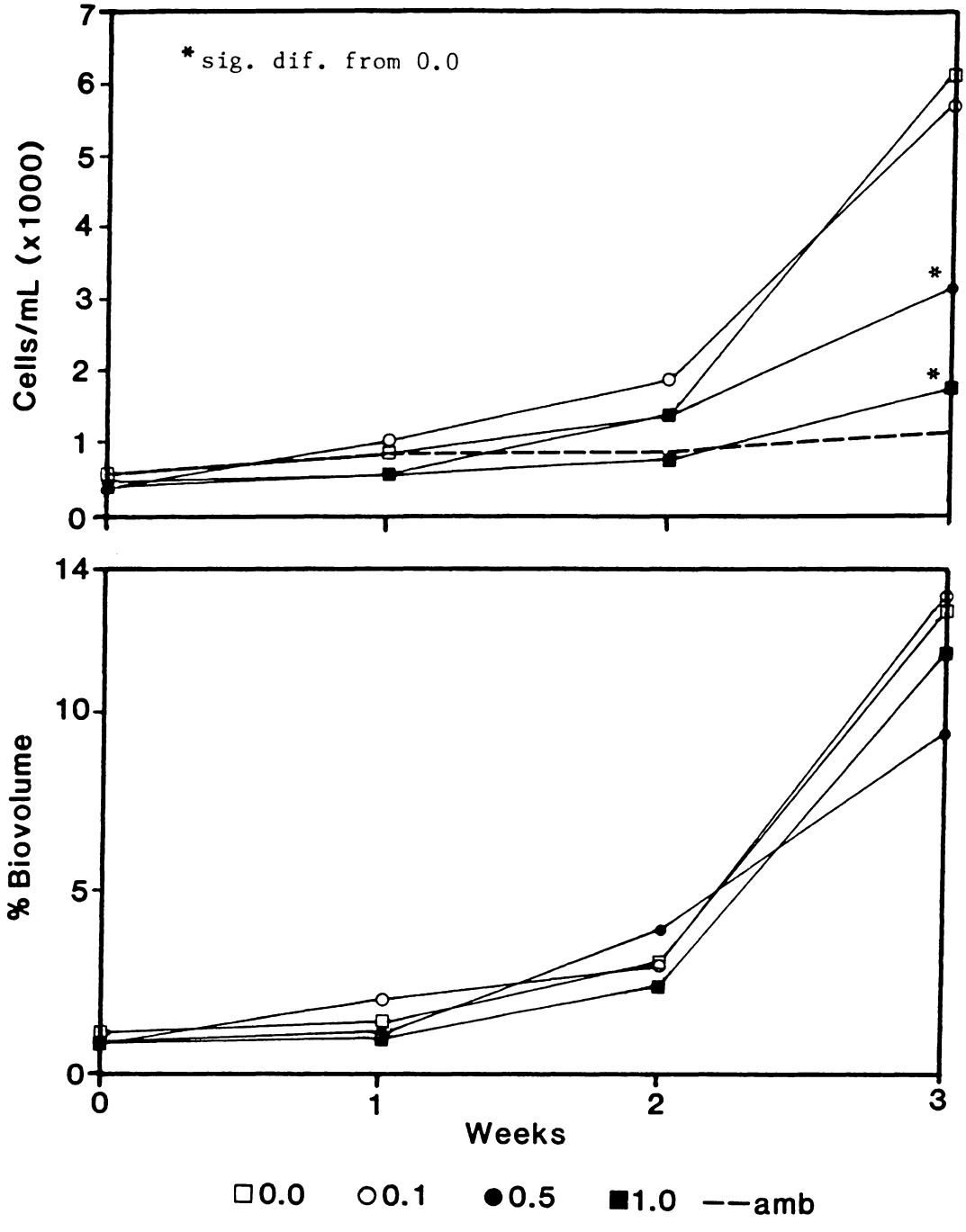

FIGURE 11. Diatoms (50-80 um) a) mean densities and b) mean % biovolumes per dose

or increased slightly, throughout the experiment (Figure 11a). Overall, % biovolumes of small diatoms increased from about 1% at Week 0 to 9-13% at Week 3.

The largest diatoms showed a trend similar to that of small diatoms (Figure 12a), although dose, time and dose-time interaction were all statistically significant ($p=0.01$). Substantial effects of doses also occurred at Week 3; all three doses were significantly different from control ($p=0.05$). The % biovolumes of the largest diatoms exhibited a dose effect similar to that of densities (Figure 12b). Control % biovolume was 7% at Week 3, while in 1.0 mg/L simazine % biovolume was about 3.5%.

The largest diatoms appeared to be more sensitive to 0.1 mg/L simazine than the small diatoms, but the higher doses similarly affected the two size classes. The largest diatoms were relatively rare, but comprised up to 7% of total algal biovolume due to their size. In combination, both size classes of diatoms comprised only about 1% of total algal biovolume at Week 1, while they ranged from 13 to 20% at Week 3.

Due to the difficulty in relating trends of numerous individual phytoplankton taxa to trends of zooplankton species, the phytoplankton taxa/groups were combined into two size classes: $\geq$ 9 um and < 9 um (see Table 3). Total algal biovolume was also analyzed with combined data.

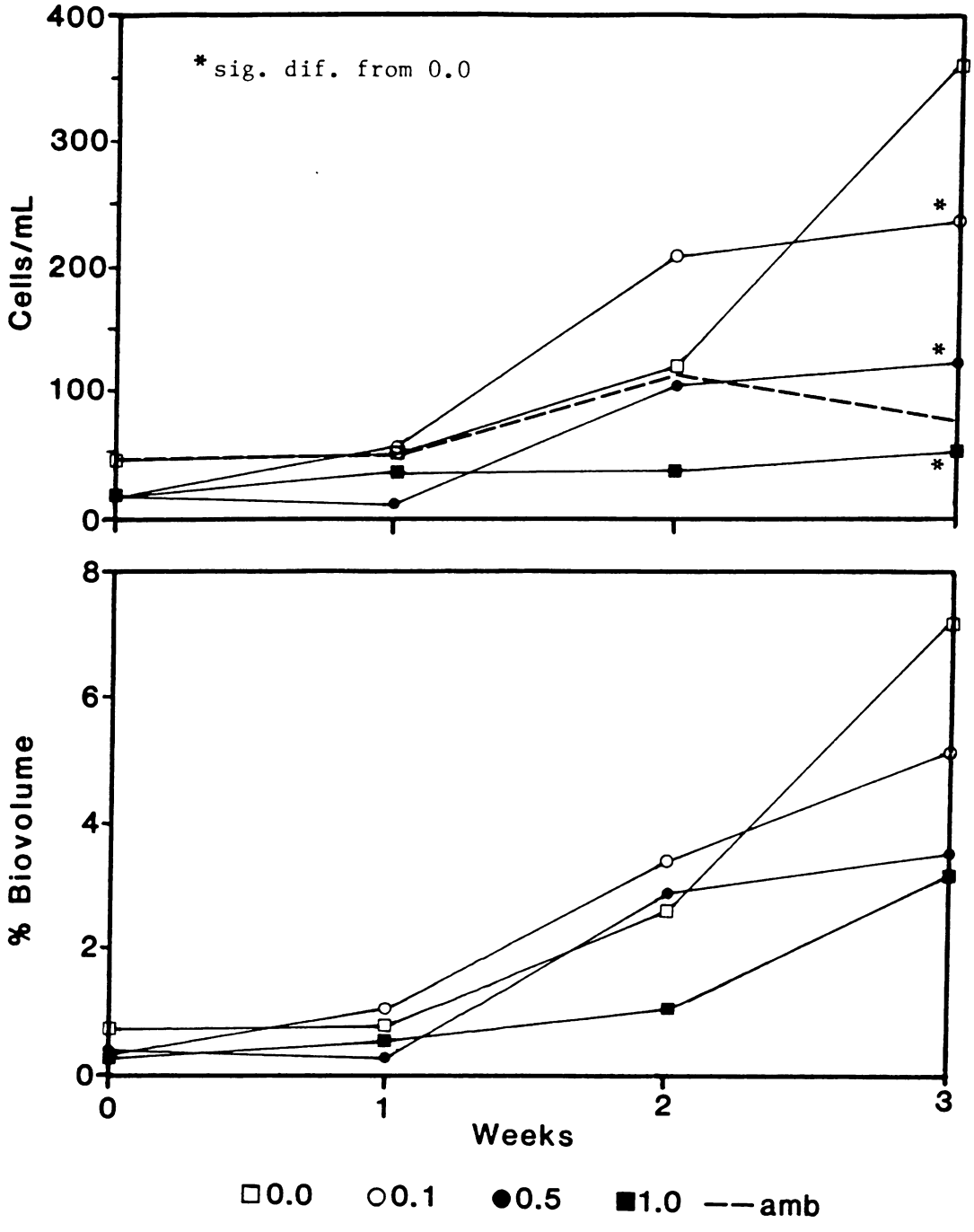

FIGURE 12. Diatoms (150 um) a) mean densities and b) mean % biovolumes per dose

All taxa/groups included in the larger size class were chlorophyllous, with the possible exception of some members of the 9-15 um miscellaneous coccoids. This group of coccoid cells probably included some colorless flagellates, capable of heterotrophic metabolism. The smaller size class includes relatively minor chlorophyllous taxa (Selenastrum minutum, Golenkinia, Micractinium) and the 3-8 um miscellaneous coccoids. It is also likely that these coccoid cells included non-pigmented flagellates.

Densities of phytoplankton $\geq$9 um were significantly related to time and dose-time interaction ($p=0.02$ and 0.04, respectively). An overall dose effect was not detected ($p=0.69$) for these pooled data because of the significant variance contributed by dose replicates ($p=0.02$). The dose-time interaction can be seen in Figure 13a. Simazine dosage negatively affected densities of the $\geq$9 um size class, but not until Weeks 2-3. The densities of phytoplankton $\geq$9 um in microcosms with 0.5 and 1.0 mg/L simazine were significantly less than control microcosms at Week 3 ($p=0.05$).

The densities of cells $\geq$9 um in control and low dose microcosms increased between Weeks 2 and 3. This may have been due to a diatom bloom at that time. The difference between magnitudes of the diatom increase (4-5,000 cells/mL) and the $\geq$9 um size class increase (2,5000-4,000

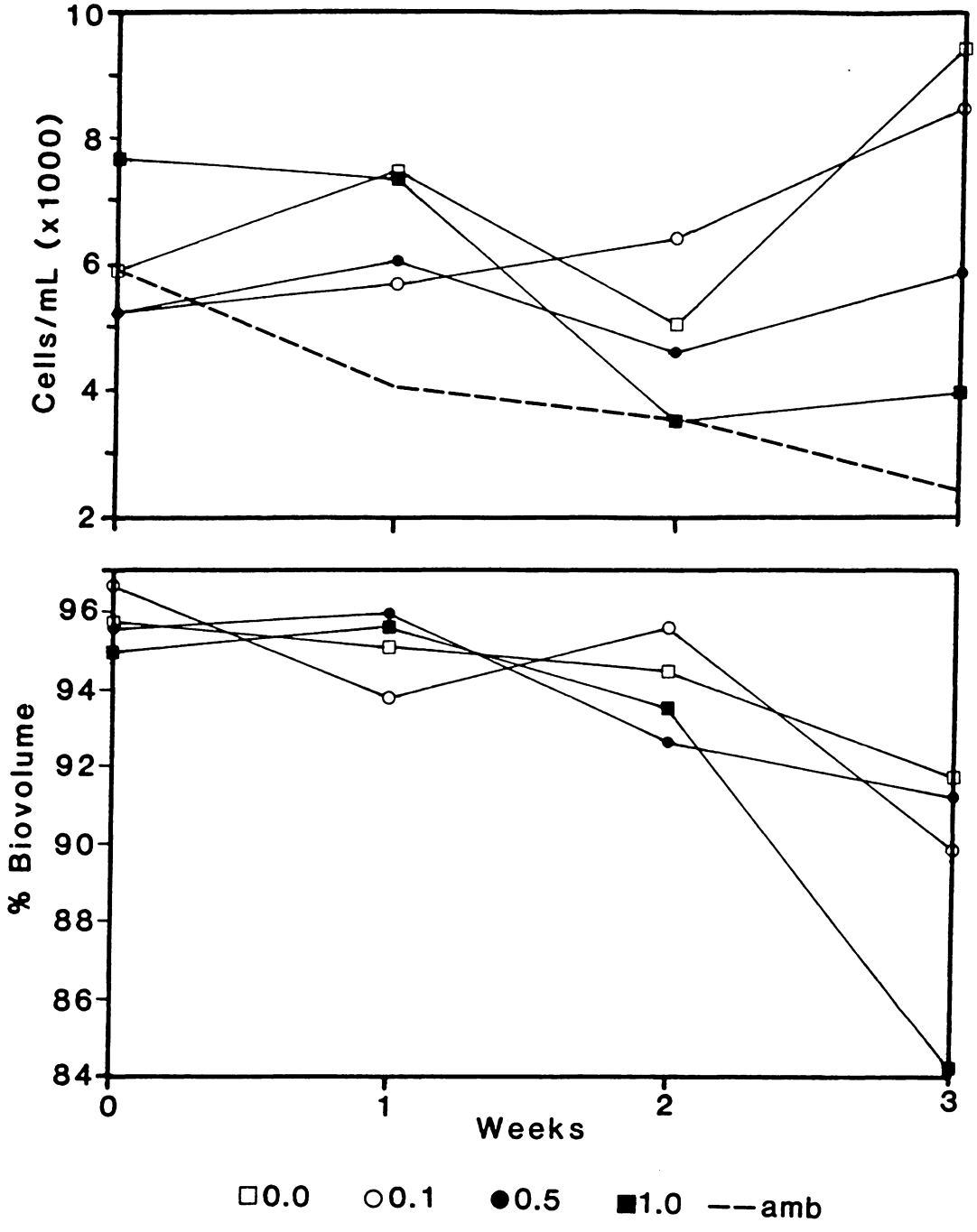

FIGURE 13. Phytoplankton (≥9um) a) mean densities and b) mean % biovolumes per dose

cells/mL) can be explained by decreases in other taxa.

Figure 13b depicts mean % biovolumes per dose for the ≥9 um size class. Except for 1.0 mg/L, there was a general decline of 4-7% biovolume at all doses. Microcosms with 1.0 mg/L followed a similar trend until Week 3, when a sharp decrease of about 10% biovolume was noted. This Week 3 value for 1.0 mg/L was significantly lower than control ($p=0.05$) and was related to death of Glenodinium and Trachelomonas. Populations of these large phytoplankters exhibited rapid decreases at this time. However, death of these cells did not appreciably affect the size class densities; their greatest numbers were only about 320 cells/mL.

Control and 0.1 mg/L densities of phytoplankton <9 um increased over time, and the higher herbicide doses limited this increase (Figure 14a). The densities of 1.0 mg/L microcosms decreased, and the value at Week 3 was significantly lower than the control ($p=0.05$).

The % biovolumes of the different doses for the <9 um size class (Figure 14b) followed an increasing trend over time. Percent biovolumes were about 4% at Week 0, and increased to about 8% at Week 3, except for 1.0 mg/L. This value was significantly higher than the control ($p=0.05$) and was due to the decline in ≥9 um cells at that time.

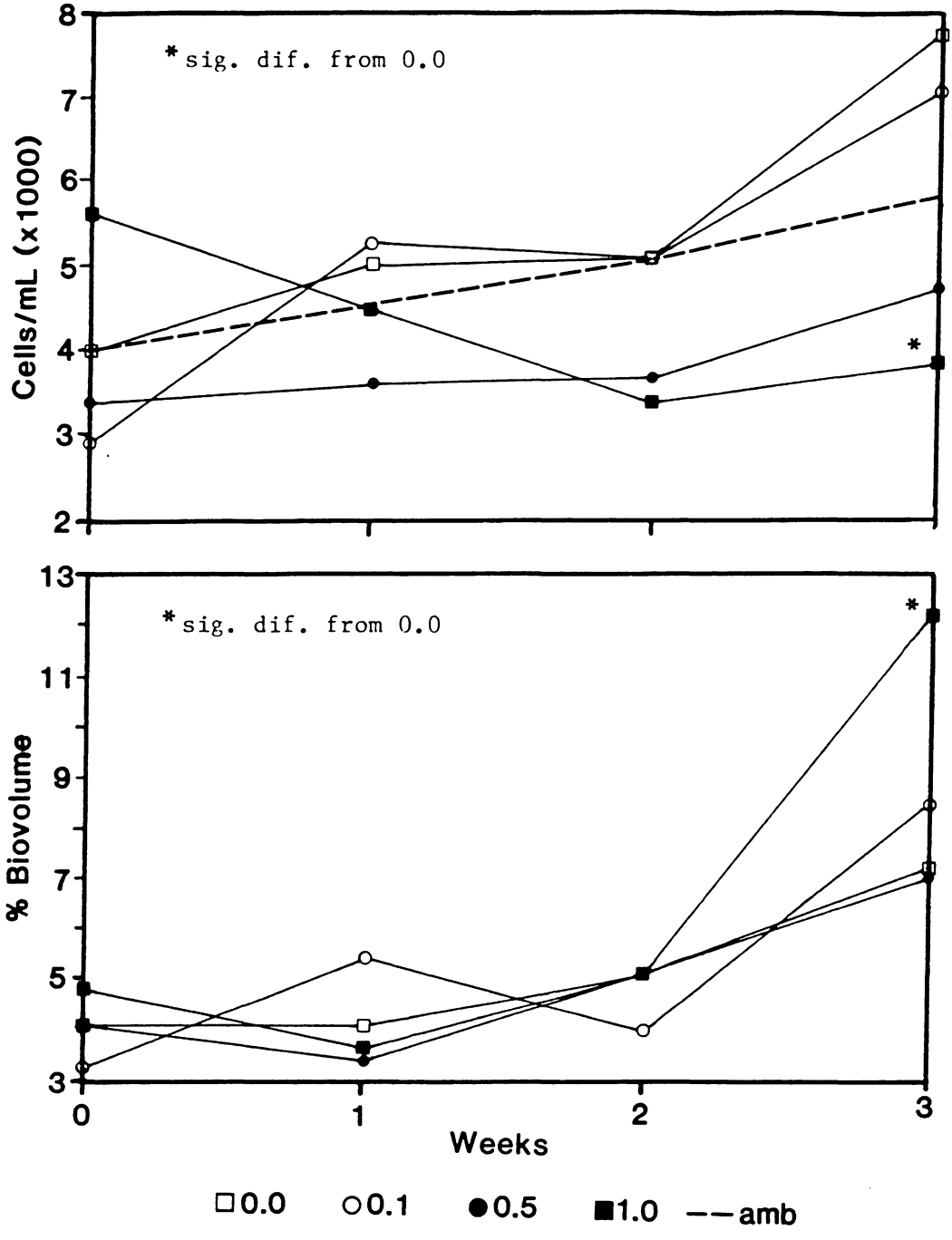

FIGURE 14. Phytoplankton (< 9 um) a) mean densities and b) mean % biovolumes

3.4 Bacteria

The results of bacteria enumeration are shown in Figure 15 and data are presented in Appendix 4. Although the plot of mean values in Figure 15 appears to indicate some differences between doses, dose was not a significant factor ($p=0.69$). Time was the significant factor ($p=0.008$); there was no significant dose-time interaction. Coefficients of variation within microcosm subsamples ranged from 1.3 to 47.1% with a mean of 22.0%. Coefficients of variation between replicate microcosms ranged from 4.3 to 54.6%, with a mean of 23.0%. This high variability within and between replicate microcosms in bacterial counts made it impossible to discern a statistically significant dose effect. In addition, the range of mean values is about 3 to 6 x 10^5 cells/mL; a relatively narrow range of a logarithmic scale.

