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RESPONSE OF A WINTER PLANKTON FOOD WEB TO SIMAZINE

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Abstract—In situ microcosms of a winter plankton community were exposed for 21 d to 0.0, 0.1, 0.5 and 1.0 mg/L of the herbicide simazine, approximating persistent levels found after application in other systems. Physical-chemical parameters, phytoplankton, bacteria and zooplankton were quantified.

Simazine induced decreases in dissolved oxygen and pH, but induced increases in nitrate and ammonia levels compared to control microcosms. Phytoplankton were differentially affected by simazine. Sensitive taxa included *Trachelomonas*, *Glenodinium* and diatoms. Others, such as *Dinobryon* and small coccoids, were not significantly affected. Bacteria data were variable and did not exhibit changes related to phytoplankton densities or simazine.

Rotifers dominated the zooplankton and were also differentially affected by simazine. The dominant species, *Kellicottia bostoniensis*, exhibited a positive response to simazine, as did *Keratella cochlearis*, due to lesser mortality in higher concentrations of simazine. *Polyarthra vulgaris* was unaffected, but *Synchaeta pectinata* was impaired by simazine at day 21. Zooplankton (primarily rotifers) may have fed on heterotrophic cells more than on autotrophic cells, and may have been more closely associated with the detrital food chain than the autotrophic food chain.

Keywords—Simazine Plankton food web Rotifers Phytoplankton

INTRODUCTION

The triazine herbicide simazine is used for selective weed control in various crops and nonselectively for vegetation control of noncropland [1]. Aquatic habitats may receive simazine in μg/L quantities via runoff [2]. Simazine is also used to control aquatic algae and macrophytes [3,4]. Manufacturer-recommended application concentrations for water range from 0.5 to 2.5 mg/L.

Simazine is persistent in aquatic systems, especially shallow, well-mixed lakes and ponds. Schwartz et al. [5] applied simazine to Ashurst Lake, Arizona (mean depth, 4.1 m) at a concentration of 0.45 mg/L. Concentrations in the water column 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. [6] applied 0.1 to 3.0 mg/L simazine to ponds and found levels of about 10% original concentrations in the water after one year. Mixing of sediments and water by wind action was considered responsible for water column concentrations.

Simazine limits carbon assimilation and oxygen production by inhibiting the Hill reaction of photosynthesis [7,8]. Strictly autotrophic cells survive only as long as stored energy reserves (starches, oils) support metabolic activities. This action would theoretically affect phytoplankton species differently depending on whether they are obligate or facultative autotrophs and how much energy they store. Differential sensitivity of phytoplankton to triazines has been observed in laboratory microcosms [9] and field studies [5,10]. However, very little information exists concerning the impact of this differential sensitivity on other components of aquatic food webs, including zooplankton.

Laboratory experiments have examined the direct toxicity of simazine to zooplankton. Fitzmayer et al. [11,12] found that the 48-h LC50 for *Daphnia pulex* exceeded 50 mg/L, but 4 mg/L simazine reduced *D. pulex* growth, delayed reproductive maturity and lowered fecundity in a 26-d chronic toxicity test.

The purpose of this study was to investigate the direct and indirect effects of simazine on the plankton community of a small pond at concentrations expected in the water column months after herbicide use. It was hypothesized that simazine would indirectly reduce winter zooplankton numbers and

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alter zooplankton composition by reducing winter phytoplankton densities and changing phytoplankton composition.

MATERIALS AND METHODS

Study site

The study was conducted in Stout's Pond, a small impounded farm pond in Montgomery County, Virginia (W 80° 22′30″ 654.5 km E, N 37° 02′30″ 4100 km N). Stout's Pond was estimated to have 0.31 ha surface area, 260 m shoreline and 1.2 m mean depth [13]. Maximum depth was about 3 m. The pond has a soft mud bottom with high turbidity and minimal macrophytic growth. The surrounding watershed is mixed woods and pasture.

Study design

The experiment used in situ microcosms to provide replicated treatments under near-natural conditions and avoid contamination of the pond. Potential limitations of microcosms for studying plankton are their relatively high surface area-to-volume ratio and lack of mixing [14,15]. However, an appropriate experimental design and enclosure period can permit comparisons to natural communities [16,17].

A split plot design was used, with main effects being simazine concentration and time. Simazine concentrations of 0.0, 0.1, 0.5 and 1.0 mg/L were selected to approximate application rates and persistent levels found in other studies [5,6]. The experiment was conducted over a three-week period (1 to 22 December 1984): Preliminary, warm-weather experiments showed longer time periods permitted periphyton growth on the outside of microcosms that interfered with light penetration.

Forty-eight microcosms (sealed, four-liter clear glass bottles) were prepared. Three replicate microcosms per treatment were randomly selected for removal after 0, 7, 14 and 21 d in situ. Seven microcosms could not be used in the experiment because of breakage that presumably occurred during the first week when the pond was ice-covered. One microcosm was available for 0.5 mg/L simazine at day 14: No other treatment–time combinations had less than two replicate microcosms. Ambient water was sampled each time microcosms were collected to evaluate enclosure effects on plankton populations.

Microcosm preparation

All bottles were sequentially washed with soap and water, sulfuric acid, rinsed in distilled water and then acetone, and air dried. A stock solution of 1.0 g simazine/L was prepared with PRINCEP 4L (Ciba Geigy Corp., Greensboro, NC: liquid emulsion, 41.9% simazine in inert ingredients). Appropriate amounts of stock solution were added to microcosms prior to being filled with pond water; control microcosms received only pond water.

Pond water was obtained with an ITT-Jabsco "Water Puppy" flexible-impeller pump, powered by a 12-V marine battery. The pump permitted rapid collection of large volumes of water, with organism collection efficiencies comparable to a plankton net [18] and no detectable pump-induced mortality (D.G. Jenkins, personal observation). 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 which enlarged the intake area and weighted the end of the hose. Water was pumped from a depth of 20 to 30 cm into three 20-gal plastic trash cans so that all three cans filled almost simultaneously. The trash cans were lined with plastic bags that had been preleached with tap water for 2 d to reduce potential contamination of sampled water by leachates (plasticizers, etc.). Water in the trash cans was then continuously stirred 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 to reduce adsorptive loss of simazine.

