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THE RESPONSES OF PLANKTON COMMUNITIES IN EXPERIMENTAL PONDS TO ATRAZINE, THE MOST HEAVILY USED PESTICIDE IN THE UNITED STATES[1]

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Abstract. Experimental ponds received single additions of the herbicide atrazine in concentrations of 20 and 500 μg/L, and were compared to control ponds for 136 d. Atrazine is an inhibitor of photosynthesis, and both concentrations depressed phytoplankton growth in the ponds within a few days. This was followed by successional changes leading to the establishment of species of phytoplankton more resistant to inhibition by atrazine. Laboratory studies verified this resistance and verified effects on other species at concentrations of atrazine as low as 1–5 μg/L. When and to what extent resistant species appeared in the phytoplankton communities differed with treatment. At the atrazine concentration of 500 μg/L, there was a delayed appearance but eventually a greater biomass and persistence of these species. The grazing zooplankton influenced these differences and were in turn affected by them. Natural interactions such as competition and predation among the species of the communities greatly affected their responses to the toxic chemical. The importance of atrazine as an environmental pollutant is suggested by these responses to concentrations of 1–5 μg/L, which are common downstream in many agricultural watersheds, 20 μg/L, which is the high level found in these waters, and 500 μg/L, which is the high level found in waters directly adjacent to treated fields.

Key words: atrazine; experimental ponds; herbicide; photosynthesis inhibitor; phytoplankton; toxicant resistance; zooplankton.

INTRODUCTION

An aquatic community exposed to a toxic chemical stress will be altered by the combined responses of its members. The response of any single species may be influenced by the responses of other species and in turn also affect these species and others. This results from the complex network of interactions and interdependencies that exists among the species of a community. Investigators have recognized this for decades (Forbes 1887), yet today most still attempt to assess and predict the effects of a toxic chemical on the environment by studying the responses of a selected organism isolated in the laboratory. These responses are likely to be only those directly induced by the toxicant and will not include secondary effects resulting from the interactions among organisms. Interactions such as competition and predation can be altered throughout the community as organisms are initially inhibited by the chemical. Because some species in the community are more resistant to the effects of the chemical, another factor in competition is introduced.

The existence of secondary effects of toxic chemicals has been shown for pesticides (Newsom 1967, Pimentel 1971, Hurlbert 1975), heavy metals (Marshall and Mellinger 1980), acid from acid rain (Ericksson et al. 1980, Schindler 1980), and other substances (Cairns et al. 1972). In order to incorporate various levels of a community response to a potentially widely spread toxic chemical, we chose to study the effects of atrazine in experimental ponds. This study will demonstrate both the immediate inhibition of certain species and the secondary effects which such a response has on other species. Species with some tolerance to the effects of the chemical will be identified in the communities, and their role in the changing conditions will also be considered.

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is the single most heavily used pesticide in the United States. In 1976, 41 million kilograms (active ingredient) of this herbicide were applied on farms in the USA, principally for weed control in corn and sorghum crops. This is compared to a total herbicide application of 252 million kilograms and a total pesticide application of 460 million kilograms, according to a 1976 United States Department of Agriculture report (Eichers et al. 1978).

Atrazine has been detected in many natural waters in concentrations of 0.1–30 μg/L (Waldron 1974, Richard et al. 1975, Muir et al. 1978) including some streams entering the Great Lakes from Ontario, Canada, where two studies report a greater than 75% frequency of detection averaging 1–5 μg/L (Frank et al. 1979, Roberts et al. 1979). In the province of Ontario at that time, over 1 million kilograms of atrazine were being applied annually (Frank et al. 1979). The highest atrazine concentrations in all of these reports were 27–69 μg/L, while in waters directly adjacent to treated fields concentrations may reach 1 mg/L (Kadoum and Mock 1978). Atrazine generally degrades in nature within 1.5 yr and does not bioconcentrate (Wagner and

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Chahal 1966, Macek et al. 1976, Klassen and Kadoum 1979).