The increase in bacterial densities in microcosms at Week 1 may represent a trend. Week 1 was significantly different from the other weeks ($p=0.05$) and Weeks 0, 2, and 3 were not significantly different. In addition, a container effect may have occurred; ambient densities declined with time, but enclosed densities exhibited fluctuations above or at original values. In conclusion, bacterial densities could not be shown to be affected by simazine dosage because the data were too variable.

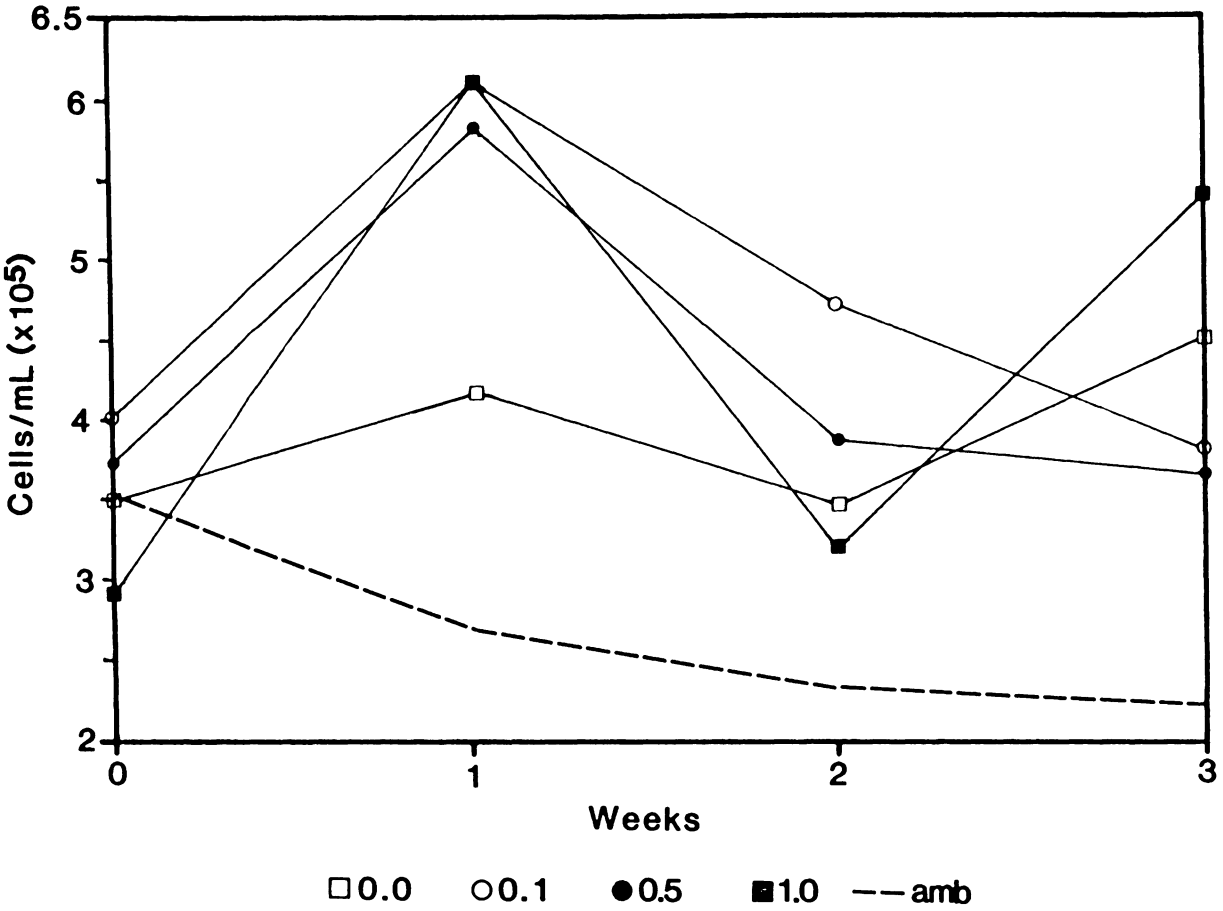

FIGURE 15. Bacteria mean densities per dose

3.5 Zooplankton

Copepods

Copepods were present in the microcosms primarily as nauplii and some copepodids; adult copepods were rare and none carried eggs. Sampling method may have contributed to the absence of adult copepods (see Section 2.4). Copepods were identified as cyclopoids and no further identification was attempted. Data are presented in Appendix 5.

Simazine dose did not significantly affect copepod populations, but time was significant ($p=0.325$ and 0.0008, respectively). There was no significant dose-time interaction. Microcosm copepod densities at Week 2 were significantly lower than other weeks, and densities at other weeks were not significantly different from each other ($p=0.05$).

Nauplii and copepodids comprised a relatively small portion of the zooplankton in the microcosms, with densities of 250-300 organisms/L at Week 0 (Figure 16). Densities generally declined to about 150 organisms/L at Week 2, and showed a slight increase to about 225 organisms/L at Week 3.

Ambient samples exhibited a sharp decrease in copepod numbers at Week 1; this was probably related to downward vertical migration and avoidance of very low surface temperature. Ambient densities remained low at Week 2, but

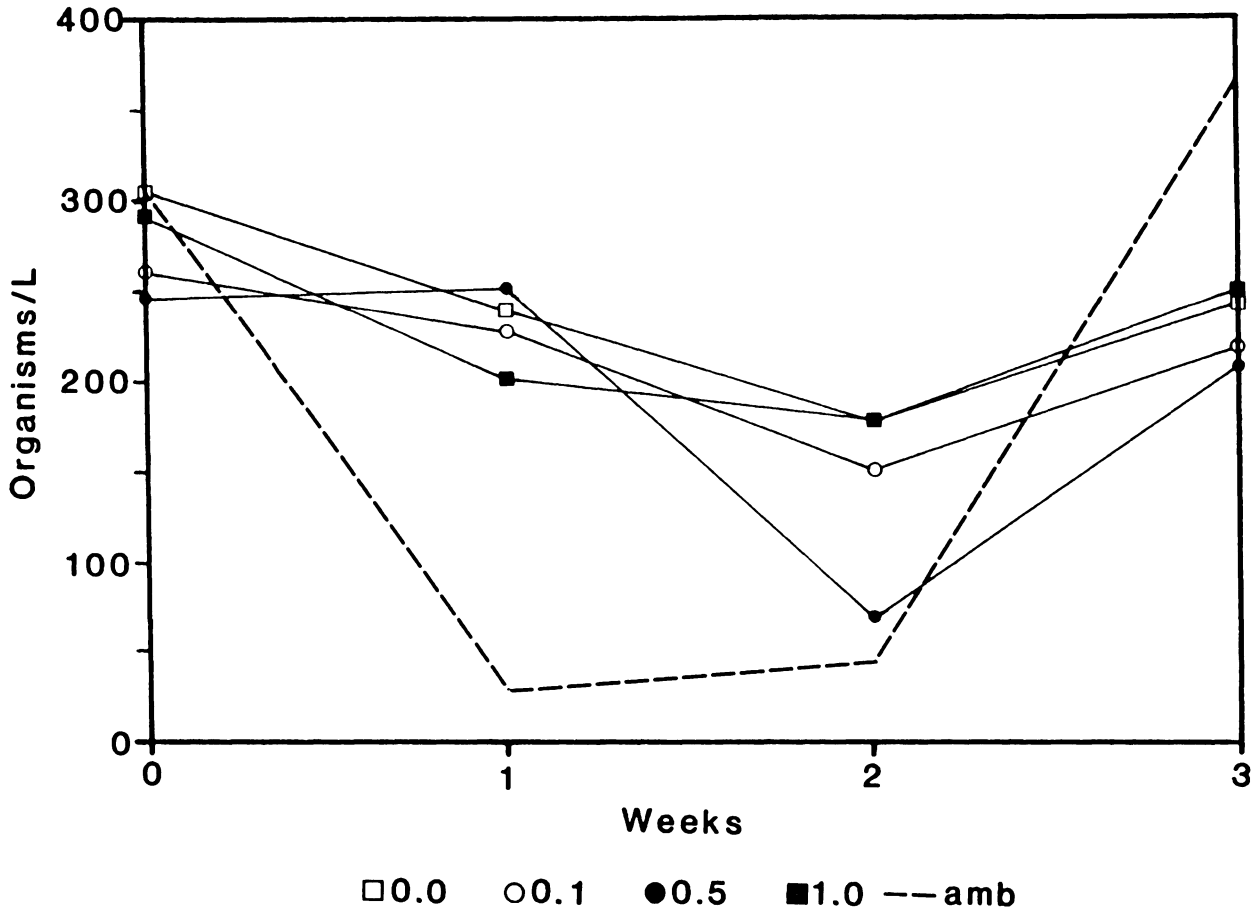

FIGURE 16. Copepod nauplii/copepodids mean densities per dose

increased to about 360 copepods/L at Week 3.

The lack of reproductively mature copepods in the microcosms meant that copepodids probably did not reach maturity during the three week experiment. It is therefore difficult to explain an increase in densities between Weeks 2 and 3. Regardless, naupliar and copepodid densities showed no response to the effects of simazine.

Cladocera

The following cladocerans were present in microcosms: Daphnia parvula, Chydorus sphaericus and Ceriodaphnia lacustris. However, these species were not present in 30% of the samples and were rare when present. Data are presented in Appendix 5, but no graphical or statistical analyses were conducted on these data.

Ciliates

The ciliate Codonella was enumerated and other miscellaneous ciliates were noted when present. Data are presented in Appendix 5. Codonella was not observed in 25% of the samples and miscellaneous ciliates were never common. Formalin preservation may have contributed to this variable presence of ciliates in the samples. This variability between replicate microcosms made the following analysis of trends inconclusive. Codonella was affected only by time ($p=0.0008$) and was not affected by simazine dose or dose-time interaction. Week 2 densities were

significantly greater than the other weeks (p=0.05). Maximum mean density at Week 2 was about 70 organisms/mL. Microcosm densities resembled ambient densities, indicating that the increase in Codonella at Weeks 1 and 2 was a natural event and not an enclosure effect. Densities generally dropped again at Week 3 to about the same numbers as Week 0 ($\leq$10 organisms/mL).

Rotifers

Four species dominated the rotifers: Kellicottia bostoniensis, Keratella cochlearis, Polyarthra vulgaris, and Synchaeta pectinata. Seven other genera were present, but were rare and never exceeded 2% of the total rotifer abundance when combined. Data for the dominant species and other genera are presented in Appendix 5. No graphical or statistical analyses were considered for rare taxa, but their numbers were included in analyses of total rotifer abundance.

Rotifers were the largest component of the zooplankton in the microcosms and pond. Total abundances at Week 0 were approximately 6,000 rotifers/L, and ranged from 2,000 - 5,000 rotifers/L at Week 3 (Figure 17). Total rotifer abundance was significantly affected by dose and time (p=0.019, 0.0001, respectively). Dose-time interaction was marginally significant (p=0.071). All doses exhibited a decline in rotifer densities with time, and an increased

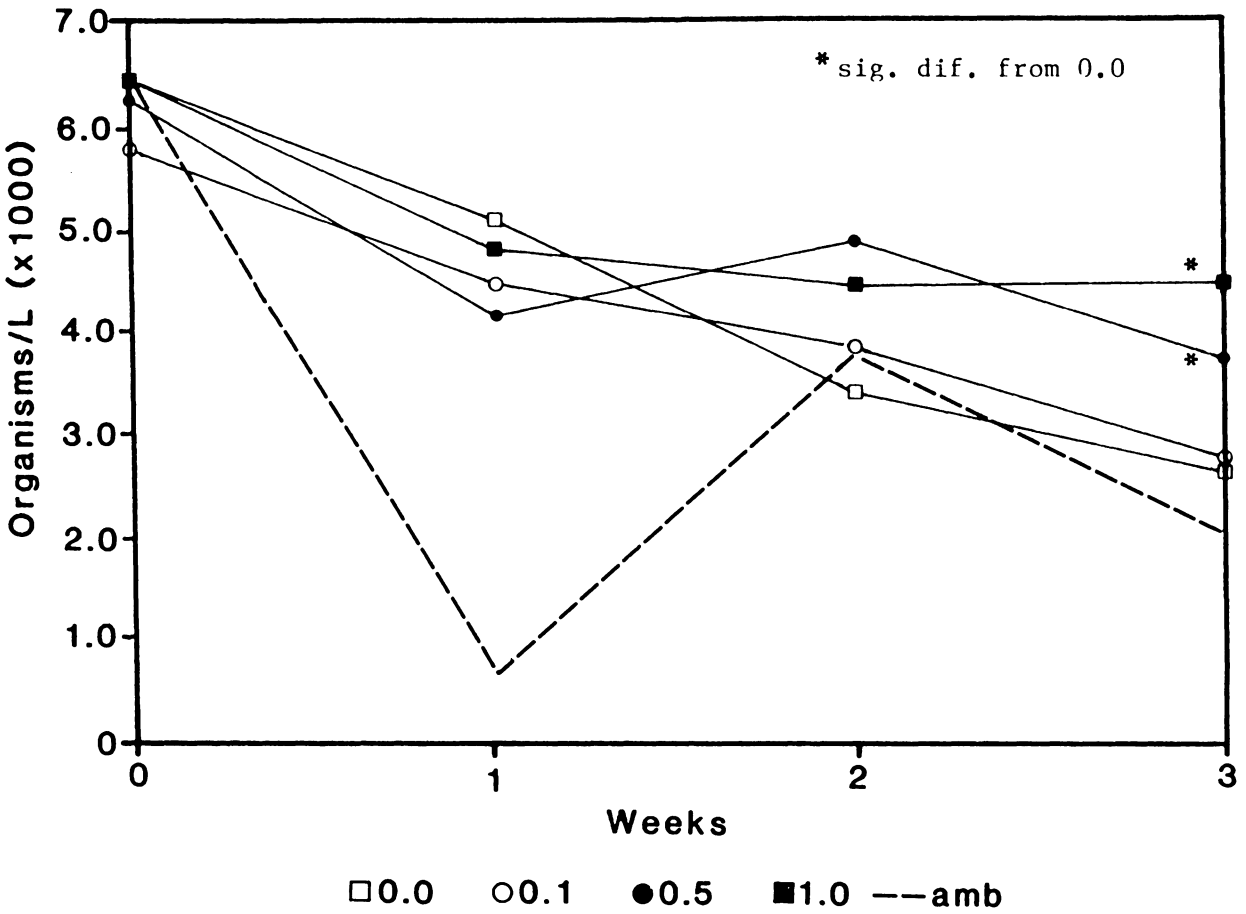

FIGURE 17. Rotifer total abundance per dose

separation among doses over time. At Week 3, the 0.5 and 1.0 mg/L microcosms had greater rotifer densities than the control and 0.1 mg/L microcosms (Figure 17). The values for 0.5 and 1.0 mg/L microcosms were significantly greater than the control ($p=0.05$).

The decline in total rotifer densities appears to have been a natural phenomenon. Ambient total densities were very similar to control and 0.1 mg/L microcosms for all weeks except Week 1 (Figure 17). The low ambient value at Week 1 was undoubtedly related to the very cold surface water at that time (about 2 C) and ice cover. The return of ambient rotifer densities to the same trend at Week 2 indicates that organisms were still present and had simply migrated back to surface waters with the onset of warmer temperatures.

