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Diet and Reproductive Success of Bluegill Recovered from Experimental Ponds Treated with Atrazine

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Studies involving whole ecosystem manipulations are rare in aquatic toxicology. The vast majority of toxicological studies consist of acute or otherwise short-term tests with organisms maintained under laboratory conditions. Extrapolation of data from these laboratory tests to natural ecosystems may or may not be appropriate (Cairns 1983). Most laboratory tests can measure only the direct, short-term responses of organisms to perturbations and preclude direct estimation of secondary responses of organisms which result from species interactions.

Atrazine herbicide (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is one of the most heavily used pesticides in the United States (USDA 1983). Atrazine enters the aquatic environment in run-off from agricultural fields and is commonly detected in aquatic habitats (Kadoum and Mock 1978; Frank et al. 1979; Wu et al. 1980; Glotfelty et al. 1984). Atrazine functions as a photosynthesis inhibitor in target weeds by blocking electron transport in the light reaction of photosynthesis (Moreland 1980). At concentrations encountered in nature, the direct toxicity of atrazine to animals appears to be minimal (Tooby et al. 1975; Macek et al. 1976), but indirect effects have been suggested (deNoyelles et al. 1982; Dewey 1986). Despite the widespread occurrence of atrazine in aquatic habitats, few studies have assessed its impact on ecosystems under natural exposure situations.

We have completed a study designed to assess the effects of atrazine on the aquatic communities of experimental ponds. The effects of atrazine on plankton communities and water quality conditions from that study have been previously reported (deNoyelles et al. 1982). From that same study we report here the effects of atrazine on macrophyte and bluegill sunfish populations and consider the direct and indirect (secondary) responses to the herbicide.

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MATERIALS AND METHODS

The pond facility used in this study was located on the Nelson Environmental Study Area, 12 km north of Lawrence, Kansas. In July 1979, six ponds (0.045 ha) were drained and then refilled with water from an adjacent reservoir pond to a depth of 2.1 m. Three species of fish were then added to each pond; bluegill sunfish (*Lepomis macrochirus*, 50 fish, average total length (TL) 85 mm), channel catfish (*Ictalurus punctatus*, 20 fish, average TL 116 mm), and gizzard shad (*Dorosoma cepedianum*, 7 fish, average TL 185 mm). All fish were obtained from local ponds and reservoirs.

On 24 July atrazine, as the commercially available herbicide (CO-OP liquid, EPA registration number 1990-381, 40.8% active ingredient), was added to four of the six ponds. A concentration (active ingredient) of 20 µg/L was created in two ponds, 500 µg/L created in two other ponds, and two ponds which received no atrazine served as controls. Atrazine concentrations were monitored throughout the study (deNoyelles et al. 1982) and 70% of the original concentration was detected in the water at the end of the study (136 days after addition).

Macrophyte vegetation in the ponds was monitored by direct visual estimates, qualitative rake hauls, and through areal analysis of photographs of individual ponds. Individual plants were collected from the rake hauls for species identification.

One hundred thirty-six days after atrazine addition the ponds were drained (overnight) to a depth of one meter and immediately seined to recover all fish originally stocked and any young that had been produced. Fish were killed and preserved in 10% formalin. The ponds were then refilled with the reservoir pond water, with no further addition of atrazine, to permit development of macrophyte communities the following spring. Stomachs of adult bluegill recaptured at the end of the study were removed and the contents analyzed. This provided an evaluation of relative difference in diet at that time but could not be used to estimate the impact of bluegill predation on particular prey throughout the 136 days.

RESULTS AND DISCUSSION

Bluegill was the only species to reproduce during the study. The number of young retrieved from atrazine-treated ponds was significantly less ($P < 0.01$) than from control ponds (Table 1). For the original fish stocked, there was no significant difference ($P > 0.05$; one-way ANOVA) among treatments in mortality. Approximately 80% of the original bluegill survived and were retrieved at the end compared to more than 95% of the gizzard shad and channel catfish.

Analysis of the stomach contents of adult bluegill revealed significant differences in diet between fish from control and

treated ponds (Tables 2, 3). Bluegill from control ponds had significantly more ($P < 0.001$) prey items in their stomachs than fish from treated ponds with control pond fish averaging 25.6 food items/stomach compared to 3.8 and 5.7 for fish from 20 and 500 µg/L treatments, respectively. Additionally, relative to treated ponds the food items in the stomachs of control pond fish represented a significantly greater ($P < 0.001$) number of taxonomic groups (Table 2).

Table 1. Reproduction of adult bluegill stocked in experimental ponds treated with 0, 20, or 500 µg/L atrazine. Values given as treatment means ± SE with values in parentheses representing individual pond measurements.

	Pond Treatment (µg/L atrazine)		
	0	20	500
# young/pond	1376 ± 132 (1507, 1244)	59 ± 59* (118, 0)	53 ± 10* (43, 63)

Treatment means significantly different from control at *$P < 0.01$; one way analysis of variance.

Table 2. Analysis[1] of stomach contents of adult bluegill from experimental ponds of 0, 20, or 500 µg/L atrazine treatments. Values given as treatment mean ± SE (n = 50) with values in parentheses representing individual pond means ± SE (n = 25).