Atrazine controls target weeds in agriculture by inhibiting photosynthesis, blocking electron transport within the Hill reaction of photosystem II (Moreland 1980). A wide variety of plants are affected by this mechanism, including species of algae shown in the laboratory to be inhibited at atrazine concentrations as low as 1 μg/L (Torres and O'Flaherty 1976, Butler 1977, Plumley and Davis 1980). The direct toxicity of atrazine to animals is generally low at concentrations found in contaminated habitats (Saunders 1970, Tooby et al. 1975, Macek et al. 1976); thus, animal responses may be secondary effects of plant responses. The extent to which the immediate effects of atrazine on phytoplankton photosynthesis can extend into longer-term, successional effects on both plant and animal communities has not previously been demonstrated in aquatic habitats. The combined responses of the plankton species will be shown to influence these changes demonstrating the importance of whole system studies for assessing the effects of toxic chemical stress.

METHODS

In July 1979 six ponds (0.045 ha) were drained and refilled with natural water and plankton from an adjacent pond maintained by well water. This was done to increase similarity among the ponds just prior to atrazine addition. To supplement the animal community, three species of fish were added to each pond: a predator, bluegill sunfish (50 fish, average length 85 mm), a benthic omnivore, channel catfish (20 fish, average length 116 mm), and a filtering omnivore, gizzard shad (7 fish, average length 185 mm). Atrazine, as the commercially available herbicide (CO-OP liquid, 41% active ingredient, EPA registration number 1990-318), was added once on 24 July to four ponds; the active ingredient was added at concentrations of 20 μg/L to two ponds and 500 μg/L to two other ponds. Two remaining ponds served as controls. The two concentrations added were chosen to simulate the higher concentrations likely to be found in direct contact with sprayed fields and waters farther downstream in watersheds.

Monitoring of the ponds was most intense for the week prior to addition and for the next 63 d, with monthly sampling thereafter to day 136. Physical and chemical conditions monitored included pond temperature and light profiles, turbidity, pH, alkalinity, and dissolved oxygen. Water samples were taken from a walkway over each pond with a column sampler at the end of a 2.4-m pole. Two water columns were taken for each sample, and three samples were taken from each pond. The concentration of atrazine was analyzed seven times from day 2 to day 92, according to the procedures of Kadoum and Mock (1978).

Samples for phytoplankton species distribution were collected as described above and preserved with acid Lugol's. Organisms were concentrated in settling chambers and counted with a Wild inverted microscope. Distributional changes of several of the more common species were recorded from counts of one sample per pond at detection limits of 2–10 organisms/mL. Phytoplankton biomass was estimated from particle counts from a Model ZB Coulter counter, using part of the water samples collected for water chemistry. Counts in seven size intervals from 8 to 64 μm were made twice from each of the three composite column samples. This range of particle sizes included most of the phytoplankton species and biomass present during the study but did not include the finer silt particles. This was verified from microscopic observation of the samples on each sampling date. Filamentous algae and macrophytes remained sparse in all ponds.

Carbon uptake by the phytoplankton was measured from incubation with $NaH^{14}CO_3$. Column samples taken as described above were incubated for 4 h in 125-mL, glass-stoppered bottles in a laboratory growth chamber. Samples were incubated at the pond surface temperature and were rotated around circular fluorescent lamps at ambient (1-m depth) pond light intensity (150 $\mu E \cdot m^{-2} \cdot s^{-1}$). After incubation, samples were processed using an acidification and bubbling method (Schindler et al. 1972). Counts per minute were used directly in the comparisons of carbon uptake for the pond phytoplankton.

To determine the immediate (first 24 h) effects of a wider range of concentrations of atrazine, phytoplankton from control and atrazine ponds, collected at various times during the 2nd and 3rd mo, were exposed to atrazine concentrations of 1, 5, 20, and 500 μg/L in a laboratory growth chamber. The responses recorded following the addition of all concentrations of atrazine were fluorescence increase after 2 min and ^{14}C uptake decrease during 24 h of continuous illumination at ambient (1-m depth) pond light intensity (150 $\mu E \cdot m^{-2} \cdot s^{-1}$). The increase in fluorescence is an indicator of electron transport blockage because absorbed light energy, which then cannot be used due to blockage, is released as fluorescent energy (Zweig et al. 1963). Before addition of atrazine to the samples, fluorescence was measured with a Turner Model 10 Rack-mount Fluorometer (Turner Designs, Mountain View, California. F4T5 blue lamp R466 infrared sensitive photomultiplier; Corning 5-60, 3-66, and 2-64 filters) calibrated against extracted chlorophyll *a* standards. Two minutes following addition, fluorescence of the sample was measured again. The atrazine itself was not fluorescent. Samples were also collected from the ponds and immediately incubated for 24 h with the same atrazine concentrations to determine changes in ^{14}C uptake.