Day 0 microcosms were prepared as above, but were transported directly to the laboratory for subsampling and analysis. All other microcosms were vertically suspended at 25 cm depth from three floats, constructed of metal pipe with a block of styrofoam at either end to minimize shading. This shallow placement was chosen because sealed microcosms containing ≥0.5 mg/L simazine and suspended below Secchi depth (46 cm on day 0) became anoxic after two weeks in preliminary, warm-weather experiments.

Microcosm sampling and chemical analyses

Dissolved oxygen, temperature and pH were measured immediately upon removal of each microcosm from the pond with a YSI 54A oxygen meter and Orion 407A pH meter, respectively. Field measurements were made between 10:00 a.m. and 12:00 noon to minimize diurnal differences in DO and pH. Upon return to the laboratory each sampling day, subsamples were collected from microcosms and frozen for subsequent water chemistry and simazine analyses.

Conductivity, total hardness and alkalinity, ni-

trate, nitrite, ammonia, soluble reactive phosphate (SRP) and sulfate were measured in the laboratory. Conductivity was measured with a YSI Model 32 conductance meter: Values were standardized to 25°C [19]. Hardness and alkalinity were measured titrimetrically [19] with modifications for smaller sample volumes. Nitrate and sulfate were measured by ion exchange chromatography on a Dionex ion chromatograph (Model 14U). Nitrite, ammonia and SRP were measured on a Perkin-Elmer Model 55E spectrophotometer [19].

Samples from the first and last weeks of the study (including controls) were analyzed for simazine to verify initial concentrations and to determine the extent of simazine degradation and loss. A 200-ml sample was extracted twice with methylene chloride. Water was removed from the methylene chloride by sodium sulfate column. The extract was then evaporated to about 1 ml and 10 ml benzene was added. Benzene was the preferred final solvent because of its low interference in analyses. The final extract volume for analyses was 2 ml benzene and was reached by a second evaporation.

Simazine analyses were conducted on a Camag high-performance thin layer chromatograph (HPTLC) with a Spectrophysics 4270 integrator. The HPTLC plates were prepared with a Camag Nanomat. Machine-integrated peak areas of the chromatograms were used to measure 0.1 mg/L samples. 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 standard curves for technical-grade simazine in benzene (simazine source: U.S. Environmental Protection Agency [EPA], Research Triangle Park, NC).

Organism analyses

Organisms were narcotized with club soda and preserved in sodium acetate-buffered 4% formalin [20]. Preserved samples were allowed to settle in the microcosm bottles for several days and concentrated by siphoning. Siphoned water was examined microscopically; on rare occasions a few phytoplankton cells were noted. No zooplankton were lost by this concentration technique.

Phytoplankton were identified and counted with a Diavert inverted microscope at 400 and 1,000×. A preserved sample was thoroughly stirred and a 1-ml aliquot removed. 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 were counted at 400×, and at least 50 fields counted at 1,000× for each sample. Only cells with intact cell membranes were counted. Densities for each taxa were calculated and expressed as number of cells/ml.

To determine the effect of simazine on food items, phytoplankton taxa were separated into two size classes: <9 and ≥9 μm diameter. It was hypothesized that this size classification might indicate changes in the distribution of available food items for major zooplankters. *Kellicottia bostoniensis* and *Keratella cochlearis* were the dominant zooplankters in this study. The upper size limit of prey items for the rotifers *Kellicottia* and *Keratella* is reported to be about 10 μm [21,22]. Other dominant species, *Polyarthra vulgaris* and *Synchaeta pectinata*, are reported to exhibit wider ranges of prey size and type [21,23]. The longest axis was used to classify taxa, such as the pennate diatoms, that are not accurately represented by diameter measurements. Also, the groups labeled as coccoids (3–8 and 9–15 μm) included various flagellate and nonflagellate cells that could not be reliably identified to genus after preservation.

Phytoplankton cell volumes were estimated using taxa dimensions and formulae of solid geometric shapes [24]. Percent algal volumes for each taxa were calculated from the ratios of taxa volume to total algal volume. Volumes and % algal volumes were also calculated for the size classes by summing taxa values within size classes.

Bacteria, another food source, were quantified by direct epifluorescent counts with a method similar to that of Porter and Feig [25]. Three replicate slides were prepared for each preserved sample, with 10 randomly selected fields of vision at 1,000× (Whipple grids) counted per slide. Total bacterial cell densities (cells/ml) were calculated, with all types of cells considered equally. The mean of these slide counts was used to represent that sample in analyses.

Zooplankton were counted with a Zeiss binocular microscope at 80 and 200× and a Sedgwick-Rafter counting chamber [26]. Only organisms judged to have been alive (based on the presence of intact internal organs) upon preservation were counted. Rotifer eggs attached to adults and separated eggs were counted. Separated eggs were identified to species by comparison with attached eggs. Three subsamples were counted from each preserved sample: The mean of these subsample

counts was used in statistical analyses. Densities of each species were calculated and expressed as numbers of organisms, or eggs, per liter. Proportions of dominant rotifer species were calculated from the ratios of each species' densities to total rotifer density. Egg ratios (*B*) per species were calculated as the number of eggs/number of adults (females). No male zooplankters were observed in any of the samples.

The instantaneous rates of population increase (*r*), birth rate (*b*) and mortality (*d*) were calculated for zooplankton according to the equations of Paloheimo [27]. The instantaneous rate of population increase (*r*) was calculated from the difference between population numbers over time and was reported per time interval. Species egg development times (*D*), necessary for calculation of (*b*), were calculated using regression data [28] and measured temperatures. The value (*b*) was calculated by using average (*B*) and (*D*) values per time interval, assuming an even change in those values during the interval [28].

Population parameters related to eggs were not calculated for *S. pectinata*. This species rarely carries its eggs [29], making enumeration of eggs very difficult.

