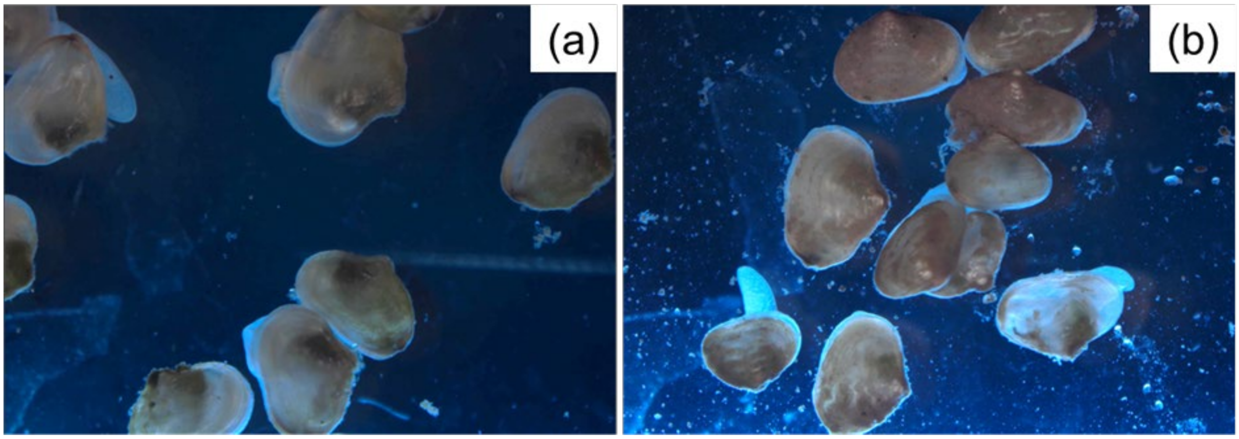
Evaluating Sedimentation Impacts to Freshwater Mussels



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16. Abstract

Transportation system construction activities can cause increased suspended and redeposited solids in adjacent water systems, which may adversely impact freshwater mussels. The objectives of this study are to evaluate the impacts of suspended solids on juvenile mussels including to establish the impact thresholds, to assess the effects of sediment deposition on adult and subadult mussels, to develop a particle tracking model to understand the particle transport behavior, and to evaluate the available mussel impact mitigation practices. The research methodology includes a literature review, a survey and interview on the current sediment management practice for mussel species protection in the nation, laboratory studies, modelling development, interviews with Missouri Department of Transportation (MoDOT) personnel, and engineering assessments. Results of the DOT surveys emphasize the need for further research to enhance understanding of the impacts of sedimentation on freshwater mussels. Laboratory experiment results suggest that (a) particles at low concentrations may benefit juveniles' growth, while higher TSS concentrations (> 1000 mg/L) inhibited their growth; (b) adult mussels' response to deposition were affected by burial depth, vertical water supply and flow direction, as well as mussel species, with the endangered Pink Mucket being the most vulnerable among all species tested. A one-dimensional model was developed to illustrate the spatial distribution of sediment transport, and results suggest that sedimentation varies with particle size, flow velocity and depth. A strategy for both streamside and instream best management practices was developed. The strategy incorporates lessons learned from other state DOTs and provides a link between both the mussel experiments and the modeling exercises and project site engineering.

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List of Abbreviations and Acronyms

ADCP	Acoustic Doppler Current Profiler
23 CFR 420	Code of Federal Regulations, Title 23, Part 420
CDF	Cumulative Distribution Function
CFD	Computational Fluid Dynamics
CRW	Continuous Random Walk
DEM	Digital Elevation Model
DOT	department of transportation
FHWA	Federal Highway Administration
GNSS	Global Navigation Satellite Systems
IQR	Interquartile Rang
LPT	Lagrangian Particle Tracking
MSDIS	Missouri Spatial Data Information Service
NTL	National Transportation Library
PDF	Probability Density Function
RANS	Reynolds-Averaged Navier-Stokes
RMS	Root Mean Square
RMSE	Root Mean Square Error
RNG	Re-Normalization Group
ROSA P	Repository & Open Science Access Portal
TIFF	Tag Image File format
TIN	Triangulated Irregular Network
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey
CERC	Columbia Environmental Research Center
DO	Dissolved Oxygen
TAN	Total Ammonia
TOC	Total Organic Carbon
TIN	Total Inorganic Carbon
TN	Total Nitrogen
PSD	Particle Size Distribution
Ctrl	Control Treatment
AB	Arkansas Brokenray
FT	Fatmucket
BF	Butterfly
PK	Pink Mucket
MK	Mucket
WB	Washboard
GF	Giant Floater
DT	Deertoe
Water-I	Water flowing into the burial layer
Water-O	Water flowing out of the burial layer

Executive Summary

Freshwater mussels of the families Margaritiferidae and Unionidae serve as “ecosystem engineers” modifying the habitat and making it more suitable for other organisms. However, freshwater mussels are one of the most imperiled faunal groups in the world. In Missouri alone, 29 species are classified as species of conservation concern, including 15 that are considered to be in danger of extirpation in Missouri or extinction throughout their range. Transportation system construction and maintenance of roads, bridges, and pipeline crossings can alter the local and regional hydraulics and water quality, leading to increases in suspended and redeposited solids in the adjacent water systems, which pose potential adverse impacts on the aquatic flora and fauna.

This project has gathered current knowledge of the means by which state departments of transportation (DOTs) across the nation work to mitigate the potential impacts of transportation project construction on mussels. State DOTs are involved in surveys to identify the locations of mussel beds to determine whether relocation is necessary. Various survey techniques are employed. Additionally, there are multiple protocols for relocating mussel beds. Relocation may be followed up with post-construction monitoring to assess the success of the effort. The activities perceived by state DOTs to most frequently cause sedimentation impacts to freshwater mussels are bridge construction, bridge removal, cofferdam removal, and culvert replacement. The factors that are the greatest challenge to DOTs’ efforts to reduce sedimentation impacts to freshwater mussels during construction are agency understaffing, coordination with other agencies, and cost. The most frequently used best management practices (BMPs) for projects impacting freshwater mussels are silt fence, seed and mulch, and limiting vegetation removal. There is significant interest among DOTs in BMPs established for bridge or culvert sites where protected species or dense mussel populations exist, such as smart silt fence, no equipment below the ordinary high-water mark (OHWM), turbidity curtains, and coconut and fiber logs. The most frequently developed DOT resources for minimizing sedimentation impacts to freshwater mussels are BMP guidelines, survey protocols, and special provisions.

A study was established to explore how elevated suspended solids associated with construction and maintenance activities may affect the survival and growth of freshwater mussels. Although no clear impact on survival was noted, effects on growth were observed, not only among different sediment/soil types, but also among different TSS concentrations from the chronic study. Unexpected but significant growth enhancement effects were observed at lower solids concentrations. However, high levels of all three sediment/soil samples could become a stressor inhibiting or stopping the growth of juvenile mussels. More studies are needed to achieve better understanding of how mussels may respond to a complex and degraded environment. The current study indicates that suspended solids may not always be a stressor to juvenile mussels. Juvenile mussels may benefit from lower levels of suspended solids originating from some types of sediments and soils. Thus, this study provides important information to policy makers to facilitate the preparation of guidelines and policies associated with construction and maintenance activities for mussel conservation purposes. Moreover, it is

suggested that although in this study the relative lower levels of suspended solids themselves may not be a threat to the juvenile mussels, it is important to consider other factors that may affect responses in natural systems.

A further study was conducted to test the responses of various mussel species to sediment burial. For the first time, a new design which allowed vertical water flow, either upwelling or downwelling, through the burial layers was developed and applied in mussel burial study. Using this flow-through design, the potential effects of vertical water flow, mimicking the hyporheic water flow connecting the underground water and surface water through the sediment in the real-life scenario, were investigated. Not surprisingly, with an increase in burial depth and a decrease of vertical water supplied, fewer mussels were capable of reemerging from the burial layers and thus increased mortality was observed. Differences between species were observed, with Pink Muckets being the most vulnerable species of those examined against a burial event, and a layer of 5 cm BBS < 5 could inhibit their capability to resurface, resulting in significant mortality. The results of this study provide essential information towards understanding the mussel response to burial and the implications for conservation purposes. Specifically, it is rational to conclude that sediment deposition on a mussel bed/habitat may be lethal depending on the thickness (and composition) of the deposition layer, the local hydraulic conditions, and the composition (and density) of the mussel bed. It is thus important to avoid sediment deposition on mussel beds/habitat during construction. If deposition is unavoidable, it is critical to make sure that the deposition layer is thin enough to allow the majority of mussels to quickly resurface, or to relocate the mussel bed to another location that would not be impacted by the sediment deposition.

To provide a quantitative tool in evaluating the impact of construction-relevant sedimentation on freshwater mussel habitats in streams, the development, validation, and application of a Lagrangian particle tracking model was completed. The model utilizes the canonical mean velocity and turbulence profiles in open channels and tracks individual sediment particles using well accepted drag equations for non-cohesive sediment within the diameter range of 100 − 104 µm. The effect of sediment exposure was modeled for three classes of sediment sizes, which indicates significant downstream distances can be affected by sediments, i.e., the mussels would be exposed to sediment clouds. For example, in the low flow condition, exposed mussels would be affected by large particles tens of meters downstream from a point source, and medium to fine particles tens to hundreds of meters downstream from a point source. Similarly, in the high flow condition, large particles would affect mussels about a hundred meters downstream, and medium and fine particles could affect mussels in the kilometer range downstream from a point source. The affected distance is strongly determined by the particle diameter, flow velocity, depth, and turbulence in the stream. This study provides quantitative measures on the locations for sediments to settle. Many other factors, however, should be considered for risk analysis of mussel burials and smothering, such as mussel tolerance and field conditions of sediments and flows. When bathymetry data is available, a detailed computational fluid dynamics model is desirable to provide more realistic simulations of hydraulics and sediment transport.

Engineering resources and practice have been utilized to develop recommendations for revisions to the MoDOT Engineering Policy Guide to mitigate the potential impacts of construction projects on freshwater mussels in Missouri. The recommendations incorporate lessons learned from other state departments of transportation and provide a link between both the mussel experiments and the modeling exercises and project site engineering. An overall strategy guides the development and implementation of stormwater management BMPs, for both streamside and instream locations. Conversations with state and federal agency personnel will be necessary to ensure that the policy recommendations discussed here are consistent with all other policies, procedures, and intentions.

1. Introduction

1.1 Background and Motivation

As required by the National Environmental Policy Act (NEPA), specifically under the Endangered Species Act [1], Missouri Department of Transportation (MoDOT) and Federal Highway Administration (FHWA) must thoroughly address the potential impacts of any transportation project to threatened and endangered species, including rare plants, animals, critical habitat and unique natural communities. Among over 1000 rare plant and animal species monitored in the State of Missouri, over 60 are listed as state endangered, of which 15 are species of native freshwater mussels. These include Slippershell (*Alasmidonta viridis*), Elephantear (*Elliptio crassidens*), Curtis Pearlymussel (*Epioblasma curtisii*), Snuffbox (*Epioblasma triquetra*), Pink Mucket (*Lampsilis abrupta*), Higgins Eye (*Lampsilis higginsii*), Neosho Mucket (*Lampsilis rafinesqueana*), Scaleshell (*Leptodea leptodon*), Spectaclecase (*Margaritifera monodonta*), Sheepnose (*Plethobasus cyphyus*), Fat Pocketbook (*Potamilus capax*), Winged Mapleleaf (*Quadrula fragosa*), Ebonyshell (*Reginaia ebenus*), Salamander Mussel (*Simpsonaias ambigua*) and Rabbitsfoot (*Theliderma cylindrica*) [2, 3].

