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UNITED STATES DEPARTMENT OF THE INTERIOR

FISH AND WILDLIFE SERVICE

# Effects of Synergized Rotenone on Nontarget Organisms in Ponds

by

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Abstract

Applications of 2- or 5-μL/L concentrations of synergized rotenone (2.5%) in the Pro-Noxfish formulation to two shallow, 0.05-ha ponds caused a temporary reduction in both total numbers and diversity of benthic invertebrates and a total mortality of caged Asiatic clams (*Corbicula manilensis*) in both ponds, and a partial mortality of a resident population of larval leopard frogs (*Rana pipiens*) in the 5-μL/L treatment. At day 7 after treatment, benthic organisms (no./$m^2$) were reduced 67% by the 2-μL/L concentration and 96% by the 5-μL/L application. The diversity index ($\bar{d}$) declined sharply in both ponds between days 3 and 7 after treatment, the lowest values being recorded on day 7 and day 37 in the 2- and 5-μL/L treatments, respectively. The equitability index (e) declined from day 3 to day 37 in both ponds. By day 69, however, total numbers of benthic organisms had more than doubled over those originally present in the 2-μL/L treatment (121% increase), had more than tripled in the 5-μL/L treatment (223% increase), and were virtually unchanged in the control pond (2% increase). Pretreatment zooplankton populations were low; no significant deleterious effects from the treatments were observed.

Formulations of rotenone have been used extensively to control fish populations. Concentrations of 0.5 to 1.0 μL/L are adequate in most situations, but rotenone treatments from 0.1 to 5.0 μL/L or more have been used, depending on the purpose of treatment, the fish species present, and conditions at the time of application (Schnick 1974). Chemical and biological conditions in eutrophic waters sometimes require use of the higher concentrations of rotenone (Orn 1962; Spitler 1970). The labels of some rotenone products were recently amended to permit use of concentrations up to 5.0 μL/L. The present study was conducted to evaluate the effects of 2.0- and 5.0-μL/L concentrations of synergized rotenone on benthic invertebrates and zooplankters in ponds. The study was conducted in three experimental ponds at the Warm Springs (Georgia) National Fish Hatchery from 23 August to 1 November 1976.

## Methods

The rotenone formulation used was Pro-Noxfish, a synergized emulsifiable concentrate containing 2.5% rotenone, furnished by S. B. Penick & Co., New York, N.Y. This product contains 2.5% sulfoxide, a synergist used to enhance the activity of the rotenone.

The ponds used in this experiment have surface areas of about 0.05 ha, average and maximum depths of about 1.1 and 1.5 m, and clay bottoms. When checked before the start of the tests, each pond supported low populations of zooplankton, moderate populations of phytoplankton and benthic invertebrates, few aquatic plants, and no fish. About 2 weeks before treatment, applications of 20–20–5 fertilizer and hydrated lime were added to each pond to stimulate plankton production, increase pH, and increase total hardness to about 20 mg/L.

On 24 August, ponds I and II were treated with 2- and 5-μL/L applications, respectively, and Pond III served as an untreated control. At the time of treatment, water temperatures were 31–32°C, dissolved oxygen concentrations were 8.5–11.5 mg/L (supersaturated), pH values were 8.6–9.6, and Secchi disk transparencies were 43–80 cm (Table 1). Concentrations of rotenone (active ingredient) applied were 0.05 and 0.125 μL/L. The required volumes of rotenone formulation were diluted in a tub filled with pond water and then dispensed into the propeller wash of an outboard motor with a boat bailer.

Plankton and benthos samples were taken from each pond before treatment and at 3, 7, 14, 37, and 69 days after treatment. Sampling was terminated after 69 days because populations of most groups of benthic organisms appeared to have recovered from the treatments.