To demonstrate the resistance of one particular species of phytoplankton to the effects of atrazine,

Cryptomonas marssonii Skuja was isolated on day 60 when it was still abundant from a pond to which atrazine at 500 μg/L had been added. It was then grown in the absence of atrazine for several months. With exposure again to an atrazine dosage of 500 μg/L, the increase in biomass of this species was compared in 10 replicate cultures to that of a species (*Oocystis* sp.) isolated at the same time from a control pond. Cultures contained a soil–water medium (Starr 1964) and were grown at 20°C and 150 $\mu E \cdot m^{-2} \cdot s^{-1}$ (L15:D9) for 19 d.

Other members of the pond communities including macrophytes, insects, amphibians, and fish were monitored (biomass beginning and end) for their responses to atrazine, but only the zooplankton showed a treatment response. Samples for zooplankton species distribution were taken as vertical net hauls at night from the walkways. One sample was taken with a 49-cm diameter, 157-μm mesh net by straining ≈400 L of pond water. In three 0.5 mL subsamples of the concentrated sample, all organisms were counted giving a detection limit of 0.07 organisms/L. Copepod nauplii and rotifers were counted in the phytoplankton samples by settling 100 mL and counting the entire assemblage.

Measurements of growth and reproduction within the zooplankton community were made for one member, the cladoceran *Simocephalus serrulatus* (Koch). *Simocephalus* was present though rare in the communities, but was chosen because of previous success in maintaining this species in in-situ enclosures (Kettle et al. 1980). Several *Simocephalus* adults were collected from one pond during the week prior to atrazine addition. On the day after addition, offspring (12 ± 12 h old) from several adults were placed individually into 100-mL plexiglass cylinders covered at both ends with 153-μm mesh nylon netting. Ten cylinders were then suspended in each pond at 1 m and examined every 2 d, continuing for the life span of all individuals. Each time offspring were counted and removed, and any mortality of the original individuals was recorded. For examination, cylinders were removed from the ponds for 1 h and held in buckets of the pond water. In an adjacent field laboratory each cylinder was opened in a dish of pond water and observed under a microscope. Growth was estimated by taking one set of length measurements (top of head to base of posterior spine) on day 10.

To separate the direct effects of atrazine from those resulting from algal changes, *Simocephalus* was also incubated in the laboratory where atrazine and food availability were both manipulated. Young were selected from the same group used for the in-situ incubation and were incubated in groups of 10 in closed 300-mL containers in a growth chamber at prevailing pond temperature and irradiance for the 1-m depth (25°C, 15L:9D, 150 $\mu E \cdot m^{-2} \cdot s^{-1}$). Water for the containers was prepared by filtering control pond water through an 86-μm mesh net to remove the larger zooplankton. This was followed by additions of atrazine at concentrations of 20 and 500 μg/L. Three replicate groups of 10 individuals were used for each treatment and the control. Each animal was removed by pipette and examined every 48 h, making the same observations as described above. Each original individual was then placed back into a freshly prepared container.

