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Effects of Esfenvalerate on the Reproductive Success of the Bluegill Sunfish, *Lepomis macrochirus* in Littoral Enclosures

D. K. Tanner, M. L. Knuth

National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Mid-Continent Ecology Division–Duluth
6201 Congdon Boulevard, Duluth, Minnesota 55804, USA

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Abstract. Adult bluegills were exposed to the synthetic pyrethroid esfenvalerate, during a reproduction study conducted in six littoral enclosures located in a 2-ha pond near Duluth, Minnesota. Bluegill reproductive success was determined after two applications of esfenvalerate at nominal concentrations of 0.0, 0.01, 0.08, 0.20, 1.0, and 5.0 μg/L. Responses measured were adult behavior and spawning, embryo hatchability, larval survival until swim-up, young-of-the-year (Y-O-Y) growth, and total biomass. All adult bluegills died within one day following the first application at 5.0 μg/L. Successful spawning and resulting hatching of offspring were observed at all esfenvalerate concentrations except 5.0 μg/L. Following exposure at 1.0 μg/L, spawning was delayed for 15 days and few or no larvae survived. Bluegill Y-O-Y growth was reduced by 62, 57, and 86% in the 0.08, 0.20, and 1.0 μg/L esfenvalerate treated enclosures. Delayed adult spawning and reduced Y-O-Y growth may result in poor Y-O-Y overwinter survival which would result in a diminished or extirpated year class.

Esfenvalerate is a synthetic pyrethroid pesticide that is used to control insects on agricultural crops, primarily apples, peaches, cotton, and almonds. Synthetic pyrethroids are broad spectrum insecticides that have relatively short half-lives in the environment, low bioaccumulation potential, and low mammalian toxicity. Esfenvalerate is known commercially as Asana® and is the common name for (*S*)-cyano(3-phenoxyphenyl) methyl-(*S*)-4-chloro-alpha-(1-methylethyl) benzeneacetate. Esfenvalerate is the most toxic of the four stereoisomers that comprise the pyrethroid pesticide fenvalerate. Bradbury *et al.* (1987) found that esfenvalerate (2S ∝ S isomer) was 5.6 times more toxic to bluegills (*Lepomis macrochirus*) than technical fenvalerate. Esfenvalerate is also toxic to nontarget organisms such as fish and aquatic invertebrates which may be exposed by spray drift or field runoff. Although there have been many single species laboratory tests using fenvalerate (Eisler 1992), very little information is available on the effects of esfenvalerate on aquatic organisms.

Littoral enclosures had been used successfully to study the direct and indirect effects of chlorpyrifos on non target biota in aquatic ecosystems (Brazner *et al.* 1989). The bluegill reproductive success study was part of a larger project which estimated the persistance, fate, and primary and secondary effects of esfenvalerate on the structure and function of lentic ecosystems (Heinis and Knuth 1992; Lozano *et al.* 1989). The objectives of the reproductive study were to determine if bluegills would spawn successfully in littoral enclosures, and if spawning was successful, to determine the effects of esfenvalerate on adult survival, spawning behavior, embryo hatchability, larval survival until swim-up, Y-O-Y growth rate, and Y-O-Y biomass sampled throughout the growing season (June–September). An accurate measure of these biological endpoints would allow for the prediction of the pesticide effects on the well-being of the populations of this important sport fish. Two other mesocosm studies using esfenvalerate were conducted concurrently by Auburn University (0.08-ha ponds) (Webber *et al.* 1992) and by the National Fisheries Contaminant Research Center, Columbia, MO (0.04 ha ponds) (Fairchild *et al.* 1993). The effects of esfenvalerate on bluegill reproductive success could then be compared using three test systems in different geographic locations with different climatic conditions.

Materials and Methods

Enclosure Description

The studies were conducted within six 5 × 10 m enclosures in the littoral zone of a 2-ha mesotrophic pond near Duluth, Minnesota. Enclosed aquatic vegetation consisted primarily of *Typha sp., Chara vulgaris,* and *Chara globularis,* and *Potamageton natans* and the sediment was characterized by unconsolidated and highly organic material. The enclosures extended from the natural shore to a depth of about 1.5 m. Each enclosure had a mean water depth of 1.1 m and a mean

Correspondence to: D. K. Tanner

water volume of 33 m^3. The littoral enclosures were constructed as described by Brazner *et al.* (1989).

Experimental Design

Esfenvalerate test concentrations were selected to encompass a wide range of the laboratory derived LC_{50} values for a variety of aquatic organisms that ranged from a no effect to a high effect level. Concentration selection was also based on microcosm and fish toxicity data provided by Stay and Jarvinen (1995). A regression approach was used for the experimental design because of the limited amount of available laboratory and field data. Six littoral enclosures were randomly assigned esfenvalerate treatment levels of 0.00, 0.01, 0.08, 0.20, 1.0, and 5.0 µg/L, respectively.

