Final Report

Natural Resource Injury to Intermittent Streams Impacted by Oil and/or Brine Spills

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Executive Summary

A sediment quality triad consisting of (1) quantification of sediment polycyclic aromatic hydrocarbons (PAHs), total petroleum hydrocarbons (TPHs), and chloride concentrations; (2) a sediment bioassay using the amphipod *Hyalella azteca*; and (3) an *in situ* study of macroinvertebrate community structure were used to assess impacts of oil and/or brine spills on intermittent streams in southern Illinois. Thirty intermittent streams and three reference streams were selected for study based on oil and/or brine spill history since 1991. Sampling sites within study streams included one location above and three locations below the reported spill site.

The sum of PAH concentrations exceeded the lower sediment quality guidelines (SQG) value for total PAHs (1.61 μg/g) in four of 130 sediment samples. All four samples exceeding the total PAH SQG were collected from locations within 40 m of where the spill entered the stream. However, there was no significant differences ($p = 0.13$) in mean sum of PAH concentrations among study and reference streams, and the reported volume of oil spilled into streams was not correlated with the sum of PAH concentrations measured in stream sediments. There was, however, a general trend toward greater PAH concentrations in sediment collected from streams where spills had occurred during the three years prior to sample collection ($\bar{x}$ = 1.01 μg/g) compared to concentrations measured in sediment collected from streams where spills had occurred during nine to twelve years prior to sample collection ($\bar{x}$ = 0.71 μg/g). No TPH concentrations were greater than the Canadian Ministry of Environment soil clean up standard of 1000 μg/g, and TPH concentrations were not significantly different between study and reference streams.

Chloride concentrations in sediment from study streams were significantly greater than concentrations in sediment from reference streams ($p < 0.028$); however, chloride concentrations only exceeded the U.S. Environmental Protection Agency (U.S. EPA) recommended water quality criterion for chloride (230 mg/L) in two streams. Chloride concentrations in sediment were not significantly correlated with the reported volume of brine spilled into streams.

The standardized sediment toxicity test component of the triad did suggest that sediment from several streams associated with oil and/or brine spills had adverse effects on *H. azteca.* However, there was no consistent indication of a corresponding association between the

observed effects and the contaminant concentrations measured in the sediment. Therefore, it was not possible to link the toxicity observed in *H. azteca* with the oil and/or brine spill.

There were no measured differences in macroinvertebrate communities among sampling locations during the *in situ* macroinvertebrate community structure component of the sediment quality triad. There were no significant correlations between contaminant concentrations, water chemistry, or physical habitat and the scores of any of the following: taxa richness; Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness; or family-level biotic index (FBI). Habitat scores, biotic indexes, and water quality evaluations of studied intermittent streams suggest a degraded environment, and the correlation between habitat scores and invertebrate FBI suggested that habitat quality was the primary factor influencing invertebrate communities. Degraded habitat quality may in part be due to agricultural activities, the nature of intermittent streams, and/or the impacts of the oil industry.

In the current study, it was not possible to separate the influences of oil and/or brine from other influences. Evaluations of some of the individual study streams did provide an indication that these streams had been exposed to oil and/or brine. However, there was little evidence to link oil and/or brine spills to effects observed in the streams studied.

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INTRODUCTION

Illinois has a rich history of oil production beginning in the early 1900's. There are over 400 active and abandoned oil fields currently in the state, encompassing approximately 42 counties in southern Illinois (Young et al. 1997). Illinois ranks eighth in the United States in the volume of resources remaining in known oil fields. There are approximately 6 billion barrels of oil remaining in Illinois, of which 1.45 billion barrels are mobile oil at a depth of less than 3,000 feet and can be recovered by conventional methods. An additional 4.4 billion barrels of oil are immobile and require advanced methodologies for removal (Oltz 1994). The majority of the remaining oil is located in two of the more than 50 producing intervals in the Illinois Basin (Figure 1). This basin has produced over a billion barrels of oil and currently support 358 oil fields (Oltz 1994).

Oil production in Illinois peaked in 1940 at 147.6 million barrels per year and dwindled to 20 million barrels in 1993. During 2002, Illinois produced 33,000 barrels/day or approximately 12 million barrels per year (Petroleum Profiles 2004). It is estimated that oil production contributes approximately $1 billion to the state's economy each year (Oltz 1994). The economic value of oil, as well as the importance placed on energy production by the United States, ensures continued oil production and will likely result in increased exploration for immobile oil.

Although economically valuable, the production of oil can be ecologically damaging when its products are released into the environment. Crude oil and produced brine, saline ground water that is extracted simultaneously with crude oil, are absorbed into the soil and sediment and are responsible for decreases in density and diversity of plant and animal communities (Shales et al. 1993). The high ion content of brine disrupts osmoregulation and may result in death of plants and animals. Crude oil is primarily composed of saturated hydrocarbons (57.2%) but may also include aromatic hydrocarbons, cycloalkanoaromatics, and aphaltenes (Gill and Robotham 1989). Some crude oils may contain as much as 28% aromatic hydrocarbons, with polycyclic aromatic hydrocarbons (PAH) posing the most significant environmental risk (Tissot and Welte 1984). Both brine and crude oil have the ability to alter community structure.

In Illinois, most oil wells occur in agricultural fields and spills may enter surface water either by emptying directly into streams or by transport via rain water runoff. Upon entering

surface water, some oil fractions and/or brine will bind to organic matter in the sediment. Benthic organisms are potentially exposed to oil and/or brine and are thus a good indicator of sediment contamination (Chapman 1986).

Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons are formed, along with other hydrocarbons, as a result of millions of years of heat and pressure acting on deposited organic matter. PAHs are composed of multiple aromatic rings that may have substituted groups attached to the rings. Various congeners of the basic structure occur and have different physical and chemical properties. PAHs generally have a low solubility in water and the solubility is inversely related to the compound's number of carbons (Gill and Robotham 1989). Therefore, larger compounds are more hydrophobic and more likely to be sorbed onto the sediment. In addition, melting and boiling points increase, and vapor pressure decrease with increasing molecular volume (Albers 1995).

Crude oil release comprises a significant portion of hydrocarbon release in southern Illinois. Spills may come from several sources including accidental spills, major accidental resurfacing of oil and brine during secondary and tertiary recovery options, and drilling fluids temporarily stored in surface impoundments (Young 1997).

In general, when crude oil is released into the aquatic environment, small saturated hydrocarbons will volatilize while larger aromatic compounds such as PAHs will settle to the bottom and sorb onto the sediment. PAHs are hydrophobic and resistant to chemical or biological degradation and therefore may accumulate in the sediment and adversely affect benthic macroinvertebrates (Verrhiest et al 2001). PAHs are carcinogenic, mutagenic, and toxic, and 16 PAHs are listed by the U.S. Environmental Protection Agency (U.S. EPA) as priority pollutants (Keith and Telliard 1979). The mode of action of PAHs is interference with cellular membrane function and binding to cellular proteins and DNA (Albers 1995). Four-, five- and six-ring PAHs are the most toxic and the addition of alkyl groups to base PAHs increases their carcinogenicity (Neff 1985). The toxicity of PAHs increases to a molecular weight of 202 (e.g., fluoranthene, pyrene) and decreases at greater molecular weights (Rand et al 1995). Albers (1995) reported that acute and chronic effects of PAHs on macroinvertebrates include reduced survival, altered physiological function, altered cellular structure and function,

inhibited reproduction, altered behavior, and changes in populations and community composition. Invertebrate egg and larval stages are more sensitive than juvenile or adult stages to PAHs (Albers 1995).

Brine

Oil-field brine is a saline groundwater closely associated with oil producing geological formations. While, brine content differs depending on location, the major ions are sulfate (SO_4^-), bicarbonate (HCO_3^-), chloride (Cl^-), sodium (Na^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) (Sours et al 1985). Because brine and oil are very closely associated, brine is pumped to the surface with oil. At the surface, oil and brine are separated by gravity in large tanks known as gun-barrel separators (Sours et al 1985). Oil is then stored until it is transported to a refinery, while injection wells are usually used to pump the brine into the geological formation from which it was originally extracted. Injection wells reduce environmental contamination and facilitate future oil production by increasing the pressure in the oil reservoir. Brine enters the environment through various means including active and abandoned storage pits, well sites and storage pits which are not properly plugged, leaking transport pipes and tank batteries, accidental and intentional spills, and improperly managed brine disposal wells (GERPDC 1982).

Today, most brine is injected, but this was not always the case. When oil production began in the early 1900's, brine was usually disposed through release into neighboring streams or rivers. Crop and plant damage was noted in areas where brine had been released into aquatic systems and therefore, storage in evaporation pits soon became the disposal method of choice. However, evaporation pits resulted in increased salt concentrations and brine frequently spilled into surface water or migrated through the soil layers to reach the ground water (Gorman 1999). Currently, brine may only be disposed by being pumped into injection wells or moved off site for treatment and burial in landfills (Sours et al. 1985).

Brine released into the environment can affect local wildlife populations either through direct contact or physical habitat alterations. Due to the high ion content, exposure to brine reduces an organism's ability to osmoregulate and may result in individual death and severely compromise species density and diversity in affected areas. The addition of brine into freshwater increases the salinity of the water and creates a hyperosmostic environment for aquatic organisms. On the cellular level, water diffuses through the cell membrane toward the

more saline water and results in cell death. Brine spills also affect the physical environment by increasing total dissolved solids (TDS) and chloride concentrations in surface waters (Thamke and Craigg 1997). Studies have demonstrated that brine spills can reduce vegetation within 500 feet of the spill (GERPDC 1982). A loss of vegetation results in increased erosion, thus affecting soil and water quality.

Sediment and Sediment Toxicity

Sediment is a semi-solid media that usually lies below a body of water and may have detrital, inorganic, and organic components. Due to heterogeneity in their composition, sediments usually vary in their physical, chemical and biological characteristics (Ingersoll 1995). Power and Chapman (1992) divided sediment into four compartments. The first compartment, interstitial water, occupies the largest volume. Interstitial water is the water between the sediment particles and usually accounts for over 50%, by volume, of surface sediments. The second compartment, the inorganic phase, includes mineral grains and rocks that enter the sediment through the erosion of terrestrial materials. The third compartment, the organic phase, is relatively small, but plays an important role in sorption and bioavailability of many contaminants. The final compartment includes all anthropogenically derived materials.

Sediment particles are a mixture of many materials derived from eroded rocks and soils, waste particles, atmospheric fall-out, and inorganic materials produced biologically. Sediment particles are categorized into two groups based on size: coarse (sand and coarse material greater than 62 μm in diameter) and fine (silts and clays less than 62 μm). Coarse particles are generally non-cohesive and are not generally associated with chemical contamination. However, fine particles have a relatively greater surface to volume ratio and are more likely to be associated with a contaminant due to surface electric charges. In addition to grain size, factors such as water movement, pH, organic carbon load, sediment movement, pore water residence time, and water chemistry may affect the sorption of contaminants to sediment (Chapman et al. 1992).

Sediments have been shown to be both sinks and sources of chemical contaminants (Larsson 1985; Schuyetma et al. 1988). Chemicals such as heavy metals, pesticides, industrial chemicals, and hydrocarbons such as PAHs and PCBs accumulate in the sediment and tend not to degrade over time (Lyman et al. 1987). However, bulk sediment concentrations of these chemicals are not correlated with bioavailability (Burton 1991). Therefore, sediment chemistry

alone cannot accurately describe the toxicity of sediments. Chapman (1986) proposed the sediment quality triad to more accurately depict sediment toxicity. In this procedure, sediment bioassays and *in situ* studies are performed, in addition to the bulk sediment chemistry, to produce toxicological data that are derived directly from the sediment. Sediment quality triad data account for interactions among multiple stressors and differences in physical and chemical properties of the sediment.

Sediment contamination may be acutely toxic to benthic organisms or may have chronic effects on growth and reproduction. Changes in growth and reproduction may, in turn, affect community structure and result in escalating changes throughout the ecosystem by altering the food web or biomagnification of contaminants (Burton and MacPherson 1995). Sibley et al. (2001) used a mesocosm study to demonstrate food web alterations in the presence of PAHs. They demonstrated that phytoplankton communities increased in the presence of creosote, a mixture of PAHs used as a wood preservative. They reported this response was due to a decrease in predatory zooplankton that resulted in increased phytoplankton abundance. The increased phytoplankton then shade out aquatic macrophytes and further reduce predatory zooplankton populations associated with them.

Test Organism

The relevance, success, and interpretation of a sediment toxicity test is influenced by the choice of test organisms. An ideal test organism should have a toxicological database demonstrating sensitivity to a range of chemicals of concern as well as a database for interlaboratory comparisons, be in contact with sediment, be readily available, be easily maintained in the laboratory, be easily identified, be ecologically or economically important, have a broad geographical distribution, be tolerant of a broad range of sediment physico-chemical characteristics, and be compatible with selected exposure methods and endpoints (U.S. EPA 2000). The U.S. EPA constructed a database comparing these criteria for several species and three species were chosen as adequate test species, *Hyalella azteca, Chironomus tentans* and *Lumbriculus variegatus*. *Hyalella azteca* was selected as the test organism for the current study.

Hyalella azteca (Amphipoda:Hyalellidae) is a small, free-living benthic organism with holarctic distribution. *H. azteca* is found in most lotic and lentic freshwater systems and occurs

in great abundance in habitats where refugia such as aquatic macrophytes are present (Covich and Thorp 2001). Reproduction in *H. azteca* starts with amplexus, in which a male grasps the female with its gnathopods while on the back of the female (Ingersoll et al. 1998). The pair remain in amplexus for one to seven days. They briefly separate while the female sheds her exoskeleton and then reunite for copulation. The pair separate again after copulation and the female releases eggs from her oviducts into the marsupium where the eggs are fertilized. Developing embryos remain in the marsupium until the next molt. The young will develop through five to eight prereproductive instars and an indefinite number of postreproductive instars.

OBJECTIVES

The objective of this study was to evaluate potential injury to natural resources from oil and/or brine spilled into intermittent streams in southern Illinois. This study used a sediment quality triad to assess this toxicity. We also characterized habitat upstream and downstream from the point where the spill entered the stream.

METHODS

Study Sites

Study streams were chosen from the Illinois Environmental Protection Agency and Illinois Department of Natural Resources databases of over 4300 recorded oil and/or brine spills since 1991. All spills were entered into a Geographic Information System to facilitate stream selection. The study streams were selected based on the following criteria: the brine and/or oil spill entered an intermittent stream (defined as a blue line on a United States Geological Survey 1:24,000 topographical map), the spill included at least one barrel of brine, and there was sufficient data to accurately locate the spill site. Large spills were preferentially chosen to account for worst case scenarios. The exact location of each spill site was determined using a combination of reports, digital orthophotography, field surveys, and a Garmin Global Positioning Satellite Receiver.

To provide a comparison to streams not impacted by oil and/or brine release, reference streams indicative of ambient background conditions were selected for inclusion in this study. Reference steams were selected based on the following criteria: the stream was not located in an oil production area, and the surrounding land use and physical habitat was similar to that of study streams.

Sediment Quality Triad

The relationship between bulk sediment chemistry and contaminant bioavailability is poorly understood and complicated by many physical and chemical variables. Therefore, a sediment quality triad was used to more accurately depict the bioavailability of chemicals in sediment (Chapman 1986). Our sediment quality triad consisted of using sediment contaminant data, a sediment bioassay using the amphipod *Hyalella azteca*, and *in situ* studies where macroinvertebrate community structure was examined to evaluate potential effects of oil and/or brine spills on intermittent streams. We also assessed both the physical and vegetative habitat of study and reference streams.