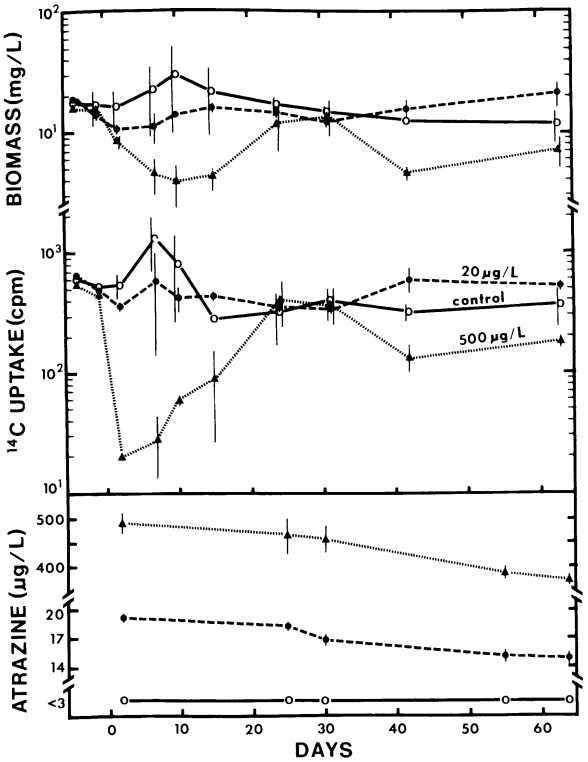

FIG. 1. Atrazine concentration (raw pond water), phytoplankton ^{14}C uptake (4-h; cpm = counts per minute), and phytoplankton biomass (live mass, Coulter counter particle counts, 6–64 μm diameter) in two control (solid line), two 20 μg/L (dashed line), and two 500 μg/L (dotted line) experimental ponds. Treatment means are plotted with vertical bars that indicate range.

RESULTS

Phytoplankton photosynthesis

The only physical or chemical conditions in the ponds affected by treatment were pH and dissolved oxygen; changes were probably caused by the effects of atrazine on phytoplankton photosynthesis. Turbidities ranged from 2 to 20 JTU (Jackson Turbidity Units), and light penetration at 1.5 m from 10 to 30% of the surface irradiance. Temperatures ranged from 20° to 30°C during the summer with bottom temperatures 3°–4° lower than those at the surface. Total alkalinity (as $CaCO_3$) remained between 100 and 140 mg/L. The pH ranged between 8.0 and 9.0, with values in the ponds to which atrazine was added at 500 μg/L 0.3 pH units lower than control pond values for the first

TABLE 1. Fluorescence increase responses of control and atrazine pond phytoplankton communities, sampled on days 91 and 93 of the field experiment, following a 2-min laboratory exposure to an addition of atrazine. Each response value is the increase in fluorescence after the 2-min exposure as a percent of the preaddition reading. The control addition represents readings after 2 min with no exposure to atrazine. Negative values are percent decreases. Each value represents the mean of 10 replicates.

Addition (μg/L)	Controls		Atrazine concentration of source pond			
			20 μg/L		500 μg/L	
	Pond 4	Pond 6	Pond 1	Pond 5	Pond 2	Pond 3
	Increase in fluorescence (%)					
Control	1.1	−0.9	−0.2	−0.8	−0.7	−0.3
1	4.3*	3.4*	−0.3	0.0	−0.3	0.0
5	11.1*	7.1*	1.7*	2.2*	−1.0	−0.4
20	36.5*	22.7*	8.1*	11.1*	−1.2	−0.7
500	148.8*	124.0*	58.2*	65.9*	11.3*	9.6*

* Fluorescence increase with addition significantly different ($P < .05$) from nonaddition (control) increases, using the Student-Newman-Keuls test.

few weeks after addition, when ^{14}C uptake by the phytoplankton was particularly low (Fig. 1). At these times dissolved O_2 also declined, generally 1–3 mg/L lower at both the surface and bottom of the ponds to which atrazine at 500 μg/L was added. At no time did early morning (1 h after sunrise) oxygen concentrations in any ponds fall below 4 mg/L. The pH and oxygen conditions in the 20-μg/L ponds showed similar declines but only ≈20% of those in the 500-μg/L ponds.

Carbon-14 uptake (4-h) by the phytoplankton communities in the ponds declined at both atrazine levels during the first 2 d following addition, indicating some inhibition of photosynthesis. In the 500-μg/L ponds, uptake significantly ($P < .05$, Student-Newman-Keuls [S-N-K] test) declined to <5% of pretreatment or control pond values (Fig. 1). Phytoplankton biomass (live mass) also declined significantly ($P < .05$) at the same time (Fig. 1). In the 20-μg/L ponds ^{14}C uptake and biomass declined significantly ($P < .05$) by day 2 but returned to control pond values by day 7, remaining there through day 63 (Fig. 1). While the phytoplankton ^{14}C uptake and biomass in the 500-μg/L ponds averaged less than in the control ponds for the entire study, after day 24 both parameters were occasionally at or above control pond values (Fig. 1). From day 63 to day 136, ^{14}C uptake and biomass were measured twice, and on both occasions control pond values were similar to those of the 20-μg/L ponds and twice those of the 500-μg/L ponds.

