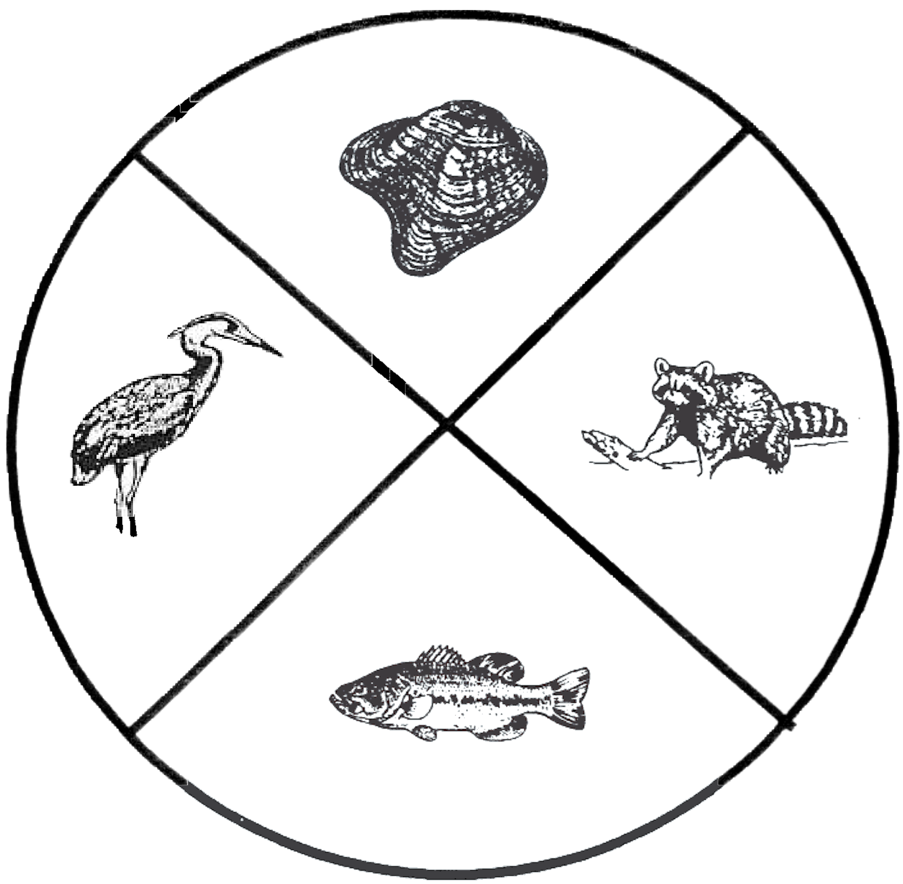
3F

Biological Report
May 1997

Bloomington Field Office
Bloomington, Indiana

FISH CREEK FEDERALLY ENDANGERED FRESHWATER MUSSEL IMPACT ASSESSMENT



U.S. Fish and Wildlife Service
U.S. Department of the Interior

INDIANA - FISH CREEK FEDERALLY ENDANGERED MUSSEL IMPACT ASSESSMENT
(FFS #3F20)

PRINCIPAL INVESTIGATOR

Cindy M. Chaffee

BLOOMINGTON, INDIANA FIELD OFFICE
U.S. FISH AND WILDLIFE SERVICE
620 SOUTH WALKER STREET
BLOOMINGTON, INDIANA 47403

ACKNOWLEDGMENTS

The Bloomington, Indiana Field Office appreciates the funding initially provided by the U.S. Fish and Wildlife Service (FWS) Division of Environmental Contaminants Off-Refuge Contaminants Investigation funding (FFS #3F20) to conduct this study. The cost of this study was later reimbursed through a negotiated settlement with ARCO Pipe Line Company and NORCO Pipeline, Inc. as a result of a Natural Resources Damage (NRD) action. The NRD actions were undertaken by the natural resource trustees under provisions of the Oil Pollution Act, following a September 1993 spill of more than 30,000 gallons of diesel fuel from a ruptured underground pipeline in DeKalb County, Indiana. The reimbursement for this study was submitted to the Natural Resource Damage Assessment Revolving Fund to support future FWS NRD activities. In addition to the contributors that Dr. Keller names in her report, Aaron Spicer and Scott Sobiech provided essential assistance in field collections and technical expertise in project development and document review, respectively.

