The Journal of the Iowa Academy of Science: JIAS

Volume 108 | Number Article 6

2001

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Jour. Iowa Acad. Sci. 108(3):90–97, 2001

Toxicity of Cedar River Water and Sediment to Larval Walleye

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Although all anthropogenic stressors affecting aquatic ecosystems have not been isolated, suspended solids, sediment, and pesticides are believed to be major facrors in agroecosystems. In the spring of 1998 and 1999, static 48-h tests were conducted to determine the toxicity of water and sediment collected from the Cedar River to prolarval, postlarval I, and postlarval II walleye (*Stizostedion vitreum*). River water and sediment were not more toxic to any larval stage of walleye than reference water and sediment. Cedar River sediments, suspended solids, and water were examined for occurrence of the most common herbicides and insecticides in Iowa. No pesticides were found in sediments or suspended solids, but metolachlor, desethyl atrazine, acetochlor, and parathion were detected in water samples. However, no adverse effects were observed in larval walleye exposed to Cedar River water containing these pesticides. In addition, cholinesterase (ChE) activity in postlarvae I walleye exposed to Cedar River water containing parathion was not significantly different from postlarvae I exposed to control water. At the time of the study, the results indicated that pesticides were not a threat to survival of larval walleye in the Cedar River.

INDEX DESCRIPTORS: larval fish, walleye, *Stizostedion vitreum*, Cedar River, toxicity, cholinesterase, pesticides.

In the United States, agriculture contributes 65% of nonpoint source pollution to rivers, lakes, and streams, which is more than three times the amount contributed by the next leading soutce (USEPA 1990). Therefore, agricultural practices pose one of the most serious threats to the continued ecological integrity of environmental systems (Thurman et al. 1991), and agriculture has been charged as the activity most responsible for loss of fish species in streams of the Midwest (Karr et al. 1985). Fish communities in the former tallgrass prairie region of the midwestern United States, which now forms the heart of the Corn Belt, a 12-state area of intensive agricultural land-use, have declined since the land was first developed for agriculture. Although all anthropogenic stressors affecting aquatic ecosystems in the Corn Belt have not been isolated, suspended solids, sediment, and pesticides are believed to be major factors.

A substantial body of research on the effects of suspended solids on salmonid fishes in the western United States has demonstrated negative effects of suspended solids on survival, growth, feeding, reproduction, and behavior (Swenson and Matson 1976, Auld and Schubel 1978, Sigler et al. 1984, Vandenbyllaardt et al. 1991). However, these studies have not attempted to characterize the nature of the inorganic components, and none evaluated the influence of clay mineralogy. Even though the most extensive damage to streams has been in the agricultural Midwest where warmwater streams have been severely degraded, little work has been done to evaluate the effects of suspended solids on warmwater fishes of the Midwest (Waters 1995).

Suspended solids at concentrations observed in nature, acting alone without pesticide adsorption, produce little or no direct mortality on juvenile and adult fish, although the same cannot be said of impacts on larval fish. Wallen (1951) evaluated the toxicity of montmorrillonite clay (aluminum silicate) on 16 species of warmwater fish; lethal concentrations of clay ranged from 69,000 to 222,000 mg/L, but most species of fish survived exposure to 100,000 mg/L of clay for a week or longer. Thus, it seems that soil particles themselves are seldom toxic. Some studies, however, indicate that suspended solids may cause sublethal effects such as reduced feeding, depressed growth, and decreased tolerance to disease and toxicants (McLeay et al. 1984, Redding et al. 1987, Goldes et al. 1988).

In Iowa, suspended solids are of major concern because the landscape has changed substantially over the past 100 yrs. For example, in the late 1800s, the Cedar River basin was mostly covered by tallgrass prairie and the stream corridors were lined with timber (Meek 1893); by the 1980s more than 90% of the Cedar River basin was used for the production of corn and soybeans (Menzel et al. 1984). As a result of intensive row-cropping, after a major storm, suspended solid concentrations as high as 3,230 mg/L can occur in Iowa rivers (USGS 1994). However, streams in the Midwest rarely have suspended solid concentrations exceeding 300 mg/L for extended periods of time (USGS 1994).

The widespread use of herbicides and insecticides for corn production (Table 1) represents an additional threat from nonpoint source contaminants in Iowa's streams. During the 1990 growing season, 95% of corn acres and 97% of soybean acres in Iowa received herbicide treatment and 35% of corn acres received insecticide treatment (Hartzler and Wintersteen 1991). Atrazine was the most widely used herbicide in Iowa; it was applied to more than 61% of the corn acres. Chlorpyrifos and terbufos (organophosphorus insecticides, OPs) were the most widely used insecticides; they were applied to more than 20% of the corn acres in Iowa (Hartzler and Wintersteen 1991).

Pesticides are most likely to represent a threat of acute mortality when spawning of fish coincides with application dates for preemergent pesticides. The timing of preemergent pesticide application is significant because high concentrations of these pesticides can occur in rivers and streams after heavy spring rains (Thurman et al. 1991). Solomon et al. (1996) reported that atrazine concentrations in streams and rivers in agricultural watersheds are episodic, with major peaks in spring and early summer following applications that typically occur in May and June. Because many species of fish spawn in the spring, larval fish may be exposed to peak concentrations of pesticides at their most sensitive life stage.