Preparation of Littoral Enclosures

Enclosure walls were installed on May 18, 1988. Four minnow traps (0.65 cm mesh, 44 cm long, 23 cm max. diam.) were placed in each enclosure on May 20 to reduce indigenous fish populations. Fish trapping efforts were terminated after three weeks when the catch rate fell to an average of one fish per day per enclosure. The indigenous species consisted of northern red belly dace (*Phoxinus eos Cope*), fathead minnows (*Pimephales promelas*), central mudminnows (*Umbra limi*), and brook sticklebacks (*Culaea inconstans*). These fishes were removed to minimize their impact on the invertebrate forage base and reduce predation on bluegill larvae.

Adult Bluegills

Adult bluegills were obtained from hatchery ponds in Minnesota and Missouri and were received at the Mid-Continent Ecology Division–Duluth in September 1987. Bluegills were held in laboratory tanks at water temperatures between 10 and 12°C, and were fed frozen brine shrimp and trout pellets daily. On May 25, 1988, brood fish were individually netted, and the sex was determined using secondary sexual characteristics described by Brauhn (1972). Adult bluegills were then transported in aerated insulated coolers to the study site. An estimated eight males and seven females were randomly assigned to each of the six enclosures. Male to female sex ratio was verified at the end of the study when the fish were captured and dissected. No supplemental foods were added to the enclosures.

Sixteen spawning substrates similar to those described by Wrenn and Granneman (1980) were placed in each enclosure two days before the addition of the adult bluegills. The substrates were black plastic pans (33 cm diam. × 10 cm deep) filled to a depth of 5 cm with washed, 4–8-mm gravel and placed in the sediment so that the rims were even with the top of the sediment. The substrates were placed at sites approximately 1.5 to 2.0 m from the shoreline at depths of 29 to 61 cm.

Spawning and Hatching

After introduction of adult bluegills, daily observations were made of their behavior. When a male bluegill began to establish territory by sweeping, circling, and guarding a nest site, a watch glass was placed in the center of the nest to obtain subsamples of the embryos. To determine percent hatchability, the watch glass was transferred to a screened 500-ml jar placed next to the nest. Both the presence of viable larvae and the time of swim-up were determined by siphoning a few of the larvae from the center of the nest.

Young Bluegill Growth and Biomass

Free-swimming Y-O-Y bluegill were sampled weekly from June 10 to September 16 with dipnets (500 to 1,000 µm). When possible, at least 10 larval bluegills were captured from each enclosure on each sampling date. Following capture, Y-O-Y bluegill were placed in ice, preserved in 10% formalin, transferred to 70% ethanol, and measured for total length (±0.1 mm) and wet weight (±0.1 mg).

At the end of the study (September 28), adult and remaining Y-O-Y bluegills were captured by seining. Rotenone (Nusyn Noxfish® 4 mg/L) was applied to the enclosures on September 29 to recover any remaining fish. Adult bluegills were measured for total length (±1 mm), wet weight (±0.1 g), and dissected for gender determination. Growth rates of larval bluegill were determined by dividing mean weight of larval bluegills captured on September 28 by the estimated days after hatching for that group of larvae. Estimates of hatching dates were based on field observation and examination of histograms prepared from larval bluegill weights and lengths throughout the study period. Product-moment correlations between larval growth rates, total biomass, and esfenvalerate concentrations were calculated.

Biomass was estimated as the total wet weight (g) of all bluegill larvae sampled from each enclosure throughout the study.

Digestive Tract Analysis

Digestive tracts of Y-O-Y bluegills were removed and the contents were counted and identified to the following categories: cladocerans, rotifers, adult copepods and copepodites, copepod nauplii, chironomids, and other invertebrates (protozoans, ostracods, mites, and nematodes). Percent frequencies for each of these categories were then calculated.

Invertebrates

Zooplankton were collected on seven dates from June 17 to September 16 to determine the effects of esfenvalerate on the primary prey of bluegill Y-O-Y and to thereby ascertain whether Y-O-Y growth effects were due to the loss or reduction of zooplankton prey. Four funnel traps (Lozano *et al.* 1989; Whiteside 1974) each holding four 10-cm diameter glass funnels were placed in each enclosure on each sample day. The contents of the traps from each enclosure were removed after 24 h, pooled, and initially preserved in a sucrose-buffered formalin solution (8% W/V). In the lab, samples were concentrated through a 35 µm mesh screen and preserved with 70% ethanol solution. Volume was adjusted to achieve a density of 150–200 organisms/ml. Five 1-ml subsamples were counted for Cladocera, Copepoda, Ostracoda, Protozoa, and Hydrachnida, and three to five subsamples were counted for Rotifera. Organisms were identified to genus with the aid of a compound microscope at 40 to 100×.

Water Temperature and Dissolved Oxygen

Water temperature and dissolved oxygen were measured throughout the study. Water temperatures were recorded daily with a Jewett recording thermometer. Dissolved oxygen was measured weekly in all enclosures using the Winkler method (American Public Health Association 1985).