Statistical analyses

Statistical analyses included analysis of variance (ANOVA) for tests of overall effects and one-tailed Dunnett's tests for pairwise comparisons of treatments to controls at sample dates [30,31]. Significant treatment-time ANOVA interaction terms are discussed below where appropriate. Analyses on percentage data were conducted with arcsine-transformed values; all other data were log-transformed before analysis [30]. ANOVA tests were conducted with the Statistical Analysis System [32].

RESULTS

Physical-chemical measurements

Five physical-chemical parameters were affected by dosage and time: temperature, dissolved oxygen (DO), pH, nitrate and ammonia. Data for these parameters and Secchi depths are presented in Table 1.

Water temperature decreased sharply at day 7 (Table 1): The pond was covered with about 10 to

Table 1. Physical-chemical parameters affected by simazine and time

Day	Simazine (mg/L)	Water temperature (°C)	pH	Dissolved oxygen % saturation	Nitrate (mg/L)	Ammonia (mg/L)
0	Ambient	8.2	7.6	87.0	0.24	ND[a]
0	0.0	9.1 ± 0.2	7.6 ± 0.0	85.7 ± 0.5	0.24 ± 0.01	ND
0	0.1	9.3 ± 0.0	7.6 ± 0.0	87.0 ± 0.0	0.25 ± 0.00	ND
0	0.5	9.0 ± 0.5	7.5 ± 0.1	84.7 ± 0.5	0.25 ± 0.00	ND
0	1.0	9.1 ± 0.2	7.5 ± 0.1	85.3 ± 0.5	0.27 ± 0.00	ND
7	Ambient	2.1	7.2	90.0	0.18	ND
7	0.0	2.2 ± 0.1	7.2 ± 0.2	95.5 ± 0.5	0.06 ± 0.00	ND
7	0.1	1.4 ± 0.1	7.6 ± 0.1	93.7 ± 1.7	0.07 ± 0.02	ND
7	0.5	1.4 ± 0.3	7.3 ± 0.3	90.0 ± 5.7	0.18 ± 0.02	ND
7	1.0	1.7 ± 0.3	7.4 ± 0.2	79.5 ± 0.5[b]	0.23 ± 0.01[c]	ND
14	Ambient	8.0	7.5	95.0	0.18	ND
14	0.0	7.7 ± 0.9	7.7 ± 0.1	87.3 ± 3.3	ND	ND
14	0.1	7.5 ± 0.5	7.7 ± 0.0	89.0 ± 9.0	ND	ND
14	0.5	7.0	7.5	68.0[b]	0.15	0.06
14	1.0	7.5 ± 0.5	7.4 ± 0.1	69.0 ± 1.0[b]	0.23 ± 0.00[b]	0.06 ± 0.00[c]
21	Ambient	10.3	7.3	88.0	0.36	ND
21	0.0	9.9 ± 0.1	8.4 ± 0.0	87.3 ± 0.9	0.55 ± 0.03	ND
21	0.1	10.0 ± 0.0	8.3 ± 0.1	84.0 ± 1.4	0.59 ± 0.24	ND
21	0.5	9.8 ± 0.2	7.7 ± 0.0[b]	68.7 ± 1.9[b]	0.70 ± 0.09	0.07 ± 0.01[b]
21	1.0	10.0 ± 0.1	7.3 ± 0.0[b]	54.5 ± 2.5[b]	0.97 ± 0.01[b]	0.10 ± 0.01[b]

Values are means ± SD. Values without SD represent single analyses. Secchi depths were as follows: day 0, 46 cm; day 7, 74 cm; day 14, 92 cm; day 21, 68 cm.
[a]Not detected at 0.05 mg/L; values set equal to 0.05 for statistical analyses.
[b]Significantly different from controls (Dunnett's test; $p = 0.01$).
[c]Significantly different from controls (Dunnett's test; $p = 0.05$).

15 cm ice with no snow cover. Temperatures at days 14 and 21 were close to initial temperature.

Dissolved oxygen data were converted to percent saturation to correct for temperature differences between measurements. Control microcosms maintained ≥85% saturation and were similar to ambient conditions (Table 1). Simazine significantly affected DO ($p = 0.0001$): 1.0 and 0.5 mg/L microcosms contained significantly less oxygen relative to controls ($p = 0.01$) within 7 and 14 d of treatment, respectively (Table 1).

Simazine significantly affected pH ($p = 0.002$), but the effect was delayed until day 21 (Table 1). The pH in control and 0.1 mg/L microcosms was elevated at day 21 whereas 0.5 and 1.0 mg/L microcosms did not exhibit this increase ($p = 0.01$).

Ammonia and nitrate were positively affected by simazine ($p = 0.002$ and 0.0006, respectively). At day 14, 1.0 mg/L microcosms had significantly more ammonia than controls ($p = 0.05$), and at day 21 both 0.5 and 1.0 mg/L microcosms exceeded control ammonia levels ($p = 0.01$). One mg/L microcosms had significantly more nitrate than controls at day 7 ($p = 0.05$) and days 14 and 21 ($p = 0.01$).

Simazine

Simazine concentrations in the microcosms did not significantly decrease during the field experiment ($p = 0.05$). Average measured concentrations (±SD) were 0.096 ± 0.025, 0.565 ± 0.068 and 1.100 ± 0.157 mg/L simazine. No simazine was detected in ambient water samples or control microcosms.

Phytoplankton

Twenty-five different algal taxa or groups were quantified. Dominant taxa were *Dinobryon*, *Glenodinium*, *Trachelomonas*, *Synedra* and coccoid cells 9 to 15 and 3 to 8 μm. Rare taxa (≤5% of total density or algal volume) were not considered in analyses other than total densities and volumes. 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%.

Dinobryon dominated the initial phytoplankton assemblage and was insensitive to simazine. A significant effect of simazine on *Dinobryon* percent total algal volume ($p = 0.003$) can be attributed to a reduction of other taxa by simazine (Table 2).

