

# **Evaluation of the Toxicity of Sediments from the Anniston PCB Site to the Mussel *Lampsilis siliquoidea***

## **Version 2.0**

Prepared for:

**U.S. Fish and Wildlife Service**  
**Alabama Field Office-Birmingham Suboffice**  
800 Lakeshore Dr., Rm. 229 Propst Hall  
Birmingham, AL 35229-2234

Prepared – *September, 2015* – by:

**MacDonald Environmental Sciences Ltd.**  
#24 - 4800 Island Highway North  
Nanaimo, British Columbia V9T 1W6

and

**U.S. Geological Survey**  
**Columbia Environmental Research Center**  
4200 New Haven Road  
Columbia, Missouri 65201



# **Evaluation of the Toxicity of Sediments from the Anniston PCB Site to the Mussel *Lampsilis siliquoidea***

**Version 2.0**

Prepared for:

**U.S. Fish and Wildlife Service  
Alabama Field Office-Birmingham Suboffice  
800 Lakeshore Dr., Rm. 229 Propst Hall  
Birmingham, AL 35229-2234**

Prepared – *September, 2015* – by:

**A. Schein, J.A. Sinclair, H.J. Prencipe, and D.D. MacDonald  
MacDonald Environmental Sciences Ltd.  
#24 - 4800 Island Highway North  
Nanaimo, British Columbia V9T 1W6**

and

**C.G. Ingersoll, N.E. Kemble, and J.L. Kunz  
U.S. Geological Survey  
Columbia Environmental Research Center  
4200 New Haven Road  
Columbia, Missouri 65201**

## **Evaluation of the Toxicity of Sediments from the Anniston PCB Site to the Mussel *Lampsilis siliquoidea* – Approval Page**

On behalf of the Natural Resource Trustees for the Anniston PCB Site, the Quality Assurance Officer has reviewed the document *Evaluation of the Toxicity of Sediments from the Anniston PCB Site to the Mussel Lampsilis siliquoidea* and found that this report is an acceptable summary of the toxicity testing with juvenile fatmucket mussels (*Lampsilis siliquoidea*) using sediments from the Anniston PCB Site.



Sept. 15, 2015

---

Carl Orazio, United States Geological Survey  
Quality Assurance Officer

Date

# Table of Contents

<b>Table of Contents.....</b>	<b>i</b>
<b>List of Tables. ....</b>	<b>iii</b>
<b>List of Figures.....</b>	<b>iv</b>
<b>List of Appendices. ....</b>	<b>vi</b>
<b>List of Acronyms. ....</b>	<b>vii</b>
<b>Preface.....</b>	<b>viii</b>
<b>Executive Summary.....</b>	<b>x</b>
<b>1.0 Introduction.....</b>	<b>1</b>
1.1 Background on Anniston Polychlorinated Biphenyl Site. ....	1
1.2 Purpose of this Study.....	2
<b>2.0 Methods.....</b>	<b>3</b>
2.1 Sample Collection and Processing.....	3
2.2 Chemical Analysis.....	4
2.3 Toxicity Testing.....	6
2.4 Data Compilation, Analysis, and Interpretation.....	8
2.4.1 Data Compilation. ....	8
2.4.2 Reference Envelope Development.....	9
2.4.3 Toxicity Designation. ....	10
2.5 Relative Endpoint Sensitivity Analysis. ....	11
2.6 Concentration-Response Models.....	12
<b>3.0 Results. ....</b>	<b>13</b>
3.1 Overlying Water Characteristics. ....	13
3.2 Sediment Characteristics. ....	14
3.3 Pore-Water Characteristics. ....	17
3.4 Sediment Toxicity. ....	19
3.5 Relative Endpoint and Relative Species Sensitivity. ....	20
3.6 Concentration-Response Models.....	21
<b>4.0 Summary and Conclusions. ....</b>	<b>23</b>
4.1 Summary. ....	23

4.2	Uncertainties. ....	24
4.3	Conclusions. ....	25
<b>5.0</b>	<b>References. ....</b>	<b>26</b>

## List of Tables

<b>Table 1</b>	General activity schedule for conducting a 28-day sediment toxicity test with <i>Lampsilis siliquoidea</i> . . . . .	T-1
<b>Table 2</b>	Test conditions for conducting a long-term sediment toxicity test with the amphipod <i>Hyaella azteca</i> , the midge <i>Chironomus dilutus</i> , or the mussel <i>Lampsilis siliquoidea</i> . . . . .	T-2
<b>Table 3</b>	Summary of test conditions for conducting reference toxicant tests. . . . .	T-4
<b>Table 4</b>	Test acceptability requirements for a 28-day sediment toxicity test with <i>Lampsilis siliquoidea</i> . . . . .	T-5
<b>Table 5</b>	Summary of the results of sediment toxicity tests conducted on the reference samples that were selected for the Anniston PCB Site. . . .	T-6
<b>Table 6</b>	Summary of the toxicity of sediment samples from the Anniston PCB Site to the mussel <i>Lampsilis siliquoidea</i> . . . . .	T-7
<b>Table 7</b>	Sediment chemistry results from the samples that were tested in both mussel and amphipod or midge exposures, unless otherwise noted. . . . .	T-9
<b>Table 8</b>	Reliability of the sediment toxicity thresholds that were derived based on the results of 28-day toxicity tests with <i>Lampsilis siliquoidea</i> (endpoint: biomass). . . . .	T-10

## List of Figures

- Figure 1** Anniston PCB site locations where sediment samples were collected from test sites and from one reference site..... F-1
- Figure 2** Locations where sediment samples used in mussel toxicity testing were collected. .... F-2
- Figure 3** Total polychlorinated biphenyls (PCBs) in pore water ( $\mu\text{g/L}$ ), as determined from SPME for exposures with the amphipod *Hyaella azteca* (*H. azteca*), the midge *Chironomus dilutus* (*C. dilutus*), the oligochaete *Lumbriculus variegatus*, and the mussel *Lampsilis siliquoidea*, compared to PCBs in sediment as grams per kilogram normalized to organic carbon (i.e., at 100% OC; g/kg OC) for cycle 1a (Figure 3A) and cycle 1b (Figure 3B)..... F-3
- Figure 4** Total polychlorinated biphenyls (PCBs) in pore water measured in whole-sediment exposures for select sediments from cycle 1a and cycle 1b for the midge *Chironomus dilutus*, the oligochaete *Lumbriculus variegatus*, and the mussel *Lampsilis siliquoidea* expressed as a fraction of the concentration measured in exposures using *Hyaella azteca*..... F-4
- Figure 5** Comparison of day 28 survival of *Lampsilis siliquoidea* to responses for other endpoints (all values are expressed as percent of control). .... F-5
- Figure 6** Comparison of day 28 biomass of *Lampsilis siliquoidea* to responses for other endpoints (all values are expressed as percent of control). .... F-6
- Figure 7** Comparison of day 28 weight of *Lampsilis siliquoidea* to responses for other endpoints (all values are expressed as percent of control). .... F-7
- Figure 8** Comparison of day 28 length of *Lampsilis siliquoidea* to responses for other endpoints (all values are expressed as percent of control). .... F-8

- 
- Figure 9** Relative endpoint sensitivity of the most responsive *Lampsilis siliquoidea* endpoint (biomass) and the most responsive *Chironomus dilutus* endpoint (adult biomass)..... F-9
- Figure 10** Relative endpoint sensitivity of the most responsive *Lampsilis siliquoidea* endpoint (biomass) and the most responsive *Hyaella azteca* endpoint (survival-normalized young per female). . . . . F-10
- Figure 11** Relative endpoint sensitivity of the most responsive *Lampsilis siliquoidea* endpoint (biomass) and *Chironomus dilutus* biomass. F-11
- Figure 12** Relative endpoint sensitivity of the most responsive *Lampsilis siliquoidea* endpoint (biomass) and *Hyaella azteca* biomass. . . . F-12
- Figure 13** Concentration-response model for total polychlorinated biphenyls (PCBs; µg/kg DW) and *Lampsilis siliquoidea* day 28 biomass... F-13
- Figure 14** Concentration-response model for total polychlorinated biphenyls (PCBs; at 1 percent OC; µg/kg) and *Lampsilis siliquoidea* day 28 biomass. . . . . F-14



## List of Appendices

**Appendix 1**    Toxicity test results from the *Lampsilis siliquoidea* exposures

## List of Acronyms

ARCADIS	ARCADIS U.S., Inc.
AVS	acid volatile sulfide
COPC	chemical of potential concern
CRM	concentration-response model
DDD	dichlorodiphenyldichloroethane
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
DOC	dissolved organic carbon
DW	dry weight
ERDC	Engineer Research and Development Center
GIS	geographic information system
GPS	global positioning system
HCH	hexachlorocyclohexane
HT	highly toxic
LC <sub>50</sub>	median lethal effect concentration
MT	moderately toxic
OC	organic carbon
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PCDDs/PCDFs	polychlorinated dibenzo- <i>p</i> -dioxins/polychlorinated dibenzofurans
PDMS	polydimethylsiloxane
PEC	probable effect concentration
PEC-Q	probable effect concentration quotient
RI/FS	remedial investigation/feasibility study
SEM	simultaneously extracted metals
SPME	solid-phase microextraction
SVOC	semivolatile organic compound
TEQ	toxicity equivalents
TOC	total organic carbon
TT	toxicity threshold
TT <sub>HR</sub>	high-risk toxicity threshold
TT <sub>LR</sub>	low-risk toxicity threshold
USEPA	U.S. Environmental Protection Agency
USGS-Columbia	U.S. Geological Survey Columbia Environmental Research Center
VOC	volatile organic compound

## Preface

This report is a companion to Ingersoll *et al.* (2014a), which presents data on the laboratory toxicity testing of polychlorinated biphenyl (PCB)-contaminated sediments from Anniston, Alabama to the amphipod, *Hyaella azteca*, and the midge, *Chironomus dilutus*, as well as data on bioaccumulation by the oligochaete, *Lumbriculus variegatus*. The same sediment samples used to assess toxicity to *Hyaella azteca* and *Chironomus dilutus* in Ingersoll *et al.* (2014a) were used to assess toxicity to the juvenile fatmucket mussel, *Lampsilis siliquoidea*, in this report. The mussel toxicity test results that are presented in this report were conducted as part of the ongoing natural resource damage assessment at the Anniston PCB Site (For more information about the natural resource damage assessment and restoration see: <http://www.fws.gov/daphne/Contaminants/index-AnnistonNRDA.html>). The natural resource trustees at the Anniston PCB Site are the U.S. Fish and Wildlife Service and the State of Alabama.

## **Acknowledgments**

Many individuals at the U.S. Geological Survey Columbia Environmental Research Center (USGS-Columbia), Columbia, Missouri contributed to the success of this project including: John Besser, Bill Brumbaugh, Eric Brunson, Rebecca Consbrock, Doug Hardesty, Jamie Hughes, Chris Ivey, Brittney King, Carl Orazio, Ning Wang, Dave Whites, Jesse Arms, Shannon Earhart, Tom May, Vanessa Melton-Silvey, and Mike Walther. We also thank Jacob Stanley (ERDC) and Robert Bringolf (University of Georgia), for providing review comments on a draft of this report. Their excellent comments and suggestions are gratefully appreciated.

## Executive Summary

The Anniston Polychlorinated Biphenyl (PCB) Site is located in the vicinity of the municipality of Anniston in Calhoun County, in the north-eastern portion of Alabama. Although there are a variety of land-use activities within the Choccolocco Creek watershed, environmental concerns in the area have focused mainly on releases of PCBs to aquatic and riparian habitats. PCBs were manufactured by Monsanto, Inc. at the Anniston facility from 1935 to 1971. The chemicals of potential concern (COPCs) in sediments at the Anniston PCB Site include: PCBs, mercury, metals, polycyclic aromatic hydrocarbons (PAHs), organochlorine and organophosphorous pesticides, volatile organic compounds (VOCs), semivolatile organic compounds (SVOCs), and polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDDs/PCDFs).

The purpose of this study was to evaluate the toxicity of PCB-contaminated sediments to the juvenile fatmucket mussel (*Lampsilis siliquoidea*) and to characterize relationships between sediment chemistry and the toxicity of sediment samples collected from the Anniston PCB Site using laboratory sediment testing. Samples were collected in August 2010 from OU-4 of the Anniston PCB Site, as well as from selected reference locations. A total of 32 samples were initially collected from six test sites and one reference site within the watershed. A total of 23 of these 32 samples were evaluated in 28-day whole-sediment toxicity tests conducted with juvenile mussels (*L. siliquoidea*). Physical and chemical characterization of whole sediment included grain size, total organic carbon (TOC), nutrients, PCBs, parent and alkylated PAHs, organochlorine pesticides, PCDD/PCDFs, total metals, simultaneously extracted metals (SEM), and acid volatile sulfide (AVS).

Sediment collected from Snow Creek and Choccolocco Creek contained a variety of COPCs. Organic contaminants detected in sediment included PCBs, organochlorine pesticides, PCDDs/PCDFs, and PAHs. In general, the highest concentrations of PCBs were associated with the highest concentrations of PAHs, PCDDs/PCDFs, and organochlorine pesticides. Specifically, sediments 08, 18, and 19 exceeded probable effect concentration quotients (PEC-Qs) of 1.0 for all organic classes of contaminants. These three sediment samples also had high concentrations of mercury and lead, which were the only metals found at elevated concentrations (i.e., above the probable effect concentration [PEC]) in the samples collected. Many sediment samples were highly contaminated with mercury, based on comparisons to samples collected from reference locations.

The whole-sediment laboratory toxicity tests conducted with *L. siliquoidea* met the test acceptability criteria (e.g., control survival was greater than or equal to 80%).

Survival of mussels was high in most samples, with 4 of 23 samples (17%) classified as toxic based on the survival endpoint. Biomass and weight were more sensitive endpoints for the *L. siliquoidea* toxicity tests, with both endpoints classifying 52% of the samples as toxic. Samples 19 and 30 were most toxic to *L. siliquoidea*, as they were classified as toxic according to all four endpoints (survival, biomass, weight, and length).

Mussels were less sensitive in toxicity tests conducted with sediments from the Anniston PCB Site than *Hyaella azteca* and *Chironomus dilutus*. Biomass of *L. siliquoidea* was less sensitive compared to biomass of *H. azteca* or biomass of larval *C. dilutus*. Based on the most sensitive endpoint for each species, 52% of the samples were toxic to *L. siliquoidea*, whereas 67% of sediments were toxic to *H. azteca* (based on reproduction) and 65% were toxic to *C. dilutus* (based on adult biomass). The low-risk toxicity threshold ( $TT_{LR}$ ) was higher for *L. siliquoidea* biomass (e.g., 20,400  $\mu\text{g/kg}$  dry weight [DW]) compared to that for *H. azteca* reproduction (e.g., 499  $\mu\text{g/kg}$  DW) or *C. dilutus* adult biomass (e.g., 1,140  $\mu\text{g/kg}$  DW; MacDonald *et al.* 2014). While mussels such as *L. siliquoidea* are known to be sensitive to some contaminants in water or in sediment (including ammonia, metals, or PAHs; e.g., Wang *et al.* 2007; 2010; 2011; 2013; Besser *et al.* 2013; 2015), *L. siliquoidea* appears to be less sensitive to PCBs associated with sediments than are *H. azteca* or *C. dilutus* (based on biomass of *L. siliquoidea*, reproduction of *H. azteca*, and adult biomass of *C. dilutus*).

## **1.0 Introduction**

### **1.1 Background on Anniston Polychlorinated Biphenyl Site**

The Anniston Polychlorinated Biphenyl (PCB) Site is located in the vicinity of the municipality of Anniston in Calhoun County, in the north-eastern portion of Alabama. Although there are a variety of land-use activities within the Choccolocco Creek watershed, environmental concerns in the area have focused mainly on releases of PCBs. PCBs were manufactured by Monsanto, Inc. at the Anniston facility from 1935 to 1971. While the facility was in operation, PCBs may have been released in production waste effluent discharges, stormwater runoff, accidental spills, uncontrolled releases from landfills, and other sources. The total mass of PCBs released from the Anniston facility is uncertain, however.

In response to public concerns, a remedial investigation/feasibility study (RI/FS) is being conducted by the U.S. Environmental Protection Agency (USEPA) to assess risks to human health and ecological receptors associated with exposure to contaminants at the Anniston PCB Site. The results of the RI/FS will be used to evaluate remedial options for addressing concerns related to environmental contamination at the Anniston PCB Site. For the purposes of the RI/FS, the USEPA has defined the Anniston PCB Site as consisting of the area where hazardous substances, including PCBs (associated with the historical and ongoing operations at the Anniston Plant by Solutia, Monsanto Company, and their predecessors), have come to be located. However, the area currently under investigation under the RI/FS extends from the Anniston facility to the mouth of Choccolocco Creek. This portion of the Anniston PCB Site was divided into four operable units, including the Solutia, Inc. facility (OU-3), the Anniston residential (OU-1) and non-residential (OU-2) areas located downstream of the facility within the Snow Creek watershed, and Choccolocco Creek from the backwater area to the Coosa River (OU-4; BBL 2003). Subsequently, OU-1 and OU-2 were combined to include all of the affected aquatic and floodplain areas within the Snow Creek watershed.

---

The chemicals of potential concern (COPCs) in sediments at the Anniston PCB Site include: PCBs, mercury, metals, polycyclic aromatic hydrocarbons (PAHs), organochlorine and organophosphorous pesticides, volatile organic compounds (VOCs), semivolatile organic compounds (SVOCs), and polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDDs/PCDFs). According to ARCADIS (2009), VOCs, SVOCs other than PAHs, and organophosphorous pesticides were not observed at frequencies or concentrations of concern. Although PAHs and organochlorine pesticides are not likely primary risk drivers, these additional compounds were included in the analyses of sediment samples in this study to help to identify other contaminants that might be associated with the observed toxicity of the Anniston PCB Site sediments.

## 1.2 Purpose of this Study

This study was conducted to evaluate the toxicity of sediments from the Anniston PCB Site to juvenile fatmucket mussels (*Lampsilis siliquoidea*) and to characterize relationships between sediment chemistry and toxicity using laboratory sediment testing. *Lampsilis siliquoidea* was the species of mussel selected for testing because this species has been routinely used to conduct water and sediment toxicity tests, is sensitive to contaminants of concern in sediments, and exhibits a sensitivity to contaminants similar to other mussels (e.g., Wang *et al.* 2007; 2010; 2011; 2013; Besser *et al.* 2013; 2015; Ingersoll *et al.* 2015). Sediment samples were collected in August 2010 from OU-4 of the Anniston PCB Site, as well as an upstream reference location. A total of 32 sediment core samples were collected from six test sites and one reference site, from locations/depths expected to represent a range of concentrations of PCBs in sediments at the Anniston PCB Site (Figure 1). A total of 23 of these 32 samples were evaluated in 28-day whole-sediment laboratory toxicity tests with juvenile *L. siliquoidea* (Figure 2), following methods adapted from ASTM International (2015a; 2015b), USEPA (2000), Wang *et al.* (2007), and Ingersoll *et al.* (2008). Samples used to conduct sediment toxicity tests with *L. siliquoidea* were selected based on measured concentrations of PCBs in the sediments and were

---



selected to represent a broad range of PCB concentrations. See Ingersoll *et al.* (2014a) for more information on this and other factors considered in the selection of sediment samples for the *L. siliquoidea* toxicity tests. Sediment samples used in the toxicity tests were analyzed for PCB Aroclors, homologs, and select congeners, as well as metals, organochlorine pesticides, PAHs, and PCDDs/PCDFs.

## **2.0 Methods**

### **2.1 Sample Collection and Processing**

A total of 32 samples of sediment were collected from six test sites within OU-4 of the Anniston PCB Site and one upstream reference site in August 2010 (Figure 1). Detailed maps identifying the locations where test and reference samples were collected are provided in ARCADIS (2010). The sampling program was designed to target six concentration ranges of organic carbon (OC)-normalized total PCBs in the sediment samples collected from the Anniston PCB Site (ARCADIS 2010), including:

- Less than 100 mg PCB/kg OC;
- 100 to 500 mg PCB/kg OC;
- 500 to 1,000 mg PCB/kg OC;
- 1,000 to 5,000 mg PCB/kg OC;
- 5,000 to 10,000 mg PCB/kg OC; and,
- More than 10,000 mg PCB/kg OC.

Data used to select the locations for sediment sampling in the current study were obtained from an Off-Site Resource Conservation and Recovery Act Facility Investigation Work Plan (BBL 2000) and the Phase 1 Field Sampling Plan for OU-4 (BBL 2006).

---

Upon arrival at a sampling site, the field crew determined if site conditions allowed for sediment sample collection (i.e., there were no concerns regarding access or safety and fine-grained sediments were available). The field sampling crew recorded the location of each sampling site on the field data-collection forms, along with a description of the physical characteristics of the site. The location was acquired with a hand-held global positioning system (GPS) unit. A boat (if used) was positioned near the sampling location within a site. A GPS reading was then taken at the location where samples were collected within a site (Table C2-1 in Ingersoll *et al.* 2014b).

A full description of the procedures used to collect sediment samples can be found in Ingersoll *et al.* (2014b). Samples of sediment were collected from the reference site using a grab sampler (posthole digger) and sediment samples from the test sites were collected using core samplers (i.e., 10-cm Lexan®<sup>1</sup> cores) in accordance with procedures outlined in ARCADIS (2010). Equipment used to collect sediment was decontaminated by brushing to remove sediment particles, washing with soapy water, and rinsing with site water between collections of each set of samples. Once all of the sediment samples were collected and processed, they were delivered to the U.S. Geological Survey Columbia Environmental Research Center (USGS-Columbia) laboratory using Federal Express Custom Critical refrigerated shipping. The sediments were logged in at USGS-Columbia and stored at 4°C in the dark under chain of custody in a secured refrigeration unit.

## 2.2 Chemical Analysis

All of the samples collected at the Anniston PCB Site were analyzed to support physical and chemical characterization. Physical characterization of whole sediment samples included analyses of grain size, total organic carbon (TOC), and nutrients. Organic contaminant chemical characterization of whole sediment samples included PCB homologs, Aroclors, and select (13) PCB congeners, parent and alkylated PAHs,

---

<sup>1</sup>Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government

---

organochlorine pesticides, and PCDD/PCDFs. The PCB Aroclors analyzed included 1016, 1221, 1232, 1242, 1248, 1254, 1260, 1262, and 1268 (which represent the complete suite of Aroclors produced in North America). Analyses of whole sediment also included total metals, simultaneously extracted metals (SEM), and acid volatile sulfide (AVS). Chemical characterization of pore water samples isolated from samples of whole sediment at the start of the sediment toxicity exposures included major cations, major anions, dissolved organic carbon (DOC), and additional water-quality characteristics.

The grain size, TOC, and chemical analyses of whole sediment were done under the direction of ARCADIS U.S., Inc. (ARCADIS; ARCADIS 2011). Also see Ingersoll *et al.* (2014b) for details on the specific methods used for the chemical analyses; Table C2-2 provides a summary of the analytical methods, responsibilities, containers, volume requirements, preservation, and holding times for sediment samples. The chemical characterization of pore water was done under the direction of the USGS-Columbia laboratory. For the *L. siliquoidea* tests, pore-water samples were collected by centrifugation of whole sediment at 5,200 revolutions per minute (7000 times relative centrifugal force) for 15 minutes at 4°C at the start of the toxicity exposures. Details on how sub-samples for major cations, major anions, sulfide, or DOC were collected, processed, and stored are provided in Ingersoll *et al.* (2014b). Centrifuged pore-water samples were also analyzed for general water-quality characteristics including pH, alkalinity, hardness, conductivity, ammonia, and hydrogen sulfide, using standard methods (APHA, AWWA, and WEF 2005). A summary of the analytical methods and targets for selected measures of accuracy and precision for these analytes is provided in Table A1-1 of Appendix 1 to Ingersoll *et al.* (2014a). More details on the pore-water analyses are also presented in the text of Ingersoll *et al.* (2014b). In addition, polydimethylsiloxane (PDMS) solid-phase microextractions (SPMEs) were used to estimate pore-water concentrations of PCBs (i.e., deployed as passive samplers in each sediment that was evaluated in the *L. siliquoidea* toxicity testing). The SPME samplers were placed in separate replicate sediment toxicity test chambers for 28 days to measure the bioavailable pore-water fraction of PCBs. Full details on the methods and data analysis used to deploy, sample, and analyze PCBs

---

that accumulated in the SPME fibers and methods used to estimate pore-water concentrations of PCBs in the toxicity sediment exposures are provided in Steevens *et al.* (2014).

## 2.3 Toxicity Testing

The sediment samples used in the *L. siliquoidea* laboratory toxicity testing described in this report were also used in the *H. azteca* and *C. dilutus* laboratory toxicity testing described in Ingersoll *et al.* (2014a). Because of the large number of samples and limited capacity of the laboratories running the tests, sediment toxicity testing with *H. azteca* and *C. dilutus* and bioaccumulation testing with *L. variegatus* was done in two cycles (cycle 1a and cycle 1b). A total of 26 of the 32 site samples were tested with *H. azteca* and *C. dilutus*, while 14 of the 32 site samples were tested with *L. variegatus*. Results of 10-day range-finding toxicity tests with *H. azteca* and with *L. variegatus* and initial characterization of TOC and total PCBs were used to select the samples for sediment toxicity testing in cycle 1a and in cycle 1b. Summaries of cycle 1a toxicity data also were used to select samples for toxicity testing in cycle 1b. See Ingersoll *et al.* (2014b) for more details on the results of range-finding tests and the selection of samples for cycle 1a and cycle 1b testing. The sediment toxicity testing with *L. siliquoidea* was done between the start of cycle 1a and cycle 1b toxicity testing with *H. azteca* and *C. dilutus*. A total of 23 of the 32 sediment samples were used in the *L. siliquoidea* toxicity tests (Table C2-1 in Ingersoll *et al.* 2014b).

Methods for preparing the sediment samples for testing with mussels were similar to those used in the midge and amphipod testing. Each sediment sample was re-homogenized with a hand-held power drill and stainless steel auger about 7 days before the start of the sediment exposures (that is Day minus 7). Exposures of test organisms were started on December 9, 2010 (Day 0). From about Day minus 7 to Day 0, the exposure chambers containing sediment and overlying water were maintained at 23°C without renewal of overlying water to allow sediments to equilibrate in the exposure chambers (Ingersoll *et al.* 2008). A general activity

---

schedule for conducting a long-term sediment toxicity test with *L. siliquoidea* is provided in Table 1. For more detailed information on the preparation of the toxicity tests see Ingersoll *et al.* (2014b) and Stanley *et al.* (2014).

The USGS-Columbia laboratory conducted 28-day sediment toxicity tests with juvenile mussels, *L. siliquoidea*, following methods adapted from ASTM International (2015a; 2015b), USEPA (2000), Wang *et al.* (2007), and Ingersoll *et al.* (2008). Exposures were done in 300 mL high-form lipless beakers with a sediment volume of 100 mL and an overlying water volume of 175 mL. The beakers were fitted with notches covered by stainless steel mesh screen to allow for daily flow-through water renewal. Testing was done at 23°C using a 16:8 light:dark cycle. A laboratory control sediment (West Bearskin Lake sediment, about 1% TOC; Ingersoll *et al.* 1998) was run with the toxicity test. More details on the test conditions are provided in Table 2. Test conditions and methods are similar to those for *H. azteca* and *C. dilutus* described in Stanley *et al.* (2014). One separate replicate SPME beaker for estimating pore-water concentrations of PCBs was included per treatment, but there were no replicate peeper beakers for measuring pore-water metals (Steevens *et al.* 2014). An acute reference toxicity test with sodium chloride was conducted according to methods outlined in Table 3 to provide an indicator of organism health at the time of testing. The 96-hour median lethal effect concentration (LC<sub>50</sub>) from this test was 4.0 g NaCl/L (no confidence intervals could be calculated due to lack of partial responses across exposure concentrations) and is representative of historic effect concentrations of NaCl for *L. siliquoidea* generated at USGS-Columbia. Test acceptability requirements for a long-term sediment toxicity test with *L. siliquoidea* are described in Table 4.

Overlying water additions were started the day before organisms were added to test beakers containing sediment. Overlying water was added three times daily using automated water renewal systems at the rate of about two volume additions per day. The water used in the toxicity tests was well water diluted with deionized water to achieve a hardness of about 100 mg/L as CaCO<sub>3</sub>, alkalinity 85 mg/L as CaCO<sub>3</sub>, and pH about 8.0. The *L. siliquoidea* used to start the exposures were about 2 months old

---

(mean starting length 2.72 mm/individual and mean starting weight of 1.0 mg dry weight [DW]/individual; Appendix 1) and were obtained from juvenile organisms transformed on Largemouth Bass (*Micropterus salmoides*) at Missouri State University in Springfield MO and cultured at USGS-Columbia (Wang *et al.* 2007; 2010). Acclimation of *L. siliquoidea* to test water and temperature occurred in one liter beakers for 48 hours before the start of the exposures. At the beginning of the exposures, 10 mussels were transferred into each of four replicate beakers per treatment. The mussels were fed a non-viable algae twice daily, as described in Wang *et al.* (2007) and ASTM International (2015b). At the end of the toxicity tests, the sediment was washed through a US #50 sieve (0.3-mm opening) and the mussels and remaining sediment were rinsed into a glass tray. In order to determine survival (foot movement within a 5-minute observation period), the mussels from each replicate were placed in a 50-mL glass beaker containing about 20 mL of water and were observed under a dissecting microscope. The *L. siliquoidea* that survived were preserved in 8% formalin to support dry weight measurement after drying for 24 hours at 60°C. The dry weight was measured to the nearest 0.001 mg with a microbalance (Model MX5, Mettler Toledo, Columbus, OH) in order to determine biomass (total dry weight of surviving mussels in a replicate).

