Keller et al. 1998

ELSEVIER

Aquatic Ecosystem Health and Management 1 (1998) 37–47

Aquatic Ecosystem Health & Management

Testing the toxicity of sediments contaminated with diesel fuel using glochidia and juvenile mussels (Bivalvia, Unionidae)

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Abstract

Sediment toxicity was evaluated for one site upstream and three sites downstream of a diesel fuel spill that occurred in Fish Creek (OH and IN) in September 1993 using glochidia and juvenile unionid mussels. This fourth order tributary of the St. Joseph River has the only known remaining population of white cat's paw pearly mussel, *Epioblasma obliquata perobliqua*, and populations of several other federal and state endangered mussels. The impact of the oil spill was of great concern because of the potential long term threat it poses to the survival of these mussels. Sediment samples collected two years after the spill contained low heavy metal concentrations, and detectable, but not quantifiable levels of organic components of diesel fuel. These levels coincided with their lack of toxicity to juvenile *Villosa villosa* and *Lampsilis siliquoidea* mussels after 9-day exposures. Fish Creek sediments may be toxic to *L. siliquoidea* glochidia.

Keywords: *Lampsilis siliquoidea*; *Epioblasma obliquata perobliqua*; Fish Creek

1. Introduction

Unionid mussels (Family Unionidae) are among the most imperiled taxa in North America (Williams et al., 1993; Master, 1990; U.S. Fish and Wildlife Service, 1994a). Of the nearly 300 species present, 200 have been identified as threatened, endangered or in decline. A number of species are currently known from only one or a few populations. Such is the case for the white cat's paw pearly mussel, *Epioblasma obliquata perobliqua*, a mussel that was once distributed throughout the state of Indiana, USA, and in parts of Ohio, but is now limited to a single population in Fish Creek, Indiana (Cummings and Mayer, 1992; Watters, 1993) (Fig. 1). This fourth order stream, a tributary of the St. Joseph River traversing northeastern Indiana and northwestern Ohio (Fig. 1), is noted for its outstanding water quality and its diverse aquatic fauna including several state and federally listed endangered mussel species (Stewart et al., 1993; U. S. Fish and Wildlife Service, 1994b; Indiana Depart-

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1463-4988/98/$19.00
PII: S1463-4988(98)00005-0