K. bostoniensis comprised about 50% of the total rotifer assemblage at Week 0, with densities of about 3,000 organisms/L. Densities declined in a linear fashion for all levels of simazine dosage, but with different slopes, so that a significant separation of doses occurred over time (Figure 18). Dose, time and dose-time interaction were statistically significant factors in K. bostoniensis population trends ($p=0.007$, 0.0001, and 0.049, respectively).

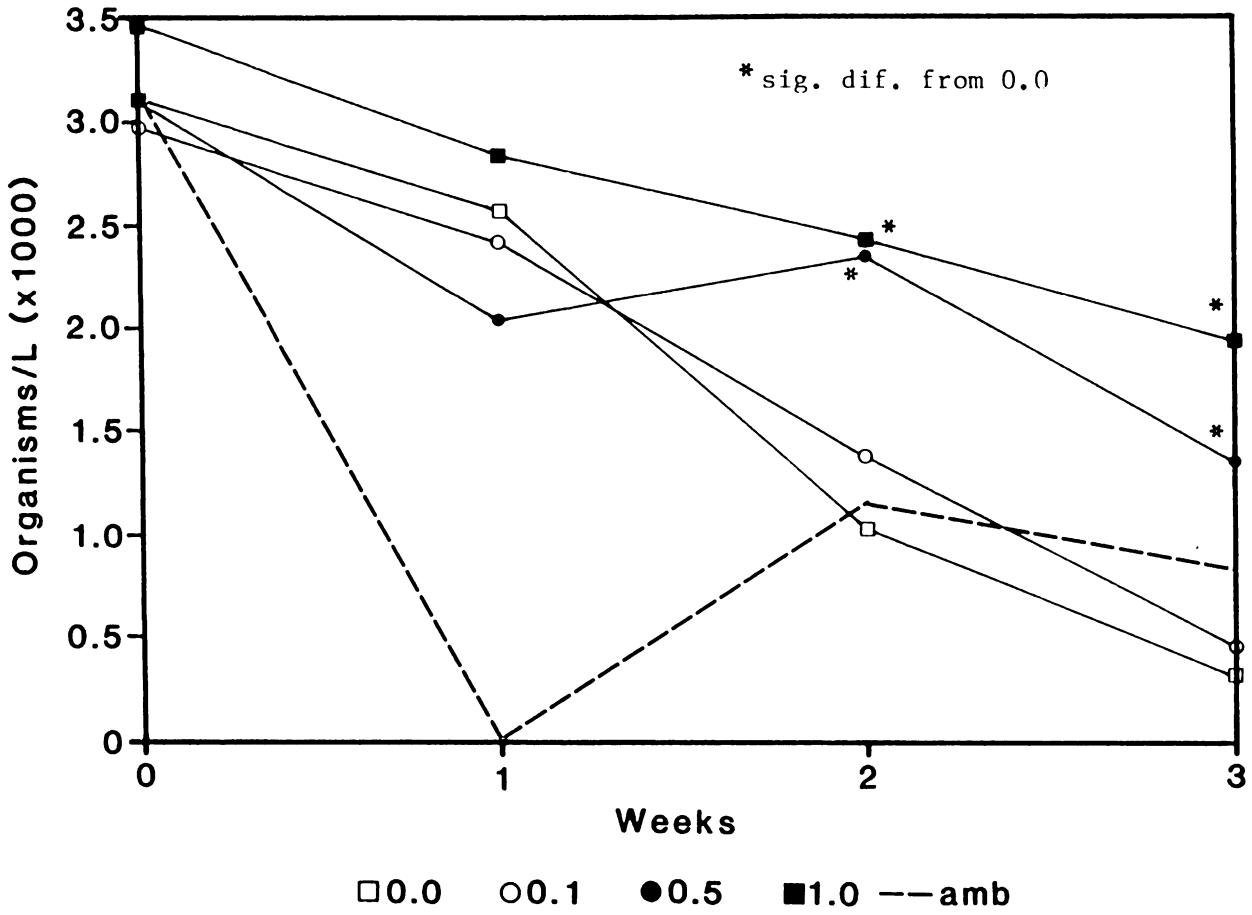

FIGURE 18. Kellicottia bostoniensis mean densities per dose

Ambient densities of K. bostoniensis were similar to control and 0.1 mg/L microcosms, except for the depression at Week 1 (Figure 18). This low value was probably due to the very low temperature at that time. No effect of enclosure on K. bostoniensis occurred beyond the restriction of vertical migration at Week 1.

Microcosms containing 0.5 and 1.0 mg/L simazine had significantly higher densities of K. bostoniensis than control microcosms at Weeks 2 and 3 ($p=0.05$). K. bostoniensis was positively affected by 0.5 and 1.0 mg/L simazine relative to control and ambient conditions.

Keratella cochlearis was abundant during the experiment and enclosed populations exhibited an overall decline from a range of 2,000 - 2,300/L at Week 0 to a range of 1,000 - 1,600/L at Week 3 (Figure 19). Samples of ambient water showed marked fluctuations in K. cochlearis densities. The low value at Week 1 can be explained by vertical migration away from the low surface temperature at that time. However, the low value at Week 3 is not so easily explained. Possible reasons include: A natural population decline; vertical stratification with lower densities in the sampled surface layer; or variation in sampling/counting methodology.

Time was the only significant factor in the population trends of K. cochlearis ($p=0.0001$); dose and dose-time

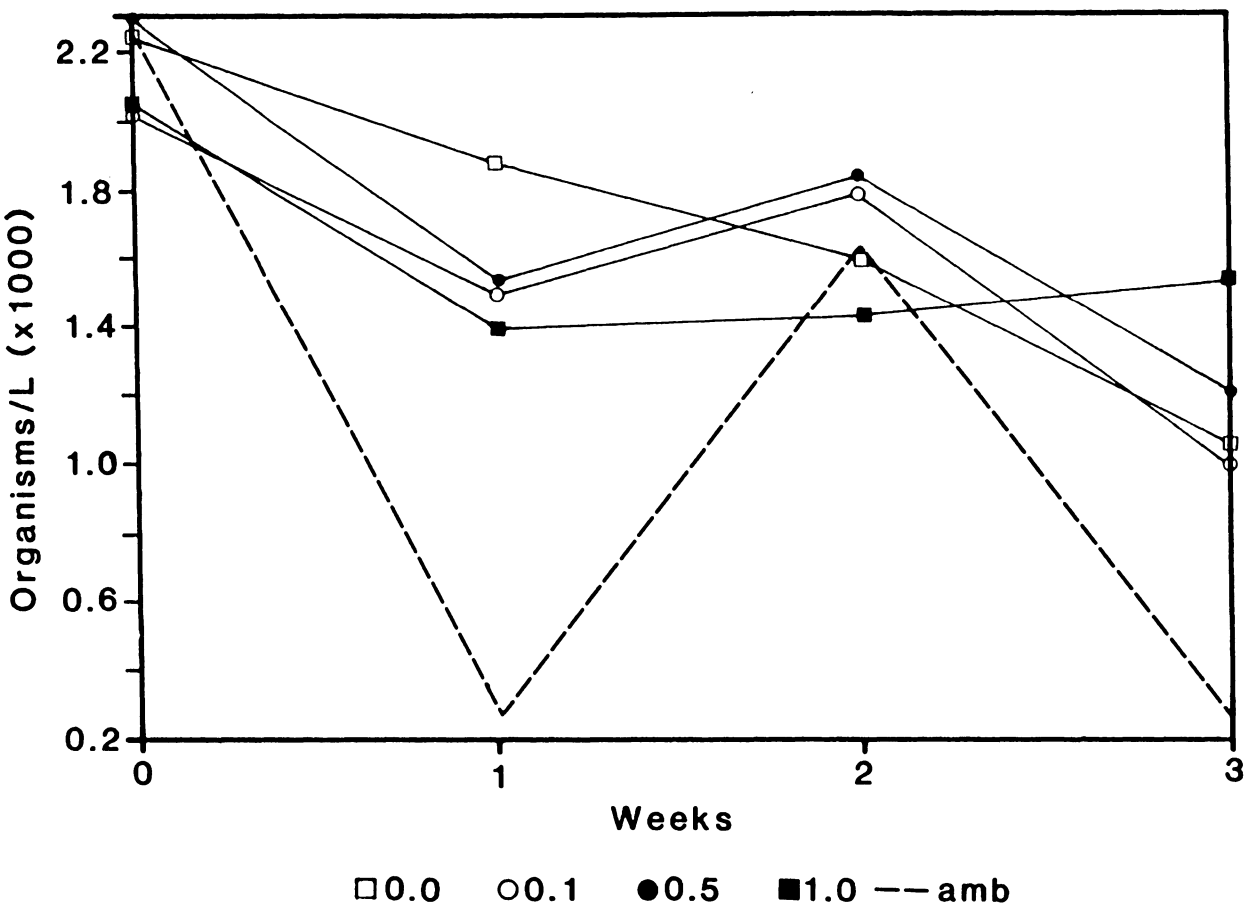

FIGURE19. Keratella cochlearis mean densities per dose

interaction were not significant. Densities at Weeks 1 and 2 were not significantly different from each other (p=0.05), but Weeks 0 and 3 were each unique. Although not statistically significant, it appeared that the K. cochlearis population in 1.0 mg/L microcosms was approaching a greater density than other doses at Week 3 (Figure 19).

Polyarthra vulgaris densities at Week 0 ranged from about 540-790 organisms/L. This species was the only one of the four dominant rotifers to exhibit an overall increase in population numbers and all of the net population increase occurred during Week 3. Densities at Week 3 were approximately 900-1,100 organisms/L (Figure 20) and were significantly greater than values of the other weeks (p=0.05).

Time was the only significant experimental factor affecting P. vulgaris populations (p=0.0001); dose and dose-time interaction were not significant. Ambient samples exhibited a trend very similar to the temperature curve (see Figure 2). Again, the low value at Week 1 was probably related to vertical migration away from very cold surface waters. Enclosed P. vulgaris populations followed the same basic trend, but with less amplitude of change than ambient populations (until Week 3). This damping of fluctuations may have been caused by enclosure and its

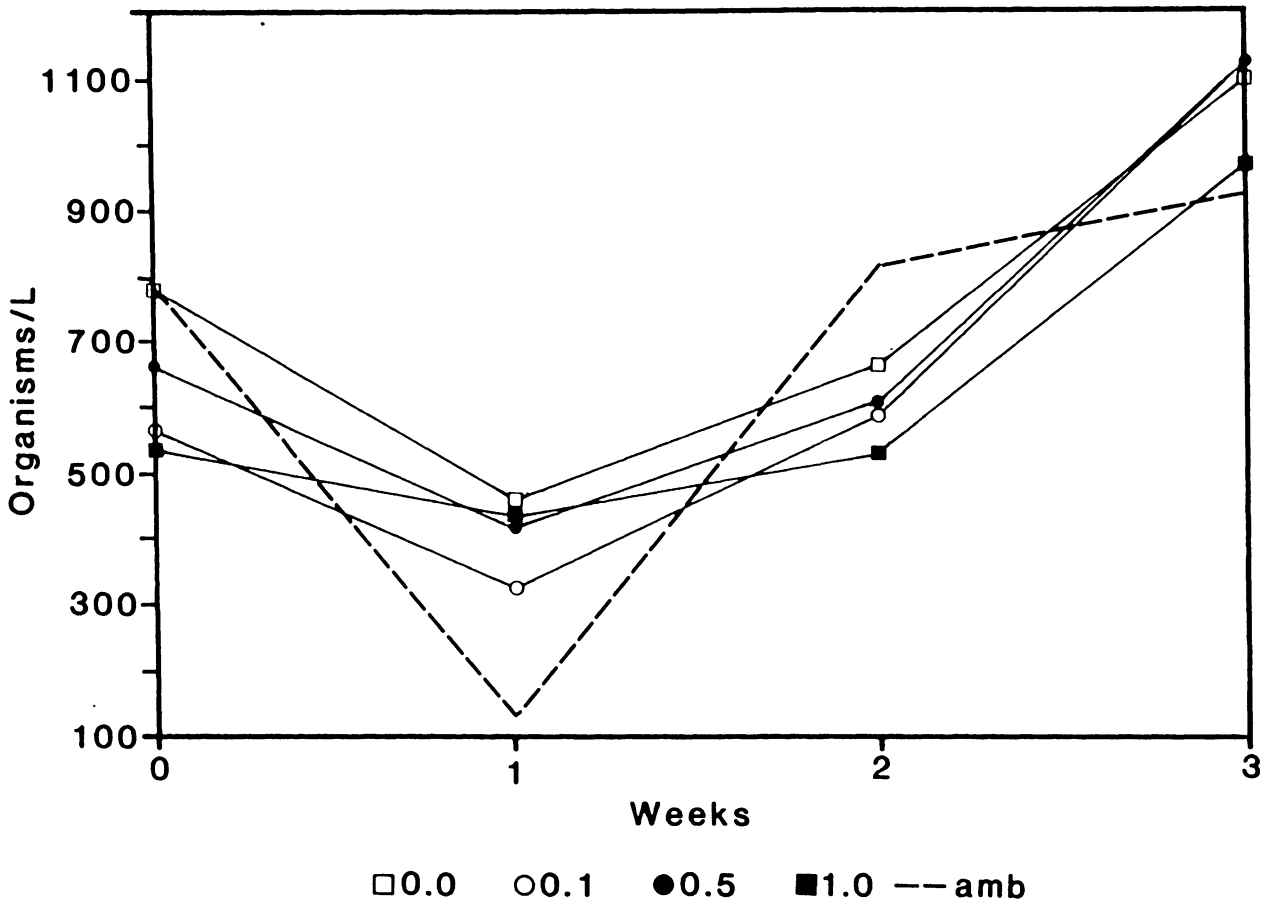

FIGURE 20. Polyarthra vulgaris mean densities per dose

restriction of the spatial heterogeneity possible in ambient samples.

Judging from the similarity of enclosed and ambient population trends (Figure 20), the increase in P. vulgaris densities during Week 3 was a natural event and was not related to effects of simazine. The greater magnitude of increase in enclosed densities (~550/L) relative to ambient (~90/L) may indicate that enclosure enhanced this natural increase in P. vulgaris.

Synchaeta pectinata was the least abundant of the four dominant rotifer species. Densities at Week 1 were about 230 organisms/L, except for the 1.0 mg/L microcosms, which contained about 370 organisms/L. This higher value may have been due to non-random distribution in the containers. Densities of S. pectinata generally declined in the experiment, so that mean values at Week 3 ranged from about 40 - 180/L (Figure 21).

Dose was not significant, but time and dose-time interaction were important factors in S.pectinata trends ($p=0.815$, 0.0001, and 0.076, respectively). The marginally significant interaction term stems from the separation of doses at Week 3, when S. pectinata densities were lower with greater simazine dose (Figure 21). This separation was not great enough to show any significant differences from controls ($p>0.05$). Therefore, some negative effect of

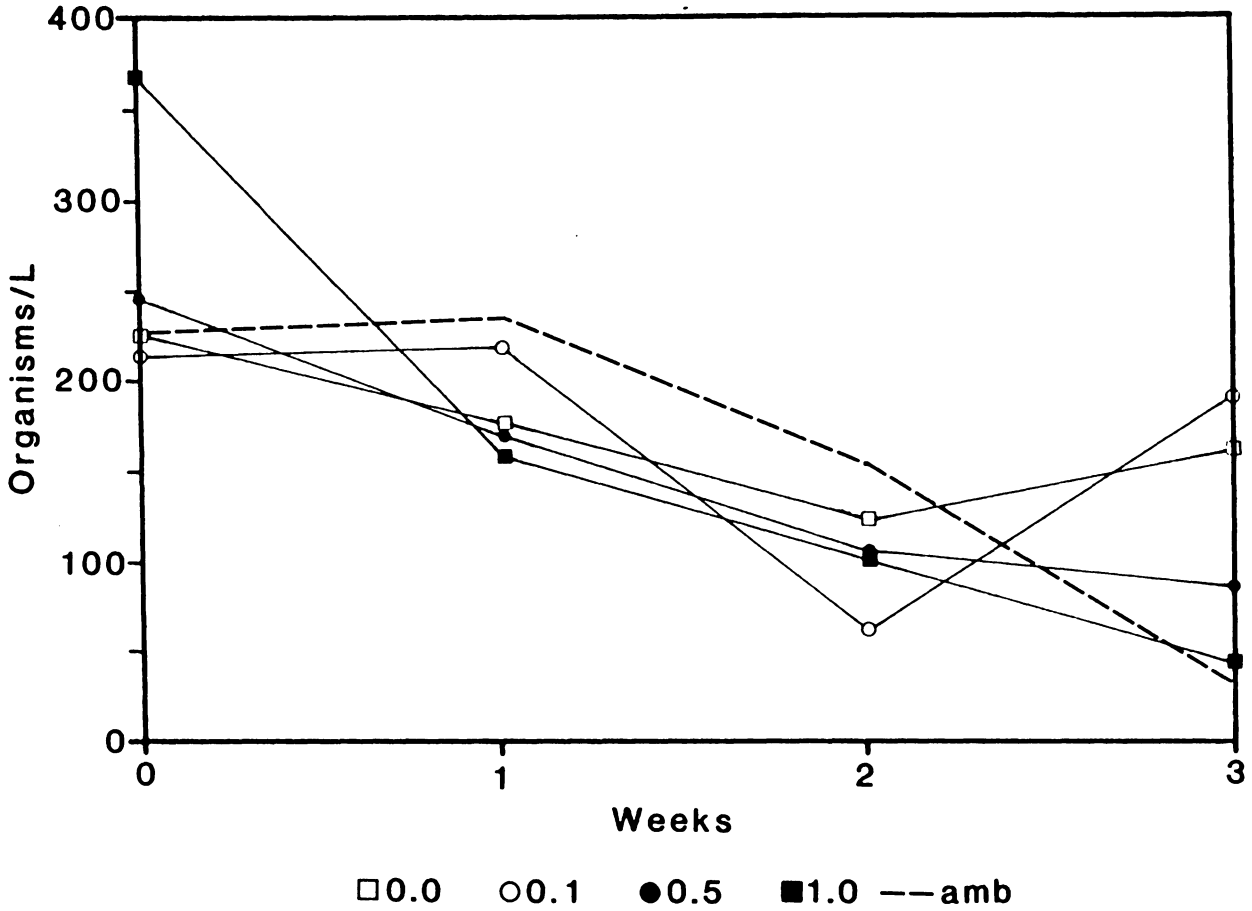

FIGURE 21. Synchaeta pectinata mean densities per dose

simazine dosage on S.pectinata may occur during Week 3, but the overriding characteristic of S. pectinata populations was the general decrease in numbers over the course of the experiment.