## **2.4 Data Compilation, Analysis, and Interpretation**

### **2.4.1 Data Compilation**

The whole-sediment and pore-water chemistry data from the 23 Anniston PCB Site sediment samples used in the *L. siliquoidea* toxicity tests were compiled in a geographic information system (GIS)-compatible relational database (Ingersoll *et al.* 2014a). In addition, the toxicity test endpoint data were also compiled in the project database so that analyses could be performed on matching chemistry and toxicity data.

---

### 2.4.2 Reference Envelope Development

A reference-envelope approach was used to classify sediments from the Anniston PCB Site as highly toxic, moderately toxic, or not toxic (Hunt *et al.* 2001; Ingersoll *et al.* 2009; Besser *et al.* 2009; Wang *et al.* 2013; Kemble *et al.* 2013; MacDonald *et al.* 2014). The reference-envelope approach is a procedure for assessing sediment toxicity that was developed to overcome the limitations associated with the use of control sediments for this purpose, including accounting for differences in the non-contaminant characteristics of test sediments and for overcoming the low statistical power associated with comparing many test results to a single control sediment with physical characteristics that may differ from site sediments.

Application of the reference envelope approach necessitates identification of reference sediment samples for each toxicity test endpoint that is evaluated. That is, all of the sediment samples in the mussel toxicity testing that met the selection criteria were considered to be candidate reference sediment samples. Candidate reference sediment samples were evaluated using chemical criteria and biological criteria. As a first step, sediment samples with chemical characteristics representative of reference conditions were identified (that is, samples substantially free of contamination). The specific chemical criteria used for reference sediment identification are outlined in Stanley *et al.* (2014). As a second step, sediment samples that met survival test acceptability requirements of a control sediment were identified (i.e., minimum of 80% survival on Day 28). If one or more chemical or biological criteria were not met, data for that sample were not used in the reference-envelope calculation. Sediment samples that met the chemical criteria and biological criteria were included in the pool of reference sediment. Of the six samples initially categorized as reference sediments before the start of sediment collection (sediments 04, 09, 10, 22, 26, and 29; Table C2-1 in Ingersoll *et al.* 2014b), five of these sediments met all of the chemical and biological criteria for inclusion in the reference envelopes for the toxicity test endpoints. One of the six reference sediments (sediment 04) was excluded as a reference sediment due to the presence of elevated sediment PAH concentrations (Table A1-3a of Appendix 1 and Table A4-1 of Appendix 4 to Ingersoll *et al.* 2014a).

---

Responses of *L. siliquoides* in test sediments were normalized to the responses in control sediment in order to account for variability in the test response data because of organism health, test procedures, and test conditions. Specifically, toxicity test response data for each endpoint were normalized to the mean response observed in the control treatment (Ingersoll *et al.* 2008).

Following the identification of reference sediment samples, the range of the responses observed in mussels exposed to these samples was determined for each endpoint measured in the *L. siliquoides* toxicity test. In this study, the reference envelope was defined as the range of responses observed for the selected reference sediment samples. Accordingly, the lower limit of the reference envelope was calculated as the minimum control-adjusted response value for each endpoint, using the data for the reference sediment samples that were selected (Table 5; Besser *et al.* 2009; Moran *et al.* 2012; MacDonald *et al.* 2012; Kemble *et al.* 2013; Wang *et al.* 2013; MacDonald *et al.* 2014).

### **2.4.3 Toxicity Designation**

The reference envelope was considered to define the normal range of responses associated with exposure of toxicity test organisms to relatively uncontaminated sediment samples. Sediment samples with effect values lower than the lower limit of the normal range of control-adjusted responses for the reference samples (that is, lower than the minimum value) were designated as toxic for the endpoint under consideration. The sediment samples also were designated as toxic or not toxic based on the results of multiple endpoints in the toxicity test (that is, survival, biomass, weight, or length). Finally, sediment samples were designated as toxic or not toxic based on the results obtained from any of the toxicity test endpoints. The toxicity designations that were assigned to each of the sediment samples that were included in the project database are listed in Table 6.

---



Classification of sediment samples as toxic or not toxic provides important information for assessing sediment quality conditions. However, data on the magnitude of toxicity can contribute to such evaluations. For this reason, toxic sediment samples were further classified to identify moderately toxic and highly toxic sediment samples. Highly toxic (HT) sediment samples were identified based on a greater than 10% reduction in survival, biomass, weight, or length, relative to the lower limit of the reference envelope (MacDonald *et al.* 2002; 2012; Table 6). Moderately toxic (MT) sediment samples were identified based on survival, biomass, weight, or length that fell less than 10% below the lower limit of the reference envelope.

## 2.5 Relative Endpoint Sensitivity Analysis

One goal of this study was to determine which *L. siliquoidea* toxicity test endpoint was most sensitive to contaminants in sediment, and whether the mussel *L. siliquoidea* was as sensitive to contaminant exposures as the more commonly tested invertebrates *H. azteca* and *C. dilutus*. Therefore, the various mussel endpoints (survival, biomass, weight, and length) were plotted against each other, after being expressed as a percentage of the control response. A line of unity was added to the plots representing equal response between the two endpoints, and this line was bracketed with red lines representing a 20-percent difference above or below the line of unity as a visualization tool to aid in the comparison between endpoints. Data points above this line of unity indicate that the endpoint on the x-axis was more responsive; whereas, data points below this line of unity indicate that the endpoint on the y-axis was more responsive. Vertical and horizontal lines on these graphs represent the lower distribution of the threshold reference-envelope response for the variable on the x and y axes. Open symbols represent reference sediments and closed symbols represent test sediments in these graphs. In addition, the most sensitive mussel endpoint was plotted against the most sensitive *H. azteca* endpoint (survival-normalized young per female) and the most sensitive *C. dilutus* endpoint (adult biomass; from Ingersoll *et al.* 2014a) in the same way described above to assess

---

relative endpoint sensitivity for the three species. Finally, *L. siliquoidea* biomass was compared to *C. dilutus* and *H. azteca* biomass.

## 2.6 Concentration-Response Models

The sediment chemistry and sediment toxicity results were used to develop concentration-response models (CRMs) for total PCBs (both DW and at 1% OC) and *L. siliquoidea* biomass. Total PCBs was chosen for the CRMs for *L. siliquoidea* because PCBs are the main COPCs at the Anniston PCB Site and total PCB concentration was found to result in one of the highest  $R^2$ -values among all COPCs evaluated with the CRMs developed in MacDonald *et al.* (2014) for *H. azteca* and *C. dilutus*. Biomass was used in CRM development because it was the most sensitive *L. siliquoidea* endpoint, based on evaluation of the relative endpoint sensitivity plots (Section 3.5). Development of the CRMs involved plotting the total PCB concentration data against the corresponding response data and determining the dependence of the toxicity test response data (dependent variables) on the total PCB concentration data (independent variables), as described in MacDonald *et al.* (2002; 2003; 2005a; 2005b; 2009; 2010; 2012; 2014). The CRMs were developed using a log-logistic CRM (Seefeldt *et al.* 1995; MacDonald *et al.* 2010) using the following equation:

$$f(x) = \frac{a}{1 + \left(\frac{x}{EC_{50}}\right)^b}$$

where:

- $a$  = Upper limit of the response (asymptote);
- $EC_{50}$  = Estimated median effect concentration; and,
- $b$  = Slope at the estimated median effect concentration.

The median effect concentration in the above model provides an estimate of the total PCB concentration where a 50% effect is predicted to be observed (for example, 50%

---

decline in survival relative to the mean reference response). The distribution of responses for *L. siliquoidea* biomass was tested for normality using the Shapiro-Wilk test (Zar 1999) before CRM development; the residuals were normally distributed (Shapiro-Wilk test;  $W = 0.951, p = 0.304$  and  $W = 0.983, p = 0.952$ ) and no bias was observed (visual assessment of the Q-Q plots). All of the relationships were described using the R environment for statistical computing and graphics (R Development Core Team 2014).

Site-specific sediment toxicity thresholds (TTs) were established for total PCBs and *L. siliquoidea* biomass, based on the site-specific CRMs that were derived. Two TTs were calculated for each total PCB-endpoint pair, including a low-risk TT ( $TT_{LR}$ ) and a high-risk TT ( $TT_{HR}$ ). The  $TT_{LR}$  values were calculated by determining the concentrations of total PCBs that corresponded to the response rates at the lower limit of the reference envelope (Besser *et al.* 2009; Kemble *et al.* 2013; MacDonald *et al.* 2012; 2014; Wang *et al.* 2013). By comparison, the  $TT_{HR}$  values were calculated by determining the concentrations of total PCBs that corresponded to the response rates at 10% below the lower limit of the reference envelope (MacDonald *et al.* 2010; 2012; 2014). These TTs were estimated using the regression equations that were developed for the corresponding CRMs.

## 3.0 Results

### 3.1 Overlying Water Characteristics

A complete summary of the overlying water quality from the *L. siliquoidea* toxicity tests is provided in Appendix 1, Tables A1-1 and A1-2. Temperature was measured daily. Dissolved oxygen and conductivity were measured the day before the toxicity test was initiated (Day minus 1) and on Days 7, 13, 21, and 27. Alkalinity, hardness, pH, and total ammonia were measured on Day minus 1 and Day 27. Means and ranges (in parentheses) of concentrations of water quality parameters from all samples over the whole exposure period were as follows: dissolved oxygen, 6.9 mg/L (5.0 -

---

8.3 mg/L); pH, 7.9 (7.6 - 8.1); alkalinity, 92 mg/L as CaCO<sub>3</sub> (86 - 100 mg/L as CaCO<sub>3</sub>); hardness, 108 mg/L as CaCO<sub>3</sub> (96 - 124 mg/L as CaCO<sub>3</sub>); conductivity, 259 µS/cm (250 - 270 µS/cm); and, total ammonia, 0.090 mg/L (0.017 - 0.511 mg/L).

## 3.2 Sediment Characteristics

A summary of the physical characteristics and concentrations of metals, PAHs, PCBs, organochlorine pesticides, and PCDDs/PCDFs in the whole-sediment samples is provided in Table A1-3a of Appendix 1 to Ingersoll *et al.* (2014a). One sample used in the *L. siliquoidea* toxicity tests (Station ID = TX60-01-P; Sample ID = 21) was not used in the *H. azteca* or *C. dilutus* toxicity tests and, thus, the sediment chemistry data were not reported in Ingersoll *et al.* (2014a). However, the sediment chemistry results from this sample fall within the ranges discussed below. Select sediment chemistry results from all the samples that were tested in the mussel, amphipod, and/or midge exposures are presented in Table 7. Briefly, the mean (and range) for the physical characteristics of the sediments tested with *H. azteca* and *C. dilutus* (Ingersoll *et al.* 2014a), were: solids, 64.4% (46.2–74.8%); TOC, 1.5% (0.22–3.99%); clay, 19.9% (0.0–41.1%); silt, 28.8% (3.2–54.3%); and sand, 50.5% (9.6–97.1%).

Organic contaminants detected in sediment included PCBs, organochlorine pesticides, PCDDs/PCDFs, and PAHs. The PCB data were reported as Aroclors, congeners, and homologs. The dominant Aroclor was 1242 (median = 15,000 µg/kg DW; maximum = 350,000 µg/kg) followed by Aroclor 1260, 1254, and 1268. The median concentration for total PCB Aroclors was 26,650 µg/kg and the maximum concentration was 476,000 µg/kg (Table A1-3a of Appendix 1 to Ingersoll *et al.* 2014a). Polychlorinated biphenyls, as Aroclors, were not detected in any of the reference or control samples. Based on the median homolog concentration, sediments were dominated, in decreasing order, by the dichloro-, trichloro-, monochloro-, and tetrachlorobiphenyl homolog groups. General trends indicated sediments with a higher concentration of total PCBs had a greater proportion of monochloro-, dichloro-, trichloro-, and tetrachlorobiphenyl homolog groups. In contrast, sediments

---

with lower concentrations of total PCBs were dominated by penta-, hexa-, and heptachlorobiphenyl. The probable effect concentration quotient (PEC-Q) for total PCBs was calculated using the total homolog concentration and dividing by the probable effect concentration (PEC) for total PCBs (expressed as  $PEC-Q_{TPCBs}$ ; MacDonald *et al.* 2000; Ingersoll *et al.* 2014a). Of the 26 sediment samples tested with *H. azteca* and *C. dilutus*, a total of 17 exceeded the  $PEC-Q_{TPCBs}$  of 1.0, and an additional three samples had a  $PEC-Q_{TPCB}$  between 0.1 and 1. Twenty-two of the 26 sediment samples tested with *H. azteca* and *C. dilutus*, plus one additional sample, were used in the *L. siliquoidea* toxicity tests. Individual PCB congeners 153 and 118 were the most abundant congeners, with concentrations as high as 5,900 and 3,200  $\mu\text{g/kg}$  measured, respectively. The PCB congeners 77, 81, 114, 157, and 169 were not detected in any sample, but the detection limits for these congeners were high in some of the samples (for example, greater than 500  $\mu\text{g/kg}$ ; Table A1-3a of Appendix 1 to Ingersoll *et al.* 2014a).

The organochlorine pesticides chlordane, dichlorodiphenyltrichloroethane (DDT) (including dichlorodiphenyldichloroethane [DDD], dichlorodiphenyldichloroethylene [DDE], and DDT), and hexachlorocyclohexane (HCH) were detected in up to half of the test sediments. When these compounds were detected, there was proportionally more HCH than total DDT and chlordane. One reference sample (sediment 09) contained minimal amounts of total DDT and HCH (less than 10  $\mu\text{g/kg}$ ). A total of three samples exceeded a PEC-Q of 1.0 for chlorinated pesticides and 12 samples exceeded a PEC-Q of 0.1 (Table A1-3a of Appendix 1 to Ingersoll *et al.* 2014a).

Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans were detected in all sediment samples (Table A1-3a of Appendix 1 to Ingersoll *et al.* 2014a). Toxicity equivalents (TEQ) were calculated using fish toxic equivalency factors reported in Van den Berg *et al.* (1998) and ranged from 0.018 to 2,730  $\text{ng/kg}$  (results reported as less than the detection limit were not included in the TEQ calculations). The median TEQ value was 6.8  $\text{ng/kg}$ , well within the range of background conditions summarized by USEPA (2007; which ranged from 0.21 to 22.9  $\text{ng/kg}$ ).

---

However, a total of 15 samples exceeded these background TEQ levels, with the exceedances ranging from 28.2 ng/kg to 2,730 ng/kg.

Concentrations of individual PAHs were generally low. The median concentration of total PAHs (calculated as the sum of 13 individual PAHs) was 1,050 µg/kg and the maximum concentration was 83,300 µg/kg. A total of four sediments exceeded a PEC-Q for total PAH of 1.0 (Table A1-3a of Appendix 1 to Ingersoll *et al.* 2014a). Eight sediments exceeded a  $\sum$ ESB-TU<sub>FCV</sub> of 1.0 (the chronic equilibrium-partitioning sediment benchmark toxic unit).

In general, the highest concentrations of PCBs were associated with the highest concentrations of PAHs, PCDDs/PCDFs, and organochlorine pesticides. Specifically, sediments 08, 18, and 19 exceeded PEC-Qs of 1.0 for all classes of organic contaminants. In general, the sediment samples that had elevated concentrations (i.e., above the PEC) of PCBs also had the highest concentrations of PCDDs/PCDFs. The samples with the highest concentrations of PCBs were sediments 19, 08, 18, and 30, with total PCB (homolog) concentrations of 1,200,000 µg/kg DW, 770,000 µg/kg DW, 740,000 µg/kg DW, and 410,000 µg/kg DW, respectively.

A summary of the concentrations of total metals in whole-sediment samples from the Anniston PCB Site is provided in Table A1-3a of Appendix 1 to Ingersoll *et al.* (2014a). Among the results for total metals provided by ARCADIS (2011), concentrations of only two were remarkable - mercury and lead. Antimony, thallium, selenium, and silver were at or below detection limits in all samples. Other metal concentrations, including barium, beryllium, cadmium, copper, nickel, vanadium and zinc were about 2-3 times greater on average in cycle 1a samples as compared with cycle 1b samples, but none of those concentrations were particularly elevated. Compared to sediment PECs (MacDonald *et al.* 2000), three of the cycle 1a sediments tested with *H. azteca* and *C. dilutus* had lead concentrations higher than the PEC value of 128 µg lead/g DW, and 12 of the 13 cycle 1a sediments and seven of the 17 cycle 1b sediments tested with *H. azteca* and *C. dilutus* had mercury concentrations higher than that PEC value of 1.1 µg mercury/g. The highest total lead concentrations

---

were in sediments 08, 18, and 19 (153, 137, and 188 µg/g DW, respectively) and these same three sediments had among the highest mercury concentrations (14, 31, and 40 µg/g DW, respectively [some values are rounded in the text for clarity]). Sediment 30 also had a high concentration of mercury (22 µg/g) and had the fourth highest concentration of lead (116 µg/g). The five sediments considered to represent reference conditions (Section 2.4.2) had lead concentrations that ranged from 4.0 to 5.8 µg/g. Six cycle 1b sediments also contained relatively high concentrations of mercury. These included sediments 02, 13, 14, 17, 20, and 27 that contained mercury concentrations of 3.2, 8.2, 7.9, 5.9, 2.0, and 2.0 µg/g, respectively. Furthermore, only seven Anniston PCB Site sediments (one from cycle 1a and six from cycle 1b) had mercury concentrations less than 0.1 µg/g. In contrast, the five sediments considered to represent reference conditions (Section 2.4.2) had mercury concentrations that ranged from 0.016 to 0.027 µg/g. Finally, the West Bearskin Lake control sediment (Table A1-3a of Appendix 1 to Ingersoll *et al.* 2014a) contained about 0.02 µg/g mercury; whereas, the maximum total mercury concentration among 17 stream sediments (less than 2-mm particle size) collected from a wide geographic distribution in the United States was reported as only 0.09 µg/g DW (Horowitz and Elrick 1987). Based on these comparisons, many of the Anniston PCB Site sediments would be classified as highly contaminated with mercury.

### 3.3 Pore-Water Characteristics

A summary of major cations and major anions measured in the pore-water samples isolated by centrifugation at the start of the *L. siliquoidea* toxicity exposures is provided in Tables A1-3f and A1-4 of Appendix 1 to Ingersoll *et al.* (2014a). Means and ranges (in parentheses) of concentrations of major cations in the Anniston PCB Site sediment pore-water samples evaluated in the *L. siliquoidea* toxicity testing (excluding the West Bearskin Lake control sediment) were as follows: calcium, 44.3 mg/L (12.3–84.1 mg/L); magnesium, 17.7 mg/L (2.2–34.2 mg/L); potassium, 2.9 mg/L (0.1–6.8 mg/L); sodium, 4.7 mg/L (3.0–10.0 mg/L); iron, 13.2 mg/L (0.1–28.3 mg/L); and manganese, 3.7 mg/L (0.2–15.8 mg/L). Analogous values for chloride

---

and sulfate were 9.2 mg/L (2.5–45.3 mg/L) and 16.7 mg/L (2.3–54.7 mg/L), respectively. Fluoride was only detected at low concentrations. Nitrate and sulfide were not measured in the pore water from the *L. siliquoidea* test, and were at or below detection limits in all pore-water samples from the cycle 1a and cycle 1b tests. DOC was not measured in pore water from the *L. siliquoidea* test. Details on the chemical characterization of pore water obtained by centrifugation or by peepers in cycle 1a and cycle 1b of testing can be found in Ingersoll *et al.* (2014b) and the corresponding tables in Appendix 1 to Ingersoll *et al.* (2014a).

A summary of the PCB congener and homolog concentrations in pore water obtained by SPME from the *L. siliquoidea* toxicity test is provided in Table A1-3f of Appendix 1 to Ingersoll *et al.* (2014a). The concentration of total PCBs (homologs) ranged from non-detect to 76 µg/L, with a mean concentration of 22.5 µg/L when non-detect results were not included. Total PCBs estimated in pore water with SPMEs deployed in selected sediments are compared for *L. siliquoidea*, *H. azteca*, *C. dilutus*, and *L. variegatus* exposures relative to PCBs in sediment (as g/kg OC; Figure 3). In general, the concentration of PCBs in pore water tended to be lower for a given concentration in sediment in the *L. siliquoidea* toxicity test compared to the *H. azteca* and *C. dilutus* tests, possibly due to *L. siliquoidea* burrowing and processing more sediment compared to *H. azteca* or *C. dilutus*. More details on the results of SPME pore-water analyses can be found in Steevens *et al.* (2014).

In addition, pore-water concentrations of PCBs in cycle 1b were remarkably lower for the *L. variegatus* exposures than for *H. azteca* and *C. dilutus* exposures (Figure 3b). This observation is not unexpected because oligochaetes differentially modify the sediments during exposure compared to *H. azteca* or *C. dilutus*. To further illustrate differences in pore-water concentration among tests, total PCBs in pore water for the midge, *C. dilutus*, and the oligochaete, *L. variegatus*, were expressed as a fraction of the concentration measured in exposures using *H. azteca* in select sediments from cycle 1a and cycle 1b (Figure 4). For the same sediment, total PCB concentrations in pore water were typically highest for *H. azteca* exposures and lowest for *L. variegatus*; pore-water concentrations for *L. siliquoidea* exposures were often

---



intermediate between those for *C. dilutus* and *L. variegatus*. However, in sediment samples 01 and 25, the total PCB concentrations in pore water were lower in the mussel exposures than in both the *C. dilutus* and *L. variegatus* exposures. Variability in pore-water PCB concentrations among tests was attributed, at least partially, to differences in species-specific test-organism/sediment interactions and test design.

### 3.4 Sediment Toxicity

Day 28 survival of *L. siliquoidea* in the control sediment was 98%, meeting the test acceptability criterion of control survival greater than or equal to 80%. In addition, in the reference toxicant test the 96-hour LC<sub>50</sub> was 4.0 g NaCl/L, which is similar to the historical LC<sub>50</sub>s for *L. siliquoidea* at USGS-Columbia.

Complete summaries of the *L. siliquoidea* toxicity test results are provided in Appendix 1 (Tables A1-3 to A1-7). The mean Day 28 survival of *L. siliquoidea* ranged from 45% (sediment 19) to 100% (sediments 01, 07, 13, 14, 17, and 20). Mean Day 28 biomass ranged from 2.52 mg (sediment 19) to 19.1 mg (sediment 20), while mean Day 28 dry weight ranged from 0.5 mg/individual (sediment 19) to 1.91 mg/individual (sediment 20). Mean Day 28 length ranged from 3.01 mm/individual (sediment 25) to 3.36 mm/individual (sediment 07). The fact that sediment 19 had the lowest survival, biomass, and individual dry weight is not surprising because it had the highest concentration of total PCBs (1,200,000 µg/kg DW). Comparing responses in the reference sediments to the control sediment, Day 28 survival ranged from 92.3% (sediment 29) to 100% (sediments 09, 10, 22, and 26) of the control response. Day 28 biomass ranged from 99.2% (sediments 10 and 29) to 119% (sediment 09) of control response, while the Day 28 individual dry weight ranged from 98.9% (sediment 10) to 119% (sediment 09) of control response. Day 28 length ranged from 102% (sediments 10 and 29) to 106% (sediment 09) of control response. Therefore, all *L. siliquoidea* responses in the reference sediments were comparable to those in the control sediment.

---

Using the reference envelope approach, test sediments were classified as highly toxic, moderately toxic, or not toxic for each endpoint (Table 6). Highly toxic (HT) sediment samples were designated based on a greater than 10% reduction in survival, biomass, weight, or length, relative to the lower limit of the reference envelope (MacDonald *et al.* 2002; 2012; 2014). Moderately toxic (MT) sediment samples were designated based on survival, biomass, weight, or length that was less than 10% below the lower limit of the reference envelope. Samples were designated as not toxic when responses fell within the reference envelope. Samples 19 and 30 were most consistently designated as toxic to *L. siliquoidea* across endpoints, with survival, biomass, weight, and length all classifying these two samples as toxic (Table 6). Samples 19 and 30 had the highest and fourth-highest sediment total PCB concentrations of 1,200,000 µg/kg DW and 410,000 µg/kg DW, respectively.

### **3.5 Relative Endpoint and Relative Species Sensitivity**

In order to determine which *L. siliquoidea* endpoint was most sensitive in exposures to contaminated sediments, the endpoints for each sample were plotted against each other in pairs after being expressed as a percentage of the control response (Figures 5 - 8). A line of unity was added representing equal response between the two endpoints and this line was bracketed with red lines representing a 20% difference above or below the line of unity as a visualization tool to aid in the comparison between endpoints (Stanley *et al.* 2014). Data points above this line of unity indicate that the endpoint on the x-axis was more responsive; whereas, data points below this line of unity indicate that the endpoint on the y-axis was more responsive. Vertical and horizontal lines on these graphs represent the lower distribution of the threshold reference-envelope response for the variable on the x and y axes. Open symbols represent reference sediments and closed symbols represent test sediments in these graphs.

Biomass and weight were the most sensitive endpoints in the mussel toxicity tests. The majority of data points fell within the 20% difference above or below the line of

---

unity in all comparisons. The responses of weight and biomass were very similar because survival in the test sediments was often 100% or more of the control response, and the biomass endpoint is calculated as the product of weight and survival. Based on toxicity designation, biomass and weight were the most sensitive endpoints, both classifying 52% of the samples as toxic (Table 6). Survival was the least sensitive endpoint, with 17% of samples classified as toxic based on survival (Table 6).

The most sensitive *L. siliquoides* endpoint (biomass) was also compared to the most sensitive *C. dilutus* (adult biomass) and *H. azteca* (survival-normalized young per female) endpoints (Figures 9 and 10, respectively). *H. azteca* and *C. dilutus* were both more sensitive to contaminants in sediment than was *L. siliquoides*. This is consistent with the classification of sediment toxicity based on the reference envelope. Survival-normalized young per female for *H. azteca* classified 67% of sediments as toxic and *C. dilutus* adult biomass classified 65% of sediments as toxic (Stanley *et al.* 2014), while *L. siliquoides* biomass classified 52% of sediments as toxic (Table 6). Finally, *L. siliquoides* biomass was compared to *C. dilutus* and *H. azteca* biomass. In general, *C. dilutus* and *H. azteca* biomass were each more sensitive than *L. siliquoides* biomass (Figures 11 and 12). Biomass of *H. azteca* classified 72% of the sediments as toxic and biomass of *C. dilutus* larvae classified 65% of the sediments as toxic. Therefore, *L. siliquoides* tended to be less responsive than either *H. azteca* or *C. dilutus* (based on biomass of *L. siliquoides*, reproduction of *H. azteca*, and adult biomass of *C. dilutus*).

### 3.6 Concentration-Response Models

The CRMs describing the relationships between total PCBs (DW or at 1% OC) and *L. siliquoides* biomass are shown in Figures 13 and 14. The goodness of fit was similar for the two CRMs, with an  $R^2$  of 0.575 obtained for dry-weight normalized PCB concentrations and an  $R^2$  of 0.581 obtained for total PCBs at 1% OC. For both relationships, a  $p$ -value of  $< 0.001$  was obtained. The TTs that were developed from

---

these CRMs are shown in Figures 13 and 14. The  $TT_{LR}$  and  $TT_{HR}$  are considered to provide a basis for identifying the total PCB concentrations that pose low risks (less than  $TT_{LR}$ ), moderate risks (between the  $TT_{LR}$  and  $TT_{HR}$ ), and high risks (greater than  $TT_{HR}$ ; MacDonald *et al.* 2010; 2012; 2014).

The  $TT_{LR}$ s for biomass of *L. siliquoidea* were 14,600 µg/kg at 1% OC and 20,400 µg/kg DW (Table 8). The  $TT_{LR}$ s for biomass of *H. azteca* were 2,350 µg/kg at 1% OC and 5,030 µg/kg DW and the  $TT_{LR}$ s for biomass of larval *C. dilutus* were 9,260 µg/kg at 1% OC and 15,000 µg/kg DW (Figures C5-7 to C5-10 in MacDonald *et al.* 2014). The  $TT_{LR}$ s for survival-normalized young per female of *H. azteca* were 659 µg/kg at 1% OC and 499 µg/kg DW and the  $TT_{LR}$ s for biomass of adult *C. dilutus* were 1,340 µg/kg at 1% OC and 1,140 µg/kg DW (Figures C5-1, C5-2, C5-5 and C5-6 in MacDonald *et al.* 2014). Hence, juvenile *L. siliquoidea* were less sensitive to the effects of the sediment-associated PCBs compared to *H. azteca* or *C. dilutus* across the endpoints evaluated in the exposures to Anniston PCB Site sediments.

All of the TTs were evaluated to determine if these thresholds would provide a reliable basis for classifying sediment samples from the Anniston PCB Site as toxic or not toxic to *L. siliquoidea* (Table 8). A TT was considered to be reliable if the following conditions were met: (1) the incidence of toxicity below the TT was less than or equal to 20%; (2) the incidence of toxicity above the TT was greater than 50%; and, (3) the overall correct classification rate was greater than or equal to 80% (MacDonald *et al.* 2014).