On each sampling date, duplicate samples of benthic organisms were taken from each pond by Ekman dredge (232 $cm^2$) at 0.3, 0.6, 0.9, 1.2, and 1.5 m in each of the ponds (a total of 10 individually preserved samples from each pond). Samples were washed on a U.S. Standard No. 30 sieve in the field and taken to the laboratory for further washing. The samples were stirred for a few minutes in a saturated aqueous solution of Epsom salts (magnesium sulfate) to facilitate separation of benthos from detritus. Floating organisms and low-density organic materials were then skimmed from the surface. This procedure was repeated at least three times and the remaining mixture of organisms and debris was preserved. Benthic organisms were removed and sorted into major taxa under a binocular microscope. I compared mean numbers of each taxon collected per grab on the several dates with pretreatment numbers, using Student's $t$ test to determine whether observed declines in numbers were significant ($P \leq 0.05$). Both mean diversity ($\bar{d}$) and equitability (e) indices for the benthic invertebrates were calculated for each sampling day in each pond, according to methods outlined by Weber (1973). The major premise on which use of the indices is based is that relatively undisturbed environments support communities having large numbers of species, with no single species present in overwhelming abundance. Declines in $\bar{d}$ and e indices are often associated with adverse influences on benthic communities.

A battery-operated pump with a capacity of 16 L/min was used to collect zooplankton samples at depths of 0.3 and 1.2 m. For each sample collection, 32 L of water were pumped through a Wisconsin plankton net (80-$\mu$m mesh) as the boat was moved slowly about the ponds. Samples collected at each depth were concentrated and preserved in 10 mL of a solution consisting of 70% ethyl alcohol, 20% water, and 10% formalin. Numbers of cladocerans, copepods, and rotifers per liter of pond water were determined as suggested by Weber (1973). In addition, 100 adult Asiatic clams, *Corbicula manilensis* (10–14 mm long, 338–985 mg wet wt), were placed in a cage in each pond before treatment and observed for mortalities. Beginning 3 days after treatment, additional cages containing 20 Asiatic clams were placed in the treated ponds on each of 4 successive days. Clams that failed to close their valves or respond to stimuli were considered to be dead.

On the fourth day after treatment, 10 bluegills, *Lepomis machrochirus* (total length, 38–50 mm; weight, 2–4 g), were placed in a cage at a depth of 0.9 m in each pond. All fish were replaced each day until at least eight of the caged fish survived for 24 h.

Temperatures and concentrations of dissolved oxygen were determined at the surface and at 1.5 m on each sampling day. The Secchi disk transparency and the pH at the surface were also recorded in each pond to help ensure that mortalities of invertebrates did not result from adverse water quality.

## Results and Discussion

Substantial phytoplankton blooms developed within 3 days after treatment in ponds I and II, and transparency readings were reduced 50% or more (Table 1). Bonn and Holbert (1961) also observed an increase in the number of phytoplankters after the addition of rotenone to lake waters.

Table 1. *Water quality measurements in ponds used to test effects of a synergized formulation of rotenone (2.5% rotenone) on aquatic invertebrates.*

| Pond, treatment ($\mu$L/L), and sampling day | Water quality measurements | | | | |
|---|---|---|---|---|---|
| | Temperature (°C) | | Dissolved oxygen (mg/L) | | Secchi disk transparency (cm) |
| | Surface | Bottom | Surface | Bottom | |
| Pond I, 2.0 | | | | | |
| 0 | 31.0 | 27.0 | 11.5 | 10.2 | 80 |
| 3 | 30.7 | 27.0 | 13.2 | 7.1 | 40 |
| 7 | 28.0 | 26.0 | >20.0 | 12.0 | 27 |
| Pond II, 5.0 | | | | | |
| 0 | 31.9 | 25.1 | 13.0 | 2.7 | 43 |
| 3 | 32.5 | 26.4 | 17.2 | 4.5 | 15 |
| 7 | 27.8 | 25.5 | >20.0 | 2.0 | 18 |
| Pond III, 0.0 (control) | | | | | |
| 0 | 31.0 | 26.8 | 8.5 | 2.0 | 80 |
| 3 | 30.3 | 26.9 | 10.1 | 6.4 | 82 |
| 7 | 28.0 | 27.0 | 17.2 | 14.3 | 80 |