Except for *Glenodinium*, statistically significant effects of simazine on phytoplankton taxa did not occur until day 21. Densities and percent total algal volumes for phytoplankton taxa are presented in Table 2.

Glenodinium densities decreased with simazine and exhibited a significant simazine-time interaction ($p = 0.0002$ and 0.0001, 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. Densities in 1.0 mg/L simazine were significantly lower than controls at days 14 and 21 ($p = 0.01$). Densities in 0.5 mg/L microcosms were also significantly lower than controls at day 21 ($p = 0.05$). The same basic trends were found in percent algal volumes (Table 2).

Trachelomonas densities were significantly affected by simazine and time (both $p = 0.02$), but only the 1.0 mg/L microcosms at day 21 exhibited *Trachelomonas* densities significantly different from control microcosms ($p = 0.01$; Table 2). Changes in *Trachelomonas* percent algal volume were not related to simazine, but were dependent on time ($p = 0.02$) and simazine-time interaction ($p = 0.04$). This effect on percent total algal volume was probably related to changes in other taxa: Values did not closely resemble the trends of *Trachelomonas* densities and were not monotonic.

Neither size class of coccoid cells (3–8 and 9–15 μm diameter) was significantly affected by simazine. Small (3–8 μm) cells were about three times more numerous than 9 to 15 μm coccoid cells and outnumbered all taxa in the experiment (except 50–80 μm diatoms in control microcosms at day 21).

Several genera of diatoms were present in two size ranges: 50 to 80 μm and 150 μm length (Table 2). The 50 to 80 μm diatoms were strongly affected by time and marginally affected by simazine ($p = 0.0001$ and 0.077, respectively). Densities of small diatoms in control and 0.1 mg/L microcosms increased sharply at day 21 relative to ambient densities. This effect of enclosure was significantly impaired by 0.5 and 1.0 mg/L simazine ($p = 0.05$ and 0.01, respectively) and was probably related to the coincident changes in pH (Table 1).

Synedra (150 μm diatom) showed a trend similar to that of small diatoms (Table 2): Densities changed with time ($p = 0.0009$) and possibly with simazine ($p = 0.073$). However, mean densities at day 21 were not significantly different from controls. *Synedra* percent algal volumes exhibited a simazine effect similar to that of densities (Table 2).

Phytoplankton taxa/groups were combined into two size classes: ≥9 μm and <9 μm (see Table 2).

Table 2. Effects of simazine on phytoplankton mean densities (cells/ml) and mean % algal volumes

Phytoplankton taxa	Simazine (mg/L)	Mean densities (mean % algal volumes) Day 0	Day 7	Day 14	Day 21
Cells >9 μm					
Coccoids (9–15 μm)	Ambient	948[a]	1,010	604	455
	0.0	948 (8)	2,217 (15)	1,455 (14)	1,115 (11)
	0.1	865 (8)	1,423 (12)	1,662 (12)	689 (8)
	0.5	1,323 (13)	1,174 (9)	946 (12)	919 (12)
	1.0	1,560 (10)	1,404 (9)	809 (12)	656 (20)
Diatoms[b] (50–80 μm)	Ambient	542	855	876	1,173
	0.0	542 (1)	854 (1)	1,383 (3)	6,163 (13)
	0.1	356 (1)	1,023 (2)	1,894 (3)	5,729 (13)
	0.5	365 (1)	586 (1)	1,386 (4)	3,174[c] (10)
	1.0	459 (1)	560 (<1)	768 (2)	1,789[d] (12)
Dinobryon sp.	Ambient	3,156	1,343	1,208	119
	0.0	3,156 (57)	2,835 (43)	922 (21)	478 (10)
	0.1	2,706 (54)	2,077 (40)	1,513 (24)	389 (10)
	0.5	2,278 (50)	2,974 (53)	1,158 (34)	517 (16)
	1.0	4,305 (56)	4,179 (61)	1,152 (36)	490 (32)
Glenodinium sp.	Ambient	131	61	175	330
	0.0	131 (18)	157 (17)	190 (32)	197 (30)
	0.1	118 (18)	170 (25)	308 (34)	211 (35)
	0.5	100 (16)	160 (21)	76 (16)	109[c] (26)
	1.0	158 (16)	120 (13)	50[d] (11)	0[d] (0)[d]
Synedra sp. (150 μm)	Ambient	44	49	114	79
	0.0	44 (<1)	51 (<1)	122 (3)	363 (7)
	0.1	14 (<1)	56 (1)	210 (3)	240 (5)
	0.5	15 (<1)	10 (<1)	107 (3)	126 (4)
	1.0	16 (<1)	36 (<1)	38 (1)	54 (3)
Trachelomonas sp.	Ambient	209	98	184	93
	0.0	209 (10)	302 (16)	328 (19)	225 (17)
	0.1	173 (14)	210 (12)	349 (17)	191 (16)
	0.5	205 (14)	250 (10)	289 (22)	193 (23)
	1.0	278 (11)	225 (10)	247 (29)	68[d] (10)
Others[e]	Ambient	844	660	403	250
	0.0	844 (2)	1,084 (3)	676 (3)	950 (4)
	0.1	960 (1)	740 (3)	512 (2)	1,095 (4)
	0.5	945 (2)	937 (3)	667 (4)	884 (4)
	1.0	891 (2)	864 (2)	430 (3)	989 (11)
Total	Ambient	5,874	4,076	3,564	2,499
	0.0	5,874 (96)	7,500 (96)	5,076 (95)	9,491 (93)
	0.1	5,192 (97)	5,699 (95)	6,448 (96)	8,544 (90)
	0.5	5,231 (96)	6,091 (97)	4,629 (95)	5,922[d] (93)
	1.0	7,667 (95)	7,388 (96)	3,494 (95)	4,046[d] (88)[c]

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Total algal volume was also analyzed with combined size class data. All taxa/groups included in the larger size class were chlorophyllous, with the possible exception of some members of the 9 to 15 μm coccoids, which may have included some nonpigmented flagellates. The smaller size class was dominated by the 3 to 8 μm coccoid cells (Table 2). It is likely that these coccoid cells also included some nonpigmented flagellates.