Freshwater mussels have a complex life cycle that includes fertilization by spermcasting, embryos brooded in marsupial gills, parasitic larvae that attach to fish, and a free-living juvenile stage that typically occupies interstitial spaces in river sediment [4, 5]. Freshwater mussels (order Unionoida) play vital ecosystem services by filtering algae, bacteria, and other particles from water, recycling nutrients and energy, and serving as food for fish, small mammals, and some birds [6]. Unfortunately, freshwater mussels are one of the most imperiled groups of animals worldwide. The population decline of many of these species can be due to various factors, including disease and water pollution as well as changes in habitat conditions such as hydrology, sedimentation, dam construction, and temperature [7]. Among human activities that have an impact on freshwater mussels and freshwater habitats in Missouri, transportation system construction and maintenance of roads, bridges, and pipeline crossings can alter the local and regional hydrology and water quality [8-11]. Bankside construction activities may result in erosion into the water system due to the disturbance of the nearby soil structures and vegetation covers [12, 13]. In-stream constructions may disturb benthic sediment and suspend the fine particles into the river flows to be redeposited downstream [11, 14, 15]. Consequently, such activities may lead to increase of suspended and redeposited solids in the adjacent water systems, which pose potential adverse impacts on the aquatic flora and fauna [16-20].

Mussels use algae, bacteria, and other small organic particles ranging from 2 to 20 µm as food, filtering a large volume of water each day [21]. Some clay particles are in sizes comparable to algae and bacterial cells and could be in relatively stable colloidal forms in freshwater systems due the strong negative charge on colloidal surfaces preventing their aggregation; as a result, they could potentially interfere with the mussel feeding process. The impact of suspended and deposited sediments on freshwater mussels is currently not well understood and quantified. The types of particles generated by road and bridge construction vary depending on activities and locations, so that it is necessary to delineate the range of particle types and the processes of suspension and deposition as well as to investigate the biology of sediment impacts on species of concern [22].

1.2 Study Objectives

The overall goal of this project is to better understand how the sedimentation relevant to the construction and maintenance of transportation infrastructure could impact freshwater mussels, especially for those endangered or threatened species, and what mitigation approaches can be developed and adapted to minimize such impacts. The specific objectives are to:

A. Evaluate the impacts of suspended solids from different sources on freshwater mussels with special focus on juveniles, the more vulnerable life stage, and explore the impact mechanisms. Construction activities necessarily disturb soils and landscapes, resulting in acute and sporadic sedimentation that could impair habitats for mussels. We will identify major mechanisms of sediment impacts to juvenile mussels.

B. Determine the impact thresholds of suspended solids to freshwater mussel juveniles. Impact threshold information is needed to ensure no or minimal impact to endangered or threatened mussel species during road construction. We will use soil and sediment samples from selective construction sites for the threshold determination.

C. Evaluate the impacts of sediment deposition on subadults and adults. Loss of subadults and adults, leading to recruitment failure of juveniles, may result in population collapse. We will study how sediment deposition may affect elder mussels, evaluate their ability to recover from different deposition layers, and accordingly estimate the threshold.

D. Investigate the possible mechanisms that result in mussel death during a bury event. Once a mussel fails to unbury itself from the deposition layer, it may eventually be killed. However, the mechanisms of mussel death remain unclear. Thus, this study strives to explore possible mechanisms for mussel kill, thus facilitating the development of mitigation approaches.

E. Develop a model to predict and estimate the particle behavior after sediments/soils enter the water column. By referring to available hydrology data, the team will use the model to predict, after sediments/soils entered the water column: how quickly they may deposit, how much may remain in the water for a long period of time, and where major deposition may be located along the river/stream.

F. Evaluate the range of available mussel impact mitigation practices according to their applicability, effectiveness, ease of implementation and cost. Using the above data and the results of the literature review, the team has assessed both physical (e.g., enhanced design of the sedimentation basins already required at construction sites, as with baffles) and chemical (e.g., the introduction of chemical coagulants in the stormwater management treatment train) options. The evaluation has considered the soil characters found at the site, construction materials brought to the site, site topography, size of the runoff contributing area, and the size and configuration of the right of way.

1.3 Report Organization

The chapters of this report are organized as follows:

- Chapter 2 discusses current DOT practices based on a survey and provides information from a review of existing literature regarding current research status and understanding towards impacts of sedimentation on freshwater mussels.
- Chapter 3 describes the methodology and results of lab experiments on impacts of suspended solids on freshwater mussel juveniles.
- Chapter 4 describes the methodology and results of lab experiments on impacts of sediment deposition on freshwater mussel adults.
- Chapter 5 describes the Lagrangian particle tracking model and applications in Missouri streams.
- Chapter 6 provides recommendations for revisions to the MoDOT Engineering Policy Guide to mitigate the impact of construction projects on freshwater mussels in Missouri.

Table 1-1 lists the supplemental information for the report included in the appendices.

Table 1-1. Report Appendices

Appendix	Title
A	DOT Survey
B	Survey Responses by DOT
C	Supplementary information on the impacts of suspended solids to freshwater mussel juveniles
D	Supplementary information on the impacts of sediment deposition to freshwater mussel juveniles
E	Model evaluation and interpretation for freshwater mussels from point-source sedimentations

Chapter 2. DOT Practices and Literature Review

2.1 DOT Practices

This chapter provides an overview of state Department of Transportation (DOT) practices for evaluating and mitigating sedimentation impacts to freshwater mussels as identified through a DOT survey and interviews.

2.1.1 DOT Survey

2.1.1.1 Methodology for DOT Survey

An online survey on sedimentation impacts to freshwater mussels was developed and administered by the researchers. The survey included 13 questions and covered various topics such as DOT experience with freshwater mussels, mitigating strategies, best management practices (BMPs), and DOT resources and partnerships. The survey questions may be found in **Appendix A**. The survey was reviewed by the project Technical Advisory Committee (TAC) before being sent to the DOTs from all 50 states and the District of Columbia. The survey was implemented using Qualtrics Survey Software (Qualtrics 2024). The survey was sent to one respondent from each state DOT using a contact list that was developed primarily from the American Association of State Highway Transportation Officials (AASHTO) Committee on Environment and Sustainability, with responses limited to one per DOT. As shown in **Figure 2-1**, responses were received from 41 agencies for a response rate of 80 percent. North Dakota Game and Fish Department completed the survey on behalf of North Dakota DOT.

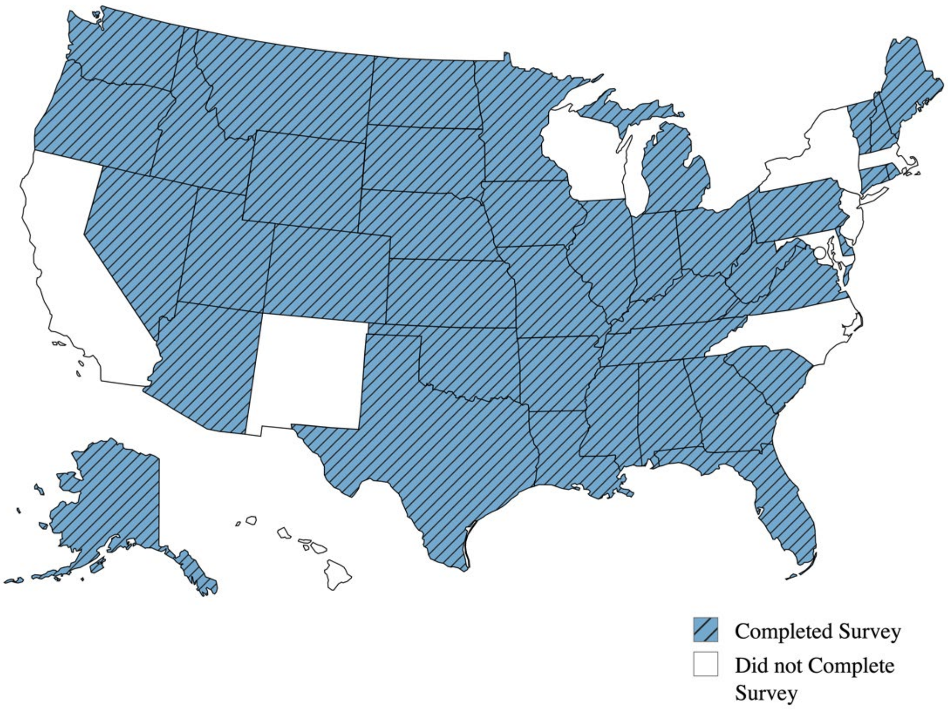


Figure 2-1. Map showing survey completion by state (created with mapchart.net).

2.1.2 Results for DOT Survey

The following sections present the survey results, organized by topic. Survey responses by DOT are provided in **Appendix B**.

2.1.2.1 DOT Experience with Freshwater Mussels

The first question of the survey asked DOTs about the number of projects they identify each year as having the potential to cause sedimentation impacts to freshwater mussels. As shown in **Table** , 29 percent of responding state DOTs do not identify any such projects, while 27 percent of responding DOTs identify 1 to 5 such projects annually. Only 17 percent of responding DOTs identify more than 25 of these projects each year.

Table 2-1. Survey results for number of projects identified annually as having the potential to cause sedimentation impacts to freshwater mussels (Question 1).

Frequency	Response
0	29%
1 to 5	27%
6 to 10	15%
11 to 25	12%
26 to 50	10%
More than 50	7%
No Response	0%

Question 4 of the survey sought information regarding the types of mussel surveys conducted by DOTs. As shown in **Table 2-2**, cells (quadrats), transect, and timed search are all conducted by 44 percent of responding DOTs. The least common method is eDNA, which is utilized by 5 percent of responding DOTs. Other methods mentioned include information from other agencies, field investigations, and personal knowledge.

Table 2-2. Survey results for types of mussel surveys conducted (Question 4).

Survey Type	Response
Cells, a.k.a. quadrats (quantitative)	44%
Transect	44%
Moving transect	10%
Timed search (qualitative)	44%
eDNA	5%
Other (Please describe)	32%
No Response	15%

NOTE: Respondents could select multiple answers.

Question 5 asked DOTs about how frequently they thought the different types of construction activities will cause sedimentation impacts to freshwater mussels at a specific site, and the results are shown in **Table 2-3**. Based on the very likely plus likely responses, the activities perceived to most frequently cause sedimentation impacts to freshwater mussels are bridge construction, bridge removal, cofferdam removal, and culvert replacement. Pile driving and temporary sheet piling installation are the activities perceived to least frequently results in sedimentation impacts to freshwater mussels. Causeway removal was mentioned in the text responses as another activity that could lead to sedimentation impacts to freshwater mussels.