Although it has been assumed that suspended solids, herbicides,

Table 1. Major herbicides and insecticides applied to Iowa farmland used for corn production: 1985 and 1990 data from Hartzler and Wintersteen (1991); 1995 data from Hallberg (G. R. Hallberg, The University of Iowa Hygienic Laboratory, personal communication).

	1985		1990		1995	
	% ha	kg. a.i. (× 10^3)	% ha	kg. a.i. (× 10^3)	% ha	kg. a.i. (× 10^3)
Herbicide						
acetochlor	N/A	N/A	N/A	N/A	26.0	2,815
alachlor	33.7	4,491	22.3	2,833	3.0	347
atrazine	49.0	4,407	61.0	3,424	62.0	2,944
cyanazine	33.9	4,702	19.6	2,322	18.0	2,402
metolachlor	37.2	4,692	34.4	4,265	33.0	3,798
Ha treated (%)[1]	97.0		95.0		99.0	
Insecticide						
chlorpyrifos	11.5	824	7.5	492	N/A	N/A
fonofos	7.0	439	4.0	195	N/A	N/A
phorate	4.7	351	2.9	197	N/A	N/A
terbufos	13.2	996	17.9	1,175	N/A	N/A
Ha treated (%)[1]	43.0		35.0		28.0	

[1]Percentage of Iowa corn ha treated with pesticides; it is not the sum of the column because some ha are treated with more than one pesticide.

and insecticides have detrimental effects on the survival of fishes in Iowa, little work has been done to quantify these factors. Several ichthyofaunistic studies in Iowa have reported a reduction in fish species in Iowa streams. Menzel (1981) reported that eight environmentally sensitive species were absent or present in only small numbers in 10 headwater streams of the Cedar River basin, and all streams examined were affected by moderate to intensive agricultural land use in their watersheds. Although the cause of the decline in the fish populations in these streams was not identified, Menzel (1981) implied that habitat degradation resulted from high levels of sediment and agricultural chemicals that were transported to the streams by runoff. Fish community composition in streams and rivers in the Eastern Iowa Basins were related to several physical and chemical factors (Sullivan 2000). Agricultural influences that caused environmental degradation and a decline in sensitive fish species included percent of rowcrops in the watershed, median total phosphorus, suspended sediment, and dissolved organic carbon concentrations.

Larval walleye (*Stizostedion vitreum*) are the subjects of this study because they have been described as "extremely sensitive species" to suspended sediment (Alabaster and Lloyd 1980), their status in Iowa's rivers has been evaluated from the late 1800s to the present, they are high on the food chain, and have economic value as a sport and food fish. Walleye is a favorite recreational species in Iowa as well as in the rest of the Midwest (Conover 1986) and is native to large river systems in Iowa (Harlan et al. 1987). A statewide angler survey in 1994 indicated that walleye were third among fish most frequently eaten by Iowa anglers (Lutz et al. 1995).

From the late 1800s to the early 1950s, walleye were commonly observed in the Iowan Surface, a geologically distinct region of the state that encompasses the Cedar River basin (Meek 1892, Cleary 1953). However, a fry-stocking program was begun in 1951 to improve river walleye fisheries because of a perceived lack of reproductive success (Cleary and Mayhew 1961). Riverine habitat degradation has affected many walleye fisheries in North America (Paragamian 1989), and an extensive statewide investigation of fish populations in the 1980s found walleye to be rare in Iowa's inland rivers (Paragamian and Kingery 1992).

The decline of walleye in inland streams of Iowa seems to be a function of poor recruitment (Kingery 1991, Paragamian and Kingery 1992). Prior to the stocking of juvenile walleye, no young-of-the-year walleye were observed, however, survival of stocked juvenile walleye was as high as 22% (Kingery 1991). Therefore, poor survival resulting from stocking of larval fish, but good survival resulting from stocking juveniles indicates that the larval stage (from hatching to juvenile) is the critical period for walleye survival. Although the eggs of walleye may settle to the bottom and be smothered by deposition of suspended solids, the reasons larval walleye are unable to survive is unknown. Therefore, the objectives of this study were to evaluate the toxicity of Cedar River sediments and water to larval walleye.

METHODS

Study Area

The Cedar River, located in the Iowan Surface, a physiographically distinct landform region (Prior 1991), was chosen for this study because it is one of the most intensively investigated inland rivers in Iowa. Walleye larvae stocking and research have been conducted in this stream for more than 30 yrs (Cleary and Mayhew 1961, Mauldin 1999). The Cedar River is also one of four rivers in the Eastern Iowa Basins study area of the U.S. Geological Survey (USGS) National Water-Quality Assessment Program (Kalkhoff 1994; Sullivan 2000). Land use in the Eastern Iowa Basins is 93% agriculture, 4% forests, 2% urban, and 1% wetlands (Sullivan 2000). Because so much of the land is used for agriculture, overland runoff of soil, nutrients, and pesticides from row-crop agriculture and animal feeding operations represents the major nonpoint source of pollution that causes eutrophication, toxic contamination, and sedimentation problems (Kalkhoff 1994).