Table 2. *Estimated total benthic invertebrates (no./m²) in bottom soil samples before and after treatment of pond I with 2 μL/L of a synergized formulation of rotenone (2.5%).*

| Taxa | Days after treatment 0 | 3 | 7 | 37 | 69 |
|---|---|---|---|---|---|
| Nematoda | 0 | 0 | 0 | 17 | 525 |
| Decapoda (*Procambarus*) | 0 | 0 | 0 | 0 | 9 |
| Ephemeroptera (*Caenis*) | 766 | 159 | 4 | 202 | 474 |
| Odonata[a] | 4 | 13 | 4 | 22 | 0 |
| Coleoptera | | | | | |
| *Agabus, Laccophilus* | 13 | 69 | 47 | 13 | 26 |
| *Dineutus* | 4 | 0 | 0 | 0 | 0 |
| *Berosus* | 211 | 603 | 56 | 715 | 577 |
| Hemiptera | | | | | |
| *Notonecta* | 17 | 116 | 0 | 4 | 17 |
| *Hesperocorixa* | 30 | 82 | 13 | 34 | 0 |
| Trichoptera (*Oecetis*) | 56 | 39 | 0 | 0 | 0 |
| Diptera | | | | | |
| *Chaoborus* | 254 | 164 | 125 | 4,585 | 3,475 |
| *Bezzia, Palpomyia* | 56 | 112 | 13 | 116 | 422 |
| *Glyptotendipes* | 3,862 | 2,644 | 1,507 | 8,284 | 6,118 |
| *Chrysops* | 0 | 0 | 0 | 0 | 4 |
| Total organisms | 5,273 | 4,001 | 1,769 | 13,992 | 11,647 |

[a]Odonata included five genera: *Didymops, Erythemis, Ischnura, Libellula,* and *Ophiogomphus.*

They believed that the increase was caused by the reduction in numbers of plankton-feeding fishes, the increased food made available by decaying organic matter, and the additional depth of light penetration. By day 7, dissolved oxygen at the surface in all ponds had increased by more than 50%. The low concentrations of dissolved oxygen (2 mg/L) at the bottom in pond III on day 0 and in II on day 7 caused no mortalities among caged clams and were not considered to have been injurious to the benthic fauna.

All Asiatic clams died within 24 h after treatment in both treated ponds, and there was a substantial mortality of a resident population of larval leopard frogs (*Rana pipiens*) in pond II (5 μL/L). However, among clams introduced into ponds I and II on the third day after treatment, none died within 25 days in pond I (2 μL/L) and only three in II (5 μL/L). No clams or bluegills died in the control pond during the study. Concentrations of rotenone were reduced to essentially nontoxic levels for clams by the fourth day after treatment, but among small bluegills placed in the treated ponds on the fourth day, mortality was 50% in pond I (2 μL/L) and 100% in pond II (5 μL/L). However, degradation proceeded rapidly and both the 2-μL/L and 5-μL/L treatments were nontoxic to bluegills by the sixth day after treatment.

Populations of benthic invertebrates were similar at the outset in that all eight of the orders of organisms were found in all three ponds. The total number of invertebrate taxa initially collected in each pond ranged from 12 to 14, and the estimated total numbers of organisms varied widely among sample days (Tables 2, 3, and 4).

Pronounced changes in total numbers and diversity of benthic organisms occurred in both ponds after treatment, and many dead insect larvae were found in the 3- and 7-day samples. In pond I (2 μL/L), the total number of organisms per unit of area was reduced by 66.5% on day 7. No whirligig beetles (*Dineutus*), backswimmers (*Notonecta*), or caddisflies (*Oecetis*) were found, reducing the total number of orders of insects collected from six to five (Table 2). In contrast, Houf and Campbell (1977) reported that a 2-mg/L application of the Noxfish (5%) formulation of rotenone produced no immediate effects on abundance of benthic organisms in a shallow (0.6 m), mud-bottomed pond that was heavily vegetated. In their study, two factors mentioned by Lindgren (1960) may have contributed to the observed reduction in toxic effects: (1) the oxidation of rotenone in the vicinity of photosynthesizing vegetation; and (2) the sorption of rotenone by gyttja, clay, or gel-mud in the bottom soils. In pond II (5 μL/L), the total numbers of organisms were reduced 96% by day 7; no nematodes, mayflies (*Caenis*), dragonflies (*Libellula, Ophiogomphis, Didymops, Erythemis,* or *Ischnura*), caddisflies, or phantom midges (*Chaoborus*) were found, and the number of orders present dropped from six to two (Table 3). In pond III (control), there was also a pronounced drop in the total number of organisms present on day 7 (Table 4). Apparently, some of this reduction resulted from an emergence of chironomids (*Glyptotendipes*), whose numbers decreased by 48% during the preceding week.