The results of this analysis indicate that some of the  $TT_{LR}$ s and  $TT_{HR}$ s based on total PCBs and *L. siliquoidea* biomass were less reliable. The incidence of toxicity below the  $TT_{HR}$  was greater than 20% for total PCBs in DW concentrations and at 1% OC. While the incidence of toxicity below the  $TT_{LR}$  was 20% for total PCBs in DW concentrations, the overall classification rate for the  $TT_{LR}$  was 78.3%, making the TT less reliable (Table 8). The  $TT_{LR}$  for total PCBs at 1% OC met the evaluation criteria, with an overall correct classification rate of 82.6% (Table 8). The TTs that were developed for *H. azteca* and *C. dilutus* biomass, *H. azteca* reproduction, and *C.*

---

*dilutus* emergence (MacDonald *et al.* 2014) tended to be more reliable than the TTs for *L. siliquoidea*.

## 4.0 Summary and Conclusions

### 4.1 Summary

Sediment samples from Snow Creek and Choccolocco Creek were contaminated by a variety of COPCs. Organic contaminants detected in sediment included PCBs, organochlorine pesticides, PCDDs/PCDFs, and PAHs. In general, the highest concentrations of PCBs were associated with the highest concentrations of PAHs, PCDDs/PCDFs, and organochlorine pesticides. Specifically, sediments 08, 18, and 19 exceeded PEC-Qs of 1.0 for all organic classes of contaminants. These three sediment samples also had high concentrations of mercury and lead, which were the only metals found at elevated concentrations (i.e., above the PEC) in the samples collected. Many sediment samples were highly contaminated with mercury. Pore-water PCBs and whole-sediment PCBs appear to be correlated in the mussel exposures (Figure 3). The concentration of PCBs in pore water was lower for a given concentration in sediment in the *L. siliquoidea* toxicity test than in the *H. azteca* and *C. dilutus* tests reported in Ingersoll *et al.* (2014a).

The whole-sediment toxicity tests conducted with *L. siliquoidea* met the test acceptability criteria (i.e., control survival greater than or equal to 80%). Survival was high in most samples, with 4 of 23 samples (17%) classified as toxic based on the survival endpoint. Biomass and weight were more sensitive *L. siliquoidea* endpoints, both of which classified 52% of the samples as toxic. Samples 19 and 30 were most toxic to *L. siliquoidea*, as they were designated as toxic for all four endpoints (survival, biomass, weight, and length). Samples 19 and 30 had the highest and fourth-highest sediment total PCB concentrations, of 1,200,000 µg/kg DW and 410,000 µg/kg DW, respectively.

---

Relative endpoint sensitivity plots were used to evaluate the sensitivity of the various mussel toxicity endpoints and to compare the sensitivity of juvenile mussels to the sensitivity of midge and amphipods. The results of this analysis indicated that biomass of *L. siliquoidea* was less sensitive compared to biomass of *H. azteca* or biomass of larval *C. dilutus*. Based on the most sensitive endpoint for each species, 52% of the samples were toxic to *L. siliquoidea*, whereas 67% of sediments were toxic to *H. azteca* and 65% were toxic to *C. dilutus*. Higher  $TT_{LR}$ s were observed for *L. siliquoidea* biomass (e.g., 20,400  $\mu\text{g/kg DW}$ ) than for *H. azteca* reproduction (e.g., 499  $\mu\text{g/kg DW}$ ) or *C. dilutus* adult biomass (e.g., 1,140  $\mu\text{g/kg DW}$ ; MacDonald *et al.* 2014). The  $TT_{LR}$  developed for *L. siliquoidea* based on total PCB concentrations at 1% OC was considered reliable, with an overall correct classification rate of 82.6%, but its corresponding  $TT_{HR}$  was classified as less reliable.

## 4.2 Uncertainties

The *L. siliquoidea* toxicity tests were conducted between the start of the cycle 1a and cycle 1b tests conducted with *H. azteca* and *C. dilutus*. The sediment chemistry was analyzed at the same time as the cycle 1a and cycle 1b *H. azteca* and *C. dilutus* tests, so there is some uncertainty as to whether the sediment chemistry at the start of the exposures with *L. siliquoidea* would directly match the measured chemistry at the start of the *H. azteca* or *C. dilutus* exposures. However, Ingersoll *et al.* (2014b) and Stanley *et al.* (2014) addressed the potential effect of sediment storage on the sediment chemistry and toxicity results by testing sample 20 in cycle 1a and cycle 1b. Responses of *C. dilutus* were similar when exposed to sample 20 in cycle 1a and cycle 1b, and Stanley *et al.* (2014) concluded that storage time likely had little effect on *C. dilutus* response to this sediment. Therefore, the reported sediment chemistry is likely an appropriate match to the sediment toxicity results from the *L. siliquoidea* exposures.

The CRMs explained more than 50% of the variability in the matching PCB chemistry and mussel toxicity data. Nevertheless, factors beyond PCB concentration may have

---

influenced the results of the toxicity tests with juvenile mussels. Although the sediment sample with the highest total PCB concentration (1,200,000 µg/kg DW) was very toxic to *L. siliquoidea*, two samples with high total PCB concentrations (sample 07 = 150,000 µg/kg DW and sample 11 = 170,000 µg/kg DW) were not toxic to *L. siliquoidea* for any endpoint. Therefore, *L. siliquoidea* may have been less sensitive to PCBs compared to *C. dilutus* or *H. azteca*. In contrast, sample 16 had a low total PCB concentration (91.2 µg/kg DW), yet was toxic to *L. siliquoidea* according to biomass, weight, and length. MacDonald *et al.* (2014) noted that sample 16 was toxic to *H. azteca*, affecting reproduction, even though there were low concentrations of COPCs in the sediment. As effects on survival or sublethal endpoints of *C. dilutus* were not observed with exposure to sediment 16, MacDonald *et al.* (2014) examined this sample in more detail. However, no specific causes of the observed toxicity in *H. azteca* were reported (MacDonald *et al.* 2014). It is possible that *L. siliquoidea* responded to the same undetermined stressor in sample 16 that affected *H. azteca*.

### 4.3 Conclusions

The mussel *L. siliquoidea* was less sensitive to exposure to Anniston PCB Site contaminants in sediment toxicity tests than were the amphipod *H. azteca* and the midge *C. dilutus*. Fewer samples were classified as toxic to *L. siliquoidea* compared to *H. azteca* or *C. dilutus*, and the TT<sub>LRs</sub> for total PCBs for *L. siliquoidea* were higher than the TT<sub>LRs</sub> for *H. azteca* and *C. dilutus*. While mussels, such as *L. siliquoidea*, are sensitive to some contaminants in water or in sediment including ammonia, metals, or PAHs (e.g., Wang *et al.* 2007; 2010; 2011; 2013; Besser *et al.* 2013; 2015), juvenile *L. siliquoidea* appear to be less sensitive to PCBs associated with sediments compared to *H. azteca* or *C. dilutus*. Accordingly, the results of toxicity tests with midge and amphipods are likely to be more relevant for assessing injury to sediment-dwelling organisms at the Anniston PCB Site.

---

## 5.0 References

- APHA, AWWA, and WEF (American Public Health Association, American Water Works Association, and the Water Environment Federation). 2005. Standard methods for the examination of water and wastewater (21<sup>st</sup> ed.): Washington, D.C. American Public Health Association. (Also available at <http://www.standardmethods.org/>).
- ARCADIS. 2009. Phase 2 field sampling plan for Operable Unit 4 for the Anniston PCB Site. Revision 1 (April 2009). Prepared by ARCADIS. Prepared for Pharmacia Corporation and Solutia Inc.
- ARCADIS. 2010. Phase 2 field sampling plan for Operable Unit 4 of the Anniston PCB Site. Revision 2 (April 2010). Attachment B: sediment toxicity testing plan. Prepared by ARCADIS. Prepared for Pharmacia Corporation and Solutia Inc.
- ARCADIS. 2011. Chemistry data report for sediment toxicity and bioaccumulation testing of Anniston PCB Site sediments. Revision 2 (June, 23, 2011; updated October 6, 2011). Prepared by ARCADIS. Prepared for Pharmacia Corporation and Solutia Inc.
- ASTM International. 2015a. Standard test method for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates. ASTM E1706-05 (2010). *In*: Annual Book of ASTM Standards v. 11.06. West Conshohocken, Pennsylvania.
- ASTM International. 2015b. Standard guide for conducting laboratory toxicity tests with freshwater mussels. ASTM E2455-06 (2013). *In*: Annual Book of ASTM Standards v. 11.06. West Conshohocken, Pennsylvania.
- ASTM International. 2015c. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. ASTM E729-96 (2014). *In*: Annual Book of ASTM Standards v. 11.06. West Conshohocken, Pennsylvania.
- BBL (Blasland, Bouck, and Lee, Inc.). 2000. Off-Site RCRA Facility Investigation (RFI) Report. Prepared for Solutia, Inc. Anniston, Alabama.
-



- BBL (Blasland, Bouck, and Lee, Inc.). 2003. Phase I - Conceptual site model report for the Anniston PCB Site. Volume 1 of 2. Prepared by BBL. Prepared for Solutia, Inc. Anniston, Alabama.
- BBL (Blasland, Bouck, and Lee, Inc.). 2006. Phase 1 field sampling plan for Operable Unit 4 of the Anniston PCB Site. Prepared by BBL. Prepared for Pharmacia Corporation and Solutia, Inc. Anniston, Alabama.
- Besser, J.M., W.G. Brumbaugh, D.K. Hardesty, J.P. Hughes, and C.G. Ingersoll. 2009. Assessment of metal-contaminated sediments from the Southeast Missouri (SEMO) mining district using sediment toxicity tests with amphipods and freshwater mussels: Administrative Report 08–NRDAR–02. Prepared for U.S. Fish and Wildlife Service. Prepared by U.S. Geological Survey. Columbia, Missouri.
- Besser, J.M., W.G. Brumbaugh, C.G. Ingersoll, C.D. Ivey, J.L. Kunz, N.E. Kemble, C.E. Schlekat, and E. Rogevich-Garman. 2013. Chronic toxicity of nickel-spiked freshwater sediments: Variation in toxicity among eight invertebrate taxa and eight sediments. *Environmental Toxicology and Chemistry* 32:2495-2506.
- Besser, J.M., C.G. Ingersoll, W.G. Brumbaugh, N.E. Kemble, T.W. May, N. Wang, D.D. MacDonald, and A.D. Roberts. 2015. Toxicity of sediments from lead-zinc mining areas to juvenile freshwater mussels (*Lampsilis siliquoidea*), compared to standard test organisms. *Environmental Toxicology and Chemistry* 34:626-639.
- Horowitz, A.J. and K.A. Elrick. 1987. The relation of stream sediment surface area, grain size and composition to trace element chemistry. *Applied Geochemistry* 2:437-451.
- Hunt, J.W., B.S. Anderson, B.M. Phillips, J. Newman, R.S. Tjeerdema, R. Fairey, H.M. Puckett, M. Stephenson, R.W. Smith, C.J. Wilson, and K.M. Taberski. 2001. Evaluation and use of sediment toxicity reference sites for statistical comparisons in regional assessments. *Environmental Toxicology and Chemistry* 20:1266-1275.
- 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 *Hyaella azteca*. *Environmental Toxicology and Chemistry* 17:508-1523.
-

- Ingersoll C.G., D.D. MacDonald, J.M. Besser, W.G. Brumbaugh, C.D. Ivey, N.E. Kemble, J.L. Kunz, T.W. May, N. Wang, and D.E. Smorong. 2008. Sediment chemistry, toxicity, and bioaccumulation data report for the US Environmental Protection Agency - Department of the Interior sampling of metal-contaminated sediment in the Tri-State Mining District in Missouri, Oklahoma, and Kansas. Prepared by USGS, Columbia Missouri and MacDonald Environmental Sciences Ltd., Nanaimo, British Columbia. Prepared for the USEPA, Kansas City, Missouri; USEPA, Dallas, Texas, and, USFWS, Columbia, Missouri.
- Ingersoll, C.G., N.E. Kemble, J.L. Kunz, W.G. Brumbaugh, D.D. MacDonald, and D. Smorong. 2009. Toxicity of sediment cores collected from the Ashtabula River in northeastern Ohio USA to the amphipod *Hyaella azteca*. Archives of Environmental Contamination and Toxicology 57:315-329 (and an erratum Archives of Environmental Contamination and Toxicology 57:826-827).
- Ingersoll, C.G., J.A. Steevens, and D.D. MacDonald (editors). 2014a. Evaluation of toxicity to the amphipod, *Hyaella azteca*, and to the midge, *Chironomus dilutus*; and bioaccumulation by the oligochaete, *Lumbriculus variegatus*, with exposure to PCB-contaminated sediments from Anniston, Alabama. U.S. Geological Survey Scientific Investigations Report 2013–5125, 122 p. (<http://pubs.usgs.gov/sir/2013/5125/>).
- Ingersoll, C.G., W.G. Brumbaugh, J.A. Steevens, G.R. Lotufo, J.K. Stanley, D.D. MacDonald, and J.A. Sinclair. 2014b. Sediment sample collection, handling, preparation, and characterization. Chapter 2 of Ingersoll, C.G., Steevens, J.A., and MacDonald, D.D., eds. Evaluation of toxicity to the amphipod, *Hyaella azteca*, and to the midge, *Chironomus dilutus*; and bioaccumulation by the oligochaete, *Lumbriculus variegatus*, with exposure to PCB-contaminated sediments from Anniston, Alabama. U.S. Geological Survey Scientific Investigations Report 2013–5125. p. 13–30 (<http://pubs.usgs.gov/sir/2013/5125/>).
- Ingersoll, C.G., J.L. Kunz, J.P. Hughes, N. Wang, D.S. Ireland, D.R. Mount, R.J. Hockett, and T.W. Valenti. 2015. Relative sensitivity of an amphipod *Hyaella azteca*, a midge *Chironomus dilutus*, and a unionid mussel *Lampsilis siliquoidea* to a toxic sediment. Environmental Toxicology and Chemistry 34:1134-1144.
- Kemble, N.E., D.K. Hardesty, C.G. Ingersoll, J.L. Kunz, P.K. Sibley, D.L. Calhoun, R.J. Gilliom, K.M. Kuivila, L.H. Nowell, and P.W. Moran. 2013. Contaminants in stream sediments from seven U.S. metropolitan areas—Part II—Sediment toxicity to the amphipod *Hyaella azteca* and the midge *Chironomus dilutus*. Archives of Environmental Contamination and Toxicology 64:52–64.
-

- MacDonald, D.D., C.G. Ingersoll, 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.
- MacDonald, D.D., C.G. Ingersoll, D.R.J. Moore, M. Bonnell, R.L. Breton, R.A. Lindscoog, D.B. MacDonald, Y.K. Muirhead, A.V. Pawlitz, D.E. Sims, D.E. Smorong, R.S. Teed, R.P. Thompson, and N. Wang. 2002. Calcasieu Estuary remedial investigation/feasibility study (RI/FS): Baseline ecological risk assessment (BERA). Technical report plus appendices. Contract No. 68-W5-0022. Prepared for CDM Federal Programs Corporation and U.S. Environmental Protection Agency. Dallas, Texas.
- MacDonald, D.D., R.L. Breton, K. Edelmann, M.S. Goldberg, C.G. Ingersoll, R.A. Lindscoog, D.B. MacDonald, D.R.J. Moore, A.V. Pawlitz, D.E. Smorong, and R.P. Thompson. 2003. Development and evaluation of preliminary remediation goals for selected contaminants of concern at the Calcasieu Estuary cooperative site, Lake Charles, Louisiana. Prepared for U.S. Environmental Protection Agency, Region 6. Dallas, Texas.
- MacDonald, D.D., C.G. Ingersoll, D.E. Smorong, L. Fisher, C. Huntington, and G. Braun. 2005a. Development and evaluation of risk-based preliminary remediation goals for selected sediment-associated contaminants of concern in the West Branch of the Grand Calumet River. Prepared for: U.S. Fish and Wildlife Service. Bloomington, Indiana.
- MacDonald, D.D., C.G. Ingersoll, A.D. Porter, S.B. Black, C. Miller, and Y.K. Muirhead. 2005b. Development and evaluation of preliminary remediation goals for aquatic receptors in the Indiana Harbor Area of Concern. Technical Report. Prepared for: U.S. Fish and Wildlife Service. Bloomington, Indiana and Indiana Department of Environmental Management. Indianapolis, Indiana.
- MacDonald, D.D., D.E. Smorong, C.G. Ingersoll, J.M. Besser, W.G. Brumbaugh, N. Kemble, T.W. May, C.D. Ivey, S. Irving, and M. O'Hare. 2009. Development and evaluation of sediment and pore-water toxicity thresholds to support sediment quality assessments in the Tri-State Mining District (TSMD), Missouri, Oklahoma, and Kansas. Draft Final Technical Report. Volume I: Text. Submitted to U.S. Environmental Protection Agency (USEPA). Region 6, Dallas, Texas, Region 7, Kansas City, Kansas, and US Fish and Wildlife Service, Columbia, Missouri. Submitted by MacDonald Environmental Sciences Ltd., Nanaimo, British Columbia. U.S. Geological Survey, Columbia, Missouri. CH2M Hill, Dallas, Texas.
-

- MacDonald, D.D., C.G. Ingersoll, M. Crawford, H. Prencipe, J.M. Besser, W.G. Brumbaugh, N. Kemble, T.W. May, C.D. Ivey, M. Meneghetti, J. Sinclair, and M. O'Hare. 2010. Advanced screening-level ecological risk assessment (SLERA) for aquatic habitats within the Tri-State Mining District, Oklahoma, Kansas, and Missouri. Draft Final Technical Report. Submitted to U.S. Environmental Protection Agency (USEPA). Region 6, Dallas, Texas, Region 7, Kansas City, Kansas, and US Fish and Wildlife Service, Columbia, Missouri. Submitted by MacDonald Environmental Sciences Ltd., Nanaimo, British Columbia. U.S. Geological Survey, Columbia, Missouri. CH2M Hill, Dallas, Texas.
- MacDonald, D.D., J. Sinclair, M.A. Crawford, C.G. Ingersoll, H. Prencipe, S. Cox, and M. Coady. 2012. Evaluation and interpretation of the sediment chemistry and sediment-toxicity data for the Upper Columbia River Site. Prepared for Washington Department of Ecology Toxic Cleanup Program, through Science Applications International Corporation, Bothell, Washington. Prepared by MacDonald Environmental Sciences Ltd., Nanaimo, BC, and U.S. Geological Survey, Columbia, Missouri and Tacoma, Washington.
- MacDonald, D.D., C.G. Ingersoll, J.A. Sinclair, J.A. Steevens, J.K. Stanley, J.D. Farrar, N.E. Kemble, J.L. Kunz, W.G. Brumbaugh, and M.R. Coady. 2014. Evaluation of relations between sediment toxicity and sediment chemistry at the Anniston PCB site. Chapter 5 of Ingersoll, C.G., Steevens, J.A., and MacDonald, D.D., eds. 2014. Evaluation of toxicity to the amphipod, *Hyalella azteca*, and to the midge, *Chironomus dilutus*; and bioaccumulation by the oligochaete, *Lumbriculus variegatus*, with exposure to PCB-contaminated sediments from Anniston, Alabama. U.S. Geological Survey Scientific Investigations Report 2013-5125. p. 66-105 (<http://pubs.usgs.gov/sir/2013/5125>).
- Moran, P.W., D.L. Calhoun, L.H. Nowell, N.E. Kemble, C.G. Ingersoll, M. Hladik, K.M. Kuivila, J. Falcone, and R.J. Gilliom. 2012. Contaminants in stream sediments from seven U.S. metropolitan areas - Data summary of a National Pilot Study. U.S. Geological Survey Scientific Investigations Report 2011-5092.
- R Development Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.r-project.org/>.
- Seefeldt, S.J., J.E. Jensen, and E.P. Fuerst, E.P. 1995. Log-logistic analysis of herbicide dose-response relationships. Weed Technology 9:218-227.
-

- Stanley, J.K., N.E. Kemble, J.D. Farrar, C.G. Ingersoll, D.D. MacDonald, J.A. Steevens, J.L. Kunz, and J.A. Sinclair. 2014. Toxicity testing with the amphipod, *Hyalella azteca*, and with the midge, *Chironomus dilutus*. Chapter 4 of Ingersoll, C.G., Steevens, J.A., and MacDonald, D.D., eds. Evaluation of toxicity to the amphipod, *Hyalella azteca*, and to the midge, *Chironomus dilutus*; and bioaccumulation by the oligochaete, *Lumbriculus variegatus*, with exposure to PCB-contaminated sediments from Anniston, Alabama. U.S. Geological Survey Scientific Investigations Report 2013–5125. p. 47–65 (<http://pubs.usgs.gov/sir/2013/5125/>).
- Steevens J.A., G.R. Lotufo, K.E. Kemble, C.G. Ingersoll, J.K. Stanley, J.D. Farrar, J.A. Sinclair, and D.D. MacDonald. 2014. Evaluation of bioaccumulation in the oligochaete, *Lumbriculus variegatus*, exposed to sediments from the Anniston PCB site. Chapter 3 of Ingersoll, C.G., Steevens, J.A., and MacDonald, D.D., eds. Evaluation of toxicity to the amphipod, *Hyalella azteca*, and to the midge, *Chironomus dilutus*; and bioaccumulation by the oligochaete, *Lumbriculus variegatus*, with exposure to PCB-contaminated sediments from Anniston, Alabama. U.S. Geological Survey Scientific Investigations Report 2013–5125. p. 31–46 (<http://pubs.usgs.gov/sir/2013/5125/>).
- USEPA (U.S. Environmental Protection Agency). 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, second edition. EPA/600/R-99/064. Washington, District of Columbia (<http://water.epa.gov/polwaste/sediments/cs/upload/freshmanual.pdf>).
- USEPA (U.S. Environmental Protection Agency). 2007. Pilot survey of levels of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, polychlorinated biphenyls, and mercury in rural soils of the United States. EPA/600/R-05/048F. Washington, District of Columbia. (Also available at <http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P100BS5N.txt>).
- Van den Berg, M., L. Birnbaum, A.T.C. Bosvald, B. Brunstrom, P. Cook, M. Feeley, J.P. Giesy, A. Hanberg, R. Hasegawa, S.W. Kennedy, T. Kubiak, J.C. Larsen, F.X.R. van Leeuwen, A.K. Djien Liern, C. Nolt, R.E. Peterson, L. Poellinger, S. Safe, D. Schrenk, D. Tillitt, M. Tysklind, M. Younes, F. Waern, and T. Zacharewski. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environmental Health Perspectives 106(12):775-792.
-

- Wang, N., C.G. Ingersoll, I.E. Greer, D.K. Hardesty, C.D. Ivey, J.L. Kunz, W.G. Brumbaugh, F.J. Dwyer, A.D. Roberts, T. Augspurger, C.M. Kane, R.J. Neves, and M.C. Barnhart. 2007. Chronic toxicity of copper and ammonia to juvenile freshwater mussels (Unionidae). *Environmental Toxicology and Chemistry* 26:2048-2056.
- Wang, N., C.G. Ingersoll, C.D. Ivey, D.K. Hardesty, T.W. May, T. Augspurger, A.D. Roberts, E. van Genderen, and M.C. Barnhart. 2010. Sensitivity of early life stages of freshwater mussels (Unionidae) to acute and chronic toxicity of lead, cadmium, and zinc in water. *Environmental Toxicology and Chemistry* 29:2053-2063.
- Wang, N., C.A. Mebane, J.L. Kunz, C.G. Ingersoll, W.G. Brumbaugh, R.C. Santore, J.W. Gorsuch, and W.R. Arnold. 2011. Influence of dissolved organic carbon on the toxicity of copper to a unionid mussel (*Villosa iris*) and a cladoceran (*Ceriodaphnia dubia*) in acute and chronic water exposures. *Environmental Toxicology and Chemistry* 30:2115-2125.
- Wang, N., C.G. Ingersoll, J.L. Kunz, W.G. Brumbaugh, C.M. Kane, R.B. Evans, S. Alexander, C. Walker, S. Bakaletz, and R.C. Lott. 2013. Toxicity of sediments potentially contaminated by coal or natural gas mining-related activities to unionid mussels and commonly tested benthic invertebrates. *Environmental Toxicology and Chemistry* 32:207-221.
- Zar, J.H. 1999. *Biostatistical analysis* (4<sup>th</sup> ed.): Upper Saddle River, N.J., Prentice Hall.
-

---

# Tables

---

**Table 1. General activity schedule for conducting a 28-day sediment toxicity test with *Lampsilis siliquoidea* .**

[Adapted from Table A6.2 in ASTM International (2015a) and from ASTM International (2015b); an analogous table is included in USEPA (2000).]

Day	Activity
Pre-Test	
Minus 7	Sample sediments for physical and chemical characteristics and sample pore water by centrifugation for water quality analyses. Place sediments into exposure beakers for about a 7-day equilibration period before the start of the toxicity tests. Add sediment into each test chamber, place chambers into exposure system, and add overlying water.
Minus 2	Isolate mussels from culture and feed and observe isolated mussels to evaluate health.
Minus 1	Check cultures.
Sediment Test	
0	Measure water quality of overlying water (pH, temperature, dissolved oxygen, hardness, alkalinity, conductivity, ammonia) on about Day 0. Transfer ten mussels into each test chamber. Release organisms under the surface of the water. Add appropriate food to each test chamber. Place SPMEs in sediment chemistry beakers.
1 to 27	Feed test organisms. Measure temperature daily, dissolved oxygen weekly, and pH at the beginning and end of the exposure. Observe behavior of test organisms.
28	Measure temperature, dissolved oxygen, pH, hardness, alkalinity, conductivity and ammonia on about Day 28. End the sediment-exposure portion of the test by collecting the test organisms with a #50 mesh sieve (300-µm mesh; U.S. standard size sieve). Sample SPMEs from chemistry beakers. Use all four mussel replicates for growth measurements: count survivors and preserve organisms in sucrose-formalin solution for growth measurements.

SPME = solid-phase microextraction.



**Table 2. Test conditions for conducting a long-term sediment toxicity test with the amphipod *Hyalella azteca*, the midge *Chironomus dilutus*, or the mussel *Lampsilis siliquoidea*.**

[Adapted from Tables A6.1 and A7.1 in ASTM International (2015a) and from ASTM International (2015b); analogous tables are included in USEPA (2000).]

Parameter	Conditions
1. Test type	Sediment toxicity test with renewal of overlying water conducted with control sediment and field-collected sediment samples from Operable Unit 4 of the Anniston Alabama Polychlorinated Biphenyl site and West Bearskin sediment as a control sediment (1.08 % total organic carbon; Ingersoll <i>et al.</i> 1998; 2014a).
2. Temperature	23 ± 1 °C.
3. Light quality	Wide-spectrum fluorescent lights.
4. Illuminance	About 200 lux.
5. Photoperiod	16L:8D.
6. Test chamber	300 mL high-form lipless beaker.
7. Sediment volume	100 mL (sediments dry sieved to <2 mm at the collection site).
8. Overlying water volume	175 mL.
9. Renewal of overlying water	2 volume additions/d.
10. Age of organisms	Amphipods and midges: about 7-day-old organisms. Mussels: 2-months post-transformation.
11. Number of organisms/chamber	Amphipods and mussels: 10. Midges: 12.
12. Number of toxicity replicate chambers/treatment	Amphipods: 12 (4 sampled on day 28 for survival and growth and 8 on day 42 for survival, growth, and reproduction). Two additional chemistry replicates/treatment (one replicate for SEM/AVS/peeper metal sampling and one replicate for SPME sampling). Midges: 16 (4 sampled on day 13 for survival and growth, 8 for emergence and reproduction, and 4 for auxiliary males [started late on day 2]). Two additional chemistry replicates/treatment (one replicate for SEM/AVS/peeper metal sampling and one replicate for SPME sampling). Mussels: 4 (sampled on Day 28 for survival and growth). One additional chemistry replicate/treatment for SPME sampling.
13. Feeding	Amphipods: Yeast Cerophyl Trout Chow food, fed 1.0 mL (1,800 mg/L stock) daily to each test chamber. Midges: Tetrafin® goldfish food, fed 1.5 mL daily to each test chamber (1.5 mL contains 6.0 mg of dry solids). Mussels: 2 mL twice daily of non-viable algae (Wang <i>et al.</i> 2007; ASTM International 2015b).
14. Aeration	None, unless dissolved oxygen in overlying water drops below 2.5 mg/L.
15. Overlying water	Well water diluted with deionized water to a hardness of about 100 mg/L (as CaCO <sub>3</sub> ), alkalinity 85 mg/L (as CaCO <sub>3</sub> ), and pH about 8.0.
16. Test chamber cleaning	If screens become clogged during a test, gently brush the outside of the screen.
17. Overlying water quality	Hardness, alkalinity, pH and ammonia at the beginning and end of a test and on the days that growth is subsampled. Temperature daily. Dissolved oxygen (DO) and pH three times/week. Concentrations of DO should be measured more often if DO has declined by more than 1 mg/L since previous measurement.

**Table 2. Test conditions for conducting a long-term sediment toxicity test with the amphipod *Hyalella azteca*, the midge *Chironomus dilutus*, or the mussel *Lampsilis siliquoidea*.**

[Adapted from Tables A6.1 and A7.1 in ASTM International (2015a) and from ASTM International (2015b); analogous tables are included in USEPA (2000).]