Pesticide Application and Water Chemistry

Esfenvalerate was applied as Asana® 1.95EC (228 g/L active ingredient) on June 20 and July 18, 1988 between 8:30 a.m. and 2:30 p.m. (CDT). The concentrate was diluted 1:100 with deionized water and

the appropriate volume of diluted formulation was mixed with 4 L of distilled water at the field site. The toxicant was applied over the entire enclosure water surface with a hand pressurized sprayer and 2.4-m wand. Depth-integrated composite water samples consisting of 5 subsamples were collected from each enclosure at 1 h, and at 1, 2, and 4 d after the first application and at 1, 3, and 9 h and 1, 2, and 4 d after the second pesticide application. Water samples were analyzed for esfenvalerate residue using capillary gas-liquid chromatography after liquid-liquid extraction with isooctane (Heinis and Knuth 1992).

Results

Water Column Residues and Dissolved Oxygen

Maximum residues of esfenvalerate in the water column were measured at one hour following the first application and at one to three hours following the second application. The measured esfenvalerate concentrations agreed reasonably well with the nominal values. Following the first application, measured and nominal (in parentheses) concentrations were $< 0.047\mu g/L$ (minimum detection limit) (control and 0.01), 0.069 (0.08), 0.14 (0.20), 0.44 (1.0), and 6.28 (5.0) μg/L. Following the second application, concentrations were < 0.047 (control and 0.01), 0.099 (0.08), 0.20 (0.20), 1.08 (1.0), and 3.61 (5.0) μg/L. Esfenvalerate residues in the water column were not detectable in any treatment enclosure after 48 h. Esfenvalerate concentrations in the sediments were not measured.

Dissolved oxygen concentrations throughout the study ranged from 2.1 to 12.1 mg/L. Control, 0.01-, and 5.0-μg/L enclosures had similar and consistently lower dissolved oxygen concentrations than 0.08-, 1.0-, and 0.20-μg/L enclosures.

Bluegill Behavioral Effects

Adverse behavioral effects were observed in adult bluegills in the 5.0-μg/L enclosure approximately 5 h following the first application of esfenvalerate, and 30 min following the second application in the 1.0-μg/L enclosure. Five hours following the first application in the 5.0-μg/L treatment enclosure bluegills rolled laterally, coughed repeatedly, and swam rapidly. After six hours these bluegills had difficulty maintaining normal orientation and position in the water column, and soon began convulsing and twitching while laying on the bottom. Within 24 h of the first application at 5.0-μg/L all adult bluegills died. Thirty minutes after the second application, 12 bluegills in the 1.0-μg/L enclosure swam rapidly around the perimeter of the enclosure near the surface, and exhibited darkened coloration, coughing and gulping responses. One hour following the second application, a male bluegill guarding a nest containing embryos in the 1.0-μg/L enclosure rim-circled (swimming around the perimeter of the nest) extremely rapidly.

Bluegill Reproduction

Before spawning, male bluegills established and defended territories above and around the artificial spawning substrates or naturally dug nest sites in all enclosures. Ninety-one percent of the nest sites were located in the artificial spawning substrates. Natural nest sites were within a meter of the artificial substrates and closely resembled them in both size and shape. The largest males were predominantly the first to establish nests and initiate spawning. Rim circling by males was observed in all enclosures before and after esfenvalerate application except at the 5.0-μg/L treatment. Water depth at nests varied from 29 to 61 cm (Table 1). The most nests in which spawning occurred (seven) were in the 0.08-μg/L enclosure, while the control enclosure had the least (one), and the remaining enclosures had from two to four nests.

Spawning occurred in all enclosures except the 5.0-μg/L enclosure. The largest number of spawns (13) were in the 0.08-μg/L enclosure, while the control enclosure had the least (two), and five or six spawns occurred in the remaining enclosures (Table 1). Bluegills spawned from May 30 to August 10 with the majority (55%) of the spawning occurring from July 4 to July 31. Spawning occurred at water temperatures of 22 to 27°C, but was most frequent at 24 to 27° C (Figure 1). There were six spawning occurrences prior to application, and 26 following pesticide exposure. However, spawning ceased for fifteen days in the 1.0-μg/L enclosure following each application.

Viable embryos were produced in all enclosures where spawning occurred. Mean percent embryo hatchability was nearly identical (67–68%) among all enclosures except the control where hatchability was 53% (Table 1). Successful hatching of embryos also occurred in all enclosures where spawning took place, even 24 h following the second application in the 1.0-μg/L enclosure. However, dead larvae were found on this nest in the 1.0-μg/L enclosure three days following embryo hatching. Bluegill larvae were regularly observed in the 0.08- and 0.20-μg/L enclosures from 21 d after the first application to the termination of the study on September 28 (79 days later). Larvae were regularly observed in the 1.0-μg/L enclosure 23 d after the first application to 1 h before the second application. Bluegill larvae were captured during weekly dip-net sampling from one day following the first application to the termination of the study in the 0.08- and 0.20-μg/L enclosure and three days prior to both the first and second applications in the 1.0-μg/L enclosure. However, larvae were not captured again in the 1.0-μg/L enclosure until 22 d following the first application, and 60 d following the second application. Although larvae were observed in the nests prior to swim-up in the 0.01-μg/L enclosure, they were not captured during dip-net sampling. Bluegill larvae were captured in the control enclosure 60 d after the first application to the termination of the study.