Densities of phytoplankton ≥9 μm were significantly related to time and simazine-time interaction ($p = 0.007$ and 0.009, respectively), but not to simazine alone. The absence of a significant simazine effect may have been a result of the significant variance among replicate microcosms ($p = 0.004$) for the pooled size-class data. However, 0.5 and 1.0 mg/L simazine significantly limited densities of the ≥9 μm size class on day 21 relative to controls ($p = 0.01$; Table 2).

Phytoplankton <9 μm were also significantly affected by time and simazine-time interaction (both $p = 0.04$). Again, no overall simazine effect

Table 2. continued.

Phytoplankton taxa	Simazine (mg/L)	Mean densities (mean % algal volumes) Day 0	Day 7	Day 14	Day 21
Cells <9 μm					
Coccoids (3–8 μm)	Ambient	3,641	3,663	3,473	4,229
	0.0	3,641 (4)	3,896 (3)	3,461 (4)	5,069 (6)
	0.1	2,538 (3)	4,632 (5)	3,670 (3)	5,388 (7)
	0.5	2,856 (4)	3,130 (3)	2,679 (4)	3,635 (6)
	1.0	5,075 (5)	3,930 (3)	2,366 (4)	2,761 (10)
Others[f]	Ambient	353	883	1,636	1,588
	0.0	353 (<1)	1,135 (<1)	1,636 (<1)	2,706 (1)
	0.1	359 (<1)	660 (<1)	1,455 (<1)	1,720 (1)
	0.5	507 (<1)	482 (<1)	998 (<1)	1,116 (1)
	1.0	551 (<1)	564 (<1)	1,018 (<1)	1,094 (2)
Total	Ambient	3,994	4,546	5,109	5,817
	0.0	3,994 (4)	5,031 (4)	5,097 (5)	7,775 (7)
	0.1	2,897 (3)	5,292 (5)	5,125 (4)	7,108 (9)
	0.5	3,363 (4)	3,612 (3)	3,677 (5)	4,751[c] (7)
	1.0	5,626 (5)	4,494 (4)	3,384 (5)	3,855[c] (12)[c]

[a]Ambient densities at day 0 were estimated from means of 0.0 mg/L microcosms at day 0. Other ambient densities represent single samples. Day 14, 0.5 mg/L densities represent single microcosm.
[b]Primarily a *Synedra* sp. and a *Nitzchia* sp., but also *Asterionella*, *Gomphonema* and naviculoid species.
[c]Significantly different from controls (Dunnett's test; $p = 0.05$).
[d]Significantly different from controls (Dunnett's test; $p = 0.01$).
[e]Included *Ankistrodesmus falcatus*, *Ankistrodesmus falcatus* var. *mirabilis*, *Euglena* sp., *Oocystis* sp. and *Scenedesmus* sp.
[f]Included *Golenkinia* sp., *Micractinium* sp., *Selenastrum minutum* and *Trachelomonas volvocina*.

was detected for this pooled data, but 0.5 and 1.0 mg/L microcosms had significantly fewer cells <9 μm than controls at day 21 ($p = 0.05$).

Bacteria

Bacteria were not significantly affected by simazine concentration or simazine-time interaction. Bacteria were present in all microcosms at a relatively narrow range of densities: 3 to 6 × 10^5 cells/ml, but data were variable. Coefficients of variation between 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 resulted from patchy distribution of bacteria in samples. For example, some microscopic fields of view revealed hundreds of bacteria clustered in *Dinobryon* tests, whereas only a few unattached bacteria were observed in other fields of view.

Zooplankton

Three cladoceran species were present in ambient samples and microcosms: *Daphnia parvula*, *Chydorus sphaericus* and *Ceriodaphnia lacustris*. However, these species were not present in 30% of all samples and were rare when present: Average densities (organisms/L ± SD) were 15.8 ± 9.6 for microcosms and 12.4 ± 9.6 for ambient samples. No analyses were conducted on these data.

Cyclopoid copepods were present in ambient samples and microcosms primarily as nauplii and some copepodids; adult copepods were rare. Nauplii and copepodids comprised a relatively small portion of zooplankton in the microcosms, with mean densities of about 281 organisms/L at day 0 (Table 3). Simazine and simazine-time interaction did not significantly affect copepod populations, but time was significant ($p = 0.0001$). This variation in copepod densities may have been caused by a sampling error during microcosm preparation.

The ciliate *Codonella* was counted and other miscellaneous ciliates were noted when present. *Codonella* was not observed in 25% of the samples and miscellaneous ciliates were never common. *Codonella* densities increased at days 7 and 14; a maximum mean density of about 70 organisms/ml occurred at day 14. Microcosm densities resembled ambient densities, indicating that the increase in *Codonella* at days 7 and 14 was a natural event

Table 3. Immature cyclopoid copepod mean densities (±SD)

Treatment (mg/L)	Nauplii and copepodids/L			
	Day 0	Day 7	Day 14	Day 21
Ambient	296[a]	44	254	365
0.0	296 (11.7)	204 (7.5)	185 (41.8)	224 (13.0)
0.1	247 (44.0)	104 (76.3)	138 (14.0)	218 (47.2)
0.5	257 (18.0)	244 (55.5)	246[b]	207 (97.5)
1.0	324 (54.8)	142 (37.5)	225 (13.0)	214 (4.5)

[a]Ambient densities at day 0 were estimated from the mean of 0.0 mg/L microcosms at day 0. Other ambient densities represent single samples.
[b]Day 14, 0.5 mg/L value represents a single microcosm.

and not an enclosure effect. Densities generally dropped again at day 21 to about the same numbers as day 0 (≤10 organisms/ml).

Rotifers were the largest component of the zooplankton in the microcosms and pond. Four rotifer species were dominant: *K. bostoniensis*, *K. cochlearis*, *P. vulgaris* and *S. pectinata*. Seven other genera were present, but were rare and never exceeded 2% of the total rotifer abundance when combined. No graphical or statistical analyses were considered for rare taxa, but their numbers were included in analyses of total rotifer abundance.