Sediment Physical and Contaminant Characterization

At study and reference streams, sediment samples were collected from four transects perpendicular to stream flow during fall 2002 and spring 2003. One transect was located 20 meters (m) upstream from the point where oil and/or brine entered the stream and three additional transects were located 0, 20, and 40 m downstream from the point where the spill entered the stream. Each transect consisted of three 50 gram (g) samples, one sample from each bank and one from the center of the stream. The three samples were combined to form one150 g composite sample. All sediment samples were collected from the top 2 cm with a stainless steel trowel. Samples were then placed in dark glass containers with Teflon®-coated lids and stored on ice during transport from the field to the Cooperative Wildlife Research Laboratory Annex (Wildlife Annex) on the campus of Southern Illinois University, Carbondale, Illinois. Samples were stored at 4^{o}C until analysis.

Sediment samples were sieved using a U.S. standard #5 (4 mm) or #6 (3.35 mm) sieve to remove rocks and large organic matter. The sieved sediment was mixed and divided into 4 subsamples for use in hydrocarbon analysis, chloride concentration determination, organic matter and percent moisture determination, and sediment size distribution analysis.

For polycyclic aromatic hydrocarbon (PAH) and total petroleum hydrocarbon (TPH) analyses, sediment sample extraction, cleanup, and quantification followed EPA methods 3541 (U.S. EPA 1992*a*) , 3630A (U.S. EPA 1990), and 8100 (U.S. EPA 1992*b*), respectively, using gas chromatography (GC) with a flame-ionization detector (FID). Initially, 50 g of sediment was blended with 25 g of sodium sulfate (Na_2SO_4) for 5 minutes. The hydrocarbons were extracted from the sediment using a Soxtec System HT 1043 extraction unit. The extract was then passed through a silica gel column that was pre-eluted with 40 mL of pentane at a rate of 2 mL/1 min. The column was then eluted with 2 mL of cyclohexane followed by 25 mL of pentane and 25 mL of methylene chloride/pentane (2:3 v/v). The last fraction, containing the hydrocarbons, was roto-evaporated and re-suspended in 1 mL of hexane prior to injecting 1 µL into a Hewlett-Packard 5890 Series II Gas Chromatograph (with a 30-m DB-5 column, 0.32-mm internal diameter and 0.25-µm film thickness; Hewlett Packard, Avondale, Pennsylvania, USA) with FID. The temperature program for the column was 40°C for 2 min, ramping at 30°C/min to 120°C, 2°C/min to 150°C, 5°C/min to 270°C, and then held at 270°C for 15 min.

Total chloride concentrations were determined via titration following EPA method 325.3 (U.S. EPA 1982). A subsample of the wet sediment (5 g) was mixed with 50 mL of distilled water for 15 minutes and centrifuged for 5 minutes at 10,000 rpm to separate water from the sediment. The overlying water was removed and added to an Erlenmeyer flask. Two drops of hydrogen peroxide, 2 drops of hydroquinone solution, 8-10 drops of diphenylcarbazone bromophenol blue indicator, and 8-10 drops of xylene cyanol FF indicator were added to the flask and mixed, thoroughly creating a purple solution. Nitric acid (0.3%) was then added drop-wise to the flask until a pH of 2.5±0.1, indicated by a pale yellow color, was reached. Once the solution turned a pale yellow, 1 mL of excess nitric acid was added. The pale yellow solution was then titrated with 0.0141 N mercuric nitrate to a definite purple endpoint. If a color change did not occur with 30 mL of titrant, the titration was discarded and repeated with 3 g of sediment.

Chloride concentrations were determined as:

$$Chloride(mg/L) = \frac{((B - A) \times N \times 35450)}{S}$$

where A = titrant volume for the blank (mL), B = titrant volume for the sample (mL), N = the normality of the titrant, and S = the sample volume (mL). Quality assurance included analyzing a blank, duplicate samples, and a matrix spike with each batch (20 samples). Chloride concentrations were compared to National Water Quality Criteria to determine the potential for chlorides to adversely affect aquatic biota (USEPA 2002).

To determine sediment moisture and organic matter content, a 2-3 g subsample was added to a preweighed aluminum weigh boat and the mass was recorded to the nearest 0.001 g. Samples were placed in an oven, dried at 60^{o}C for 24 hrs, and reweighed. Percent moisture was calculated by dividing the difference between the wet and dry weights by the wet weight. To determine percent organic matter content, the dried sediment was placed in a muffle furnace at 550^{o}C for 2 hrs and then reweighed. Percent organic matter content was calculated by dividing the difference between the dry and ashed weight by the dry weight.

An hydrometer method was used to determine sediment particle size distribution (Foth et al. 1971). A 50 g subsample of sediment was oven dried for 48 hrs at 60^{o}C. The dried sediment was mixed with 100 mL of deionized water and 5 mL of 1 N sodium hexametaphosphate, a dispersing agent. The sediment solution was added to a 1 L graduated cylinder and brought to a final volume of 1 L. Upon thoroughly mixing the solution, a hydrometer and thermometer were immediately placed in the solution and readings were recorded after 40 s. Corrections were made to the hydrometer reading to account for temperature effects. For each degree above 68^{o}C, 0.2 was added to the hydrometer reading, and for each degree below 68^{o}C, 0.2 was subtracted from the hydrometer reading. The hydrometer reading and temperature were again recorded after 2 hrs. Percentages of sand, silt, and clay were calculated using the following formula:

$$P = \frac{(10000 / WG)(R_c - G_L)}{(G - G_L)}$$

where P = percent of sample in suspension, W = oven dry weight of sample, G = specific gravity of sediment particles (2.65), G_L = specific gravity of liquid (1.00), and Rc = corrected hydrometer reading. The percent calculated using the hydrometer readings recorded after 40 s represents the combined percent clay and silt in the sample. The percent sand was calculated by subtracting the percent clay and silt in the suspension recorded after 40 s from 100. The percent

silt was calculated using the hydrometer reading recorded after 2 hrs. Finally, the percent clay was determined by subtracting the percent silt determined after 2 hrs from the percent silt and clay determined after 40 s. Sediment texture class was determined following procedures described by Foth et al. (1971).

Macroinvertebrate community structure

Macroinvertebrate samples were collected during fall 2002 and spring 2003 following USEPA rapid bioassessment protocols (Barbour et al. 1999). A 50 m reach immediately downstream from the location where the oil and/or brine spill entered the stream was sampled with a standard unit of effort. Twenty jabs with a D-frame dip net were taken over the 50 m reach in relative proportion to the available habitat, which was determined by visual estimation. For example, if 50% of the habitat was riffles, 10 jabs would be taken in riffles. The contents were emptied into a bucket, elutriated through a 250 μm sieve and placed in a pollination bag containing a 10% formalin solution and Phloxine-b dye. The same procedure was repeated for each study and reference stream. Streams not containing sufficient water for 20 jabs were not sampled or included in analysis.

Macroinvertebrate samples were subsampled using a gridded pan and sorted prior to identification. Subsampling reduces the effort required for identification and accurately reflects time expenditure (Barbour and Gerritsen 1996). The samples were rinsed in a 250 μm mesh sieve to remove preservative and fine sediment. Large organic matter (leaves, twigs, algal mats, etc.) were rinsed, inspected, and discarded. After rinsing, the samples were spread evenly across a pan marked with 6 cm x 6 cm grids. A random number table was used to select four numbers corresponding to grids within the gridded pan. Subsampling continued until a sample of 200 macroinvertebrates was achieved. Any organism lying over a line separating two grids was included in the grid containing the head. The organism was considered in the grid that contained the majority of the body in situations in which the location of the head could not be determined. When less than 200 individuals per sample were present, all organisms were collected. The samples were stored in glass vials containing 70% ethanol until identification. Identification and taxonomic classification of macroinvertebrates followed Pennak (1989) and Merritt and Cummins (1996). Annelids were identified to Class, Crustaceans to Order or Family, and Molluscs and Insects to Family.

Macroinvertebrate community structure was evaluated by assigning tolerance values to each Family (Hilsenhoff 1988, Barbour et al. 1999) and computing a Family-level biotic index (FBI), which takes into account numbers of species, number of individuals of each species and pollution tolerance (Hilsenhoff 1988). A FBI was calculated for each sample as:

$$FBI = \sum \frac{x_i t_i}{n}$$

where x_i = number of individuals within a taxon, t_i = tolerance value of a taxon (0 = least tolerant, 10 = most tolerant, Hilsenhoff 1988), and n = total number of organisms in a sample. Increased FBI scores are indicative of increased impairment. Taxa richness (total number of different taxonomic categories collected at a sampling site) and the number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa collected at each sampling site were also used to compare macroinvertebrate community structure.

Toxicity Testing

Sediment samples were collected during March and April of 2003 concurrent with sediment collection for chemical analysis. The top 2 cm of sediment were collected from a 20 cm^2 area at the center of the stream at the site of the spill using a stainless steel trowel and each sample was placed in glass containers with Teflon®-coated lids and stored on ice for transport to the Wildlife Annex. Samples were stored at 4°C until toxicity testing began (no longer than 12 months).

The toxicity tests were conducted in a modified version of an exposure system described by Leppanen and Maier (1998) with three plastic laundry sinks (64-cm long x 64-cm wide x 82-cm high) acting as the main compartments. Water was circulated through a water heater and diverted three ways using plastic "T" splitters and rubber tubing. Inlet and outlet tubes were placed into each sink to ensure a constant temperature of 23°C and to prevent thermal gradients within and among sinks. A plastic ceiling grid (49 cm x 47 cm) was placed in the bottom of each sink to provide a flat surface to support 48 300 mL glass jars. A rectangular notch approximately 1 cm^2 was cut into the lip of each jar, and covered with a stainless steel cloth to prevent test organisms from escaping during water renewal. A PVC stand pipe was glued into the laundry sink to maintain a water level below the lips of the glass jars. Another plastic ceiling grid (56-cm x 55-cm) was placed over the sink and portions removed to allow 60 mL disposable

plastic syringes (total volume 75 mL) to rest above each jar. The syringes were fitted with 1 inch, 18-gauge needles to allow steady water flow. A 100 mL aliquot of sediment was added to each jar two days before test commencement and overlying water renewal began. Water was renewed twice a day by filling the corresponding syringe body twice with spring water and allowing the water to drain into each glass jar by gravity while the replaced water overflowed through the notch cut in the lip of the jar.

The *H. azteca* used in toxicity tests were obtained from a culture maintained at the Wildlife Annex and were from cultures originally obtained from the U. S. Geological Survey Columbia Environmental Research Center, Columbia, Missouri. *H. azteca* cultures were maintained in two-40 L glass aquaria containing dechlorinated tap water and several pieces of nylon coiled mesh (3M Corp., St. Paul, Minnesota, USA). Twenty-five milliliters of a yeast-Cerophyl®-trout chow mixture were added to each *H. azteca* culture every two days. Maple leaves previously soaked in salt water were added to serve as additional substrate and as a food source.

Mixed-age amphipods of a similar size were isolated using a stack of U.S. Standard sieves: #30 (600 µm), #40 (425 µm), and #60 (250 µm). Artificial substrates and leaves were placed in a bucket containing culture water. The contents of the bucket were then poured through the stacked sieves. Amphipods stopped by the #60 sieve were washed into a collecting pan and the larger amphipods in the #30 and #40 sieves were returned to the culture aquaria. The smaller amphipods were placed in a 1-L beaker containing spring water. Food was supplied and the water was gentle aerated (Ingersoll and Nelson 1990).

Test conditions for the 42-day *H. azteca* toxicity test have been standardized (U.S. EPA 2000). Briefly, ten mixed-age *H. azteca* of a similar size were placed in test chambers containing 100 mL of sediment and 150 mL of overlying spring water. Each sample was replicated 12 times and survival determined at day 28. Four of the 12 replicates were removed on day 28 to determine growth (measured as length), and individuals from the remaining eight replicates were transferred to beakers containing spring water only and allowed to reproduce. Surviving organisms were counted on day 35, and the test concluded on day 42 when adult survivors were counted, weighed, and their gender determined. The number of juveniles from each replicate also was determined at day 35 and 42. Overlying water quality measurements

such as dissolved oxygen, pH, conductivity, and temperature were determined three times per week.

Growth of *H. azteca* was determined using a photo microsystem equipped with a Leica MZ9.5 microscope (Leica Microsystems, Wetzlar, Germany) and Fuji 6800 Zoom digital camera (Elmsford, NY, USA). Digital photos were taken of each amphipod and length was determined using ImageJ 1.29 image analysis software (National Institute of Health, Washington D.C., USA). Amphipod length was measured on the dorsal side from the base of the first antenna to the end of the third uropod (Ingersoll and Nelson 1990).

Physical and Vegetative Habitat Assessment

Habitat assessment and physicochemical parameters followed U.S. EPA standard methods described by Barbour et al. (1999). Standard water quality data including temperature, dissolved oxygen, conductivity, and pH were recorded using standard handheld meters at each site concurrent with macroinvertebrate collections. Habitat quality was quantitatively assessed based on the following ten reach characteristics: epifaunal substrate/available cover, pool substrate characterization, pool variability, sediment deposition, channel flow status, channel alteration, channel sinuosity, bank stability, bank vegetative protection, and riparian vegetative zone width. Individual reach characteristics were scored from 0 to 20 and a composite habitat score ranging from 0 (poorest habitat quality) to 200 (optimal habitat quality) was calculated for each reach. Physical habitat scores were averaged from two independent observers to reduce personal bias.

The density of vegetation (measured as percent ground cover) was determined using visual estimation for a 100 m^2 area located upstream and downstream from the site of the oil and/or brine spill. A 50 m transect was established 1 m from the bottom edge of each stream and the density of vegetation for 1 m on either side of the transect was determined (i.e., estimated for a 2 m x 50 m area). The plant communities also were assessed using a Floristic Quality Index (FQI). The FQI was calculated as:

$$FQI = (\sum C / N) \times \sqrt{N}$$

Where C, the coefficients of conservatism, are integers from 0 to 10 assigned to each taxon in Illinois flora based on the species tolerance to the duration, frequency, and magnitude of disturbance, and the degree of habitat fidelity (Taft et al. 1997). Values near 0 are assigned to non-native species and species most successful in damaged habitats. Species that are disturbance intolerant and are generally restricted to natural areas have C values of 10. Therefore, higher FQI values are representative of relatively undisturbed habitat.

DATA ANALYSIS

All data were analyzed using JMP 4.0 statistical software (SAS Institute, Cary, NC) or Systat 10 software (SPSS Inc., Chicago, IL). Results were considered significant at $p \leq 0.05$. Data not exhibiting a normal distribution were transformed using the most adequate method. Data were compared using a nonparametric Kruskal-Wallis test when transformations did not improve normality. The Tukey-Kramer HSD test was used for separation of means when significant differences were determined using ANOVA.

Contaminant Analyses

If measured concentrations were 0, the concentration was reported as 0; if measured concentrations were greater than 0, but less than the minimum detection limit (MDL), they were reported as half the MDL; and measured concentrations greater than the MDL were reported as the measured concentration. Means of individual PAHs were calculated using concentrations that were greater than the MDL. Analysis of variance (ANOVA) and/or non-parametric statistical analyses were used to compare differences in sediment total PAH concentration among streams.