Ambient S. pectinata densities also exhibited this decline, and there does not appear to have been any enclosure effect on S. pectinata until Week 3. The microcosms most similar to ambient at Week 3 were those with 1.0 mg/L simazine (Figure 21). Organisms in control and 0.1 mg/L enclosures seem to have benefited from enclosure during Week 3.

S. pectinata was the only rotifer that did not decrease in numbers in the 2 C surface water at Week 1. Its abundance during Week 1 was about the same or slightly greater than that of Week 0.

Rotifers: Percent Compositions

The trends of individual rotifer species can be considered relative to each other as a proportion of total rotifer numbers. Each weekly mean density value was expressed as a percentage of the total rotifer abundance for that week. Figure 22 shows changes in the percent compositions of the four dominant species by simazine dose.

In control and 0.1 mg/L microcosms, K. bostoniensis and P. vulgaris changed positions of dominance by Week 3. This change in dominance was delayed as simazine dose

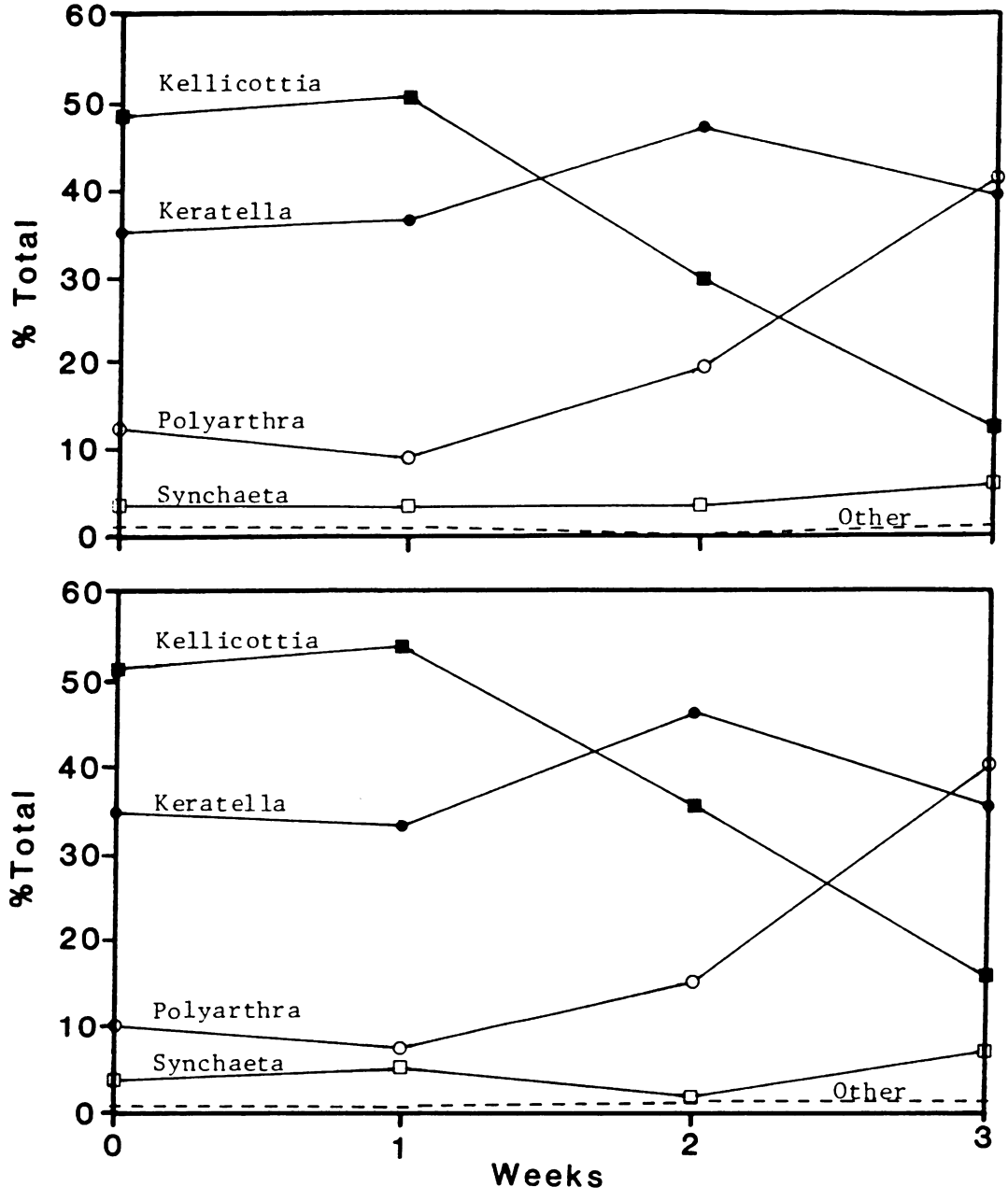

FIGURE 22. Rotifer percent compositions a) 0.0 and b) 0.1 mg/L

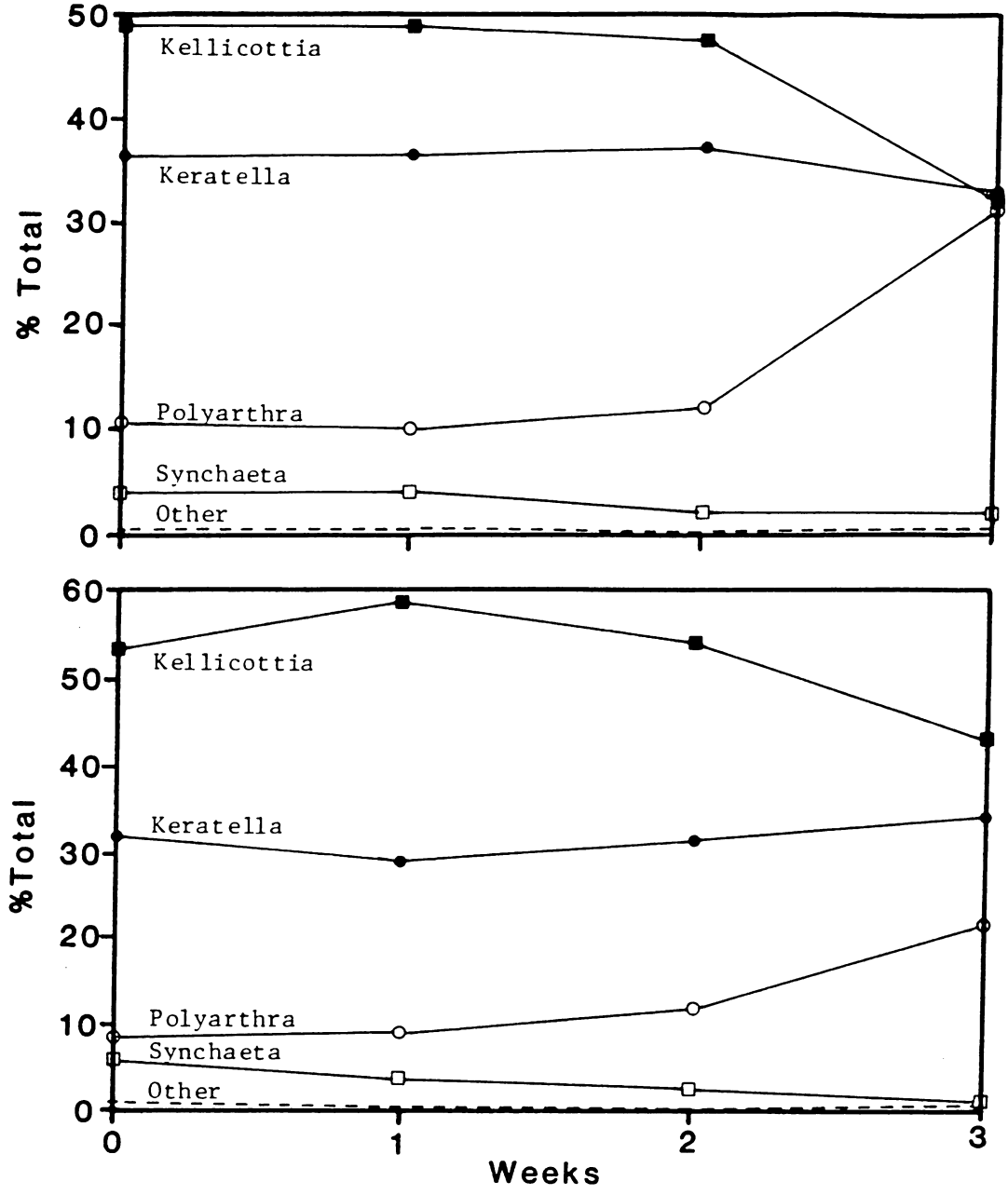

FIGURE 22, cont'd. c) 0.5 and d) 1.0 mg/L

increased. K. bostoniensis and P. vulgaris converged on about the same proportion (~33%) at the end of Week 3 in 0.5 mg/L simazine. The % composition plots of the two species did not converge at the end of Week 3 in 1.0 mg/L, but appeared to converge at a later time. Keratella cochlearis and Synchaeta pectinata did not change in percent composition throughout the experiment. Because P. vulgaris populations did not vary with dose or dose-time interaction, changes in K. bostoniensis alone were responsible for the changes in percent composition. The positive effect of simazine on K. bostoniensis maintained its dominance in the rotifer assemblage.

Rotifers: Population Parameters

The following parameters were calculated in an effort to understand the rotifer species trends discussed above: instantaneous rate of population increase (r); instantaneous birth rate (b); and instantaneous death rate (d). The values (r) and (b) were calculated from species densities, eggs/female and egg development time. The value (d) was calculated from the difference of (b) (potential population growth rate) - (r) (actual population growth rate). The values (r), (b) and (d) per dose-time combination are listed in Table 4 for K. bostoniensis, K. cochlearis and P. vulgaris.

The instantaneous birth rate (b) followed a very

TABLE 4. Rotifer Species Population Parameters(a)

Weekly Intvl.	Dose	Kellicottia bostoniensis b	d	r	Keratella cochlearis b	d	r	Polyarthra vulgaris b	d	r
0-1	amb.	0.14	0.97	-0.83	0.16	0.46	-0.30	0.20	0.46	-0.26
0-1	0.0	0.13	0.16	-0.03	0.16	0.18	-0.03	0.21	0.29	-0.08
0-1	0.1	0.13	0.16	-0.03	0.15	0.20	-0.04	0.20	0.28	-0.08
0-1	0.5	0.13	0.18	-0.06	0.16	0.21	-0.05	0.21	0.28	-0.06
0-1	1.0	0.13	0.16	-0.03	0.16	0.22	-0.06	0.21	0.24	-0.03
1-2	amb.	0.10	-0.59	0.69	0.10	-0.16	0.26	0.17	-0.09	0.26
1-2	0.0	0.08	0.21	-0.13	0.10	0.12	-0.02	0.18	0.13	0.05
1-2	0.1	0.08	0.16	-0.08	0.10	0.07	0.03	0.16	0.07	0.09
1-2	0.5	0.08	0.06	0.02	0.10	0.07	0.03	0.18	0.13	0.05
1-2	1.0	0.08	0.10	-0.02	0.10	0.10	0.00	0.18	0.15	0.03
2-3	amb.	0.30	0.35	-0.05	0.29	0.55	-0.26	0.37	0.35	0.02
2-3	0.0	0.30	0.46	-0.17	0.34	0.40	-0.06	0.37	0.29	0.07
2-3	0.1	0.30	0.46	-0.16	0.33	0.42	-0.09	0.34	0.25	0.09
2-3	0.5	0.30	0.38	-0.08	0.33	0.40	-0.06	0.36	0.27	0.09
2-3	1.0	0.29	0.32	-0.03	0.32	0.31	0.01	0.35	0.27	0.09

(a) b = instantaneous birth rate, d = instantaneous death rate, r = instantaneous population growth rate (Paloheimo 1974).

similar trend for all three rotifer species (e.g., Figure 23a for K. bostoniensis). Statistics could be calculated for these (b) values because they were ultimately derived from counts of eggs and adults per microcosm. This was not true for the other parameters (r and d); analyses of these values were limited to graphical interpretation.

Time was highly significant for (b) of all three species ($p=0.0001$), and dose and dose-time interaction were not significant. This means that the positive effect of simazine on K. bostoniensis densities was not related to increased production of young. Birth rate was influenced only by time (=temperature). The birth rates of weekly intervals 0-1 and 1-2 were comparatively low due to the low temperature at Week 1.

Figure 23b is a plot of the calculated K. bostoniensis death rate (d), over weekly intervals for each simazine dose. All doses exhibited increased death rate between weekly intervals 1-2 and 2-3. Higher doses exhibited lower instantaneous death rates (Table 4). The positive effect of simazine dose on K. bostoniensis was due to a reduction in the death rate of this species.

The data in Table 4 indicate no dose-related differences in death rate for K. cochlearis with the possible exception of the 2-3 week interval. The (d) value for 1.0 mg/L dose appeared to be lower than the other doses

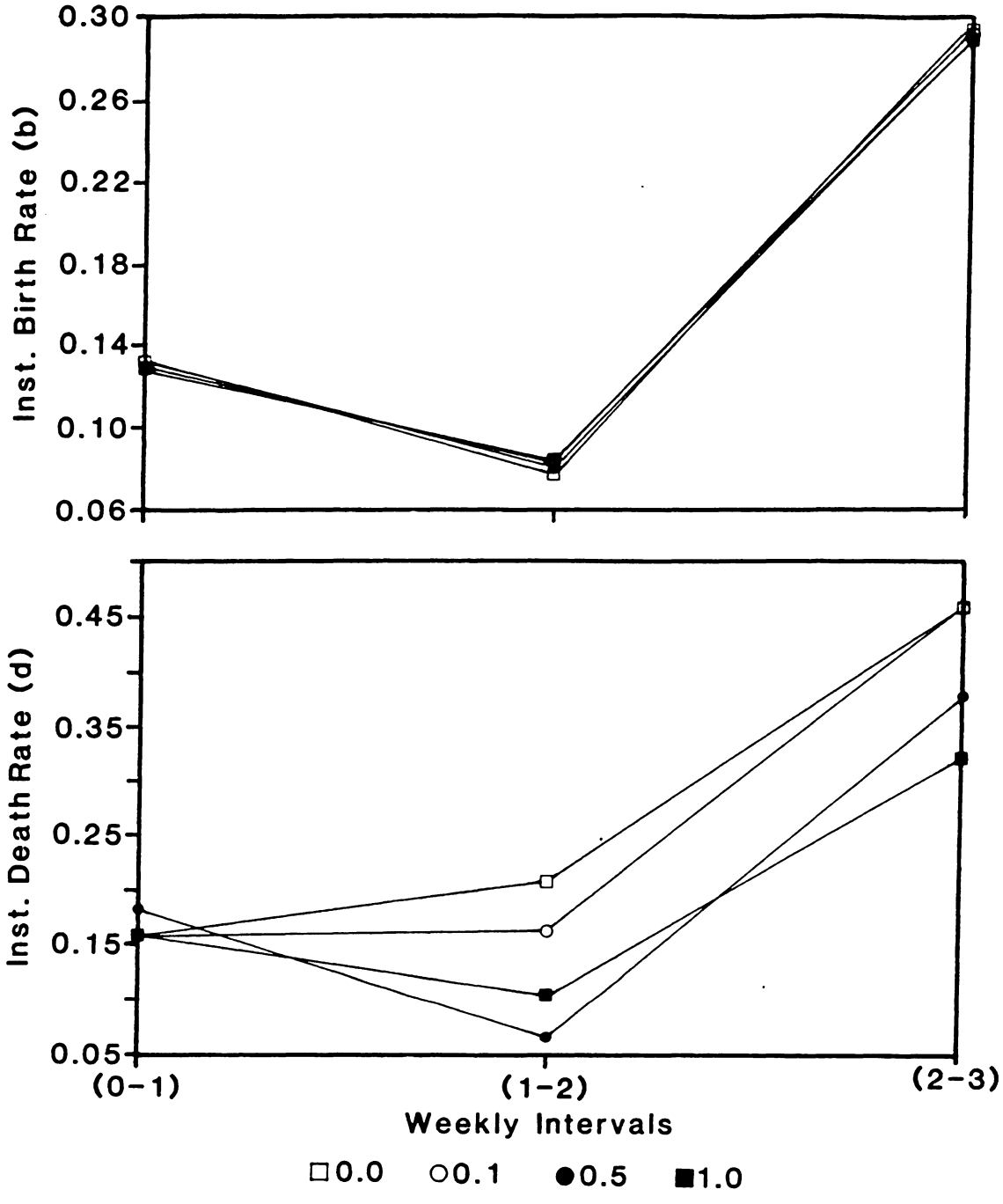

FIGURE 23. Kellicottia bostoniensis a) birth and b) mortality rates per dose

at this time. This lower mortality rate was probably responsible for the slightly greater abundance of K. cochlearis in 1.0 mg/L simazine at Week 3. No dose-related differences in any of the three parameters existed for P. vulgaris, which varied only with time (=temperature).