Parameter	Conditions
18. Chemistry sampling of pore water and sediment (see additional detail in Ingersoll <i>et al.</i> 2014a)	Pore water (sampled on about day minus 7 of the <i>H. azteca</i> exposures): centrifugation at 4 °C for 15 minutes at 5,200 rpm; 0.5 L sediment/treatment): Ammonia, pH, free sulfide, hardness, alkalinity, conductivity, dissolved oxygen, major cations and anions, dissolved organic carbon. Sediment (sampled on about day minus 7 of the <i>H. azteca</i> exposures): (1) PCB Aroclors, homologs and congeners, (2) TAL metals, (3) OCl pesticides, (4) PAHs, and (5) PCDDs/PCDFs. Sediment (sampled during exposures): (1) Simultaneously extracted metals, acid volatile sulfide, peeper pore-water metals (sampled about day 21 in separate chemistry beakers [in the <i>H. azteca</i> exposures]) and (2) Solid-phase microextraction sampling of PCBs (about day 28 in separate chemistry beakers).
19. Endpoints	Amphipods: 28-day survival and growth (length, weight, total biomass), 35-day reproduction, and 42-day survival, growth, reproduction. Midges: 13-day survival and ash-free-dry weight, female and male emergence, adult mortality, the number of egg cases oviposited, the number of eggs produced, the number of hatched eggs, and adult biomass. Mussel: 28-day survival (foot movement) and growth (shell length, dry weight, total biomass).
20. Test acceptability	Amphipods: Minimum mean control survival of 80% on day 28. Midges: No test acceptability requirements have been established for long-term tests starting with 7-day-old larvae. Average survival of <i>C. dilutus</i> in the control sediment should be greater than or equal to 70% at day 20 and greater than or equal to 65% at the end of the test. Emergence should be greater than or equal to 50%. Mussels: Minimum mean control survival of 80% on day 28. Additional performance-based criteria specifications are outlined in Table 4.

AVS = acid volatile sulfide; DO = dissolved oxygen; OCl = organochlorine; PAHs = polycyclic aromatic hydrocarbons; PCB = polychlorinated biphenyl; PCDDs/PCDFs = polychlorinated dibenzo- *p* -dioxins/ polychlorinated dibenzofurans; SEM = simultaneously extracted metals; SPME = solid-phase microextraction; TAL = target analyte list.

**Table 3. Summary of test conditions for conducting reference toxicant tests.**

[Adapted from ASTM International (2015a,c) and USEPA (2000).]

Parameter	Conditions
1. Test chemical	Sodium chloride (NaCl).
2. Test type	Static.
3. Test duration	Amphipods and midges: 48 h. Mussels: 96 h.
4. Temperature	23 °C.
5. Light quality	Ambient laboratory light.
6. Light intensity	200 lux.
7. Photoperiod	16L:8D.
8. Test chamber size	50 mL.
9. Test solution volume	30 mL.
10. Renewal of solution	None.
11. Age of test organism	See Table 2.
12. No. organisms/test chamber	5
13. No. replicate	4
14. Feeding	None.
15. Chamber cleaning	None.
16. Aeration	None.
17. Dilution water	ASTM International (2015c) reconstituted hard water (170 mg/L as CaCO <sub>3</sub> ).
18. Dilution factor	0.5.
19. Test concentration	0, 0.5, 1, 2, 4, and 8 g NaCl/L.
20. Chemical residues	Salinity in each NaCl solution will be measured at the beginning and the end of test.
21. Water quality	Dissolved oxygen, pH, hardness, and alkalinity will be determined at the control, medium, and high NaCl concentrations at the beginning and the end of test.
22. Endpoint	Survival.
23. Test acceptability	≥90% control survival.

**Table 4. Test acceptability requirements for a 28-day sediment toxicity test with *Lampsilis siliquoidea*.**  
 [Adapted from Table A6.3 in ASTM International (2015a) and from ASTM International (2015b); an analogous table is included in USEPA (2000).]

- 
- A. It is recommended for conducting the 28-day test with *Lampsilis siliquoidea* that the following performance criteria be met:
1. Age of *Lampsilis siliquoidea* at the start of the test should be about 2 months post-transformation.
  2. Average survival of *Lampsilis siliquoidea* in the control sediment on day 28 should be greater than or equal to 80%.
  3. Hardness, alkalinity, and ammonia in the overlying water typically should not vary by more than 50% during the sediment exposure, and dissolved oxygen should be maintained above 2.5 mg/L in the overlying water.
- B. Performance-based criteria for culturing *Lampsilis siliquoidea* include the following:
1. It may be desirable for laboratories to periodically perform 96-h water-only reference-toxicity tests to assess the sensitivity of culture organisms (section 11.16.2 of ASTM International [2015a]). Data from these reference toxicity tests could be used to assess genetic strain or life-stage sensitivity of test organisms to select chemicals.
  2. Laboratories should track juvenile survival and growth in the cultures and record this information using control charts if known-age cultures are maintained. Records should also be kept on the frequency of restarting cultures and the age of brood organisms.
  3. Laboratories should record the following water-quality characteristics of the cultures at least quarterly: pH, hardness, alkalinity, and ammonia. Dissolved oxygen in the cultures should be measured weekly. Temperature in the cultures should be recorded daily. If static cultures are used, it may be desirable to measure water quality more frequently.
- C. Additional requirements:
1. All organisms in a test must be from the same source.
  2. Storage of sediments collected from the field should follow guidance outlined in section 10.2 of ASTM International (2015a).
  3. All test chambers (and compartments) should be identical and should contain the same amount of sediment and overlying water.
  4. Negative-control sediment and appropriate solvent controls must be included in a test. The concentration of solvent used must not adversely affect test organisms.
  5. Test organisms must be cultured and tested at 23 °C ( $\pm 1$  °C).
  6. The mean of the daily test temperature must be within  $\pm 1$  °C of 23 °C. The instantaneous temperature must always be within  $\pm 3$  °C of 23 °C.
  7. Natural physico-chemical characteristics of test sediment collected from the field should be within the tolerance limits of the test organisms.
-

**Table 5. Summary of the results of sediment toxicity tests conducted on the reference samples that were selected for the Anniston PCB Site.**

<b>Station ID:</b>	<b>TXR1-01-P</b>	<b>TXR1-02-P</b>	<b>TXR1-04-P</b>	<b>TXR1-05-P</b>	<b>TXR1-06-P</b>	<b>Mean of reference samples</b>	<b>5th percentile</b>	<b>Minimum of reference samples</b>	<b>Maximum of reference samples</b>	<b>Threshold for toxicity designation</b>
<b>Mussel (<i>Lampsilis siliquoidea</i>) endpoints; normalized to percent of control response</b>										
Day 28 survival	100	100	100	92.3	100	98.5	93.8	92.3	100	92.3
Day 28 biomass	113	119	112	99.2	99.2	108	99.2	99.2	119	99.2
Day 28 weight	112	119	112	105	98.9	109	100	98.9	119	98.9
Day 28 length	103	106	103	102	102	103	102	102	106	102

**Table 6. Summary of the toxicity of sediment samples from the Anniston PCB Site to the mussel *Lampsilis siliquoidea* (Note: Replicate toxicity test results for all endpoints are presented in Appendix 1).**

Station ID	Sample ID	Cycle <sup>1</sup>	ΣPCB (µg/kg DW) <sup>2</sup>	Survival (%)	Biomass (mg)	Growth (mg; weight)	Growth (mm; length)	Not Toxic	Moderately Toxic	Highly Toxic	Toxic (Moderately and highly toxic)
TXR1-03-P	04 <sup>3</sup>	1b	<16	NT	NT	NT	NT	100% (4 of 4)	0% (0 of 4)	0% (0 of 4)	0% (0 of 4)
TXR1-04-P	22	1b	<32	NT	NT	NT	NT	100% (4 of 4)	0% (0 of 4)	0% (0 of 4)	0% (0 of 4)
TXR1-06-P	10	1b	<33	NT	NT	NT	NT	100% (4 of 4)	0% (0 of 4)	0% (0 of 4)	0% (0 of 4)
TXR1-01-P	26	1b	<33	NT	NT	NT	NT	100% (4 of 4)	0% (0 of 4)	0% (0 of 4)	0% (0 of 4)
TXR1-05-P	29	1b	<33	NT	NT	NT	NT	100% (4 of 4)	0% (0 of 4)	0% (0 of 4)	0% (0 of 4)
TXR1-02-P	09	1a	62	NT	NT	NT	NT	100% (4 of 4)	0% (0 of 4)	0% (0 of 4)	0% (0 of 4)
TX10-01-P	16	1b	91.2	NT	<b>HT*</b>	<b>HT</b>	<b>MT</b>	25% (1 of 4)	25% (1 of 4)	50% (2 of 4)	75% (3 of 4)
TX60-02-P	20	1b <sup>4</sup>	6,000	NT	NT	NT	NT	100% (4 of 4)	0% (0 of 4)	0% (0 of 4)	0% (0 of 4)
TX60-01-P	21	M <sup>5</sup>	8,600	NT	<b>HT</b>	<b>MT</b>	<b>MT</b>	25% (1 of 4)	50% (2 of 4)	25% (1 of 4)	75% (3 of 4)
TX40-01-P	27	1b <sup>4</sup>	13,000	NT	NT	NT	<b>MT</b>	75% (3 of 4)	25% (1 of 4)	0% (0 of 4)	25% (1 of 4)
TX60-04-P	13	1b <sup>4</sup>	25,000	NT	<b>MT</b>	<b>MT</b>	NT	50% (2 of 4)	50% (2 of 4)	0% (0 of 4)	50% (2 of 4)
TX30-01-P	25	1a	60,000	NT	<b>HT</b>	<b>HT</b>	<b>MT</b>	25% (1 of 4)	25% (1 of 4)	50% (2 of 4)	75% (3 of 4)
TX40-02-P	17	1b	64,000	NT	NT	NT	NT	100% (4 of 4)	0% (0 of 4)	0% (0 of 4)	0% (0 of 4)
TX40-04-P	01	1a	68,000	NT	<b>HT</b>	<b>HT</b>	<b>MT</b>	25% (1 of 4)	25% (1 of 4)	50% (2 of 4)	75% (3 of 4)
TX40-05-P	14	1b	68,000	NT	<b>MT</b>	<b>MT</b>	<b>MT</b>	25% (1 of 4)	75% (3 of 4)	0% (0 of 4)	75% (3 of 4)
TX60-03-P	06	1a	100,000	NT	<b>HT</b>	<b>HT</b>	<b>MT</b>	25% (1 of 4)	25% (1 of 4)	50% (2 of 4)	75% (3 of 4)
TX30-05-P	02	1b	120,000	<b>HT</b>	<b>HT</b>	<b>HT</b>	NT	25% (1 of 4)	0% (0 of 4)	75% (3 of 4)	75% (3 of 4)
TX30-03-P	07	1a	150,000	NT	NT	NT	NT	100% (4 of 4)	0% (0 of 4)	0% (0 of 4)	0% (0 of 4)
TX50-04-P	11	1b <sup>4</sup>	170,000	NT	NT	NT	NT	100% (4 of 4)	0% (0 of 4)	0% (0 of 4)	0% (0 of 4)
TX50-05-P	30	1a	410,000	<b>HT</b>	<b>HT</b>	<b>HT</b>	<b>MT</b>	0% (0 of 4)	25% (1 of 4)	75% (3 of 4)	100% (4 of 4)
TX30-02-P	18	1a	740,000	<b>HT</b>	<b>HT</b>	<b>HT</b>	NT	25% (1 of 4)	0% (0 of 4)	75% (3 of 4)	75% (3 of 4)
TX50-01-P	08	1a	770,000	NT	<b>HT</b>	<b>HT</b>	<b>MT</b>	25% (1 of 4)	25% (1 of 4)	50% (2 of 4)	75% (3 of 4)
TX50-02-P	19	1a	1,200,000	<b>HT</b>	<b>HT</b>	<b>HT</b>	<b>MT</b>	0% (0 of 4)	25% (1 of 4)	75% (3 of 4)	100% (4 of 4)
<b>Not Toxic</b>				83% (19 of 23)	48% (11 of 23)	48% (11 of 23)	57% (13 of 23)				
<b>Moderately Toxic</b>				0% (0 of 23)	8.7% (2 of 23)	13% (3 of 23)	43% (10 of 23)				
<b>Highly Toxic</b>				17% (4 of 23)	43% (10 of 23)	39% (9 of 23)	0% (0 of 23)				
<b>Toxic (Moderately and highly toxic)</b>				17% (4 of 23)	52% (12 of 23)	52% (12 of 23)	43% (10 of 23)				

Footnotes on next page...

**Table 6. Summary of the toxicity of sediment samples from the Anniston PCB Site to the mussel *Lampsilis siliquoidea* (Note: Replicate toxicity test results for all endpoints are presented in Appendix 1).**

Station ID	Sample ID	Cycle <sup>1</sup>	ΣPCB (µg/kg DW) <sup>2</sup>	Survival (%)	Biomass (mg)	Growth (mg; weight)	Growth (mm; length)	Not Toxic	Moderately Toxic	Highly Toxic	Toxic (Moderately and highly toxic)
------------	-----------	--------------------	---------------------------------	-----------------	--------------	------------------------	------------------------	-----------	---------------------	--------------	--

DW = dry weight; ID = identifier; PCB = polychlorinated biphenyl; NT = not toxic (within the reference envelope); MT = moderately toxic (within 10 % of the reference envelope); HT = highly toxic (> 10 % below the reference envelope); The percentage and number of samples classified as not toxic, moderately toxic, and highly toxic for a given endpoint are shown at the bottom of the table. The percentage and number of endpoints included in the table classified as not toxic, moderately toxic, and highly toxic for a given sample are shown at the right of the table.

\* Bolded results represent sediment samples classified as toxic.

<sup>1</sup> Cycle that the sediment chemistry result is associated with.

<sup>2</sup> ΣPCBs are calculated as the sum of the 10 homolog groups.

<sup>3</sup> This sample was excluded from the reference envelope because of elevated sediment polycyclic aromatic hydrocarbon (PAH) concentrations.

<sup>4</sup> This sample had sediment chemistry results from cycles 1a and 1b. The cycle 1b results are presented because they were obtained closer in time to when the mussel toxicity test was run.

<sup>5</sup> M = mussel; this sample was only used with the mussel exposures and was not used in cycles 1a or 1b.

**Table 7. Sediment chemistry results from the samples that were tested in both mussel and amphipod or midge exposures, unless otherwise noted.**

Station ID	Sample ID	Cycle <sup>1</sup>	ΣPCB (µg/kg DW) <sup>2</sup>	ΣPCB (µg/kg at 1% OC) <sup>2</sup>	Solids (%)	TOC (%)	Clay (%)	Silt (%)	Sand (%)
TX10-01-P	16	1b	91.2	344	70.1	0.265	20.4	20.6	59
TX20-01-P	28	1b <sup>3,4</sup>	220	303	69.7	0.726	2.8	7.5	89.7
TX20-03-P	24	1b <sup>3</sup>	310	1,410	74.8	0.22	0	3.2	97.1
TX30-01-P	25	1a	60,000	23,200	60	2.59	25.5	37.8	36.7
TX30-02-P	18	1a	740,000	280,000	55	2.64	41.1	49.3	9.6
TX30-03-P	07	1a	150,000	37,600	57	3.99	31.9	42.9	25.2
TX30-04-P	23	1b <sup>3</sup>	15,000	7,210	60.4	2.08	27	29.1	43.9
TX30-05-P	02	1b	120,000	39,200	46.2	3.06	28.8	42	29.2
TX40-01-P	27	1b <sup>4</sup>	13,000	11,900	64.1	1.09	34.4	42.9	22.7
TX40-02-P	17	1b	64,000	59,300	62.2	1.08	31	39.9	29.1
TX40-03-P	15	1b <sup>3</sup>	990	673	62.7	1.47	34.6	54.3	11.1
TX40-04-P	01	1a	68,000	36,200	63	1.88	29.7	40.7	29.6
TX40-05-P	14	1b	68,000	35,600	60.1	1.91	36.9	43.4	19.7
TX50-01-P	08	1a	770,000	279,000	60	2.76	33.7	41.9	24.4
TX50-02-P	19	1a	1,200,000	463,000	59	2.59	39.5	45.8	14.7
TX50-04-P	11	1b <sup>4</sup>	170,000	45,200	59.4	3.76	32.2	41.3	26.5
TX50-05-P	30	1a	410,000	178,000	59	2.3	25.8	30.9	43.3
TX60-01-P	21	M <sup>5</sup>	8,600	30,500	72.8	0.282	6.2	13.4	80.4
TX60-02-P	20	1b <sup>4</sup>	6,000	8,680	66.8	0.691	13	15.9	71.1
TX60-03-P	06	1a	100,000	75,200	65	1.33	17.6	25.7	56.7
TX60-04-P	13	1b <sup>4</sup>	25,000	16,900	65.7	1.48	21.3	25.9	52.8
TXR1-01-P	26	1b	<33	<109	68.2	0.303	3	8.3	88.7
TXR1-02-P	09	1a	62	86.4	71	0.718	4.4	10.1	85.5
TXR1-03-P	04	1b	<16	<26	68.6	0.617	2.9	9.3	87.8
TXR1-04-P	22	1b	<32	<67.6	70.6	0.474	5.8	6.8	87.4
TXR1-05-P	29	1b	<33	<70.6	68.1	0.468	6.4	12.8	80.8
TXR1-06-P	10	1b	<33	<62.6	67	0.527	6.8	19.8	73.4

DW = dry weight; OC = organic carbon; PCB = polychlorinated biphenyls; TOC = total organic carbon.

<sup>1</sup> Cycle that the sediment chemistry result is associated with.

<sup>2</sup> ΣPCBs are calculated as the sum of the 10 homolog groups.

<sup>3</sup> This sample was tested with amphipod and/or midge in cycle 1b and was not tested with mussels.

<sup>4</sup> This sample had sediment chemistry results from cycles 1a and 1b. The cycle 1b results are presented because they were obtained closer in time to when the mussel toxicity test was run.

<sup>5</sup> M = mussel; this sample was only used with the mussel exposures and was not used in cycles 1a or 1b.



**Table 8. Reliability of the sediment toxicity thresholds that were derived based on the results of 28-day toxicity tests with *Lampsilis siliquoidea* (endpoint: biomass).**

Chemical of Potential Concern	n	TT <sub>LR</sub>	TT <sub>HR</sub>	Percent incidence of toxicity (number of samples in parentheses)						
				< TT <sub>LR</sub>	≥ TT <sub>LR</sub>	Correct Classification Rate for TT <sub>LR</sub>	TT <sub>LR</sub> - TT <sub>HR</sub>	≤ TT <sub>HR</sub>	> TT <sub>HR</sub>	Correct Classification Rate for TT <sub>HR</sub>
Total PCBs (µg/kg DW)	23	20,400	89,700	20.0 (2 of 10)	76.9 (10 of 13)	78.3	80.0 (4 of 5)	40.0 (6 of 15)	75.0 (6 of 8)	65.2
Total PCBs (µg/kg; at 1% OC)	23	14,600	49,500	<b>11.1 (1 of 9)</b>	<b>78.6 (11 of 14)</b>	<b>82.6</b>	85.7 (6 of 7)	43.8 (7 of 16)	71.4 (5 of 7)	60.9

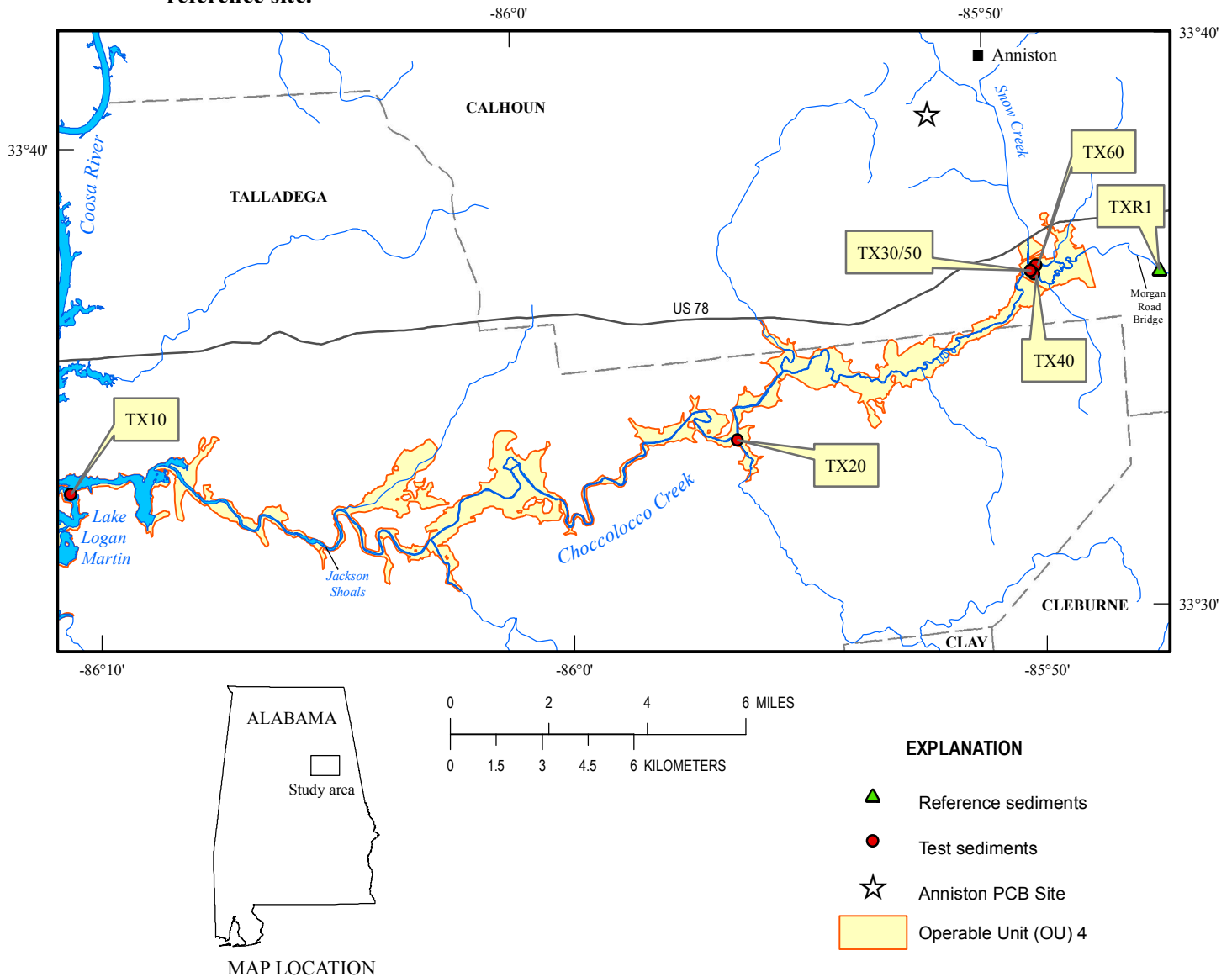
DW = dry weight; HR = high risk; LR = low risk; n = number of samples; OC = organic carbon; TT = toxicity threshold; TT<sub>HR</sub> = high-risk toxicity threshold; TT<sub>LR</sub> = low-risk toxicity threshold. Toxicity thresholds were considered to be reliable if the incidence of toxicity was ≤ 20% below the TT, if the incidence of toxicity was > 50% above the TT, and if the overall correct classification rate was > 80%. Bolded values represent reliable TTs.

---

## **Figures**

---

**Figure 1. Anniston PCB site locations where sediment samples were collected from test sites and from one reference site.**



TXR1: Choccolocco Creek, about 500 meters upstream from Morgan Road Bridge; collected along left bank within about a 10-meter radius of the designated sampling coordinates

TX10: Choccolocco Creek downstream of Jackson Shoals; collected along the lacustrine portion of the site (Lake Logan Martin) near an exposed island within about 50 meters of the designated sampling coordinates

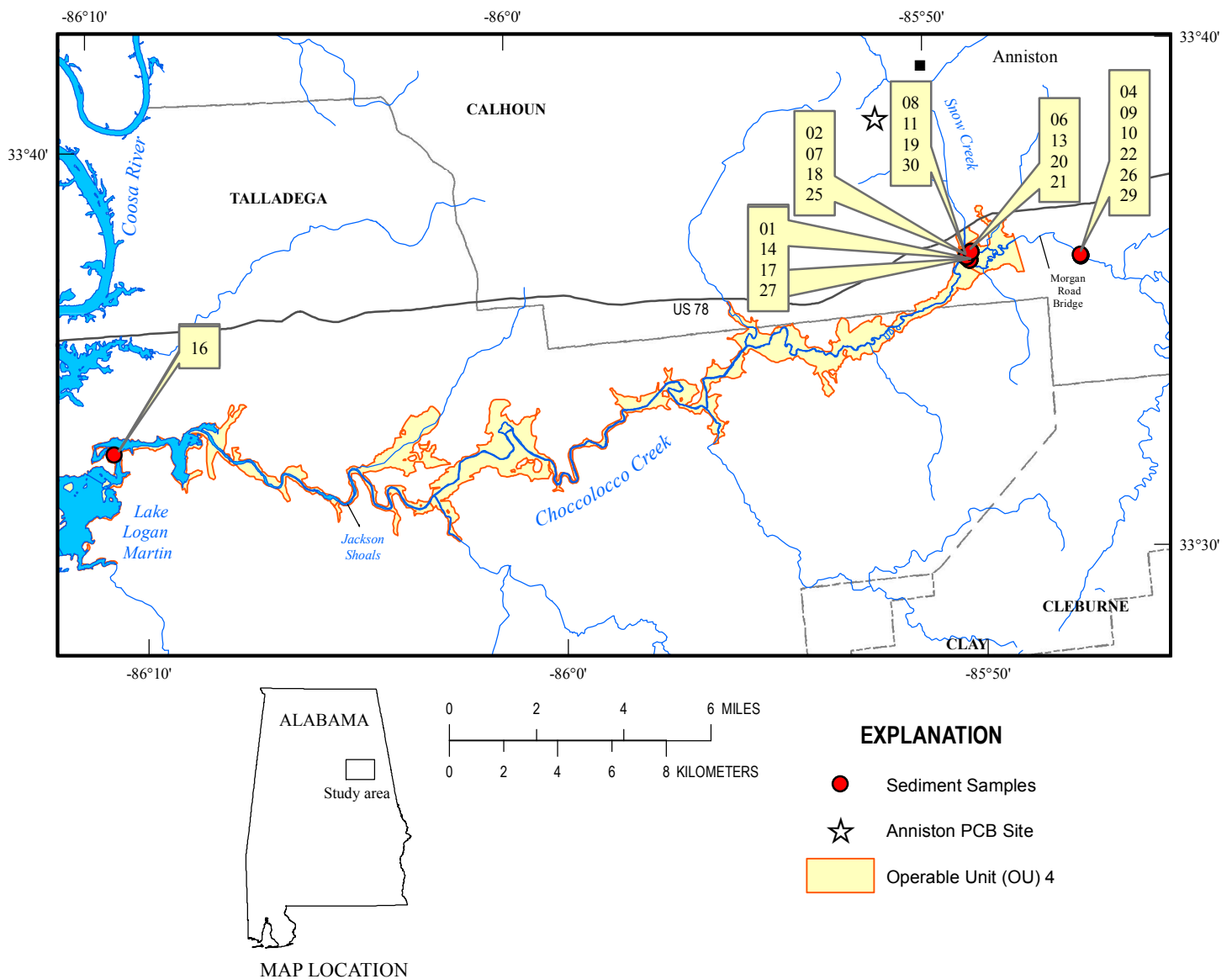
TX20: Choccolocco Creek near Jackson Shoals; collected on the inside portion of a river bend (right bank) across the river from the designated sampling coordinates

TX30: Choccolocco Creek about 125 meters upstream from the confluence with Snow Creek; collected left, middle, right bank within about a 20-meter radius of the designated sampling coordinates

TX40: Choccolocco Creek about 300 meters upstream from the confluence with Snow Creek; collected left, middle, right bank within about a 15-meter radius of the designated sampling coordinates

TX50: Choccolocco Creek about 125 meters upstream from the confluence with Snow Creek; collected left, middle, right bank within about a 20-meter radius of the designated sampling coordinates

**Figure 2. Locations where sediment samples used in mussel toxicity testing were collected.**



TXR1: (Samples 04, 09, 10, 22, 26, 29) Choccolocco Creek, about 500 meters upstream from Morgan Road Bridge; collected along left bank within about a 10-meter radius of the designated sampling coordinates

TX10: (Sample 16) Choccolocco Creek downstream of Jackson Shoals; collected along the lacustrine portion of the site (Lake Logan Martin) near an exposed island within about 50 meters of the designated sampling coordinates