PREFACE

The following is a report entitled "Testing the Recovery of Sediments in Fish Creek, Indiana and Ohio, USA, Following a Diesel Fuel Spill Using Glochidia and Juvenile Mussels (Bivalvia, Unionidae)" authored by Dr. Anne Keller and D. Shane Ruessler of the National Biological Service, Southeastern Biological Science Center in Gainseville, Florida. The Bloomington, Indiana Field Office (BFO) entered into an inter-agency cooperative agreement with the National Biological Service (NBS) in Gainesville, Florida (FWS agreement #14-48-0003-95-1041) to assess the toxicity of Fish Creek sediments to early life stages of freshwater mussels that reside in Fish Creek. The toxicological information provided in the NBS final report fulfills the intent of this study to determine the acute toxicity of No. 2 diesel fuel contaminated sediments to unionid mussels in laboratory exposures.

Dr. Keller's report addresses the management objectives of this study by providing much needed and useful unionid toxicity information, continuing towards standardizing toxicity testing for unionids, and supporting management decisions for the Natural Resource Damage Restoration and mussel recovery activities in Fish Creek. Dr. Keller's paper will be published in a 1997 edition of the *Aquatic Ecosystem Health and Restoration* scientific journal.

We have the following comments and clarifications to add to Dr. Keller's report:

In the abstract and throughout the document, Dr. Keller refers to the quantifiable but not detectable organic components in the sediment, however, the contract laboratory that conducted the analyses had relatively high detection limits (535 ppb) when compared to FWS contract laboratories (1 ppb).

On page 2 of the report, it is stated that *Epioblasma obliquata perobliqua* is limited to a single population in Fish Creek, Indiana. The *E.o. perobliqua* is found exclusively in the Ohio waters of Fish Creek.

Dr. Keller discusses the death of *Elliptio dilatata* within month after the spill on pages 2 and 3 of her report. *E. dilatata* was the most common mussel species found dead immediately following the spill, perhaps because it is very prevalent in Fish Creek. However, there were several other mussel species that suffered acute lethality, as well. On page 3, she also states that; "Initial impacts may have been minimized because the spill occurred during leaf fall which provided a large adsorptive capacity for the oil...". Our opinion is that impacts to endangered mussels were elevated due to the oil soaked detritus in Fish Creek because freshwater mussels utilize detritus and plankton as a food source. As Dr. Keller stated, mussels are sedentary benthic animals, and therefore, mussels in Fish Creek may continue to be exposed to sediment-sorbed hydrocarbons through their general environment as well as their food sources.

On pages 11 and 12, Dr. Keller states that the Ohio Environmental Protection Agency's (OEPA) invertebrate community index (ICI) scores for River Mile 5.4 returned to its pre-spill value of 50 in 1995. However, OEPA's September 30, 1996 report (page 10) states that even though this site achieved its pre-spill ICI score, it continued to show greatly reduced numbers of caddisflies (a pollution sensitive taxa) as a percent of the total sample, when compared to prespill numbers.

In the first sentence of Dr. Keller's conclusions, she states that, "Fish Creek sediments collected 2 years after the diesel oil spill were not toxic to juvenile unionid mussels or amphipods in 9-day toxicity tests". We commented on Dr. Keller's draft report that we believe this to be an overstatement. During our review and comment of the draft document, we requested that she refrain from generalizing the toxicity to entire faunas and define the toxicity results relative to the specific species utilized and that she clarify that this conclusion was made from laboratory results.

Dr. Keller discusses the problems of predation on juvenile mussels in sediment. Dr. Neves at Virginia Technical Institute (personal communication) also recognizes this as a concern. On page 12, Dr. Keller states that because the only significant toxicity was measured at CR-16 (the reference site), that this may indicate a toxic effect, however, she also states that further testing is necessary to corroborate this conclusion. Since the organic content was so high at CR-16 (2.13-2.25% compared to 0.25 - 1.31% at the other sites) it is possible that predation from bacteria and protozoa would be higher at this site, as well. The low survival rate at this site could potentially be due to predation, not chemical toxicity. Table 4 indicates that CR-16 is by far the cleanest site when compared to the organic contaminants of the other sites.