Table 2-3. Survey results for perceptions of construction activities that lead to sedimentation impacts to freshwater mussels (Question 5).

Factor	Very Likely	Somewhat Likely	Neutral	Somewhat Unlikely	Very Unlikely	No Response
Bridge Construction	39%	37%	2%	2%	2%	17%
Bridge Rehabilitation	2%	34%	7%	20%	12%	24%
Bridge Removal	44%	32%	0%	5%	2%	17%
Cofferdam Construction	27%	24%	15%	10%	5%	20%
Cofferdam Removal	29%	34%	7%	5%	2%	22%
Culvert Replacement	22%	41%	7%	10%	2%	17%
Drilled Shafts	2%	34%	22%	12%	5%	24%
General Soil Disturbance of Overall Construction Site	17%	34%	7%	20%	2%	20%
Grading	12%	27%	15%	22%	2%	22%
Pile Driving	2%	20%	29%	22%	7%	20%
Riprap Placement	7%	41%	12%	15%	5%	20%
Temporary Causeway Construction	24%	29%	12%	7%	2%	24%
Temporary Sheet Piling Installation	0%	32%	29%	15%	2%	22%
Other (Please describe)	2%	0%	0%	0%	0%	98%

Table 2-4. Survey results for factors that hinder efforts to reduce sedimentation impacts to freshwater mussels during construction (Question 6).

Concern	Strongly Agree	Somewhat Agree	Neither Agree Nor Disagree	Somewhat Disagree	Strongly Disagree	No Response
Agency Understaffed	5%	39%	22%	7%	7%	20%
Coordination with Other Agencies	22%	20%	24%	2%	12%	20%
Cost	10%	29%	22%	10%	10%	20%
Lack of Agency Buy-In	2%	32%	22%	12%	12%	20%
Lack of Available Data	12%	22%	17%	17%	12%	20%
Lack of Available Guidance	12%	22%	29%	10%	7%	20%
Lack of Contractor Buy-In	7%	22%	22%	17%	12%	20%
Need for Ground-Truthing for Assessment of Impacts	7%	22%	39%	5%	7%	20%
Proper Expertise for Evaluation and Mitigation	2%	27%	24%	15%	12%	20%
Public Awareness	0%	17%	29%	22%	12%	20%
Staff Awareness	0%	7%	22%	29%	22%	20%
Other (Please Describe)	2%	0%	2%	0%	2%	93%

Question 6 sought information from DOTs regarding factors that hinder their efforts to reduce sedimentation impacts to freshwater mussels during construction, and the results are shown in **Table 2-4**. Based on the strongly agree plus somewhat agree responses, the factors that are the greatest challenge to DOTs' efforts to reduce sedimentation impacts to freshwater mussels during construction are agency understaffing, coordination with other agencies, and cost. Only

7 percent of responding DOTs agreed that staff awareness limits these efforts. Regulatory concerns were also mentioned in the text responses.

In response to Question 8, 39 percent of responding DOTs indicated that they have performed post-construction monitoring to assess construction impacts to freshwater mussels (**Table 2-5**).

Table 2-5. Survey results regarding whether DOTs have performed post-construction monitoring to assess construction impacts to freshwater mussels.

Answer Choice	Response
Yes	39%
No	59%
No Response	2%

2.1.2.2 Mitigating Strategies and Best Management Practices (BMPs)

Table 2-6. Survey results for frequency of use of mitigation strategies or BMPs (Question 2).

Factor	Always	Almost Always	Sometimes	Rarely	Never	No Response
Brush Barriers	0%	2%	10%	15%	22%	51%
Design Causeways	7%	5%	22%	15%	32%	20%
Ditch Checks	12%	22%	22%	5%	22%	17%
Flocculants	0%	2%	10%	17%	51%	20%
Gabion Baskets	0%	0%	24%	12%	44%	20%
In-Water Turbidity Barriers	5%	17%	22%	17%	22%	17%
Limit Vegetation Removal	17%	24%	17%	2%	22%	17%
Monetary Compensation	0%	2%	12%	10%	56%	20%
No Equipment Below Ordinary High Water Mark (OHWM)	2%	24%	29%	12%	20%	12%
Relocate Mussel Beds	10%	15%	24%	10%	24%	17%
Seed and Mulch	46%	10%	7%	2%	17%	17%
Silt Bags	5%	17%	27%	5%	27%	20%
Silt Fence	39%	27%	2%	5%	12%	15%
Triangular Silt Dikes	2%	7%	7%	20%	44%	20%
Work During Specific Times of Year (e.g., No/Low Flow, No Spawning)	10%	27%	24%	15%	15%	10%
Work Pads	15%	15%	22%	10%	20%	20%
Other (Please describe)	2%	0%	2%	0%	10%	85%

Two survey questions asked DOTs about their use of mitigating strategies and BMPs on projects identified as having potential sedimentation impacts to freshwater mussels during construction. The results for Question 2, regarding frequency of use of these BMPs, are shown in

Table 2-6. The results indicate that, based on the always plus almost always responses, the most frequently used BMPs for these types of projects are silt fence, seed and mulch, and limiting vegetation removal. Based on the never responses, the least frequently implemented BMPs are monetary compensation, flocculants, gabion baskets, and triangular silt dikes. Other BMPs mentioned in the text responses include design modifications to avoid mussel beds, cofferdams, protective fence to separate work from mussel beds, and pollution control measures.

In Question 3, respondents rated the performance of mitigation strategies or BMPs used by their DOT in reducing sedimentation impacts to freshwater mussels on a scale of 1 (Poor) to 5 (Outstanding). As shown by the results in **Table 2-7**, respondents rated no equipment below ordinary high water mark (OHWM), working during specific times of year, and limiting vegetation removal as the most effective strategies and monetary compensation, brush barriers, and flocculants as the least effective strategies.

Table 2-7. Survey results for performance ratings of mitigation strategies or BMPs (1 = Poor, 5 = Outstanding) (Question 3).

Method	Average Rating	Standard Deviation	Lowest Rating	Highest rating	Number of Ratings
Brush Barriers	2.44	1.24	1	4	9
Design Causeways	3.06	1.35	1	5	18
Ditch Checks	3.22	1.00	1	5	23
Flocculants	2.56	1.24	1	4	9
Gabion Baskets	3.00	0.95	2	4	12
In-Water Turbidity Barriers	3.52	0.90	2	5	23
Limit Vegetation Removal	3.96	1.02	2	5	25
Monetary Compensation	2.11	1.54	1	5	9
No Equipment Below Ordinary High Water Mark (OHWM)	4.12	0.83	3	5	25
Relocate Mussel Beds	3.73	0.88	2	5	22
Seed and Mulch	3.68	0.90	2	5	25
Silt Bags	3.30	0.92	1	5	20
Silt Fence	3.59	0.98	1	5	29
Silt Dikes	2.75	0.97	1	4	12
Work During Specific Times of Year (e.g., No/Low Flow, No Spawning)	4.11	0.83	1	5	28
Work Pads	3.55	0.74	2	5	22
Other	4.00	1.41	3	5	2

2.1.2.3 DOT Resources and Collaborations

The survey included three questions related to DOT resources and partnerships. As shown in **Table 2-8**, approximately two thirds of responding DOTs have access to a database of mussel beds either through an internal or external database. The results for Question 9, shown in **Table 2-9**, indicate that the most frequently developed DOT resources for minimizing sedimentation impacts to freshwater mussels are BMP guidelines, survey protocols, and special provisions. DOTs submitted various resources, such as standard specifications, survey protocols, and special provisions in response to this question. As shown in **Table 2-10**, DOTs most frequently collaborate with other state agencies and US Fish and Wildlife Service and least frequently collaborate with non-profit organizations and US Geological Survey to evaluate and minimize sedimentation impacts to freshwater mussels.

Table 2-8. Survey results for access to data regarding mussel beds (Question 7).

Answer Choice	Response
Yes, my agency maintains a database with this information	10%
Yes, my agency has access to an external database with this information	56%
No	32%
No Response	2%

Table 2-9. Survey results for development of resources (Question 9).

Resource	Response
Survey protocol	29%
BMP guidelines	37%
Specifications	22%
Special provisions	27%
Evaluation studies	20%
Other (please describe)	29%
No Response	29%

NOTE: Respondents could select multiple answers.

Table 2-10. Survey results for collaborations with other organizations (Question 10).

Organization	Response
Consultants	46%
Non-profit organizations	2%
Other state agencies	63%
Universities	27%
U.S. Department of Agriculture	0%
U.S. Environmental Protection Agency	7%
U.S. Fish and Wildlife Service	61%
U.S. Geological Survey	2%
U.S. Army Corps of Engineers	29%
Other (please describe)	10%
None	15%
No Response	10%

NOTE: Respondents could select multiple answers.

2.1.2.4 Other Survey Feedback

The remaining questions sought additional information from state DOTs regarding their practices for evaluating and minimizing sedimentation impacts to freshwater mussels. As shown in **Table 2-11**, 61 percent of responding DOTs were willing to participate in a follow-up interview. In addition, 49 percent of responding DOTs indicated an interest in learning more about reducing sedimentation impacts for freshwater mussels (**Table 2-12**).

Table 2-11. Survey results for willingness to participate in a follow-up interview (Question 11).

Answer Choice	Response
Yes	61%
No	32%
No Response	7%

Table 2-12. Survey results for interest in learning more about reducing sedimentation impacts for freshwater mussels (Question 12).

Answer Choice	Response
Yes	49%
No	41%
No Response	10%

The final question of the survey provided an opportunity for DOTs to provide other feedback on reducing sedimentation impacts for freshwater mussels. A few notable comments are summarized below, and other comments may be found in **Appendix B**.

- Surveys in turbid waters can be costly, and it would be beneficial to develop thresholds for turbidity levels to warrant a survey.
- Additional research could investigate the utilization of BMPs to improve connectivity within riverine systems.
- One DOT uses erosion and sediment BMPs with plan notes for aquatic invasive species.
- Some DOTs rarely deal with mussels.

2.1.3 DOT Interviews

This section summarizes the results of interviews that were conducted with six DOTs.

2.1.3.1 Georgia DOT

The mussel survey protocol for the Georgia Department of Transportation (GDOT) [23] presents procedures for wadeable streams (at least 75 percent of survey reach with depth of 1.5 m or less) and non-wadeable streams (at least 25 percent of the survey reach with depth of 1.5 m ft or more). As shown in **Figure 2-2**, the survey area is divided into eight 50-meter segments (two upstream segments and six downstream segments). GDOT's protocol also describes relocation processes for relocation survey, distribution within the relocation site, and monitoring. Annual monitoring surveys are required for five years after the end of construction activity.