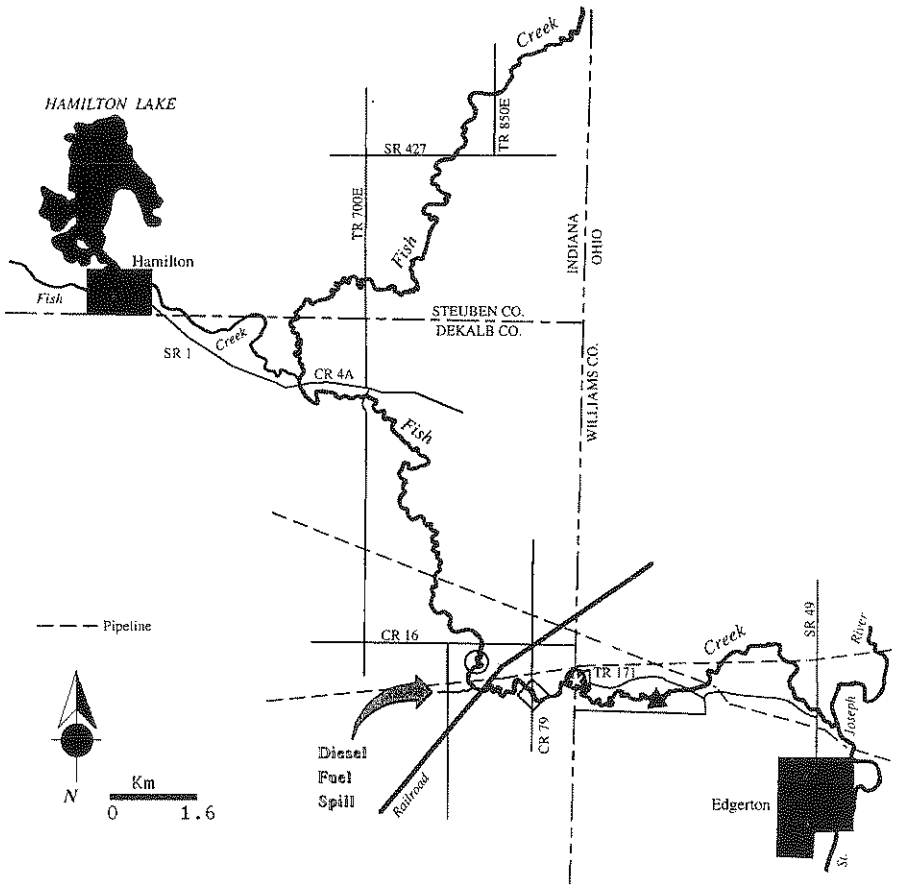

Fig. 1. Map of Fish Creek, Indiana and Ohio, USA, showing the location of the oil spill and sediment sampling sites. ○ = CR-16, ◊ = CR-79, ☆ = SL-95, and ▲ = QUA-95, SR = State Road, CR = County Road, TR = Township Range. Fish Creek flows north to south then west to east into the St. Joseph River.

ment of Natural Resources, 1990; U.S. Fish and Wildlife Service, 1990).

While many potential causes for the loss of unionid species have been identified, chemical contaminants are suspected as a major contributor (Master, 1990; Williams et al., 1993; U.S. Fish and Wildlife Service, 1990, 1994b). There are some data on the toxicity of common metal and pesticide pollutants to unionid mussels, but there is virtually no information about the toxicity of petroleum products to these mollusks. This became a serious issue in September 1993 when approximately 115,000 liters of No. 2 diesel fuel were spilled into Fish Creek (Fig. 1) 2.09 km west of the Ohio state line (40°27′ 56″N, 83°50′10″W). The states of Indiana and Ohio, and the U.S. Fish and Wildlife Service (USFWS) were concerned about the effects of the oil spill on the biota of this small stream (mean annual flow in spill area of 0.77 cm) (Stewart et al., 1993), particularly the endangered unionid mussels. The total distribution of *Epioblasma. o. perobliqua* lies within the area downstream of the oil spill (Watters, 1993).

Many adult individuals of the common mussel species *Elliptio dilatata*, and several other unionids inhabiting the spill area died within one month after the spill. Survival rates of rarer mussel species such as the endangered white cat's paw mussel, or

early life stages of any mussel species are unknown. Initial impacts of the spill may have been minimized because it occurred about the time of leaf fall, which could provide a large adsorptive capacity for the oil, and the onset of cooler weather, which stimulates mussels to burrow into the sediment (Balfour and Smock, 1995) and slows their metabolic rate (McMahon, 1991). However, there is a great potential for long-term effects on mussels because hydrocarbons that comprise diesel fuel partition quickly to the sediments or other organic media where they may persist for years (Blumer and Sass, 1972). Since mussels are sedentary benthic animals that inhabit the sediments, they may continue to be exposed to sediment-sorbed hydrocarbons even after the overlying water is relatively free of contamination. The results of this exposure could include not only adult mortality, but also decreased reproductive success due to the toxicity of sediments to the larval or juvenile mussel life stages.

Toxicity tests generally require the use of over a hundred animals making the use of early life stages in sediment tests preferable over adult mussels, especially for species that are rare. Hundreds of thousands of glochidia can be harvested from one adult for use in toxicity tests directly, or to produce juvenile mussels in the laboratory. In addition, early life stages are usually more sensitive than adults of a species, and can produce test results in a shorter period of time (McKim, 1985). No standard United States Environmental Protection Agency (U.S.E.P.A.) or American Society for Testing and Materials (ASTM) toxicity test methods have been developed for unionid mussels because the presence of a parasitic larval stage (glochidia) in the unionid life cycle has made laboratory culture and the development of standard test methods difficult. However, test methods for fathead minnows, the cladoceran *Ceriodaphnia dubia*, and the freshwater amphipod *Hyalella azteca* (Lewis et al., 1994; ASTM 1993) have been modified for use with unionids (Keller and Zam, 1991; McKinney and Wade, 1996; U.S.E.P.A. and Department of the Army, 1994) and were utilized in this study.

This study was performed to determine if the sediments in Fish Creek, Ohio and Indiana, USA are presently toxic to unionid mussels. Such information is needed by resource trustees to plan restoration activities in the stream reaches that were impacted by the 1993 diesel fuel spill.