Analysis of Eggs of Walleye Collected from the Cedar River

One female walleye (length = 480 mm; weight = 1,056 g) was collected from the Cedar River by angling on 16 April 1998, and one female walleye (length = 523 mm; weight = 1,243 g) was collected by angling on 9 April 1999. Eggs were stripped from both females, placed in glass jars, and sent to the University of Iowa Hygienic Laboratory (Iowa City, IA) for analysis of chlorinated hydrocarbon insecticides. Eggs were evaluated for chlorinated hydrocarbons because these insecticides are extremely persistent and have high biomagnification factors (Pait et al. 1992, Richmonds and Dutta 1992).

Collection and Analysis of Sediment and Water Samples from the Cedar River

River sediment and water samples were collected once in the fall of 1997 (18 September), five times in spring 1998 (31 March; 16 April; 3 May; 6 May; 13 May), and five times in spring 1999 (9 April; 16 April; 29 April; 4 May; 10 May) from Janesville, Iowa (USGS Station number 05458500). On each sampling date, the top 3 cm of sediment was collected with a petite ponar dredge from 3 areas of the river where sediment had been deposited. The samples were pooled together, placed in 2, 1-L glass jars, and stored in a cooler for transport to our laboratory at Iowa State University. Upon arrival to the laboratory, one jar was placed in a cooler with ice and sent to the University of Iowa Hygienic Laboratory for analysis of pesticides within 24-h of collection. The other jar containing sediment was used for toxicity testing. All toxicity tests were started

immediately upon arrival to our laboratory at Iowa State University (within 4-h of sediment collection).

River water, including suspended solids, was collected in two 20-L carboys and six 1-L glass jars, placed in a cooler, and transported to our laboratory at Iowa State University. Upon arrival to our laboratory, six jars containing river water were placed in a cooler with ice and sent to the Iowa Hygienic Laboratory for analysis of pesticides within 24-h of collection. At the Iowa Hygienic Laboratory, the river water was filtered through a glass microfiber filter (1.5-μm pore size), and the suspended solids were collected. After the water was filtered, the suspended solids, water, and sediment were analyzed for the most common herbicides and used in Iowa. River water collected in the 20-L carboys was used for toxicity testing. All toxicity tests were started within 4 h of collection.

Test Organisms and Culture Conditions

Eyed walleye eggs were obtained from Rathbun Fish Hatchery, Moravia, Iowa, on 28 April 1998 and from Spirit Lake Fish Hatchery, Spirit Lake, Iowa, on 21 April 1999. Eggs were incubated at 14.0°C for 4 days and 13.0°C for 5 days in standard hatching jars before hatching began in Rathbun and Spirit Lake fish, respectively. To maintain uniformity of age, only larvae that hatched within a 24-h interval were used in the experiments. Mean length ± SE of 20 larvae at hatching was 7.8 ± 0.04 and 7.4 ± 0.05 mm for Rathbun and Spirit Lake fish, respectively. For both groups of fish, larvae were stocked at a density of 20 larvae/L (3,000 larvae) in a 150-L tank at 3 days posthatch. Fish stocked in this tank were used for later toxicity tests.

Rathbun and Spirit Lake larval walleye were raised at 16.9 ± 0.4°C and 16.5 ± 0.3°C for 30 days, respectively, following procedures described by Summerfelt (1996). Both groups of walleye were fed Fry Feed Kyowa B-400 and C-700 diets (BioKyowa, Inc., Chesterfield, MO) every 5 min, 22 h/day during the larval stages of development.

Water Quality of Experimental Test Chambers

At the beginning and end of each static 48-h toxicity test, temperature, dissolved oxygen (DO), hardness, alkalinity, pH, and ammonia were measured in each test chamber. Temperature (± 0.1°C) was measured using a glass thermometer, and DO was measured to the nearest 0.1 mg/L using an oxygen-sensitive membrane electrode (polarographic). Total ammonia-nitrogen (NH_3-N; TAN) was measured to the nearest 0.01 mg/L using the Nesslerization method (APHA 1998) and a spectrophotometer, and pH was measured to the nearest 0.1 with a standard combination electrode and meter standardized with pH 4.0, 7.0, and 10.0 buffers. Hardness was measured to the nearest 1 mg/L using the Man Ver 2 burette titration method (HACH Company, Loveland, CO), and total alkalinity was measured to the nearest 1 mg/L by titration with 0.02 N H_2SO_4 (APHA 1998). Quality control samples (HACH Company, Loveland, CO) were analyzed along with water samples to verify the accuracy of the procedures used to measure TAN. Measured concentrations for the externally supplied TAN quality assurance samples were always within the certified 95% confidence interval.