GDOT sponsored a research study on protection of imperiled aquatic species [24]. The study resulted in the development of a Total Effect Score which uses a risk-based methodology to evaluate construction and post construction impacts to imperiled freshwater species for a time horizon of 50 years. The process for calculation of the Total Effect Score is shown in **Figure 2-3**. The methodology was developed based on evaluation of characteristics of 111 freshwater species (including mussel species), and a spreadsheet tool to determine this score was also created. Other study deliverables included a template for a programmatic agreement to assess projects based on Total Effect Score and recommendations for project special provisions. GDOT is working towards a programmatic agreement (anticipated completion in 2025) that would slightly adapt the Total Effect Score to facilitate implementation.

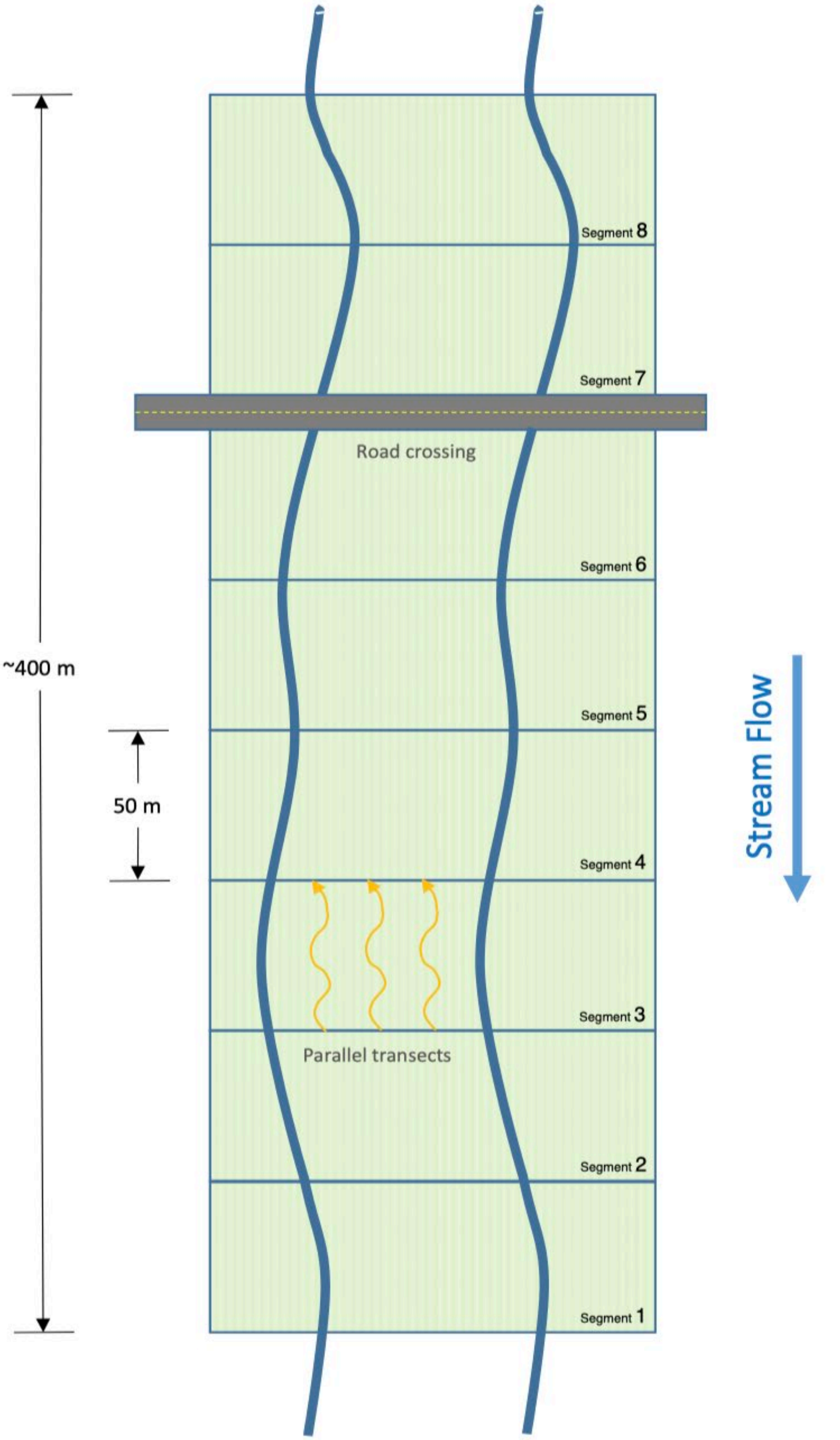


Figure 2-2. Layout of mussel sampling methodology for GDOT [23].

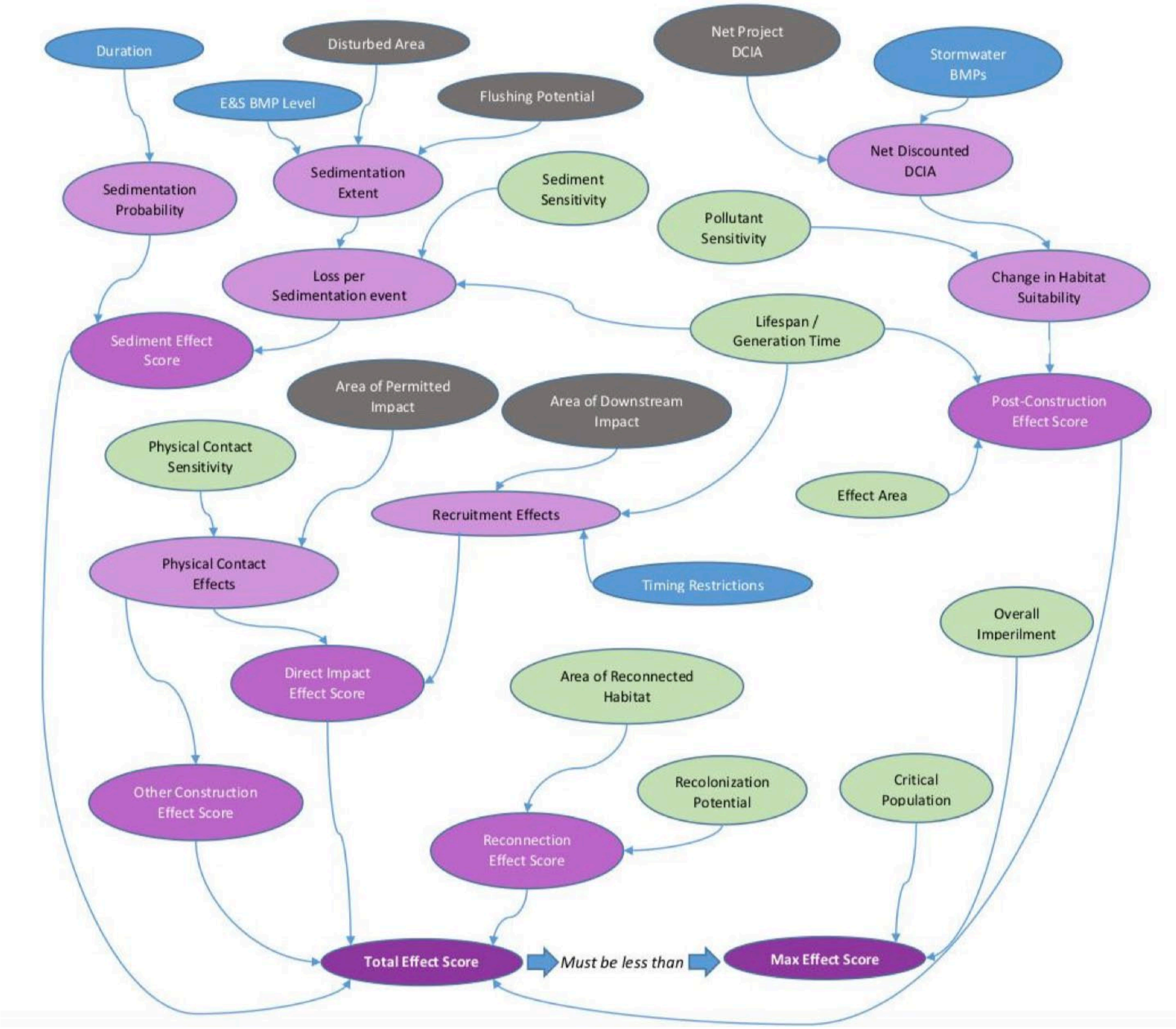
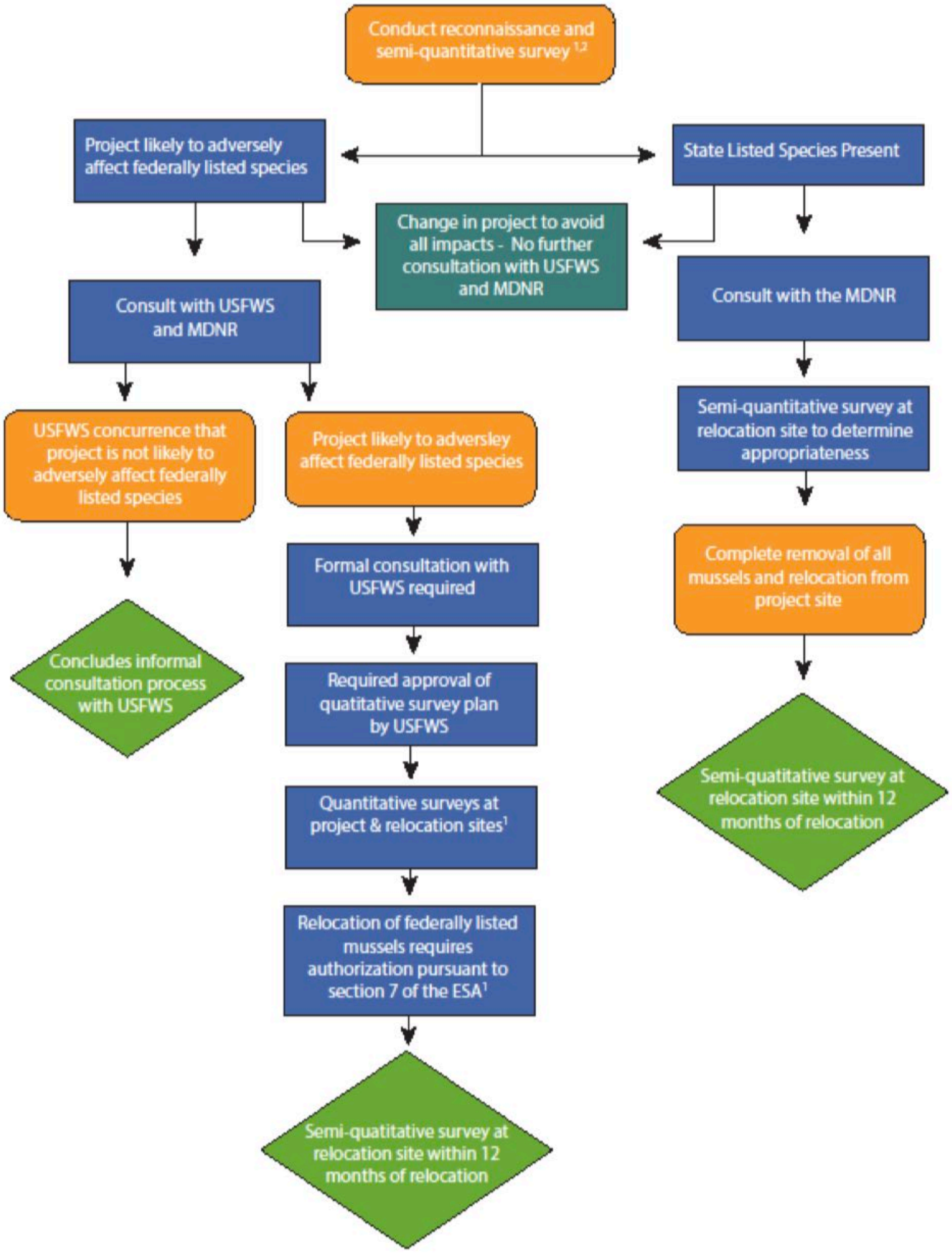


Figure 2-3. Diagram showing process of determining the Total Effect Score for a species [24].