Reductions in abundance of several taxa were observed at various intervals after treatment, but most were not

*Table 3. Estimated total benthic invertebrates (no./m$^2$) in bottom soil samples before and after treatment of pond II with 5 µL/L of a synergized formulation of rotenone (2.5%).*

| | Days after treatment | | | | |
|---|---|---|---|---|---|
| Taxa | 0 | 3 | 7 | 37 | 69 |
| Nematoda | 13 | 0 | 0 | 17 | 784 |
| Decapoda (*Procambarus*) | 0 | 0 | 0 | 4 | 0 |
| Ephemeroptera (*Caenis*) | 2,174 | 164 | 0 | 125 | 82 |
| Odonata[a] | 168 | 34 | 0 | 138 | 4 |
| Coleoptera | | | | | |
| *Agabus, Laccophilus* | 30 | 4 | 13 | 0 | 0 |
| *Dineutus* | 8 | 0 | 4 | 0 | 0 |
| *Berosus* | 499 | 133 | 116 | 112 | 52 |
| Hemiptera (*Hesperocorixa*) | 0 | 0 | 0 | 4 | 0 |
| Trichoptera (*Oecetis*) | 4 | 0 | 0 | 0 | 0 |
| Diptera | | | | | |
| *Chaoborus* | 392 | 56 | 0 | 2,458 | 2,665 |
| *Bezzia, Palpomyia* | 4 | 4 | 9 | 39 | 215 |
| *Glyptotendipes* | 560 | 65 | 17 | 8,925 | 8,641 |
| Total organisms | 3,852 | 460 | 159 | 11,822 | 12,443 |

[a]Odonata included five genera: *Didymops, Erythemis, Ischnura, Libellula,* and *Ophiogomphus.*

statistically significant. In pond I (2 µL/L), a mortality of mayflies was readily evident, and significant reductions in their numbers were observed on days 3, 7, and 37 (Table 5). Inasmuch as water boatmen (Corixidae) were present in only small numbers on the first four sampling dates, failure to collect them on day 69 was regarded as a sampling anomaly rather than a result of treatment. In pond II (5 µL/L), mayflies and dragonflies were significantly reduced on day 3; on day 7 no mayflies or dragonflies were taken (Table 6). Although both populations increased thereafter, the reduction in their numbers remained significant on all later sampling dates until day 37, when numerous dragonflies were taken (82% of the original number). In the control pond, the numbers of organisms in different taxa fluctuated widely, but no reductions were statistically significant (Table 7).

Recovery from the effects of treatment varied among the different groups of organisms, but was especially rapid

Table 4. *Estimated total benthic invertebrates (no./m$^2$) in bottom soil samples in pond III (control) on days when treated ponds were sampled.*

| | Days after treatment | | | | |
|---|---|---|---|---|---|
| Taxa | 0 | 3 | 7 | 37 | 69 |
| Nematoda | 22 | 17 | 99 | 56 | 263 |
| Decapoda | | | | | |
| *Procambarus* | 0 | 0 | 0 | 4 | 0 |
| *Palaemonetes* | 73 | 73 | 164 | 69 | 103 |
| Ephemeroptera (*Caenis*) | 0 | 0 | 0 | 4 | 0 |
| Odonata[a] | 9 | 13 | 0 | 4 | 4 |
| Coleoptera (*Berosus*) | 4 | 0 | 4 | 4 | 9 |
| Hemiptera | | | | | |
| *Notonecta* | 4 | 0 | 0 | 13 | 4 |
| *Hesperocorixa* | 0 | 17 | 22 | 0 | 0 |
| Trichoptera (*Oecetis*) | 0 | 0 | 0 | 4 | 0 |
| Diptera | | | | | |
| *Chaoborus* | 1,085 | 288 | 2,316 | 112 | 4,994 |
| *Bezzia, Palpomyia* | 99 | 43 | 146 | 336 | 633 |
| *Glyptotendipes* | 17,110 | 11,814 | 8,852 | 7,552 | 12,731 |
| Total organisms | 18,406 | 12,265 | 11,603 | 8,158 | 18,741 |

[a]Odonata included five genera: *Didymops, Erythemis, Ischnura, Libellula,* and *Ophiogomphus.*