Phytoplankton were collected from all ponds at various times during the 2nd and 3rd mo of the study and

TABLE 2. Twenty-four-hour carbon-14 uptake (CPM) responses of samples of the control and atrazine pond phytoplankton communities, collected at the indicated times during the field experiment, to additions of atrazine. Each response value is the percent decline in ^{14}C uptake comparing treated and untreated samples. The three values marked with a plus sign are percent increase. Each value is the mean of 5–10 replicates.

Addition (μg/L)	Sampling day	Controls		Atrazine concentration of source pond			
				20 μg/L		500 μg/L	
		Pond 4	Pond 6	Pond 1	Pond 5	Pond 2	Pond 3
				Decline in ^{14}C uptake (%)			
1	85	+3.25					
	98	+7.0	0.5				
5	49	5.4*					
	85	4.1					
	98	6.9*	6.7*				
20	49	12.4*					
	56	18.0*	2.4	21.2*	20.2*	3.3	1.0
	70	19.5*	13.9*	10.6*	14.4*	+1.8	0.6
	85	11.8*					
500	49	83.1*					
	56	86.8*	87.4*	89.6*	88.0*	20.8*	0.9
	70	90.3*	88.2*	89.7*	87.6*	18.7*	9.5
	85	91.6*					

* ^{14}C uptake with atrazine addition significantly different ($P < .05$) from nonaddition uptake, using the Student-Newman-Keuls test.

exposed to atrazine in the laboratory. For communities sampled from control ponds, significant ($P < .05$, S-N-K test with 10 replicates) fluorescence increases were recorded at atrazine concentrations as low as 1 μg/L (Table 1) and significant ($P < .05$) ^{14}C uptake decreases at concentrations as low as 5 μg/L (Table 2). At atrazine concentrations of 1, 5, 20, and 500 μg/L, respectively, fluorescence increases over controls averaged 4, 9, 30, and 136%, and ^{14}C uptake decreases from controls averaged 0, 8, 12, and 88%.

Phytoplankton collected from the 20-μg/L ponds and exposed to additional atrazine experienced significant ($P < .05$) fluorescence increase only at concentrations of 5 μg/L and above (Table 1). The increases were all significantly ($P < .05$) less intense than those described above for the control-pond phytoplankton. For phytoplankton from the 500-μg/L ponds, only an atrazine concentration of 500 μg/L produced a significant ($P < .05$) fluorescence increase, but the mean increase was only 10% compared to the 136% mean recorded for control-pond phytoplankton (Table 1). Decreases in ^{14}C uptake compared to those of control-pond phytoplankton were significant ($P < .05$) only for the 500-μg/L pond phytoplankton (Table 2). They experienced no decline at 20 μg/L and a mean 12% decline at 500 μg/L. This is compared to mean declines of 14 and 88% with 20 μg/L and 500 μg/L additions, respectively, for the phytoplankton from all control and 20-μg/L ponds (Table 2). These reduced responses by the phytoplankton from the atrazine ponds to further atrazine addition were considered the first indication of some resistance to the effects of atrazine. It is noted that phytoplankton from the atrazine ponds on days 42, 63, 91, and 114 had the same or greater ^{14}C/live biomass compared to control pond phytoplankton. A reduced response to further atrazine addition due only to an already inhibited condition from the pond atrazine therefore seemed unlikely.

As further evidence of resistance, a species of phytoplankton that dominated the 500-μg/L ponds was isolated into culture; it showed less response to atrazine than a species from a control pond (Table 3). Atrazine-treated *Cryptomonas* cultures increased in biomass by 50 fold in the presence of atrazine concentrations of 500 μg/L over a 19-d incubation. Untreated cultures increased 100 fold. Atrazine-treated *Oocystis* cultures showed no increase in biomass, while untreated cultures increased 100 fold, indicating no resistance for *Oocystis* but some for *Cryptomonas*.

TABLE 3. Biomass changes in cultures of *Cryptomonas marssonii* (500 μg/L pond isolate) and *Oocystis* sp. (control pond isolate) incubated with atrazine at 500 μg/L for 19 d. Biomass expressed as micrograms fresh mass per litre calculated from microscope cell counts and cell volume measurements.