Fish Sampling

For both groups of fish, a sample of five fish was netted every day and euthanized with 300 mg/L tricaine merhanesulfonate (Finquel®). Walleye were then measured (mm) and observed microscopically for gas bladder inflation (GBI) and presence of food in the gut. Observations of the day when the yolk sac and oil globule disappeared were used to describe their larval stage (prolarvae, postlarvae I and postlarvae II) as well as when GBI began and first feeding occurred. The three larval stages of walleye are prolarval (yolk sac present: 1 to 5 days posthatch); postlarval I (yolk sac absent and oil globule present: 6 to 14 days posthatch); and postlarval II (oil globule absent: 15 to 21 days posthatch).

Acute Toxicity Assays

Static 48-h acute toxicity tests were conducted on three stages of larval walleye to determine the toxicity of river sediment and river water collected from the Cedar River in 1998 and 1999 using standard methods (APHA 1998). Food was withheld 24 h preceding and during the 48-h exposure. Also, sediment and water used in the toxicity tests were collected at the same time and same site as the sediment analyzed by the University of Iowa Hygienic Laboratory. Therefore, the concentration of pesticides in river sediment and water that larval walleye were exposed to was known.

Toxicity of Cedar River Sediments. In both 1998 and 1999, the toxicity of resuspended Cedar River sediments to three stages of larval walleye was determined by stocking 14 prolarvae and postlarvae I walleye/L (50 fish/test chamber) and 7 postlarvae II walleye/L into 12, 4-L test chambers. Test chambers described by Schmidt-Dallmier et al. (1992) were used; however, several modifications were made to the chambers. Glass baffles and a small-mesh stainless steel wire screen were removed, and a 1-mm mesh screen was used to cover the notches cut at the top and bottom of the funnel to prevent larval walleye from passing through the notches. Sediment suspension was maintained with a propeller-tipped stirring rod driven by an electric motor with a rheostat. The revolution rates of all stir rods were synchronized at 1,500 rpm with a stroboscope before additions of sediment to the chambers.

A control (no riverine sediment) and three concentrations (high, medium, and low) of Cedar River sediment were used to determine the toxicity of the sediment (three replicates per treatment). To obtain the desired concentrations of suspended solids, Cedar River sediment was added to 3.5 L of reference water (water used in the university aquaculture laboratories) until turbidity levels of 500, 250, and 100 nephelometric turbidity units (NTUs), measured with a 90° light-scattering turbidimeter (HACH model 2100P, HACH Company, Loveland, CO), were obtained.

In 1998, suspended solid concentrations were significantly lower than desired after the 48-h experiment because coarse materials settled to the bottom of the test chambers. Therefore, in 1999, Cedar River sediment was added to 10 L of reference water in a 20-L bucket, stirred, and allowed to settle for 5 min. This method was repeated until the desired turbidities were obtained. This allowed finer particles of suspended solids to be used in the tests and resulted in increased suspended solid concentrations in 1999. In addition, in 1999, test chambers were incubated in a water bath to maintain constant water temperatures. Fish survival for each treatment was derermined from counrs at the end of the static 48-h test.

Toxicity of Cedar River Water. In 1998 and 1999, static 48-h tests were conducted to determine the toxicity of water collected from the Cedar River to prolarval, postlarval I, and postlarval II walleye. The water used to accomplish this objective was collected from the Janesville site three times in the postspawning interval as described. This experiment was run simultaneously with the sediment toxicity tests, and fish from the same hatch were used in both experiments. Reference water (water used in the university aquaculture laboratories) was used as the control, and two concentrations of river water were used: 50:50 river water:reference water and 100% river water (3 replicates per treatment). Larvae were stocked into nine 4-L test chambers as described. Survival for each treatment was determined from counts at the end of the static 48-h test.

Cholinesterase Inhibition and Analysis

Parathion, an OP, was detected in a water sample collected from the Cedar River on 4 May 1999. Therefore, because OPs inhibit cholinesterase (ChE) activity, postlarvae I walleye exposed to this treatment were analyzed for ChE activity. Five postlarvae I walleye from each test chamber containing Cedar River water and from reference water that survived the 48-h static acute toxicity tests were analyzed to determine total ChE activity. Larval fish that died during the 48-h exposure were not analyzed for ChE activity because rapid decomposition made the analysis unreliable.

A colorimetric method for analyzing whole body ChE activity modified for use on a THERMOmax microplate reader and SOFTmax software (Molecular Devices Corporation, Sunnyvale, CA) was used to monitor the rate of formation of 5-thio-2-nitrobenzoate, a yellow-colored anion. Hydrolysis of acetylthiocholine (AThCh) by ChE results in an acetate ion, and a negatively charged thiocholine complex reacts with 5,5-dithiobis-2-nitrobenzoic acid (DTNB) to form 5-thio-2-nitrobenzoate (Ellman et al. 1961, Hill and Fleming 1982, Gard and Hooper 1993). The microplate reader was set in the kinetic mode to monitor increases in absorbance at 405 nm for 2 min, read at 8-s intervals with a 0-s lag time, and a final volume of 250 µl/well at 25°C (Gard and Hooper 1993, Beauvais 1997). The optimal substrate concentration, AThCh, for larval walleye determined prior to analysis with non-test samples was 0.001 M AThCh. The Vmax and dilution factors were used to calculate ChE activities, reported as micromoles AThCh hydrolyzed per min per g of tissue (hereafter abbreviated as µM AThCh).