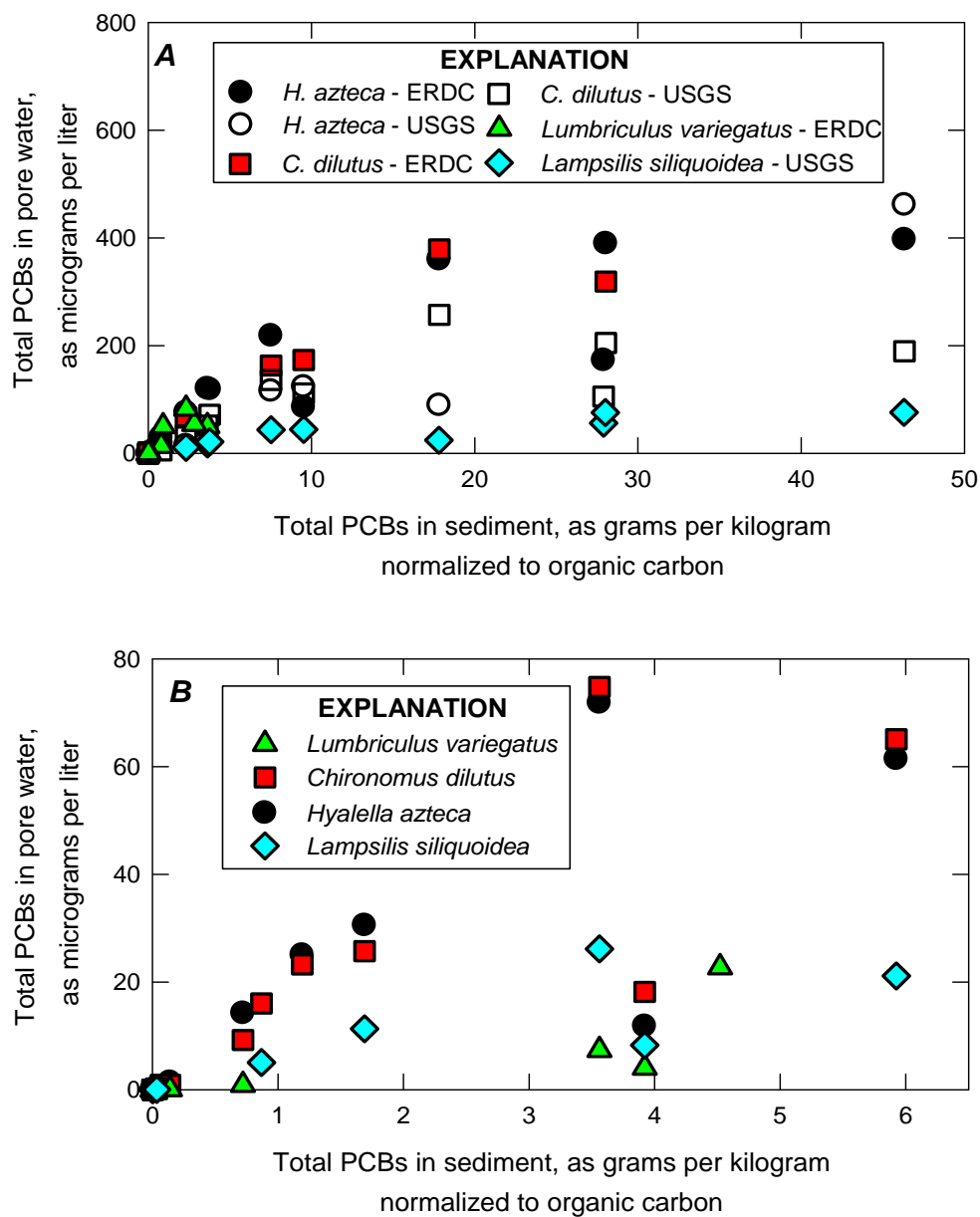
TX30: (Samples 02, 07, 18, 25) Choccolocco Creek about 125 meters upstream from the confluence with Snow Creek; collected left, middle, right bank within about a 20-meter radius of the designated sampling coordinates

TX40: (Samples 01, 14, 17, 27) Choccolocco Creek about 300 meters upstream from the confluence with Snow Creek; collected left, middle, right bank within about a 15-meter radius of the designated sampling coordinates

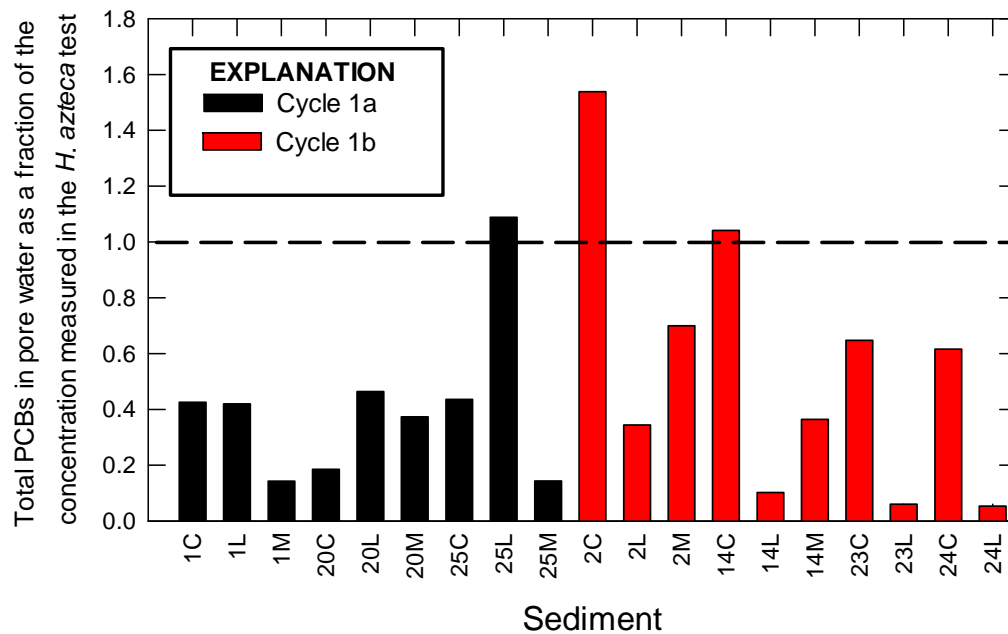
TX50: (Samples 08, 11, 19, 30) Choccolocco Creek about 125 meters upstream from the confluence with Snow Creek; collected left, middle, right bank within about a 20-meter radius of the designated sampling coordinates

TX60: (Samples 06, 13, 20, 21) Choccolocco Creek about 175 meters upstream from the confluence with Snow Creek; collected along the left bank within about a 15-meter radius of the designated sampling coordinates

**Figure 3. Total polychlorinated biphenyls (PCBs) in pore water ( $\mu\text{g/L}$ ), as determined from SPME for exposures with the amphipod *Hyalella azteca* (*H. azteca*), the midge *Chironomus dilutus* (*C. dilutus*), the oligochaete *Lumbriculus variegatus*, and the mussel *Lampsilis siliquioidea*, compared to PCBs in sediment as grams per kilogram normalized to organic carbon (i.e., at 100% OC; g/kg OC) for cycle 1a (Figure 3A) and cycle 1b (Figure 3B). [ERDC, U.S. Army Engineer Research and Development Center; USGS, U.S. Geological Survey]**

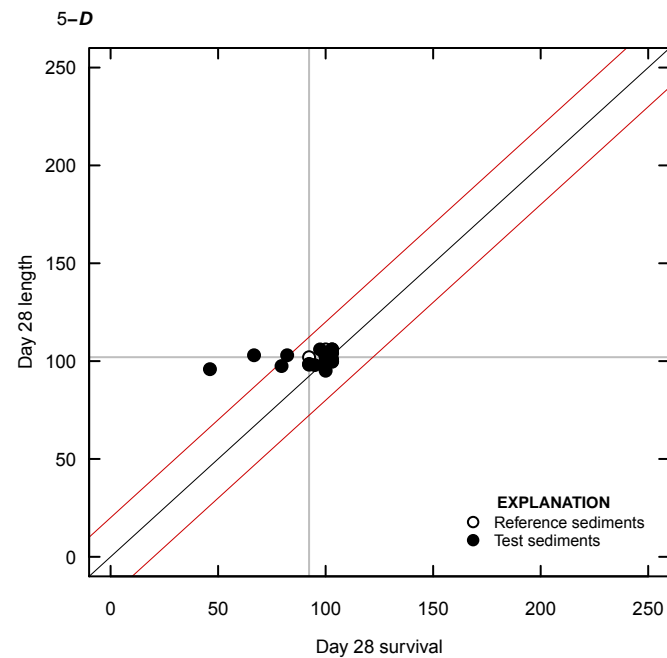
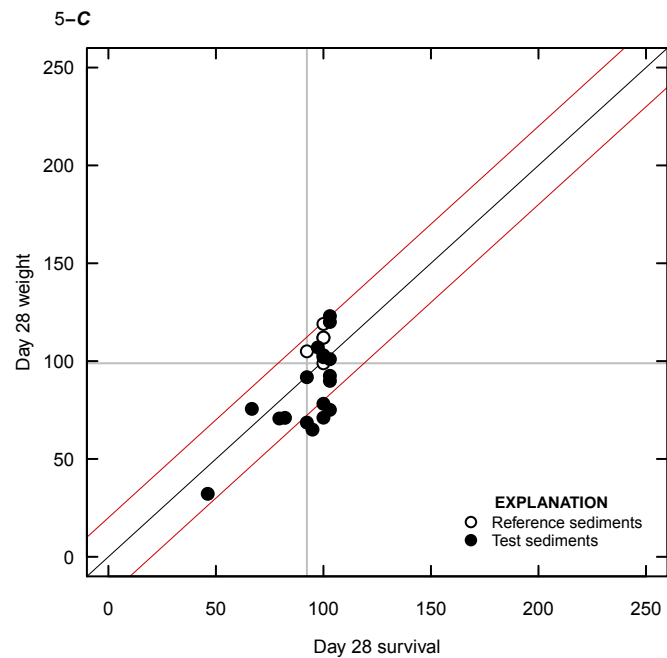
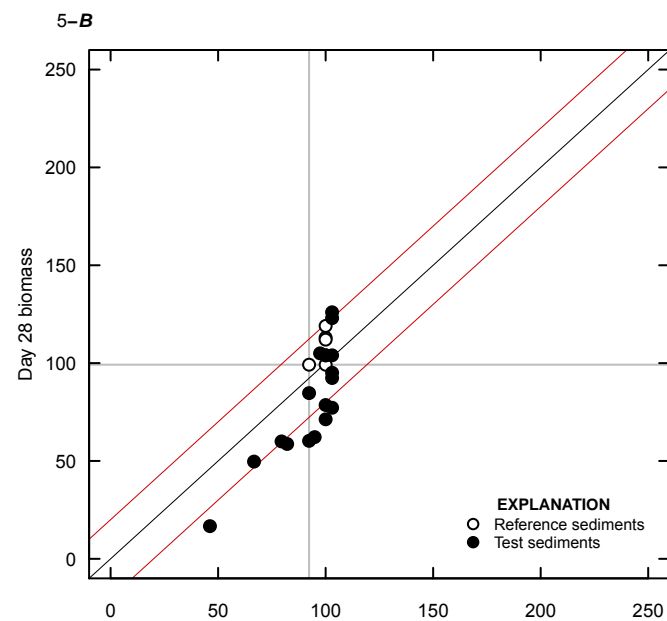
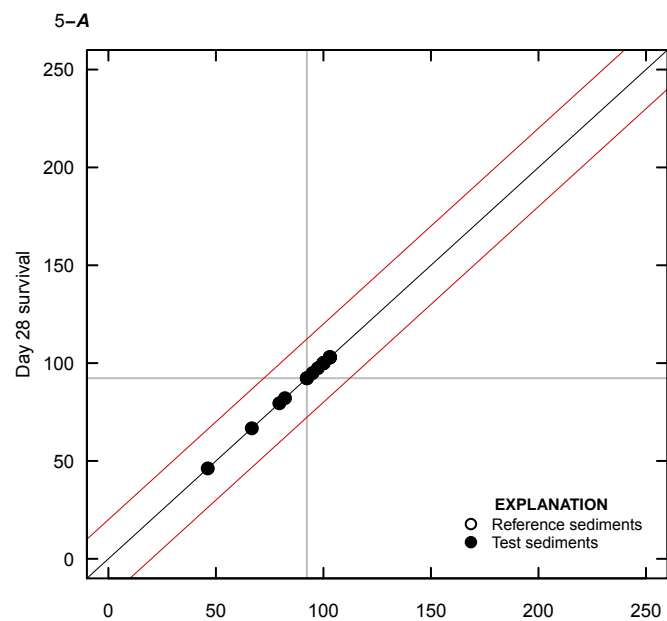


**Figure 4.** Total polychlorinated biphenyls (PCBs) in pore water measured in whole-sediment exposures for select sediments from cycle 1a and cycle 1b for the midge *Chironomus dilutus*, the oligochaete *Lumbriculus variegatus*, and the mussel *Lampsilis siliquioidea* expressed as a fraction of the concentration measured in exposures using *Hyaella azteca*. For example, if the total PCB concentration in pore water for sample 02 in the mussel test was 8.26 µg/L and a total PCB concentration of 11.8 µg/L was measured in sample 02 in the *H. azteca* test, a value of  $8.26 / 11.8 = 0.7$  is reported for that sample. Abbreviations on x-axis are for sample number and *Chironomus dilutus* test (#C), sample number and *Lumbriculus variegatus* test (#L), and sample number and *Lampsilis siliquioidea* test (#M).



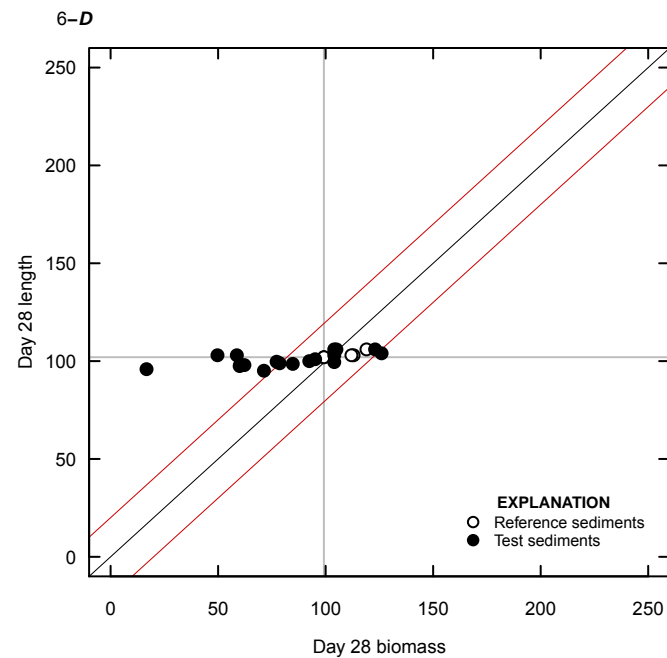
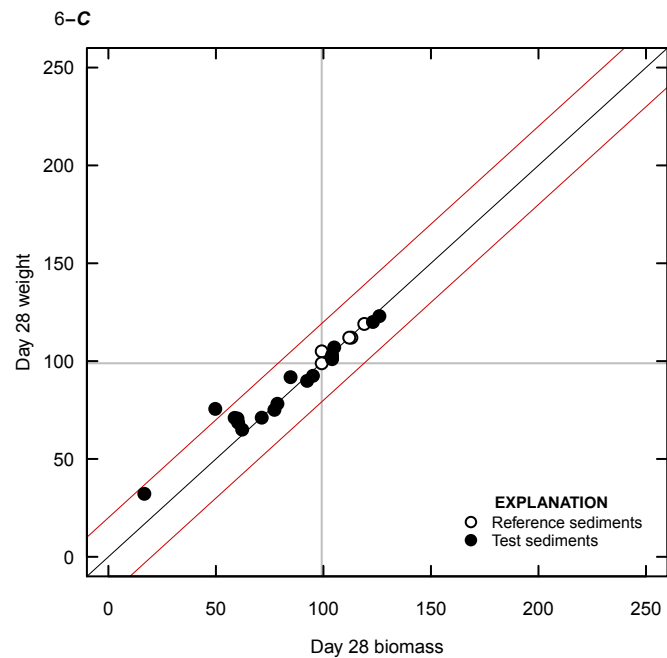
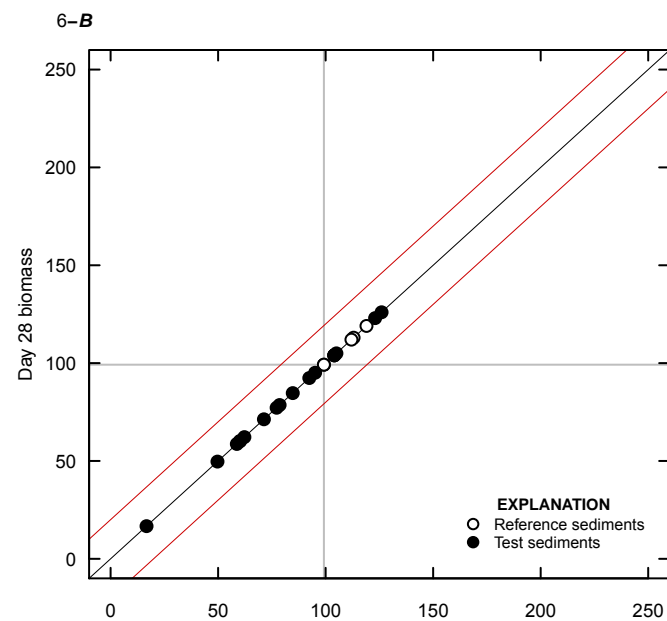
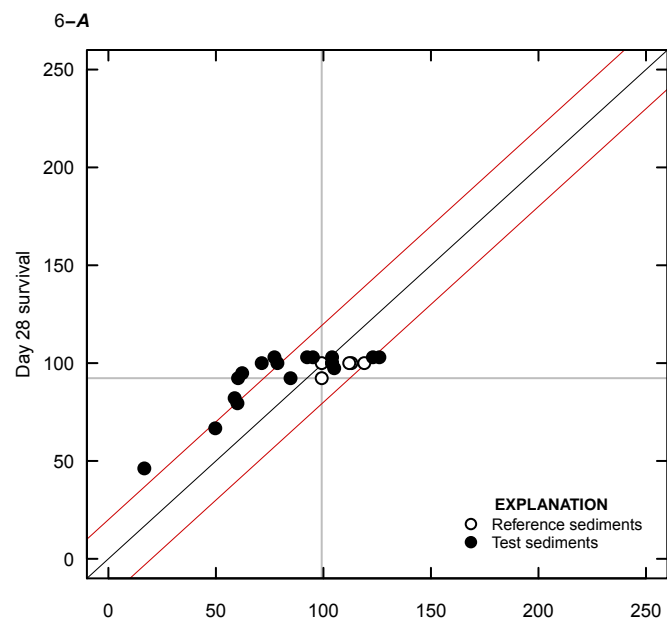
**Figure 5. Comparison of day 28 survival of *Lampsilis siliquioidea* to responses for other endpoints (all values are expressed as percent of control).**

[Solid line represents line of unity and red lines represent plus or minus 20 percent of unity; gray lines represent the minimum value of the reference envelope.]



**Figure 6. Comparison of day 28 biomass of *Lampsilis siliquioidea* to responses for other endpoints (all values are expressed as percent of control).**

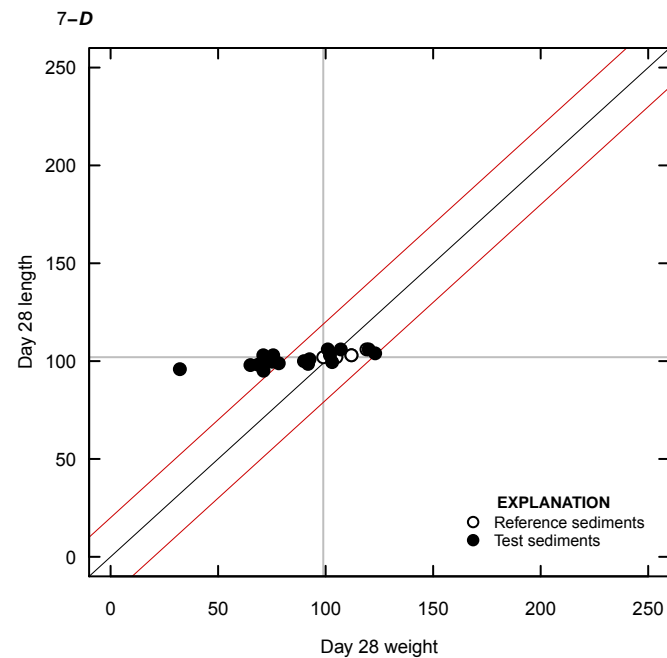
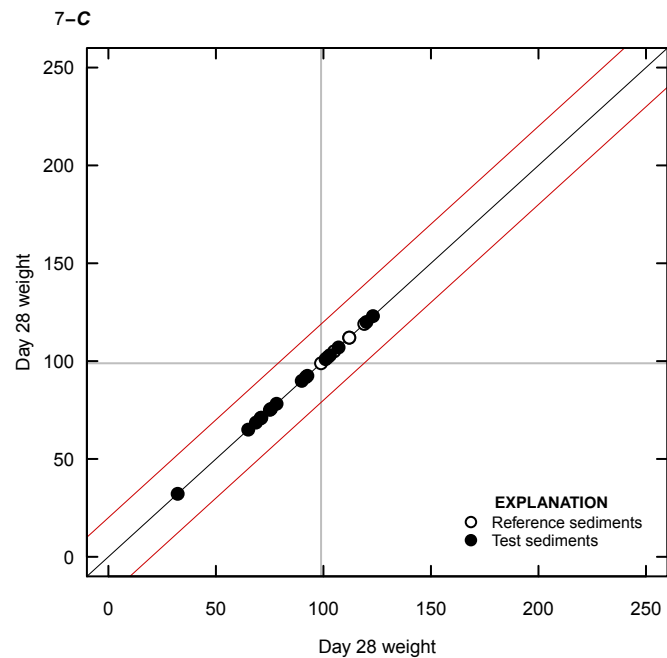
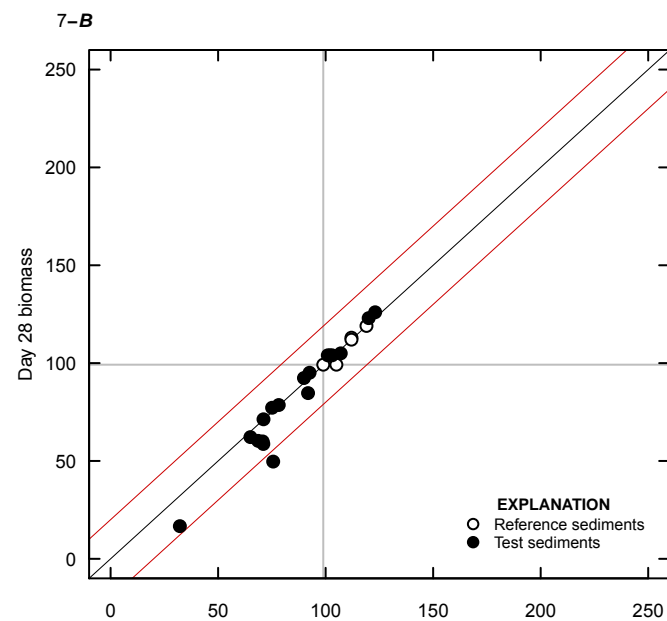
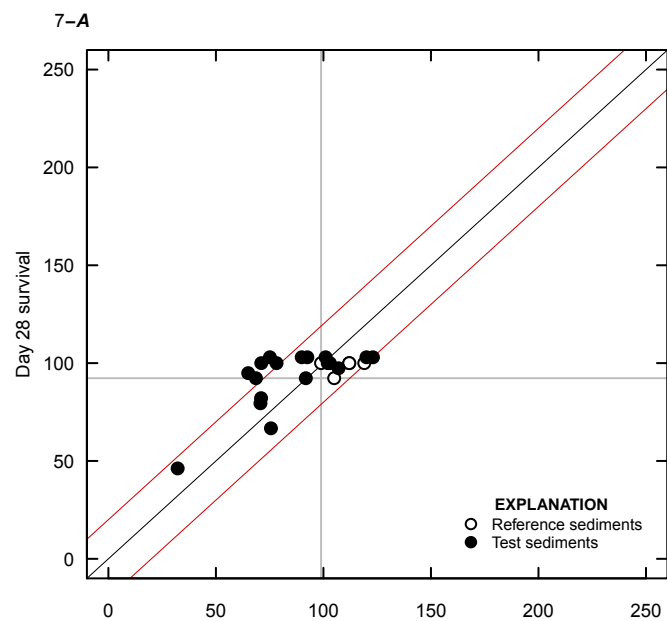
[Solid line represents line of unity and red lines represent plus or minus 20 percent of unity; gray lines represent the minimum value of the reference envelope.]





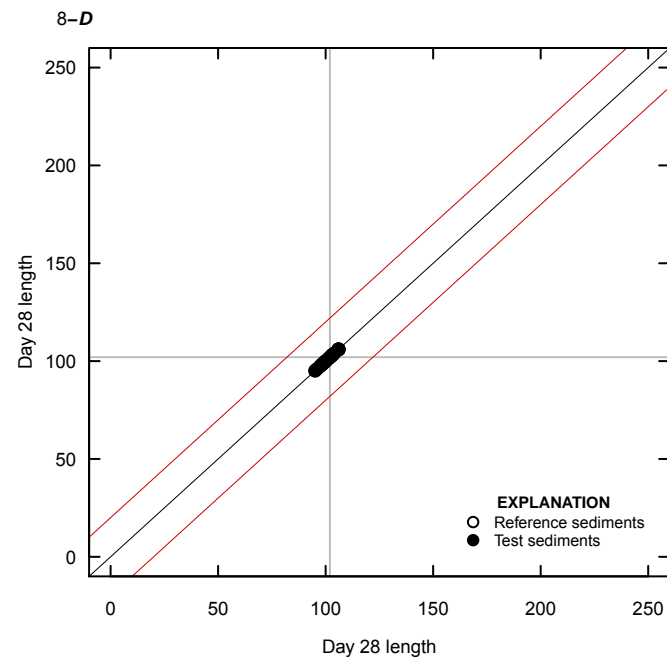
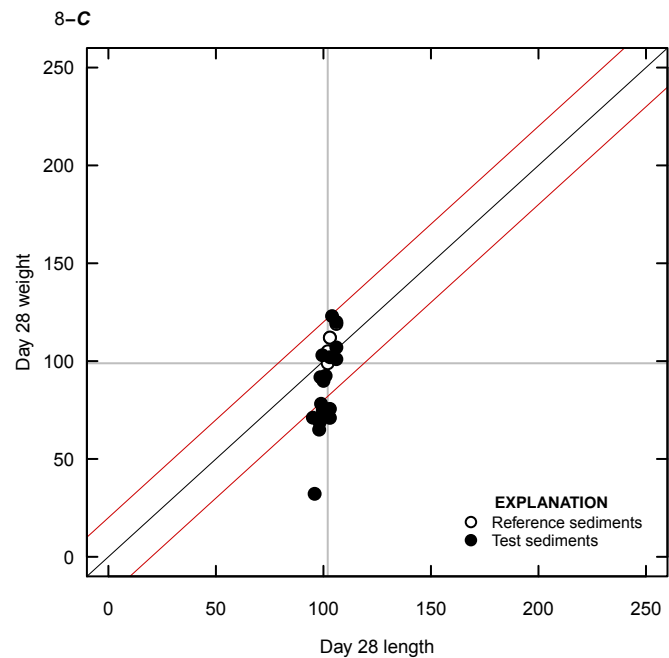
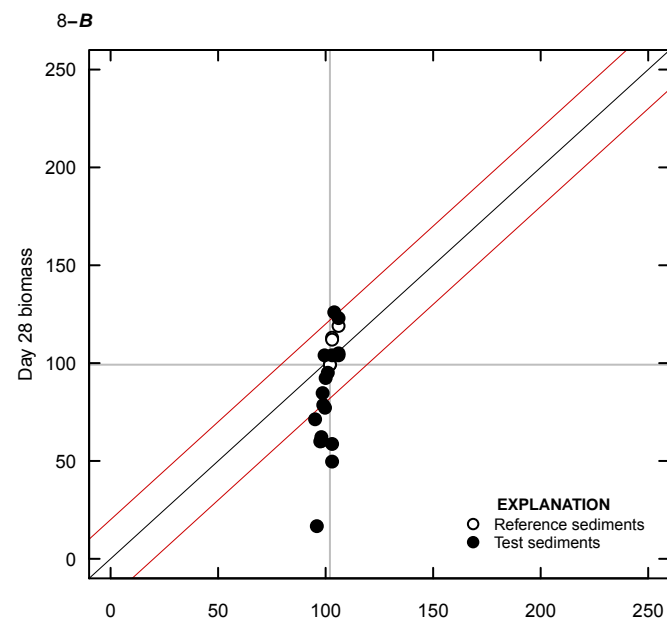
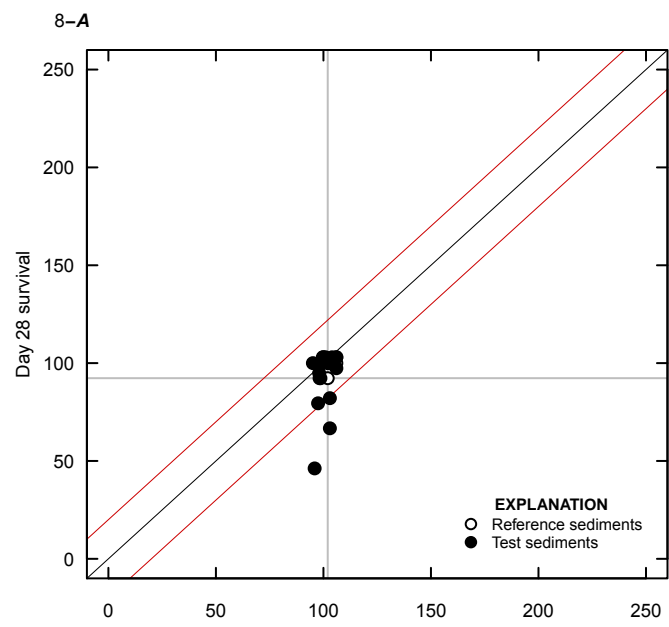
**Figure 7. Comparison of day 28 weight of *Lampsilis siliquioidea* to responses for other endpoints (all values are expressed as percent of control).**

[Solid line represents line of unity and red lines represent plus or minus 20 percent of unity; gray lines represent the minimum value of the reference envelope.]