	Cryptomonas		*Oocystis*	
	Control	Atrazine	Control	Atrazine
Initial				
$\bar{x}$	60	60	216	216
Range	(58–62)	(58–62)	(158–274)	(158–174)
Final				
$\bar{x}$	5829	2915	25 120	149
Range	(1011–16 686)	(76–6136)	(7770–47 073)	(17–767)
SE	1648	650	4142	73
t*	1.644 ($P < .2$)		6.028 ($P < .001$)	

* Two-tailed t test comparing final values of treatment and control for each species, with 10 replicate cultures.

Phytoplankton succession

The succession of phytoplankton species in the ponds was altered by the addition of atrazine in amounts of 20 and 500 μg/L as shown in Fig. 2 for some of the more abundant species. The inhibitory effects of atrazine on certain species were most obvious for the 500-μg/L ponds. *Peridinium inconspicuum* Lemm., a small dinoflagellate, was rare or absent after 7 d, while in the 20-μg/L ponds its abundance remained similar to that in the control ponds. All of the other dominant species in the 500-μg/L ponds, as shown by total biomass in Fig. 1, also rapidly declined by day 7, but some later increased by day 15 (Figs. 1 and 2). Species that increased were several species of the flagellates *Mallomonas* (predominantly *M. pseudocoronata* Prescott) and *Cryptomonas* (predominantly *C. marssonii* and *C. erosa* Ehrenberg; Fig. 2). When ^{14}C uptake and biomass began to increase in the 500-μg/L ponds by day 15, these genera represented over 95% of the phytoplankton biomass in both ponds. At this time the concentration of atrazine in the water column had declined little from the original 500 μg/L (Fig. 1). While declines of *Mallomonas* and *Cryptomonas* occurred during the first 2 wk in the 500-μg/L ponds, increases of the same organisms were occurring in both 20-μg/L ponds. During the same period their abundance remained relatively unchanged in the control ponds. These species made up ≈50% of the phytoplankton biomass in the 20-μg/L ponds at the peak of their increase. After day 63 the abundance of the species in Fig. 2 changed very little in all ponds.

Other species of phytoplankton were noted for their successional responses according to treatment. Those that declined in abundance, only apparent in 500-μg/L ponds, were *Coelastrum* spp., *Dictyosphaerium pulchellum* Wood, *Glenodinium* sp., *Oocystis* spp., *Scenedesmus* spp., *Staurastrum tetracerum* Ralfs and *Tetraëdron minimum* (A. Braun) Hansgirg. Species present in all ponds and occasionally abundant at both atrazine levels were *Dinobryon divergens* v. *Schauinslandii* (Lemmermann), *Kirchneriella lunaris* v. *irregularis* G. M. Smith, *Rhodomonas pusilla* (Bachm.) Javorn, *Synedra acus* Kutz, *S. radians* Kutz, and *Uroglenopsis americana* Catkins.

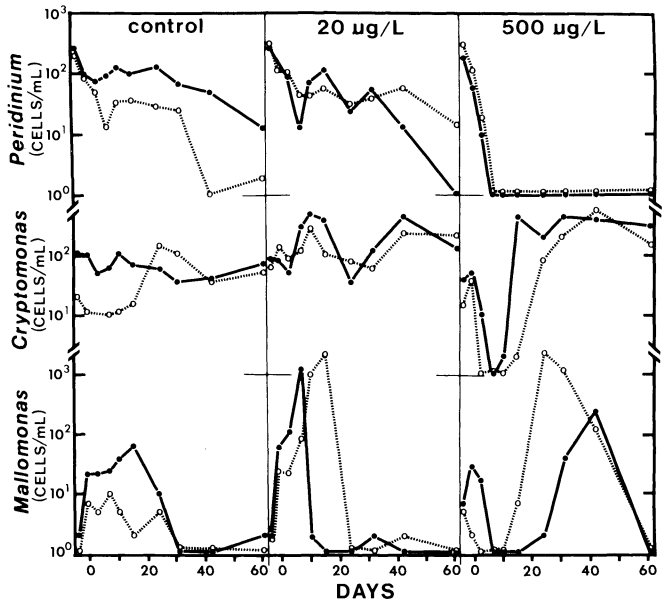

FIG. 2. Abundance of selected phytoplankton species in each of two control, two 20 μg/L, and two 500 μg/L experimental ponds. Dotted and dashed lines represent replicate ponds. Cells were counted microscopically at detection limits of 2–10 cells/mL.