2. Methods

2.1 Sediment sampling

Two six-litre sediment samples were collected to a depth of approximately 7.5 cm from each of four sites in Fish Creek, Indiana and Ohio in August 1993 (23 months after the diesel fuel spill), using a stainless steel scoop. The sites were located as follows: CR-16, the reference site, 1.2 km upstream of the oil spill; CR-79, 1.6 km downstream of the oil spill site in Indiana; SL-95, at the Ohio/Indiana state line, 3.2 km downstream of the spill; and QUA-95, in Ohio 6.4 km downstream of the spill site (Fig. 1). When possible, samples were collected in depositional zones where oil was visible in the sediment or at the water surface. Sediments were placed in insulated coolers and shipped overnight to our laboratory in Gainesville, FL. Upon receipt, samples were refrigerated at 4°C until tests were initiated three days later.

2.2 Sediment chemical analyses

A subsample of sediment was taken for each site and frozen at –30°C until analyzed for polyaromatic hydrocarbons (PAH), pesticide and metal contaminants, grain size, percent dry weight, and percent organic content. Metals, except mercury, were analyzed using EPA method 6010 (U.S.E.P.A., 1986). Mercury was analyzed by EPA method 7471 (U.S.E.P.A., 1986). Determination of organic contaminants was performed as prescribed in EPA method 8270 (U.S.E.P.A., 1986). The Walkley–Black method, a dichromate oxidation and titration method, was used to determine organic content (Allison, 1965). Percent moisture was determined by ASTM method D2216 (ASTM, 1994). Sediment size fractionation was performed by wet sieving the sediment through U.S. Standard screens (500 μm, 250 μm, 125 μm, 68 μm openings), drying to a constant weight at 100°C, and weighing the individual fractions.

Table 1
Mean of nine daily test chamber water quality measurements (mean ± s.d.) for mussel toxicity tests compared to Fish Creek water quality

Site	pH (mean ± s.d.)	Conductivity (mean ± µS ± s.d.)	Dissolved oxygen (mean ppm ± s.d.)	Initial hardness[b] (mg/l as $CaCO_3$)
CR-16	8.3 ± 0.17	438 ± 47	6.0 ± 0.57	250
CR-79	8.1 ± 0.21	381 ± 37	5.9 ± 0.62	
SL-95	8.3 ± 0.16	352 ± 13	6.9 ± 0.45	
QUA-95	8.3 ± 0.22	396 ± 24	6.2 ± 0.51	
Fish Creek water quality[a]				
	7.4–8.1	270–770	7.15–15.90	144–422

[a]Stewart et al. (1992), measurements taken within Fish Creek sampling area.
[b]Hardness of well water used in the test.

2.3 Mussel toxicity tests

For this study, 5-liter aquaria were used as test chambers. Twenty-four hours prior to the introduction of test organisms, sediment was added to aquaria to a depth of 3 cm and overlain with 5 cm of well water (hardness ≈250 mg l^{-1} as $CaCO_3$) having characteristics similar to those of Fish Creek water (Table 1) (Stewart et al., 1993). Test animals were contained in glass exposure chambers consisting of glass cylinders 5 cm in diameter and 7.5 cm in height closed on one end with 100 µm Nitex screen. Aquaria were aerated, kept at a constant 22°C in a water bath and exposed to a 12L:12D photoperiod. Dissolved oxygen, pH, conductivity and temperature were measured daily in all aquaria.

The concern about the effects of the oil spill centered around the survival of the last population of *E. o. perobliqua*, and other endangered mussels. Since these mussels are protected by state or federal law and are very limited in number, surrogate mussel species were selected for use in toxicity tests. Surrogates were selected based on their presumed sensitivity, having similar habitat preferences to the species of concern, taxonomic relatedness and availability, and our knowledge of host fish requirements. Based on these criteria, the mussels *Lampsilis siliquoidea* and *Lasmigona costata* were selected for use in the Fish Creek study (Kevin Cummings, Illinois Natural History Survey; and Tom Watters, Ohio Department of Natural Resources; pers. comm.). Gravid female *Lampsilis siliquoidea* mussels, collected from Spain Creek, Ohio (40°13′73″N, 83°31′49″W), and *Lasmigona costata* mussels, collected from the Wild River, Minnesota (45° 34′N, 92°52′W) were shipped overnight in cooled containers for use in glochidia tests.