An overall decline in total rotifer densities occurred in ambient samples and microcosms and thus was not an enclosure effect (Fig. 1). Low ambient densities at day 7 (collected near the surface) were probably related to the very cold surface water (about 2°C) and ice cover at that time.

Total rotifer abundance was significantly affected by simazine and time ($p = 0.014$, 0.0001, respectively). Simazine-time interaction was marginally significant ($p = 0.058$). All treatment levels exhibited a decline in rotifer densities with time, and an increased separation among levels over time (Fig. 1). However, the densities in control and 0.1 mg/L microcosms declined more than densities in 0.5 and 1.0 mg/L microcosms. At days 14 ($p = 0.05$) and 21 ($p = 0.01$), the 0.5 and 1.0 mg/L microcosms had significantly greater rotifer densities than the control and 0.1 mg/L microcosms (Fig. 1).

K. bostoniensis comprised almost 50% of the total rotifer assemblage at day 0, with densities of about 3,000 organisms/L. Densities declined in a linear fashion for all simazine levels, but with different slopes, so that a significant separation of doses occurred over time (Fig. 1). Simazine, time and simazine-time interaction were statistically significant factors in *K. bostoniensis* population trends ($p = 0.006$, 0.0001 and 0.014, respectively).

Microcosms containing 0.5 and 1.0 mg/L simazine had significantly higher densities of *K. bostoniensis* than control microcosms at days 14 ($p = 0.05$) and 21 ($p = 0.01$). *K. bostoniensis* was positively affected by 0.5 and 1.0 mg/L simazine relative to control and ambient conditions and was responsible for the positive effect of simazine on total rotifer densities.

K. cochlearis was abundant during the experiment but enclosed populations exhibited an overall decline during the experiment (Fig. 1). Time was the only significant factor in the population trends of *K. cochlearis* ($p = 0.0001$); simazine and simazine-time interaction were not significant. However, *K. cochlearis* in 1.0 mg/L microcosms were significantly more abundant than in control microcosms at day 21 ($p = 0.01$).

P. vulgaris was the only one of the four dominant rotifers to exhibit an overall increase in population numbers (Fig. 1). Time was the only significant experimental factor affecting *P. vulgaris* populations ($p = 0.0001$). Ambient and enclosed populations exhibited trends very similar to the temperature trend (Fig. 1).

S. pectinata was the least abundant of the four dominant rotifer species. Densities of *S. pectinata* were affected by time ($p = 0.0001$) and generally declined during the experiment (Fig. 1). Simazine-time interaction was significant ($p = 0.007$), because of depressed densities in 0.5 and 1.0 mg/L microcosms ($p = 0.01$) at day 21.

Rotifer species trends were compared as proportions of total rotifer numbers (Fig. 2). The trends in 0.1 mg/L microcosms were virtually identical to those in control microcosms and are omitted in Figure 2. In control and 0.1 mg/L microcosms, *K.*

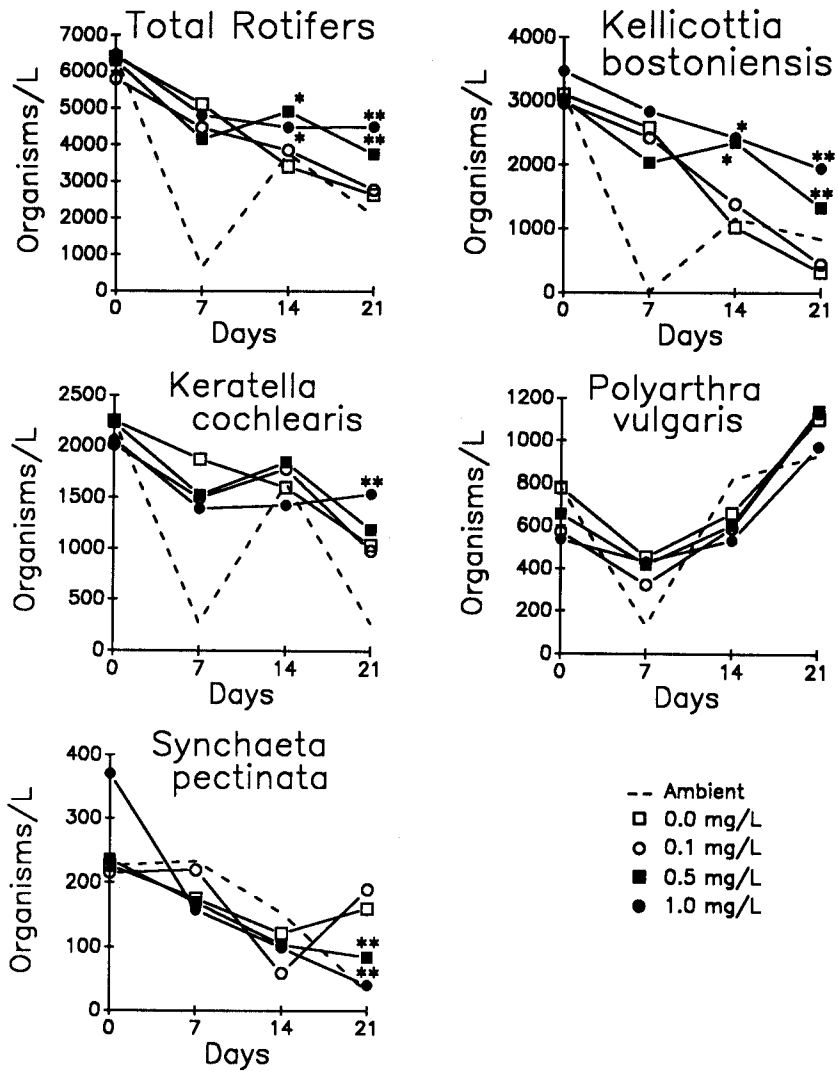

Fig. 1. Rotifer mean densities as a function of simazine concentration. *, significantly different from controls ($p = 0.05$); **, significantly different from controls ($p = 0.01$).

bostoniensis and *P. vulgaris* changed positions of dominance by day 21. This change in dominance was delayed as simazine increased (Fig. 2). Because *P. vulgaris* populations did not vary with simazine or simazine-time interaction, the response of *K. bostoniensis* to simazine treatment was responsible for maintained rotifer composition. *K. cochlearis* and *S. pectinata* maintained about the same percent composition throughout the experiment.