GDOT looks closely at special provisions for projects (e.g., enhanced erosion control in channel) to mitigate impacts to freshwater mussels and other aquatic species. GDOT finds low cost and low maintenance BMPS easier to implement. In GDOT's experience, the most frequent construction activity that affects freshwater mussels is bridge replacement.

2.1.3.2 Michigan DOT

Michigan's mussel survey protocol [25] is based on the protocols for West Virginia [26] and Ohio [27]. Michigan's protocol divides streams into four groups (1, 2, 3a, 3b) based on size and whether federal listed mussel species are expected. The size of the buffer area is determined individually for each project based on factors such as substrate particle size, indirect impacts, and construction methods. As shown in **Figure 2-4**, Michigan's survey protocol includes a reconnaissance survey and, under certain conditions, a quantitative survey. For Group 3a and 3b streams, a quantitative survey is required if an initial survey identifies the presence of federally listed species, a mussel density of at least $0.25/m^2$, and/or the presence of at least four different mussel species.



[1] ESA Section 10(a)1(A) permit required for Group 3 waters

[2] MDNR Scientific Collectors permit and State Threatened and Endangered Species permit required for Group 2 waters

Figure 2-4. Overview of Michigan's mussel survey process [25].

Michigan's survey protocol provides a checklist of data to be recorded, such as methods and results. Mussel relocation procedures are outlined for site selection, relocation methods, transporting and placement, post relocation monitoring, and reporting.

The Michigan Department of Transportation (MDOT) reviews projects for impacts to wildlife, and some projects are tagged for potential impacts to freshwater mussels. MDOT finds ground truthing and determining if plans match exactly what is happening in the field to be challenging. MDOT is interested in learning if other DOTs have BMPs established for bridge or culvert sites where protected species or dense mussel populations exist.

2.1.3.3 Minnesota DOT

The Minnesota Department of Transportation (MnDOT) follows a freshwater mussel survey and relocation protocol developed by the Minnesota Department of Natural Resources and U.S. Fish and Wildlife Service [28]. The protocol provides guidance regarding methods and reporting requirements for Level I (qualitative) and Level II (quantitative) surveys. An example screenshot from a Level I survey is shown in **Figure 2-5**. Level II surveys are performed when a Level I survey encounters at least one mussel per minute or state or federally listed species. The protocol also presents requirements for moving mussels to a suitable habitat located at least 30 meters upstream from the area of project impact.

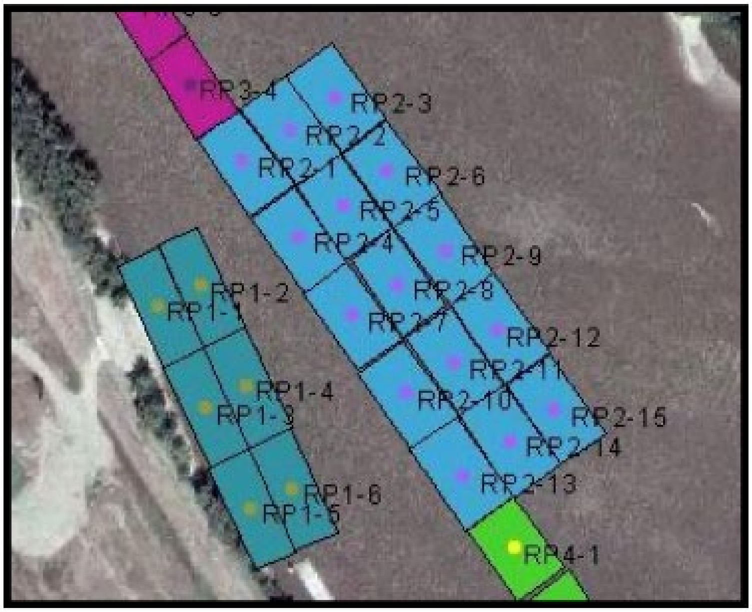


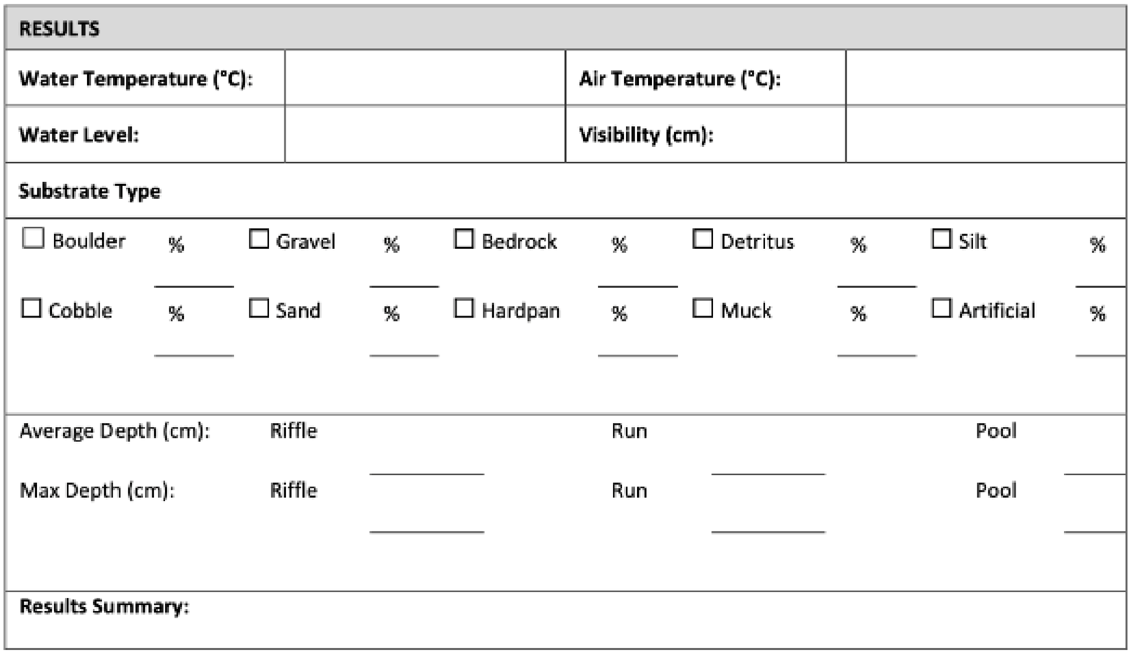
Figure 2-5. Screenshot of Level I survey for MnDOT [28].

MnDOT's preferred strategy is to avoid impacts to freshwater mussels and host fish. If that is not possible, MnDOT will seek to minimize and mitigate those impacts. For mitigating impacts, projects can relocate mussels from the area of in-stream impact and/or pay compensatory mitigation that will be used to further freshwater mussel conservation efforts. MnDOT encourages designers to think about constructability and mitigation of impacts to freshwater mussels during the design phase. Some considerations during the design phase to avoid and minimize impacts to freshwater mussels include limiting work below OHWM, alternative locations, designing to limit the number of in-water piers, and detours versus temporary bridges. Strategies utilized during construction include timing to avoid fish spawning, staging barges and other equipment outside of known mussel areas, inspections, not placing dredged material back in the river, sediment and erosion control measures, and the use of barges versus causeways.

Challenges that MnDOT faces in mitigating impacts to freshwater mussels include the need for contractor access and/or buy-in, issues encountered with larger bridges, diver safety in deeper and swifter waters, and coordination with other state DOTs and other agencies when a river forms a border between two states. MnDOT is interested in seeing research regarding how long it takes for mussels to recolonize areas where they were removed from.

2.1.3.4 Ohio DOT

The mussel survey protocol for Ohio [27] is based on the mussel survey protocol for West Virginia [26]. Ohio's protocol divides streams into five groups (Unlisted, Groups 1-4) based on size and whether federally listed mussel species are expected. The protocol includes a table of survey buffer area based on stream group and type of work. Reconnaissance of Group 1 and unlisted streams (minimum watershed 5 mi^2) can be used to determine the presence of mussels. If mussels are present, they must be relocated prior to construction. Relocation surveys prior to construction are also required for Group 3 streams. A mussel survey is required for systems in Group 2 and 4 streams to determine if federally listed species are present. Based on consultation with U.S. Fish and Wildlife Service (USFWS), a mussel relocation is generally required for these streams prior to construction. An excerpt from the survey reporting form for Group 1 and Group 3 systems is shown in **Figure 2-6**.



RESULTS

Water Temperature (°C):		**Air Temperature (°C):**	
Water Level:		**Visibility (cm):**	

Substrate Type

☐ Boulder	%	☐ Gravel	%	☐ Bedrock	%	☐ Detritus	%	☐ Silt	%
☐ Cobble	%	☐ Sand	%	☐ Hardpan	%	☐ Muck	%	☐ Artificial	%

Average Depth (cm):	Riffle	Run	Pool
Max Depth (cm):	Riffle	Run	Pool

Results Summary:

Figure 2-6. Excerpt from Ohio reporting form for Group 1 and Group 3 systems [27].

The protocol notes that the preference is to avoid and minimize impacts to waters of the United States, including impacts to mussels. Minor design modifications or changes in location can help to avoid impacts.

The Ohio Department of Transportation (ODOT) uses various BMPs (e.g., silt fence, ditch checks) to mitigate sedimentation impacts to freshwater mussels. In ODOT's experience, the cost of the survey can be challenging. ODOT finds that various types of construction activities can lead to impacts to mussels, and work pads with pipes in big streams can be especially challenging. In the future, ODOT would like to streamline mussel survey efforts through programmatic agreements and work towards making survey buffers more tailored to each site.

2.1.3.5 Texas DOT

The mussel survey protocol for Texas [29] divides streams into five groups (Groups 1-5) based on size and whether federally listed mussel species are expected. The protocol includes a table of survey buffer area based on stream group and type of work. As shown in **Figure 2-7**, Texas' process includes an instream survey for Groups 1, 2, and 4 for proposed projects and for Groups 3 and 5 for proposed projects if a reconnaissance survey determines the presence of mussels. For instream surveys for Groups 1 and 3, the preferred method for sampling is cells, while transect and timed search surveys are preferred for Groups 2 and 4. The protocol also provides guidance on relocation and specifies that relocation sites should be at least 100 m upstream from the project impact area. The salvage zone (area from which mussels must be moved prior to the beginning of in-stream construction) is determined based on project type, stream group, and dominant substrate type.