Following the methods described in Lopes and Furlong (2001) and MacDonald et al. (2000), upper and lower sediment-quality guideline (SQG) values were chosen from available freshwater and marine sediment quality guidelines to evaluate the potential for adverse effects on aquatic biota. Polycyclic aromatic hydrocarbon concentrations were compared to SQG values to distinguish where toxicity to aquatic organisms was unlikely, possible, or probable. Adverse effects were unlikely for concentrations less than the lower SQG, possible for concentrations above the lower SQG, and probable for concentrations above the upper SQG. Sediment quality guidelines do not exist for TPHs, therefore, TPH concentrations were compared to Canadian

Ministry of Environment soil cleanup standards (Persaud et al. 1993). Chloride concentrations were compared to the U.S. EPA national recommended water quality criterion for chlorides (U.S. EPA 2002).

Macroinvertebrate Community Structure

Family-level biotic index scores were log transformed and evaluated using a one-way analysis of variance (ANOVA). Taxa richness and Ephemeroptera, Plecoptera, Trichoptera (EPT) richness data were evaluated using a nonparametric Kruskal-Wallis test. Spearman rank correlation and stepwise multiple regression were used to examine relationships between physical habitat data, water chemistry, and macroinvertebrate community indices. Statistical significance for the correlations was adjusted using the Bonferroni method to account for experiment-wise error.

Toxicity Tests

Survival and reproduction data were analyzed using a one-way ANOVA, and length was analyzed using a one-way nested ANOVA. Percent survival data were arcsin transformed, and length and reproduction data were log transformed prior to analysis. Dunnett's test was performed to compare study and reference streams pairwise. Spearman rank correlations were used to evaluate the relationships between amphipod responses and the physical characteristics of the sediment and the overlying water quality characteristics. Statistical significance for the correlations was adjusted using the Bonferroni method to account for experiment-wise error.

RESULTS

Site Selection

Although the Illinois Environmental Protection Agency and Illinois Department of Natural Resources (IDNR) databases contained oil and/or brine spill data, most of the records were incomplete and/or spill amounts were rough estimates, making selection of specific study streams difficult and precluding an analysis of temporal trends. Intermittent steams that appeared to be good candidates for evaluating impacts associated with oil and/or brine spills were chosen using the available data, along with site visits and discussions with IDNR personnel (Table 1). Thirty intermittent streams associated with oil and/or brine spills and two reference steams were selected for study during 2002 (Figure 2). During 2003, sampling at site 8 was

precluded by flooding beyond the stream bank, and an additional reference stream was added to more accurately depict the range of habitat quality associated with intermittent streams. Samples were collected during October 2002, and during March and April, 2003, respectively.

Sediment Physical and Chemical Characterization

Physical characterization

Mean organic matter content ($\bar{x}$ = 3.0%, range 0.8 - 11.3%) and percent moisture ($\bar{x}$ = 31.6%, range 22.1 - 47.6%) in sediment collected from streams associated with oil and/or brine spills were significantly lower ($p \leq 0.05$) than mean organic matter content ($\bar{x}$ = 5.4%, range 4.0 - 6.3%) and percent moisture ($\bar{x}$ = 44.4%, range 43.2 - 45.6%) in sediment collected from reference streams (Table 2). Sediments were characterized as sand, sandy loam, sandy clay, clay, clay loam, and loam based on particle size distribution (Table 2).

Polycyclic aromatic hydrocarbons

Thirteen individual PAHs were analyzed in 242 sediment samples for a total of 2662 measurements. Of the 2662 individual PAH measurements, 502 (19%) had concentrations that were above detection limits. The most prevalent PAHs quantified were anthracene, benzo(ghi)perylene, fluorene, and pyrene, while chrysene and indeno(1,2,3-cd)pyrene did not exceed detection limits in any samples. The method detection limit (MDL) ranged from 0.013 μg/g for anthracene to 0.406 μg/g for benzo(a)anthracene (Table 3), and recoveries ranged from 74 ± 37% for benzo(ghi)perylene to 120 ± 38% for benzo(*a*)anthracene.

Fifty of the 502 PAH measurements (10%) that exceeded the detection limits were above lower SQG concentrations. This represents 2% (50 of 2662) of the total number of measurements (Table 4). The majority (70%) of samples with individual PAHs exceeding the lower SQG were collected from locations within 40 m of where the spill entered the stream (locations D1, D2, and D3, Table 4). Thirty percent (15/50) of the individual concentrations that exceeded the lower SQG value were measured in samples collected either above the location where the spill entered the stream (12/50) or from samples collected from reference streams (3/50).

Acenaphthylene was detected at concentrations greater than the MDL at 22 of 242 samples (9%) and, because the MDL was greater than the SQG, all concentrations also were

greater than the SQG. Anthracene was detected in 84 of 242 samples (35%). Of the 84 samples in which Anthracene was detected, the concentration was greater than the SQG in 9 samples (11%). Benzo(a)anthracene was detected at concentrations greater than the MDL at 16 of 242 samples (6%) and, because the MDL was greater than the SQG, all concentrations also were greater than the SQG. Fluorene was detected in 56 of 242 samples (23%). Two samples were detected at concentrations greater than the SQG (1%). Phenanthrene was detected in 42 of 242 sample (17%) and exceeded the SQG in 1 sample (<1%). Pyrene was detected in 136 of 242 samples (56%) and at concentrations greater than the SQG in 1 sample (<1%). Benzo(b)fluoranthene, Benzo(a)pyrene, Benzo(ghi)perylene, Fluoranthene, and Naphthalene were detected in 16, 64, 36, and 28 samples, respectively. These PAHs were not detected at concentrations greater than the SQG.

The sum of PAH concentrations exceeded the lower SQG value for total PAHs (1.61 µg/g) in four of 130 samples collected during fall 2002 and in none of the samples collected in spring 2003 (Figure 3). All four samples exceeding the total PAH SQG were collected from locations within 20 m of where the spill entered the stream: two-were from D1 locations (streams 20, 25) and two were from D2 locations (streams 16 and 19).

There was no significant differences ($p = 0.13$) in mean sum of PAH concentrations among streams. The stream with the greatest mean sum of PAH concentration ($\bar{x} = 0.948$ µg/g, stream 16) was a stream with an oil spill in 2001 (Table 1). Similarly, the stream with the greatest sum of PAH concentration at a specific location (2.814 µg/g, 20 m downstream from where oil entered stream 19) was a stream with an oil spill in 2001 (Table 1). The mean sum of PAH concentrations measured in sediment collected at the reference sites was 0.640 µg/g (range = 0.097 – 1.052 µg/g). When all samples were considered, the sum of PAH concentrations was significantly greater ($p < 0.001$) in 2002 ($\bar{x} = 0.768$ µg/g, range 0.148 - 2.814 µg/g) than 2003 ($\bar{x} = 0.458$ µg/g, range 0.045 - 1.433 µg/g). The reported volume of oil spilled into the stream was not correlated with mean sum of PAH concentrations in sediment collected during 2002 ($r^2 = 0.08$, $p = 0.663$) or 2003 ($r^2 = 0.22$, $p = 0.265$, Figure 4). Additionally, the year of the spill was not correlated with mean sum of PAH concentrations in sediment collected during 2002 ($r^2 = 0.08$, $p = 0.689$) or 2003 ($r^2 = -0.30$, $p = 0.128$). However, there was a general trend toward greater PAH concentrations in sediment collected from streams where spills had occurred during the three years prior to sample collection ($\bar{x} = 1.01$ µg/g, range 0.69 – 2.12) compared to

concentrations measured in sediment collected from streams where spills had occurred during nine to twelve years prior to sample collection ($\bar{x}$ = 0.71 µg/g, range 0.49 – 0.92).

Total petroleum hydrocarbons

No locations sampled in either fall 2002 or spring 2003 had TPH concentrations greater than the Canadian Ministry of Environment soil clean up standard of 1000 µg/g (Persaud et al. 1993, Figure 5). Total petroleum hydrocarbon concentrations were significantly greater (p = 0.001) in sediments collected during 2002 than 2003; however, concentrations were not significantly different between study and reference locations (p = 0.140) during fall 2002 or during spring 2003 (p = 0.090). The MDL for TPH analysis was 43 µg/g.

Chloride

During fall 2002 and spring 2003, chloride concentrations in sediment from study streams were significantly greater than concentrations in sediment from reference streams (p < 0.028). However, chloride concentrations only exceeded the U.S. EPA recommended water quality criterion for chloride (230 mg/L) at one collection site (D2) in intermittent stream 8 during 2002 and at three of the four collection sites in intermittent stream 15 during 2003 (Figure 6). Neither chloride concentrations in sediment collected during 2002 (r^2 = -0.25, p = 0.193) nor 2003 (r^2 = -0.11, p = 0.556) were significantly correlated with the reported brine spill volume in streams (Figure 7).

PHYSICAL HABITAT ASSESSMENT

Mean physical habitat scores were similar for study streams during 2002 ($\bar{x}$ = 95, range 53 - 123) and 2003 ($\bar{x}$ = 93, range 58 - 123), and reference streams ($\bar{x}$ = 113, range 91 - 128) (Tables 5-6). There was no significant difference (p > 0.005) in FQI values among upstream ($\bar{x}$ = 5.5, N = 28), downstream ($\bar{x}$ = 5.7, N = 29), and reference ($\bar{x}$ = 5.8, N = 3) locations. Similarly, there were no significant difference (p > 0.05) in percent vegetation cover among upstream ($\bar{x}$ = 75%, range 30 - 95%), downstream ($\bar{x}$ = 77%, range 30 - 95%), and reference ($\bar{x}$ = 70%, range 50 - 90%) locations.

MACROINVERTEBRATE COMMUNITY STRUCTURE

A total of 39 taxa were identified in communities collected throughout the study. There were no significant correlations between contaminant concentrations, water chemistry, or physical habitat and either taxa richness, EPT richness, or FBI scores.

In 2002, mean taxa richness, mean EPT richness, and mean FBI scores were similar at upstream sites (taxa richness: 6.4, EPT richness: 0.8, FBI: 7.21), downstream sites (taxa richness: 6.3, EPT richness: 0.6, FBI: 7.28), and the reference site (taxa richness: 5, EPT richness: 0, FBI: 7.67, Table 5). Individual site analysis did not indicate large differences in macroinvertebrate community composition between upstream and downstream locations. Similarly for 2003, mean taxa richness, mean EPT richness, and mean FBI scores were similar at upstream sites (taxa richness: 5.6, EPT richness: 0.8, FBI: 7.41), downstream sites (taxa richness: 5.4, EPT richness: 0.7, FBI: 7.20), and the reference sites (taxa richness: 4.7, EPT richness: 0.3, FBI: 6.68, Table 6). When individual streams were evaluated, macroinvertebrate community composition was similar between upstream and downstream locations for each stream.

Mean FBI scores for communities collected during spring 2003 had a significant negative correlation with habitat scores ($r^2 = -0.51$, $p = 0.005$, Figure 8). Stepwise multiple regression analysis with mean FBI scores and habitat score, total dissolved solids, and mean TPH concentration (model: $p = 0.020$) indicated that habitat score was the primary variable driving the relationship with the FBI scores ($p = 0.018$).

SEDIMENT TOXICITY TESTS

Due to limitations in space and resources, not all study streams could be included in toxicity testing. Therefore, toxicity tests were conducted with sediment collected from 23 randomly selected streams of the 30 streams exposed to oil and/or brine spills selected for study and from two of three reference streams not associated with oil or brine spills.

Mean amphipod (*H. azteca*) survival was significantly different ($p < 0.001$) among streams after 28-d exposure to sediment (Table 7). Survival at streams 13 (53%) and 24 (67%) was significantly lower than survival at the reference stream (92%). Amphipod survival also was significantly different ($p < 0.001$) at day 35 and day 42 (water only endpoints). For both

time points, streams 13 and 24 had amphipod survival that was significantly lower than the reference stream (Table 7).

Streams 13 and 24 were not included in growth and reproduction analysis due to significant mortality. Significant differences ($p < 0.001$) in mean lengths of amphipods were evident among streams after both the 28-d and 42-d exposures. Mean lengths of amphipods exposed to sediment from streams 2, 7, and 30 were significantly less ($p < 0.05$) than mean length of amphipods exposed to reference sediment after the 28-d sediment exposure (Table 7). After 42-d, mean length was significantly less ($p < 0.05$) for streams 7, 14, 16, 20, and 29 compared to mean length in reference sediments (Table 7).

Mean reproduction ranged from 0.1 to 10.2 young/female and was significantly different among streams ($p < 0.001$). However, no streams had significantly lower reproduction than the reference stream (Table 7). Significant correlations were observed between mean length after 28-d and mean young/female ($R^2 = 0.70$, $p < 0.001$), as well as, mean length after 42-d and mean young/female ($R^2 = 0.71$, $p < 0.001$, Figure 9). Overlying water chemistry in the test beakers was similar for all streams (Table 8).

DISCUSSION

In the current study, a sediment quality triad was used to assess toxicity of oil and/or brine released into intermittent streams in southern Illinois. The triad consisted of chemical analysis of sediment, evaluation of macroinvertebrate communities, and a standardized sediment bioassay. Each component of this triad was evaluated independently of the others prior to an overall assessment of potential toxic effects.

The first component of the triad was chemical analysis of sediments. For study and reference streams, sediment samples were collected from one location 20 m upstream from the point where the spill was reported to have entered the stream, and from three downstream locations, each 20 m apart starting at the point where the spill entered the stream. Results of chemical analysis indicated that low concentrations of some PAHs were present in sediment collected from intermittent streams, and in some cases, quantified concentrations were above lower SQG values (Table 4). The majority (70%) of sampling locations where PAHs were above the lower SQG, were from locations between 0 and 40 m downstream from where the spill

had entered the stream. This indicates that, in the majority of the cases where PAHs were quantified, the greatest PAH concentrations were in sampling locations below the point of the reported oil spill. However, to keep this in perspective, it should be remembered that only 2% of all PAH measurements were above lower SQG concentrations, and that no PAH concentrations were above the upper SQG. Similarly, only 3% (4/130) of the sum of PAH concentrations exceeded lower SQG concentrations. Therefore, sediment contaminant analysis provided little evidence of the potential for adverse effects due to PAH concentrations. This was further supported by the fact that there were no statistical differences in PAH concentrations among sampling locations within streams, and that there was no correlation between the amount of oil spilled and PAH concentrations measured in sediment.

We also measured TPH and chloride concentrations in sediment. There was no significant differences in mean TPH concentrations among streams and all TPH concentrations were below the Canadian Ministry of Environment Soil Cleanup Standard (1000 μg/g).

During 2003, mean chloride concentrations were significantly greater in sediments collected from study streams compared to reference streams, and the concentrations in one study stream exceeded the U.S. EPA national recommended water quality criterion. An abandoned tank battery located uphill from this stream is an obvious point source for brine contamination at this site. The area downhill of the tank battery was completely devoid of vegetation including riparian vegetation. It was obvious that brine had adversely effected vegetation at this site and that concentrations in stream sediments reflected this exposure. The chloride data suggest that, at least during 2003, intermittent streams had greater exposure to brine. However, only results from one stream indicated potential adverse effects and there was no correlation between chloride concentrations and the volume of brine reportedly spilled in streams.

The following is a summary of our findings for the chemical analysis phase of our sediment quality triad: (1) there was no significant differences in mean sum of PAH concentrations among streams, and no consistent pattern for quantified PAH concentrations among stream locations, (2) although there was a trend towards decreasing sediment PAH concentrations over time, this trend was not statistically significant, (3) the majority of the PAH concentrations that exceeded lower SQG values were from locations immediately downstream from the point where the spill entered the stream, (4) TPH concentrations were below soil cleanup standards, (5) PAH and TPH concentrations were not correlated with reported amounts

of oil spilled, (6) chloride concentrations were greater in study streams relative to reference streams during 2003, (7) chloride concentrations exceeded the national water quality criterion in only one stream, and (8) chloride concentrations were not correlated with reported amounts of brine spilled into streams. Although these results suggest that chemical analysis provided some evidence that oil and/or brine had entered some intermittent streams, there was little evidence to support a conclusion of lingering potential adverse impacts one to twelve years after the spill events.