For each mussel species, glochidia viability was tested by mixing larvae from two or three females, collecting a subsample, adding several drops of NaCl solution, and counting the number of glochidia that closed. Glochidia were considered viable if ≥90% closed (Jones, 1950). If the batch was viable, a sample of 50–100 microscopic larvae (0.25–0.33 mm) was placed into each of three replicate test chambers using an eyedropper. Each species was tested in separate chambers that were placed at the surface of Fish Creek sediment in aquaria. The toxicity endpoint, viability, was determined after 24-hour and 48-hour exposures to Fish Creek sediments. This test duration was selected because in the wild, glochidia from many mussel species attach to host fish within one to two days, encyst, and are then more protected from further exposure to contaminants (U.S. Fish and Wildlife Service, 1994; Jacobson, 1990).

Juvenile mussels were produced by transformation of glochidia on host fish (Coker, et al., 1921). *Villosa villosa* was substituted for *Lasmigona.*

costata after attempts to culture juveniles of the latter species failed twice and sediment samples were reaching their storage time limit. To accomplish this transformation, glochidia (suspended in well water) of *V. villosa* (adults collected in Suwannee River, FL at 29°36′N, 82°40′30″W) and *Lampsilis siliquoidea* were pipetted onto the gills of largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*). The fish were then held in flow-through aquaria containing well water at 22°C for approximately three weeks until the juvenile mussels excysted and dropped to the aquarium bottom. Once transformed, juveniles were collected by siphoning the aquaria, examined, and distributed 10 per chamber into each of three replicates per sediment sample. Animals were fed a tri-algal mixture during the tests consisting of *Chlorella* sp., *Selenastrum capricornutum*, and *Neochloris oleoabundans*, at a final concentration of 3.0 $\times 10^7$ cells ml^{-1}, and had access to detritus in the sediments via the mesh bottom. Juvenile survival was recorded each day for nine days. Juvenile mussels were counted as 'dead', if upon microscopic examination, pedal movement, ciliary activity, and heartbeat had ceased (Keller and Zam, 1991).

2.4 Statistical analyses

Survival data were arc sine square root transformed to improve normality. Analysis of variance and Duncan's test ($\alpha = 0.05$) (SAS ANOVA procedure, SAS Institute, Inc., 1982) were used to assess whether survival varied significantly between sites.

3. Results

3.1 Chemical characterization of sediments

Silt fractions (<68 μm) constituted from 5% (QUA-95) to 50% (CR-16) of the Fish Creek sediments used in toxicity tests (Table 2), while total organic (TO) values ranged from 0.25% (QUA-95) to 2.25% (CR-16) in dried samples (Table 3). Metal contaminants were present in low concentrations, but several exceeded the 'effects range low' range (Table 3). Only four organic contaminants, i.e., dimethyl phenanthrene, trimethyl phenanthrene, tetramethyl phenanthrene and tetrahydro indacene dione were detected in test sediments, but they were unquantifiable based on the detection level of 535 ppb. Sediments collected by USFWS one year after the diesel spill were found to contain low concentrations of several PAHs based on a minimum detection limit of 1 ppb (Table 4).

Table 2
Particle size distribution for Fish Creek sediments by site. Samples A and B for each site were collected at the same time but in slightly different locations at the site

Site	% > 250 μm	% 250–125 μm	%124–68 μm	% < 68 μm (silt)
CR-16A	17.33	12.19	20.04	50.44
CR-16B	20.91	15.37	26.87	36.84
CR-79A	18.38	22.18	23.33	36.84
CR-79B	18.72	19.97	17.43	43.87
SL-95A	55.64	12.75	10.56	21.03
SL-95B	49.24	13.99	12.79	23.98
QUA-95A	79.16	11.37	4.13	5.34
QUA-95B	79.88	11.21	3.38	5.52