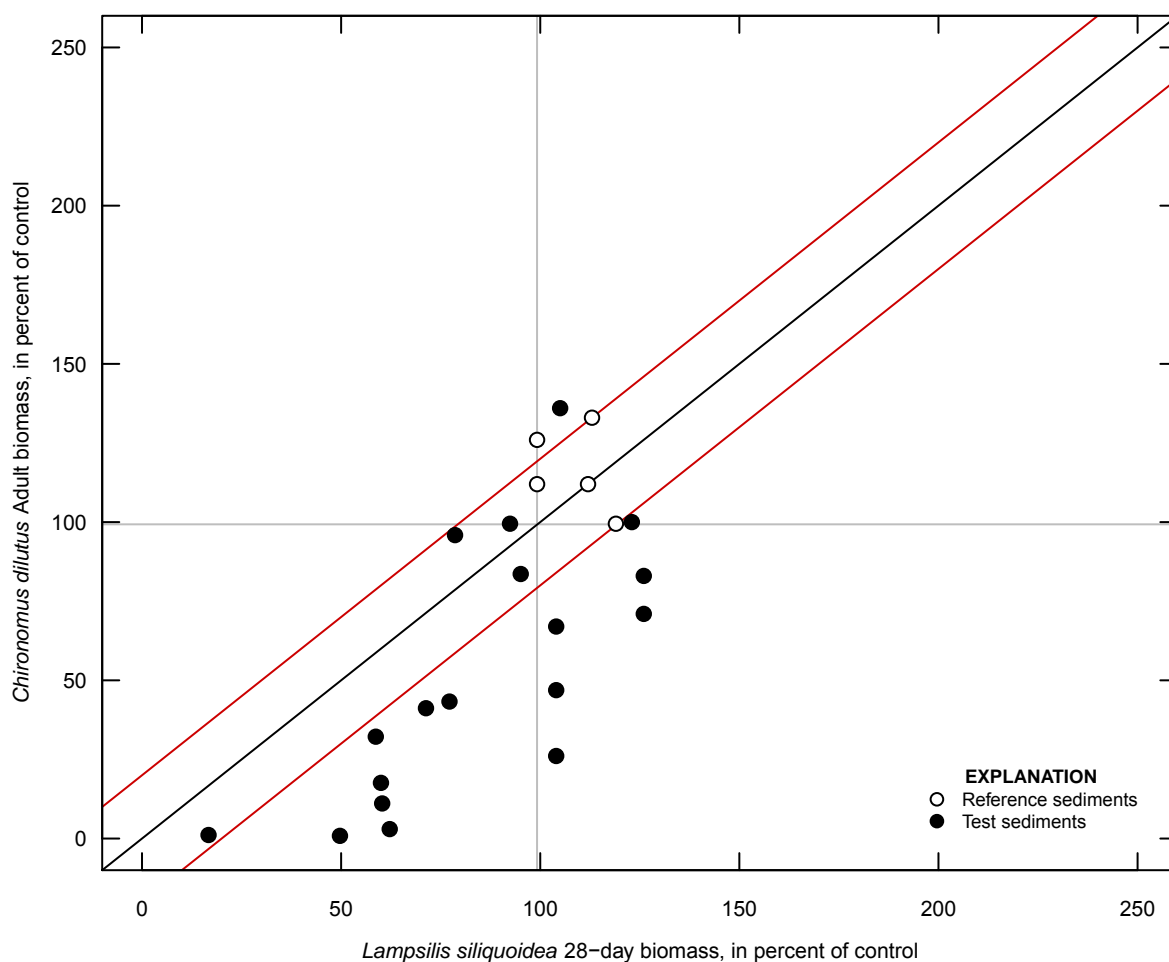
**Figure 8. Comparison of day 28 length of *Lampsilis siliquoidea* to responses for other endpoints (all values are expressed as percent of control).**

[Solid line represents line of unity and red lines represent plus or minus 20 percent of unity; gray lines represent the minimum value of the reference envelope.]



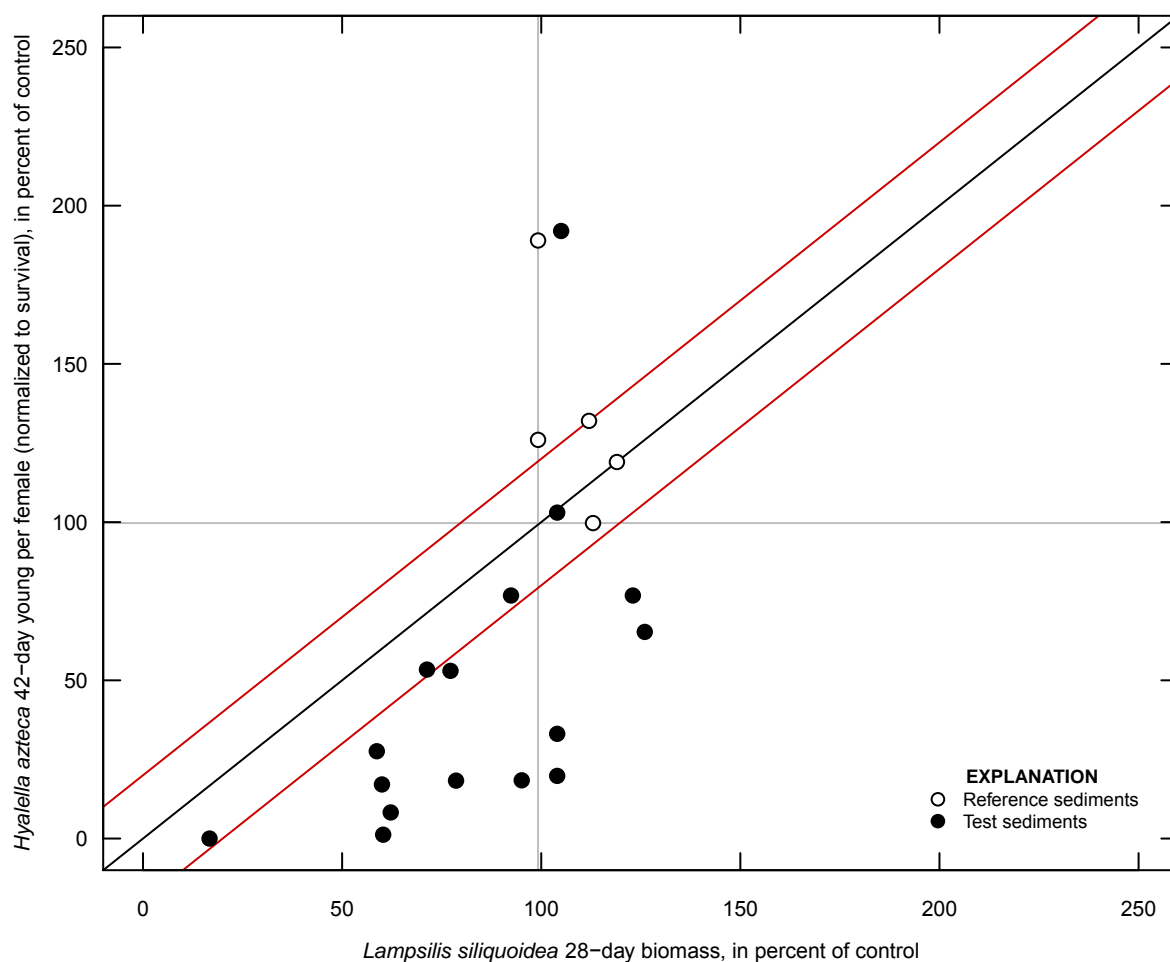
**Figure 9. Relative endpoint sensitivity of the most responsive *Lampsilis siliquoidea* endpoint (biomass) and the most responsive *Chironomus dilutus* endpoint (adult biomass).**

[Solid line represents line of unity and red lines represent plus or minus 20 percent of unity; gray lines represent the minimum value of the reference envelope.]



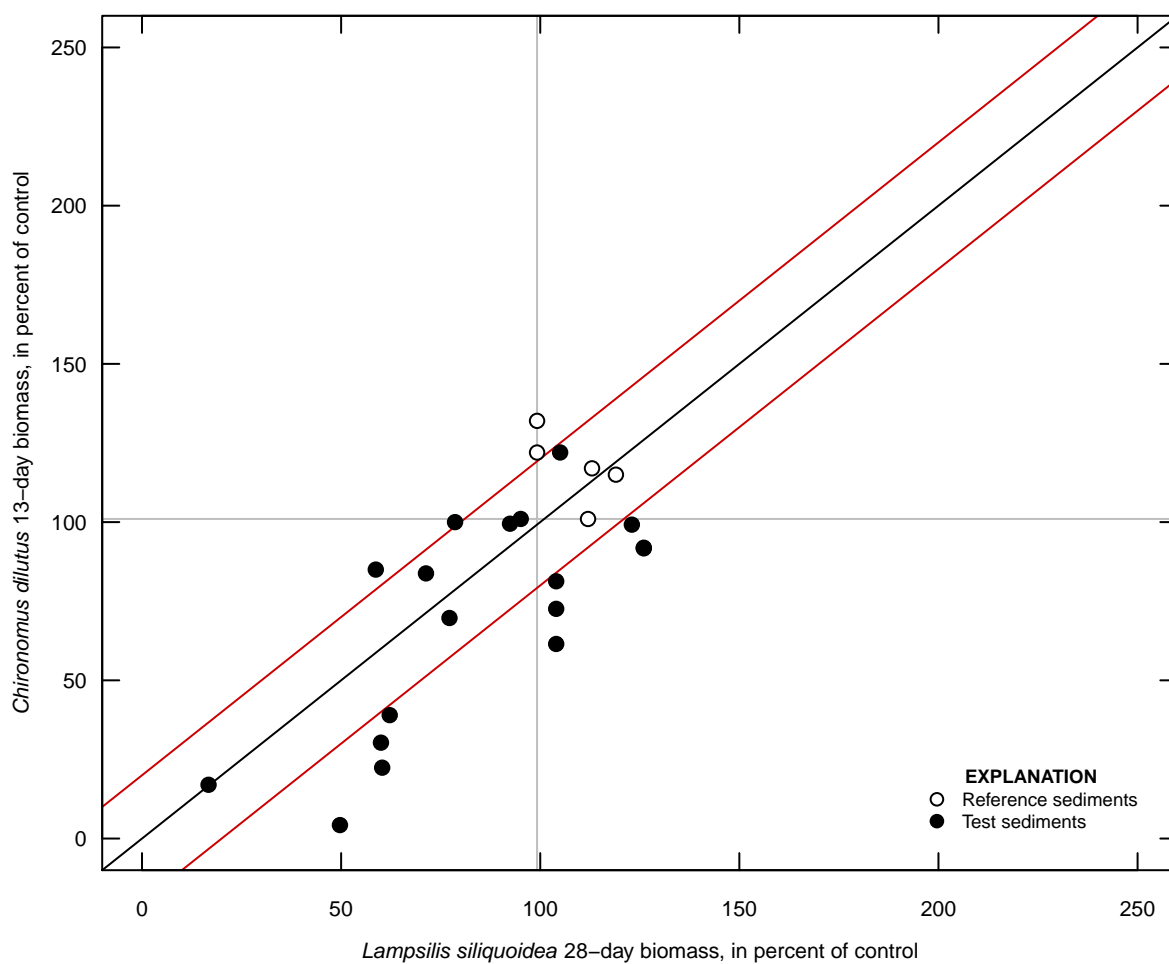
**Figure 10. Relative endpoint sensitivity of the most responsive *Lampsilis siliquoidea* endpoint (biomass) and the most responsive *Hyalella azteca* endpoint (survival–normalized young per female).**

[Solid line represents line of unity and red lines represent plus or minus 20 percent of unity; gray lines represent the minimum value of the reference envelope.]



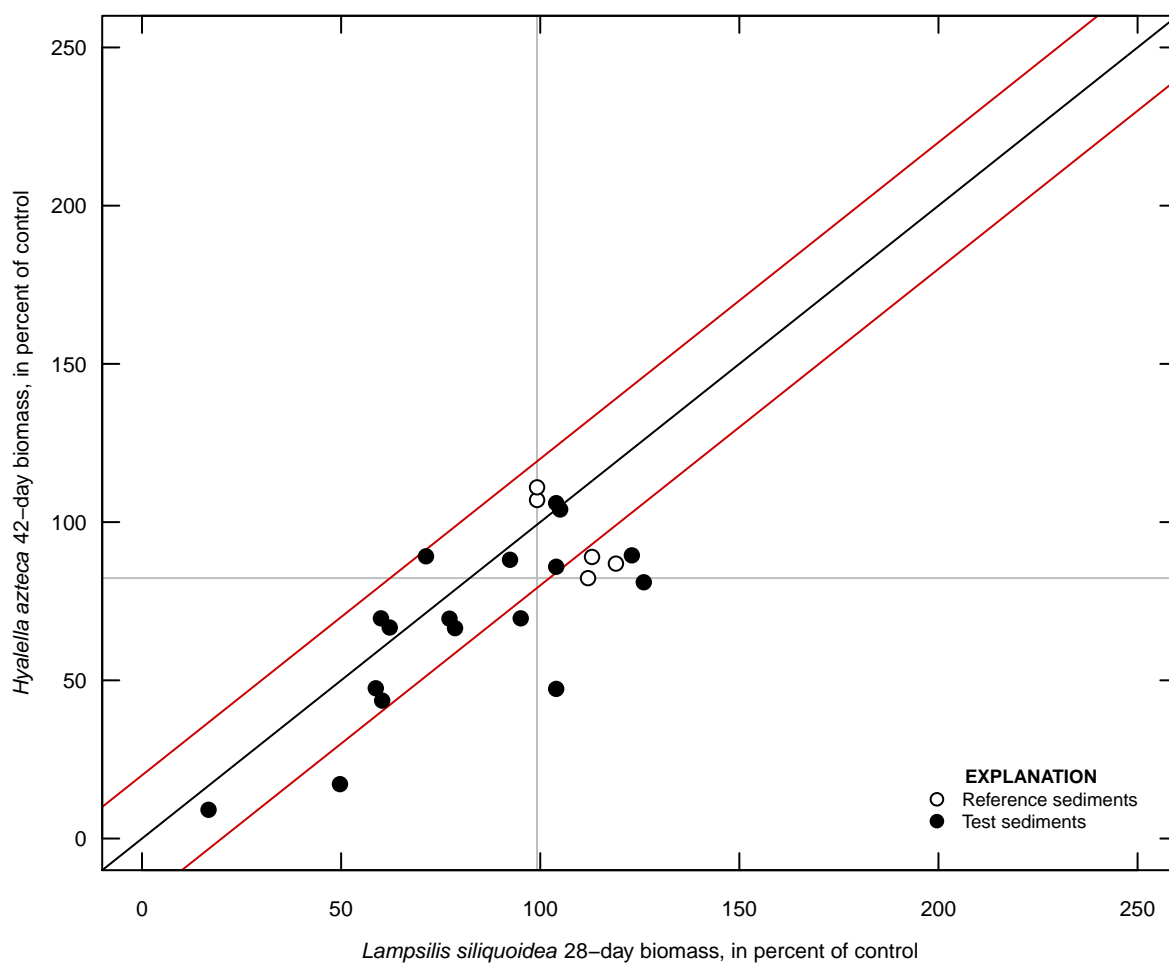
**Figure 11. Relative endpoint sensitivity of the most responsive *Lampsilis siliquoidea* endpoint (biomass) and *Chironomus dilutus* biomass.**

[Solid line represents line of unity and red lines represent plus or minus 20 percent of unity; gray lines represent the minimum value of the reference envelope.]



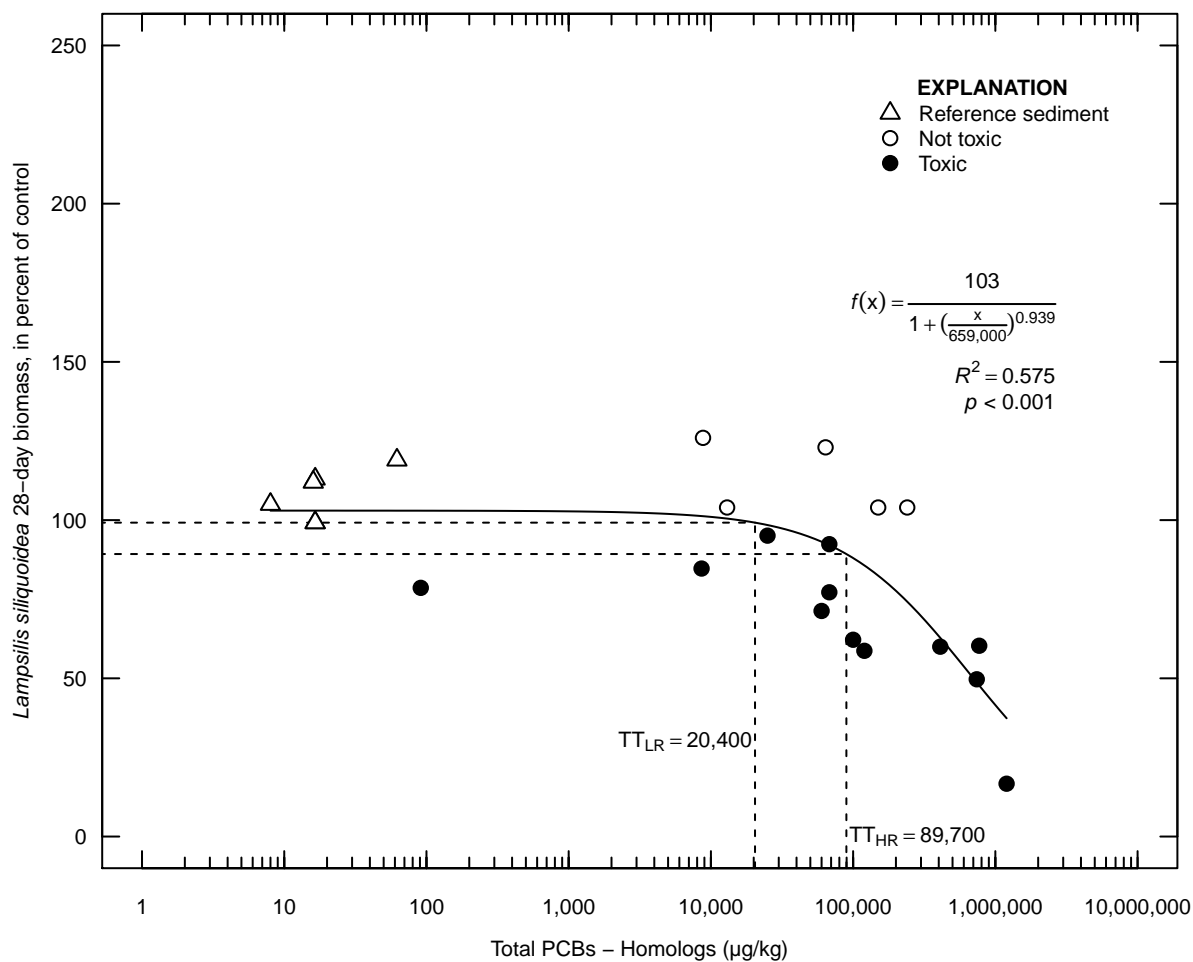
**Figure 12. Relative endpoint sensitivity of the most responsive *Lampsilis siliquoidea* endpoint (biomass) and *Hyalella azteca* biomass.**

[Solid line represents line of unity and red lines represent plus or minus 20 percent of unity; gray lines represent the minimum value of the reference envelope.]



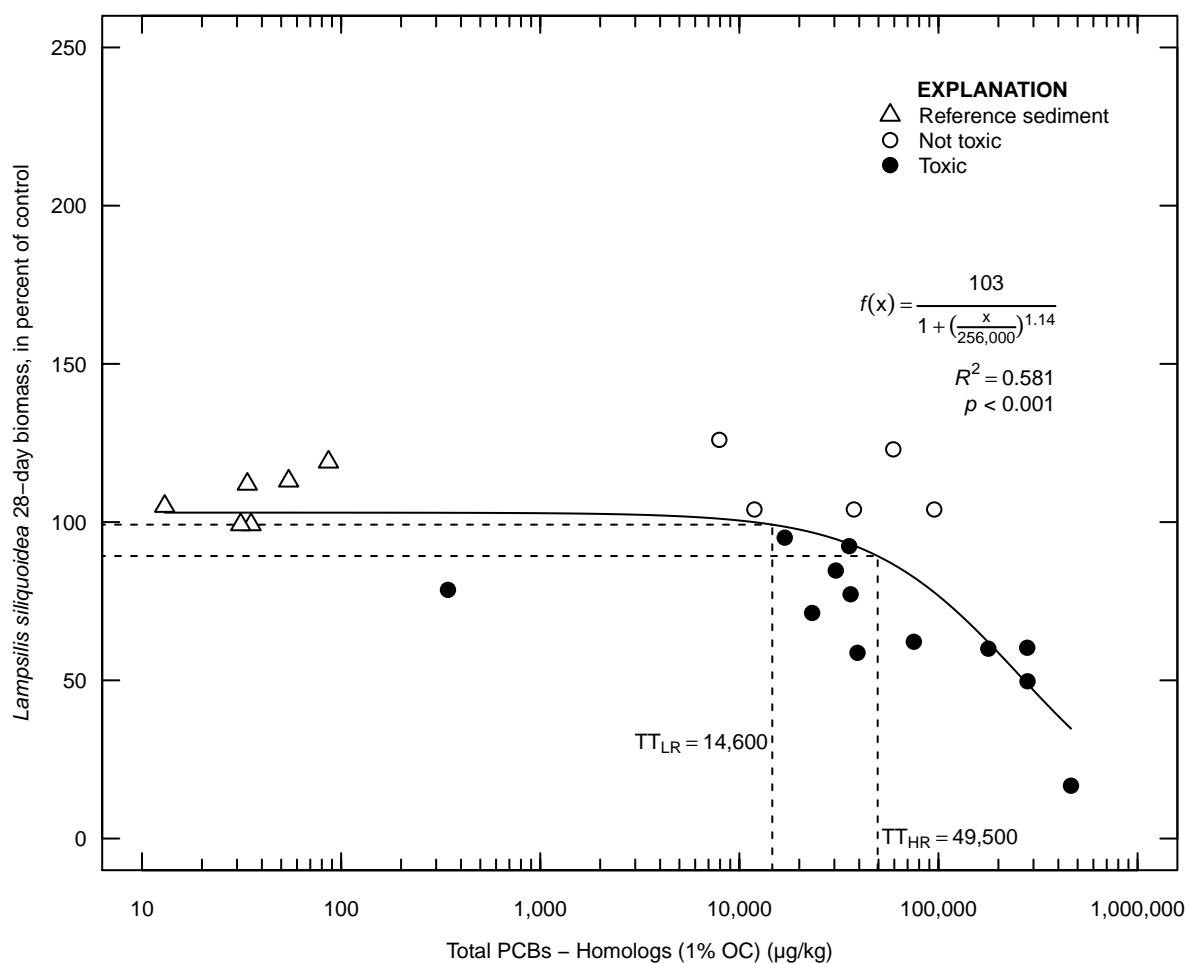
**Figure 13. Concentration–response model for total polychlorinated biphenyls (PCBs; µg/kg DW) and *Lampsilis siligoidea* day 28 biomass.**

[µg/kg, micrograms per kilogram; DW, dry weight; TT<sub>LR</sub>, low risk toxicity threshold; TT<sub>HR</sub>, high risk toxicity threshold]



**Figure 14. Concentration–response model for total polychlorinated biphenyls (PCBs; at 1 percent OC; µg/kg) and *Lampsilis siliquoidea* day 28 biomass.**

[OC, organic carbon; µg/kg, micrograms per kilogram; TT<sub>LR</sub>, low risk toxicity threshold; TT<sub>HR</sub>, high risk toxicity threshold]





---

## **Appendix 1**

**Toxicity test results  
from the *Lampsilis*  
*siliquoidea* exposures**

---

## Table of Contents

<b>Table A1-1</b>	Overlying water quality data for 28-day toxicity tests with <i>Lampsilis siliquidea</i> . . . . .	A1-1
<b>Table A1-2</b>	Summary of overlying water quality data collected during the 28-day whole-sediment toxicity tests with <i>Lampsilis siliquidea</i> ..	A1-6
<b>Table A1-3</b>	Results of 28-day whole-sediment toxicity tests with <i>Lampsilis siliquidea</i> : survival. . . . .	A1-8
<b>Table A1-4</b>	Results of 28-day whole-sediment toxicity tests with <i>Lampsilis siliquidea</i> : weight and biomass. . . . .	A1-11
<b>Table A1-5</b>	Results of 28-day whole-sediment toxicity tests with <i>Lampsilis siliquidea</i> : length. . . . .	A1-15
<b>Table A1-6</b>	Summary of results of 28-day whole-sediment toxicity tests with <i>Lampsilis siliquidea</i> . . . . .	A1-37
<b>Table A1-7</b>	Summary of control-adjusted responses observed in <i>Lampsilis siliquidea</i> exposures during toxicity testing of whole sediment collected from the Anniston PCB Site. . . . .	A1-38

**Table A1-1. Overlying water quality data for 28-day toxicity tests with *Lampsilis siliquidea*.**

Sediment ID	Cycle	Test Day	Date of Measurment	Dissolved Oxygen (mg/L)	pH	Alkalinity (mg/L CaCO <sub>3</sub> )	Hardness (mg/L CaCO <sub>3</sub> )	Conductivity (µS/cm)	Total Ammonia (mg/L)
01	M	-1	2010-12-08	7.7	7.9	94	108	255	0.217
01	M	7	2010-12-16	7.1				258	
01	M	13	2010-12-22	8.0				257	
01	M	21	2010-12-30	7.6				258	
01	M	27	2011-01-05	5.4	7.8	90	110	258	0.0361
02	M	-1	2010-12-08	7.6	7.9	94	104	257	0.154
02	M	7	2010-12-16	6.5				256	
02	M	13	2010-12-22	7.6				257	
02	M	21	2010-12-30	6.6				255	
02	M	27	2011-01-05	5.1	7.8	92	112	258	0.0278
04	M	-1	2010-12-08	7.7	7.9	96	114	262	0.0318
04	M	7	2010-12-16	7.4				259	
04	M	13	2010-12-22	6.5				270	
04	M	21	2010-12-30	7.1				260	
04	M	27	2011-01-05	5.1	8.0	100	112	267	0.0222
06	M	-1	2010-12-08	7.7	7.9	92	110	260	0.0879
06	M	7	2010-12-16	7.8				258	
06	M	13	2010-12-22	7.0				265	
06	M	21	2010-12-30	6.9				257	
06	M	27	2011-01-05	5.2	7.8	92	110	257	0.0199
07	M	-1	2010-12-08	7.4	7.9	94	100	260	0.319
07	M	7	2010-12-16	6.2				261	
07	M	13	2010-12-22	7.9				255	
07	M	21	2010-12-30	6.0				252	
07	M	27	2011-01-05	5.1	7.8	94	112	255	0.0228

**Table A1-1. Overlying water quality data for 28-day toxicity tests with *Lampsilis siliquidea*.**

Sediment ID	Cycle	Test Day	Date of Measurment	Dissolved Oxygen (mg/L)	pH	Alkalinity (mg/L CaCO <sub>3</sub> )	Hardness (mg/L CaCO <sub>3</sub> )	Conductivity (µS/cm)	Total Ammonia (mg/L)
08	M	-1	2010-12-08	7.2	7.9	96	110	258	0.207
08	M	7	2010-12-16	6.3				264	
08	M	13	2010-12-22	7.7				257	
08	M	21	2010-12-30	6.0				253	
08	M	27	2011-01-05	5.2	7.9	94	110	256	0.0196
09	M	-1	2010-12-08	7.7	7.9	90	108	263	0.0651
09	M	7	2010-12-16	7.6				258	
09	M	13	2010-12-22	6.8				266	
09	M	21	2010-12-30	6.9				260	
09	M	27	2011-01-05	5.1	7.9	96	112	256	0.0203
10	M	-1	2010-12-08	7.3	7.6	90	96	256	0.0328
10	M	7	2010-12-16	7.5				258	
10	M	13	2010-12-22	6.4				270	
10	M	21	2010-12-30	7.0				261	
10	M	27	2011-01-05	5.1	7.9	94	110	256	0.0215
11	M	-1	2010-12-08	7.3	7.8	90	110	260	0.156
11	M	7	2010-12-16	7.4				256	
11	M	13	2010-12-22	6.6				261	
11	M	21	2010-12-30	7.0				257	
11	M	27	2011-01-05	5.4	7.9	96	106	256	0.0303
13	M	-1	2010-12-08	7.5	7.9	86	110	256	0.145
13	M	7	2010-12-16	7.6				257	
13	M	13	2010-12-22	6.8				260	
13	M	21	2010-12-30	7.2				257	
13	M	27	2011-01-05	5.4	7.9	90	104	255	0.0335

**Table A1-1. Overlying water quality data for 28-day toxicity tests with *Lampsilis siliquidea*.**

Sediment ID	Cycle	Test Day	Date of Measurment	Dissolved Oxygen (mg/L)	pH	Alkalinity (mg/L CaCO <sub>3</sub> )	Hardness (mg/L CaCO <sub>3</sub> )	Conductivity (µS/cm)	Total Ammonia (mg/L)
14	M	-1	2010-12-08	7.4	7.9	96	110	252	0.251
14	M	7	2010-12-16	6.8				256	
14	M	13	2010-12-22	7.8				256	
14	M	21	2010-12-30	6.5				256	
14	M	27	2011-01-05	5.3	8.0	90	106	254	0.0266
16	M	-1	2010-12-08	8.2	8.0	88	100	253	0.0402
16	M	7	2010-12-16	7.8				257	
16	M	13	2010-12-22	8.3				261	
16	M	21	2010-12-30	7.8				257	
16	M	27	2011-01-05	5.4	8.0	90	108	258	0.0320
17	M	-1	2010-12-08	7.7	8.0	94	110	255	0.335
17	M	7	2010-12-16	6.7				254	
17	M	13	2010-12-22	7.8				255	
17	M	21	2010-12-30	6.7				257	
17	M	27	2011-01-05	5.1	8.0	90	100	250	0.0241
18	M	-1	2010-12-08	7.1	7.9	98	110	269	0.511
18	M	7	2010-12-16	6.1				266	
18	M	13	2010-12-22	7.8				258	
18	M	21	2010-12-30	6.0				251	
18	M	27	2011-01-05	5.0	8.1	92	108	258	0.0339
19	M	-1	2010-12-08	7.4	8.0	96	124	270	0.481
19	M	7	2010-12-16	6.1				265	
19	M	13	2010-12-22	7.8				264	
19	M	21	2010-12-30	7.3				257	
19	M	27	2011-01-05	5.3	8.1	92	112	256	0.0257

**Table A1-1. Overlying water quality data for 28-day toxicity tests with *Lampsilis siliquidea*.**

Sediment ID	Cycle	Test Day	Date of Measurment	Dissolved Oxygen (mg/L)	pH	Alkalinity (mg/L CaCO <sub>3</sub> )	Hardness (mg/L CaCO <sub>3</sub> )	Conductivity (µS/cm)	Total Ammonia (mg/L)
20	M	-1	2010-12-08	7.6	8.0	94	124	269	0.0744
20	M	7	2010-12-16	7.2				259	
20	M	13	2010-12-22	8.2				259	
20	M	21	2010-12-30	7.6				257	
20	M	27	2011-01-05	5.5	8.1	92	102	257	0.0309
21	M	-1	2010-12-08	7.7	7.9	92	110	264	0.0277
21	M	7	2010-12-16	7.8				259	
21	M	13	2010-12-22	7.3				266	
21	M	21	2010-12-30	7.2				258	
21	M	27	2011-01-05	5.7	8.1	90	102	255	0.0223
22	M	-1	2010-12-08	7.5	7.8	92	120	260	0.0280
22	M	7	2010-12-16	7.6				257	
22	M	13	2010-12-22	6.6				265	
22	M	21	2010-12-30	6.8				260	
22	M	27	2011-01-05	5.5	8.1	90	106	259	0.0173
25	M	-1	2010-12-08	7.4	7.9	92	100	255	0.0296
25	M	7	2010-12-16	7.1				260	
25	M	13	2010-12-22	8.0				261	
25	M	21	2010-12-30	7.4				257	
25	M	27	2011-01-05	5.5	8.1	90	106	261	0.0237
26	M	-1	2010-12-08	7.7	7.8	92	122	259	0.0296
26	M	7	2010-12-16	7.6				256	
26	M	13	2010-12-22	6.8				263	
26	M	21	2010-12-30	7.2				259	
26	M	27	2011-01-05	5.5	8.1	92	106	261	0.0237
27	M	-1	2010-12-08	7.7	7.9	94	104	251	0.307
27	M	7	2010-12-16	7.9				251	
27	M	13	2010-12-22	7.9				256	
27	M	21	2010-12-30	7.3				257	
27	M	27	2011-01-05	5.2	8.1	90	104	254	0.0199

**Table A1-1. Overlying water quality data for 28-day toxicity tests with *Lampsilis siliquidea*.**

Sediment ID	Cycle	Test Day	Date of Measurment	Dissolved Oxygen (mg/L)	pH	Alkalinity (mg/L CaCO <sub>3</sub> )	Hardness (mg/L CaCO <sub>3</sub> )	Conductivity (µS/cm)	Total Ammonia (mg/L)
29	M	-1	2010-12-08	7.9	8.1	94	110	259	0.0416
29	M	7	2010-12-16	7.2				256	
29	M	13	2010-12-22	7.0				266	
29	M	21	2010-12-30	7.0				260	
29	M	27	2011-01-05	5.2	8.1	90	104	254	0.0206
30	M	-1	2010-12-08	7.2	7.9	92	110	264	0.0879
30	M	7	2010-12-16	7.4				259	
30	M	13	2010-12-22	6.7				266	
30	M	21	2010-12-30	7.0				261	
30	M	27	2011-01-05	5.4	8.0	90	106	260	0.0227
33	M	-1	2010-12-08	7.7	7.6	90	110	257	0.0513
33	M	7	2010-12-16	7.8				257	
33	M	13	2010-12-22	7.0				264	
33	M	21	2010-12-30	7.9				256	
33	M	27	2011-01-05	5.5	8.1	90	104	251	0.0219

M = mussel exposure.