Zooplankton

The zooplankton community in each pond before atrazine addition was dominated in biomass by the cladoceran *Diaphanosoma brachyurum* (Lieven) and the cyclopoid copepod *Tropocyclops prasinus mexicanus* Kiefer. These species continued as the dominant crustaceans throughout the study but were replaced as the dominant zooplankton by rotifers, principally *Keratella cochlearis* (Gosse), after day 31. Other zooplankton present but in low numbers included *Simocephalus serrulatus* and *Daphnia pulex* Leydig and species of *Chydorus*, *Pleuroxus*, and *Diaptomus*. All are grazers on phytoplankton, though in the case of *T. prasinus mexicanus* this is assumed only from knowing that the closely related *T. prasinus* (Fischer) is a grazer (McNaught et al. 1980).

Before atrazine addition zooplankton abundance was high and similar in all ponds, with 100 adult crustaceans/L, 1000 copepod nauplii/L, and 1000 rotifers/L. *Tropocyclops prasinus mexicanus*, which represented over 70% of the zooplankton biomass at this time, declined in the 500-μg/L ponds within 14 d to <25% of the adult and nauplius biomass remaining in each of the other ponds. *Simocephalus serrulatus*, suspended in the ponds in perforated chambers, experienced both atrazine exposure and changing food conditions in the ponds, and individuals responded with growth and reproduction changes correlated only with food abundance (Table 4). Individuals reared in the laboratory at the same atrazine concentrations but with food renewed showed no response correlated with atrazine concentration.

DISCUSSION

Atrazine is a toxicant which has direct effects on just certain members of the aquatic community. However, by the responses of these members, others are also affected. In the plankton communities studied here, some algae were inhibited immediately such that within 2 d ^{14}C uptake and biomass declined with both the atrazine additions of 20 and 500 μg/L (Fig. 1). Such immediate growth responses of the phytoplankton at these atrazine concentrations are consistent with the findings of investigators who exposed isolated species to atrazine in the laboratory (Gramlick and Frans 1964, Walsh 1972, Butler et al. 1975, Torres and O'Flaherty 1976, Butler 1977).

Lower concentrations of atrazine, down to 1 μg/L, though not studied as pond additions, were shown to affect the photosynthesis of samples of phytoplankton communities from control ponds (Tables 1 and 2). Responses to these lower concentrations have only been occasionally reported in the literature (Butler et al. 1975, Torres and O'Flaherty 1976, Plumley and Davis 1980), again with isolated species. Such studies with isolated species do correctly predict the existence and immediate severity of the effects of this toxicant. However, as initial effects extend into longer-term ones involving phytoplankton succession and zooplankton growth and reproduction, it becomes impossible to incorporate the causes (e.g., altered competition and predation) for such changes into exposure experiments that involve only one species.

The successional changes within the phytoplankton communities were in part a consequence of the exis-

TABLE 4. Reproduction and growth of *Simocephalus serrulatus* compared to phytoplankton biomass and ^{14}C uptake. Ten juveniles (12 ± 12 h old) were suspended singly in each pond in perforated chambers where they were exposed to both the atrazine and changing phytoplankton conditions for the first 20 d of the pond experiment. Individuals were examined every 2 d throughout their life span, counting and removing offspring and, on day 10, measuring length as the growth estimate. Only the data for the in-situ exposures are shown here, as individuals that received renewed food in the laboratory showed no response to atrazine alone.

		Simocephalus		Phytoplankton	
Pond	Atrazine addition (μg/L)	Young per ♀ per day A	Growth B	Biomass* (mg/L) C	^{14}C uptake* (CPM) D
2	500	0.81	1.090	4.2	28.7
3	500	2.50	1.229	6.1	39.0
1	20	4.06	1.429	14.3	522.3
5	20	2.87	1.360	12.4	376.3
4	0	2.96	1.349	10.0	422.0
6	0	6.70	1.520	36.2	1395.7

Comparison	r_s†	Comparison	r_s†
AC	.94	AD	1.00
BC	1.00	BD	.97

* Biomass and ^{14}C uptake were averaged for the period during which at least 90% of the young were recorded.