3.2 Mussel tests

Following a 24-hour exposure to Fish Creek sediments, *Lampsilis siliquoidea* glochidia had mean (± s.d.) survival rates of 40 ± 13% and 47 ± 4% at QUA-95 and SL-95, respectively, and 42 ± 2% and 32 ± 22% at CR-79 and CR-16. There were no significant differences in glochidia survival among sites after 24 hours (ANOVA, $p = 0.05, f = 3.35$). After 48 hours of exposure, these glochidia had mean survival rates of 11 ± 6% and 8 ± 8% at sites CR-16 and CR-79, respectively, and 35 ± 13% and 31 ± 4% at QUA-95 and SL-95, respectively (Table 5). Higher survival rates at sites QUA-95 and SL-95 were significantly different from those at CR-16 and CR-79 after 48 hours ($p = 0.05, F = 6.01, df = 3$). A separate set of glochidia held in well water alone (no sediment) had a 79% survival

Table 3
Description of the appearance and heavy metal content of each sediment used in Fish Creek toxicity tests

Site	Description	TO (% dry)	As	Cd	Cr	Cu	Fe	Pb	Hg	Ni	Zn
		ppm dry weight									
CR-16A	Black, fine with sticks.	2.13	7.52*	<0.845	9.40*	14.0	13100	<16.9	<0.174	13.4*	49.5
CR-16B	Slight sulfur odor.	2.25	8.11*	<0.851	12.0*	16.2*	15500	<17.0	<0.171	16.8*	59.2
CR-79A	Fine, brown and black.	1.21	7.08*	<0.736	8.27*	11.0	12000	<14.7	<0.151	13.0*	39.4
CR-79B	Lots of detritus. No odor	1.31	5.45	<0.744	7.94*	10.7	11100	<14.9	<0.149	12.6*	36.9
SL-95A	Light brown with some	0.49	5.27	<0.579	6.00*	9.27	9290	17.7*	<0.122	9.33*	48.7
SL-95B	gravel. No odor	0.39	5.58	<0.625	7.56*	9.83	9520	18.9*	<0.127	10.0*	64.7
QUA-95A	Sand, coarse. Leaf and	0.48	5.02	<0.684	3.86*	4.45	6110	<13.7	<0.138	5.87*	15.8
QUA-95B	stem particles. Oily odor with dark patches.	0.25	3.71	<0.667	3.83*	6.33	5630	<13.3	<0.134	5.46*	17.5

*These values exceed the 'effect range low' values as determined by Ingersoll et al. (1996) and Persaud et al. (1992).

rate at 24 hours and a 69% survival rate at 48 hours, significantly higher than all sediment sites.

Lasmigona costata glochidia had mean survival rates ranging from 20 ± 3% at SL-95 to 37 ± 19% at CR-16 after 24 hours of exposure, decreasing to a low of 5 ± 2% at SL-95 and a high of 8 ± 5% at CR-79 after 48 hours (Table 5). All survival rates for glochidia of this species were lower than for *Lampsilis siliquoidea*. ANOVA and Duncan's test indicated that there were no significant differences in the survival of *Lasmigona costata* glochidia among the sites after 24 hours ($p = 0.05$, $F = 0.33$, $df = 3$) or after 48 hours ($p = 0.05$, $F = 0.88$, $df = 3$). The water-only controls for this species were accidentally destroyed.

There were no significant differences in survival of juvenile *V. villosa* or *Lampsilis siliquoidea* for sediments collected at the four sample sites in Fish Creek, with one exception, CR-16 (Table 5). While both juvenile *V. villosa* and *Lampsilis siliquoidea* had 90–100% survival rates for sites CR-79, SL-95 and QUA-95 after 9 d exposures, sediments from CR-16 were toxic to 23% of juvenile *Lampsilis siliquoidea,* a significant difference from other sites ($p = 0.05$, $F = 3.36$, $df = 3$). All controls for juvenile tests had ≥90% survival after 9 d.

4. Discussion

4.1 Glochidia and juveniles as sediment toxicity test organisms

While *in situ* tests are sometimes preferable to laboratory methods because they assess toxicity including physical, chemical and biological stressors present at the site of interest, they also can be complicated by the vulnerability of test containers to vandalism, the potential loss of vessels during high stream flows, and the difficulty of working with microscopic test animals in field conditions. The selected laboratory approach minimized the complications of field testing with mussel early life stages, and was similar to the more common *Hyalella azteca* and *Chironomus tentans* tests.