The second component of our sediment quality triad was an evaluation of macroinvertebrate communities located 50 m downstream from the point where oil and/or brine had entered the stream and from reference streams. Macroinvertebrate communities collected during 2002 and 2003 did not differ between study and reference streams. There were no significant differences in taxa richness, EPT richness, or FBI scores between study and reference streams, and sediment contaminant concentrations were not correlated with any of these three factors. These results do not support a conclusion that oil and/or brine adversely effected macroinvertebrate community structure. The macroinvertebrate community composition appeared to be primarily influenced by physical habitat in this study. Water quality evaluations (Table 5) indicated that 77% of the evaluations of study streams were rated fairly poor or worse, as were 75% of the evaluations of reference streams. Similarly, habitat scores averaged 94 for study streams and 113 for reference streams. Since habitat scores near 200 represent optimal habitat quality, our results indicate that the habitats associated with intermittent streams in southern Illinois are of poor quality. Therefore, it is not surprising that the macroinvertebrate communities in our study streams were not "robust" and probably did not serve as good indicators of the impacts of oil and/or brine contamination. Similar to our findings, physical habitat also was the most descriptive parameter for describing macroinvertebrate responses in a study of watershed impacts of small acid mine drainage (Schmidt et al. 2002).

In the current study, plant species richness, the floristic quality index (FQI), and the coefficient of conservatism, were not significantly different between upstream, downstream, and reference locations. The coefficients of conservatism for all three locations were low (below 2.5) indicating a plant community that is tolerant of disturbance (a coefficient of conservatism of 10 would indicate less tolerant plant communities).

It is possible that oil production activities may have influenced macroinvertebrate community structure in the intermittent streams studied. Loss of vegetation due to brine contamination is known to result in increased stream sedimentation and increased total dissolved solids (GERPDC 1982). However, most streams used in the current study have been altered, usually for agricultural purposes, and therefore habitat degradation due to oil production cannot be isolated as a cause. Stone (2003) reported that in-stream physical habitat was the most important variable determining macroinvertebrate community structure in highly degraded agricultural streams; therefore it was not possible to separate the impacts of oil production from the impacts of agriculture. In addition to degradation, the intermittent nature of the streams used in the current study may have influenced macroinvertebrate community structure. Macroinvertebrates were collected from less than half of the study sites in fall 2002 due to lack of water. Intermittent habitats are more variable than perennial habitats and differences in flow regime may confound responses to contamination. Additionally, streams recover at different rates depending on the intensity and duration of the dry period and how many drying periods occur per year (Niemi et al. 1990). Whiles and Goldowitz (2001) reported that streams with intermediate hydroperiods, and a fewer number of drying events per year, supported more taxa, and intermittent streams have been reported to support different communities than perennial stream (del Rosario and Resh 2000). Due to the variable physical conditions, whether associated with oil production activities (brine spills) or physical alterations associated with agricultural activities, the USEPA rapid bioassessment protocols may not be suitable for bioassessment of intermittent streams such as those evaluated during the current study.

The following is a summary of our findings for the macroinvertebrate community structure phase of our sediment quality triad: (1) there were no significant differences in taxa richness, EPT richness, or FBI between study and reference streams; and (2) there were no correlations between sediment contaminant concentrations and either taxa richness, EPT richness, or FBI scores. The results of our evaluation of macroinvertebrate community structure provide no evidence to support a conclusion of potential adverse impacts from oil and/or brine spills in intermittent streams. However, there are several confounding factors, mainly habitat degradation, time since the spill, and the nature of intermittent streams, that may be masking efforts to evaluate the impacts of oil and/or brine in these streams.

The third component of our sediment quality triad was an evaluation of sediment toxicity following standard EPA protocols. Sediment was collected from study streams at the point where the oil and/or brine spill had entered the stream and from reference streams. Sediments from streams 13 and 24 were acutely toxic to *H. azteca*; however, low survival recorded from stream 24 may be a result of difficulties associated with recovering the amphipods rather than contamination. The sediment from stream 24 was composed of large course particles, mostly pebble size, and very fine silt that clouded the water. The combination of many hiding spots and decreased visibility made recovering amphipods from the test beakers difficult and therefore survival may have been underestimated. At stream 13, approximately 8000 L of brine was reported to have spilled in 2000 (Table 1). However, the reported spill volume is an estimate and may not reflect the actual volume released into the stream. Several dead trees were present at the spill site, suggesting a relatively large brine spill, which also would suggest that the observed toxicity was related to exposure to brine. However, observed toxicity from stream 13 did not correspond with chloride concentrations measured in sediment and the reported volume of brine spilled in other study streams greatly exceeded the volume reported for stream 13 (e.g., >40,000 L reported for streams 9 and 10, see Table 1).

Growth was significantly lower for amphipods exposed to sediment from three streams (streams 2, 7, and 30) after the 28-d sediment exposure and five streams (streams 7, 14, 16, 20, and 29) after the water only exposure (day 42 of test) suggesting that sediments from these streams were marginally contaminated. Reproduction from day 28 to 42 was not significantly lower in amphipods exposed to sediments from study streams compared to reference streams. However, there were significant differences among some study streams.

Reproduction and growth endpoints after 28 and 42 d were significantly correlated in the current study. Stressors, either natural or anthropogenic, that affect growth can also affect reproduction in *H. azteca* due to the minimum body size required for reproduction (Rees and Crawley 1989, Ernsting et al 1993, Moore and Dillon 1993, Enserink et al 1995, Moore and Farrar 1996, Sibley et al 1996, Sibley et al 1997). Ingersoll et al (1998) also reported significant correlations between reproduction from day 28 to 42 and amphipod growth on day 28 and 42 using formulated and field-collected sediments with low to moderate concentrations of contaminants.

The elevated chloride contamination measured in sediment collected from site 15 did not result in increased toxicity in *H. azteca.* The lack of toxicity may be explained by the organism's ability to tolerate saline waters up to 29 ‰ (Ingersoll et al 1992), a concentration much greater than any reported in the current study. Decreased amphipod growth did not correspond with contaminants measured in this study, nor with physical characteristics of the sediment or overlying water chemistry.

The following is a summary of our findings for the sediment toxicity test phase of our sediment quality triad: (1) mean amphipod survival was significantly lower following exposure to sediments collected from streams 13 and 24 compared to exposure to sediments collected from reference streams; (2) growth (mean length) of amphipods exposed to sediment collected from streams 2, 7, 14, 16, 20, 29, and 30 were significantly less than growth of amphipods exposed to reference sediments; and (3) reproduction was significantly different among some study streams, but there were no differences among study streams and reference streams. Sediment toxicity test results provide evidence to support a conclusion of potential adverse impacts in study streams. However, it was not possible to conclude that oil and/or brine spills were associated with causation. We did not observe an association between sediment contaminant concentrations and survival, growth, or reproduction in amphipods.

CONCLUSIONS AND RECOMMENDATIONS

A sediment quality triad consisting of a quantification of sediment contaminant concentrations, a sediment bioassay using the amphipod *Hyalella azteca*, and an *in situ* study of macroinvertebrate community structure were used to assess impacts of oil and/or brine spills on intermittent streams in southern Illinois. Sampling sites within streams included locations above and below the reported spill site and reference streams with no reported oil or brine spills. Polycyclic aromatic hydrocarbons, TPHs, and chloride concentrations were quantified in sediments, and except for chloride concentrations measured in sediment collected during 2003, there were no significant differences among collection locations; however, there were several streams where individual PAH concentrations or the sum of PAH concentration exceeded SQG values (Table 4). The individual PAHs that exceeded the SQG values varied among streams and in some cases were measured in sediment collected above the site of the reported spill.

Therefore this component of the triad is inconclusive. Although PAHs were measured in sediment, there is little evidence to demonstrate that sediment PAH concentrations were associated with a specific oil spill. However, the sum of PAH concentrations that exceeded the SQG values were all from locations directly downstream from the location of the reported spill, providing some evidence linking the spilling of oil to the sum of PAHs in sediment.

The lack of measured differences in macroinvertebrate communities among sampling locations suggests that this component of the triad provided little detectable effect from the spilling of oil and/or brine in the streams studied. The standardized sediment toxicity test did suggest that sediment from several streams associated with oil and/or brine spills had adverse effects on *H. azteca*; however, there was no consistent indication of a corresponding association between the observed effects and the contaminant concentrations we measured in the sediment. Therefore, it was not possible to link the toxicity observed in *H. azteca* with the oil and/or brine spill.

The habitat associated with intermittent streams in southern Illinois does not offer a quality environment for biotic communities. Habitat scores, biotic indexes, and water quality evaluations suggest a degraded environment, and the correlation between habitat scores and invertebrate FBI (Figure 8) suggested that habitat quality is the factor influencing invertebrate communities. This degradation may in part be due to agricultural activities, the nature of intermittent streams, and/or the impacts of the oil industry. Because of the poor quality of the environment and the community associated with that environment, it was not possible to separate the influences of oil and/or brine from other influences. Evaluations of some of the individual study streams did provide an indication that these streams had been exposed to oil and/or brine; however, there was little evidence from our study that oil and/or brine spills had resulted in significant impacts.

It is possible that the effects of oil and/or brine spilled in streams during periods of water flow would be masked by movement of material downstream and that during periods of rapid water movement, as is common in intermittent streams, that deposited material may be washed further downstream. To address these potential impacts, it would be desirable to sample sediment and macroinvertebrate communities immediately after, and for several months following, an oil or brine spill to determine the fate of the oil and brine associated with the spill and the temporal and spatial response of macroinvertebrate communities.

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LITERATURE CITED

Albers, P. H. 1995. Petroleum and individual polycyclic aromatic hydrocarbons. Pages 330-355 *in* D.H. Hoffman, B.A. Rattner, G.A. Burton, Jr., and J.C. Cairns, Jr., editors. Handbook of ecotoxicology. Lewis Publishers, Boca Raton, Florida, USA.

Barbour, M. T., and J. Gerritsen. 1996. Subsampling of benthic samples: A defense of the fixed-count method. Journal of the North American Benthological Society 15:386-391.

Barbour, M. T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Washington, D.C., USA.

Burton, G. A., Jr. 1991. Assessing the toxicity of freshwater sediments. Environmental Toxicology and Chemistry 10:1585-1627.

Burton, G. A., Jr., and C. MacPherson. 1995. Sediment toxicity testing issues and methods *in* D.H. Hoffman, B.A. Rattner, G.A. Burton, Jr., and J.C. Cairns, Jr., editors. Handbook of ecotoxicology. Lewis Publishers, Boca Raton, Florida, USA.

Chapman, P. M. 1986. Sediment quality criteria from the sediment quality triad: an example. Environmental Toxicology and Chemistry 5:957-964.

Chapman, P., E. A. Power, and G. A. Burton, Jr. 1992. Integrative assessments in aquatic ecosystems, *in* Burton, G. A., Jr., editor. Sediment toxicity assessment. Lewis Publishers, Boca Raton, Florida, USA.

Covich, A. P., and J. H. Thorp. 2001. Introduction to the subphylum Crustacea *in* J.H. Thorp and A.P. Covich, editors. Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego, California, USA.

del Rosario, R. B., and V. H. Resh. 2000. Invertebrates in intermittent and perennial streams: is the hyporheic zone a refuge from drying? Journal of the North American Benthological Society 19: 680-696.

Enserink, E. L., M. J. J. Kerkhofs, C. A. M. Baltus, and J. H. Koeman. 1995. Influence of food quantity and lead exposure on maturation in *Daphnia magna*: Evidence for a trade-off mechanism. Functional Ecology 9:175-185.

Ernsting, G., C. Zonneveld, J. A. Isaaks, and A. Kroon. 1993. Size at maturity and patterns of growth and reproduction in an insect with indeterminate growth. Oikos 66:17-26.

Foth, H. D., H. S. Jacobs, and L. V. Withee. 1971. Laboratory manual for introductory soil science. Third edition. Wm. C. Brown Company Publishers, Dubuque, Iowa, USA.

Gill, R. A., and P. W. J. Robotham. 1989. Composition, sources and source identification of petroleum hydrocarbons and their residues. Pages 11-40 *in* J. Green and M.W. Trett, editors. The fate and effects of oil in freshwater. Elseveir Science Publishing Co., New York, USA.

GERPDC. 1982. An overview of oil field brine problems in three Illinois Counties. Greater Egypt Regional Planning and Development Commission, Report Number GERPDC-82-626, Carbondale, Illinois.

Gorman, H. S. 1999. Efficiency, environmental quality, and oil field brines: the success and failure of pollution control by self-regulation. Business History. 73:601-640.

Hilsenhoff, W. L. 1988. Rapid field assessment of organic pollution with a family-level biotic index. Journal of the North American Benthological Society 7:65-68.

Hilsenhoff, W.L. 1987. An improved biotic index of organic stream pollution. The Great Lakes Entomologist 20:31-39.

Ingersoll, C. G. 1995. Sediment tests *in* G.M Rand, editor. Fundamentals of aquatic toxicology; effects, environmental fate, and risk assessment. Taylor and Francis, Bristol, Pennsylvania, USA.

Ingersoll, C. J., and M. K. Nelson. 1990. Testing sediment toxicity with *Hyalella azteca* (Amphipoda) and *Chironomus tentans* (Diptera) *in* W. G. Landis and W. H. van der Schalie, editors. Aquatic Toxicology and Risk Assessment, 13th Volume. ASTM, Philadelphia, Pennsylvania, USA.

Ingersoll, C. J., F. J. Dwyer, S. A. Burch, M. K. Nelson, D. R. Buckler, and J. B. Hunn. 1992. The use of freshwater and saltwater animals to distinguish between the toxic effects of salinity and contaminants in irrigation drainwater. Environmental Toxicology and Chemistry 11:503-511.

Ingersoll, C. G., E. L. Brunson, F. J. Dwyer, D. K. Hardesty, and N. E. Kemble. 1998. Use of sublethal endpoints in sediment toxicity tests with the amphipod *Hyalella azteca*. Environmental Toxicology and Chemistry 17:1508-1523.

Keith, L. H., and W. A. Telliard. 1979. Priority pollutants, I - A perspective view. Environmental Science and Technology 13:416-423.

Larsson, P. 1985. Contaminated sediments of lakes and oceans act as sources of chlorinated hydrocarbons for release to water and atmosphere. Nature 317:347-349.

Leppanen, C. J., and K. H. Maier. 1998. An inexpensive and efficient modular water-renewal system for bulk sediment toxicity testing. Environmental Toxicology and Chemistry 17:969-971.

Lopes, T. J., and E. T. Furlong. 2001. Occurrence and potential adverse effects of semivolatile organic compounds in streambed sediment, United States, 1992-1995. Environmental Toxicology and Chemistry 20:727-737.

Lyman, W. J., A. E. Glazer, J. H. Ong, and S. F. Coons. 1987. An overview of sediment quality in the United States. EPA-905/9-88-002. Washington, D.C., USA.

MacDonald, D.D., Ingersoll, G.G., and T.A. Berger. 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Archives of Environmental Contamination and Toxicology 39:20-31.