† Correlation coefficients were calculated using the Spearman rank method (Zar 1974). All pairings significantly correlated ($P < .05$), exceeding the critical value for significance ($r_s = .885$; two-tailed test, $n = 6$). Life span averaged from 11 to 16 d and did not correlate with either atrazine treatment or food availability.

tence in the communities of species with some resistance to the effects of atrazine on photosynthesis. Evidence for the resistance of some species is given by their growth in the atrazine ponds (Figs. 1 and 2) and reduced response to additional atrazine exposure (Tables 1, 2, and 3). Verified cases of resistance in the field have only been reported for terrestrial plants (Bandeen and McLaren 1976, Pfister et al. 1979).

Phytoplankton succession was also affected by the direct inhibition of some organisms causing changes in others. Such chains of events are inevitable with the disturbance of natural ecosystems because they are characterized as having interacting and interdependent parts. With the atrazine addition of 500 μg/L, photosynthesis was reduced by 95% within 2 d, directly by atrazine inhibition. However, the rapid biomass declines which occurred would quite likely result in part from a concurrent natural death rate factor such as grazing pressure, which the now-inhibited species could no longer outgrow. Grazing by the zooplankton could quickly reduce their biomass, leaving the originally small biomass of resistant species (Fig. 2) as an increasing proportion of the food source, hence more heavily grazed upon. Thus the initial decline and later increase of some species may not have been due to delayed resistance to the higher atrazine concentrations, as might occur with selection of resistant varieties of these species.

In the 20-μg/L ponds the more resistant species were not exposed to this increased grazing pressure since the other species declined only slightly in ^{14}C uptake and biomass (Fig. 1). Instead with still some initial inhibition of the phytoplankton with atrazine additions of 20 μg/L (Fig. 1, Tables 1 and 2), the more resistant species may have experienced enough relief in competition to allow their increased growth during the 1st wk (Fig. 2). These same species later increased in the 500 μg/L ponds after day 10 when the grazing zooplankton declined in abundance, perhaps in response to reduced food supply as shown for *Simocephalus* (Table 4).

Responses that could definitely be attributed to the effects of atrazine were not recorded higher up the food chain above the zooplankton during this 136-d study. Studies are in progress through 1982 to determine the longer-term effects. It can be concluded now that atrazine can affect both phytoplankton growth and succession. This in turn can begin affecting higher levels of the food chain beginning with the zooplankton. The importance of atrazine as an environmental pollutant is suggested by these effects at concentrations found in nature. In the laboratory, atrazine concentrations as low as 1–5 μg/L affected phytoplankton photosynthesis. The literature indicates that such concentrations are common downstream in many agricultural watersheds. Atrazine concentrations of 20 μg/L were shown in the laboratory and field to affect both photosynthesis and succession, including the establishment of resistant species within the phytoplankton community. Again in studies listed in the literature cited, this concentration has been detected in downstream waters generally as the highest level recorded. An atrazine concentration of 500 μg/L caused an almost complete inhibition of the photosynthesis of all but the resistant species, reduced zooplankton growth and reproduction due to declining food, and established the dominance of resistant phytoplankton species in the community for the 136-d duration of the study. Even this concentration has been detected in natural habitats but only those in direct contact with sprayed fields.

It should be apparent how interactions such as competition and predation can affect the response of a plankton community to a toxic chemical. These natural interactions exist in all communities, aquatic or terrestrial, and clearly many effects of toxic chemicals will not be evident from just the responses of isolated species. The responses of isolated species can provide valuable information, particularly in predicting the most immediate effects on certain types of organisms, such as reduced photosynthesis for the phytoplankton as shown here. Some recent studies have extended the use of isolated species, constructing particular combinations of species which provide the opportunity to

incorporate some types of interactions (Taub and Crow 1978). Here we can only begin to approximate natural communities and must realize that there are interactions still so little understood in the field that it is impossible to ensure their incorporation in the laboratory. Therefore to determine the effects which a toxic chemical can have on natural communities we must continue, though not restrict ourselves to, the study of these stresses in natural communities.

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