TESTING THE RECOVERY OF SEDIMENTS IN FISH CREEK, INDIANA AND OHIO, FOLLOWING A DIESEL FUEL SPILL USING GLOCHIDIA AND JUVENILE MUSSELS (BIVALVIA, UNIONIDAE)

FINAL REPORT TO:

U.S. FISH AND WILDLIFE SERVICE
BLOOMINGTON FIELD OFFICE
620 S. WALKER
BLOOMINGTON, IN 47403

BY:

ANNE E. KELLER, Ph.D.
U.S. GEOLOGICAL SURVEY
7920 N.W. 71st STREET
GAINESVILLE, FL 32653

MAY 19, 1997

(This Final Report was originally submitted August 1, 1996. This is the revised and final version that was submitted to *Aquatic Ecosystem Health and Restoration*, but which has not been accepted yet.)

Testing the Recovery of Sediments in Fish Creek, Indiana and Ohio, USA, Following a Diesel Fuel Spill Using Glochidia and Juvenile Mussels (Bivalvia, Unionidae)

Anne E. Keller*[1], D. Shane Ruessler[1] and Cindy Chaffee[2]

[1]U.S. Geological Survey, Biological Resources Division,
Florida Carribean Science Center
7920 NW 71 St., Gainesville, FL, USA 32653

[2]U.S. Fish and Wildlife Service, Bloomington Field Office
620 S. Walker
Bloomington, IN, USA 47403

Key words: toxicity testing, diesel, *Hyalella azteca*, unionids

Summary. 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 radiata luteola* mussels, and the amphipod *Hyalella azteca* after 9-day exposures. Fish Creek sediments may be toxic to *L. r. luteola* glochidia.

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 *Epioblasma obliquata perobliqua*, a mussel that was once distributed throughout the state of Indiana, USA, and in portions of Ohio, but is now limited to a single population in Fish Creek, Indiana (Cummings & Mayer, 1992; Watters 1993). This fourth order stream, a tributary of the St. Joseph River traversing northeastern Indiana and northwestern Ohio (Figure 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, 1994; Indiana Department 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 and 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 L of No. 2 diesel fuel were spilled into Fish Creek (Figure 1) 2.09 km west of the Ohio state line. The states of Indiana and Ohio, and the U.S. Fish and Wildlife Service 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 cms) (Stewart *et al.*, 1993), particularly the endangered unionid mussels. The total distribution of *E. 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 & 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 & 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. Prior to initiating any mussel restoration activities in Fish Creek, natural resource trustees needed to know the potential impact of diesel fuel remaining in the sediment.

Because toxicity tests generally require the use of over a hundred animals, the early life stages were selected for use in sediment tests. Hundreds of thousands of glochidia can be harvested from one adult for use in toxicity tests directly, and to produce juvenile mussels in the laboratory. This avoids the use of a large number of adults which are declining in number. 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). There are no standard USEPA or ASTM toxicity test methods for unionid mussels largely 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, existing 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 & Zam, 1991; McKinney and Wade, 1996; USEPA & Department of the Army, 1994). 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.

2. Methods

2. *Sediment sampling*

Two sediment samples were collected from each of four sites in Fish Creek, Indiana and Ohio in August 1995 (23 months after the diesel fuel spill), using a stainless steel scoop. The sites were located as follows: CR-16, the reference site, .2 km upstream of the oil spill; CR-79, .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 (Figure 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 PAHs, pesticide and metal contaminants, grain size, per cent dry weight, and per cent organic content. Metals, except mercury, were analyzed using EPA method 6010 (USEPA, 1986). Mercury was analyzed by EPA method 7471

(USEPA, 1986). Determination of organic contaminants was performed as prescribed in EPA method 8270 (USEPA, 1986). The Walkley-Black method, a dichromate oxidation and titration method, was used to determine organic content (Allison, 1965). Per cent moisture was determined by ASTM method D2216 (ASTM, 1994). Sediment size fractionation was performed by wet sieving the sediment through U.S. Standard screen (500 um, 250 um, 125 um, 68 um), drying to a constant weight at 100°C, and weighing the individual fractions.