Merritt, R. W., and K. W. Cummins. 1996. An introduction to the aquatic insects of North America. Third edition. Kendall/Hunt Publishing Co. Dubuque, Iowa, USA.

Moore, D. W., and T. M. Dillon. 1993. The relationship between growth and reproduction in the marine polychaete *Nereis (Neanthes) arenaceodentata* (Moore): Implications for chronic sublethal sediment bioassays. Journal of Experimental Marine Biology and Ecology 173:231-246.

Moore, D. W., and J. D. Farrar. 1996. Effect of growth on reproduction in the freshwater amphipod *Hyalella azteca*. Hydrobiologia 328:127-134.

Neff, J. M. 1985. Polycyclic aromatic hydrocarbons *in* G.M Rand, editor. Fundamentals of aquatic toxicology; effects, environmental fate, and risk assessment. Taylor and Francis, Bristol, Pennsylvania, USA.

Niemi, G. J., P. DeVore, N. Detenbeck, D. Taylor, A. Lima, J. Pastor, J. D. Yount, R. J. Naiman. 1990. Overview of case studies on recovery of aquatic systems from disturbance. Environmental Management 14:571-587.

Oltz, D. F. 1994. Improved and enhanced oil recovery in Illinois through reservoir characterization. U.S. Department of Energy, Report DOE/BC/14250-19, Washington, D.C., USA.

Pennak, R. W. 1989. Fresh-water invertebrates of the United States. Third edition. John Wiley and Sons, Inc. New York, New York, USA.

Persaud, D., R. Jaagumagi, and A. Hayton August. 1993. Guidelines for the protection and management of aquatic sediment quality in Ontario. Ontario Ministry of the Environment and Energy, Ottawa, Ontario, Canada.

Petroleum Profile. 2004. Petroleum profile: Illinois. Energy Information Administration. Online at http://www.tonto.eia.doe.gov/oog/info/state/il.html (accessed 24 June 2004).

Power, E. A., and P. M. Chapman. 1992. Assessing sediment quality *in* G.A. Burton, editor. Sediment toxicity assessment. Lewis Publishers, Boca Raton, Florida, USA.

Rand, G. M., P. G. Wells, and L. S. McCarty. 1995. Introduction to aquatic toxicology. Pages 3-67 *in* G.M. Rand, editor. Fundamentals of aquatic toxicology. Taylor and Francis, Bristol, Pennsylvania, USA.

Rees, M., and M. J. Crawly. 1989. Growth, reproduction and population dynamics. Functional Ecology 3:645-653.

Schmidt, T. S., D. J. Soucek, and D. S. Cherry. 2002. Modification of an ecotoxicological rating to bioassess small acid mine drainage-impacted watershed exclusive of benthic macroinvertebrate analysis. Environmental Toxicology and Chemistry 21:1091-1097.

Shales, S., B. A. Thake, B. Frankland, S. H. Khan, J. D. Hutchinson, and C. F. Mason. 1989. Biological and ecological effects of oils. Pages 81-172 *in* J. Green and M.W. Trett, editors. The fate and effects of oil in freshwater. Elsevier Science Publishing, New York, USA.

Schuytema, G. S., D. F. Krawczyk, W. L. Grirris, A. V. Nebeker, M. L. Robideaux, B. J. Brownawell, and J. C. Westall. 1988. Comparative uptake of hexachlorobenzene by fathead minnows, amphipods and oligochaete worms from water and sediment. Environmental Toxicology and Chemistry 7:1035-1045.

Sibley, P. K., G. T. Ankley, A. M. Cotter, and E. N. Lenoard. 1996. Predicting chronic toxicity of sediment spiked with zinc: An evaluation of the acid-volatile sulfide (AVS) model using a life-cycle test with the midge *Chironomus tentans*. Environmental Toxicology and Chemistry 15:2102-2112.

Sibley, P. K., D. A. Benoit, and G. T. Ankley. 1997. The significance of growth in *Chironomus tentans* sediment toxicity tests: Relationship to reproduction and demographic endpoints. Environmental Toxicology and Chemistry 16:336-345.

Sibley, P. K., M. L. Harris, K. T. Bestari, T. A. Stelle, R. D. Robinson, R. W. Gensemer, K. E. Day, and K. R. Solomon. 2001. Response of phytoplankton communities to liquid creosote in freshwater microcosms. Environmental Toxicology and Chemistry 20:2785-2793.

Sours, D., F. Kulfinski, and J. DeWitt. 1985. Status of the brine problem in Illinois. Illinois Department of Energy and Natural Resources, RE-EA-85/05. Springfield, Illinois, USA.

Stone, M. L. 2003. The influence of riparian vegetation on water quality, in-stream habitat, and biotic integrity in agriculturally impacted southern Illinois streams. Master's Thesis. Southern Illinois University - Carbondale. Carbondale, Illinois, USA.

Taft, J. B., G. S. Wilhelm, D. M. Ladd, and L. A. Masters. 1997. Floristic quality assessment for vegetation in Illinois: A method for assessing vegetation integrity. Erigenia 15:3-95.

Tissot, B. P., and D. H. Welte. 1984. Petroleum formation and occurrence. Springer-Verlag, New York, USA.

Thamke, J. N., and S. D. Craigg. 1997. Saline-water contamination in quaternary deposits and the Poplar River, East Poplar Oil Field, Northeastern Montana. United States Geological Survey, Water-Resources Investigations Report 97-4000. Helena, Montana, USA.

U.S. EPA. 1982. Method 325.3. Chloride (Titrimetric, Mecuric Nitrate). U.S. EPA. Methods for the Chemical Analysis of Water and Wastes (MCAWW) (EPA/600/4-79/020).

_____ 1990. Method 3640A. Revision 1. Silica Gel cleanup. U.S. EPA. Test methods for evaluating solid waste, physical/chemical methods, SW-846. Third edition. United States Environmental Protection Agency, Washington, D.C., USA.

_____ 1992a. Method 3541. Automated soxhlet extraction. Pages 3541-1 to 3541-10 *in* U.S. EPA. Test methods for evaluating solid waste, physical/chemical methods, SW-846. Third edition. United States Environmental Protection Agency, Washington, D.C., USA.

_____ 1992b. Method 8100. Polynuclear Aromatic Hydrocarbons. U.S. EPA. Test methods for evaluating solid waste, physical/chemical methods, SW-846. Third edition. United States Environmental Protection Agency, Washington, D.C., USA.

_____ 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. United States Environmental Protection Agency Publication EPA/600/R-94/024.

_____ 2002. National recommended water quality criteria: 2002. United States Environmental Protection Agency Publication EPA/822/R/02/047.

Verrhiest, G., B. Clement, and G. Blake. 2001. Single and combined effects of sediment-associated PAHs on three species of freshwater macroinvertebrates. Ecotoxicology 10:363-372.

Whiles M. R., and B. S. Goldowitz. 2001. Hydrologic influences on insect emergence production from central Platte River wetlands. Ecological Applications 11:1829-1842.

Young, M. Y. 1997. *In situ* biological assessment of the photoinduced toxicity of environmental releases of crude oil. U.S. Fish and Wildlife Service, Rock Island, Illinois, USA.

Yong, M. Y., D. Jascheke, and M. G. Henry. 1997. Laboratory assessment of the photoinduced toxicity of crude oil. U.S. Fish and Wildlife Service, Rock Island, Illinois, USA.

Table 1. Location information and oil and brine spill amounts (liters L) for study and reference streams sampled during fall 2002 and spring 2003 in southern Illinois.

Site	IDNR spill number	Year of spill	UTM coordinates[a]	County	Brine spill amount (L)	Oil spill a mount (L)
1	1000037	2000	406530 4237220	Edwards	11129	0
2	1000053	2000	390333 4206366	White	2385	477
3	1000141	2001	359484 4207185	Hamilton	1590	159
4	1000154	2001	412989 4242895	Edwards	NR[b]	477
5	1000159	2001	394172 4247018	Wayne	1590	795
6	1003062	Unknown	350863 4205290	Hamilton	9539	0
7	1003212	1997	387531 4215865	White	1590	0
8	1005298	2002	394172 4247018	Wayne	3180	2385
9	2000277	2000	324858 4269165	Marion	47696	3975

Table 1. Continued.

Site	IDNR spill number	Year of spill	UTM coordinates[a]	County	Brine spill amount (L)	Oil spill amount (L)
10	2000290	2000	341572 43337181	Fayette	44516	0
11	2000318	2000	326155 4272324	Marion	795	159
12	2000319	2000	326366 4271892	Marion	15899	1590
13	2000354	2000	341465 4334343	Fayette	7949	0
14	2000355	2000	337892 4327277	Fayette	7949	0
15	2000451	2001	316134 4288586	Marion	2348	79
16	2000455	2001	335058 4322339	Fayette	795	159
17	2000459	2001	340633 4336727	Fayette	1590	636
18	2000484	2001	336498 4330592	Fayette	1590	397
19	2000501	2001	335891 4325131	Fayette	795	159

Table 1. Continued.

Site	IDNR spill number	Year of spill	UTM coordinates[a]	County	Brine spill amount (L)	Oil spill amount (L)
20	2000523	2001	336349 4328110	Fayette	4770	0
21	2000633	1992	341130 4336736	Fayette	25438	0
22	2900099	1992	325846 4270962	Marion	3180	159
23	2900161	1991	336499 4327510	Fayette	10334	0
24	3000032	2000	431848 4289806	Lawrence	15899	477
25	3000112	2000	432374 4286390	Lawrence	1590	636
26	3000139	2000	423477 4318958	Crawford	795	318
27	3000162	Unknown	411779 4271090	Richland	795	159
28	3002118	1994	401851 4301882	Jasper	31797	0
29	3002331	1999	429902 4305035	Crawford	NR	NR

Table 1. Continued.

Site	IDNR spill number	Year of spill	UTM coordinates[a]	County	Brine spill amount (L)	Oil spill amount (L)
30	3900149	1991	438009 4278422	Lawrence	1590	0
R1	N/A[c]	N/A	319831 4288485	Marion	N/A	N/A
R2	N/A	N/A	321716 4282399	Marion	N/A	N/A
R3	N/A	N/A	433954 4282215	Lawrence	N/A	N/A

[a] Zone 16
[b] Not reported
[c] Not applicable

Table 2. Physical characteristics of sediment collected from streams exposed to oil and/or brine and reference streams during fall 2002 and spring 2003 in southern Illinois.

Site	% moisture	% organic matter	Particle size distribution			Texture class
			% sand	% silt	% clay	
1	43.9	5.16	74	16	11	Sandy loam
2	30.9	3.36	59	18	23	Sandy loam
3	30.6	6.63	78	13	9	Sandy loam
4	35.6	11.27	53	27	20	Sandy loam
5	41.9	3.23	82	17	1	Sandy loam
6	34.1	3.98	62	24	14	Sandy clay
7	36.3	2.60	64	25	11	Sandy clay
8	32.9	2.40	59	18	23	Sandy loam
9	24.7	1.65	46	22	31	Loam
10	45.3	1.04	33	40	27	Clay loam
11	46.0	2.40	92	8	0	Sand
12	23.0	0.94	90	9	1	Sand
13	24.5	0.84	91	9	1	Sand
14	30.4	2.73	83	14	3	Sandy loam
15	28.3	1.65	74	16	10	Sandy loam
16	30.7	2.50	95	1	4	Sand
17	22.2	3.57	38	31	31	Clay loam
18	27.2	1.34	7	54	39	Clay
19	22.1	1.16	40	38	22	Clay loam
20	30.8	2.58	97	1	1	Sand
21	26.6	2.61	40	23	37	Loam

Table 2. Continued.

Site	% moisture	% organic matter	Particle size distribution			Texture class
			% sand	% silt	% clay	
22	24.2	1.21	96	2	2	Sand
23	33.8	4.81	30	41	29	Clay loam
24	29.5	3.33	89	4	7	Sandy loam
25	31.2	5.03	45	25	30	Clay loam
26	29.9	2.32	73	17	10	Sandy loam
27	22.2	3.76	62	15	23	Sandy loam
28	29.8	2.16	95	2	2	Sand
29	47.6	1.45	82	10	9	Sandy loam
30	30.4	1.91	78	17	5	Sandy loam
Mean	31.6	2.99	67	19	15	
(SE)	(1.3)	(0.4)	(4.3)	(2.3)	(2.2)	
R1	45.6	5.90	96	2	2	Sand
R2	44.5	4.01	59	21	20	Sandy loam
R3	43.2	6.30	399	27	34	Clay loam
Mean	44.4	5.40	65	17	20	
(SE)	(0.7)	(0.7)	(16.7)	(7.5)	(9.4)	
p-value[a]	< 0.001	0.05				

[a] Student's t-test

Table 3. Polycyclic aromatic hydrocarbon (PAH), lower sediment-quality guidelines (SQG)[a] and method detection limits (MDL).

PAH	Lower SQG (µg/g)	MDL (µg/g)
Acenaphthylene [b]	0.006	0.035
Anthracene [c]	0.057	0.013
Benzo(a)anthracene [c]	0.108	0.406
Benzo(b)fluoranthene [b]	3.600	0.068
Benzo(a)pyrene [c]	0.150	0.104
Benzo(ghi)perylene [b]	0.720	0.035
Chrysene [c]	0.166	0.171
Fluoranthene [c]	0.423	0.050
Fluorene [c]	0.077	0.028
Indeno[1,2,3-*cd*]pyrene [b]	0.690	0.189
Napthalene [c]	0.176	0.031
Phenanthrene [c]	0.204	0.051
Pyrene [c]	0.195	0.059
Total PAHs [c]	1.610	

[a] PAH concentrations greater than lower SQGs suggest possible effects (Lopes and Furlong 2001).
[b] Marine sediment-quality guidelines.
[c] Freshwater sediment-quality guidelines.

Table 4. Mean (SE)[a] and range of concentrations (μg/g) of polycyclic aromatic hydrocarbons (PAHs) measured in sediment collected during 2002 and 2003 from intermittent streams in southern Illinois exposed to oil and/or brine spills and from reference streams.