The change of dominance between K. bostoniensis and P. vulgaris may be explained by consideration of the death rate values for these species (Table 4). The birth rates of these two species did not change relative to each other, but death rates did change. The death rate for K. bostoniensis was approximately 60% of the rate for P. vulgaris at interval 0-1 week. At interval 2-3 weeks, the (d) for K. bostoniensis was 159% of the P. vulgaris value in controls and 118% in microcosms with 1.0 mg/L. The mortality rate of K. bostoniensis surpassed that of P. vulgaris over the course of the experiment. Simazine reduced the difference between species death rates by the end of the experiment because the rate for K. bostoniensis decreased.

4.0 DISCUSSION

4.1 Physical/Chemical Parameters

Stout's Pond can be characterized as a shallow, soft-water, low alkalinity impoundment, with an abundance of planktonic organisms. The pH was neutral to slightly basic. Limited macrophytic growth occurred at the shoreline.

Conducted during December 1984, this study was subject to winter conditions, i.e., short daylength and low temperatures. The low temperatures were especially important during the first week of the experiment, when the pond froze over and surface water temperatures were 1-2 C. Thereafter, temperatures returned to the range of Week 0 (8-10 C).

This temperature change had a profound effect on the abundance of some organisms in the ambient surface waters. Copepod nauplii/copepodids, and the rotifers Kellicottia bostoniensis, Keratella cochlearis and Polyarthra vulgaris all exhibited a sharp decline in surface densities at Week 1. This effect was probably related to the vertical migration of these organisms to a greater depth and relatively higher temperatures. The rotifer Synchaeta pectinata did not exhibit this response and maintained stable surface densities. Ruttner-Kolisko (1974) stated that S. pectinata are cold-adapted and have population

maxima at low temperatures; the same is true for S. lakowitziana which exhibit largest population numbers at 0-5 C (Edmondson 1946).

Bacteria and most small phytoplankton did not exhibit the same response as the zooplankton to reduced surface temperatures. These plankters remain suspended in the water column primarily by turbulence and usually do not exhibit marked vertical migration patterns. The larger flagellated forms Dinobryon, Glenodinium and Trachelomonas did decline in ambient densities at Week 1, indicating that they also migrated down. Ambient densities of the ciliate, Codonella, increased slightly at Week 1.

Exposure to cold does not appear to have had any immediate effects upon the enclosed communities other than slowing rotifer metabolic rates. Indirect evidence of this metabolic slow-down was the birth and death rates of the dominant rotifers (Table 4; Week 1). Edmondson (1946) found rotifer feeding and reproductive rates were depressed in cold water, and duration of egg development and lifespan were increased.

Microcosm densities of phytoplankton, bacteria, ciliates and copepods did not appear to be negatively affected by the cold at Week 1. Some taxa may have benefited from the low temperatures (i.e., Trachelomonas, miscellaneous coccoids 9-15 um).

Percent saturation of DO in the microcosms was inversely related to simazine dose over time (Figure 3). This was probably related to the inhibition of photosynthesis in phytoplankton. The trends of DO % saturation appeared to be more closely related to total algal biovolume than to total algal densities (Figure 6a,b). The control microcosms remained virtually identical to the ambient samples in DO % saturation and never fell below 86% saturation. This indicated that sufficient light entered the microcosms to maintain photosynthesis in the controls. Schwartz et al. (1981) reported a rapid depression in oxygen saturation from about 100% to 57% within 3 days of treating a lake with 0.45 mg/L simazine. This oxygen depression was considered a result of both decomposition and photosynthetic inhibition. Similar results were observed in other studies with simazine and related s-triazine herbicides (Tucker et al. 1983; deNoyelles et al. 1982).

The dose-related decline in DO had no apparent negative effect on zooplankton; K. bostoniensis responded positively to simazine. Ruttner-Kolisko (1974) considered K. bostoniensis "probably eurythermous, requiring little oxygen," and also stated that it is often hypolimnetic. Edmunds (1974) reported a "large population" of K. bostoniensis present at an oxygen concentration of 0.2 mg/L

in Pandapas Pond, but considered it a "fortuitous" phenomenon, related to the seasonal change in temperature/oxygen content. Conversely, K. bostoniensis does quite well at a high DO. The greatest densities of K. bostoniensis in this study occurred at Week 0, when DO was 87% saturation. Potter (1978) reported a K. bostoniensis population maximum in Pandapas Pond during October 1977, when DO was about 77% saturation or greater. Knauer (1979), also working on Pandapas Pond, reported two population maxima of K. bostoniensis during September 1976 and May 1977, when DO was in excess of 90% saturation.

Ambient pH did not vary significantly during the experiment (Figure 4). Higher simazine dose nullified the increase in pH observed in control and 0.1 mg/L microcosms at Week 3. This pH increase may have been affected by the increase in total algal densities at Week 3. Theoretically, increased utilization of CO_2 by the growing algal populations in control and 0.1 mg/L microcosms would have increased pH, and at the higher doses, simazine inhibition of photosynthesis reduced CO_2 uptake. Consequently, pH did not increase in higher simazine doses. However, one would expect DO to follow a similar trend due to oxygen production by increased algal densities: the lack of resemblance between DO and pH trends is puzzling and can not be adequately explained.

The trends in pH did not influence zooplankton populations. They were within the natural range of pH for productive waters and dose-related changes in pH occurred after changes in zooplankton densities (K. bostoniensis).

A similar effect of simazine on pH was reported by Schwartz et al. (1981). A temporary depression in pH occurred (from 9.0 to 7.4) within one week of treating a lake with 0.45 mg/L simazine.

Nitrogenous compounds (nitrate and ammonia) increased late in the experiment and were positively influenced by simazine. These trends may have been related to lysis of cells during natural phytoplankton population decline (Dinobryon) and simazine-induced mortality (Glenodinium, etc.). These changes did not influence zooplankton populations.

Ammonia is the primary nitrogenous end-product of decomposition by heterotrophic bacteria (Wetzel 1983). The dose-related nitrate increase may have been partially due to bacterial nitrification of ammonium ions, but this probably did not contribute significant amounts of nitrate. A dose-related increase in bacterial densities did not occur at Weeks 2 and 3 (see below). Ammonia concentrations were an order of magnitude lower than nitrate concentrations, so that it was unlikely that ammonium ion concentrations were driving the nitrification reaction. In addition,

if nitrification was significant, the intermediate of the reaction, nitrite, should have been present in a pattern similar to nitrate. Nitrite was not found in any such pattern and was usually in very low concentrations (below detection limit). Finally, the primary agent of nitrification in freshwater is Nitrobacter, which is less tolerant of low temperatures (Wetzel 1983). No further explanation was developed for the observed nitrate trends, however.

4.2 Phytoplankton

Total algal density and biovolume were negatively affected as simazine dose increased, but the effects varied among algal groups. Dinobryon spp. was by far the dominant phytoplankter at Week 0, with densities of about 2-4,000 cells/mL and about 50-55% of algal biovolume. Dinobryon was not affected by simazine. Densities of Dinobryon declined during the experiment to about 500 cells/mL by Week 3 and were very similar to densities in ambient water samples. This tolerance was noted by others; Dinobryon divergens was apparently insensitive to 0.50 mg/L atrazine (deNoyelles et al. 1982).

The ability of Dinobryon to ingest bacteria could explain the absence of an herbicide effect. Bird and Kalff (1986) found four Dinobryon species to be phagotrophic and major consumers of bacteria in a Canadian lake. These phagotrophic Dinobryon were concentrated at the metalimnion

with dim light. Dinobryon considered in the present study were collected and enclosed in the epilimnion with ample light. If Dinobryon autotrophy/phagotrophy is facultative, it might be expected that photosynthesis would dominate in ample light, and bactivory would dominate in low light or other conditions not amenable to autotrophy. If this is true, inhibition of photosynthesis by simazine may have had little effect on Dinobryon because energy and nutrients were simply obtained by bactivory.

Glenodinium and Trachelomonas were not in great abundance ($\leq$350 cells/mL), but were important to total biovolume because of their relatively large individual sizes (Figures 8 and 9). These two genera comprised about 30% of the algal biovolume at Week 0. Glenodinium was quite sensitive to simazine. Increasing dosage cancelled a natural increase in Glenodinium densities, so that the 1.0 mg/L microcosms contained significantly less cells than controls at Week 3. Abundance of Glenodinium spp. was found to decrease with atrazine concentration (deNoyelles et al. 1982).

Trachelomonas was not affected by simazine until Week 3, when densities in 1.0 mg/L decreased (Figure 9). Other simazine concentrations did not affect Trachelomonas. Simazine inhibition of autotrophy may have forced Trachelomonas to depend on storage products (paramylum)

until Week 3, when energy stores were depleted, and organisms died. Chlorophyllous euglenids, including Trachelomonas, are considered capable of saprobic nutrition (Kudo 1977). Assuming that autotrophy was completely inhibited by 1.0 mg/L simazine, an exclusively saprobic Trachelomonas population would not be expected to decrease at Week 3. Dissolved organic materials from phytoplankton cell death were probably abundant, as evidenced by nitrate and ammonia concentrations. The Trachelomonas population in 1.0 mg/L may have relied heavily on its storage products and could not continue on saprobic nutrition alone, once those storage products were depleted.

Miscellaneous coccoids were a major contributor to total algal numbers, but did not comprise as great a role in biovolumes due to their small individual size (Figure 10). The miscellaneous coccoids were counted as two separate size groups: 9-15 and 3-8 um diameter. The 9-15 um miscellaneous coccoids exhibited an overall decreasing trend in densities, but were not significantly affected by simazine. The 3-8 um miscellaneous coccoids remained at about the same densities through Week 2 and increased at Week 3. The lone exception was the 3-8 um cells in 1.0 mg/L simazine; these decreased about 50% between Weeks 0 and 2, and then increased slightly at Week 3.

Small miscellaneous coccoids were more abundant than the larger miscellaneous coccoids throughout the experiment. This difference between coccoid size groups increased over the course of the experiment at all doses. The larger miscellaneous coccoids were more severely affected by enclosure but the smaller miscellaneous coccoids may have been more affected by simazine dose (Figure 10).

The miscellaneous coccoids in this study probably included cryptomonads and other small flagellates capable of heterotrophic metabolism (Morgan and Kalff 1978, Fenchel 1982, Haas and Webb 1979). Heterotrophic flagellates are considered important bacterial grazers (Sherr and Sherr 1983) and would not be expected to be greatly affected by a photosynthesis inhibitor. Schwartz et al. (1981) reported a bloom of cryptomonad densities immediately following treatment of Ashurst Lake with 0.45 mg/L simazine. The authors suggested a relationship between the bloom and increased soluble organics from lysis of other cells and/or tolerance to simazine. Cryptomonas marsonii isolated from treatment ponds was found to be resistant to 0.50 mg/L atrazine after a 19 d incubation (deNoyelles et al. 1982). The relative insensitivity of the miscellaneous coccoids to simazine may have been related to heterotrophic metabolism of these cells.

Diatom densities exhibited a late bloom in the microcosms; this bloom did not occur in the ambient waters and was probably an artifact of enclosure. This enclosure effect may have been related to several factors, including: a) static conditions, b)increased availability of nutrients, presumably from cell lysis of other taxa (i.e., Dinobryon), c) availability of silica from the glass bottles, and d) provision of substrate for attachment. Because no visible growth occurred on the inner surfaces of the bottles, this last factor was probably not significant.

Diatoms 50-80 um in length had a density of about 500-1000 cells/mL until Week 3, when they increased to about 6000 cells/mL in control and 0.1 mg/L microcosms. This increase in enclosed diatom densities was greatly limited by the two higher concentrations of simazine. The larger Synedra spp. (~150 um in length) showed a similar trend. Other workers have reported an effect of 0.45 mg/L simazine on diatoms (Schwartz et al. 1981).

4.3 Bacteria

It was hypothesized that the herbicide-induced death of phytoplankton cells would have enhanced bacterial production. Bacterial densities were expected to be inversely related to phytoplankton densities and directly related to simazine concentration. However, bacterial densities did not appear to vary with either phytoplankton

or simazine. Densities in the microcosms ranged from 3 to 6 x 10^5 cells/mL during the experiment. Bacterial populations enclosed in microcosms did appear to maintain higher densities than ambient bacteria.

Large numbers of bacteria (several hundred) were occasionally observed in the loricas of dead Dinobryon and other cells. This clumping of bacteria contributed to data variability and the subsequent inability to discern any significant effects of simazine dose.

4.4 Zooplankton

Copepods

Cyclopoid nauplii and copepodid densities did not vary among simazine doses (Figure 16). Excluding ambient densities and the Week 2, 0.5 mg/L datum (one replicate microcosm), nauplii/copepodid densities ranged between about 160-300 organisms/L. No effect on copepod reproduction was anticipated because of the long life cycle and the lack of adult organisms. However, there was a potential for an effect on the nauplii/copepodid population. Hargrave and Geen (1970) reported that calanoid nauplii ingested cells no larger than 5 um in diameter. If this size range also applied to cyclopoid nauplii, the miscellaneous coccoids (3-8 um) and bacteria should have been an important food supply. The lack of a dose effect

on copepod nauplii and copepodids may have been due to the relative insensitivity of food items and/or copepods to simazine.

Adult copepods may have been excluded from the microcosms by use of a pump for sample collection. Nauplii and copepodids in microcosms did not mature to adulthood during the experiment; copepods have an extended development time. In addition, adult copepods may have been relatively rare in Stout's Pond during the experiment. Cyclopoid copepods undergo diapause in response to factors such as decreased temperatures and photoperiod (Wetzel 1983). The absence of adult cyclopoid copepods may have greatly reduced the potential for rotifer predation in the microcosms (Gilbert and Williamson 1978).

Ciliates

Ciliates were not present in all microcosms and were not affected by simazine dose. Codonella populations exhibited a temporary increase in density at Weeks 1 and 2 and may have been important at this time. The increase occurred in both ambient samples and microcosms, indicating that it was in response to natural conditions (temperature, photoperiod, etc.) and not to enclosure in microcosms. Codonella was the dominant zooplankter of Lake Oglethorpe in the winter (Pace and Orcutt 1981). Codonella has been reported to graze on small diatoms and chrysomonads (Bick

1972), but no obviously related trends were observed.

Rotifers

Numerically, rotifers were the major zooplankters in the microcosms. The four dominant species, Kellicottia bostoniensis, Keratella cochlearis, Polyarthra vulgaris, and Synchaeta pectinata are common in temperate fresh waters. Information exists regarding rotifer prey selectivity, food sizes, etc. and is summarized in Table 5. Some of this information is contradictory, and differences may be related to the various seasonal, habitat and experimental conditions of the cited studies.

The food size ranges and apparent preferences presented in Table 5 indicate some degree of resource partitioning between the dominant rotifers. Functional specialization for different foods is one of the natural mechanisms known to alleviate competition in coexisting rotifers (Dumont 1977). Keratella cochlearis is the only species capable of feeding on detritus/bacteria and apparently relies on it as an important food source; it can also eat small chrysomonads, cryptomonads and diatoms. Kellicottia bostoniensis eats these same cells but does not appear to feed on detritus/bacteria, and both species share an upper size limit of 10-12 um. Edmondson (1965) considered Kellicottia longispina and Keratella cochlearis to have a potential for competition, based on similar algal

TABLE 5. Rotifer Prey Items and Related Notes

Rotifer Spp.	Trophi Type(a)	Summary of Literature Information (b)	Literature Cited (c)
Kellicottia bostoniensis	M	-- (K. longispina reproduction) Chlorococcales 0; Volvocales 0; Chrysomonadales +; Cryptomonadales (+); diatoms +; detritus/bacteria ? Upper size limit = 10-12 um.	(1)
		-- (K. longispina reproduction) Stichococcus ++; Chrysochromulina ++. Potential for competition between K. longispina and Keratella cochlearis. Bacteria not considered important to reproduction.	(2)
		-- (K. longispina) Small Chrysomonads eaten, detritus/bacteria avoided.	(3)
		-- Filtration rate in mixed rotifer population = 0.25 uL/animal/hr.	(4)
		-- Filtration rate = 0.3 uL/animal/hr.	(5)
		-- Filtration rates = 0.85 uL/animal/hr (Chlamydomonas: 7.5 um); 0.77 uL/animal/hr (Rhodotorula: 6.0 x 2.5 um); 0.18 uL/animal/hr (Aerobacter: 2.5 x 0.5 um).	(6)
		-- Ingested heat-killed and live Chlamydomonas equally (no preference for live/dead).	(7)
Keratella cochlearis	M	-- (reproduction) Chlorococcales 0; Volvocales 0; Chrysomonadales ++; Cryptomonadales +; diatoms +?; detritus/bacteria ++ Upper size limit = 10-12 um. Green flagellates/microalgae occasionally ingested but of little nutritive value. One of species that consume mainly detritus with its associated bacteria.	(1)
		-- (reproduction) Stichococcus ++; Chrysochromulina ++; misc. colorless flagellates +. Bacteria of very little influence on reproduction.	(2)
		-- Eats small chrysomonads.	(3)
		-- Preferentially consumed heat-killed over live Chlamydomonas and exceeded Kellicottia longispina filtration rates by an order of magnitude.	(7)
		-- Filtration rates primarily determined by temperature, ingested wide range of chlorophyllous and achlorophyllous particles.	(8)
		-- No selection for/against flagellated/de-flagellated cells and fed on variety of cell types.	(9)

(a) M = Malleate (grinding, crushing), V = Virgate (piercing, sucking, grasping).