All tissue samples analyzed for ChE activity were run in triplicate on the microtiter plate. If the coefficient of variance (CV) among the triplicates was greater than 10%, samples were rerun (less than 5% of the samples had to be rerun). Because a commercial ChE standard for walleye tissue was not available, a check standard was used. The check standard was made by pooling larval walleye diluted 100-fold in pH 7.4 trizma buffer. The pooled tissue was homogenized and divided into 1-mL aliquots that were stored in 2 mL cryovials and placed in liquid nitrogen. These aliquots were run as check standards in triplicate along with each plate of treated samples. If the CV of the check standard was greater than 10%, all samples were rerun (less than 5% of the samples had to be rerun).

Statistical Analysis

Differences in survival and/or ChE activity due to treatment effects were assessed by ANOVA (SAS 1998). When the F-value for the overall test was significant ($P < 0.05$), Fisher's least significant difference test was used to determine significance among treatments. Survival data were transformed to a normal distribution before analysis using the following formula: transformed survival = arcsine (survival proportion)$^{1/2}$ (Zar 1984).

RESULTS

Analysis of Eggs of Walleye Collected from the Cedar River

In 1998, DDE was the only chlorinated hydrocarbon present in walleye eggs (Table 2). In 1999, DDD, DDE, and dieldrin were present in walleye eggs (Table 2).

Pesticide Analyses of Water, Suspended Solids, and Sediment

Suspended solids, water samples, and river sediment were analyzed for the most common herbicides and most common OPs used in Iowa. No detectable concentrations of these pesticides were found in the 18 September 1997 sample when flow rates are normally low (Fig. 1). In 1998, metolachlor was present in water samples collected on 31 March (0.31 µg/L) and 3 May (0.35 µg/L) when flow rates in the river were at their highest (Fig. 2); desethyl atrazine was detected on 16 April (0.12 µg/L) and 6 May (0.10 µg/L; Fig. 2). Pesticides were not found in the suspended solids or river sediment samples on any date.

Table 2. Concentrations of chlorinated hydrocarbon insecticides in eggs of walleye collected from the Cedar River on 16 April 1998 and 9 April 1999.

Organochlorine Insecticide	1998 (mg/kg)	1999 (mg/kg)	Quantitation Limit (mg/kg)
Aldrin	<0.01	<0.01	0.01
alpha-BHC	<0.01	<0.01	0.01
beta-BHC	<0.01	<0.01	0.01
delta-BHC	<0.01	<0.01	0.01
Lindane (gamma-BHC)	<0.01	<0.01	0.01
DDD	<0.01	0.02	0.01
DDE	0.034	0.16	0.01
DDT	<0.01	<0.01	0.01
Dieldrin	<0.01	0.04	0.01
Eudosulfan I	<0.01	<0.01	0.01
Endosulfan II	<0.01	<0.01	0.01
Endosulfan sulfate	<0.01	<0.01	0.01
Endrin	<0.01	<0.01	0.01
Endrin aldehyde	<0.01	<0.01	0.01
Endrin ketone	<0.01	<0.01	0.01
Heptachlor	<0.01	<0.01	0.01
Heptachlor epoxide	<0.01	<0.01	0.01
Methoxychlor	<0.01	<0.01	0.01
Chlordane	<0.04	<0.04	0.04
Toxaphene	<2.00	<2.00	2.00
alpha-Chlordane	<0.01	<0.01	0.01
gamma-Chlordane	<0.01	<0.01	0.01
cis-Nonachlor	<0.01	<0.01	0.01
tans-Nonachlor	<0.01	<0.01	0.01
Oxychlordane	<0.01	<0.01	0.01

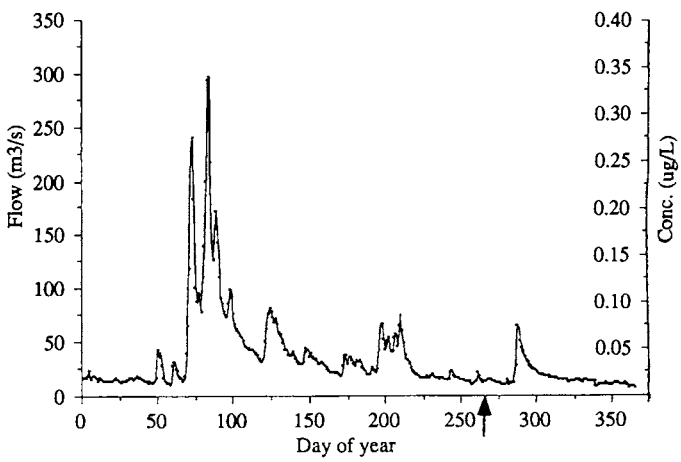

Fig. 1. Total flow (m^3/s) in the Cedar River at Janesville, Iowa (Station 05458500) in 1997. No detectable concentrations of pesticides were found in water, suspended solids, or sediment samples (arrow indicates sampling date).