Table 5. *Mean numbers of numerically important groups of benthic invertebrates per grab (standard error of means in parentheses) and percent change from pretreatment numbers after treatment with 2 μL/L of a synergized formulation of rotenone (2.5%) in pond I.*

| | Pretreatment | Days after treatment: 3 | | 7 | | 37 | | 69 | |
|---|---|---|---|---|---|---|---|---|---|
| Taxa | Mean number | Mean number | Change (%) | Mean number | Change (%) | Mean number | Change (%) | Mean number | Change (%) |
| Ephemeroptera (*Caenis*) | 17.8 (5.2) | 3.7[a] (1.3) | -79 | 0.1[a] (0.1) | -99 | 4.7[a] (2.1) | -74 | 11.0 (3.9) | -38 |
| Odonata[b] | 0.1 (0.1) | 0.3 (0.2) | 200 | 0.1 (0.1) | 0 | 0.5 (0.5) | 400 | 0.0 (0.0) | -100 |
| Coleoptera | | | | | | | | | |
| *Agabus, Laccophilus* | 0.3 (0.2) | 1.6 (0.7) | 433 | 1.1 (0.6) | 267 | 0.3 (0.3) | 0 | 0.6 (0.3) | 100 |
| *Berosus* | 4.9 (3.0) | 17.6 (8.0) | 259 | 1.3 (0.6) | -73 | 16.6 (5.3) | 239 | 13.4 (5.2) | 173 |
| Hemiptera | | | | | | | | | |
| *Notonecta* | 0.4 (0.2) | 2.7 (0.7) | 575 | 0.0 (0.0) | -100 | 0.1 (0.1) | -75 | 0.4 (0.2) | 0 |
| *Hesperocorixa* | 0.7 (0.3) | 1.9 (1.3) | 171 | 0.3 (0.3) | -57 | 0.8 (0.5) | 14 | 0.0[a] (0.0) | -100 |
| Diptera | | | | | | | | | |
| *Chaoborus* | 5.9 (4.7) | 3.8 (1.4) | -36 | 2.9 (2.2) | -51 | 106.5 (23.3) | 1,705 | 80.7 (40.0) | 1,268 |
| *Bezzia, Palpomyia* | 1.3 (0.5) | 2.6 (0.9) | 100 | 0.3 (0.2) | -77 | 2.7 (1.7) | 108 | 9.8 (5.3) | 654 |
| *Glyptotendipes* | 89.7 (42.6) | 61.4 (16.5) | -32 | 35.0 (8.0) | -61 | 192.4 (105.6) | 114 | 142.1 (56.2) | 58 |
| Total organisms | 121.1 (46.0) | 95.5 (21.7) | -21 | 41.1 (7.2) | -66 | 324.6 (112.3) | 168 | 245.8 (64.9) | 103 |

[a]Significant reduction in numbers from pretreatment samples.
[b]Odonata included five genera: *Didymops*, *Erythemis*, *Ischnura*, *Libellula*, and *Ophiogomphus*.

among dipterans. The density of organisms (no./m$^2$) in treated ponds on day 37 after treatment exceeded pretreatment populations by 165% in pond I and by 207% in II. In contrast, the numbers in the control pond on day 37 were 56% less than before treatment. One month later (day 69), total numbers of organisms collected in each pond were greater than those before treatment by the following margins: 2-μL/L treatment, 121%; 5-μL/L treatment, 223%; and control, 2%. This finding corresponds with that of Cook and Moore (1969), who observed a rapid and explosive resurgence in insect numbers within 2 to 6 weeks after a late-summer rotenone treatment of a California creek.

Calculation of mean diversity ($\bar{d}$) and equitability (e) indices for the benthic invertebrates showed that treatment caused a substantial decline in $\bar{d}$ values in both ponds I and II by day 7 (Table 8). In pond I (2 μL/L), $\bar{d}$ had increased markedly by day 37, and was slightly higher than the pretreatment value by day 69. In pond II, however, $\bar{d}$ on day 69 remained depressed but the dipteran population (largely *Chaoborus* and *Glyptotendipes*) had increased so much that the total numbers of organisms per square meter greatly exceeded the numbers before treatment. In the control pond, $\bar{d}$ values fluctuated considerably, but were highest on day 69.