The Texas Department of Transportation (TxDOT) has a standard list of sediment and erosion control measures to minimize impacts of sediment. The agency is interested in seeing more data on the impacts of sediment on freshwater mussels and has sponsored research investigating downstream ecological impacts of sediment due to bridge construction [30]. TxDOT is interested in seeing if other state DOTs implement BMPs or practices for construction access specifically for mitigating sedimentation impacts to freshwater mussels.

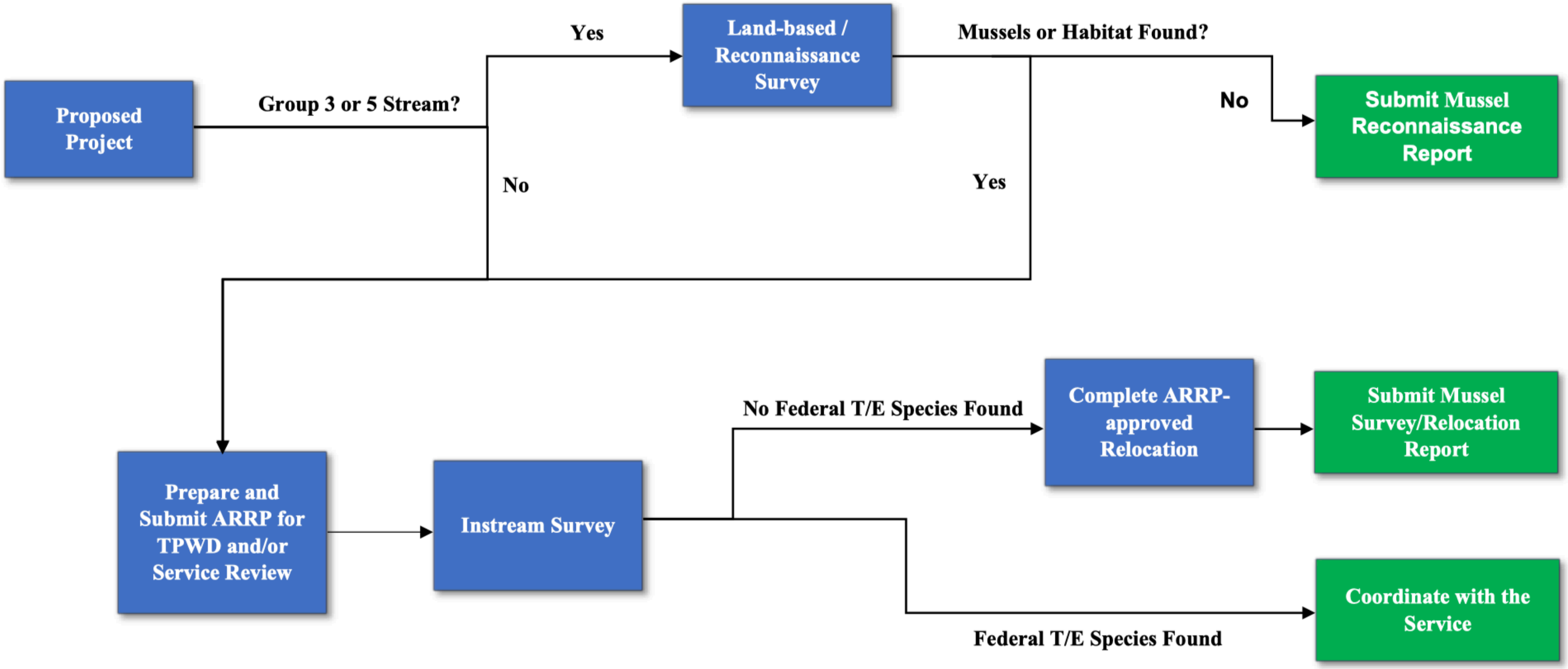


Figure 2-7. Flowchart for Texas mussels survey protocol [29].

2.1.3.6 West Virginia DOT

West Virginia's mussel survey protocol [31] divides streams into four groups (1, 2, 3, 4) based on size and whether federal listed mussel species are expected. The protocol includes a table of survey buffer area based on stream group and type of work (see **Figure 2-8**). Special considerations for various types of activities, such as maintenance dredging, bridge projects, and shoreline structures, are presented. The protocol describes procedures for various types of surveys, including timed search, cells, and transect surveys. **Figure 2-9** shows an example survey layout for a Group 1 stream. All survey proposals submitted to the USFWS and West Virginia Department of Natural Resources require an alternatives analysis and justification for the proposed work taking place below the ordinary high water mark (OHWM), and a description of the work that is planned below the OHWM.

West Virginia DOT performs approximately 30 to 40 mussel surveys per year, mostly for bridge projects. West Virginia DOT utilizes GIS and the West Virginia DNR to determine if the project falls on a known mussel stream. If so, Google Earth is used to help determine survey limits. West Virginia DOT has developed a list of avoidance and minimization measures (see **Figure 2-10**), such as smart silt fence, no equipment below OHWM, turbidity curtains, and coconut and fiber logs, for projects around streams with federally listed species [32]. Mitigation typically involves relocating the mussels. If a Group 1 survey indicates the presence of mussels, in-stream work must begin before July 15th of the following year. If in-stream work has not started by that date, a mussel relocation is necessary (another survey to move any mussels that may be present in the original survey limits). Mitigation measures sometimes include limitations on construction during spawning (April 15 to June 30) which can be challenging to the construction process.

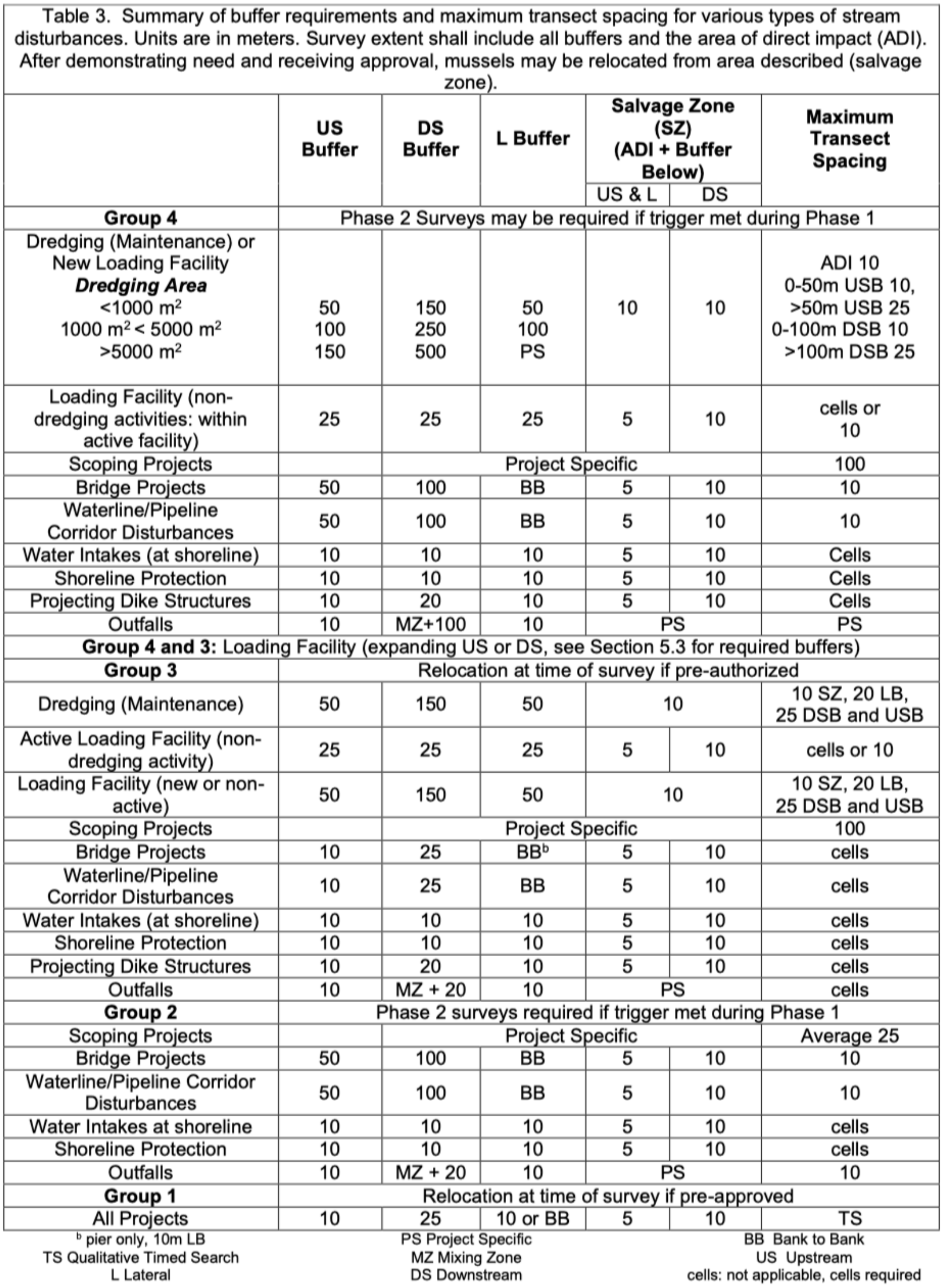


Table 3. Summary of buffer requirements and maximum transect spacing for various types of stream disturbances. Units are in meters. Survey extent shall include all buffers and the area of direct impact (ADI). After demonstrating need and receiving approval, mussels may be relocated from area described (salvage zone).

	US Buffer	**DS Buffer**	**L Buffer**	**Salvage Zone (SZ) (ADI + Buffer Below)**		**Maximum Transect Spacing**
				US & L	DS	
Group 4	Phase 2 Surveys may be required if trigger met during Phase 1					
Dredging (Maintenance) or New Loading Facility ***Dredging Area*** <1000 m^2 1000 m^2 < 5000 m^2 >5000 m^2	50 100 150	150 250 500	50 100 PS	10	10	ADI 10 0-50m USB 10, >50m USB 25 0-100m DSB 10 >100m DSB 25
Loading Facility (non-dredging activities: within active facility)	25	25	25	5	10	cells or 10
Scoping Projects		Project Specific				100
Bridge Projects	50	100	BB	5	10	10
Waterline/Pipeline Corridor Disturbances	50	100	BB	5	10	10
Water Intakes (at shoreline)	10	10	10	5	10	Cells
Shoreline Protection	10	10	10	5	10	Cells
Projecting Dike Structures	10	20	10	5	10	Cells
Outfalls	10	MZ+100	10	PS		PS
Group 4 and 3: Loading Facility (expanding US or DS, see Section 5.3 for required buffers)						
Group 3	Relocation at time of survey if pre-authorized					
Dredging (Maintenance)	50	150	50	10		10 SZ, 20 LB, 25 DSB and USB
Active Loading Facility (non-dredging activity)	25	25	25	5	10	cells or 10
Loading Facility (new or non-active)	50	150	50	10		10 SZ, 20 LB, 25 DSB and USB
Scoping Projects		Project Specific				100
Bridge Projects	10	25	BB[b]	5	10	cells
Waterline/Pipeline Corridor Disturbances	10	25	BB	5	10	cells
Water Intakes (at shoreline)	10	10	10	5	10	cells
Shoreline Protection	10	10	10	5	10	cells
Projecting Dike Structures	10	20	10	5	10	cells
Outfalls	10	MZ + 20	10	PS		cells
Group 2	Phase 2 surveys required if trigger met during Phase 1					
Scoping Projects		Project Specific				Average 25
Bridge Projects	50	100	BB	5	10	10
Waterline/Pipeline Corridor Disturbances	50	100	BB	5	10	10
Water Intakes at shoreline	10	10	10	5	10	cells
Shoreline Protection	10	10	10	5	10	cells
Outfalls	10	MZ + 20	10	PS		10
Group 1	Relocation at time of survey if pre-approved					
All Projects	10	25	10 or BB	5	10	TS

[b] pier only, 10m LB
TS Qualitative Timed Search
L Lateral
PS Project Specific
MZ Mixing Zone
DS Downstream
BB Bank to Bank
US Upstream
cells: not applicable, cells required

Figure 2-8. Buffer requirements and maximum transect spacing for West Virginia mussel survey protocol [31].