(b) Symbols related to reproductive studies: 0 = not ingested; (+) = ingested but little/no reproduction; + = medium reproduction; ++ = good reproduction. (After Pourriot 1977).

(c) (1) Pourriot (1977); (2) Edmondson (1965); (3) Nauwerck (1963); (4) Nauwerck (1959); (5) Haney (1973); (6) Bogdan et al. (1980); (7) Starkweather and Bogdan (1980); (8) Bogdan and Gilbert (1982); (9) Gilbert and Bogdan (1981); (10) Buikema et al. (1978); (11) Buikema et al. (1977); (12) Edmondson (1946); (13) Hutchinson (1967).

continued-

TABLE 5 (Cont'd.). Rotifer Prey Items and Related Notes

Rotifer Spp.	Trophi Type(a)	Summary of Literature Information (b)	Literature Cited (c)
Polyarthra vulgaris	V	-- (reproduction) Chlorococcales 0; Volvocales 0; Cryptomonadales ++; Chrysomonadales +; diatoms +; detritus/bacteria 0. One of the rotifer species considered exclusively phytophagous.	(1)
		-- (reproduction) Cryptomonas spp. ++, although not totally dependent on it. Cells smaller than Cryptomonas (<15 um) not stat. sig. to reproduction.	(2)
		-- Eats chrysomonads, flagellates and small Chlorococcales.	(3)
		-- (P. dolichoptera) Filtration rate in mixed rotifer populations = 1.79 uL/animal/hr.	(4)
		-- Ingested only Chlamydomonas and Euglena, with slight preference for Euglena.	(8)
		-- Did not ingest Aerobacter, Rhodotorulla (yeast) or Chlorella.	
		-- Fed about 2x more efficiently on flagellated cells than unflagellated cells (approx. 4-6 um diameter) and was more selective than Keratella.	(9)
		-- Dinobryon considered major food item, with Cryptomonas erosa and Chilomonas spp. Occasionally fed on Mallomonas and Euglena rubra. Cryptomonas preferred over Dinobryon, but Cryptomonas was relatively rare.	(10)
		-- Cultured on mixture of Chilomonas paramecium, Cyathomonas truncata and 3 Bodo spp. Vitamin B12, thiamine, biotin and pantothenic acid necessary. Correlations of Cryptomonas with P. vulgaris may be illusory and based on common vitamin requirements, not predator-prey relationship. Observed eating: Cyathomonas truncata, Chilomonas paramecium, Bodo variabilis, Bodo minimus, Bodo mutabilis. Also ate less of Euglena viridis and Chlamydomonas reinhardii. Fed most frequently on colorless flagellates and reproduction occurred when these were present.	(11)

(a) M = Malleate (grinding, crushing), V = Virgate (piercing, sucking, grasping).

(b) Symbols related to reproductive studies: 0 = not ingested; (+) = ingested but little/no reproduction; + = medium reproduction; ++ = good reproduction. (After Pourriot 1977).

(c) (1) Pourriot (1977); (2) Edmondson (1965); (3) Naumerck (1963); (4) Naumerck (1959); (5) Haney (1973); (6) Bogdan et al. (1980); (7) Starkweather and Bogdan (1980); (8) Bogdan and Gilbert (1982); (9) Gilbert and Bogdan (1981); (10) Buikema et al. (1978); (11) Buikema et al. (1977); (12) Edmondson (1946); (13) Hutchinson (1967).

continued-

TABLE 5 (Cont'd.). Rotifer Prey Items and Related Notes

Rotifer Spp.	Trophi Type(a)	Summary of Literature Information (b)	Literature Cited (c)
Synchaeta pectinata	V	-- (reproduction) Chlorococcales 0; Volvocales 0; Cryptomonadales ++; Chrysomonadales +; diatoms +; detritus/bacteria 0. One of the rotifer species considered exclusively phytophagous. Planktonic Synchaeta feed on a range from few um to >50 um.	(1)
		-- Eats small chrysomonads and small diatoms.	(3)
		-- Filtration rates zero or near zero on Aerobacter and Rhodotorula.	(6)
		-- (S. lakowitziana) Largest numbers occur at lowest temperatures (0-5 C) and males occur in populations at higher temperatures (>5 C).	(12)
		-- Eats Mallomonas, various protists and Keratella, according to cited papers.	(13)

(a) M = Malleate (grinding, crushing), V = Virgate (piercing, sucking, grasping).
(b) Symbols related to reproductive studies: 0 = not ingested; (+) = ingested but little/no reproduction; + = medium reproduction; ++ = good reproduction. (After Pourriot 1977).
(c) (1) Pourriot (1977); (2) Edmondson (1965); (3) Nauwerck (1963); (4) Nauwerck (1959); (5) Haney (1973); (6) Bogdan et al. (1980); (7) Starkweather and Bogdan (1980); (8) Bogdan and Gilbert (1982); (9) Gilbert and Bogdan (1981); (10) Buikema et al. (1978); (11) Buikema et al. (1977); (12) Edmondson (1946); (13) Hutchinson (1967).

food types. However, the apparent dependence of K. cochlearis on detritus/bacteria may serve to separate these species and permit the coexistence observed in this and other studies (i.e., Carlin 1943, as discussed in Hutchinson 1967).

Polyarthra and Synchaeta may be potential competitors, because they apparently ingest a wide range of similar sized cells. A difference between these two species is the potential predation of Keratella by Synchaeta. Conflicting evidence exists regarding Synchaeta's predatory abilities. The two species may also have distinctive preferences for cell types that are as yet unreported.

There is some suggestion that Polyarthra preferentially feeds on cells 15 um (i.e., Cryptomonas) or larger (Edmondson 1965). This may indicate a partial separation of Polyarthra from Kellicottia and Keratella in preferred foods.

Most of the information on Kellicottia is for the species longispina, but this information was considered applicable to K. bostoniensis. A general consensus is that Kellicottia feeds upon small chrysomonads; small diatoms and cryptomonads may also be important food items. Detritus and its associated bacteria do not seem to be part of Kellicottia's diet. However, a distinction can be made between fresh and old detritus, and Kellicottia was

reported to ingest freshly heat-killed and live Chlamydomonas equally well (Starkweather and Bogdan 1980).

Kellicottia bostoniensis densities declined over time for all simazine doses, but populations in higher simazine concentrations exhibited greater densities than controls; this effect was not related to reproduction. Population birth rates varied only with temperature during the experiment (Figure 23). K. bostoniensis mortality rates were lowered in the presence of simazine, meaning that the length of life for these organisms was greater than those in control microcosms. Simazine increased adult K. bostoniensis lifespans, but did not appreciably increase reproductive rates relative to organisms in the control microcosms.

The potential food items of K. bostoniensis were the miscellaneous coccoids (3-8 um). Comparison of miscellaneous coccoids (3-8 um) densities with K. bostoniensis (Figures 10b and 18) showed them to be unrelated. The 3-8 um miscellaneous coccoids were adversely affected by simazine, and cannot explain the beneficial effect upon K. bostoniensis. Comparisons of trends from other groups (bacteria, large miscellaneous coccoids) that might have been eaten by K. bostoniensis also do not provide any other obvious explanations.

The positive response of K. bostoniensis to 0.5 and

1.0 mg/L simazine can not be satisfactorily explained by the data collected in this study. Possible reasons for this response may include:

1. A response to oxygen levels. K. bostoniensis has been observed in almost anoxic conditions, is considered eurythermous and often hypolimnetic. K. bostoniensis may be best adapted to conditions of lower dissolved oxygen. Measured filtration rates for Kellicottia are generally an order of magnitude lower than those of Keratella and Polyarthra, despite its greater size. This indicates a lower metabolic rate, necessary for existence in low oxygen conditions. Population maxima observed at high dissolved oxygen may be responses to other factors (temperature, food, etc.).

2. Algal antibiosis. Various phytoplankton species have been demonstrated to release toxic extracellular materials (Fogg 1971), including various dinoflagellates (Taylor and Seliger 1979, Schantz 1979). Most notable of the poisonous dinoflagellates are marine species (i.e., Gonyaulax) responsible for "red tides." Marine Glenodinium species also contribute to red tides (Mountford 1979); this genus occurred in this study. However, no literature evidence regarding toxic freshwater Glenodinium was found.

If K. bostoniensis was sensitive to an extracellular product of Glenodinium in this study, the simazine-related

decline in Glenodinium would have benefited K. bostoniensis survival. Circumstantial evidence for such a relationship is the enhanced survival (decreased death rate) of K. bostoniensis coupled with the simazine dose effect on Glenodinium in this study.

The lack of a dose effect on other members of the zooplankton may indicate differential sensitivity of zooplankters to such a toxin, with K. bostoniensis being most sensitive. No effect of a Glenodinium toxin on other dominant phytoplankton seems to have occurred.

3. Unmeasured parameter(s). Simazine may have affected an unmeasured parameter that was beneficial to longevity of K. bostoniensis. Dissolved vitamins have been shown to be important for Polyarthra vulgaris reproduction (Buikema et al. 1977). Perhaps vitamins (and/or other compounds) play a similar role in K. bostoniensis nutrition and longevity.

If the K. bostoniensis trend was due to an effect of simazine on a non-food related parameter (oxygen, etc.), the resultant higher densities of K. bostoniensis would theoretically permit a greater grazing pressure on its food organisms relative to controls. A combined effect of photosynthetic inhibition by simazine and greater grazing pressure would then act upon the portion of the phytoplankton in the diet of K. bostoniensis. A dose-

related decline in mean values of miscellaneous coccoids (3-8 um) occurred, but was only slightly significant (p=0.09, Figure 10). The size class of phytoplankton <9 um had a similar dose-related trend that was marginally significant. It is not clear if an increased grazing pressure of K. bostoniensis contributed to these trends.

Keratella cochlearis feeds on the same types of cells as K. bostoniensis (chrysomonads, cryptomonads and diatoms <10-12 um), but also appears to rely on detritus and its associated bacteria. K. cochlearis showed no preference for flagellated or aflagellated cells, but did prefer recently heat-killed Chlamydomonas to live cells (Starkweather and Bogdan 1980, Gilbert and Bogdan 1981). K. cochlearis appears to be a more general grazer, ingesting a variety of cells and particles, especially detritus/bacteria, within an appropriate size range.

Keratella cochlearis was not significantly affected by simazine. Densities declined by roughly one half over the course of the experiment for all doses (Figure 19). This may have been a natural decline in K. cochlearis densities, caused by seasonal conditions (shortened photoperiod and lower temperatures). Ambient densities were too variable between weeks to adequately validate this hypothesis. The Week 3 ambient density was very low despite warmer temperature, and may indicate this natural decline.

The relative constancy of K. cochlearis densities between simazine dose levels was probably due to the emphasis of detritus/bacteria in the diet of K. cochlearis. Bacteria did not appreciably vary with dose. This may have reduced the potential for an effect of simazine on K. cochlearis via changes in photosynthetic food items.

Several authors have considered Cryptomonas to be the major food item of Polyarthra vulgaris (i.e., Pourriot 1977, Edmondson 1965), although Edmondson (1965) stated that P. vulgaris was not totally dependent on cryptomonads for reproduction to occur.

P. vulgaris rejected Cryptomonas ovata (15-18 x 20-80 um) (Buikema et al. 1977), but was observed eating Cyathomonas truncata (10-15 x 15-25 um), Chilomonas paramecium (10-15 x 15-25 um) and three Bodo species (5-15 um). P. vulgaris also fed less often on Euglena viridis (14-20 x 40-65 um) and Chlamydomonas reinhardii (3-5 x 10-15 um). The colorless flagellates were eaten most frequently and reproduction occurred when these species were available. The authors suggested that the correlation between Cryptomonas and P. vulgaris from field data (i.e., Edmondson 1965) is not solely due to trophic relations, but is due to a common requirement of the organisms for vitamin B_{12}.

Buikema et al. (1978) found P. vulgaris to prefer Cryptomonas to the flagellate Dinobryon, but the relative rarity of Cryptomonas could only explain two population peaks of P. vulgaris over a one year study. The authors concluded that Dinobryon was the major food for P. vulgaris and that Cryptomonas and Chilomonas were also eaten when available.

Polyarthra vulgaris seemed to be selective, in that it preferred flagellated cells (Gilbert and Bogdan 1981), especially Cryptomonas and Dinobryon, as well as others. Pouriott (1977) stated that P. dolichoptera-vulgaris also consumed diatoms, but that detritus/bacteria were not eaten. This avoidance of bacteria was also noted by Bogdan and Gilbert (1982).

Because Polyarthra have virgate trophi, their mode of feeding is less dependent on prey size than Kellicottia or Keratella. The coronal cilia create currents to bring in small organisms to the mouth, where they are consumed whole, but the trophi are used for piercing and sucking larger prey items. The upper size limit of cells ingested by P. vulgaris has not been determined, but is certainly greater than the 10-12 um limit of Keratella and Kellicottia. Edmondson (1965) stated that 15-20 um cells are ingested whole, while cells up to 35 um may be pierced and emptied by pumping. Edmondson also considered cells

smaller than 15 um to be of no statistical significance to P. vulgaris reproduction.

Polyarthra vulgaris was the only dominant rotifer to generally increase in densities during the experiment (Figure 20). Simazine-induced changes in the phytoplankton did not significantly affect P. vulgaris population densities. As mentioned earlier, the trends for P. vulgaris in the microcosms appeared to be a smoothed-out version of ambient densities and both were very similar to the temperature plot (Figure 2). It appeared that the P. vulgaris population trend was determined almost exclusively by temperature, and was not significantly affected by changes in the composition or densities of phytoplankton. Food item abundances may have been sufficient, even with a simazine effect, to not have affected P. vulgaris reproduction or mortality.

If Dinobryon was a major prey item of P.vulgaris in Stout's Pond, as it was in Pandapas Pond (Buikema et al. 1978), the increase in P. vulgaris during Week 3 may also have been related to this abundant food supply before or early in the experiment. The time lag of several weeks would have been due to the extended duration of development of P. vulgaris eggs in the low temperatures.

Synchaeta also has virgate trophi and feeds like Polyarthra. Where Polyarthra appears to be entirely

phytophagous (Pourriot 1977), Synchaeta has the potential for predation on other rotifers, although there is conflicting evidence on this issue. Pourriot (1977) considered Synchaeta to be exclusively phytophagous, while Hutchinson (1967) cited several papers stating that S. pectinata eats Mallomonas, various protists and Keratella. For purposes of this discussion, Synchaeta pectinata was considered to have the potential to prey on other rotifers.

Little information regarding phytoplankton in the diet of S. pectinata is available. Pourriot (1977) stated that Synchaeta species eat cryptomonads, chrysomonads and diatoms, but do not ingest detritus/bacteria (similar to Polyarthra), and that they feed on cells ranging from a few to >50 um in size.

Synchaeta pectinata was in relatively low abundance during the study, and demonstrated a general decline of approximately 50%. K. cochlearis followed a similar overall trend (50% decline), but this cannot be considered as proof of S. pectinata predation on K. cochlearis. Both species may simply have been responding to seasonal conditions and/or changes in other food items.

Simazine seems to have affected S.pectinata late in the experiment, with higher doses having fewer organisms. This may have been due to grazing by S. pectinata on phytoplankton (e.g., diatoms) sensitive to simazine. This

is also inconclusive and S. pectinata showed no statistically significant response to the phytoplankton changes induced by simazine.

5.0 SUMMARY

Physical/chemical parameters affected by simazine dosage were dissolved oxygen, pH, and concentrations of nitrate and ammonia. Temperature decreased greatly during the first week of the experiment, but returned to higher values thereafter.

The herbicide simazine had a differential effect on the phytoplankton. Some species were clearly not affected by the photosynthesis inhibitor (e.g., Dinobryon); others were definitely affected (Glenodinium, Trachelomonas, and diatoms); and some may or may not have been affected (miscellaneous coccoids). The larger phytoplankton cells (>9 um size class) were more severely affected by simazine than those <9 um in diameter (Figures 13 and 14). Comparison of the % biovolumes for these two size classes indicated a trend toward a greater percentage of cells <9 um, especially in the highest dose of simazine. The phytoplankters most responsible for this size class effect were the diatoms 50-80 um in length, Dinobryon, and the miscellaneous coccoids 9-15 um in length.

Bacterial densities did not increase in response to simazine-induced death of phytoplankton. The ciliate Codonella, copepod nauplii and copepodids, and the rotifers, Keratella cochlearis, Polyarthra vulgaris and Synchaeta pectinata did not show any significant response

to induced changes in phytoplankton composition. Rotifers were far more affected by temperature. Cladocera were rare during the experiment and were not analyzed.

The dominant rotifer Kellicottia bostoniensis exhibited a positive response to simazine dose. Possible explanations for this event included: Kellicottia's ability to tolerate lower oxygen levels present in microcosms at higher simazine concentrations; inhibition of the potentially toxic dinoflagellate Glenodinium by simazine; and release of beneficial materials (e.g., vitamins) upon induced death and lysis of phytoplankton.