Young Bluegill Growth and Biomass

Larval bluegill growth rates decreased with increasing concentrations of esfenvalerate with the exception of growth rates from the 0.08- and 0.20-μg/L enclosures, which were nearly identical (Figure 2). Growth (mg/day) was reduced by 62, 57, and 86% in the 0.08-, 0.20-, and 1.0-μg/L enclosures, respectively, relative to the control enclosure. A significant correlation ($r = 0.96$) existed between growth rate and the log of the nominal treatment concentration, $\log(x + 0.01)$.

Young-of-the-year bluegill biomass varied between enclosures (Figure 3). The largest biomass (144 g) was produced in the 0.08-μg/L enclosure, and the smallest biomass (0.28 g) was produced in the 1.0-μg/L enclosure. Bluegill biomass in the 0.20-μg/L and control enclosures were 79 and 3.0 g, respec-

Table 1. Number of bluegill spawnings, range of spawning dates, temperature at spawning, hatching success, male:female ratio, and depth of water over nests in littoral enclosures. Esfenvalerate was applied in the morning on June 20 and July 18, 1988

Nominal esfenvalerate concentration (μg/L)	Number of spawnings (Number of nests)	Range of spawning dates (Mo/day)	Range of spawning temperature (°C)	Mean percent embryo hatching (Range)	Male/Female Ratio	Depth of water over nest site (cm)
Control	2 (1)	7/11–7/30	23.4–26.4	53 (6–100)	9:4	61
0.01	5 (2)	6/22–8/1	23.5–26.0	68 (25–92)	—[a]	38–39
0.08	14 (7)	5/30–8/10	22.4–27.0	68 (8–98)	7:6	29–43
0.2	6 (3)	5/31–8/10	22.0–27.0	68 (0–100)	6:8	47–51
1.0	6 (4)	6/8–8/2	22.0–25.5	67 (20–100)	9:5	34–44
5.0	—[b]	—	—	—	—	—

[a] Unknown; all adult fish escaped approximately August 9
[b] All adult fish died after first application of esfenvalerate

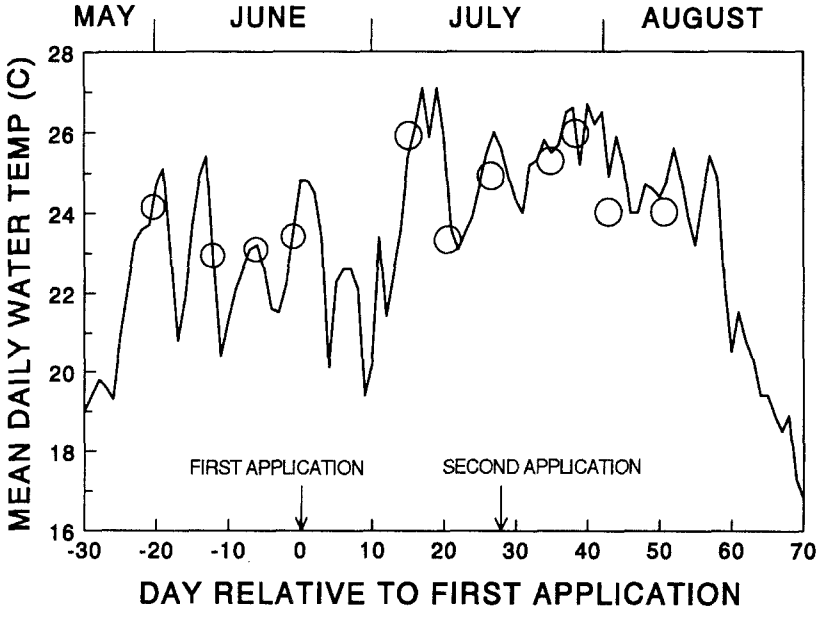

Fig. 1. Bluegill spawning periods (circled areas) in relation to mean daily water temperature in the enclosures and day relative to two applications of esfenvalerate

tively. Total biomass was not significantly correlated to the log of the nominal treatment concentration log(x + 0.01) (r = 0.0%).

Invertebrate Abundance

For most of the study period, abundance (organisms/m^2) of cladocerans, copepods, and rotifers from the 0.08- and 0.20-μg/L enclosures were similar to the control, whereas zooplankton numbers in the 1.0-μg/L enclosure were notedly different (Figure 4). Eighty-eight days after the first esfenvalerate application cladoceran, copepod (adult and copepodite), and copepod nauplii populations were inversely correlated with treatment concentrations. Cladocerans in the 1.0-μg/L enclosure peaked on day 43 after the first application with a population of 166,000, whereas the control cladocerans reached their highest numbers of 108,000 on day 88. Cladoceran populations remained greater in the 1.0-μg/L enclosure compared to populations in the control enclosure, 3 d before the first application to 15 d after the second application. However, by day 88 after the first application, cladoceran numbers in the 1.0-μg/L enclosures were reduced by 87% compared to the control cladocerans.

Copepod (adults and copepodites) from all enclosures had similar populations from 3 days before to 50 d after the first application. By day 88, however, copepod numbers in the 1.0-μg/L enclosure were reduced by 99% compared to the control copepods (adults and copepodites).