The positive response of *K. bostoniensis*, and to a lesser extent *K. cochlearis*, was caused by a reduction in the death rate of this species as simazine concentration increased (Table 4). *K. bostoniensis* birth rates were influenced only by changes in temperature (time; Table 4). Lower mortality provided *K. bostoniensis* a relative advantage over rotifer species that exhibited less response to simazine, enabling *K. bostoniensis* to maintain its dominance of the zooplankton assemblage.

DISCUSSION

Physical-chemical parameters affected by simazine were dissolved oxygen, pH, nitrate and ammonia. Simazine-related decreases in dissolved oxygen

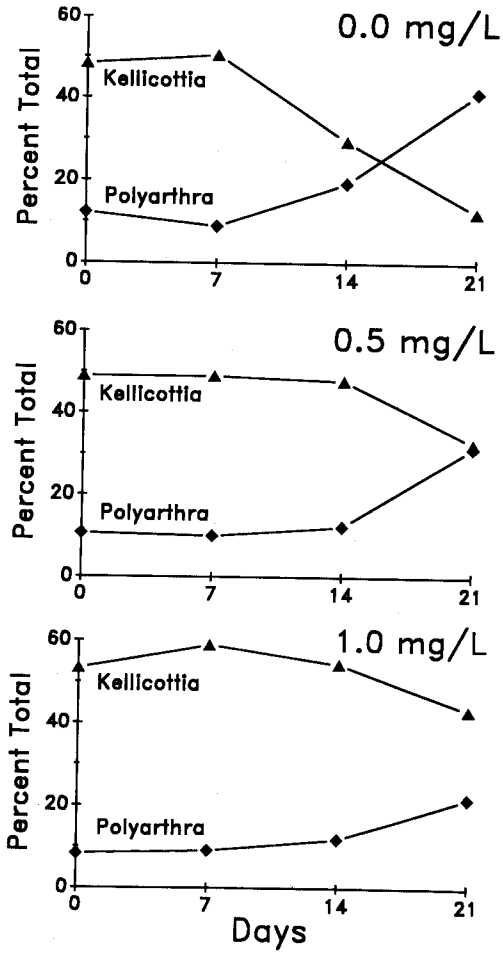

Fig. 2. Percent total rotifer densities for *K. bostoniensis* and *P. vulgaris*. 0.1 mg/L % densities were virtually identical to 0.0 mg/L.

indicate photosynthesis was inhibited. Changes in pH, nitrate and ammonia relative to control microcosms were probably related to photosynthesis inhibition, phytoplankton cell death and lysis.

Similar results have been observed in simazine treatments of ponds and a lake [4,5], indicating that the microcosms were comparable to natural systems for this three-week experiment. Plankton communities enclosed in small, closed containers will not mimic planktonic conditions indefinitely [14,15] because of the absence of mixing and the presence of a large surface area. These limitations were taken into account in the design of this experiment by collecting ambient samples and replicated treatments through time and by ending the experiment at an appropriate point as indicated by preliminary experiments. The effect of enclosure on some phytoplankton densities at day 21 and the coinciding changes in pH indicate that the microcosms were becoming less representative of ambient, planktonic conditions by the end of the experiment. We feel that this late divergence from ambient conditions for some enclosed populations does not impair interpretation of the effects of simazine on these populations.

Simazine differentially affected phytoplankton species. *Glenodinium* was severely affected by simazine, with no viable cells present in 1.0 mg/L microcosms by day 21. Other taxa were less sensitive, exhibiting significant effects only at day 21, or not at all (Table 2). This mixed response may be related to heterotrophic abilities of some phytoplankters (e.g., *Dinobryon* [33]), presumably enabling them to be relatively insensitive to photosynthesis inhibitors. In addition, the delayed response of some phytoplankton taxa to simazine may have been related to low temperatures during the first week of the experiment.

Phytoplankton size classes (≥ 9 and <9 μm) reflected this mixed response, exhibiting significant effects of simazine only at day 21. Cells <9 μm gained in their proportion of algal biovolume at day 21 in 1.0 mg/L microcosms, despite a significant reduction of densities due to simazine at that time (Table 2). This result was apparently due to reductions in large taxa such as *Glenodinium*.

The phytoplankters most important as food to the dominant zooplankton (*K. bostoniensis* and *K. cochlearis*) were the cells <9 μm. Bacteria were also potential food for these zooplankters. Coccoid cells (3–8 μm) and others <9 μm did not exhibit significant simazine effects; effects were significant only at day 21 for the pooled category of total cells <9 μm (Table 2). This relative absence of effects on phytoplankton prey items, in combination with the lack of measurable response in bacterial densities, should have contributed to an absence of indirect effects of simazine on the dominant zooplankters.

However, a mixed and unexpected response was observed in the rotifer-dominated zooplankton. *K. bostoniensis* and, to a lesser extent, *K. cochlearis* exhibited positive responses to simazine. Instantaneous death rates were reduced with greater simazine levels, permitting *K. bostoniensis* to maintain its numerical dominance of the zooplankton assemblage (Fig. 2, Table 4). The same phenomenon occurred with *K. cochlearis*, but not until day 21 and only in the 1.0 mg/L microcosms (Table 4).