**Table A1-2. Summary of overlying water quality data collected during the 28-day whole-sediment toxicity tests with *Lampsilis siliquidea*.**

Sediment ID	Dissolved Oxygen (mg/L)					pH					Alkalinity (mg/L CaCO <sub>3</sub> )				
	Mean	SD	Min	Max	n	Mean	SD	Min	Max	n	Mean	SD	Min	Max	n
01	7.2	1.0	5.4	8.0	5	7.9	0.0	7.8	7.9	2	92.0	2.8	90.0	94.0	2
02	6.7	1.0	5.1	7.6	5	7.9	0.1	7.8	7.9	2	93.0	1.4	92.0	94.0	2
04	6.8	1.0	5.1	7.7	5	8.0	0.0	7.9	8.0	2	98.0	2.8	96.0	100.0	2
06	6.9	1.0	5.2	7.8	5	7.8	0.0	7.8	7.9	2	92.0	0.0	92.0	92.0	2
07	6.5	1.1	5.1	7.9	5	7.8	0.1	7.8	7.9	2	94.0	0.0	94.0	94.0	2
08	6.5	1.0	5.2	7.7	5	7.9	0.0	7.9	7.9	2	95.0	1.4	94.0	96.0	2
09	6.8	1.0	5.1	7.7	5	7.9	0.0	7.9	7.9	2	93.0	4.2	90.0	96.0	2
10	6.7	1.0	5.1	7.5	5	7.8	0.2	7.6	7.9	2	92.0	2.8	90.0	94.0	2
11	6.7	0.8	5.4	7.4	5	7.9	0.1	7.8	7.9	2	93.0	4.2	90.0	96.0	2
13	6.9	0.9	5.4	7.6	5	7.9	0.0	7.9	7.9	2	88.0	2.8	86.0	90.0	2
14	6.8	1.0	5.3	7.8	5	7.9	0.0	7.9	8.0	2	93.0	4.2	90.0	96.0	2
16	7.5	1.2	5.4	8.3	5	8.0	0.0	8.0	8.0	2	89.0	1.4	88.0	90.0	2
17	6.8	1.1	5.1	7.8	5	8.0	0.0	8.0	8.0	2	92.0	2.8	90.0	94.0	2
18	6.4	1.1	5.0	7.8	5	8.0	0.1	7.9	8.1	2	95.0	4.2	92.0	98.0	2
19	6.8	1.0	5.3	7.8	5	8.0	0.1	8.0	8.1	2	94.0	2.8	92.0	96.0	2
20	7.2	1.0	5.5	8.2	5	8.0	0.1	8.0	8.1	2	93.0	1.4	92.0	94.0	2
21	7.1	0.8	5.7	7.8	5	8.0	0.1	7.9	8.1	2	91.0	1.4	90.0	92.0	2
22	6.8	0.8	5.5	7.6	5	7.9	0.2	7.8	8.1	2	91.0	1.4	90.0	92.0	2
25	7.1	0.9	5.5	8.0	5	8.0	0.1	7.9	8.1	2	91.0	1.4	90.0	92.0	2
26	6.9	0.9	5.5	7.7	5	8.0	0.2	7.8	8.1	2	92.0	0.0	92.0	92.0	2
27	7.2	1.1	5.2	7.9	5	8.0	0.1	7.9	8.1	2	92.0	2.8	90.0	94.0	2
29	6.9	1.0	5.2	7.9	5	8.1	0.0	8.1	8.1	2	92.0	2.8	90.0	94.0	2
30	6.7	0.8	5.4	7.4	5	8.0	0.1	7.9	8.0	2	91.0	1.4	90.0	92.0	2
33	7.2	1.0	5.5	7.9	5	7.8	0.3	7.6	8.1	2	90.0	0.0	90.0	90.0	2



**Table A1-2. Summary of overlying water quality data collected during the 28-day whole-sediment toxicity tests with *Lampsilis siliquidea*.**

Sediment ID	Hardness (mg/L CaCO <sub>3</sub> )					Specific Conductivity (µS/cm)					Total Ammonia (mg/L)				
	Mean	SD	Min	Max	n	Mean	SD	Min	Max	n	Mean	SD	Min	Max	n
01	109.0	1.4	108.0	110.0	2	257.2	1.3	255.0	258.0	5	0.127	0.128	0.036	0.217	2
02	108.0	5.7	104.0	112.0	2	256.6	1.1	255.0	258.0	5	0.091	0.089	0.028	0.154	2
04	113.0	1.4	112.0	114.0	2	263.6	4.7	259.0	270.0	5	0.027	0.007	0.022	0.032	2
06	110.0	0.0	110.0	110.0	2	259.4	3.4	257.0	265.0	5	0.054	0.048	0.020	0.088	2
07	106.0	8.5	100.0	112.0	2	256.6	3.8	252.0	261.0	5	0.171	0.209	0.023	0.319	2
08	110.0	0.0	110.0	110.0	2	257.6	4.0	253.0	264.0	5	0.113	0.133	0.020	0.207	2
09	110.0	2.8	108.0	112.0	2	260.6	4.0	256.0	266.0	5	0.043	0.032	0.020	0.065	2
10	103.0	9.9	96.0	110.0	2	260.2	5.8	256.0	270.0	5	0.027	0.008	0.022	0.033	2
11	108.0	2.8	106.0	110.0	2	258.0	2.3	256.0	261.0	5	0.093	0.089	0.030	0.156	2
13	107.0	4.2	104.0	110.0	2	257.0	1.9	255.0	260.0	5	0.089	0.079	0.034	0.145	2
14	108.0	2.8	106.0	110.0	2	254.8	1.8	252.0	256.0	5	0.139	0.159	0.027	0.251	2
16	104.0	5.7	100.0	108.0	2	257.2	2.9	253.0	261.0	5	0.036	0.006	0.032	0.040	2
17	105.0	7.1	100.0	110.0	2	254.2	2.6	250.0	257.0	5	0.180	0.220	0.024	0.335	2
18	109.0	1.4	108.0	110.0	2	260.4	7.2	251.0	269.0	5	0.272	0.337	0.034	0.511	2
19	118.0	8.5	112.0	124.0	2	262.4	5.9	256.0	270.0	5	0.253	0.322	0.026	0.481	2
20	113.0	15.6	102.0	124.0	2	260.2	5.0	257.0	269.0	5	0.053	0.031	0.031	0.074	2
21	106.0	5.7	102.0	110.0	2	260.4	4.5	255.0	266.0	5	0.025	0.004	0.022	0.028	2
22	113.0	9.9	106.0	120.0	2	260.2	2.9	257.0	265.0	5	0.023	0.008	0.017	0.028	2
25	103.0	4.2	100.0	106.0	2	258.8	2.7	255.0	261.0	5	0.027	0.004	0.024	0.030	2
26	114.0	11.0	106.0	122.0	2	260.0	2.6	256.0	263.0	5	0.027	0.004	0.024	0.030	2
27	104.0	0.0	104.0	104.0	2	253.8	2.8	251.0	257.0	5	0.163	0.203	0.020	0.307	2
29	107.0	4.2	104.0	110.0	2	259.0	4.6	254.0	266.0	5	0.031	0.015	0.021	0.042	2
30	108.0	2.8	106.0	110.0	2	262.0	2.9	259.0	266.0	5	0.055	0.046	0.023	0.088	2
33	107.0	4.2	104.0	110.0	2	257.0	4.6	251.0	264.0	5	0.037	0.021	0.022	0.051	2

max = maximum; min = minimum; n = number of samples; SD = standard deviation.

**Table A1-3. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: survival.**

Sample Name	Replicate	Number Adults Recovered	Number Adults recovered (Corrected)	% Survival
01	1	10	10	100%
01	2	10	10	100%
01	3	10	10	100%
01	4	10	10	100%
02	1	7	7	70%
02	2	10	10	100%
02	3	7	7	70%
02	4	8	8	80%
04	1	9	9	90%
04	2	10	10	100%
04	3	9	9	90%
04	4	10	10	100%
06	1	9	9	90%
06	2	10	10	100%
06	3	8	8	80%
06	4	10	10	100%
07	1	11	10	100%
07	2	10	10	100%
07	3	10	10	100%
07	4	10	10	100%
08	1	9	9	90%
08	2	14	10	100%
08	3	7	7	70%
08	4	10	10	100%
09	1	10	10	100%
09	2	9	9	90%
09	3	10	10	100%
09	4	10	10	100%
10	1	9	9	90%
10	2	10	10	100%
10	3	10	10	100%
10	4	10	10	100%
11	1	9	9	90%
11	2	10	10	100%
11	3	10	10	100%
11	4	10	10	100%
13	1	10	10	100%
13	2	10	10	100%
13	3	10	10	100%
13	4	10	10	100%
14	1	10	10	100%
14	2	10	10	100%
14	3	10	10	100%
14	4	10	10	100%

**Table A1-3. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: survival.**

Sample Name	Replicate	Number Adults Recovered	Number Adults recovered (Corrected)	% Survival
16	1	10	10	100%
16	2	10	10	100%
16	3	9	9	90%
16	4	10	10	100%
17	1	10	10	100%
17	2	10	10	100%
17	3	10	10	100%
17	4	10	10	100%
18	1	5	5	50%
18	2	7	7	70%
18	3	8	8	80%
18	4	6	6	60%
19	1	2	2	20%
19	2	5	5	50%
19	3	7	7	70%
19	4	4	4	40%
20	1	10	10	100%
20	2	10	10	100%
20	3	10	10	100%
20	4	10	10	100%
21	1	6	6	60%
21	2	10	10	100%
21	3	10	10	100%
21	4	10	10	100%
22	1	10	10	100%
22	2	9	9	90%
22	3	10	10	100%
22	4	10	10	100%
25	1	10	10	100%
25	2	10	10	100%
25	3	9	9	90%
25	4	10	10	100%
26	1	10	10	100%
26	2	10	10	100%
26	3	10	10	100%
26	4	9	9	90%
27	1	10	10	100%
27	2	11	10	100%
27	3	9	9	90%
27	4	10	10	100%
29	1	6	6	60%
29	2	10	10	100%
29	3	10	10	100%
29	4	10	10	100%

**Table A1-3. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: survival.**

Sample Name	Replicate	Number Adults Recovered	Number Adults recovered (Corrected)	% Survival
30	1	9	9	90%
30	2	9	9	90%
30	3	6	6	60%
30	4	7	7	70%
33	1	10	10	100%
33	2	9	9	90%
33	3	10	10	100%
33	4	10	10	100%

**Table A1-4. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: weight and biomass.**

Sample Name	Replicate	Day 28 Number Adults Recovered	Day 28 Number Adults Recovered (Corrected)	Day 28 Number Animals on Pan	Pan Weight (mg)	Pan & Animal Weight (mg)	Replicate Total Biomass (mg)	Recovery Based Replicate Total Biomass (mg)	Mean Individual Dry Weight (mg)
01	1	10	10	10	82.298	97.066	14.768	14.768	1.477
01	2	10	10	10	84.871	93.878	9.007	9.007	0.901
01	3	10	10	10	81.725	91.357	9.632	9.632	0.963
01	4	10	10	10	81.438	94.638	13.200	13.200	1.320
02	1	7	7	7	80.777	87.765	6.988	6.988	0.998
02	2	10	10	10	81.557	93.553	11.996	11.996	1.200
02	3	7	7	7	81.468	90.159	8.691	8.691	1.242
02	4	8	8	8	83.299	91.035	7.736	7.736	0.967
04	1	9	9	9	81.793	96.416	14.623	14.623	1.625
04	2	10	10	10	82.442	100.263	17.821	17.821	1.782
04	3	9	9	8	80.484	92.181	11.697	13.159	1.462
04	4	10	10	10	80.743	98.679	17.936	17.936	1.794
06	1	9	9	9	81.316	90.045	8.729	8.729	0.970
06	2	10	10	9	82.861	90.633	7.772	8.636	0.864
06	3	8	8	8	81.665	88.948	7.283	7.283	0.910
06	4	10	10	10	82.570	95.467	12.897	12.897	1.290
07	1	11	10	11	82.537	101.883	19.346	17.587	1.759
07	2	10	10	10	83.154	96.522	13.368	13.368	1.337
07	3	10	10	10	81.387	97.710	16.323	16.323	1.632
07	4	10	10	10	82.718	97.965	15.247	15.247	1.525
08	1	9	9	9	81.712	94.667	12.955	12.955	1.439
08	2	14	10				ND	ND	
08	3	7	7	7	82.931	90.386	7.455	7.455	1.065
08	4	10	10	10	81.674	88.551	6.877	6.877	0.688
09	1	10	10	10	82.512	100.595	18.083	18.083	1.808
09	2	9	9	9	82.355	102.033	19.678	19.678	2.186
09	3	10	10	10	82.149	101.277	19.128	19.128	1.913
09	4	10	10	10	81.076	95.982	14.906	14.906	1.491

**Table A1-4. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: weight and biomass.**

Sample Name	Replicate	Day 28 Number Adults Recovered	Day 28 Number Adults Recovered (Corrected)	Day 28 Number Animals on Pan	Pan Weight (mg)	Pan & Animal Weight (mg)	Replicate Total Biomass (mg)	Recovery Based Replicate Total Biomass (mg)	Mean Individual Dry Weight (mg)
10	1	9	9	9	80.166	93.257	13.091	13.091	1.455
10	2	10	10	10	82.228	99.068	16.840	16.840	1.684
10	3	10	10	10	81.222	98.281	17.059	17.059	1.706
10	4	10	10	10	83.459	96.368	12.909	12.909	1.291
11	1	9	9	9	83.927	92.768	8.841	8.841	0.982
11	2	10	10	10	81.786	99.668	17.882	17.882	1.788
11	3	10	10	10	82.590	101.285	18.695	18.695	1.870
11	4	10	10	10	81.756	98.853	17.097	17.097	1.710
13	1	10	10	10	81.188	96.559	15.371	15.371	1.537
13	2	10	10	10	82.722	95.192	12.470	12.470	1.247
13	3	10	10	10	83.520	101.028	17.508	17.508	1.751
13	4	10	10	9	81.790	92.632	10.842	12.047	1.205
14	1	10	10	10	80.709	90.873	10.164	10.164	1.016
14	2	10	10	10	82.105	96.957	14.852	14.852	1.485
14	3	10	10	10	81.339	98.786	17.447	17.447	1.745
14	4	10	10	10	82.644	95.957	13.313	13.313	1.331
16	1	10	10	10	82.474	90.780	8.306	8.306	0.831
16	2	10	10	10	81.896	95.882	13.986	13.986	1.399
16	3	9	9	9	82.079	91.970	9.891	9.891	1.099
16	4	10	10	10	81.422	96.696	15.274	15.274	1.527
17	1	10	10	10	82.587	101.135	18.548	18.548	1.855
17	2	10	10	10	82.392	96.392	14.000	14.000	1.400
17	3	10	10	10	82.536	103.882	21.346	21.346	2.135
17	4	10	10	10	81.194	101.511	20.317	20.317	2.032
18	1	5	5	5	82.311	88.429	6.118	6.118	1.224
18	2	7	7	7	82.295	90.158	7.863	7.863	1.123
18	3	8	8	8	82.028	89.681	7.653	7.653	0.957
18	4	6	6	6	81.361	89.705	8.344	8.344	1.391

**Table A1-4. Results of 28-day whole-sediment toxicity tests with *Lampsilis siligoidea*: weight and biomass.**

Sample Name	Replicate	Day 28 Number Adults Recovered	Day 28 Number Adults Recovered (Corrected)	Day 28 Number Animals on Pan	Pan Weight (mg)	Pan & Animal Weight (mg)	Replicate Total Biomass (mg)	Recovery Based Replicate Total Biomass (mg)	Mean Individual Dry Weight (mg)
19	1	2	2	2	81.849	82.442	0.593	0.593	0.296
19	2	5	5	5	80.352	83.103	2.751	2.751	0.550
19	3	7	7	7	81.915	86.851	4.936	4.936	0.705
19	4	4	4	4	82.732	84.526	1.794	1.794	0.448
20	1	10	10	10	82.588	103.251	20.663	20.663	2.066
20	2	10	10	10	82.108	100.310	18.202	18.202	1.820
20	3	10	10	10	82.403	100.343	17.940	17.940	1.794
20	4	10	10	10	82.954	102.441	19.487	19.487	1.949
21	1	6	6	9	81.799	94.965	13.166	8.777	1.463
21	2	10	10	10	80.231	92.078	11.847	11.847	1.185
21	3	10	10	10	80.473	93.559	13.086	13.086	1.309
21	4	10	10	10	82.056	99.477	17.421	17.421	1.742
22	1	10	10	10	82.621	97.946	15.325	15.325	1.533
22	2	9	9	9	81.121	96.140	15.019	15.019	1.669
22	3	10	10	10	82.051	101.862	19.811	19.811	1.981
22	4	10	10	10	81.596	99.195	17.599	17.599	1.760
25	1	10	10	10	82.172	92.943	10.771	10.771	1.077
25	2	10	10	10	80.309	87.671	7.362	7.362	0.736
25	3	9	9	9	80.415	89.903	9.488	9.488	1.054
25	4	10	10	10	80.269	95.700	15.431	15.431	1.543
26	1	10	10	10	82.575	101.385	18.810	18.810	1.881
26	2	10	10	10	81.706	100.733	19.027	19.027	1.903
26	3	10	10	10	82.354	99.051	16.697	16.697	1.670
26	4	9	9	9	82.179	95.709	13.530	13.530	1.503
27	1	10	10	10	81.540	96.874	15.334	15.334	1.533
27	2	11	10	11	81.673	103.834	22.161	20.146	2.015
27	3	9	9	9	84.296	95.883	11.587	11.587	1.287
27	4	10	10	10	82.764	98.187	15.423	15.423	1.542

**Table A1-4. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: weight and biomass.**

Sample Name	Replicate	Day 28 Number Adults Recovered	Day 28 Number Adults Recovered (Corrected)	Day 28 Number Animals on Pan	Pan Weight (mg)	Pan & Animal Weight (mg)	Replicate Total Biomass (mg)	Recovery Based Replicate Total Biomass (mg)	Mean Individual Dry Weight (mg)
29	1	6	6	6	82.115	90.126	8.011	8.011	1.335
29	2	10	10	10	83.755	102.145	18.390	18.390	1.839
29	3	10	10	10	81.342	97.315	15.973	15.973	1.597
29	4	10	10	10	82.166	99.712	17.546	17.546	1.755
30	1	9	9	9	81.519	92.356	10.837	10.837	1.204
30	2	9	9	9	82.483	98.034	15.551	15.551	1.728
30	3	6	6	6	82.792	84.818	2.026	2.026	0.338
30	4	7	7	7	81.823	89.628	7.805	7.805	1.115
33	1	10	10	10	81.492	96.507	15.015	15.015	1.502
33	2	9	9	9	82.784	97.944	15.160	15.160	1.684
33	3	10	10	10	82.038	96.883	14.845	14.845	1.485
33	4	10	10	10	83.012	98.368	15.356	15.356	1.536



**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siligoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
Initial Length		1	3.1954	2.719
Initial Length		2	2.7443	
Initial Length		3	2.4075	
Initial Length		4	2.9072	
Initial Length		5	2.4703	
Initial Length		6	3.1595	
Initial Length		7	2.1963	
Initial Length		8	1.652	
Initial Length		9	2.6118	
Initial Length		10	2.4732	
Initial Length		11	2.7492	
Initial Length		12	2.7859	
Initial Length		13	2.8986	
Initial Length		14	3.0321	
Initial Length		15	2.9074	
Initial Length		16	3.437	
Initial Length		17	2.6396	
Initial Length		18	2.6538	
Initial Length		19	2.6338	
Initial Length		20	3.0741	
Initial Length		21	2.4642	
Initial Length		22	2.7251	
01	1	1	3.507	3.285
01	1	2	3.409	
01	1	3	3.890	
01	1	4	3.477	
01	1	5	3.575	
01	1	6	3.049	
01	1	7	2.645	
01	1	8	2.734	
01	1	9	2.613	
01	1	10	3.953	
01	2	1	2.739	2.916
01	2	2	2.741	
01	2	3	2.988	
01	2	4	3.290	
01	2	5	3.265	
01	2	6	2.505	
01	2	7	3.140	
01	2	8	3.187	
01	2	9	2.320	
01	2	10	2.986	
01	3	1	3.477	3.030
01	3	2	3.379	
01	3	3	2.961	
01	3	4	3.124	

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
01	3	5	3.504	
01	3	6	3.162	
01	3	7	2.270	
01	3	8	2.660	
01	3	9	2.720	
01	3	10	3.040	
01	4	1	3.183	3.374
01	4	2	3.206	
01	4	3	3.329	
01	4	4	3.144	
01	4	5	2.547	
01	4	6	4.126	
01	4	7	3.961	
01	4	8	3.486	
01	4	9	3.033	
01	4	10	3.721	
02	1	1	3.311	3.110
02	1	2	2.794	
02	1	3	3.120	
02	1	4	2.108	
02	1	5	3.287	
02	1	6	3.453	
02	1	7	3.693	
02	1	8		
02	1	9		
02	1	10		
02	2	1	2.996	3.125
02	2	2	3.060	
02	2	3	2.291	
02	2	4	3.587	
02	2	5	2.710	
02	2	6	2.648	
02	2	7	4.145	
02	2	8	2.928	
02	2	9	3.230	
02	2	10	3.655	
02	3	1	3.892	3.527
02	3	2	3.330	
02	3	3	3.338	
02	3	4	3.822	
02	3	5	2.969	
02	3	6	3.312	
02	3	7	4.029	
02	3	8		
02	3	9		
02	3	10		

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
02	4	1	3.728	3.284
02	4	2	3.544	
02	4	3	3.270	
02	4	4	2.666	
02	4	5	4.280	
02	4	6	3.035	
02	4	7	3.271	
02	4	8	2.476	
02	4	9		
02	4	10		
04	1	1	3.561	3.424
04	1	2	3.019	
04	1	3	4.416	
04	1	4	3.603	
04	1	5	3.361	
04	1	6	3.013	
04	1	7	3.589	
04	1	8	3.313	
04	1	9	2.936	
04	1	10		
04	2	1	3.677	3.388
04	2	2	3.659	
04	2	3	3.118	
04	2	4	3.278	
04	2	5	3.615	
04	2	6	3.107	
04	2	7	3.488	
04	2	8	4.388	
04	2	9	2.781	
04	2	10	2.771	
04	3	1	3.639	3.152
04	3	2	3.131	
04	3	3	2.445	
04	3	4	3.278	
04	3	5	3.532	
04	3	6	3.160	
04	3	7	2.776	
04	3	8	2.784	
04	3	9	3.624	
04	3	10		
04	4	1	3.382	3.419
04	4	2	3.024	
04	4	3	4.162	
04	4	4	3.565	
04	4	5	2.113	
04	4	6	3.523	

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
04	4	7	4.077	
04	4	8	3.589	
04	4	9	3.301	
04	4	10	3.455	
06	1	1	2.746	3.044
06	1	2	3.370	
06	1	3	2.924	
06	1	4	3.040	
06	1	5	3.002	
06	1	6	3.062	
06	1	7	3.079	
06	1	8	2.972	
06	1	9	3.202	
06	1	10		
06	2	1	3.059	3.011
06	2	2	3.742	
06	2	3	3.912	
06	2	4	2.955	
06	2	5	2.639	
06	2	6	3.257	
06	2	7	2.484	
06	2	8	2.589	
06	2	9	2.639	
06	2	10	2.837	
06	3	1	2.941	3.162
06	3	2	3.108	
06	3	3	2.875	
06	3	4	3.522	
06	3	5	3.030	
06	3	6	3.350	
06	3	7	3.134	
06	3	8	3.336	
06	3	9		
06	3	10		
06	4	1	3.882	3.173
06	4	2	2.721	
06	4	3	2.364	
06	4	4	3.824	
06	4	5	3.501	
06	4	6	3.518	
06	4	7	3.217	
06	4	8	2.998	
06	4	9	2.729	
06	4	10	2.978	
07	1	1	3.675	3.502
07	1	2	3.902	

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
07	1	3	3.778	3.236
07	1	4	4.302	
07	1	5	2.947	
07	1	6	4.165	
07	1	7	3.612	
07	1	8	3.878	
07	1	9	3.212	
07	1	10	2.486	
07	1	11	2.568	
07	2	1	3.516	3.444
07	2	2	2.866	
07	2	3	4.027	
07	2	4	3.910	
07	2	5	3.223	
07	2	6	2.473	
07	2	7	3.259	
07	2	8	3.318	
07	2	9	3.051	
07	2	10	2.718	
07	3	1	2.417	3.270
07	3	2	2.795	
07	3	3	3.781	
07	3	4	3.626	
07	3	5	3.064	
07	3	6	3.776	
07	3	7	4.013	
07	3	8	3.301	
07	3	9	4.025	
07	3	10	3.642	
07	4	1	3.246	3.156
07	4	2	3.168	
07	4	3	2.711	
07	4	4	3.576	
07	4	5	3.153	
07	4	6	3.254	
07	4	7	3.302	
07	4	8	3.407	
07	4	9	2.988	
07	4	10	3.899	
08	1	1	3.485	3.156
08	1	2	3.258	
08	1	3	3.093	
08	1	4	2.982	
08	1	5	3.175	
08	1	6	3.234	
08	1	7	2.729	

