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BEHAVIOR OF SUNFISH EXPOSED TO HERBICIDES: A FIELD STUDY

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Abstract — An underwater closed-circuit video system was used to remotely monitor and record the behavior of bluegills (*Lepomis macrochirus*) and redear sunfish (*Lepomis microlophus*) guarding their nests before, during, and after applications of aquatic herbicides. Nests were sprayed to achieve a nominal concentration of 4 mg/L of either a dipotassium salt of endothall (Aquathol-K®), a dimethylamine salt of 2,4-dichlorophenoxyacetic acid (2,4-D), or water (control). No significant differences in rates of nest abandonment existed among the three treatments ($p > 0.10$). Abandonment averaged 5.17 min for both species and herbicide treatments, whereas the herbicides persisted in the water column for at least 45 min. When abandonment occurred, congeners nearly always intruded on the nest to feed on eggs or fry. After spraying, the adjusted mean frequency of rim circling, fanning, and agonistic behaviors exhibited by bluegills guarding eggs did not differ among the three treatments ($p \geq 0.35$). These results suggest the two herbicides will not elicit pronounced shifts in reproductive behavior of sunfish when they are properly applied.

Keywords — Herbicides Toxicity Sunfish reproduction Behavior

INTRODUCTION

Herbicides are commonly used to control nuisance aquatic vegetation when alternative controls are not feasible. Two extensively applied aquatic herbicides in southeastern U.S. impoundments are Aquathol-K® and 2,4-dichlorophenoxyacetic acid (2,4-D). The direct effects on fish are considered minimal when these herbicides are properly applied because only low concentrations (≤4 mg/L) are needed to control aquatic macrophytes [1]. The 96-h LC50 values for Aquathol-K and the dimethylamine (DMA) formulation of 2,4-D are ≥100 mg/L [2], and other research substantiates that low concentrations of these herbicides are not acutely toxic to fish [3,4]. However, little is known about the sublethal effects of herbicides on fish.

Anecdotal reports by anglers and fishery biologists suggested that sunfishes (*Lepomis* spp.) alter their behavior by abandoning nests they are guarding when littoral areas are treated with herbicides. The degree to which male bluegills (*L. macrochirus*) defend their nests is affected by factors such as brood size and level of past investment in the brood [5], but the role environmental contaminants might play in the parental decision-making process of sunfishes is unknown. The behavior of an animal represents cumulative changes in physiological and biochemical functioning [6]; therefore, behavioral studies often provide data that are more sensitive and meaningful when assessing potential toxicity of a compound. In laboratory studies, contaminant-induced changes in sunfish behavior and social structure were documented [7-9]. Small behavioral shifts induced by contaminants can be particularly damaging to a population if they occur during the reproduction cycle [10]. Because fish do not always avoid toxic substances [11], the behavior of fish exposed to a contaminant may be an important factor in determining the toxicity of a compound in nature [11-14].

Few studies have examined the effects of aquatic herbicides on fish behavior or physiology. Acute exposure to glyphosphate and triclopyr herbicides did not produce physiological stress in coho salmon (*Oncorhynchus kisutch*) [15], and behavioral shifts occurred only at concentrations well above those likely to be experienced by salmon in streams receiving drift from aerial herbicide applications [14]. Hildebrand et al. [16] reported no

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mortality of rainbow trout (*O. mykiss*) held in aquaria placed in streams and subjected to one hundred times the recommended field dose of glyphosphate; they also reported that rainbow trout tested in avoidance-preference chambers would avoid lethal concentrations of glyphosphate. The only examination of in situ behavior of fish exposed to herbicides [17] consisted of sampling and radio-tracking largemouth bass (*Micropterus salmoides*) in herbicide-treated areas of Guntersville Reservoir, Alabama. The investigators detected no change in largemouth bass density in areas treated with either 2,4-D or a mixture of dibromide diquat and Komeen® (a copper-based compound). Furthermore, the herbicide treatments had no detectable effect on the movement patterns of individual largemouth bass. Cope et al. [18] reported that bluegill spawning was delayed for several weeks when 10 mg/L of a 2,4-D ester formulation was applied to small ponds, but no change in spawning behavior was noted at concentrations ≤5 mg/L. Ester formulations of 2,4-D are known to be more toxic than DMA formulations [3]; potential effects of DMA formulations of 2,4-D on sunfish reproductive behavior are unknown.