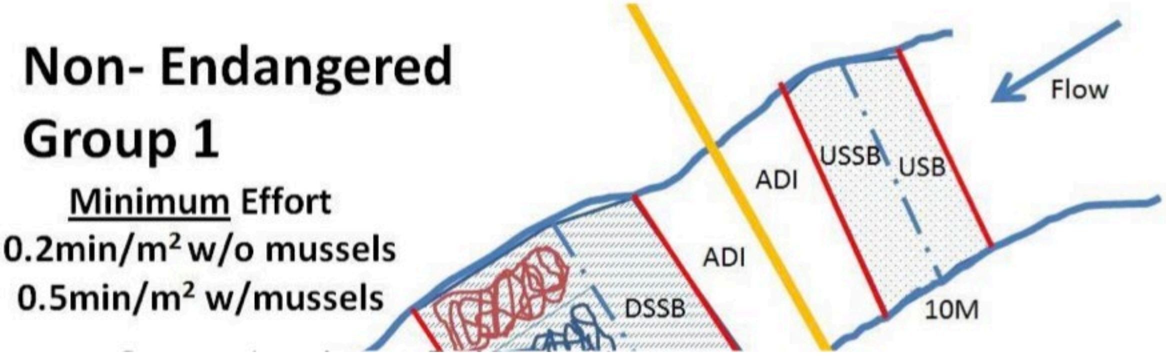
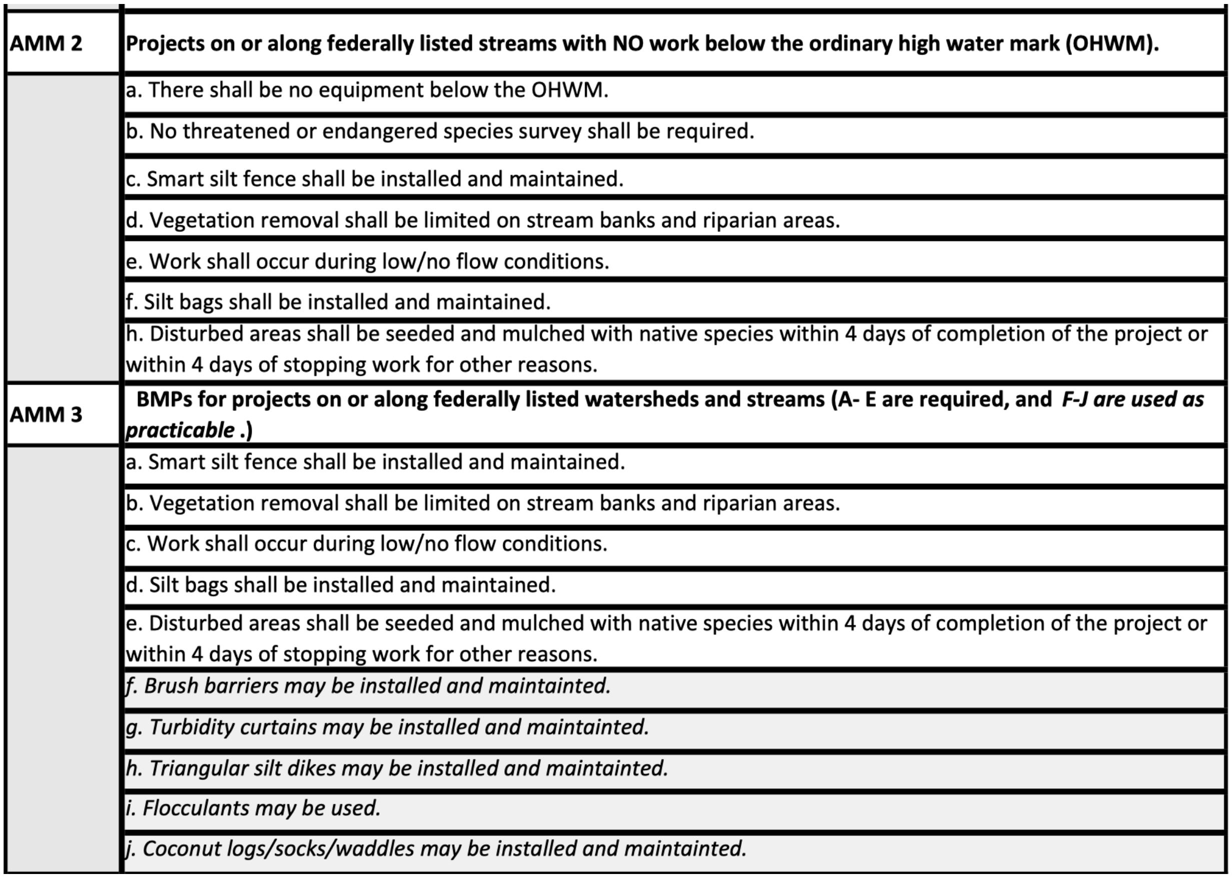


Figure 2-9. Example survey layout for Group 1 stream for West Virginia [31].



AMM 2	**Projects on or along federally listed streams with NO work below the ordinary high water mark (OHWM).**
	a. There shall be no equipment below the OHWM.
	b. No threatened or endangered species survey shall be required.
	c. Smart silt fence shall be installed and maintained.
	d. Vegetation removal shall be limited on stream banks and riparian areas.
	e. Work shall occur during low/no flow conditions.
	f. Silt bags shall be installed and maintained.
	h. Disturbed areas shall be seeded and mulched with native species within 4 days of completion of the project or within 4 days of stopping work for other reasons.
AMM 3	**BMPs for projects on or along federally listed watersheds and streams (A- E are required, and *F-J are used as practicable* .)**
	a. Smart silt fence shall be installed and maintained.
	b. Vegetation removal shall be limited on stream banks and riparian areas.
	c. Work shall occur during low/no flow conditions.
	d. Silt bags shall be installed and maintained.
	e. Disturbed areas shall be seeded and mulched with native species within 4 days of completion of the project or within 4 days of stopping work for other reasons.
	f. Brush barriers may be installed and maintainted.
	g. Turbidity curtains may be installed and maintainted.
	h. Triangular silt dikes may be installed and maintainted.
	i. Flocculants may be used.
	j. Coconut logs/socks/waddles may be installed and maintainted.

Figure 2-10. Avoidance and mitigation measures for West Virginia DOT [32].

2.1.4 Summary of DOT Practices

State DOT practices for evaluating and mitigating sedimentation impacts to freshwater mussels are summarized below.

- State DOTs have a wide range of experience with projects identified as having the potential to cause sedimentation impacts to freshwater mussels. 29 percent of responding state DOTs do not identify any such projects, while 17 percent of responding DOTs identify more than 25 of these projects annually.
- Cells (quadrats), transect, and timed search are the most frequently used types of surveys.
- Survey protocols typically group streams based on stream characteristics and/or whether federal listed mussel species are expected. The protocols often include provisions for initial surveys and quantitative surveys. The protocols also generally prescribe procedures for relocation of mussels.
- The activities perceived by state DOTs to most frequently cause sedimentation impacts to freshwater mussels are bridge construction, bridge removal, cofferdam removal, and culvert replacement.
- The factors that are the greatest challenge to DOTs' efforts to reduce sedimentation impacts to freshwater mussels during construction are agency understaffing, coordination with other agencies, and cost.
- 39 percent of responding DOTs indicated in the survey that they have performed post-construction monitoring to assess construction impacts to freshwater mussels. An example practice for post-construction monitoring of mussel relocation is to conduct a monitoring survey annually for five years after the end of construction activity.
- The most frequently used BMPs for projects impacting freshwater mussels are silt fence, seed and mulch, and limiting vegetation removal. There is significant interest among DOTs in BMPs established for bridge or culvert sites where protected species or dense mussel populations exist, such as smart silt fence, no equipment below OHWM, turbidity curtains, and coconut and fiber logs.
- Approximately two thirds of responding DOTs have access to a database of mussel beds either through an internal or external database.
- The most frequently developed DOT resources for minimizing sedimentation impacts to freshwater mussels are BMP guidelines, survey protocols, and special provisions.
- DOTs most frequently collaborate with other state agencies and US Fish and Wildlife Service to evaluate and minimize sedimentation impacts to freshwater mussels.

2.2 Current Status of Sedimentation on Freshwater Mussels

Freshwater mussels of the family Unionidae serve as "ecosystem engineers" modifying the habitat and making it more suitable for other organisms [21, 33, 34]. Freshwater mussels perform many important functions in aquatic ecosystems, such as filtering algae, bacteria, and other particles from water, recycling nutrients and energy, and serving as food for fish, small mammals, and some birds [6, 35]. There are approximately 300 species of freshwater mussel in North America [36], and about 69 species in Missouri, the United States [37]. However, freshwater mussels are one of the most imperiled faunal groups in the world [6, 7, 38, 39]. In Missouri alone, 29 species are classified as species of conservation concern, including 15 that are considered to be in danger of extirpation in Missouri or extinction throughout their range [37]. The rapid decline of freshwater mussel populations has been observed not only in North America but all over the world [38, 40-42]. Many factors are suggested to contribute to mussel decline, including toxic contamination, climate change, invasive species, and habitat alteration, including increased levels of suspended solids and turbidity [7, 38, 43-45].

Freshwater mussels have a complex life cycle that includes fertilization by spermcasting, embryos brooded in marsupial gills, parasitic larvae that attach to fish, and a free-living juvenile stage that typically occupies interstitial spaces in river sediment [4, 5]. In some cases, suspended solids may adversely affect fertilization and brooding of some species [46, 47]. Most freshwater mussel species require years to reach maturity[48]. Both juvenile and adult mussels are suspension feeders, and feeding can be inhibited by suspended solids [49, 50]. Because of their small size and relatively rapid metabolism and growth, juvenile mussels are often more sensitive than adults to stressors in laboratory experiments [5]. Juvenile mussels living in interstitial spaces are sensitive to infilling of those spaces with fine sediments. For example, Österling *et al.* associated turbidity and sedimentation with recruitment failure in 14 of 24 populations of freshwater pearl mussel (*Margaritifera margaritifera*) that they investigated [51]. Absence of juvenile mussels from a lack of recruitment is considered to be a characteristic feature of many declining mussel populations, which may eventually cause population collapse [6, 52]. Thus, it is of great importance to investigate how elevated suspended solids may affect juvenile mussels.