In terms of the original hypothesis, the effect of simazine on phytoplankton did not indirectly reduce zooplankton numbers and maintained rather than altered zooplankton composition. All four of the major rotifer species were reported to feed on cells <9 um, and the two dominant species, Kellicottia bostoniensis and Keratella cochlearis, graze exclusively on this size class. Heterotrophic organisms (cells <9 um, bacteria) were probably the major food for these rotifers, linking the zooplankton more closely to the detrital food chain than to the autotrophic food chain. The effect of simazine on autotrophic phytoplankton was thus of little consequence to the rotifer-dominated zooplankton assemblage.

Related literature information is incomplete and variable. deNoyelles et al. (1982) studied the responses of plankton communities in experimental ponds to 0.02 and 0.5 mg/L atrazine, an herbicide very similar to simazine. The zooplankton communities were dominated in biomass by the cladoceran Diaphanosoma brachyurum and the cyclopoid copepod Tropocyclops prasinus mexicanus prior to atrazine addition. These species remained the dominant crustaceans during the study (136 days), "but were replaced as the dominant zooplankton by rotifers, principally Keratella cochlearis (Gosse), after day 31." No further data or analysis of these dominant zooplankton were presented by the authors. Nonetheless, it seems that the effect of atrazine on the phytoplankton community was of less significance to the rotifers than to the copepods and cladocerans.

Schwartz et al. (1981) reported no measurable effects on a zooplankton community of 0.45 mg/L simazine administered to an Arizona lake. Emphasis of the study was on phytoplankton and water chemistry: zooplankton data were inadequate for determining the presence of effects.

Dewey (1986) treated experimental ponds with a series of atrazine concentrations, the greatest being 0.5 mg/L. The structure and emergence of the aquatic insect community was examined. Nonpredatory insects were greatly reduced in

abundance with the addition of 0.02 mg/L atrazine, but predatory insects showed no response to the herbicide. The response was apparently indirect, and related to the close association and dependence of the nonpredatory (herbivorous-detritivorous) insects on the affected periphyton and macrophytes.

The present study and the above cited papers indicate that changes in the composition and abundance of primary producers may or may not affect populations of higher trophic levels (grazers, predators). This is dependent on the predator-prey relationships of the organisms in the higher trophic levels, i.e., the dependence of the grazers (and predators) on the affected prey organism(s). Cladocerans and copepods, traditionally considered to dominate the zooplankton, seem to be closely linked by their predator-prey relationships with the autotrophic phytoplankton. When the autotrophic phytoplankton are selectively impacted by a photosynthetic inhibitor, these crustaceans are also affected. However, zooplankton communities are a heterogenous composite of numerous organisms, variable in time and space. Rotifers and protozoans can also comprise a major portion of the zooplankton, and may not be closely linked by predator-prey interactions to autotrophic food items. The smaller prey items (colorless flagellates, cryptomonads, bacteria, etc.)

of planktonic "herbivorous" rotifers tend to have great capacity for heterotrophic metabolism and may not be significantly affected by autotrophic inhibition.

6.0 CONCLUSIONS

The following conclusions were made based on analysis of data from this study:

1. The inhibition of photosynthesis by simazine reduced dissolved oxygen content in the clear glass microcosms. A pH increase during the last week of the experiment was nullified by simazine, and dose-related increases in nitrate and ammonia concentrations occurred during the last week.

2. Phytoplankton were differentially sensitive to simazine. Sensitive taxa included Trachelomonas, Glenodinium, and diatoms, as well as several taxa of relatively minor significance. Dinobryon and miscellaneous coccoids were not significantly affected by simazine. The larger phytoplankton (>9 um) were more affected by simazine than the small (<9 um) phytoplankton.

3. Rotifers were the major zooplankters and are reported to feed on cells <9 um. The two dominant species (Kellicottia bostoniensis and Keratella cochlearis) graze exclusively in this size class. Cells of this size tend to be capable of heterotrophic metabolism and may not have been affected by simazine. The greatest effect of simazine, on the larger, autotrophic phytoplankton, was not transmitted via predator-prey relationships to the dominant zooplankton.

4. The trophic relationships of the plankton community resisted herbicide-induced changes to phytoplankton numbers and composition. Ciliates, copepod nauplii/copepodids, the rotifers Keratella cochlearis, Polyarthra vulgaris and Synchaeta pectinata, and bacteria did not respond significantly to the induced changes in the phytoplankton. Population parameters of the three dominant rotifer species were primarily affected by temperature. One rotifer species, Kellicottia bostoniensis, actually had lesser mortality in higher concentrations of simazine. This enhanced survival may have been due to: the ability of K. bostoniensis to tolerate low oxygen levels; release of essential materials (e.g., vitamins) upon death and lysis of phytoplankton and; simazine-induced death of Glenodinium if this freshwater dinoflagellate released toxic extracellular products.

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APPENDIX 1

TABLE A1.1 Physical/Chemical Data: Field Measurements[a]

Week	Dose[b]	Water Temp. (C)	pH	Dissolved Oxygen[c]	D.O. % Saturation
0	A	6.0 (---)	8.0 (---)	10.4 (---)	86.0 (---)
0	B	8.2 (---)	7.6 (---)	9.9 (---)	87.0 (---)
0	0.0	9.1 (0.2)	7.6 (0.0)	9.6 (0.1)	85.7 (0.5)
0	0.1	9.3 (0.0)	7.6 (0.0)	9.6 (0.0)	87.0 (0.0)
0	0.5	9.0 (0.5)	7.5 (0.1)	9.5 (0.1)	84.7 (0.5)
0	1.0	9.1 (0.2)	7.5 (0.1)	9.5 (0.0)	85.3 (0.5)
1	A	1.0 (---)	6.8 (---)	12.4 (---)	90.0 (---)
1	B	2.1 (---)	7.2 (---)	12.0 (---)	90.0 (---)
1	0.0	2.2 (0.1)	7.2 (0.2)	12.7 (0.1)	95.5 (0.5)
1	0.1	1.4 (0.1)	7.6 (0.1)	12.8 (0.2)	93.7 (1.7)
1	0.5	1.4 (0.3)	7.3 (0.3)	12.2 (0.8)	90.0 (5.7)
1	1.0	1.7 (0.3)	7.4 (0.2)	10.8 (0.2)	79.5 (0.5)
2	A	6.5 (---)	7.5 (---)	11.6 (---)	97.0 (---)
2	B	8.0 (---)	7.5 (---)	10.9 (---)	95.0 (---)
2	0.0	7.7 (0.9)	7.7 (0.1)	10.1 (0.2)	87.3 (3.3)
2	0.1	7.5 (0.5)	7.7 (0.0)	10.3 (0.9)	89.0 (9.0)
2	0.5	7.0 (---)	7.5 (---)	8.0 (---)	68.0 (---)
2	1.0	7.5 (0.5)	7.4 (0.1)	8.0 (0.2)	69.0 (1.0)
3	A	10.0 (---)	7.3 (---)	9.5 (---)	87.0 (---)
3	B	10.3 (---)	7.3 (---)	9.6 (---)	88.0 (---)
3	0.0	9.9 (0.1)	8.4 (0.0)	9.5 (0.1)	87.3 (0.9)
3	0.1	10.0 (0.0)	8.3 (0.0)	9.2 (0.1)	84.0 (1.4)
3	0.5	9.8 (0.2)	7.7 (0.0)	7.5 (0.2)	68.7 (1.9)
3	1.0	10.0 (0.1)	7.3 (0.0)	6.0 (0.2)	54.5 (2.5)

(a) Numbers in parentheses = std. dev. of replicate microcosms per dose per week. (---) indicates no std. dev. was calculated (single sample).
(b) Simazine concentration (mg/L); "A" = ambient surface water, "B" = sampled (bottled) ambient surface water.
(c) units = mg/L.

APPENDIX 1

TABLE A1.2 Physical/Chemical Data: Laboratory Measurements(a)

Week	Dose(b)	Conductivity (micromhos)	Hardness (mg/L as CaCO3)	Total Alkalinity (mg/L as CaCO3)	ortho-Phosphate (mg/L)	Nitrate (mg/L)	Nitrite (mg/L)	Ammonia (mg/L)	Sulfate (mg/L)
0	amb	68.0 (---)	25.0 (---)	28.1 (---)	<0.05 (---)	0.24 (---)	0.07 (---)	<0.05 (---)	1.60 (---)
0	0.0	70.4 (0.4)	25.0 (0.0)	29.1 (1.5)	<0.05 (---)	0.24 (0.01)	0.07 (0.00)	<0.05 (---)	1.60 (0.00)
0	0.1	67.3 (0.0)	25.0 (0.0)	25.5 (0.0)	<0.05 (---)	0.25 (0.00)	<0.05 (---)	<0.05 (---)	1.64 (0.00)
0	0.5	69.3 (1.5)	25.0 (0.0)	27.6 (0.7)	<0.05 (---)	0.25 (0.00)	<0.05 (---)	<0.05 (---)	1.57 (0.04)
0	1.0	70.7 (0.7)	25.0 (0.0)	28.1 (0.0)	0.05 (0.04)	0.27 (0.00)	0.08 (0.00)	<0.05 (---)	1.91 (0.41)
1	amb	65.0 (---)	25.0 (---)	28.1 (---)	<0.05 (---)	0.18 (---)	0.06 (---)	<0.05 (---)	1.60 (---)
1	0.0	70.6 (0.2)	25.0 (0.0)	29.7 (0.0)	<0.05 (---)	0.06 (0.00)	0.06 (0.00)	<0.05 (---)	1.60 (0.00)
1	0.1	70.3 (0.4)	25.0 (0.0)	29.7 (1.3)	<0.05 (---)	0.07 (0.02)	0.06 (0.01)	<0.05 (---)	1.61 (0.05)
1	0.5	69.8 (1.1)	25.0 (0.0)	29.2 (0.8)	<0.05 (---)	0.18 (0.02)	0.05 (0.00)	<0.05 (---)	1.64 (0.02)
1	1.0	71.2 (0.7)	27.5 (2.5)	29.7 (1.5)	<0.05 (---)	0.23 (0.01)	0.05 (0.00)	<0.05 (---)	1.68 (0.01)
2	amb	63.0 (---)	25.0 (---)	28.1 (---)	<0.05 (---)	0.18 (---)	0.06 (---)	<0.05 (---)	1.68 (---)
2	0.0	66.2 (0.3)	25.0 (0.0)	29.7 (0.0)	<0.05 (---)	<0.05 (---)	<0.05 (---)	<0.05 (---)	1.51 (0.01)
2	0.1	66.2 (0.1)	25.0 (0.0)	28.2 (1.5)	<0.05 (---)	<0.05 (---)	0.05 (0.00)	<0.05 (---)	1.62 (0.00)
2	0.5	65.9 (---)	25.0 (---)	29.7 (---)	<0.05 (---)	0.15 (---)	0.06 (---)	0.06 (---)	1.50 (---)
2	1.0	67.8 (0.5)	25.0 (0.0)	28.1 (0.0)	<0.05 (---)	0.23 (0.00)	0.06 (0.00)	0.06 (0.00)	1.62 (0.04)
3	amb	63.2 (---)	25.0 (---)	25.0 (---)	<0.05 (---)	0.36 (---)	<0.05 (---)	<0.05 (---)	1.52 (---)
3	0.0	67.2 (0.1)	25.0 (0.0)	26.7 (2.4)	<0.05 (---)	0.55 (0.03)	<0.05 (---)	<0.05 (---)	1.56 (0.05)
3	0.1	67.0 (0.6)	25.0 (0.0)	25.8 (3.1)	<0.05 (---)	0.59 (0.24)	<0.05 (---)	<0.05 (---)	1.59 (0.03)
3	0.5	67.8 (0.4)	25.0 (0.0)	26.7 (2.4)	<0.05 (---)	0.70 (0.09)	<0.05 (---)	0.07 (0.01)	1.69 (0.04)
3	1.0	67.9 (0.3)	25.0 (0.0)	27.5 (2.5)	<0.05 (---)	0.97 (0.01)	<0.05 (---)	0.10 (0.01)	1.59 (0.07)

(a) Numbers in parentheses = std. dev. of replicate microcosms per dose per week. (---) indicates no std. dev. was calculated (single sample or below detection limit).

(b) Simazine concentration (mg/L); "amb" indicates samples from ambient water at each week, collected by the same method used for microcosm preparations.

APPENDIX 2. Simazine Data

TABLE A2.1 Simazine Concentrations (mg/L)[a]

Target	Week 0	Week 3	Week 0+3 Mean	"Spiked"[b]
0.1[c]	0.077 (0.018)	0.115 (0.013)	0.096 (0.025)	0.129 / 129%
0.5[d]	0.535 (0.078)	0.595 (0.064)	0.565 (0.068)	0.700 / 140%
1.0[e]	1.157 (0.193)	1.015 (0.007)	1.100 (0.157)	1.170 / 117%

(a) Numbers in parentheses = std. deviations of samples from replicate microcosms. Concentrations were calculated against std. curve.
(b) Concentration / percent recovery.
(c) Measured from integrated peak areas. Std. curve correlation coefficient (r) = 0.983.
(d) Measured from peak heights. Std. curve (r) = 0.970.
(e) Measured from peak heights. Std. curve (r) = 0.995.

APPENDIX 3. Phytoplankton Data

TABLE A3.1 Phytoplankton Mean Densities (Cells/mL)(a)

Week	Dose(b)	Ankistrodesmus falcatus	Ankistrodesmus falcatus var. mirabilis	Diatoms (50-80 um long)	Diatoms (150 um long) (Synedra)	Dinobryon spp.	Glenodinium	Golenkinia
0	0.0	28.7 (11.0)	653.3 (208.7)	542.0 (36.0)	44.3 (31.8)	3156.3 (813.0)	131.0 (20.7)	32.3 (45.7)
0	0.1	13.7 (11.0)	724.0 (428.0)	356.3 (180.3)	14.0 (0.0)	2706.3 (998.9)	116.3 (25.7)	66.0 (24.7)
0	0.5	24.7 (6.9)	810.3 (214.8)	364.7 (35.6)	15.3 (12.7)	2277.7 (139.9)	100.3 (28.0)	50.3 (40.1)
1	1.0	57.7 (16.6)	628.3 (65.3)	459.0 (140.3)	15.7 (13.1)	4305.3 (2235.0)	158.3 (52.0)	91.0 (28.3)
1	amb	49.0 (---)	562.0 (---)	855.0 (---)	49.0 (---)	1343.0 (---)	61.0 (---)	126.0 (---)
1	0.0	77.5 (48.5)	864.5 (26.5)	854.0 (197.0)	51.0 (37.0)	2635.0 (309.0)	157.0 (11.0)	346.0 (57.0)
1	0.1	56.7 (2.4)	585.3 (96.4)	1023.3 (196.4)	55.7 (18.2)	2076.7 (337.8)	169.7 (46.0)	213.7 (51.4)
1	0.5	84.3 (16.2)	550.7 (398.3)	585.7 (233.7)	10.3 (14.6)	2973.7 (270.5)	159.7 (49.4)	102.7 (43.9)
1	1.0	28.5 (15.5)	722.0 (153.0)	560.5 (180.5)	35.5 (6.5)	4179.0 (435.0)	120.0 (26.0)	93.0 (93.0)
2	amb	18.0 (---)	350.0 (---)	876.0 (---)	114.0 (---)	1208.0 (---)	175.0 (---)	50.0 (---)
2	0.0	98.3 (34.0)	330.0 (58.9)	1383.3 (262.0)	122.0 (36.3)	922.3 (119.0)	190.0 (11.5)	135.0 (106.1)
2	0.1	84.5 (3.5)	318.5 (49.5)	1893.5 (535.5)	210.5 (4.5)	1513.0 (371.0)	306.5 (133.5)	72.0 (21.0)
2	0.5	46.0 (---)	411.0 (---)	1386.0 (---)	107.0 (---)	1158.0 (---)	76.0 (---)	210.0 (---)
2	1.0	32.0 (7.0)	353.0 (107.0)	768.0 (238.0)	37.5 (24.5)	1152.0 (363.0)	50.0 (24.0)	0.0 (0.0)
3	amb	92.0 (---)	132.0 (---)	1173.0 (---)	79.0 (---)	119.0 (---)	330.0 (---)	0.0 (---)
3	0.0	102.0 (46.4)	741.0 (256.0)	6162.7 (461.1)	363.3 (34.0)	477.7 (164.3)	197.0 (64.2)	148.3 (135.6)
3	0.1	54.7 (31.4)	965.0 (605.2)	5729.3 (2793.6)	239.7 (117.9)	387.7 (63.3)	210.7 (88.5)	114.3 (28.1)
3	0.5	25.7 (2.5)	773.7 (219.7)	3174.3 (584.5)	125.7 (27.9)	516.7 (27.4)	109.0 (26.7)	30.0 (21.6)
3	1.0	55.5 (28.5)	836.5 (361.5)	1789.0 (269.0)	54.5 (26.5)	489.5 (80.5)	0.0 (0.0)	30.5 (16.5)

(a) Numbers in parentheses = std. deviations of replicate microcosms per dose per week. (---) indicates no std. dev. was calculated (single sample).