This study was initiated to determine whether nesting sunfishes abandoned habitats treated with Aquathol-K or 2,4-D DMA and whether sunfish behavior is altered by exposure to these herbicides. This field experiment represents a novel approach to observing individual fish in behavioral toxicology studies.

MATERIALS AND METHODS

Our research was conducted in Old Monterey Lake, a 26-ha impoundment in Monterey, Tennessee. This system is a headwater impoundment, and inflow and outflow were negligible during the field trials. Observations of nesting bluegills and redear sunfish were made throughout the spawning season (late May–mid-August) in 1989 and 1990. Aquatic vegetation in Old Monterey Lake is abundant and consists primarily of *Brasenia schrebei*, *Nuphar luteum*, and *Nymphaea odorata*. Water-quality parameters for Old Monterey Lake in March of 1988 were alkalinity = 24 mg/L $CaCO_3$; pH = 7.5; conductivity = 80 μmho/cm. In December 1990, the following parameters were measured: total dissolved solids = 0.8 mg/L; total nitrogen = 0.61 mg/L; total phosphorus = 0.022 mg/L.

Nest monitoring

Observations of sunfish behavior before, during, and after herbicide applications were made with an underwater video-recording system. The commercially available system (Fuhrman Diversified, Inc., La Porte, TX) consisted of an 8-mm-format video camcorder, waterproof external camera system, and weatherproof viewing monitor. Due to the external camera's small size (53 × 33 × 99 mm), we assumed the behavior of the spawning sunfishes would not change when the camera was placed near the nest. Camouflage netting was wrapped around the housing of the camera to further reduce the likelihood that the camera would affect the males' behavior. A 40-m coaxial cable connected the external camera with the land-based video system. The video image was recorded from shore while the external camera was positioned near the nesting sunfishes, thus keeping movements of the experimenter from affecting the behavior of the fish. The monitor was used to observe the behavior of the fish in real time while the camcorder made a permanent recording of the observations. The complete system could function for several hours on battery power. Using a 500-W portable generator and a battery charger, the nests could be monitored indefinitely.

In several redear sunfish trials, the nest was observed using binoculars while sitting on a 3-m stepladder set near the shoreline. If abandonment occurred, the time spent away from the nest and the presence of intruding congeners was recorded.

Test procedure

Nests were located and defined as active if a male sunfish was guarding the nest. Highest sampling priority was given to testing males guarding eggs. To eliminate dissimilarities in behavior from social ranking and nest location [7,19], the nest belonging to the most dominant male in each colony was chosen for observation. The nest belonging to the dominant male was assumed to be the most centrally located nest in the colony [7–9]. Most of the nests selected for testing were in small colonies (<10 active nests) located in shallow water (≤0.75 m). Aquatic vegetation was never abundant within a colony's boundary; however, vegetation and woody debris were usually present near the borders of each colony. Over the two sampling seasons, 23 nests with bluegills guarding eggs and 12 nests with redear sunfish guarding eggs or fry were treated.

A 230-m^2 test area was measured so that the nest was approximately centered in the area. Depth measurements for each test area were taken at the front, middle, and back of each test plot and averaged so that the volume of the test area could be estimated. The desired amount of herbicide was then measured out and diluted with water in a 9.46-L pressurized spray can.

A 15-min adjustment period was allowed to habituate sunfish to the presence of the camera before video-recording began. Each 1-h trial consisted of a 15-min pretreatment (control) period and three consecutive 15-min post-treatment periods. The herbicide (or water) was applied at the end of the control period using the pressurized spray can. The person applying the herbicide stood in a small boat positioned at an offshore corner of the test plot; a trolling motor or the wind moved the boat slowly along the edge of the test plot while the herbicide was sprayed uniformly over the area. The spraying took about 2.5 min to complete.

The spraying procedure was expected to cause some fish to abandon the nests, at least temporarily. Of greatest interest was whether the rates at which nests were abandoned (and times spent away from the nest) differed among treatments. Herbicides are normally applied from outboard motorboats, airboats, or helicopters, and fish nesting in shallow water would be expected to abandon their nests temporarily in those circumstances. This experiment sought to simulate a routine application of herbicides and was not designed to eliminate the disturbance variable. Instead, every attempt was made to remain consistent among trials in the amount of disturbance imposed on the nesting sunfish. Such consistency allowed treatment effects to be evaluated in the context of an actual vegetation management procedure.