2.3 *Mussel toxicity tests*

For this study, 5 L aquaria were used as test chambers. Test animals were contained in screen-bottomed glass exposure chambers. Sediment added to a depth of 3 cm was overlain with 5 cm of well water (hardness ~250 mg as $CaCO_3$) with characteristics similar to those of Fish Creek water (Table 1) (Stewart *et al.*, 1993). Exposure chambers consisted of glass cylinders 5 cm in diameter and 7.5 cm in height closed on one end with 100 um 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 (Table 1).

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, *Lampsilis radiata luteola* and *Lasmigona costata* mussels 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 *L. 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 $\geq$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). To accomplish this transformation, drops of *Villosa villosa* (adults collected in Suwannee River, FL at 29°36' N, 82°40'30" W) and *L. r. luteola* glochidia in well water 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. *V. villosa* was substituted for *L. costata* after attempts to culture juvenile *L. costata* failed twice and sediment samples were reaching their storage time limit. 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 3.0×10^7 cells/mL, 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 & Zam, 1991

2.4 *Amphipod toxicity tests*

Amphipod (*H. azteca*) tests were also performed with Fish Creek sediments because amphipods are more widely used toxicity test organisms than mussels, and their sensitivities to contaminants are better described. The amphipods were collected from a local pond, identified to species, and cultured in the laboratory in a flow-through well water system using flaked fish food and oak leaves as the food source. Test procedures were modified from ASTM method E1383 (ASTM, 1993) in the following ways: *H. azteca* were randomly distributed five (rather than 10-20) to each of three replicates per sediment test chamber; they were not fed, but had access to organic matter and bacteria attached to the sediment through the mesh at the bottom of the glass exposure chamber; and amphipod survival was recorded daily, but size was not measured at the end of the test since survival was the only endpoint for the glochidia and juvenile mussels.

2.5 *Statistical analyses*

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

3. Results

Chemical characterization of sediments

Silt fractions (<68 um) constituted from 5% (QUA-95) to 50% (CR-16) of the Fish Creek sediments used in toxicity tests (Table 2), while TOC values ranged from 0.25% (QUA-95) to 2.25% (CR-16) in dried samples (Table 3). Metal contaminants were present in low concentrations (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 ppb (Table 4).

3.2 *Mussel tests*

Following a 24-hour exposure to Fish Creek sediments, *L. r. luteola* 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, *L. r. luteola* 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 rate at 24 hours and a 69% survival rate at 48 hours, significantly higher than all sediment sites.

L. 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 *L. r. luteola*. ANOVA and Duncan's test indicated that there were no significant differences in the survival of *L. 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 accidently destroyed.

There were no significant differences in survival of juvenile *V. villosa* or *L. r. luteola* for sediments collected at the four sample sites in Fish Creek, with one exception, CR-16 (Table 5). While both juvenile *V. villosa* and *L. r. luteola* 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 *L. r. luteola*, a significant difference from other sites (p=0.05, F=3.36, df=3).

3.3 *Amphipod tests*

One hundred per cent of the adult *H. azteca* survived the nine day test.

4. Discussion

4. *Glochidia and juveniles as sediment toxicity test organisms*

Fish Creek sediments may be acutely toxic to *Lampsilis radiata luteola* glochidia, but generally not to glochidia of *Lasmigona costata* glochidia, juvenile *Villosa villosa* or *L. r. luteola* mussels, or adult Hyalella azteca. Results for tests with the latter three organisms are in accord with relatively low contaminant levels detected in test sediments. 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 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 & Zam, 1991; Keller & Ruessler, 1997; Hansten *et al.*, 1996). 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 *Villosa 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. Eighty-three per cent of *Epioblasma triquetra* glochidia survived in water-only controls after 24 hours, compared to 83.8% in control sediment (A. Keller and S.D. Ruessler, pers. comm.). After 48 hours, survival in sediment had decreased to 49%, while water-only survival was 86%. 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*, *L. straminea claibornensis*, *Villosa 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 & 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 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, and neither include data on unionid mussels. Ingersoll *et al.* (1996) calculated guidelines from test results for *H. 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, juvenile mussels and amphipods 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 yr. 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 yr. post-spill) were also below the "lowest effect level" at all sites (detection limit=1 ppb), except for fluorene at QUA-95 (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 & 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 to 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 or amphipods in 9-day toxicity tests. The only significant toxicity was measured for *L. r. luteola* juveniles in CR-16 sediment, the designated reference site. Low survival rates were recorded for glochidia of both *L. costata* and *L. r. luteola* in all sediments, however, these low survival rates were statistically significant only for *L. r. luteola* 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.