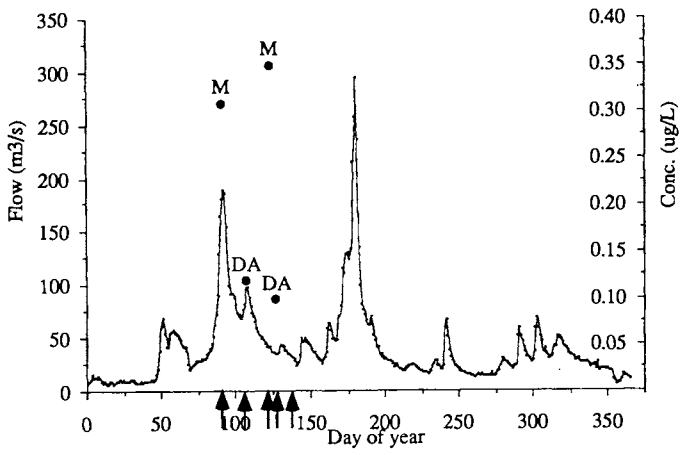

Fig. 2. Total flow (m^3/s) in the Cedar River at Janesville, Iowa (Station 05458500) in 1998. Metolachlor (M) and desethyl atrazine (DA) were detected in water samples. No detectable concentrations of pesticides were found in suspended solid or sediment samples (arrows indicate sampling dates).

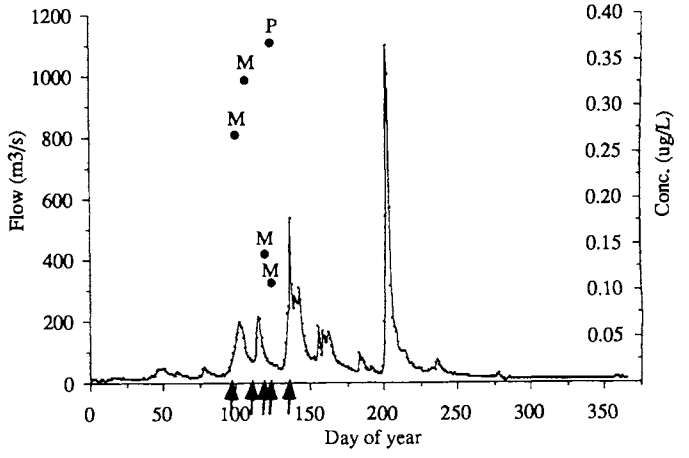

Fig. 3. Total flow (m^3/s) in the Cedar River at Janesville, Iowa (Station 05458500) in 1999. Metolachlor (M) and parathion (P) were detected in water samples. No detectable concentrations of pesticides were found in suspended solid or sediment samples (arrows indicate sampling dates).

Five spring 1999 samples (9 April, 16 April, 29 April, 4 May, 10 May) of suspended solids, bottom sediment, and water samples were analyzed for the most common herbicides and OPs when flow rates in the river were at some of their highest levels (Fig. 3). No detectable concentrations of these pesticides were found in suspended solids and sediment samples collected on any of the five dates. Metolachlor was present in water samples collected on four of the five collections: 9 April (0.27 μg/L), 16 April (0.33 μg/L), 29 April (0.14 μg/L), and 4 May (0.11 μg/L; Fig. 3). Acetochlor was present in a water sample collected on 10 May (0.16 μg/L). Parathion, an organophosphorus insecticide, was detected in a water sample collected on 4 May (0.37 μg/L; Fig. 3). No other pesticides were detected in water samples on any date.

Toxicity of Cedar River Water and Sediments

Prolarvae. In 1998, survival of 3-day-old prolarvae (mean length ± SE = 9.0 ± 0.03 mm) exposed for 48 h to reference water, a 50:50 ratio of river water:reference water, and 100% river water did not differ from larval survival in the reference water. Likewise, differences in survival of prolarvae exposed to Cedar River sediment did not differ from survival of fish exposed to the reference (clay) treatment (Table 3). Also, differences in measures of water quality (temperature, DO, TAN, hardness, and alkalinity) among treatments were not significantly different at the beginning and end of the experiment.

In 1999, survival of 3-day-old prolarvae walleye (mean length ± SE = 8.8 ± 0.05 mm) exposed for 48 h to reference water, a 50:50 ratio of river water:reference water, and 100% river water did not differ from larval survival in reference water. Likewise, differences in survival of prolarvae exposed to Cedar River sediment did not differ from survival of fish exposed to the reference (clay) treatment (Table 4). Differences in measures of water quality (temperature, DO, TAN, hardness, and alkalinity) among treatments were not significantly different at the beginning and end of the experiment.

Postlarvae I. In 1998, differences in survival of 6-day-old postlarvae I (mean length ± SE = 9.6 ± 0.06 mm) after a 48-h exposure to reference water, a 50:50 ratio of reference water:Cedar River water,

Table 3. Survival of three larval stages (prolarval, postlarval I, and postlarval II) of walleye in 48-h static toxicity tests in Cedar River water and in several concentrations of Cedar River sediment (sed.) collected in 1998.