Herbicides and sampling analytical methods

The Aquathol-K formulation (Penwalt Corporation, Philadelphia, PA) was a liquid dipotassium salt of endothall [7-oxabicyclo(2.2.1) heptane-2,3-dicarboxylic acid] with 40.3% active ingredient (a.i.). The 2,4-D formulation (Red Panther Company, Clarksdale, MS) was a liquid dimethylamine salt of 2,4-dichlorophenoxyacetic acid with 47.4% a.i. Aquathol-K and 2,4-D are typically applied at concentrations of 0.1 to 4.0 mg/L a.i. in submersed applications, depending on the herbicide and the plant species [1]. In our tests, enough herbicide was sprayed on the test plot to achieve a nominal concentration of 4 mg/L a.i. Some nests were treated with water to establish a control response of the fish to the method of application. All other testing procedures were identical during the control trials.

Post-treatment water samples collected during three 2,4-D trials were analyzed to estimate how long the herbicide persisted in the immediate vicinity of the nest. Aquathol-K concentrations were not measured in any trials, but the dispersal of Aquathol-K was assumed to be similar to 2,4-D dispersal. In each of three 2,4-D trials, water samples were collected immediately following the application of the herbicides, as well as 15, 30, and 45 min after application. The samples were collected through a 6.4-mm Nalgene® (Rochester, NY) hose that was taped to the coaxial cable running to the external camera. In this manner, water samples were withdrawn from the immediate vicinity of the nest without disturbing the male guarding the nest. The hose passed through a peristaltic pump head and into a collection jar. Methyl esters of 2,4-D were then analyzed with a Hewlett Packard (Avondale, PA) 5890 GC ([20], method 6640B).

Data analyses

The videotape recordings were returned to the laboratory, where an analysis of the behavior of each test fish was conducted. Specific behaviors were listed to create an ethogram (Table 1). Trials were examined to determine whether the male left the nest when the herbicide (or water) was sprayed. Abandonment was defined as any instance when the male left the nest and did not engage in agonistic behaviors. If the male left the nest, the time spent away from the nest was recorded. Videotapes of the trials in which abandonment occurred were also examined to determine whether other fish intruded on the nest while the male was away. A contingency table and the chi-square statistic were used

Table 1. Ethogram of sunfish behaviors tallied

Behavior	Description
Display	Male extends all fins and orients toward a perceived threat
Chase	Male pursues another fish
Rim circling	Male swims in circles about the rim of the nest
Fanning	Male hovers over substrate and uses pectoral fins to fan eggs
Spawning	Male and female engage in releasing eggs and milt
Tail sweeping	Male uses tail to excavate or clean out the nest
Substrate biting	Male bites at the substrate
Float-up	Male floats up in the water column and remains suspended
Abandonment	Male leaves the nest for reasons other than a chase

to test whether rates of abandonment were significantly higher for sunfishes sprayed with either herbicide than for sunfishes sprayed with water. Yates' correction for a 2 × 2 contingency table was used [21], and significance was declared at $p \leq 0.10$.

An instantaneous sampling scheme [22] was employed to quantify behavioral states of bluegills guarding eggs. The behavior of each bluegill at 25 randomly preselected moments in time was recorded during each 15-min period. Differences among treatments in the frequency of certain behaviors were evaluated with analysis of covariance (ANCOVA). The frequencies of three different behaviors during the last 30 min of each trial (15–45 min after spraying) were tallied for all bluegills guarding eggs, irrespective of whether they abandoned the nests or not. All fish that exhibited an abandonment response returned to the nests within 15 min; therefore, observations of these fish were included in the analysis to increase the sample size. Insufficient numbers of redear sunfish at a similar reproductive stage were treated; therefore, no behavioral analysis was conducted for that species.

The three behaviors that served as the dependent variables in each of the ANCOVA tests were rim circling, fanning, and agonistic behaviors (agonistic included both display and chase behaviors). Frequency of each of the behaviors during the 15-min control period served as the covariable in the ANCOVA to reduce experimental error, and herbicide served as the independent variable. Before testing the adjusted means, homogeneity of slopes was tested.

Table 2. Concentration of 2,4-D (mg/L) at the nest

Trial	Time of sample (min)	Concentration (mg/L)	Grand mean (SE)
1	0	6.46	
	15	0.18	
	30	2.46	
	45	9.47	4.6 (2.1)
2	0	1.91	
	15	8.24	
	30	9.64	
	45	7.95	6.9 (1.7)
3	0	11.10	
	15	0.02	
	30	4.44	
	45	5.34	5.2 (2.2)

Time of sample refers to minutes after spraying was completed.