REFERENCES

Allison, L.E., 1965. Organic carbon. In: C.A. Black (ed.), Methods of Soil Analysis, Part 2. American Society of Agronomy, Madison, WI. pp. 1372- 1376.

ASTM, 1994. Annual Book of ASTM Standards. Volume 04.08. American Society for Testing and Materials, Philadelphia, PA. pp. 177-180.

ASTM, 1993. Annual Book of ASTM Standards. Section 11. American Society of Testing and Materials, Philadelphia, PA. pp. 1173-1199.

Balfour, D.L. & L.A. Smock, 1995. Distribution, age structure, and movements of the freshwater mussel *Elliptio complanata* (Mollusca: Unionidae) in a headwater stream. J. Fresh. Ecol., 10:255-268.

Blumer, M. & J. Sass, 1972. Oil pollution: persistence and degradation of spilled fuel oil. Science, 176:1129-112.

Coker, R.E., A.F. Shira, H.W. Clark & A.D. Howard, 1921. Natural history and propagation of fresh-water mussels. Bull Bur. Fish., 37:76-181.

Cummings, K.S. & C.A. Mayer, 1992. Field Guide to Freshwater Mussels of the Midwest. Illinois Natural History Survey Manual 5, Champaign, IL. 189 pp.

Fausch, D.O., J.R. Karr & P.R. Yant, 1984. Regional application of an index of biotic integrity based on stream fish communities. Trans. Am. Fish. Soc., 113:39-55.

Guiney, P. D., J. L. Sykora & G. Keleti, 1987. Environmental impact of an aviation kerosene spill on stream water quality in Cambria County, Pennsylvania. Environ. Toxicol. Chem., 6:977-988.

Haag, W.R., R.S. Butler & P.D. Hartfield, 1995. An extraordinary reproductive strategy in freshwater bivalves: prey mimicry to facilitate larval dispersal. Freshwater Biol., 34:471-476.

Hansten, C., M. Heino & K. Pynnonen, 1996. Viability of glochidia of *Anodonta anatina* (Unionidae) exposed to selected metals and chelating agents. Aquatic Toxicology, 34:1-12.

Indiana Department of Natural Resources, 1990. Indiana's rare plants and animals. A checklist of endangered and threatened species. Division of Nature Preserves. Indianapolis, IN.

Ingersoll, C.G., P.S. Haverland, E.L. Brunson, T.J. Canfield, F.J. Dwyer, C.E. Henke, N.E. Kemble, D.R. Mount & R.G. Fox, 1996. Calculation and evaluation of sediment effect concentrations for the amphipod *Hyalella azteca* and the midge *Chironomus riparius*. J. Great Lakes Research, 22:602-623.

Jacobson, P.J., 1990. Sensitivity of early life stages of freshwater mussels (Bivalvia: Unionidae) to copper. Master's Thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA. 162 pp.

Jones, R.O., 1950. Propagation of fresh-water mussels. Prog. Fish Cult. 1:13-25.

Karr, J.R., 1981. Assessment of biotic integrity using fish communities. *Fisheries* 6:21-27.

Keller, A.E. & S.G. Zam, 1991. The toxicity of selected metals to the freshwater mussel Anodonta imbecilis. Environ. Toxicol. Chem., 10:539-546.

Keller, A.E. & D.S. Ruessler, 1997. The toxicity of malathion to unionid mussels: Relationship to expected environmental concentrations. Environ. Toxicol. Chem., 16: (in press).

Lewis, P.A., D.J. Klemm, J. M. Lazorchak, T.J. Norberg-King, W.H. Peltier & M.A. Heber, 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Third edition. Environmental Monitoring Systems Laboratory--Cincinnati, U.S. Environ. Protect. Agency, EPA/600/4-91/002 OH.

Long, E.R., D.D. MacDonald, S.L. Smith & F.D. Calder, 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ. Manag., 19:81-97.