Copepod nauplii from the control, 0.08-, and 0.20-μg/L enclosures had similar populations from 3 days before application to 50 d after the first application. Copepod nauplii from the 1.0-μg/L enclosure declined steadily from one to 29 d after the first application, increased to peak at 211,000 on day 43, and then plummeted to 1274 on day 88. On this day copepod nauplii (#/m^2) were reduced by 94% compared to control nauplii.

Rotifers populations in the control, 0.08-, and 0.20-μg/L enclosures were also similar for the entire study period. However, populations in the 1.0-μg/L enclosures were again noticeably different, with rotifer numbers falling an order of magnitude below the levels of the other enclosures by day 29 after the first application, and increasing by 48% above control numbers on day 88.

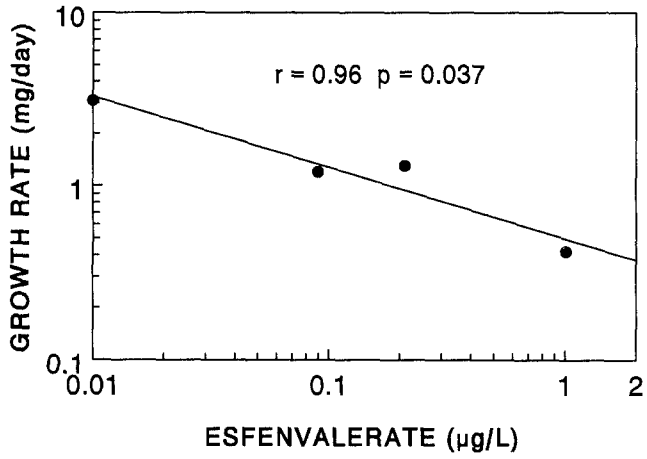

Fig. 2. Y-O-Y bluegill growth rate (mg/day) in each enclosure in relation to the log of the nominal + 0.01 esfenvalerate concentration (µg/L)

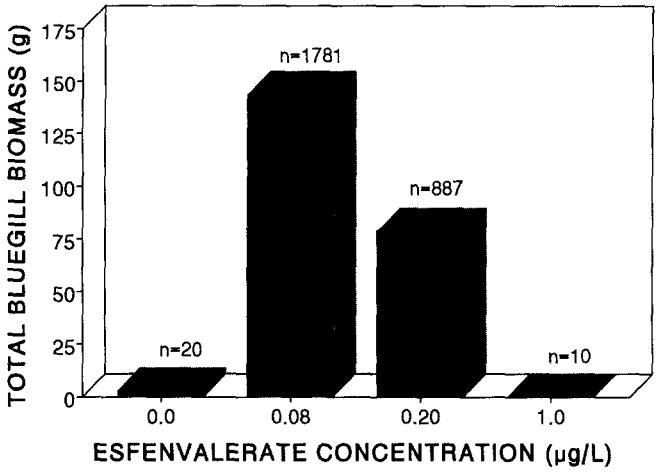

Fig. 3. Y-O-Y bluegill biomass (g) calculated as the total wet weight of larvae captured from June 17 to October 6 from four enclosures

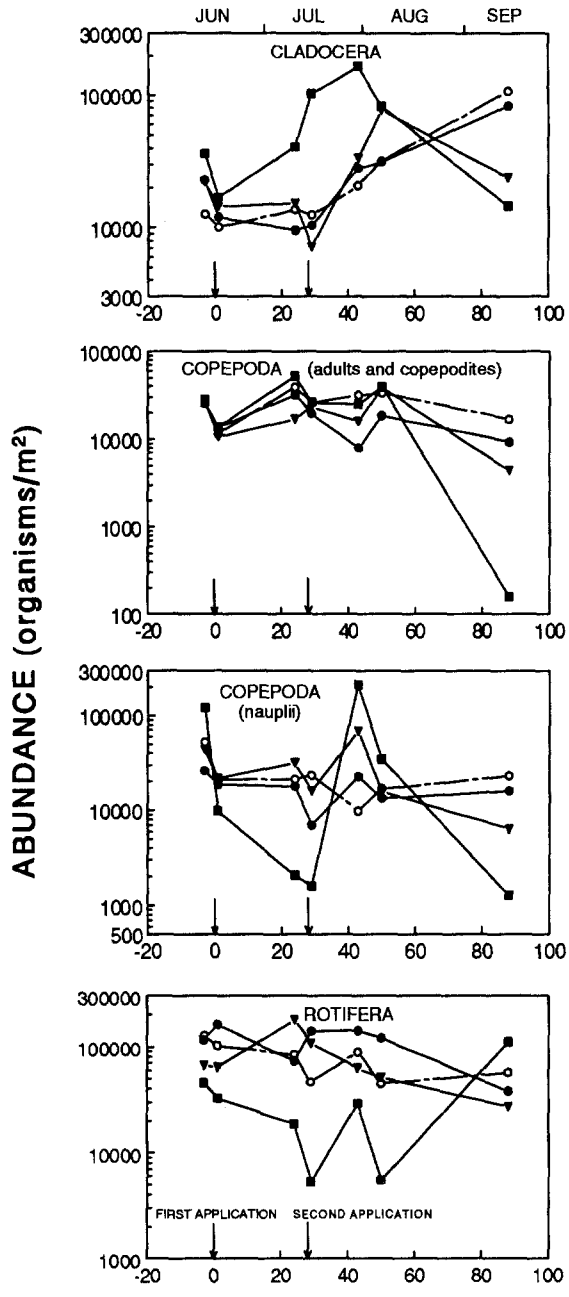

Fig. 4. Cladocera, copepoda (adults and copepodites), copepoda (nauplii), and rotifera abundance (organisms/m^2) relative to esfenvalerate application day for control (○), 0.08- (●), 0.20- (▼), and 1.0-µg/L (■) exposures