RESULTS

The remote video-monitoring system performed well. Males commonly engaged in agonistic behaviors directed toward the external camera when it was first placed near their nests, but their behavior seemed to return to normal before the 15-min habituation period was over. Water clarity averaged about 1 m (Secchi disk transparency), but the test animals (and often neighboring males guarding their nests) were clearly visible in most trials. The technology we employed to observe and record animal behavior in situ clearly represents an improvement over using scuba- or snorkel-equipped divers, in terms of efficiency, safety, and minimizing disturbance of the test animal.

Herbicide concentrations that sunfish would experience during large-scale herbicide applications (i.e., 2–4 mg/L) were exceeded most of the time (Table 2). In each of three trials in which 2,4-D was measured, mean concentrations over 45 min were within 3 ppm of the nominal concentration (4 mg/L). These results also revealed that herbicide dispersal was nonuniform within each trial, but no pattern was evident.

Aquathol-K appeared to elicit a greater response from nesting bluegills than 2,4-D when applied as a surface spray (Table 3). Sixty-six percent of all

Table 3. Incidence of nest abandonment and percentage of trials in which congeners intruded if the male abandoned the nest

Species	Treatment	Stage of reproduction	N	Abandonment? Yes	Abandonment? No	χ^2	Percent with intrusions
Bluegill	2,4-D	Eggs	9	6	3	0.01	100%
Bluegill	Aquathol-K	Eggs	8	7	1	0.88	86
Bluegill	Control	Eggs	6	3	3		33
Redear	2,4-D	All stages	7	4	3	0.48	100
Redear	Control	All stages	5	1	4		100

The chi-square statistic tested whether abandonment rate for each species and herbicide was higher than the abandonment rate for the same species treated with water. Critical value of the chi-square statistic (1 $d.f.$; $p = 0.10$) = 2.71.

bluegills exposed to 2,4-D while guarding their eggs temporarily abandoned their nests (six of nine trials), whereas 88% of all bluegills exposed to Aquathol-K while guarding their eggs abandoned their nests (seven of eight trials). The differences, however, were not significant. Abandonment typically occurred soon (≤30 s) after the herbicide (or water) was sprayed near the nests. When exposed to 2,4-D, redear sunfish guarding eggs abandoned their nests at a slightly lower rate (50%; three of six trials) than bluegills.

When the nests were sprayed with water, abandonment occurred in 20% of all the redear sunfish trials and 50% of the bluegill trials in which the male was defending eggs or fry. The null hypothesis that the herbicides had no effect on abandonment rate could not be rejected for either species or herbicide ($p > 0.10$; Table 3).

Congeners usually intruded on the sunfish nests when the males left the nests (Table 3). The intruders arrived soon after the males left and fed on undetermined numbers of eggs or fry until the males returned. Because herbicide concentrations ≥2 mg/L were present at each nest most of the time, congeners intruding on the nest were also exposed to the herbicides.

For each tested behavior or group of behaviors (rim circling, fanning, agonistic), slopes among treatments were declared similar ($p > 0.10$). Adjusted mean rates of rim circling, fanning, and agonistic behaviors exhibited by fish sprayed with 2,4-D, Aquathol-K, or water were similar among the three treatments ($p \geq 0.35$; Table 4). The hypothesis that 2,4-D had no effect on bluegill behavior was further supported by the observation that bluegill spawning occurred in one trial 10 min after 2,4-D was applied.

Table 4. Frequency of behaviors exhibited by bluegills guarding eggs after being sprayed with 2,4-D, Aquathol-K, or water

Behavior	p	2,4-D	Aquathol-K	Water
Rim circling	0.35	12.9 (1.9)	8.8 (2.0)	10.6 (2.6)
Fanning	0.84	18.0 (2.7)	16.4 (3.0)	15.5 (3.8)
Agonistic	0.67	10.0 (2.0)	7.5 (2.1)	7.6 (2.5)

Values represent adjusted mean frequency of behaviors during the last 30 min of each trial; values are adjusted for frequency of same behavior during each 15-min control period. Values in parentheses are standard errors. Probabilities associated with testing the hypothesis that adjusted means did not differ (analysis of covariance with 2, 18 *d.f.*) are listed. Sample size per treatment = 9 (2,4-D), 8 (Aquathol-K), and 5 (control).