Long, E.R. & L.G. Morgan, 1991. The potential for biological effects of sediment-sorbed contaminants tested in the National Status and Trends Program. NOAA Technical Memorandum NOS OMA 52. National Oceanic and Atmospheric Administration. Seattle, WA. 175 pp.

MacDonald Environmental Sciences Ltd., 1994. Approach to the assessment of sediment quality in Florida coastal waters. Vol. I-IV. Ladysmith, BC.

Masnik, M.T., J.R. Stauffer, C.H. Hocutt & J.H. Wilson, 1976. The effects of an oil spill on the macro invertebrates and fish in a small southwestern Virginia creek. J. Environ. Sci. Health, 4-5:281-296.

Master, L., 1990. The imperiled status of North American aquatic animals. Biodiversity Network News, 3:1-2,7-8.

McCann, M. T., 1993. Toxicity of zinc, copper, and sediments to early life stages of freshwater mussels in the Powell River, Virginia. Master's Thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA.

McKim, J.M., 1985. Early life stage toxicity tests. In: G.M. Rand and S.R. Petrocelli (eds.), Fundamentals of Aquatic Toxicology, Hemisphere Publishing Corp., New York. p. 59.

McMahon, R.F., 1991. Mollusca: Bivalvia. In: J.H. Thorp and A.P. Covich (ed.). Ecology and Classification of North American Freshwater Invertebrates. Academic Press, Inc., New York. pp. 315-399.

McKinney, A.D. & D.C. Wade, 1996. Comparative response of *Ceriodaphnia dubia* and juvenile *Anodonta imbecillis* to pulp and paper mill effluents discharged to the Tennessee River and its tributaries. Environ. Toxicol. Chem. 15:514-517.

Ohio EPA, 1987. Biological criteria for the protection of aquatic life: Volume II: users manual for biological field assessment of Ohio surface waters. Columbus, OH.

Ohio EPA, 1994. Summary of fish community assessment results for Fish Creek: 1991-1993. Ohio Environmental Protection Agency, Columbus, OH. 18 pp.

Ohio EPA, 1995. Fish and macroinvertebrate study of Fish Creek 1994. Ohio Environmental Protection Agency, Columbus. OH. 37 pp.

Ohio EPA, 1996. Addendum to fish and macroinvertebrate study of Fish Creek 1994. Ohio Environmental Protection Agency, Columbus, OH. 39 pp.

Persaud, D., R. Jaagumagi & A. Hayton, 1992. Guidelines for the protection and management of aquatic sediment quality in Ontario. Water Resources Branch, Ontario Ministry of the Environment. 26 pp.

Ryck, F.M. & R.M. Duchrow, 1974. Oil pollution and the aquatic environment in Missouri, 1960-1972. Trans. Mo. Acad. Sci., 7-8:164-171.

SAS Institute, Inc., 1982. SAS User's Guide: Statistics, 1982 Edition. SAS Institute Inc., Cary, NC. 584 pp.

Stewart, P.M., R. Duffey & T.O. Swinford, 1993. Water quality of Fish Creek in reference to the endangered white cat's paw pearly mussel. Final Report to the Indiana Department of Natural Resources, Nongame and Endangered Wildlife Program, Indianapolis, IN.

USEPA, 1986. Test methods for evaluating solid waste. November 1986 SW846. 3rd Edition, Volume 1A.

USEPA & Department of the Army, 1994. Evaluation of dredged material proposed for discharge in waters of the U.S.--Testing Manual (Draft). EPA-823-B-94-002.

U.S. Fish and Wildlife Service, 1990. White cat's paw pearly mussel--Recovery plan. USFWS, Region 3, Twin Cities, MN.

U.S. Fish and Wildlife Service, 1994a. Endangered and threatened wildlife and plants. Fed. Register, 59:58982-59028.

U.S. Fish and Wildlife Service, 1994b. Clubshell and northern riffleshell--Recovery plan. USFWS, Region 5, Hadley, MA.

Watters, G.T., 1993. A guide to the freshwater mussels of Ohio. Ohio Department of Natural Resources, Columbus, OH.

Williams, J.D., M.L. Warren, Jr., K.S. Cummings, J.L. Harris & R.J. Neves, 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries, 18:6-22.