Bluegill Digestive-tract Analysis

Bluegill digestive-tract analysis on day 100 after the first application showed reasonably similar diets among all enclosures, however some differences in prey preference were noted in the control and 1.0-µg/L exposed Y-O-Y. Cladocerans comprised the largest percentage of food organisms found in the Y-O-Y bluegill digestive tracts in all but the 1.0-µg/L enclosure, where rotifers were the most abundant food organisms (Figure 5). Control Y-O-Y consumed the largest percentage of copepod nauplii (42%), while no nauplii were found in the tracts of the 1.0-µg/L Y-O-Y. Additionally, the percentage of rotifers in Y-O-Y diets increased with increasing esfenvalerate concentrations; the controls and 0.08-µg/L Y-O-Y consumed the least (1.5 and 1.2% respectively), while the 0.20-µg/L Y-O-Y ingested 13%, and the 1.0-µg/L Y-O-Y consumed the largest percentage of rotifers (48.6%). Chironomids, copepod (adults and copepodites), and others (protozoans, ostracods, mites, and nematodes) made up a small but similar percentage of the diet of Y-O-Y bluegills from all enclosures.

Discussion

Esfenvalerate adversely affected bluegill reproductive success directly through adult and larval mortality, delayed spawning, and reduced Y-O-Y growth. A single application of 5.0 µg/L caused 100% mortality of adult bluegills within 24 h. Two applications of 1.0 µg/L 28 d apart delayed bluegill spawning for 15 d after each application, caused mortality of larvae after hatching and before swim-up, and resulted in an 86% reduction in the Y-O-Y rate growth (mg/day). Bluegill growth (Y-O-Y)

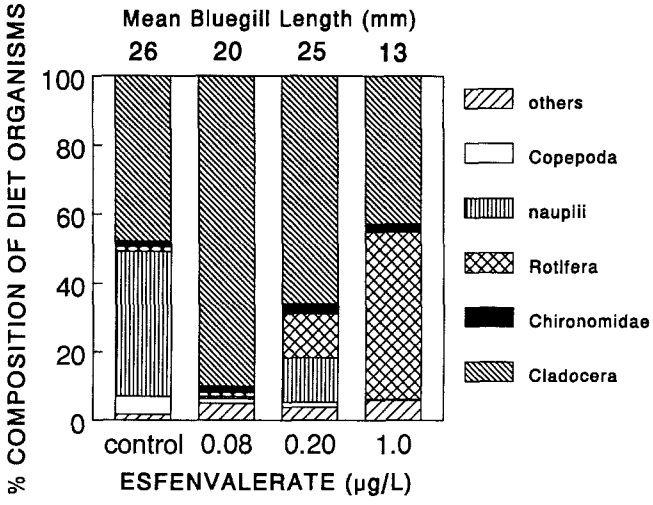

Fig. 5. Percent composition of invertebrates in stomach and digestive tracts of bluegill larvae from each enclosure 100 days following the first application of esfenvalerate. The mean total length (mm) of bluegill larvae in each enclosure on each day is also presented

was also reduced by 62 and 57% in the 0.08- and 0.20-µg/L enclosures, respectively.

No adult bluegills survived following the first application in the 5.0-µg/L enclosure, while following the second application of esfenvalerate in the 1.0-µg/L enclosure bluegill larvae were apparently eliminated. Symptoms of esfenvalerate intoxication at 5.0-µg/L in adult bluegills were nearly identical to those observed by Bradbury *et al.* (1985) in fathead minnows exposed to fenvalerate. Although some bluegill Y-O-Y survived the first application in the 1.0-µg/L enclosure, complete Y-O-Y bluegill mortality occurred after the second application at 1.0-µg/L (based on observation in the nest and the lack of observation or capture of larvae in the enclosure from shortly after application until new larvae were produced). This conclusion is also supported by the mean weight of bluegill larvae following the first and second application in the 1.0-µg/L enclosure. The mean weight of bluegill larvae that were captured in the 1.0-µg/L enclosure 22 d after the first application was 0.06 g, while 72 d after the second application bluegill mean weight was 0.02 g. Since spawning occurred 15 d after each application in the 1.0-µg/L enclosure, that would imply that the larvae captured on day 22 following the first application were only seven days old if all larvae from previous spawnings had died. Since seven-day-old bluegill larvae have an approximate mean weight of 0.0006 g, and the mean larvae weight on day 22 was 0.06 g, this indicates that some larvae survived the first application in the 1.0-µg/L enclosure. However, bluegill mean weights (0.02 g) at the end of the study strongly imply that no larvae survived the second esfenvalerate treatment, and all larvae in the 1.0-µg/L enclosure were from spawnings that occurred 15 d or later following the second application. This may be explained by the measured esfenvalerate water concentrations, which was 0.44 µg/L after the first application, but reached 1.08 µg/L following the second application, a sufficient increase to cause complete larval mortality. Stay and Jarvinen (1995) reported a 48-h LC_{50} of 0.44 µg/L for juvenile bluegills exposed to esfenvalerate in a laboratory study, while Lozano *et al.* (1992) found complete mortality among caged juvenile bluegills in littoral enclosures 53 d following application of 1.0 µg/L esfenvalerate. Fairchild *et al.* (1994) also found 90–92% mortality after 48 h in caged juvenile bluegills exposed to 1.71 µg/L esfenvalerate in a mesocosm study.