Results for tests with juvenile *V. villosa* and *Lampsilis siliquoidea* mussels are in accord with relatively low contaminant levels detected in test sediments. Fish Creek sediments may be acutely toxic to *Lampsilis siliquoidea* glochidia, but were not toxic to glochidia of *Lasmigona costata*. The lower survival of glochidia in all sediments compared to juvenile mussels may indicate that glochidia are more sensitive to low contaminant levels in the

Table 4
Contaminant concentrations (ppb dry weight) is sediment samples collected at three sites, one year after the September 15, 1993 oil spill in Fish Creek, Indiana

Contaminant	Cr-16*	SL-95a	SL-95b	QUA-95a	QUA-95b
Fluoranthene	bdl	11.26	12.30	10.77	6.46
Fluorene	bdl	5.84	4.19	104.71[a]	10.31[a]
Indeno(1,2,3-cd)pyrene	bdl	<1.38	<2.10	<1.37	<1.37
n-Decane	nm	107.34	45.15	nm	nm
Biphenyl	nm	3.99	2.40	67.07	9.07
Chrysene	bdl	9.55	9.30	8.90	7.15
Dibenzothiophene	nm	8.41	6.90	111.45	10.72
Benzo(e)pyrene	nm	3.71	4.05	2.44	2.89
Benzo(g,h,i)perylene	bdl	4.13	3.45	2.30	2.47
Benzo(k)fluoranthene	bdl	2.71	3.74	1.58	1.51
Anthracene	bdl	2.71	4.50	20.68[a]	2.20
Benzo(a)pyrene	bdl	<1.38	2.10	<1.37	<1.37
Benzo(b)fluoranthene	bdl	2.70	3.74	1.58	1.51
c4-Naphthalenes	nm	215.54	257.83	2483.27	123.33
Acenaphthylene	bdl	<1.384	<1.42	<1.37	<1.37
Acenaphthene	bdl	1.99	<1.42	35.76	3.57
c3-Naphthalenes	nm	129.86	126.74	2399.56	180.53
c4-Phenanthrenes and anthracenes	nm	68.99	105.60	166.74	44.13
c4-Chrysenes	nm	3.71	2.40	3.73	2.61
c3-Phenanthrenes and anthracenes	nm	150.25	230.99	532.53	84.01
c3-Chrysenes	nm	6.98	3.90	3.59	4.81
c3-Dibenzothiophenes	nm	65.57	96.29	281.92	30.94
c3-Fluorenes	bdl	81.97	167.09	595.43	46.74
c2-Fluorenes	nm	57.73	80.24	618.70	47.02
c2-Naphthalenes	nm	65.43	50.25	2388.05	165.82
c1-Naphthalenes	nm	43.33	22.50	957.92	105.18
c2-Phenanthrenes and anthracenes	nm	132.43	168.74	772.80	75.48
c2-Chrysenes	nm	19.24	18.30	18.09	27.77
c2-Dibenzothiophenes	nm	61.44	80.24	386.76	32.17
c1-Chrysenes	nm	12.97	12.90	17.66	18.84
c1-Dibenzothiophenes	nm	32.36	31.80	298.15	26.95
c1-Fluorenes	nm	16.53	25.05	345.68	27.50
2-Methylnaphthalene	bdl	25.80	13.95	567.86	61.05
c1-Fluoranthenes and pyrenes	nm	24.95	37.95	85.59	16.91
c1-Phenanthrenes and anthracenes	nm	66.43	72.60	650.00	54.86
1-Methylnaphthalene	nm	17.39	8.70	390.06	44.13
1-Methylphenanthrene	nm	22.95	20.10	113.74	12.23
2,6-Dimethylnaphthalene	nm	24.23	20.55	85.59	78.10
1,2,5,6-Dibenzathracene	nm	<1.38	<1.42	<1.37	<1.37
1,2-Benzathracene	nm	3.14	2.40	2.15	1.79
1,6,7-Trimethyl-naphthalene	nm	28.22	27.75	615.52	41.93

*CR-16 = reference site; bdl = below detection limit; nm = compound not measured.
[a]Above "effects range low" (Ingersoll et al., 1996).