Table 4. Rotifer species population parameters[a]

Time interval (d)	Treatment (mg/L)	*K. bostoniensis*			*K. cochlearis*			*P. vulgaris*		
		b	*d*	*r*	*b*	*d*	*r*	*b*	*d*	*r*
0–7	Ambient	0.14	0.97	−0.83	0.16	0.46	−0.30	0.20	0.46	−0.26
0–7	0.0	0.13	0.16	−0.03	0.16	0.18	−0.03	0.21	0.29	−0.08
0–7	0.1	0.13	0.16	−0.03	0.15	0.20	−0.04	0.20	0.28	−0.08
0–7	0.5	0.13	0.18	−0.06	0.16	0.21	−0.05	0.21	0.28	−0.06
0–7	1.0	0.13	0.16	−0.03	0.16	0.22	−0.06	0.21	0.24	−0.03
7–14	Ambient	0.10	−0.59	0.69	0.10	−0.16	0.26	0.17	−0.09	0.26
7–14	0.0	0.08	0.21	−0.13	0.10	0.12	−0.02	0.18	0.13	0.05
7–14	0.1	0.08	0.16	−0.08	0.10	0.07	0.03	0.16	0.07	0.09
7–14	0.5	0.08	0.06	0.02	0.10	0.07	0.03	0.18	0.13	0.05
7–14	1.0	0.08	0.10	−0.02	0.10	0.10	0.00	0.18	0.15	0.03
14–21	Ambient	0.30	0.35	−0.05	0.29	0.55	−0.26	0.37	0.35	0.02
14–21	0.0	0.30	0.46	−0.17	0.34	0.40	−0.06	0.37	0.29	0.07
14–21	0.1	0.30	0.46	−0.16	0.33	0.42	−0.09	0.34	0.25	0.09
14–21	0.5	0.30	0.38	−0.08	0.33	0.40	−0.06	0.36	0.27	0.09
14–21	1.0	0.29	0.32	−0.03	0.32	0.31	0.01	0.35	0.27	0.09

[a]Population parameters: *b*, instantaneous birth rate; *d*, instantaneous death rate; *r*, instantaneous population growth rate [27].

Two speculative explanations for this unexpected result are:

1. Inhibition of algal antibiosis. *Glenodinium* is a close relative of *Gonyaulux* and other marine dinoflagellates known to produce extracellular toxins. If freshwater *Glenodinium* also produced a toxin, and simazine decreased production of that toxin, mortality rates of *K. bostoniensis* and *K. cochlearis* would decrease. However, no similar effect on other zooplankton species was observed, and no evidence was found in the literature indicating toxin production by *Glenodinium*.
2. Release of beneficial materials (e.g., vitamins, dissolved organics) upon induced death and lysis of phytoplankton. Rotifers need dissolved nutrients, as evidenced in culture [34] and field results [35] with *P. vulgaris*. Again, no similar effect was observed on rotifers other than *K. bostoniensis* and *K. cochlearis*.

S. pectinata was impaired by simazine at day 21, whereas *P. vulgaris* did not show any significant response to simazine. These results may indicate more dietary flexibility in *P. vulgaris* than *S. pectinata*, although both are reported to feed on a variety of cell types [21–23,34–36]. The ciliate *Codonella* and immature cyclopoid copepods were present but were not affected by simazine. Cladocera were rare during the experiment and were not analyzed.

In terms of the original hypothesis, the effect of simazine on phytoplankton indirectly reduced numbers of only one zooplankton species (*S. pectinata*). The two dominant zooplankters, *K. bostoniensis* and *K. cochlearis*, exhibited greater densities due to decreased mortality rates. This mixed response served to maintain total rotifer densities and composition relative to controls.

All four of the major rotifer species were reported to feed on cells <9 μm, and the two dominant species, *K. bostoniensis* and *K. cochlearis*, graze primarily on this size class. Facultative and obligate heterotrophic organisms (small flagellates and bacteria) may have been 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 did not significantly impair the rotifer-dominated winter zooplankton assemblage.

Comparable results have been reported for simazine and another triazine herbicide, atrazine. Atrazine, a widely used compound, is very similar to simazine in its mode of action and effects.

Schwartz et al. [5] reported no measurable effects on a zooplankton community of 0.45 mg/L simazine administered to an Arizona lake. However, their study emphasized phytoplankton and water chemistry and published zooplankton data were inadequate to determine the presence of ef-

fects. Our study detected significant effects on zooplankton, but the effects were either beneficial or deleterious, depending on the species.

deNoyelles et al. [10] treated experimental ponds with 0.02 and 0.5 mg/L atrazine and studied the responses of plankton communities. The cladoceran *Diaphanosoma brachyurum* and the cyclopoid copepod *Tropocyclops prasinus mexicanus* dominated zooplankton biomass prior to atrazine addition. These species remained the dominant crustaceans during the study (136 d), "but were replaced as the dominant zooplankton by rotifers, principally *K. cochlearis* (Gosse), after day 31." No further data or analyses 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. This result is consistent with the results of our study.

The present study and the above cited papers indicate that herbicide-induced changes in phytoplankton composition and abundance may or may not impair zooplankton populations. The presence of indirect effects to zooplankton via shifts in food web structure depends on the taxonomic compositions and strengths of interactions between zooplankton and phytoplankton. Cladocerans and copepods, traditionally considered to dominate the zooplankton, seem to be closely linked by their predator-prey relationships with the autotrophic phytoplankton [10]. When the autotrophic phytoplankton are selectively impacted by a photosynthesis inhibitor, these crustaceans are also affected [10]. However, zooplankton communities are a heterogeneous composite of numerous organisms, variable in time and space. Rotifers and protozoans can also comprise a major portion of the zooplankton [37,38, this study], and may not be closely linked by predator-prey interactions to autotrophic food items. Thus, photosynthesis inhibitors may not impair zooplankton communities dominated by these organisms.

In conclusion, persistent levels of simazine may not have a deleterious impact on winter zooplankton communities dominated by noncrustacean taxa (rotifers and protozoans). Phytoplankton may be differentially affected, but the relative lack of dependence on autotrophs by winter zooplankton communities may mitigate indirect effects via food webs. An unexpected positive response to simazine treatment was observed for the dominant rotifers *K. bostoniensis* and *K. cochlearis*, an effect for which we have only speculative explanations. This study quantitatively evaluated the chain of events induced by simazine treatment, but was not able (nor intended) to elucidate the underlying mechanisms responsible for that chain of events. Evaluation of those mechanisms will require different techniques.

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