The e values in the control pond were consistently lower than those in the treated ponds (Table 8). The e values in both treated ponds were lowest on day 37, and the decline was greatest in the 5-μL/L treatment. The reduction of both $\bar{d}$ and e values indicated that the rotenone treatments disturbed community structure, but a strong trend toward recovery was evident at 69 days after treatment.

Zooplankton populations in all three ponds remained consistently low throughout the study (Table 9). Minor fluctuations in abundance occurred, but the treatments did not produce the pronounced deleterious effects sometimes associated with rotenone applications (Almquist 1959; Kiser et al. 1963). Wollitz (1962) found that a treatment with 0.95 μL/L Pro-Noxfish in one experimental pond greatly reduced the zooplankton population, whereas a 0.70-μL/L treatment in another pond apparently had little effect on most zooplankters. The reason for such differences is unclear. Kiser et al. (1963) suggested that the application of rotenone in spring and early summer, during zooplankton population pulses, appears to have more severe impacts and more lingering effects than applications made in autumn.

Table 6. *Mean numbers of numerically important groups of benthic invertebrates per grab (standard error of means in parentheses) and percent change from pretreatment numbers after treatment with 5 μL/L of a synergized formulation of rotenone (2.5%) in pond II.*

| | | Days after treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pretreatment | 3 | | 7 | | 37 | | 69 | |
| Taxa | Mean number | Mean number | Change (%) | Mean number | Change (%) | Mean number | Change (%) | Mean number | Change (%) |
| Nematoda | 0.3 | 0.0 | -100 | 0.0 | -100 | 0.4 | 33 | 18.2 | 5,967 |
| | (0.2) | (0.0) | | (0.0) | | (0.2) | | (10.0) | |
| Ephemeroptera (*Caenis*) | 50.5 | 3.8[a] | -92 | 0.0[a] | -100 | 2.9[a] | -94 | 1.9[a] | -96 |
| | (9.6) | (1.5) | | (0.0) | | (16.6) | | (0.6) | |
| Odonata[b] | 3.9 | 0.8[a] | -79 | 0.0[a] | -100 | 3.2 | -18 | 0.1[a] | -97 |
| | (1.3) | (0.4) | | (0.0) | | (2.2) | | (0.1) | |
| Coleoptera | | | | | | | | | |
| *Agabus, Laccophilus* | 0.7 | 0.1 | -86 | 0.3 | -57 | 0.0 | -100 | 0.0 | -100 |
| | (0.4) | (0.1) | | (0.2) | | (0.0) | | (0.0) | |
| *Berosus* | 11.6 | 3.1 | -73 | 2.7 | -77 | 2.6 | -78 | 1.2 | -90 |
| | (5.9) | (1.9) | | (1.2) | | (1.1) | | (0.4) | |
| Diptera | | | | | | | | | |
| *Chaoborus* | 9.1 | 1.3 | -86 | 0.0 | -100 | 57.1 | 527 | 61.9 | 580 |
| | (5.7) | (0.7) | | (0.0) | | (16.8) | | (40.8) | |
| *Bezzia, Palpomyia* | 0.1 | 0.1 | 0 | 0.2 | 100 | 0.9 | 800 | 5.0 | 4,900 |
| | (0.1) | (0.1) | | (0.2) | | (0.3) | | (1.3) | |
| *Glyptotendipes* | 13.0 | 1.5 | -88 | 0.4 | -97 | 207.3 | 1,495 | 200.7 | 1,444 |
| | (9.5) | (0.6) | | (0.2) | | (51.6) | | (45.3) | |
| Total organisms | 89.2 | 10.7 | -88 | 4.0 | -96 | 274.5 | 208 | 289.0 | 224 |
| | (18.0) | (3.9) | | (1.2) | | (63.5) | | (37.4) | |

[a]Significant reduction in numbers from pretreatment samples.
[b]Odonata included five genera: *Didymops, Erythemis, Ischnura, Libellula,* and *Ophiogomphus.*