Table 5
Percent survival of glochidia and juvenile mussels after 48-hour and 9-day exposures, respectively, to Fish Creek sediments (mean ± s.d.). Survival data with the same letters are not significantly different from other sites ($p = 0.05$)

Site	Glochidia 24-hour and 48-hour tests			
	Lasmigona costata		*Lampsilis siliquoidea*	
	24 hours	48 hours	24 hours	48 hours
CR-16*	37 ± 19[a]	4 ± 4[a]	32 ± 22[a]	11 ± 6[a]
CR-79	35 ± 4[a]	8 ± 6[a]	42 ± 12[a]	8 ± 8[a]
SL-95	20 ± 3[a]	2 ± 2[a]	47 ± 4[a]	30 ± 4[b]
QUA-95	22 ± 5[a]	5 ± 2[a]	40 ± 13[a]	35 ± 13[b]
Water only	–	–	79 ± 5[a]	69 ± 4[c]

Site	Juvenile 9-day tests	
	Villosa villosa	*Lampsilis siliquoidea*
CR-16	93 ± 8[a]	77 ± 12[a]
CR-79	98 ± 4[a]	93 ± 5[b]
SL-95	93 ± 12[a]	92 ± 12[b]
QUA-95	88 ± 20[a]	93 ± 8[b]

*Reference site.

tested sediments than are juveniles. Literature values for glochidia and juvenile mussel sensitivity to contaminants include those that demonstrate both similar and different degrees of sensitivity for these two life stages (McCann, 1993; Jacobson, 1990; Keller and Zam, 1991; Hansten et al., 1996; Keller and Ruessler, 1997). However, other factors may also be responsible for the lower glochidia survival rate. For example, unpublished studies from this laboratory have found that *Megalonaias nervosa*, *Lampsilis teres* and *V. lienosa* glochidia survive in water for only a few hours after removal from the female's marsupia. Exposure to sediments appear to decrease the survival rate of glochidia. While 83% of *Epioblasma triquetra* glochidia survived in water-only controls after 24 hours, compared to 83.8% in control sediment, after 48 hours, survival in sediment had decreased to 49%, and water-only survival was 86% (A. Keller and S.D. Ruessler, pers. comm.). This short-duration glochidia phase may be an adaptation in some species of mussels. Many lampsiline (tribe Lampsilini) mussels use lures to attract hosts to bite the marsupia which then rupture, releasing a cloud of larvae to attach to gills of the fish host (McMahon, 1991; Haag et al. 1995). If the glochidia do not attach to the fish immediately, it is unlikely that they will find their way to a fish's gills later. Examples of this type of mussel are *Lampsilis teres*, *Lampsilis straminea claibornensis*, *V. vibex*, and *V. lienosa*.

Another reason that glochidia may die more quickly once on the sediment is that they are exposed to protozoa, bacteria and other microbes that can attack them. Large numbers of protozoa have been observed consuming glochidia and juvenile mussels in toxicity tests at this laboratory. Therefore, higher 'water only' control survival may represent the survival rate of glochidia in the absence of predators and omnivores (protozoa, bacteria) that inhabit sediments rather than their survival in the absence of contaminants. More research is needed on this issue.

4.2 Mussel sensitivity compared to sediment guidelines

In contrast to the large database on marine and estuarine sediment toxicity (Long and Morgan, 1991; Long et al., 1995; MacDonald Environmental Sciences Ltd., 1994), little is known about what contaminant levels constitute a threat to freshwater organisms. Only two sets of sediment quality

Table 6
Range of values identified as "Effects Range Low" and "Lowest Effect Level" values for selected contaminants in freshwater sediments in ppb (dry weight) (Ingersoll et al., 1996, and Persaud et al., 1992)

Contaminant	Ingersoll et al. Effects range low (ppb dry)	Persaud et al. Lowest effect level (ppb dry)
Fluoranthene	31–234	na
Fluorene	10–91	na
Chrysene	27–367	na
Anthracene	10–130	na
Benzo-α-pyrene	32–220	na
Arsenic	7,400–13,100	6,000
Cadmium	340–9,100	600
Chromium	1,300–274,000	26,000
Copper	17,000–96,500	16,000
Iron	3.6×10^7–2.0×10^8	2×10^7
Nickel	4,870–40,000	16,000
Lead	13,890–99,000	31,000

na: data not provided.

guidelines for freshwater have been published (Ingersoll et al., 1996; Persaud et al., 1992). Both contain only a few of the thousands of contaminants that may be present in the environment, but neither include data on unionid mussels. Ingersoll et al. (1996) calculated guidelines from test results for *Hyallela azteca* and *Chironomus tentans*. Persaud et al. (1992) derived their guidelines from results of field surveys of species distribution versus contaminant concentrations, and laboratory toxicity tests with a number of species.