Behavioral effects affecting reproductive success were noted in our study after both the first and second treatment of 1.0-µg/L esfenvalerate. In addition to the rapid rim circling and excited swimming behavior, spawning ceased for 15 d following both 1.0-µg/L applications. Fairchild *et al.* (1992) also surmized that bluegill reproduction was eliminated during the 12 weeks that esfenvalerate was applied at 1.71 µg/L biweekly in mesocosms in Columbia, Missouri. Therefore bluegill Y-O-Y produced from spawning prior to application were a least 12 weeks older than Y-O-Y produced after application resulting in significantly larger and smaller bluegill size classes. A notable difference between the results of our study and the Columbia mesocosm study was that a larger size class of Y-O-Y bluegill was found in Columbia mesocosms treated with 1.71 µg/L esfenvalerate, whereas no larger size classes of Y-O-Y were found in the 1.0-µg/L enclosure in the present study. Possible explanations for this larger size class are: 1) concentrations did not reach 1.0 µg/L everywhere in the Columbia mesocosms, and 2) Y-O-Y were larger prior to application at Columbia and therefore not as sensitive to esfenvalerate, or 3) esfenvalerate may not be as toxic to aquatic organisms in warmer locations. Fenvalerate was found to be twice as toxic to mosquito larvae *Aedes aegypti* at 20° than at 30° C (Cutkomp and Subramanyam 1986). Webber *et al.* (1992) also observed differences in Y-O-Y bluegill size classes in Auburn, Alabama, treated for ten weeks with a mean measured esfenvalerate water concentration of 0.69 µg/L compared to control mesocosms. The mesocosms treated with 0.69 µg/L esfenvalerate had 1- and 3-cm bluegill Y-O-Y, but lacked 2-cm Y-O-Y. Possibly, in the Auburn study the 3-cm Y-O-Y were produced from spawning prior to treatment, and the 1-cm Y-O-Y class was from spawning that occurred after treatment. Similarly, as in the Columbia study, the missing middle size class Y-O-Y at Auburn may have resulted from the elimination of bluegill reproduction during the treatment period. The results of our study imply that whenever esfenvalerate is applied repeatedly biweekly at concentrations $\geq$ 0.44 µg/L, spawning will cease thereby eliminating further Y-O-Y production. Adult reproductive behavior may be even more sensitive to esfenvalerate than larval survival, since spawning ceased for 15 d after the first application, yet some larvae survived in the 1.0-µg/L enclosure where measured esfenvalerate concentrations were 0.44 µg/L. Little *et al.* (1993) observed bluegill behavioral responses (decreased aggression and increased body tremors) at 0.025 µg/L esfenvalerate, an order of magnitude less than concentrations that affected growth or survival.

In northern regions bluegill spawning may begin as late as June 20 and successful bluegill year classes are often determined by July 7 (Beard 1982). Two applications of esfenvalerate at 0.44 µg/L on June 15 and June 30 could delay spawning until July 15, thereby resulting in a poor year class. We have also observed that spawning that occurred after July 9 provided only 12% of the total biomass of Y-O-Y bluegill (Tanner and Moffett 1995) and spawning that occurred after July 8 contributed only 0.8% to the total biomass of Y-O-Y bluegill (Tanner and Knuth 1995). This may partially explain the low numbers of bluegill Y-O-Y (20) in the control enclosure in the present study since spawning did not occur in the control enclosure until July 30.