Table 7. *Mean numbers of numerically important groups of benthic invertebrates per grab (standard error of means in parentheses) and percent change in numbers present in pond III (control) on various days after ponds I and II were treated.*

| | | Days after treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pretreatment | 3 | | 7 | | 37 | | 69 | |
| Taxa | Mean number | Mean number | Change (%) | Mean number | Change (%) | Mean number | Change (%) | Mean number | Change (%) |
| Nematoda | 0.5 | 0.4 | -20 | 2.3 | 0 | 2.3 | 160 | 6.1 | 1,120 |
| | (0.2) | (0.3) | | (0.9) | | (0.6) | | (1.6) | |
| Odonata[a] | 0.2 | 0.3 | 50 | 0.0 | -100 | 0.1 | -50 | 0.1 | -50 |
| | (0.2) | (0.2) | | (0.0) | | (0.1) | | (0.1) | |
| Coleoptera (*Berosus*) | 0.1 | 0.0 | -100 | 0.1 | 0 | 0.1 | 0 | 0.2 | 100 |
| | (0.1) | (0.0) | | (0.1) | | (0.1) | | (0.2) | |
| Hemiptera (*Notonecta*) | 0.1 | 0.0 | -100 | 0.0 | -100 | 0.3 | 200 | 0.1 | 0 |
| | (0.1) | (0.0) | | (0.0) | | (0.3) | | (0.1) | |
| Diptera | | | | | | | | | |
| *Chaoborus* | 25.2 | 6.7 | -73 | 54.8 | 117 | 2.6 | -90 | 116.0 | 360 |
| | (20.2) | (3.0) | | (27.2) | | (0.9) | | (54.6) | |
| *Bezzia, Palpomyia* | 2.3 | 1.0 | -57 | 3.4 | 48 | 7.8 | 239 | 14.7 | 539 |
| | (0.5) | (0.4) | | (1.3) | | (1.8) | | (3.3) | |
| *Glyptotendipes* | 397.4 | 274.4 | -31 | 205.7 | -48 | 175.4 | -56 | 295.7 | -26 |
| | (99.0) | (82.2) | | (81.0) | | (41.6) | | (68.4) | |
| Total organisms | 425.8 | 282.8 | -34 | 266.3 | -37 | 187.9 | -56 | 432.9 | -38 |
| | (89.8) | (62.7) | | (65.8) | | (39.9) | | (81.3) | |

[a]Odonata included five genera: *Didymops, Erythemis, Ischnura, Libellula,* and *Ophiogomphus.*

Table 8. *Mean indices of diversity ($\bar{d}$) and equitability (e) for benthic invertebrate samples collected at designated times from two ponds treated with a synergized formulation of rotenone and a control pond.*

| Pond no. | Concentration of Pro-Noxfish (μL/L) | Index | Pretreatment | Days after treatment 3 | 7 | 37 | 69 |
|---|---|---|---|---|---|---|---|
| I | 2.0 | $\bar{d}$ | 1.6229 | 1.8061 | 0.8459 | 1.3656 | 1.6050 |
| | | e | 0.4885 | 0.5038 | 0.5177 | 0.4550 | 0.5511 |
| II | 5.0 | $\bar{d}$ | 1.8424 | 2.1075 | 0.5548 | 1.0257 | 1.2744 |
| | | e | 0.7771 | 1.1455 | 0.8419 | 0.3484 | 0.4924 |
| III | 0.0 | $\bar{d}$ | 0.4214 | 0.2625 | 0.9905 | 0.4810 | 1.1799 |
| | (control) | e | 0.3039 | 0.2647 | 0.4734 | 0.3286 | 0.4360 |

Table 9. *Estimated numbers of zooplankters per liter of water taken at selected intervals from pond III (control) and from ponds I and II, which were treated with 2.0- and 5.0-μL/L applications of rotenone, respectively.*

| | Cladocera | | | Copepoda | | | Rotifera | | |
|---|---|---|---|---|---|---|---|---|---|
| Sampling day | Pond I | Pond II | Pond III | Pond I | Pond II | Pond III | Pond I | Pond II | Pond III |
| Pretreatment | 0.47 | 0.00 | 0.00 | 0.16 | 0.16 | 7.03 | 0.00 | 0.16 | 0.00 |
| 7 | 0.00 | 0.00 | 0.00 | 0.00 | 0.16 | 2.66 | 0.63 | 0.16 | 0.00 |
| 37 | 0.00 | 9.84 | 3.44 | 0.16 | 0.16 | 0.16 | 0.00 | 0.00 | 0.00 |
| 69 | 0.00 | 1.09 | 0.16 | 3.13 | 2.19 | 9.84 | 0.31 | 0.16 | 2.03 |

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