	Pond Treatment (µg/L atrazine)		
	0	20	500
# food items/ fish stomach[2]	25.6 ± 4.6 (32.8±8.8,18.3±2.5)	3.8 ± 1.2* (3.9±1.1,3.7±2.0)	5.7 ± 1.9* (9.0±3.4,2.4±1.0)
# prey taxa/ fish stomach[2,3]	2.1 ± 0.2 (1.9±0.3,2.3±0.2)	0.9 ± 0.1* (1.3±0.2,0.5±0.1)	1.0 ± 0.1* (1.0±0.2,0.9±0.1)

[1]Kruskal-Wallis test with group separation through nonparametric multiple comparison (Zar 1974); treatment means significantly different from control at *$P < 0.001$.
[2]Excludes consideration of crustacean zooplankton.
[3]Taxa denotes order classification or above.

Bluegill stomachs from control ponds generally contained more insect orders and a significantly greater number of individuals per given order than did bluegill stomachs from treated ponds (Table 3). The Ephemeroptera was the most frequently represented order in all fish; however, a greater percentage of fish from control ponds (86%) contained Ephemeropterans than did fish from 20 µg/L or 500 µg/L treatments (50% and 38% respectively). Bluegill from control ponds also contained a significantly greater ($P < 0.001$) number of Ephemeropterans than did fish from 20 µg/L or 500 µg/L treatments. The Odonata were commonly found in control bluegill (42% of fish) but were not found in the stomachs of fish from treated ponds. Significantly more ($P < 0.01$) Coleoptera were found in the stomachs of fish from control ponds than from treated ponds where they were rare or absent from stomachs. The Diptera were commonly observed in fish stomachs from one of the control ponds but were rare or absent in fish from all other ponds.

Table 3. Analysis[1] of number of individuals of a given insect order represented in stomachs of adult bluegill from experimental ponds of 0, 20, or 500 µg/L atrazine treatment. Values given as treatment means ± SE (n = 50) with values in parentheses representing individual pond means + SE (n = 25).

	Pond Treatment (µg/L atrazine)		
Insect Order	0	20	500
Ephemeroptera	15.9 ± 2.7 (18.3±5.1,13.4±2.1)	2.7 ± 1.0** (1.8±0.5,3.7±2.0)	4.8 ± 1.9** (8.3±3.5,1.2±1.0)
Odonata	1.9 ± 0.5 (0.6±0.3,3.2±0.9)	0**	0**
Coleoptera	0.9 ± 0.2 (1.0±0.4,0.9±0.3)	<0.1* (<0.1,0)	<0.1* (<0.1,<0.1)
Diptera	6.6 ± 2.7 (13.1±5.2, <0.1)	<0.1ns (<0.1,0)	<0.1ns (<0.1,0)

[1]Kruskal-Wallis test with group separation through nonparametric multiple comparison (Zar 1974); treatment means significantly different from control at *$P < 0.01$, **$P < 0.001$ or ns: $P > 0.05$.

The macrophyte community in treated ponds was noticeably reduced, relative to controls, throughout the summer. Visual estimates of the macrophyte communities of the ponds showed roughly a 60% decline in the 20 µg/L ponds and a 90% decline in the 500 µg/L

ponds two months after atrazine addition. This was verified by rake hauls which produced these same relative differences. In May, when macrophytes are normally well established in Kansas ponds and 10 months after atrazine addition, the ponds were drained completely and the inhibitory effects of atrazine on macrophyte communities were further quantified through estimates from aerial photographs. Relative to control ponds, 20 µg/L ponds had a 90% reduction in macrophyte coverage and 500 µg/L ponds had a >95% reduction in macrophyte coverage. Differences were also noted in the macrophyte species present in control and treated ponds. Control ponds contained *Potamogeton pusillus* and *P. nodosus*, *Najas guadalupensis*, and small amounts of *Chara globularis*, whereas treated ponds contained mostly *C. globularis*.

The pond responses we observed were likely the result of both the direct and indirect (secondary) effects of atrazine herbicide on pond ecosystems. The macrophyte communities of the treated ponds were directly affected by atrazine, a photosynthesis inhibitor (Moreland 1980). The observed responses of the bluegill, however, were not likely due to direct atrazine toxicity. Macek et al. (1976), in chronic toxicity tests with bluegill, reported no effect (relative to controls) of atrazine concentrations as high as 95 µg/L on fish growth, spawning, or development of young. In our study, the differences in bluegill diet and reproductive success between control and treated ponds were more likely due to an indirect effect of atrazine related to reduction of macrophytes in treated ponds. Macrophytes provide habitat (substrate and food) for a diverse invertebrate fauna which in turn serves as prey for bluegill. The density of this invertebrate fauna is known to be directly proportional to the density of the macrophyte vegetation (Gerking 1957). The reduction of the macrophyte community in the treated ponds, and consequently an invertebrate fauna associated with it, was likely reflected in the relatively depauparate diet of fish from treated ponds. Littoral vegetation also provides protection for prey from predators (Cooper and Crowder 1979). A reduced macrophyte refugia in the treated ponds would have exposed bluegill young in those ponds to greater predation pressure, principally from bluegill adults. Additionally, Carlander (1977) reports that shortage of other suitable prey will result in bluegill eating most or all of the eggs and young produced. This may further explain the reduced number of bluegill young recovered from treated ponds.

This study has demonstrated that atrazine herbicide, at concentrations as low as 20 µg/L, can significantly affect the diet and reproductive success of bluegill. Research is needed to conclusively determine the mechanism responsible for these effects. This research should involve considerations of both direct and secondary effects of the herbicide on the ecosystem.

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