Reduced Y-O-Y growth rates (mg/day) were observed in the

0.08-, 0.20-, and 1.0-μg/L esfenvalerate treated enclosures. The similarity between the reduced Y-O-Y growth rates in the 0.08(57%)- and 0.20(62%)-μg/L enclosures may be in part due to the larger Y-O-Y biomass in the 0.08-μg/L enclosure. Biomass in the 0.08-μg/L enclosure was 144 g, while biomass in the 0.20-μg/L enclosure was 79 g. Possibly some of the Y-O-Y growth reduction in the 0.08-μg/L enclosure was due to competitive stress resulting from overcrowding. However, Lozano *et al.* (1989) also observed that caged juvenile bluegills in 0.08 and 0.20 μg/L esfenvalerate treated enclosures had growth reduced by 42 and 47%, respectively, compared to caged bluegills in control enclosures. Growth reductions of Y-O-Y were not a secondary effect as a result of reduced invertebrate bluegill prey as was found in the diflubenzuron littoral enclosure study (Tanner and Moffett 1995), nor was growth related to density-dependent food limitations as in the Columbia (Fairchild *et al.* 1992) or Auburn (Webber *et al.* 1992) mesocosms studies. In our study both the zooplankton abundances in the enclosures and the digestive tract analysis of bluegill Y-O-Y further confirm that observed growth effects were not due to decreased prey availability. Cladoceran, copepod, and rotifer populations were similar for most of the study period in the control, 0.08-, and 0.20-μg/L enclosures. In addition, the cladocerans in the 1.0-μg/L enclosure had higher abundances than any of the other enclosures for most of the study period. Although copepod nauplii numbers following the first application were depressed until day 29 in the 1.0-μg/L enclosure, nauplii populations exceeded those in the control and 0.08-μg/L enclosures on days 43 and 50. When the cladoceran and copepod numbers plummeted down two orders of magnitude from day 50 to day 88 in the 1.0-μg/L enclosure, rotifers increased two orders of magnitude (Figure 4). This increase in rotifer numbers is reflected in the Y-O-Y diet in the 1.0-μg/L enclosure. This diet consisted of 49% rotifers on day 100, while cladocerans comprised 43% of the digestive tract contents. In contrast, rotifers made up only 1.5% of the Y-O-Y diet in the control enclosure, while cladocerans constituted 48%. Preferred prey of bluegill Y-O-Y 10–20 mm in length is typically cladocerans or copepods (Siefert 1972; Keast 1980). When diflubenzuron was applied to littoral enclosures (Tanner and Moffett 1995) cladocerans were virtually eliminated after two applications of 30 μg/L. Near the end of the diflubenzuron study (day 70) control Y-O-Y diet consisted of 75% cladocerans and no rotifers, whereas Y-O-Y diet in the 30-μg/L enclosure diet consisted of 36% rotifers, 57% chironomids, and no cladocerans. Diflubenzuron greatly reduced or eliminated cladocerans causing Y-O-Y bluegills to switch to rotifers and chironomids. Esfenvalerate did not eliminate cladocerans, abundances of cladocerans following treatment were actually higher in the 1.0-μg/L enclosure than in the other enclosures for most of the study. Following esfenvalerate treatment, copepod (adults and copepodites) abundances in the 1.0-μg/L enclosure remained similar to copepod abundances in the control enclosure. This implies that Y-O-Y growth reductions at least in the 1.0-μg/L enclosure were not caused by the reduction or elimination of preferred prey species.

Growth reductions were observed in Y-O-Y bluegills in enclosures where esfenvalerate water concentrations were 0.08 μg/L or greater. These growth reductions will directly affect recruitment success since slower growing larvae are more subject to starvation (Miller *et al.* 1988), predation (Chevalier 1973), and higher overwinter mortality (Post and Evans 1989).

The high productivity of the mesocosms at Columbia (Fairchild *et al.* 1992) and Auburn (Webber *et al.* 1992) coupled with the protracted and prolific spawning habits of the bluegill led to overproduction which somewhat masked the effects of esfenvalerate in their studies. Webber *et al.* (1992) observed overcrowded bluegill populations in all treatments, while Fairchild *et al.* (1992) observed density dependent food limitations in the control and low treatment mesocosms. Y-O-Y bluegills in our study had adequate food in the control and treatment enclosures. Although the Y-O-Y biomass (144 g) in the 0.08-μg/L enclosure was much greater than the Y-O-Y biomass (3 g) of the control, cladoceran and copepod abundances were nearly identical. Furthermore-Y-O-Y biomass in the 1.0-μg/L enclosure was only 0.28 g, therefore Y-O-Y from the 1.0-μg/L enclosure had the greatest opportunity for growth if growth was density dependent in the littoral enclosure study. Y-O-Y growth may have been poorest in the 1.0-μg/L enclosure because esfenvalerate affected Y-O-Y directly by impairing their motor abilities to find and capture prey. Finger *et al.* (1985) observed that bluegills exposed to fluorene at 0.062, 0.12, 0.25, 0.50, and 1.0 μg/L were significantly less successful in capturing chironomid prey than control bluegills and growth was subsequently inhibited at 0.25, 0.5, and 1.0 μg/L flourene.

Conclusion

This study confirms that littoral enclosure studies can be sensitive and effective tools in measuring the effects of pesticides on critical life-cycle events (including reproduction) in fishes. This study measured the effects of two applications of esfenvalerate on adults and the early life stages of the bluegill. Whenever concentrations of esfenvalerate reach 5.0 μg/L in the water column, bluegills will not survive. If exposure to 5.0 μg/L esfenvalerate occurs prior to reproduction, bluegills would be eliminated from that body of water until bluegills are reintroduced. Esfenvalerate may reduce Y-O-Y bluegill growth at concentrations as low as 0.08 μg/L. Biomass was the least sensitive endpoint in this study, whereas adult reproductive behavior, Y-O-Y survival, and Y-O-Y growth were found to be the most sensitive.

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