PAH	Collection Location[b]				
	D1 n = 59[c]	D2 n = 57	D3 n = 58	U n = 58	R n = 10
Acenaphthylene	0.085(0.020) 0.043-0.140 4/4[d]	0.079(0.024) 0.038-0.186 6/6	0.041(0.004) 0.037-0.053 4/4	0.060(0.007) 0.040-0.098 7/7	---- 0.080 1/1
Anthracene	0.038(0.007) 0.015-0.129 22/4	0.024(0.004) 0.015-0.087 19/1	0.022(0.002) 0.015-0.053 21/0	0.031(0.005) 0.014-0.087 17/2	0.039(0.012) 0.018-0.069 5/2
Benzo(a)anthracene	1.022(0.149) 0.657-1.575 6/6	0.986(0.189) 0.492-1.815 7/7	---- 0.842 1/1	---- 0.625-0.687 2/2	---- ---- 0
Benzo(b)fluoranthene	0.090(0.008) 0.072-0.110 4/0	0.107(0.033) 0.072-0.172 3/0	0.079(0.004) 0.072-0.085 3/0	0.127(0.039) 0.075-0.241 4/0	---- 0.075-0.090 2/0
Benzo(a)pyrene	---- ---- 0	0.111(0.001) 0.110-0.113 3/0	0.110 1/0	0.117(0.003) 0.110-0.122 4/0	---- ---- 0
Benzo(ghi)perylene	0.152(0.009) 0.149-0.180 13/0	0.139(0.015) 0.045-0.245 13/0	0.152(0.009) 0.143-0.215 18/0	0.132(0.020) 0.037-0.345 16/0	0.161(0.003) 0.155-0.168 4/0

Table 4. Continued

Fluoranthene	0.087(0.013) 0.051-0.122 14/0	0.067(0.005) 0.053-0.091 9/0	0.067(0.006) 0.053-0.087 7/0	0.066(0.006) 0.053-0.115 13/0	0.072(0.012) 0.052-0.092 3/0
Fluorene	0.050(0.028) 0.028-0.122 17/1	0.052(0.037) 0.028-0.140 12/1	0.035(0.011) 0.028-0.057 13/0	0.065(0.048) 0.033-0.171 13/2	 0.090 1/0
Naphthalene	0.048(0.008) 0.033-0.113 9/0	0.042(0.006) 0.033-0.060 4/0	0.036(0.001) 0.033-0.040 5/0	0.044(0.006) 0.031-0.087 10/0	---- ---- 0
Phenanthrene	0.091(0.016) 0.055-0.214 11/1	0.090(0.017) 0.060-0.187 7/0	0.059(0.002) 0.053-0.068 11/0	0.079(0.009) 0.055-0.126 9/0	0.074(0.017) 0.055–0.125 4/0
Pyrene	0.095(0.005) 0.059-0.170 38/0	0.095(0.006) 0.059-0.165 25/0	0.090(0.004) 0.060-0.172 35/0	0.100(0.008) 0.060-0.226 32/1	0.135(0.013) 0.085-0.167 6/0
Total PAH	0.651(0.045) 0.127-2.124 2	0.650(0.046) 0.089-2.814 2	0.567(0.045) 0.045-1.408 0	0.590(0.046) 0.103-1.262 0	0.971(0.172) 0.097-1.052 0

[a] Mean and range are calculated from concentrations above the detection limit.
[b] Collection locations: D1 = at the location where the spill entered the stream, D2 = 20 m downstream from D1, D3 = 40 m downstream from D1, U = 20 m upstream from D1, and R = samples collected from reference streams.
[c] Number of samples analyzed.
[d] Number of samples above the detection limits/number of samples above sediment quality guidelines (SQG) (see Table 3). Number of samples above detections limits does not apply for total PAH and only the number of samples above sediment SQGs are listed.

Table 5. Habitat scores and mean (± SE) benthic macroinvertebrate index values from sediment collected during 2002 from intermittent streams exposed to oil and/or brine and reference streams in southern Illinois.

		Upstream				Downstream			
Site	Habitat Score	Taxa Richness	EPT [a] Richness	FBI[b]	Water Quality Evaluation[c]	Taxa Richness	EPT Richness	FBI	Water Quality Evaluation
5	92	2	0	5.67	Fair	3	0	7.96	Very poor
7	72	4	0	7.67	Very poor	1	0	9.00	Very poor
8	53	5	0	7.66	Very poor	8	1	6.31	Fairly poor
13	117	12	2	6.50	Fairly poor	10	1	4.58	Good
14	121	10	2	7.89	Very poor	8	1	4.80	Good
17	123	9	2	7.84	Very poor	11	1	7.39	Very poor
18	109	9	3	6.61	Poor	7	2	6.06	Fairly poor
23	91	2	0	6.88	Poor	3	1	5.98	Fairly poor
24	117	2	0	6.22	Fairly poor	0	0	8.56	Very poor
25	92	6	0	8.58	Very poor	8	0	9.23	Very poor
26	88	8	0	7.92	Very poor	9	0	7.14	Very poor
27	101	NC[d]	NC	NC	---	10	0	7.84	Very poor
30	95	8	0	7.05	Poor	4	1	9.84	Very poor
Mean	95	6.4	0.8	7.21	Poor	6.3	0.6	7.28	Very poor

Table 5. Continued.

	Reference				
Site	Habitat Score	Taxa Richness	EPT [a] Richness	FBI[b]	Water Quality Evaluation[c]
R2	120	5	0	7.67	Fairly poor

[a] Ephemeroptera-Plecoptera-Trichoptera
[b] Family-level Biotic Index
[c]Water quality evaluation = categorization by range of FBI Values (Hilsenhoff 1987)
[d]Not Calculated

Table 6. Habitat scores and mean (± SE) benthic macroinvertebrate index values from sediment collected during 2003 from intermittent streams exposed to oil and/or brine and reference streams in southern Illinois.

		Upstream				Downstream			
Site	Habitat Score	Taxa Richness	EPT [a] Richness	FBI [b]	Water Quality Evaluation[c]	Taxa Richness	EPT Richness	FBI	Water Quality Evaluation
2	70	5	0	8.57	Very poor	2	0	8.00	Very poor
4	58	5	0	7.77	Very poor	4	0	7.93	Very poor
5	92	11	3	7.41	Very poor	6	2	3.25	Excellent
6	86	7	0	8.17	Very poor	9	2	8.09	Very poor
7	72	6	2	8.67	Very poor	9	2	8.18	Very poor
10	97	5	0	7.46	Very poor	6	0	8.12	Very poor
11	78	3	0	8.83	Very poor	4	0	7.38	Very poor
12	74	2	0	6.67	Poor	2	0	6.67	Poor
13	117	3	1	7.14	Poor	7	2	6.30	Fairly poor
14	121	8	2	6.76	Poor	7	2	7.41	Very poor
15	79	2	0	9.43	Very poor	2	0	5.33	Fair
16	99	2	0	8.27	Very poor	2	0	8.00	Very poor
17	123	6	1	6.62	Poor	7	1	8.11	Very poor

Table 6. Continued.

		Upstream				Downstream			
Site	Habitat Score	Taxa Richness	EPT[a] Richness	FBI[b]	Water Quality Evaluation[c]	Taxa Richness	EPT Richness	FBI	Water Quality Evaluation
18	109	5	1	5.89	Fairly poor	8	3	6.10	Fairly poor
19	109	13	4	6.06	Fairly poor	9	1	6.28	Fairly poor
20	79	7	1	7.66	Very poor	11	2	7.02	Poor
21	105	6	0	7.19	Poor	4	0	7.64	Very poor
22	88	2	0	8.00	Very poor	2	0	7.79	Very poor
23	91	5	3	6.03	Fairly poor	2	0	7.13	Poor
24	117	4	0	6.48	Fairly poor	5	0	6.38	Fairly poor
25	92	9	1	6.50	Fairly poor	7	0	7.75	Very poor
26	88	8	0	6.14	Fairly poor	6	0	6.06	Fairly poor
27	101	4	0	7.51	Very poor	5	0	7.21	Poor
28	95	7	1	6.61	Poor	3	0	7.89	Very poor
29	89	9	2	7.08	Poor	7	1	7.28	Very poor
30	95	2	0	9.71	Very poor	4	0	9.77	Very poor
Mean	93	5.6	0.8	7.41	Very poor	5.4	0.7	7.20	Poor

Table 6. Continued.

	Reference				
Site	Habitat Score	Taxa Richness	EPT[a] Richness	FBI[b]	Water Quality Evaluation[c]
R1	91	5	1	8.00	Very poor
R2	120	3	0	6.53	Poor
R3	128	6	0	5.51	Fair
Mean	113	4.7	0.3	6.68	Poor

[a] Ephemeroptera-Plecoptera-Trichoptera
[b] Family-level Biotic Index
[c]Water quality evaluation = categorization by range of FBI Values (Hilsenhoff 1987)

Table 7. Mean (±SE) response of *Hyalella azteca* in chronic toxicity tests following exposure to sediment collected in 2003 from intermittent streams exposed to oil and/or brine and reference streams in southern Illinois. Asterisks indicate study streams that are significantly lower than reference streams.

	Survival (%)			Length (mm)		Number of young/female
Site	Day 28	Day 35	Day 42	Day 28	Day 42	Day 28 to Day 42
2	90 (2.1)	82 (3.5)	82 (3.5)	3.2 (0.11)*	4.0 (0.06)	0.7 (0.47)
4	92 (2.1)	85 (4.6)	85 (4.6)	3.7 (0.07)	4.0 (0.05)	0.3 (0.10)
5	95 (1.9)	90 (3.8)	90 (3.8)	4.0 (0.10)	4.5 (0.05)	1.9 (0.37)
6	96 (1.5)	93 (2.5)	91 (3.0)	4.0 (0.08)	4.5 (0.05)	2.5 (0.33)
7	89 (2.6)	84 (3.2)	80 (4.2)	3.1 (0.16)*	3.7 (0.06)*	0.1 (0.14)
10	89 (2.6)	82 (5.5)	81 (5.5)	3.7 (0.05)	4.3 (0.06)	3.5 (1.21)
11	94 (3.4)	83 (4.9)	81 (4.4)	4.3 (0.06)	5.0 (0.06)	8.4 (1.24)
12	94 (1.9)	95 (5.3)	91 (2.2)	3.5 (0.09)	4.2 (0.04)	1.0 (0.72)
13	53 (9.9)*	56 (9.8)*	55 (9.6)*	NM[a]	NM	NM
14	98 (1.1)	94 (3.8)	93 (4.1)	3.4 (0.08)	3.9 (0.07)*	0.2 (0.09)
15	99 (0.8)	99 (1.4)	96 (3.0)	4.1 (0.09)	4.2 (0.05)	2.2 (0.42)
16	96 (1.9)	96 (2.6)	94 (3.2)	3.4 (0.07)	3.9 (0.07)*	1.5 (0.39)
18	94 (3.4)	93 (8.9)	90 (4.2)	3.8 (0.07)	4.0 (0.06)	2.4 (0.36)

Table 7. Continued.

Site	Survival (%)			Length (mm)		Number of young/female
	Day 28	Day 35	Day 42	Day 28	Day 42	Day 28 to Day 42
19	86 (4.2)	78 (6.2)	78 (6.2)	3.7 (0.07)	4.0 (0.04)	1.9 (0.53)
20	92 (2.4)	90 (2.7)	86 (4.2)	3.5 (0.06)	3.8 (0.06)*	0.3 (0.12)
22	89 (3.4)	87 (4.2)	87 (4.2)	4.1 (0.10)	4.4 (0.06)	10.2 (6.41)
24	67 (7.5)*	68 (7.7)*	66 (7.0)*	NM	NM	NM
25	93 (3.7)	88 (5.6)	84 (5.3)	3.7 (0.08)	4.5 (0.06)	2.3 (1.02)
26	94 (1.5)	88 (1.6)	83 (4.1)	3.9 (0.06)	4.2 (0.05)	4.0 (0.76)
27	79 (3.7)	83 (3.7)	83 (3.7)	3.6 (0.11)	4.1 (0.04)	0.7 (0.27)
28	90 (2.4)	79 (4.4)	79 (4.4)	3.7 (0.11)	4.0 (0.04)	0.1 (0.10)
29	98 (1.3)	94 (3.2)	91 (4.0)	3.3 (0.06)	3.9 (0.04)*	0.8 (0.54)
30	86 (5.3)	86 (3.2)	86 (3.2)	3.2 (0.12)*	4.4 (0.05)	1.2 (0.31)
Reference	92 (2.1)	90 (2.9)	89 (3.0)	3.6 (0.05)	4.3 (0.04)	1.4 (0.35)
p value [b]	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

[a] NM = not measured
[b] ANOVA

Table 8. Mean dissolved oxygen (DO), temperature, pH and conductivity (±SE) of water overlying sediment used in chronic toxicity tests. Sediment collected from intermittent streams exposed to oil and/or brine and reference streams in southern Illinois.

Stream	Dissolved oxygen (mg/L)	Temperature (^{o} C)	pH	Conductivity (µS/cm)
2	5.2 (0.49)	20.8 (1.62)	8.9 (0.18)	695 (45.1)
4	4.7 (0.40)	20.6 (1.32)	9.0 (0.15)	706 (36.8)
5	6.0 (0.49)	24.2 (1.62)	8.7 (0.18)	705 (45.1)
6	4.7 (0.49)	20.0 (1.62)	8.9 (0.18)	530 (45.1)
7	5.5 (0.69)	20.7 (2.29)	8.6 (0.25)	660 (63.8)
10	5.4 (0.31)	21.9 (1.02)	9.1 (0.11)	636 (28.5)
11	5.6 (0.49)	23.4 (1.62)	8.9 (0.18)	685 (45.1)
12	4.7 (0.28)	21.2 (0.93)	9.4 (0.11)	672 (28.5)
13	5.2 (0.35)	22.2 (1.14)	8.9 (0.13)	643 (21.9)
14	4.7 (0.49)	21.0 (1.62)	9.2 (0.18)	665 (45.1)
15	6.4 (0.49)	23.2 (1.60)	8.8 (0.19)	715 (45.0)
16	5.1 (0.35)	22.5 (1.14)	9.0 (0.13)	690 (31.9)
18	5.8 (0.50)	19.7 (1.62)	9.0 (0.18)	665 (45.1)
19	5.2 (0.35)	21.8 (1.14)	8.9 (0.13)	653 (31.9)
20	5.9 (0.31)	24.4 (1.02)	9.0 (0.11)	710 (28.5)
22	5.1 (0.40)	24.0 (1.32)	8.9 (0.15)	710 (36.8)
24	5.7 (0.31)	24.0 (1.02)	8.9 (0.11)	690 (28.5)
25	5.4 (0.35)	23.7 (1.14)	8.9 (0.13)	725 (31.9)
26	4.7 (0.28)	22.3 (0.93)	9.2 (0.10)	677 (26.0)
27	5.5 (0.28)	20.9 (0.91)	8.9 (0.11)	688 (28.5)
28	5.2 (0.35)	22.1 (1.14)	9.0 (0.13)	615 (31.9)

Table 8. Continued.