	Prolarval		Postlarval I		Postlarval II	
Treatment	Water (%)	% Survival ± SE	Water (%)	% Survival ± SE	Water (%)	% Survival ± SE
Reference water	100	96 ± 1.2	100	56 ± 13.1	100	48 ± 4.2
Cedar R.	50/50	92 ± 4.2	50/50	52 ± 14.0	50/50	40 ± 1.2
Cedar R.	100	87 ± 11.7	100	61 ± 5.8	1000	44 ± 5.5
P-value of ANOVA		0.45		0.88		0.29
	Conc. clay (mg/L)	% Survival ± SE	Clay (mg/L)	% Survival ± SE	Clay (mg/L)	% Survival ± SE
Reference clay	132 ± 6.7	84 ± 3.1	32 ± 0.9	38 ± 10.1	74 ± 20.4	44 ± 2.4
Cedar R. sed.	25 ± 3.2	91 ± 2.9	12 ± 3.1	55 ± 12.2	12 ± 6.7	52 ± 2.4
Cedar R. sed.	50 ± 2.8	94 ± 2.0	20 ± 1.5	39 ± 11.4	34 ± 0.9	48 ± 3.5
Cedar R. *sed.*	85 ± 11.9	90 ± 6.1	27 ± 1.8	17 ± 5.2	42 ± 2.6	44 ± 5.3
P-value of ANOVA		0.37		0.11		0.31

Table 4. Survival of three larval stages (prolarval, postlarval I, and postlarval II) of walleye in 48-h static toxicity tests in Cedar River water and in several concentrations of Cedar River sediment (sed.) collected in 1999.

	Prolarvae		Postlarvae I		Postlarvae II	
Treatment	Water (%)	% Survival ± SE	Water (%)	% Survival ± SE	Water (%)	% Survival ± SE
Reference water	100	91 ± 4.1	100	83 ± 1.8	100	88 ± 3.1
Cedar R.	50/50	91 ± 3.3	50/50	77 ± 5.5	50/50	81 ± 2.4
Cedar R.	100	85 ± 2.7	100	69 ± 10.1	1000	96 ± 1.2
P-value of ANOVA		0.39		0.45		0.12
	Conc. clay (mg/L)	% Survival ± SE	Clay (mg/L)	% Survival ± SE	Clay (mg/L)	% Survival ± SE
Reference clay	340 ± 82	93 ± 4.4	250 ± 17	57 ± 2.4	288 ± 26	93 ± 1.8
Cedar R. sed.	83 ± 14	94 ± 3.5	56 ± 15	73 ± 5.9	81 ± 13	95 ± 1.8
Cedar R. sed.	280 ± 86	96 ± 1.2	169 ± 18	56 ± 12.9	155 ± 12	92 ± 1.2
Cedar R. sed.	392 ± 55	87 ± 3.7	291 ± 46	43 ± 16.3	366 ± 12	80 ± 5.0
P-value of ANOVA		0.40		0.35		0.33

and 100% Cedar River water were not significant. Likewise, differences in survival of postlarvae I exposed to Cedar River sediments did not differ from survival of fish exposed to the reference (clay) treatment (Table 3). Also, differences in measures of water quality (temperature, DO, TAN, hardness, and alkalinity) among treatments were not significantly different at the beginning and end of the experiment.

In 1999, differences in survival of 8-day-old postlarvae I (mean length ± SE = 10.2 ± 0.10 mm) after a 48-h exposure to reference water, a 50:50 ratio of reference water:Cedar River water, and 100% Cedar River water were not significant (Table 4). Likewise, differences in survival of postlarvae I exposed to Cedar River sediments did not differ significantly from survival of fish exposed to the reference (clay) treatment (Table 4). Also, differences in measures of water quality (temperature, DO, TAN, hardness, and alkalinity) among treatments were not significantly different at the beginning and end of the experiment.

Because parathion, an OP insecticide, was found in river water, ChE activity was measured in postlarvae I walleye exposed to the reference water, 50:50 Cedar River:reference water, and 100% Cedar River water treatments. Mean ChE activity was 8.18 ± 0.29 μM AThCh for postlarvae I exposed to reference water and 8.34 ± 0.44 and 9.32 ± 0.40 μM AThCh for the Cedar River/reference water and 100% Cedar River water treatments, respectively. Cholinesterase activities did not differ among treatments.

Postlarvae II. In 1998, differences in survival of 13-day-old postlarvae II (mean length ± SE = 12.3 ± 0.15 mm) after a 48-h exposure to reference water, a 50:50 ratio of Cedar River water and reference water, and 100% Cedar River water were not significant (Table 3). Likewise, differences in survival of postlarvae II exposed to Cedar River sediments did not differ from survival of fish exposed to the reference (clay) treatment (Table 3). Also, differences in measures of water quality (temperature, DO, TAN, hardness, and alkalinity) among treatments were not significantly different at the beginning and end of the experiment.