(b) Simazine concentration (ng/L); "amb" indicates sampled ambient water from each week. Week 0 controls were used to represent Week 0 ambient.

continued -

APPENDIX 3. Phytoplankton Data

TABLE A3.1 (Cont'd.) Phytoplankton Mean Densities (Cells/mL) (a)

Week	Dose(b)	Micractinium	Misc. Coccoids (3-8 um dia.)	Misc. Coccoids (9-15 um dia.)	Oocystis	Scenedesmus	Selenastrum minutum	Trachelomonas
0	0.0	97.0 (137.2)	3640.7 (770.2)	948.0 (368.5)	48.0 (12.8)	104.7 (110.8)	156.3 (68.3)	208.7 (19.0)
0	0.1	143.7 (62.7)	2538.0 (671.3)	865.3 (52.6)	160.7 (102.8)	55.7 (60.6)	91.0 (55.4)	173.0 (12.0)
0	0.5	166.0 (165.6)	2856.0 (370.5)	1323.3 (540.1)	39.3 (27.9)	70.7 (50.0)	206.3 (35.6)	204.7 (69.1)
0	1.0	163.7 (71.0)	5074.7 (630.3)	1559.7 (690.7)	41.0 (29.1)	164.3 (122.4)	149.3 (16.4)	278.0 (120.2)
1	amb	547.0 (---)	3663.0 (---)	1010.0 (---)	0.0 (0.0)	49.0 (0.0)	168.0 (0.0)	98.0 (---)
1	0.0	392.0 (90.0)	3895.5 (233.5)	2217.0 (49.0)	28.5 (0.5)	106.5 (10.5)	372.5 (131.5)	301.5 (63.5)
1	0.1	331.3 (235.1)	4632.0 (1773.9)	1423.3 (723.8)	20.0 (28.3)	29.3 (24.5)	115.3 (48.6)	209.5 (1.5)
1	0.5	185.7 (131.7)	3130.3 (1262.4)	1173.7 (588.9)	140.7 (99.5)	158.3 (114.2)	161.3 (196.5)	249.7 (36.6)
1	1.0	186.0 (186.0)	3930.0 (204.0)	1403.5 (56.5)	13.5 (13.5)	100.5 (100.5)	215.0 (64.0)	225.0 (23.0)
2	amb	0.0 (---)	3473.0 (---)	604.0 (---)	35.0 (0.0)	0.0 (0.0)	26.0 (0.0)	184.0 (---)
2	0.0	226.0 (246.6)	3461.3 (1031.5)	1455.3 (533.2)	181.0 (256.0)	57.7 (81.6)	118.0 (66.4)	328.0 (13.0)
2	0.1	0.0 (0.0)	3669.5 (239.5)	1662.0 (318.0)	32.0 (19.0)	76.0 (17.0)	72.0 (21.0)	349.0 (107.0)
2	0.5	0.0 (---)	2679.0 (---)	946.0 (---)	105.0 (0.0)	105.0 (0.0)	105.0 (0.0)	289.0 (---)
2	1.0	67.0 (67.0)	2366.5 (353.5)	809.0 (38.0)	0.0 (0.0)	44.5 (44.5)	75.5 (13.5)	247.0 (12.0)
3	amb	0.0 (---)	4229.0 (---)	455.0 (---)	0.0 (0.0)	26.0 (0.0)	224.0 (0.0)	93.0 (---)
3	0.0	1183.0 (279.7)	5068.7 (802.7)	1114.7 (242.6)	61.0 (48.0)	32.3 (45.7)	218.7 (189.3)	225.3 (39.1)
3	0.1	378.3 (274.4)	5388.3 (2428.5)	688.7 (167.3)	33.0 (36.0)	31.7 (44.8)	397.7 (397.8)	191.3 (106.2)
3	0.5	79.3 (112.2)	3635.0 (949.1)	919.3 (439.7)	33.7 (47.6)	34.7 (33.0)	289.0 (175.9)	193.0 (34.7)
3	1.0	93.5 (93.5)	2761.0 (47.0)	655.5 (140.5)	28.0 (28.0)	68.5 (12.5)	244.5 (136.5)	68.5 (40.5)

(a) Numbers in parentheses = std. deviations of replicate microcosms per dose per week. (---) indicates no std. dev. was calculated (single sample).

(b) Simazine concentration (ug/L); "amb" indicates sampled ambient water from each week. Week 0 controls were used to represent Week 0 ambient.

APPENDIX 3. Phytoplankton Data

TABLE A3.2 Phytoplankton Mean Percent Biovolumes

Week	Dose(a)	Ankistro-desmus falcatus	A. falcatus var. mirabilis	Diatoms (50-80 um long)	Diatoms (150 um long)	Dinobryon spp.	Glenodinium	Golenkinia
0	0.0	0.49	0.83	1.00	0.68	56.93	17.74	0.04
0	0.1	0.29	0.97	0.70	0.28	54.14	18.25	0.08
0	0.5	0.48	1.23	0.79	0.33	50.15	15.89	0.06
0	1.0	0.71	0.71	0.65	0.20	55.68	16.32	0.07
1	0.0	0.98	0.89	1.27	0.73	42.73	17.34	0.29
1	0.1	0.95	0.78	1.95	1.01	40.54	24.76	0.23
1	0.5	1.31	0.65	1.00	0.19	52.98	20.86	0.10
1	1.0	0.34	0.74	0.83	0.48	61.20	12.73	0.08
2	0.0	1.87	0.51	3.06	2.60	21.16	31.78	0.16
2	0.1	1.24	0.36	2.93	3.41	24.33	34.08	0.07
2	0.5	1.14	0.82	3.93	2.89	33.70	16.18	0.34
2	1.0	0.94	0.77	2.38	1.04	36.53	11.30	0.00
3	0.0	1.92	1.08	12.91	7.21	10.41	30.24	0.17
3	0.1	1.14	1.73	13.33	5.16	10.49	34.84	0.19
3	0.5	0.69	1.62	9.46	3.53	15.81	25.55	0.05
3	1.0	3.29	3.94	11.74	3.21	32.02	0.00	0.12

(a) Simazine concentration (mg/L).

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APPENDIX 3. Phytoplankton Data

TABLE A3.2 (Cont'd.). Phytoplankton Mean Percent Biovolumes

Week	Dose(a)	Micractinium	Misc. coccoids (3-8 um)	Misc. coccoids (9-15 um)	Scenedesmus	Selenastrum minutum	Trachelomonas	Other(b)
0	0.0	0.04	3.75	8.00	0.04	0.21	10.00	0.25
0	0.1	0.05	2.88	8.18	0.02	0.14	13.89	0.14
0	0.5	0.06	3.53	12.71	0.04	0.34	13.95	0.44
0	1.0	0.04	4.48	9.73	0.06	0.17	10.92	0.25
1	0.0	0.09	3.26	14.82	0.04	0.42	16.30	0.83
1	0.1	0.11	4.93	12.05	0.01	0.16	11.72	0.78
1	0.5	0.05	3.00	8.93	0.07	0.19	9.98	0.67
1	1.0	0.05	3.22	9.13	0.03	0.24	10.20	0.73
2	0.0	0.09	4.27	14.41	0.03	0.19	19.08	0.78
2	0.1	0.00	3.44	12.00	0.03	0.10	17.27	0.73
2	0.5	0.00	4.30	12.15	0.07	0.22	21.75	2.51
2	1.0	0.04	4.44	11.97	0.04	0.19	28.58	1.79
3	0.0	0.41	6.01	10.69	0.01	0.33	17.30	1.30
3	0.1	0.14	7.23	7.60	0.01	0.65	15.52	1.95
3	0.5	0.04	6.05	11.92	0.02	0.61	22.61	2.02
3	1.0	0.11	10.12	19.57	0.10	1.25	10.35	4.21

(a) Simazine concentration (mg/L).
(b) Included Euglena, Chlorogonium, Mallomonas, Oocystis, Pediastrum, Phacus, Spirulina, Staurastrum, Treubaria. Also included Salpingoeca, a heterotrophic microflagellate attached to diatoms and Dinobryon.

APPENDIX 4. Bacteria Data

TABLE A4.1 Bacterial Mean Densities[a]

Week	Dose(b)	Mean Cells/mL
0	0.0	350333 (35270)
0	0.1	400474 (73898)
0	0.5	370963 (27986)
0	1.0	407986 (181873)
1	amb	266190 (---)
1	0.0	416004 (21295)
1	0.1	611390 (100530)
1	0.5	585649 (23149)
1	1.0	616252 (229177)
2	amb	229550 (---)
2	0.0	363984 (161873)
2	0.1	470049 (47242)
2	0.5	385830 (---)
2	1.0	317599 (9621)
3	amb	220880 (---)
3	0.0	451438 (66901)
3	0.1	380909 (132762)
3	0.5	333937 (60570)
3	1.0	542530 (69779)

(a) Numbers in parentheses = std. deviations of replicate microcosms per dose per week. (---) indicates no std. dev. was calculated (single sample).
(b) Simazine concentration (mg/L); "amb" indicates sampled ambient water from each week. Week 0 controls were used to represent Week 0 ambient.

APPENDIX 5. Zooplankton Data

TABLE A5.1 Copepod, Cladoceran, and Ciliate Mean Data[a]

Week	Dose(b)	Nauplii/ Copepodids Organisms/L	Total Cladocerans(c) Organisms/L	Codonella(d)
0	0.0	304.7 (73.5)	17.7 (17.8)	0.0 (0.0)
0	0.1	260.0 (56.4)	13.3 (9.4)	31.7 (25.0)
0	0.5	246.7 (20.9)	29.0 (17.7)	0.0 (0.0)
0	1.0	292.3 (24.9)	15.7 (5.9)	0.0 (0.0)
1	amb	28 (---)	0.0 (---)	37.0 (---)
1	0.0	239.5 (42.5)	21.5 (0.5)	36.0 (8.0)
1	0.1	227.0 (46.7)	17.7 (10.8)	24.3 (14.6)
1	0.5	251.0 (50.2)	7.3 (5.2)	24.7 (6.9)
1	1.0	201.0 (50.0)	5.5 (5.5)	28.0 (1.0)
2	amb	44 (---)	22.0 (---)	44.0 (---)
2	0.0	176.7 (25.5)	14.0 (7.1)	52.3 (29.6)
2	0.1	150.0 (26.0)	5.5 (5.5)	62.5 (4.5)
2	0.5	68.0 (---)	34.0 (---)	30.0 (---)
2	1.0	177.0 (19.0)	0.0 (0.0)	69.0 (30.0)
3	amb	365 (---)	10.0 (---)	0.0 (---)
3	0.0	242.0 (3.3)	17.0 (9.9)	9.3 (13.2)
3	0.1	218.0 (47.2)	25.0 (10.7)	3.7 (5.2)
3	0.5	207.3 (39.5)	25.7 (9.0)	9.7 (13.7)
3	1.0	249.0 (5.0)	5.0 (11.0)	47.0 (47.0)

(a) Numbers in parentheses = std. deviations of replicate microcosms per dose per week. (---) indicates no std. dev. was calculated (single sample).
(b) Simazine concentration (mg/L); "amb" indicates sampled ambient water from each week. Week 0 controls were used to represent Week 0 ambient.
(c) Primarily Daphnia parvula, but also Ceriodaphnia lacustris and Chydorus sphaericus.
(b) Miscellaneous ciliates were present in only six microcosms; all at different dose-time combinations.

APPENDIX 5. Zooplankton Data

TABLE A5.2 Rotifer Mean Data (a)

		Kellicottia bostoniensis			Keratella cochlearis			Polyarthra vulgaris		
Week	Dose(b)	Organisms/L	Eggs/L	Eggs/ Female	Organisms/L	Eggs/L	Eggs/ Female	Organisms/L	Eggs/L	Eggs/ Female
0	0.0	3093.3 (221.9)	817.0 (87.1)	0.26	2247.7 (53.1)	521.7 (42.0)	0.23	779.0 (33.4)	160.00 (7.3)	0.21
0	0.1	2974.3 (122.8)	793.0 (17.7)	0.27	2014.3 (124.2)	427.7 (64.5)	0.21	573.7 (54.3)	99.00 (5.7)	0.17
0	0.5	3079.0 (236.8)	768.8 (103.6)	0.25	2283.3 (187.3)	577.8 (80.4)	0.25	667.0 (30.3)	182.75 (67.9)	0.27
0	1.0	3464.3 (539.4)	871.3 (203.0)	0.25	2060.7 (365.3)	527.0 (118.6)	0.26	538.0 (147.9)	121.33 (76.4)	0.23
1	amb	9.0 (---)	0.0 (---)	0.00	270.0 (---)	19.0 (---)	0.07	130.0 (---)	0.00 (---)	0.00
1	0.0	2573.0 (323.0)	720.5 (43.5)	0.28	1872.5 (377.5)	398.0 (126.0)	0.21	454.5 (47.5)	140.00 (46.0)	0.31
1	0.1	2421.0 (360.2)	654.7 (84.3)	0.27	1493.7 (246.3)	249.7 (44.3)	0.17	324.7 (15.6)	80.67 (36.8)	0.25
1	0.5	2032.0 (333.3)	687.0 (69.5)	0.34	1524.0 (301.8)	270.3 (60.0)	0.18	418.7 (130.4)	86.00 (7.8)	0.21
1	1.0	2627.0 (24.0)	845.0 (18.0)	0.30	1390.0 (112.0)	247.0 (15.0)	0.18	432.0 (49.0)	121.00 (10.0)	0.28
2	amb	1158.0 (---)	393.0 (---)	0.34	1638.0 (---)	142.0 (---)	0.09	819.0 (---)	197.00 (---)	0.24
2	0.0	1022.3 (342.5)	143.0 (50.2)	0.14	1599.0 (315.4)	225.7 (57.4)	0.14	660.7 (46.6)	166.00 (48.1)	0.25
2	0.1	1385.5 (288.5)	272.5 (13.5)	0.20	1781.5 (135.5)	197.0 (10.0)	0.11	589.5 (71.5)	133.50 (1.5)	0.23
2	0.5	2348.0 (---)	558.0 (---)	0.24	1846.0 (---)	171.0 (---)	0.09	604.0 (---)	160.00 (---)	0.26
2	1.0	2425.0 (108.0)	463.5 (54.5)	0.19	1427.0 (226.0)	177.0 (9.0)	0.12	532.5 (113.5)	143.00 (13.0)	0.27
3	amb	830.0 (---)	118.0 (---)	0.14	267.0 (---)	10.0 (---)	0.04	928.0 (---)	267.00 (---)	0.29
3	0.0	319.0 (72.8)	65.7 (46.6)	0.21	1039.0 (156.1)	339.0 (53.7)	0.33	1101.7 (259.5)	263.33 (79.0)	0.24
3	0.1	443.3 (96.9)	87.0 (28.6)	0.20	982.3 (78.0)	326.3 (41.0)	0.33	1130.7 (208.5)	261.00 (64.3)	0.23
3	0.5	1328.0 (679.0)	225.3 (110.5)	0.17	1193.0 (83.4)	361.7 (74.6)	0.30	1139.3 (148.1)	234.00 (24.1)	0.21
3	1.0	1940.5 (20.5)	300.0 (5.0)	0.15	1539.5 (45.5)	289.5 (35.5)	0.19	970.5 (25.5)	147.50 (86.5)	0.15

(a) Numbers in parentheses = std. deviations of replicate microcosms per dose per week. (---) indicates no std. dev. was calculated (single sample).

(b) Simazine concentration (ug/L); "amb" indicates sampled ambient water from each week. Week 0 controls were used to represent Week 0 ambient.

continued -

TABLE A5.2 Rotifer Mean Data (a)

Week	Dose(b)	Synchaeta pectinata Organisms/L	Other (c) Organisms/L	Total Rotifers Organisms/L	Total Rotifer Eggs/L
0	0.0	226.0 (37.6)	47.0 (9.9)	6398.0 (258.9)	1529.3 (132.1)
0	0.1	215.0 (31.8)	20.0 (14.1)	5794.3 (170.7)	1320.0 (82.1)
0	0.5	245.5 (29.5)	11.7 (9.8)	6147.0 (404.7)	1465.3 (188.6)
0	1.0	370.3 (125.6)	40.3 (42.4)	6473.7 (427.8)	1519.7 (294.5)
1	amb	233.0 (---)	0.0 (---)	642.0 (---)	19.0 (---)
1	0.0	175.0 (55.0)	37.5 (6.5)	5102.5 (63.5)	1296.0 (135.0)
1	0.1	219.3 (57.2)	3.3 (4.7)	4465.3 (585.4)	988.3 (149.2)
1	0.5	169.0 (43.2)	15.0 (10.6)	3785.3 (445.9)	1043.3 (86.2)
1	1.0	157.0 (4.0)	5.0 (5.0)	4806.0 (43.0)	1213.0 (43.0)
2	amb	153.0 (---)	0.0 (---)	3768.0 (---)	732.0 (---)
2	0.0	121.0 (47.3)	3.0 (4.2)	3412.3 (317.1)	534.7 (56.6)
2	0.1	59.0 (7.0)	32.0 (12.0)	3853.0 (520.0)	613.5 (8.5)
2	0.5	103.0 (---)	0.0 (---)	4912.0 (---)	889.0 (---)
2	1.0	99.0 (31.0)	10.0 (0.0)	3733.5 (333.5)	783.5 (76.5)
3	amb	30.0 (---)	0.0 (----)	2065.0 (---)	395.0 (---)
3	0.0	159.7 (31.1)	36.5 (18.5)	2610.5 (270.5)	674.3 (36.0)
3	0.1	189.3 (27.5)	41.0 (1.0)	2939.0 (15.0)	650.7 (81.3)
3	0.5	84.3 (25.4)	13.0 (13.0)	3533.0 (958.0)	837.0 (12.8)
3	1.0	40.5 (10.5)	10.0 (10.0)	4501.5 (50.5)	737.0 (127.0)

(a) Numbers in parentheses = std. deviations of replicate microcosms per dose per week. (---) indicates no std. dev. was calculated (single sample).

(b) Simazine concentration (mg/L); "amb" indicates sampled ambient water from each week. Week 0 controls were used to represent Week 0 ambient.

(c) Included Anuraeopsis fissa, Brachionus calyciflorus, Euchlanis dilatata, Filinia terminalis, Lecane bulla, Trichocerca birostris, Lepadella ovalis.

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