Stream	Dissolved oxygen (mg/L)	Temperature (^{o} C)	pH	Conductivity (µS/cm)
29	5.4 (0.69)	20.3 (2.29)	9.5 (0.25)	680 (33.8)
30	5.4 (0.28)	21.1 (0.93)	9.2 (0.11)	680 (28.5)
R2	4.7 (0.28)	21.0 (0.93)	8.9 (0.10)	688 (28.5)
R3	4.8 (0.35)	25.7 (1.14)	8.8 (0.13)	705 (31.9)

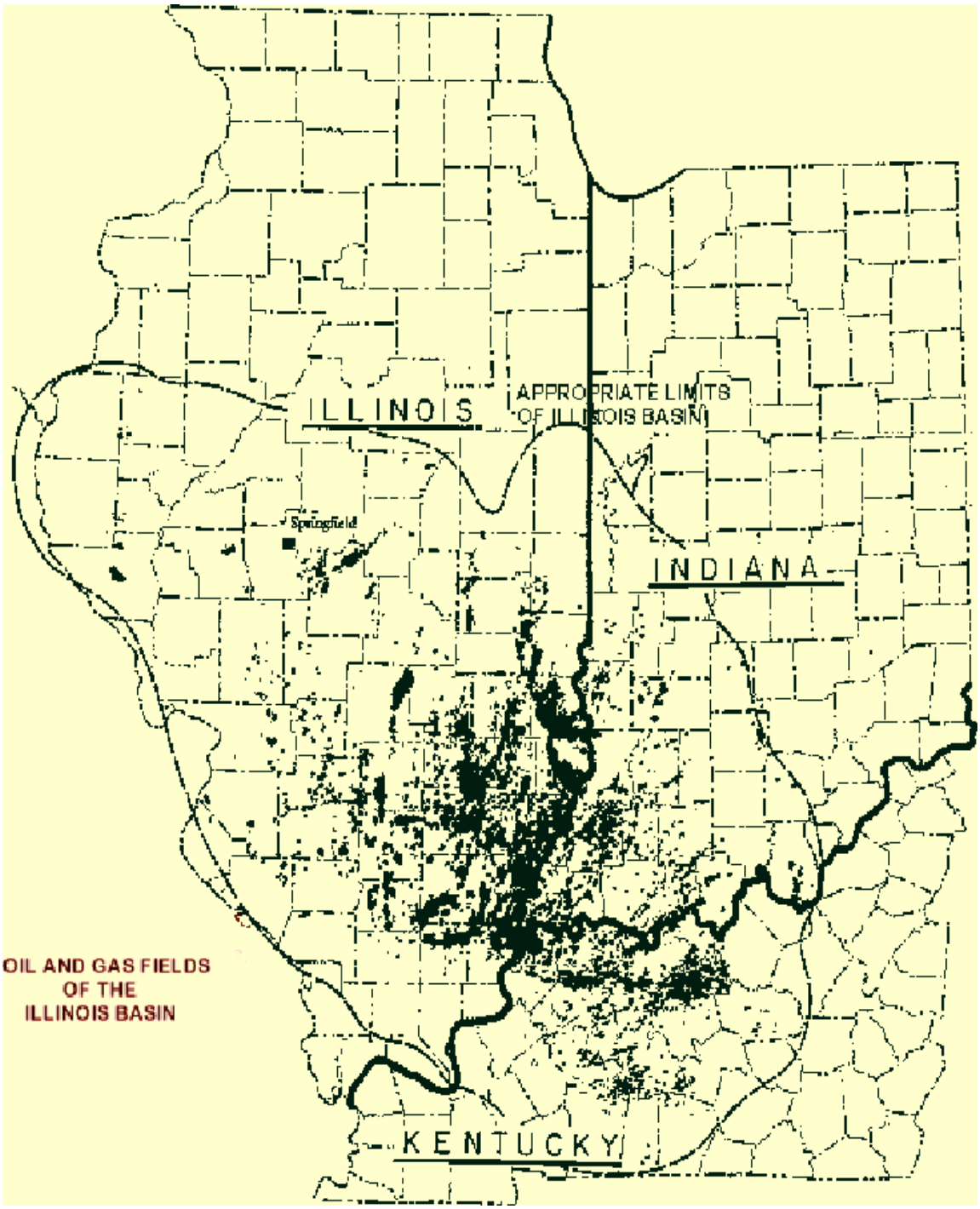

Figure 1. Oil and gas fields of the Illinois Basin (Oltz 1994).

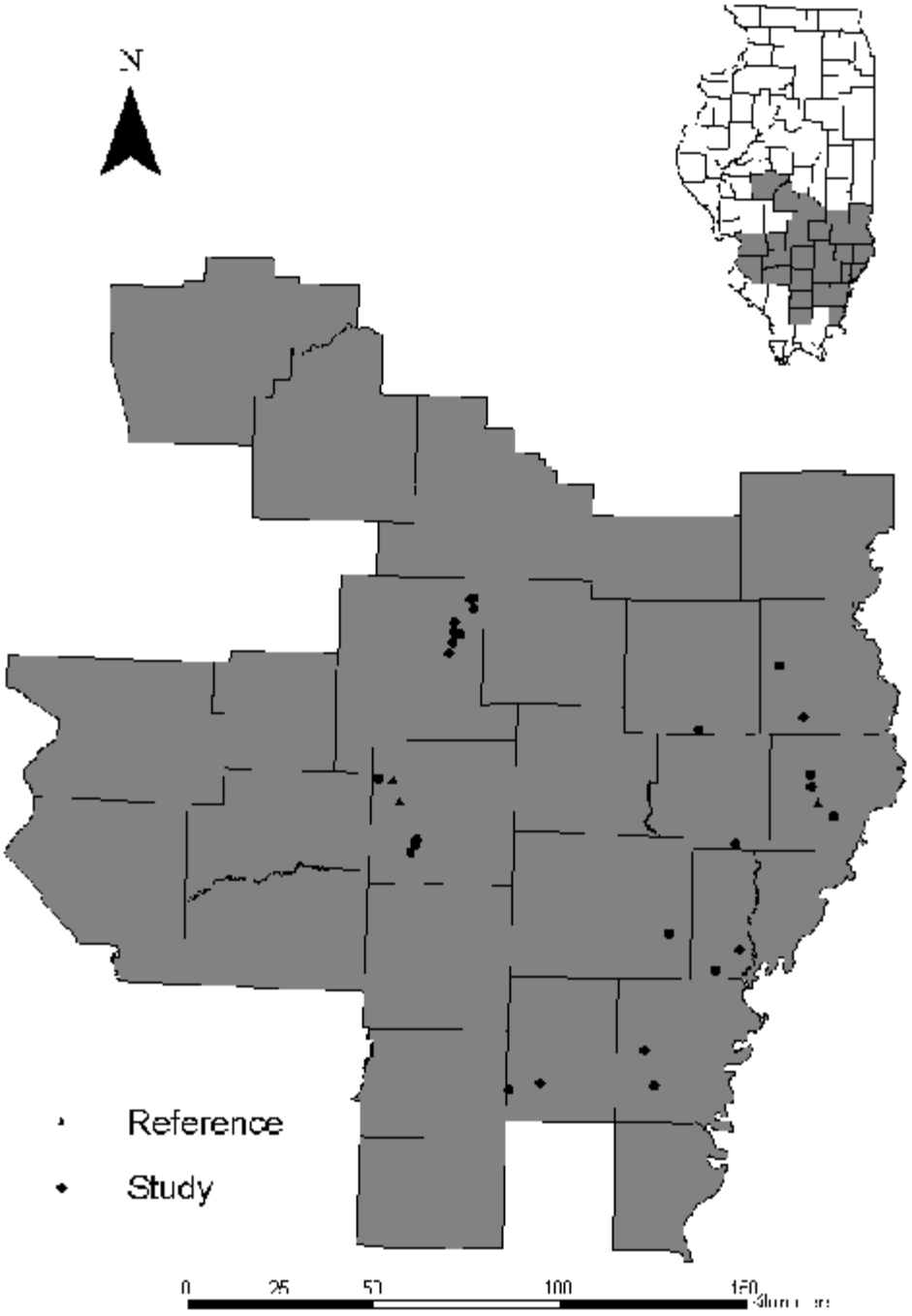

Figure 2. Locations of study intermittent streams exposed to oil and/or brine and reference intermittent streams sampled during fall 2002 and spring 2003 in southern Illinois.

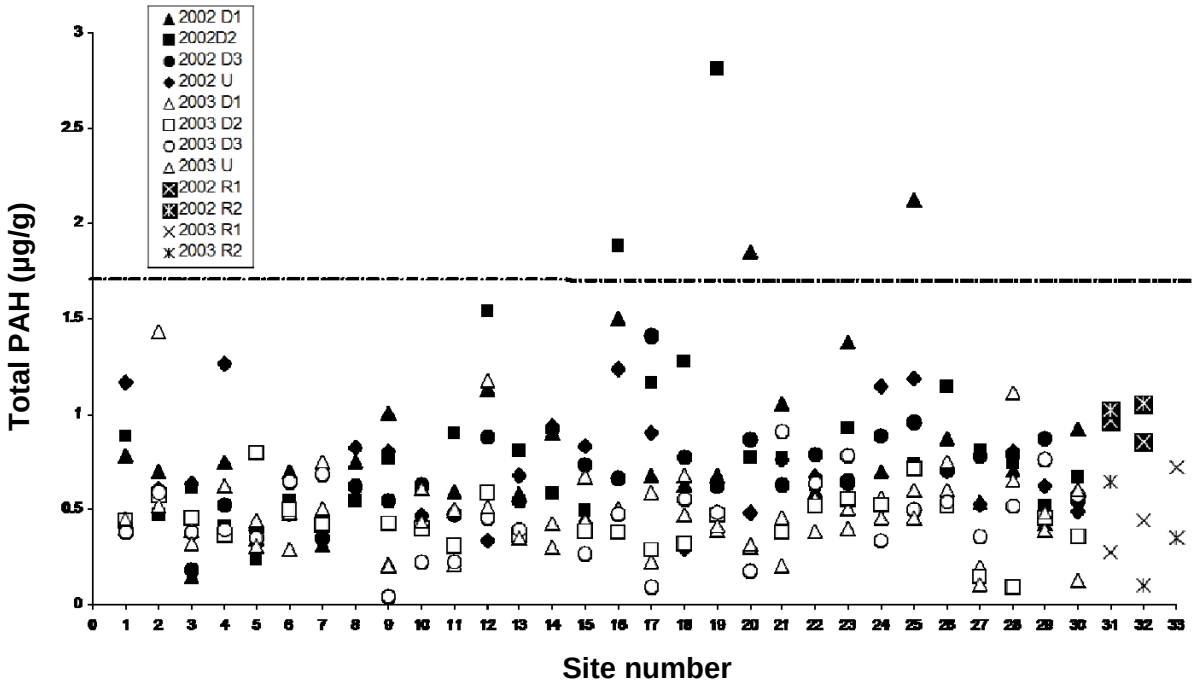

Figure 3. Total polycyclic aromatic hydrocarbon (PAH) concentrations in sediment collected during fall 2002 and spring 2003 from intermittent streams (site numbers 1 - 30) and reference streams (site numbers 31 - 33) in southern Illinois. Symbols represent the year and locations within each stream where sediment samples were collected (U = 20 m above spill site, D1 = spill site, D2 and D3 were 20 and 40 m, respectively, downstream from spill site. The dashed line represents the sediment quality guideline for total PAHs (1.61 µg/g; Lopes and Furlong 2001).

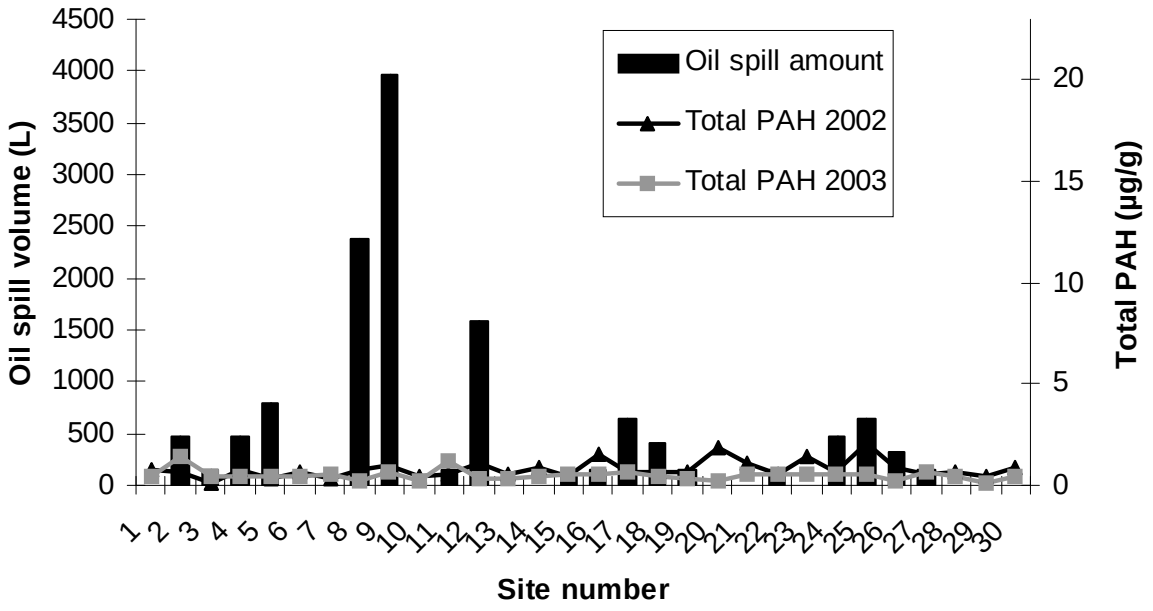

Figure 4. The relationship between the volume of oil spilled into intermittent stream study sites and the total PAH concentrations detected in sediment collected during fall 2002 and spring 2003.

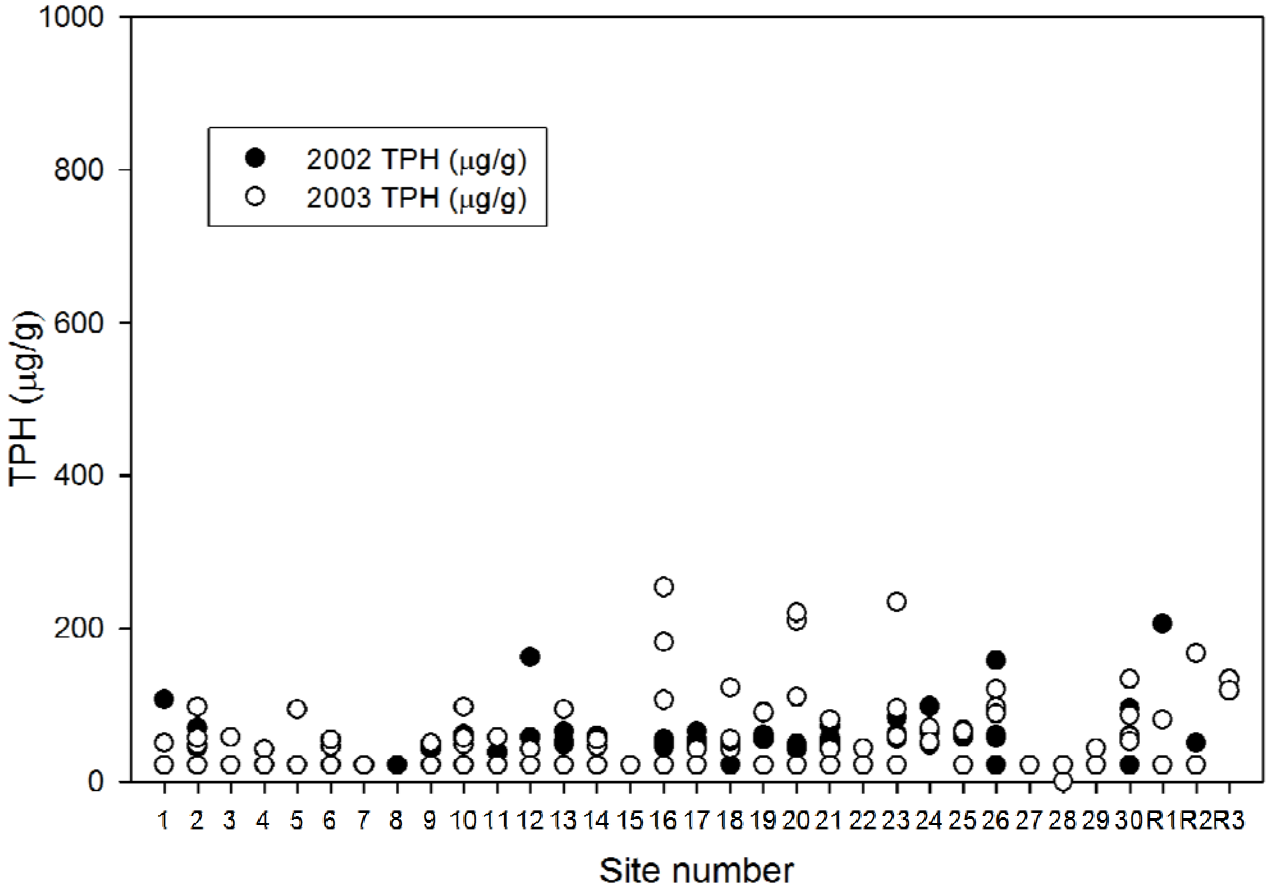

Figure 5. Total petroleum hydrocarbon (TPH) concentrations in sediment collected during fall 2002 and spring 2003 from intermittent streams (site numbers 1 - 30) and reference streams (site numbers R1 - R3) in southern Illinois.