DISCUSSION

Permanent nest abandonment by bluegills results in higher mortality rates for eggs and fry [23], but the mean times of abandonment observed in this research did not seem to be biologically significant. No reports were found that indicated abandonment times of only 5 to 6 min have an adverse effect on the nesting success of sunfishes, despite the fact that nest intrusion and egg predation were observed. Egg predation would be noteworthy only if it caused permanent nest abandonment and resulted in fewer numbers of juveniles and adults. Male sunfish sometimes abandon their nests when the brood size is reduced, in order to maintain themselves for future spawning opportunities [5,24,25]. Most nests were observed after they were sprayed, and none had been permanently abandoned; therefore, egg and fry losses due to short-term predation by intruders were probably not excessive. It is unknown whether similar egg losses resulting from herbicide-induced, temporary nest abandonment would adversely affect reproductive success and recruitment by other nest-building fish species.

The high rate at which other sunfish intruded on abandoned nests suggests that congeners were also unaffected by the herbicide applications. Water sampling revealed that congeners intruded (and the males returned to their nests) before the herbicides had dissipated. From the intruder's perspective, benefits associated with nest predation outweighed any risks from being exposed to the herbicide that might have been detected.

When sunfishes are initially exposed to toxicants such as Cd and Zn, behavioral shifts occur [13]. In this study, substantive behavioral responses by sunfishes exposed to the two tested herbicides could not be detected. Similarly, Bain et al. [17] were unable to detect significant responses by largemouth bass exposed to aquatic herbicides in a field experiment. The conclusion that proper application of Aquathol-K and 2,4-D herbicides will not result in behavioral aberrations agrees with the findings of Hildebrand et al. [16] and Morgan et al. [14], who worked with other herbicide formulations and salmonids.

The reservoir system where this study was conducted is a relatively soft body of water. Because the toxicity of many herbicides is higher in soft water [3,26], this study of behavioral responses to herbicides represented a worst-case scenario. Therefore, the likelihood of behavioral shifts resulting from applications of 2,4-D or Aquathol-K in hard-water systems appears to be minimal. Given the scarcity of such studies, additional field studies of the interaction between these and other herbicides and survival-dependent behaviors are required to confirm the absence of deleterious side effects associated with herbicide treatments.

Aquatic herbicides are often applied premixed with various adjuvants that are added to improve the performance of the herbicides. Adjuvants may modify the dispersion of the herbicide formulation or may increase the ability of the herbicide to penetrate plant surfaces [27]. Some adjuvants are known to increase the toxicity of herbicide formulations [14] and may be toxic by themselves, but data about chronic or acute effects of adjuvants are rare. Worldwide multiple-use demands on lentic resources will increase in the years ahead, and more effective use of herbicides and adjuvants will likely be explored. Increased emphasis should be placed on examining the response, in the laboratory and in situ, of nontarget aquatic organisms to any potentially toxic substances *intentionally* introduced into aquatic ecosystems.

Underwater video technology is improving rapidly and will facilitate future in situ behavioral toxicology studies. Incorporating an intervalometer (which controls the duration and frequency of video recording) and additional remote cameras into the basic system described in this paper will reduce the time (and ultimately, the cost) involved in data collection. For instance, this study could observe only one nesting male per trial, even though an entire colony was treated. With additional cameras and the means to preprogram the recording of video images, larger sample sizes would be possible, with subsequent increases in the power of statistical tests.

Interest in behavioral responses to aquatic toxicants is expected to increase [6]. As in other areas of environmental impact assessment (e.g., thermal pollution and fish movements), increased emphasis will also be placed on relating laboratory-derived data to the systems being managed or degraded. The methods outlined in this paper will be appropriate when the specific location of fish in wild or semiwild situations can be predicted with reasonable confidence. For instance, once a freshly excavated sunfish nest is located, confidence is high that a particular individual will be present at that location for at least a few days. Similarly, some stream fishes exhibit strong affinity for discrete foraging sites within their home range (e.g., brown trout *Salmo trutta* [28]), thus facilitating camera placement and data acquisition. Underwater video apparatus is useless in turbid situations; however, direct observation of fish behavior at night is possible. Infrared-sensitive underwater cameras and infrared light sources are commercially available, thus expanding the possibilities for conducting in situ behavioral toxicology studies.

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