In 1999, differences in survival after a 48-h exposure, starting with 14-day-old postlarvae II (mean length ± SE = 13.8 ± 0.14 mm) to reference water, a 50:50 ratio of Cedar River water and reference water, and 100% Cedar River water were not significant (Table 4). Likewise, differences in survival of postlarvae II exposed to Cedar River sediments did not differ from survival of fish exposed to the reference (clay) treatment (Table 4). Also, differences in measures of water quality (temperature, DO, TAN, hardness, and alkalinity) among treatments were not significantly different at the beginning and end of the experiment.

DISCUSSION

The experiments conducted on three larval stages of walleye in 1998 and 1999 indicate that Cedar River water and sediment are not more toxic to larval walleye, at any larval stage, than reference water and sediment. In both years, survival of postlarvae I walleye was lower than survival of prolarvae and postlarvae II walleye, but survival of postlarvae I walleye exposed to Cedar River sediment and water did not differ significantly from survival in the reference clay and water. We attribute the lower survival of postlarvae I compared with prolarvae and postlarvae II to the higher mortality that naturally occurs in postlarvae I as they switch from endogenous to exogenous feeding (Summerfelt 1996).

Most investigators have found that suspended solids have a low toxicity to fish (Wallen 1951). Auld and Schubel (1978) determined that striped bass (*Morone saxatilis*) and yellow perch (*Perca flavescens*) were able to tolerate high concentrations of suspended sediment containing illite, chlorite, and kaolinite (1 to 4 μm particles) collected from the Chesapeake Bay (≥ 500 mg/L). Similarly, Phillips (1996) found that a 28-day exposure of larval and early juvenile walleye to a ball clay (56% SiO_2 with 72% of the particles <1.0 μm) was not harmful at concentrations as high as 360 mg/L. However, Panther Creek clay (64.6% SiO_2), a bentonite clay, was found to be highly toxic to postlarval I and II walleye (Phillips 2000). This indicates that suspended solids may be acutely toxic to fish, depending on the physical and chemical characteristics of the suspended solid.

No pesticides were detected in Cedar River sediments or suspended solids in this study, but metolachlor, desethyl atrazine, acetochlor, and parathion were detected in Cedar River water. We have no explanation for the presence of parathion and this finding was not confirmed with mass spectrometry. However, no adverse effects were observed in larval walleye exposed to Cedar River water containing these pesticides. In addition, ChE activity in postlarvae I walleye exposed to Cedar River water containing parathion was not significantly different from postlarvae I exposed to control water.

The lack of toxicity of Cedar River sediment and water in April and May 1998 and 1999, the time that walleye eggs and larvae were collected in the Cedar River (Mauldin 1999), does not completely

eliminate pesticides as a possible contributor to mortality of larval fish in this river because concentrations of pesticides may vary with the nature of runoff events and time of year. However, at the time these experiments were conducted, the results do not implicate pesticides as a problem to survival of larval walleye in the Cedar River. Other factors limiting natural recruitment of walleye in the Cedar River may include a lack of riparian nursery habitat and scarcity of zooplankton (Mauldin 1999).

Although Cedar River water and sediment were not acutely toxic to the three stages of larval walleye, eggs collected from walleye of the Cedar River in 1998 and 1999 were found to contain DDE, a metabolites of DDT, and dieldrin (a cyclodiene insecticide). The extreme persistence (years), biomagnification, and lipophilic characteristics of these three compounds are well established (Ware 1994). As a result, a federal ban on the use of DDT was declared by the U.S. Environmental Protection Agency (EPA) in 1973 and most agricultural uses of the cyclodiene insecticides were canceled by the EPA between 1975 and 1980 (Ware 1994). However, the presence of organochlorine insecticides in aquatic organisms is not uncommon. For example, although the concentrations were low (4.22 to 111.9 μg/kg), dieldrin, heptachlor epoxide, and chlordane were detected in whole 3-yr-old common carp (*Cyprinus carpio*) collected from the Des Moines River in Iowa (Lutz and Cavender 1997). Newsome et al. (1993) found mean organochlorine contents (including hexachlorobenzenes, chlordane, DDTs, HCH, nonachlor, coctachlorostyrene, heptachlor, heptachlor epoxide, dieldrin, mirex, and toxaphene) in several species of commercial fish from the Great Lakes ranged from 44 to 138 mg/kg (lipid basis).

ACKNOWLEDGMENTS

This is journal paper J-18871 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa (Project No. 3480), and was supported by Hatch Act and State of Iowa funds. This research was funded by the Iowa Science Foundation, USDA, NRICGP (grant 9700882), the Leopold Center for Sustainable Agriculture (grant 98–08), and the Carver Trust Grant. We thank Randy Esser and Tracy Williams for their assistance in water quality analysis and fish husbandry. We also thank Louise Mauldin and Aaron Griffith for their help in collecting Cedar River water and sediment samples. Andy Moore, Rathbun Fish Hatchery, and Wally Jorgenson, Spirit Lake Fish Hatchery, Iowa Department of Natural Resources, provided walleye eggs.

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