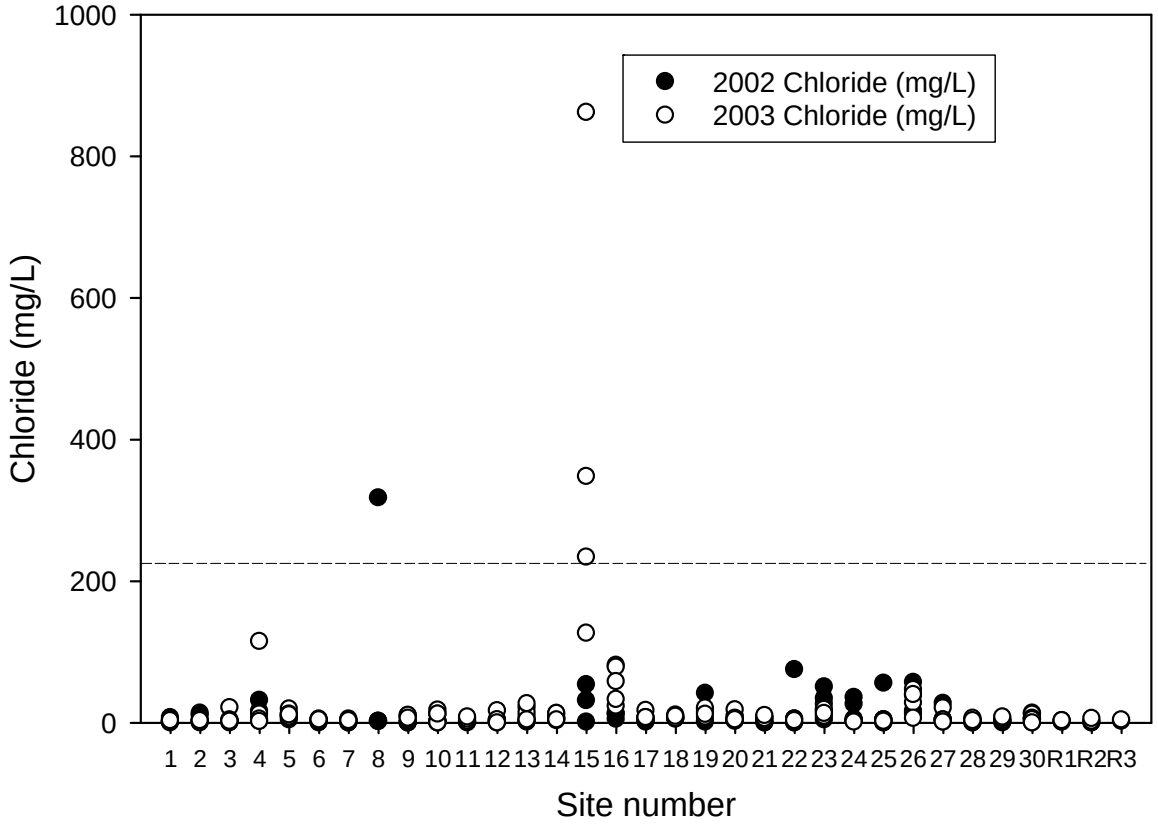

Figure 6. Chloride concentrations in sediment collected during fall 2002 and spring 2003 from intermittent streams (site numbers 1 - 30) and reference streams (site numbers R1 - R3) in southern Illinois. The dashed line represents the water quality criteria for chloride (230 mg/l; U.S.EPA 2002).

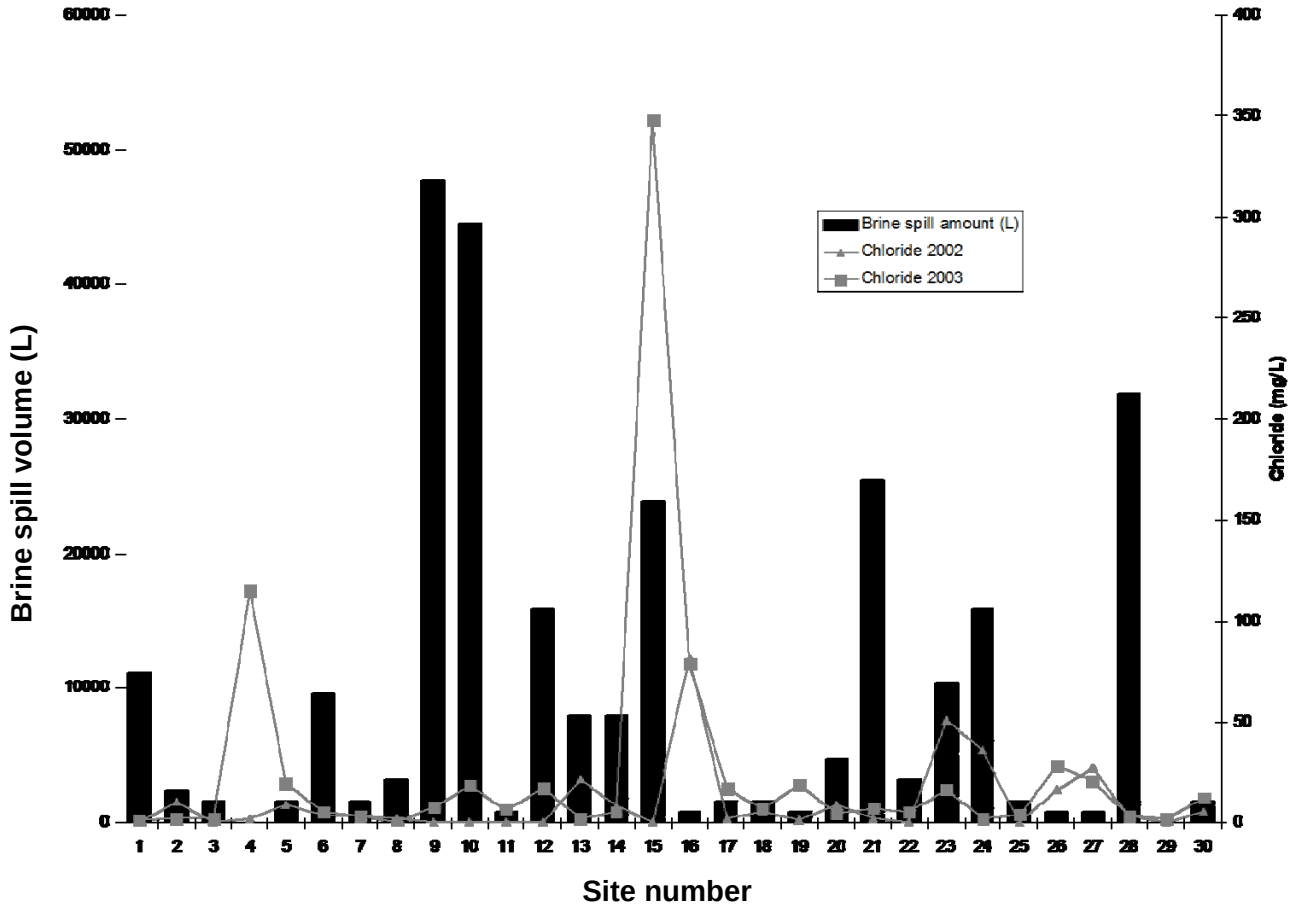

Figure 7. Relationship between the volume of brine spilled into intermittent streams and the chloride concentrations detected in sediment collected during 2002 and 2003 from intermittent stream study sites in southern Illinois.

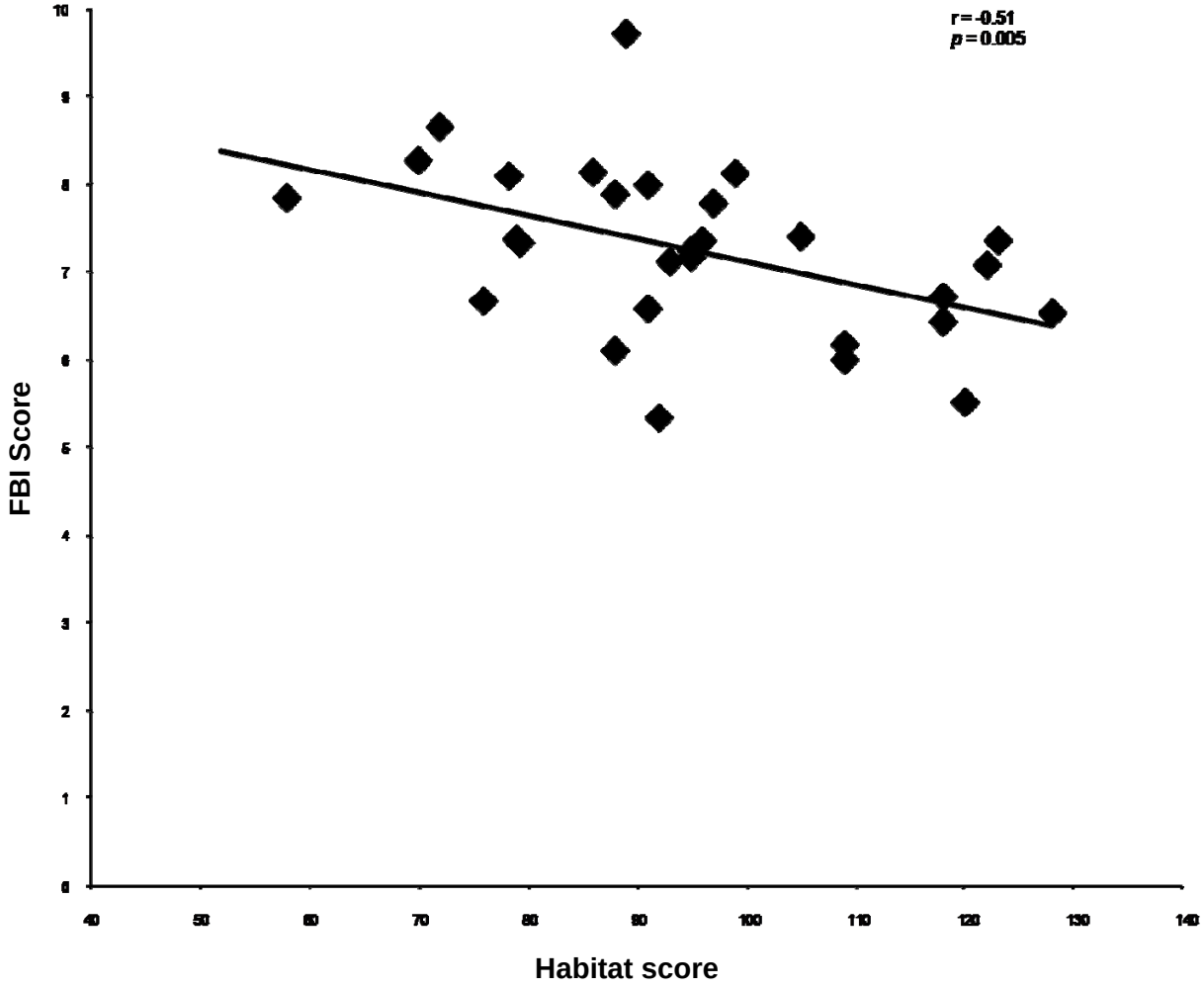

Figure 8. Spearman correlation between habitat scores and Family-level biotic index (FBI) scores from macroinvertebrate communities collected during 2003 from study and reference intermittent streams in southern Illinois. Increasing FBI scores indicated more pollution tolerant communities.

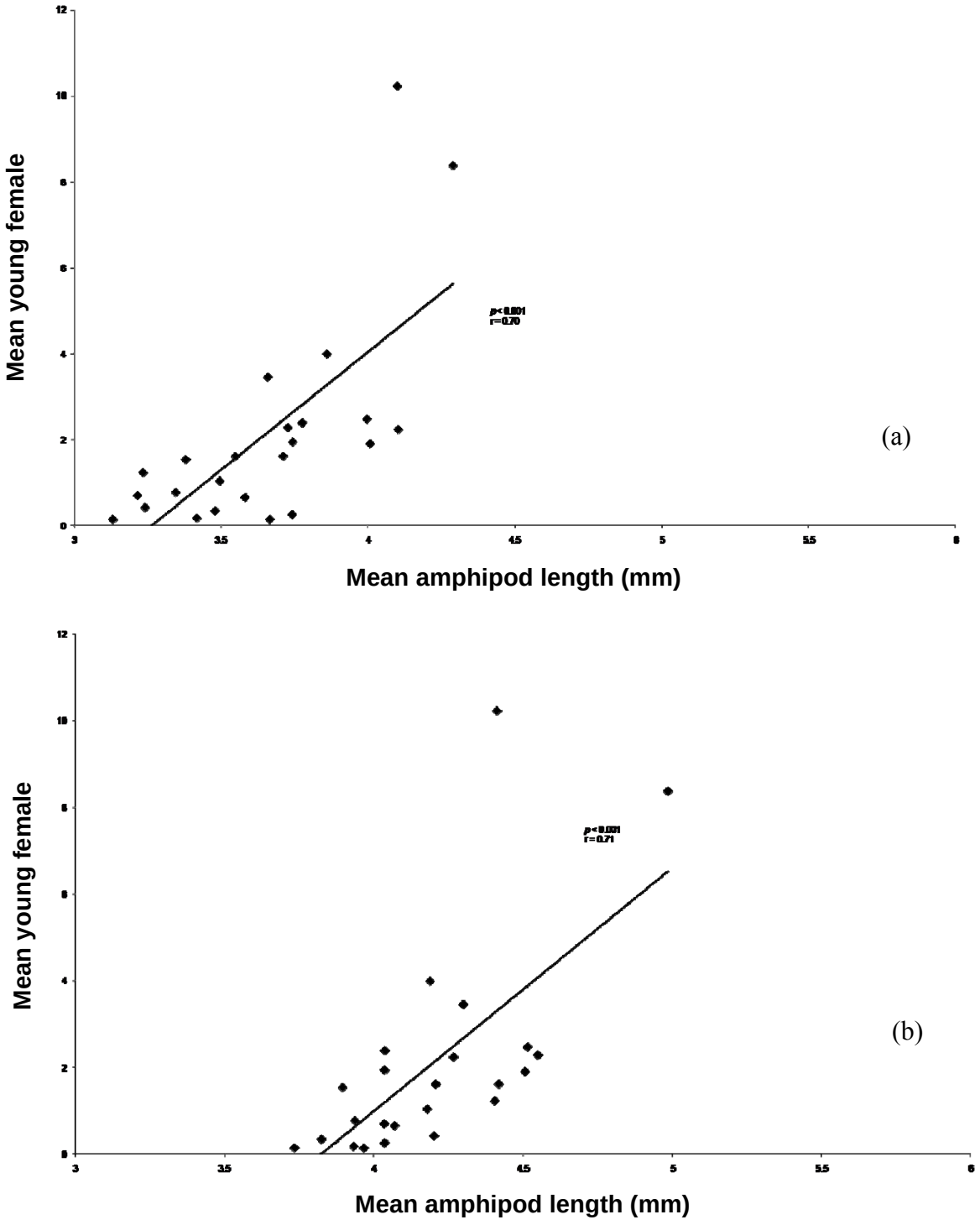

Figure 9. Relationships between mean amphipod length and reproduction after 28 (a) and 42 (b) days exposure to sediment collected during spring 2003 from study and reference intermittent streams in Southern Illinois.