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siligoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
08	1	8	2.920	3.312
08	1	9	3.532	
08	1	10		
08	2	1	4.068	3.216
08	2	2	2.862	
08	2	3	2.849	
08	2	4	3.178	
08	2	5	3.044	
08	2	6	2.967	
08	2	7	2.897	
08	2	8	3.917	
08	2	9	3.136	
08	2	10	2.729	
08	2	11	3.828	
08	2	12	3.780	
08	2	13	3.243	
08	2	14	3.870	
08	3	1	3.673	3.216
08	3	2	3.297	
08	3	3	2.420	
08	3	4	3.057	
08	3	5	3.360	
08	3	6	3.673	
08	3	7	3.034	
08	3	8		
08	3	9		
08	3	10		
08	4	1	2.220	2.728
08	4	2	2.898	
08	4	3	2.652	
08	4	4	3.042	
08	4	5	2.482	
08	4	6	2.614	
08	4	7	2.723	
08	4	8	2.836	
08	4	9	2.822	
08	4	10	2.990	
09	1	1	3.204	3.322
09	1	2	3.364	
09	1	3	4.081	
09	1	4	3.138	
09	1	5	3.335	
09	1	6	2.947	
09	1	7	3.593	
09	1	8	3.285	
09	1	9	3.568	

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
09	1	10	2.708	3.533
09	2	1	3.343	
09	2	2	3.745	
09	2	3	3.994	
09	2	4	3.333	
09	2	5	4.086	
09	2	6	3.860	
09	2	7	3.722	
09	2	8	2.541	
09	2	9	3.169	
09	2	10		
09	3	1	2.838	3.351
09	3	2	2.771	
09	3	3	3.583	
09	3	4	3.216	
09	3	5	3.753	
09	3	6	3.227	
09	3	7	2.918	
09	3	8	3.217	
09	3	9	3.956	
09	3	10	4.034	
09	4	1	3.080	3.136
09	4	2	4.003	
09	4	3	2.442	
09	4	4	2.681	
09	4	5	3.436	
09	4	6	2.182	
09	4	7	3.603	
09	4	8	2.932	
09	4	9	4.006	
09	4	10	2.999	
10	1	1	3.202	3.272
10	1	2	2.731	
10	1	3	3.923	
10	1	4	3.171	
10	1	5	3.311	
10	1	6	3.316	
10	1	7	3.373	
10	1	8	3.126	
10	1	9	3.296	
10	1	10		
10	2	1	3.479	3.218
10	2	2	3.477	
10	2	3	2.567	
10	2	4	3.580	
10	2	5	2.941	

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siligoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
10	2	6	3.035	
10	2	7	3.355	
10	2	8	3.253	
10	2	9	3.586	
10	2	10	2.906	
10	3	1	3.366	3.412
10	3	2	3.973	
10	3	3	3.446	
10	3	4	3.473	
10	3	5	3.717	
10	3	6	4.189	
10	3	7	2.614	
10	3	8	3.375	
10	3	9	3.274	
10	3	10	2.694	
10	4	1	2.945	3.044
10	4	2	2.484	
10	4	3	2.479	
10	4	4	2.644	
10	4	5	3.499	
10	4	6	2.482	
10	4	7	4.222	
10	4	8	3.345	
10	4	9	2.854	
10	4	10	3.485	
11	1	1	3.420	2.977
11	1	2	2.726	
11	1	3	2.517	
11	1	4	2.785	
11	1	5	3.026	
11	1	6	3.411	
11	1	7	3.035	
11	1	8	3.149	
11	1	9	2.725	
11	1	10		
11	2	1	3.450	3.369
11	2	2	2.926	
11	2	3	3.405	
11	2	4	3.655	
11	2	5	4.170	
11	2	6	3.345	
11	2	7	3.139	
11	2	8	3.821	
11	2	9	3.365	
11	2	10	2.419	



**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siligoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
11	3	1	3.566	3.407
11	3	2	3.193	
11	3	3	3.949	
11	3	4	3.178	
11	3	5	3.751	
11	3	6	3.438	
11	3	7	4.082	
11	3	8	2.570	
11	3	9	3.554	
11	3	10	2.795	
11	4	1	3.721	3.246
11	4	2	3.236	
11	4	3	3.008	
11	4	4	2.863	
11	4	5	3.049	
11	4	6	3.011	
11	4	7	2.888	
11	4	8	4.127	
11	4	9	3.378	
11	4	10	3.174	
13	1	1	2.507	3.245
13	1	2	3.370	
13	1	3	4.103	
13	1	4	2.525	
13	1	5	3.622	
13	1	6	2.699	
13	1	7	3.364	
13	1	8	3.491	
13	1	9	3.226	
13	1	10	3.549	
13	2	1	3.274	3.159
13	2	2	3.174	
13	2	3	3.310	
13	2	4	2.609	
13	2	5	3.333	
13	2	6	3.102	
13	2	7	2.681	
13	2	8	3.749	
13	2	9	3.029	
13	2	10	3.323	
13	3	1	3.713	3.376
13	3	2	3.610	
13	3	3	3.916	
13	3	4	2.797	
13	3	5	3.469	
13	3	6	3.020	

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
13	3	7	3.434	3.052
13	3	8	3.234	
13	3	9	3.154	
13	3	10	3.415	
13	4	1	3.552	2.930
13	4	2	3.045	
13	4	3	4.025	
13	4	4	2.933	
13	4	5	3.338	
13	4	6	2.826	
13	4	7	2.765	
13	4	8	1.938	
13	4	9	3.479	
13	4	10	2.617	
14	1	1	3.144	2.930
14	1	2	2.767	
14	1	3	3.855	
14	1	4	2.234	
14	1	5	2.738	
14	1	6	2.884	
14	1	7	2.489	
14	1	8	3.272	
14	1	9	2.981	
14	1	10	2.937	
14	2	1	3.038	3.314
14	2	2	3.105	
14	2	3	3.002	
14	2	4	4.267	
14	2	5	3.536	
14	2	6	3.901	
14	2	7	3.090	
14	2	8	3.299	
14	2	9	3.255	
14	2	10	2.650	
14	3	1	3.426	3.297
14	3	2	3.218	
14	3	3	3.404	
14	3	4	2.811	
14	3	5	3.939	
14	3	6	3.369	
14	3	7	3.962	
14	3	8	3.215	
14	3	9	3.061	
14	3	10	2.568	
14	4	1	3.555	3.158
14	4	2	2.893	

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siligoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
14	4	3	3.421	2.679
14	4	4	3.517	
14	4	5	2.863	
14	4	6	3.159	
14	4	7	3.635	
14	4	8	2.489	
14	4	9	3.326	
14	4	10	2.722	
16	1	1	2.580	3.311
16	1	2	2.775	
16	1	3	2.856	
16	1	4	2.462	
16	1	5	2.751	
16	1	6	2.638	
16	1	7	2.673	
16	1	8	2.740	
16	1	9	2.935	
16	1	10	2.379	
16	2	1	3.483	3.195
16	2	2	3.972	
16	2	3	3.323	
16	2	4	3.582	
16	2	5	3.207	
16	2	6	2.898	
16	2	7	3.261	
16	2	8	2.941	
16	2	9	3.430	
16	2	10	3.011	
16	3	1	3.083	3.323
16	3	2	3.891	
16	3	3	3.919	
16	3	4	2.881	
16	3	5	3.111	
16	3	6	2.875	
16	3	7	2.550	
16	3	8	3.744	
16	3	9	2.699	
16	3	10		
16	4	1	4.162	3.323
16	4	2	2.980	
16	4	3	3.643	
16	4	4	3.484	
16	4	5	3.426	
16	4	6	3.285	
16	4	7	2.482	
16	4	8	2.929	

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siligoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
16	4	9	2.818	
16	4	10	4.023	
17	1	1	3.024	3.409
17	1	2	3.572	
17	1	3	3.900	
17	1	4	3.921	
17	1	5	3.746	
17	1	6	2.679	
17	1	7	2.844	
17	1	8	3.540	
17	1	9	3.710	
17	1	10	3.155	
17	2	1	2.821	3.238
17	2	2	2.937	
17	2	3	3.173	
17	2	4	3.421	
17	2	5	3.576	
17	2	6	2.977	
17	2	7	3.429	
17	2	8	3.999	
17	2	9	2.721	
17	2	10	3.321	
17	3	1	3.207	3.267
17	3	2	4.105	
17	3	3	2.981	
17	3	4	3.366	
17	3	5	2.737	
17	3	6	2.651	
17	3	7	3.465	
17	3	8	3.100	
17	3	9	3.641	
17	3	10	3.415	
17	4	1	3.899	3.432
17	4	2	2.999	
17	4	3	4.145	
17	4	4	3.445	
17	4	5	3.518	
17	4	6	2.829	
17	4	7	3.190	
17	4	8	2.644	
17	4	9	4.000	
17	4	10	3.646	
18	1	1	3.713	3.515
18	1	2	3.587	
18	1	3	3.016	
18	1	4	4.115	

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
18	1	5	3.142	3.092
18	1	6		
18	1	7		
18	1	8		
18	1	9		
18	1	10		
18	2	1	3.104	3.003
18	2	2	2.768	
18	2	3	3.456	
18	2	4	2.724	
18	2	5	2.664	
18	2	6	3.074	
18	2	7	3.856	
18	2	8		
18	2	9		
18	2	10		
18	3	1	2.487	3.460
18	3	2	3.127	
18	3	3	3.803	
18	3	4	3.175	
18	3	5	2.456	
18	3	6	3.575	
18	3	7	3.149	
18	3	8	2.250	
18	3	9		
18	3	10		
18	4	1	2.708	3.223
18	4	2	4.107	
18	4	3	3.407	
18	4	4	3.997	
18	4	5	3.016	
18	4	6	3.528	
18	4	7		
18	4	8		
18	4	9		
18	4	10		
19	1	1	3.640	3.223
19	1	2	2.806	
19	1	3		
19	1	4		
19	1	5		
19	1	6		
19	1	7		
19	1	8		
19	1	9		
19	1	10		

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
19	2	1	3.248	3.018
19	2	2	3.202	
19	2	3	2.543	
19	2	4	2.991	
19	2	5	3.105	
19	2	6		
19	2	7		
19	2	8		
19	2	9		
19	2	10		
19	3	1	2.972	2.941
19	3	2	2.784	
19	3	3	3.130	
19	3	4	3.674	
19	3	5	2.575	
19	3	6	2.799	
19	3	7	2.656	
19	3	8		
19	3	9		
19	3	10		
19	4	1	3.556	2.938
19	4	2	2.423	
19	4	3	2.835	
19	4	4	2.938	
19	4	5		
19	4	6		
19	4	7		
19	4	8		
19	4	9		
19	4	10		
20	1	1	3.070	3.454
20	1	2	3.147	
20	1	3	3.127	
20	1	4	4.261	
20	1	5	2.950	
20	1	6	2.885	
20	1	7	3.716	
20	1	8	4.349	
20	1	9	3.811	
20	1	10	3.223	
20	2	1	2.935	3.154
20	2	2	3.620	
20	2	3	3.729	
20	2	4	3.043	
20	2	5	2.993	
20	2	6	2.511	

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
20	2	7	3.226	3.245
20	2	8	3.399	
20	2	9	3.070	
20	2	10	3.015	
20	3	1	3.515	3.282
20	3	2	2.746	
20	3	3	2.376	
20	3	4	3.361	
20	3	5	4.223	
20	3	6	3.769	
20	3	7	3.014	
20	3	8	3.957	
20	3	9	2.784	
20	3	10	2.708	
20	4	1	2.816	3.124
20	4	2	3.067	
20	4	3	3.472	
20	4	4	3.900	
20	4	5	2.823	
20	4	6	2.910	
20	4	7	2.556	
20	4	8	3.341	
20	4	9	3.876	
20	4	10	4.059	
21	1	1	3.202	3.104
21	1	2	3.022	
21	1	3	3.327	
21	1	4	3.689	
21	1	5	3.119	
21	1	6	2.300	
21	1	7	2.438	
21	1	8	3.702	
21	1	9	3.319	
21	1	10		
21	2	1	3.235	3.059
21	2	2	2.898	
21	2	3	2.907	
21	2	4	3.011	
21	2	5	2.576	
21	2	6	3.367	
21	2	7	3.427	
21	2	8	3.282	
21	2	9	2.960	
21	2	10	3.378	
21	3	1	3.042	3.440
21	3	2	3.440	

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
21	3	3	3.698	3.181
21	3	4	2.902	
21	3	5	2.884	
21	3	6	2.865	
21	3	7	2.479	
21	3	8	3.399	
21	3	9	3.363	
21	3	10	2.517	
21	4	1	3.388	3.142
21	4	2	3.499	
21	4	3	2.634	
21	4	4	3.629	
21	4	5	2.649	
21	4	6	2.508	
21	4	7	3.139	
21	4	8	4.614	
21	4	9	3.464	
21	4	10	2.283	
22	1	1	3.158	3.128
22	1	2	3.552	
22	1	3	3.415	
22	1	4	3.197	
22	1	5	3.276	
22	1	6	3.420	
22	1	7	3.491	
22	1	8	1.971	
22	1	9	3.474	
22	1	10	2.463	
22	2	1	3.618	3.352
22	2	2	3.045	
22	2	3	3.722	
22	2	4	4.046	
22	2	5	2.316	
22	2	6	3.167	
22	2	7	3.225	
22	2	8	2.278	
22	2	9	2.737	
22	2	10		
22	3	1	3.768	3.352
22	3	2	3.175	
22	3	3	3.720	
22	3	4	3.392	
22	3	5	3.631	
22	3	6	2.043	
22	3	7	2.749	
22	3	8	4.092	



**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siligoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
22	3	9	2.986	
22	3	10	3.962	
22	4	1	3.934	3.345
22	4	2	3.443	
22	4	3	3.457	
22	4	4	3.297	
22	4	5	4.323	
22	4	6	3.306	
22	4	7	2.732	
22	4	8	3.066	
22	4	9	2.622	
22	4	10	3.275	
25	1	1	3.111	2.992
25	1	2	2.838	
25	1	3	3.470	
25	1	4	2.800	
25	1	5	2.629	
25	1	6	2.914	
25	1	7	3.059	
25	1	8	3.078	
25	1	9	3.181	
25	1	10	2.843	
25	2	1	2.984	2.853
25	2	2	2.681	
25	2	3	3.534	
25	2	4	3.232	
25	2	5	2.952	
25	2	6	2.647	
25	2	7	3.357	
25	2	8	1.937	
25	2	9	2.356	
25	2	10		
25	3	1	2.949	2.969
25	3	2	2.758	
25	3	3	3.167	
25	3	4	3.717	
25	3	5	3.081	
25	3	6	2.759	
25	3	7	2.890	
25	3	8	2.433	
25	3	9	2.963	
25	3	10		
25	4	1	4.320	3.207
25	4	2	2.940	
25	4	3	3.160	
25	4	4	4.315	

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siligoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
25	4	5	2.141	3.336
25	4	6	2.943	
25	4	7	2.342	
25	4	8	3.551	
25	4	9	2.104	
25	4	10	4.258	
26	1	1	3.474	3.430
26	1	2	3.756	
26	1	3	3.178	
26	1	4	3.258	
26	1	5	3.382	
26	1	6	2.625	
26	1	7	3.324	
26	1	8	3.535	
26	1	9	3.416	
26	1	10	3.415	
26	2	1	3.289	3.430
26	2	2	4.216	
26	2	3	3.744	
26	2	4	2.996	
26	2	5	3.449	
26	2	6	3.489	
26	2	7	3.044	
26	2	8	3.567	
26	2	9	2.705	
26	2	10	3.804	
26	3	1	2.727	3.198
26	3	2	3.244	
26	3	3	3.799	
26	3	4	2.946	
26	3	5	3.073	
26	3	6	3.116	
26	3	7	4.035	
26	3	8	2.837	
26	3	9	2.781	
26	3	10	3.425	
26	4	1	4.087	3.095
26	4	2	3.020	
26	4	3	3.536	
26	4	4	3.475	
26	4	5	2.795	
26	4	6	2.551	
26	4	7	2.280	
26	4	8	2.971	
26	4	9	3.139	
26	4	10		

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
27	1	1	3.879	3.133
27	1	2	2.754	
27	1	3	3.932	
27	1	4	2.660	
27	1	5	3.277	
27	1	6	3.553	
27	1	7	2.838	
27	1	8	3.365	
27	1	9	2.926	
27	1	10	2.143	
27	2	1	2.740	3.221
27	2	2	3.282	
27	2	3	3.056	
27	2	4	2.836	
27	2	5	3.348	
27	2	6	3.024	
27	2	7	3.258	
27	2	8	2.905	
27	2	9	3.397	
27	2	10	3.678	
27	2	11	3.912	
27	3	1	2.949	3.059
27	3	2	2.889	
27	3	3	3.050	
27	3	4	2.622	
27	3	5	3.164	
27	3	6	3.119	
27	3	7	3.214	
27	3	8	3.404	
27	3	9	3.119	
27	3	10		
27	4	1	3.012	3.172
27	4	2	2.927	
27	4	3	3.538	
27	4	4	3.825	
27	4	5	3.424	
27	4	6	4.087	
27	4	7	1.918	
27	4	8	3.564	
27	4	9	2.461	
27	4	10	2.958	
29	1	1	3.317	3.163
29	1	2	2.842	
29	1	3	2.495	
29	1	4	2.834	
29	1	5	4.432	

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
29	1	6	3.057	
29	1	7		
29	1	8		
29	1	9		
29	1	10		
29	2	1	3.380	3.280
29	2	2	3.257	
29	2	3	3.275	
29	2	4	2.925	
29	2	5	3.779	
29	2	6	3.363	
29	2	7	3.301	
29	2	8	2.931	
29	2	9	3.535	
29	2	10	3.052	
29	3	1	3.585	3.181
29	3	2	4.418	
29	3	3	3.178	
29	3	4	2.588	
29	3	5	3.505	
29	3	6	3.354	
29	3	7	3.261	
29	3	8	2.552	
29	3	9	3.021	
29	3	10	2.345	
29	4	1	3.405	3.282
29	4	2	4.093	
29	4	3	4.289	
29	4	4	2.983	
29	4	5	3.582	
29	4	6	3.424	
29	4	7	2.841	
29	4	8	2.491	
29	4	9	3.348	
29	4	10	2.362	
30	1	1	3.226	3.214
30	1	2	2.872	
30	1	3	2.530	
30	1	4	3.472	
30	1	5	3.297	
30	1	6	3.862	
30	1	7	3.269	
30	1	8	3.378	
30	1	9	3.023	
30	1	10		

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
30	2	1	3.414	3.349
30	2	2	3.376	
30	2	3	2.354	
30	2	4	3.436	
30	2	5	3.936	
30	2	6	3.742	
30	2	7	3.092	
30	2	8	3.584	
30	2	9	3.211	
30	2	10		
30	3	1	3.057	2.625
30	3	2	2.626	
30	3	3	2.533	
30	3	4	2.687	
30	3	5	2.308	
30	3	6	2.539	
30	3	7		
30	3	8		
30	3	9		
30	3	10		
30	4	1	2.824	3.139
30	4	2	3.146	
30	4	3	2.892	
30	4	4	3.115	
30	4	5	3.266	
30	4	6	3.373	
30	4	7	3.357	
30	4	8		
30	4	9		
30	4	10		
33	1	1	3.169	3.198
33	1	2	3.963	
33	1	3	3.248	
33	1	4	3.346	
33	1	5	2.584	
33	1	6	3.439	
33	1	7	2.905	
33	1	8	2.783	
33	1	9	3.323	
33	1	10	3.218	
33	2	1	3.623	3.255
33	2	2	3.676	
33	2	3	3.880	
33	2	4	3.035	
33	2	5	3.099	
33	2	6	2.759	

**Table A1-5. Results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*: length.**

Sample Name	Replicate	Organism	Length (mm)	Mean Length per Replicate (mm)
33	2	7	3.017	3.130
33	2	8	3.064	
33	2	9	3.145	
33	2	10		
33	3	1	3.600	3.130
33	3	2	3.003	
33	3	3	3.228	
33	3	4	2.580	
33	3	5	2.924	
33	3	6	3.001	
33	3	7	2.845	
33	3	8	3.125	
33	3	9	3.808	
33	3	10	3.185	
33	4	1	3.568	3.061
33	4	2	2.803	
33	4	3	2.464	
33	4	4	3.399	
33	4	5	3.203	
33	4	6	3.177	
33	4	7	3.535	
33	4	8	3.505	
33	4	9	2.975	
33	4	10	1.979	

**Table A1-6. Summary of results of 28-day whole-sediment toxicity tests with *Lampsilis siliquoidea*.**

Sample Name	28-day Survival (%)						28-day Total Biomass (mg)						28-day Individual Dry Weight (mg)						28-day Length (mm)					
					Percent						Percent						Percent						Percent	
	Mean	SD	SEM	CV	of Control	n	Mean	SD	SEM	CV	of Control	n	Mean	SD	SEM	CV	of Control	n	Mean	SD	SEM	CV	of Control	n
Initials	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2.72	0.38	0.08	13.9%	N/A	22
01	100	0	0	0.0%	103%	4	11.7	2.78	1.39	23.9%	77.2%	4	1.17	0.28	0.14	23.9%	75.1%	4	3.15	0.21	0.11	6.8%	99.7%	4
02	80	14.1	7.1	17.7%	82.1%	4	8.85	2.21	1.1	24.9%	58.7%	4	1.1	0.14	0.07	12.6%	71.0%	4	3.26	0.19	0.1	5.9%	103%	4
04	95	5.8	2.9	6.1%	97.4%	4	15.9	2.38	1.19	15.0%	105%	4	1.67	0.16	0.08	9.4%	107%	4	3.35	0.13	0.06	3.9%	106%	4
06	92.5	9.6	4.8	10.4%	94.9%	4	9.39	2.43	1.22	25.9%	62.2%	4	1.01	0.19	0.1	19.1%	65.0%	4	3.1	0.08	0.04	2.6%	98.0%	4
07	100	0	0	0.0%	103%	4	15.6	1.79	0.89	11.4%	104%	4	1.56	0.18	0.09	11.4%	101%	4	3.36	0.13	0.06	3.9%	106%	4
08	90	14.1	7.1	15.7%	92.3%	4	9.1	3.35	1.94	36.9%	60.3%	3	1.06	0.38	0.22	35.3%	68.6%	3	3.1	0.26	0.13	8.3%	98.2%	4
09	97.5	5	2.5	5.1%	100%	4	18	2.13	1.07	11.9%	119%	4	1.85	0.29	0.14	15.5%	119%	4	3.34	0.16	0.08	4.9%	106%	4
10	97.5	5	2.5	5.1%	100%	4	15	2.28	1.14	15.2%	99.2%	4	1.53	0.2	0.1	12.9%	98.9%	4	3.24	0.15	0.08	4.7%	102%	4
11	97.5	5	2.5	5.1%	100%	4	15.6	4.57	2.29	29.3%	104%	4	1.59	0.41	0.2	25.7%	102%	4	3.25	0.19	0.1	6.0%	103%	4
13	100	0	0	0.0%	103%	4	14.4	2.57	1.29	17.9%	95.1%	4	1.43	0.26	0.13	17.9%	92.5%	4	3.21	0.14	0.07	4.3%	102%	4
14	100	0	0	0.0%	103%	4	13.9	3.04	1.52	21.8%	92.4%	4	1.39	0.3	0.15	21.8%	89.9%	4	3.17	0.18	0.09	5.6%	100%	4
16	97.5	5	2.5	5.1%	100%	4	11.9	3.3	1.65	27.8%	78.6%	4	1.21	0.31	0.16	25.7%	78.2%	4	3.13	0.3	0.15	9.7%	98.9%	4
17	100	0	0	0.0%	103%	4	18.6	3.25	1.62	17.5%	123%	4	1.86	0.32	0.16	17.5%	120%	4	3.34	0.1	0.05	2.9%	106%	4
18	65	12.9	6.5	19.9%	66.7%	4	7.49	0.96	0.48	12.8%	49.7%	4	1.17	0.18	0.09	15.5%	75.6%	4	3.27	0.26	0.13	7.9%	103%	4
19	45	20.8	10.4	46.3%	46.2%	4	2.52	1.84	0.92	73.0%	16.7%	4	0.5	0.17	0.09	34.4%	32.2%	4	3.03	0.13	0.07	4.4%	95.9%	4
20	100	0	0	0.0%	103%	4	19.1	1.26	0.63	6.6%	126%	4	1.91	0.13	0.06	6.6%	123%	4	3.28	0.13	0.06	3.8%	104%	4
21	90	20	10	22.2%	92.3%	4	12.8	3.58	1.79	28.0%	84.7%	4	1.42	0.24	0.12	16.9%	91.8%	4	3.12	0.05	0.03	1.6%	98.6%	4
22	97.5	5	2.5	5.1%	100%	4	16.9	2.23	1.12	13.2%	112%	4	1.74	0.19	0.09	10.9%	112%	4	3.24	0.12	0.06	3.8%	103%	4
25	97.5	5	2.5	5.1%	100%	4	10.8	3.41	1.71	31.7%	71.3%	4	1.1	0.33	0.17	30.1%	71.1%	4	3.01	0.15	0.07	4.9%	95.1%	4
26	97.5	5	2.5	5.1%	100%	4	17	2.55	1.28	15.0%	113%	4	1.74	0.19	0.09	10.9%	112%	4	3.26	0.15	0.07	4.5%	103%	4
27	97.5	5	2.5	5.1%	100%	4	15.6	3.51	1.75	22.4%	104%	4	1.59	0.3	0.15	19.1%	103%	4	3.15	0.07	0.03	2.2%	99.5%	4
29	90	20	10	22.2%	92.3%	4	15	4.75	2.38	31.7%	99.2%	4	1.63	0.22	0.11	13.6%	105%	4	3.23	0.06	0.03	2.0%	102%	4
30	77.5	15	7.5	19.4%	79.5%	4	9.05	5.67	2.83	62.6%	60.0%	4	1.1	0.57	0.29	52.3%	70.7%	4	3.08	0.32	0.16	10.3%	97.5%	4
33	97.5	5	2.5	5.1%	100%	4	15.1	0.22	0.11	1.4%	100%	4	1.55	0.09	0.05	5.9%	100%	4	3.16	0.08	0.04	2.7%	100%	4

CV = coefficient of variation; n = number of replicates; SD = standard deviation; SEM = standard error of the mean.

**Table A1-7. Summary of control-adjusted responses observed in *Lampsilis siliquoidea* exposures during toxicity testing of whole sediment collected from the Anniston PCB Site.**

Station ID	Sample ID	Cycle <sup>1</sup>	TOC (%)	Fines (%)	Total PCBs (µg/kg DW) <sup>2</sup>	28-day Survival	28-day Total Biomass	28-day Individual Dry Weight	28-day Length
TX10-01-P	16	1b	0.265	41	91.2	100%	78.6%	78.2%	98.9%
TX30-01-P	25	1a	2.59	63.3	60,000	100%	71.3%	71.1%	95.1%
TX30-02-P	18	1a	2.64	90.4	740,000	66.7%	49.7%	75.6%	103%
TX30-03-P	07	1a	3.99	74.8	150,000	103%	104%	101%	106%
TX30-05-P	02	1b	3.06	70.8	120,000	82.1%	58.7%	71.0%	103%
TX40-01-P	27	1b <sup>3</sup>	1.09	77.3	13,000	100%	104%	103%	99.5%
TX40-02-P	17	1b	1.08	70.9	64,000	103%	123%	120%	106%
TX40-04-P	01	1a	1.88	70.4	68,000	103%	77.2%	75.1%	99.7%
TX40-05-P	14	1b	1.91	80.3	68,000	103%	92.4%	89.9%	100%
TX50-01-P	08	1a	2.76	75.6	770,000	92.3%	60.3%	68.6%	98.2%
TX50-02-P	19	1a	2.59	85.3	1,200,000	46.2%	16.7%	32.2%	95.9%
TX50-04-P	11	1b <sup>3</sup>	3.76	73.5	170,000	100%	104%	102%	103%
TX50-05-P	30	1a	2.3	56.7	410,000	79.5%	60.0%	70.7%	97.5%
TX60-01-P	21	M <sup>4</sup>	0.282	19.6	8,600	92.3%	84.7%	91.8%	98.6%
TX60-02-P	20	1b <sup>3</sup>	0.691	28.9	6,000	103%	126%	123%	104%
TX60-03-P	06	1a	1.33	43.3	100,000	94.9%	62.2%	65.0%	98.0%
TX60-04-P	13	1b <sup>3</sup>	1.48	47.2	25,000	103%	95.1%	92.5%	102%
TXR1-01-P	26	1b	0.303	11.3	<33	100%	113%	112%	103%
TXR1-02-P	09	1a	0.718	14.5	62	100%	119%	119%	106%
TXR1-03-P	04	1b	0.617	12.2	<16	97.4%	105%	107%	106%
TXR1-04-P	22	1b	0.474	12.6	<32	100%	112%	112%	103%
TXR1-05-P	29	1b	0.468	19.2	<33	92.3%	99.2%	105%	102%
TXR1-06-P	10	1b	0.527	26.6	<33	100%	99.2%	98.9%	102%

DW = dry weight; PCB = polychlorinated biphenyl; TOC = total organic carbon.

<sup>1</sup> Cycle that the sediment chemistry result is associated with.

<sup>2</sup> Total PCBs are calculated as the sum of the 10 homolog groups.

<sup>3</sup> This sample had sediment chemistry results from cycles 1a and 1b. The cycle 1b results are presented because they were obtained closer in time to when the mussel toxicity test was run.

<sup>4</sup> M = mussel; this sample was only used with the mussel exposures and was not used in cycles 1a or 1b.