The fact that Fish Creek sediments were generally not acutely toxic to the glochidia or juvenile mussels appears to be consistent with the relatively low contaminant levels detected in Fish Creek (Table 3), and with published sediment toxicity guidelines (Table 6). Test sediments were found to contain a few metals at concentrations above the 'lowest effect level' (Persaud et al., 1992) or the 'effects range low' (Ingersoll et al., 1996). These include arsenic, chromium, copper, lead and zinc. The 'effects range low' is defined as a concentration representing the lower fifteenth percentile where biological effects were recorded (Ingersoll et al., 1996), while the similar 'lowest effect level' is defined as the concentration at which biotoxic effects become apparent (Persaud et al., 1992). Sediments used in toxicity tests (2 years post-spill) did not contain quantifiable levels of any measured organic contaminant (PAHs, chlorinated pesticides, PCBs, phenols, base-neutral extractables), but minimum detection limits were fairly high (380–2,670 ppb). Concentrations of organic contaminants in sediments collected a year earlier (1 year post-spill) were also below the 'lowest effect level' at all sites (detection limit = 1 ppb), except for fluorene at QUA-95a and QUA-95b and anthracene at QUA95a (Table 4).

Three of our sample sites, CR-16, CR-79 and SL-95 coincide with locations sampled by the Ohio Environmental Protection Agency to assess the health of the fish and macroinvertebrate communities of Fish Creek annually using the Index of Biotic Integrity (IBI) (Karr, 1981; Fausch et al., 1984), and an Invertebrate Community Index (ICI) (Ohio EPA, 1987). Their corresponding sampling sites are designated RM 8.3, RM 6.5 and RM 5.4, respectively. Sites RM 5.4 and RM 6.5 are closest to and downstream of the origin of the diesel fuel spill. IBI scores calculated for RM 5.4 and RM 6.5 for 1994, 41 vs 52 and 43 vs 46, respectively, were lower than those calculated for the same locations during the pre-oil spill period of 1991–1993. Although improvements were seen in 1995, the fish community still had not totally recovered from the impacts of the diesel oil spill (Ohio EPA, 1994, 1995). Macroinvertebrate communities at all sites showed some recovery and ranked good to exceptional in 1994. The 1995 ICI for RM 5.4 returned to its pre-spill value of 50 (Ohio EPA, 1996) indicating that the macroinvertebrate community had recovered from the effects of the oil spill (Ohio EPA, 1996). This difference in recovery time for fish and macroinvertebrate communities following exposure to petroleum contamination has been noted in other streams (Masnik et al., 1976; Guiney et al., 1987; Ryck and Duchrow, 1974), and is related to the greater mobility and shorter turn-over time of aquatic insects compared to fish. The recovery of unionid mussel communities after perturbations may take even longer than fish since they are less mobile and must rely on fish hosts for reproduction.

5. Conclusions

In general, Fish Creek sediments collected two years after the diesel oil spill (September 1993) were not toxic to juvenile unionid mussels in 9-day toxicity tests. The only significant toxicity was measured for *Lampsilis siliquoidea* juveniles in CR-16 sediment, the designated reference site. Low survival rates were recorded for glochidia of both *Lasmigona costata* and *Lampsilis siliquoidea* in all sediments. However, these low survival rates were statistically significant only for *Lampsilis siliquoidea* in sediments from the reference site, CR-16, and the first site downstream of the oil spill, CR-79, after 48-hour exposures. The fact that test results were inconclusive for glochidia and demonstrated juvenile toxicity at only one site suggests that further evaluation is needed before the potential threat to the unionid fauna of this important creek can be fully characterized.

Acknowledgments

The authors wish to thank: Tom Watters and Mark Hove for providing mussels for this study; Celeste South (USFWS-Daphne, AL) for reviewing the manuscript; and Dave Altfater (Ohio EPA) for permitting us to use his map of Fish Creek.

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