Sediment is an essential component of aquatic ecosystems [53], and the changes of sediment composition and quantity directly influence the aquatic life on various scales. The balance of sediment transport and deposition have strong impacts on aquatic biota, [54] and excessive sediment loads is regarded as a global concern which negatively affect the aquatic ecosystems by affecting channel formation and stream productivity [55, 56]. Sediment can be introduced to the rivers and streams via various natural processes (such as bank erosion [57-59] and precipitation runoff [60]) and human activities (such as agriculture [61-63], mining [61, 64], dam removal [65, 66], and constructions [12, 13]). Excessive sediment loads can result in increased suspended solids and deposition of sediment in the rivers and streams and subsequently leading to habitat degradation [67-69].

Particularly, although suspended solids exist under natural conditions, the increased suspended solids can alter the physical (such as reduce penetration of light and change temperature), chemical (such as release of contaminants and nutrients), and biological properties (act as stressor directly affecting the aquatic biota, including primary producers, macroinvertebrates and fish) of the waterbody [70, 71]. Deposition of sediment may change the properties of the substrate, reduce habitat space, clog the interstitial space within the sediment [67, 69, 72]. It has been pointed out that both the increased suspended solids and sedimentation are possible factors contributing to the decline of freshwater mussels [46, 51, 73].

2.2.1 Impacts of High Suspended Solid on Freshwater Mussels

Although effects of high suspended solids on freshwater mussels are still not well understood, there have been some studies available focusing on various aspects, including reproduction, growth, and clearance rates, where controversial results are sometime found.

Several studies observed that increased suspended solids might negatively affect the reproduction of freshwater mussels. In 2013, Landis *et al.* linked the reproductive failure of Pondmussel (*Ligumia subrostrate*) females to the elevated suspended solids [46]. At lowest TSS concentration (~8 mg/L), 88% of females were gravid. However, the percent of gravid females were found to quickly decline with increased TSS levels, where complete reproductive failure observed at TSS > 20 mg/L. Interestingly, they found that the fertilization was an all-or-nothing phenomenon, where, for those gravid females, (a) 98-99% of eggs were fertilized regardless of TSS levels and (b) total fecundity was not related to TSS levels. Interestingly, sperm production was not correlated with TSS concentrations, and mature sperm cells account for > 90% of all cells in each gamete extract for 97% of males. Accordingly, they proposed two possible mechanisms explaining the reproductive failure due to TSS interference: (a) reduced clearance rate (> ~8 mg/L TSS) might lower the chance of females encountering suspended sperm during filter feeding, or (b) increased pseudofeces production at higher TSS levels might bind sperm in mucus causing its egestion before fertilization.

In another study, Landis *et al.* assessed the stage-specific disruption of reproduction due to high TSS levels in two mussels species, including a short-term brooding mussel species (Ebonyshell, *Reginaia ebenus*) and a long-term brooding species (Pondmussel, *Ligumia subrostrata*) [47]. Although reduced reproductive success was observed in both species at high TSS levels, the effects were different. More female *Reginaia ebenus* (33-93%) were found fertilized across the TSS gradient (11-92 mg/L) tested, but few glochidia developed when TSS was higher than 20 mg/L. In the case of female *Ligumia subrostrate*, similar to the 2013 study, much less female (0-28%) were fertilized at higher TSS levels, but all those gravid successfully produced fully developed glochidia. As they pointed out earlier, Landis *et al.* suggested that the declined reproduction was due to the physical interference with sperm capture at high TSS level. Especially, the observed differences of species might be explained by the differences in gill structure (the density of cilia on the gills) and gill function related to habitat use and/or brooding strategies. Species with low cilia density (usually lentic species), like *Ligumia subrostrata*, might not be able to simultaneously use their gills and capture sperm at high TSS concentrations. Another mechanism may work for *Reginaia ebenus*, a short-term brooder with a high density of gill cilia (usually lotic species). Long-term brooders like *Ligumia subrostrate* use the posterior portion of the two outer gills to brood glochidia for around 6-8 months brooding period. On the contrary, short-term brooders like *Reginaia ebenus* use all their four gills to hold developing eggs and glochidia for only 2-6 weeks. Thus, it is more likely that high TSS could cause respiratory stress for short-term brooders, particularly, during brooding when a female attempts to meet the respiratory demands for herself as well as her brood leading to subsequent declines in condition for both [74].

Many previous studies focused the effects of high suspended solids on the growth of freshwater mussels from different aspects including feeding, respiration, and metabolism. As filter feeder, high suspended solids in the environment may affect mussels' growth by decreasing the feeding and respiration, and in return affect their energy metabolism [75]. Specifically, increased suspended solids may result in declined clearance rates (CR) to prevent clogging of gill filaments. For example, Tuttle-Raycraft *et al.* evaluated the changes of CR of four species (adults and newly transformed juveniles) including *Lampsilis fasciola*, *Lampsilis siliquoidea*, *Ligumia nasuta*, and *Villosa iris* under different TSS levels [49]. The highest TSS concentration was set at 15 mg/L for juvenile mussels and at 100 mg/L for adults. One-week-old juveniles showed an increased CR with increased TSS levels; however, older juveniles (two to four weeks old) presented a declining CR trend with elevated TSS concentrations. In the case of adults, significantly reduced CR was observed at TSS ≥ 8 mg/L. Moreover, differences among species were noticed where *Lampsilis fasciola* showed the most remarkable drop of CR (46% vs no-TSS control) while CR of *Villosa iris* only decreased by 21%. Tuttle-Raycraft *et al.* suggested that the observed differences among species may be related to the differences in the TSS levels and substrates of their source rivers as the mussels collected from rivers with lower TSS showed more notable CR decline. Another important finding was that juveniles might be more vulnerable compared to adults as their decrease in feeding was more significant than the adults.

In another study that used much higher suspended solids levels (up to 8000 mg/L of bentonite clay), Tokumon *et al.* examined the impacts of suspended inorganic solids on filtration rate (FR) and grazing rate (GR) of an invasive species *Limnoperna fortune* (Golden mussel, adults with shell length 15-20 mm) [76]. Both FR and GR were strongly affected by inorganic sediment loads. Interestingly, they discovered that maximum FR occurred at the lowest concentrations (100 mg/L and 0 mg/L), while it dropped by 50% at 1000 mg/L and became negligible at concentrations ≥ 4000 mg/L. A similar trend was noticed in the case of GR, and GR (highest at 0 mg/L) gradually decrease with increase of concentrations. Thus, they suggested that high inorganic suspended solids concentration (> 1000 mg/L) would inhibit the feeding of Golden mussel, which would subsequently affect their growth and even survivorship in the long term. Although studies like Tuttle-Raycraft and Tokumon indicated that freshwater mussels would be affected in feeding and subsequently in growth by reduced CR, there have been other studies pointing out that reduced CR might not necessarily result in automatic reduction in feeding and growth. For example, in studies by Landis, increased TSS levels were not related to the growth of *Ligumia subrostrate* and *Reginaia ebenus* [46, 47].

Thus, how high suspended solids concentrations affect freshwater mussels may be quite complex, and changes of CR, FR or GR may not fully explain the observed effects on growth. Still, research continues regarding the effects of increased suspended solids on the respiration and metabolism of freshwater mussels. In 1987, Aldridge *et al.* evaluated the oxygen uptake of three species including *Cyclonaias pustulosa* (Pimpleback), *Fusconaia cerina* (Gulf Pigtoe) and *Pleurobema beadleanum* (Mississippi Pigtoe) under intermittent exposure to suspended solids for 9 days [77]. Specifically, mussels were exposed to suspended inorganic solids under two different conditions: (a) infrequent exposure with an average ~750 mg/L for 7 minutes every 3 hours and (b) frequent exposure with an average ~600 mg/L every 0.5 h. For mussels infrequently exposed to suspended inorganic solids, reduced CR was observed and 2 of the 3 tested species (except Gulf Pigtoe) had reduced oxygen uptake and nitrogen excretion rates. However, although the metabolic rate was reduced, they did not shift from the mainly protein-based catabolism of controls. On the other hand, mussels frequently exposed were found to have reduced CR, oxygen update, and nitrogen excretion rates. More importantly, they shifted their metabolism to non-protein body stores indicating that those mussels were more seriously affected compared to those that were infrequently exposed. Accordingly, they stated that the changes in catabolism were a possible result of starvation as mussels that were unable to feed shifted to stored carbohydrates (such as glycogen) and lipids which would be typically used in reproduction or overwintering. Thus, the growth and reproduction could be affected and may result in long-term negative consequences to population persistence. In 1998, Madon *et al.* thoroughly investigated the impacts of elevated inorganic suspended sediment (up to 100 mg/L) and food concentrations (up to 2.0 mg/L particulate organic matter) on energetic processes of Zebra mussel (*Dreissena polymorpha*). They found that inorganic sediments could negatively affect Zebra mussels' energetic processes and thus concluded that zebra mussels in turbid rivers may have low growth potential [78].

So far, available data on impacts of suspended solids on freshwater mussels are still limited, reported mortality caused by increased suspended solids are generally very low, and conflicting results about mussel growth are frequently seen. Differences among studies may be due to a variety of reasons, including different species and ages, different suspended solids sources and properties, different suspended solids concentrations, and different experiment methods. More importantly, studies focusing on juvenile mussels, the more sensitive life stage compared to adults, were rare, and such data gaps should be filled to better understand responses of mussels of different life stages towards elevated suspended solid levels.

2.2.2 Impacts of Sediment Deposition on Freshwater Mussels

As early as 1898, it was summarized by Kunz that "covering with mud" resulted in destruction of mussel shells, making it a possible cause of mussel declines [79]. Some surveys also linked mussel decline with deposited sediments. For example, some studies focused on the influence of dams and dam removal on freshwater mussels. It has been well known that dams have a wide range of adverse effects on freshwater mussels in many different ways, such as altering the natural cycle of flow, changing sediment cycles, limiting host fish distribution, and isolating small mussel populations between dammed water river segment [65, 80, 81]. However, on the other hand, dam removal may also negatively impact freshwater mussels, for example, by releasing large amounts of sediment stored in the impoundment to the downstream, and it is possible that the persistent siltation may cause mussel death [81-83]. Sethi *et al.* worked on the impacts of a small dam removal on freshwater mussels. Specifically, they conducted mussel surveys at three sites near the dam. They reported that dead mussels, buried 10-20 cm in deposited silt, were found at a site of 1.7 km below the dam [80]. It is possible that their failure to unbury themselves eventually resulted in their death.

In the case of lab work, very few studies have been conducted thus far. In the 1930s, Ellis investigated the effects of erosion silt on aquatic complexes, combing field work and in lab experimental work. It was pointed out that erosion silt entering the water could negatively affect the living organisms via two pathways: (a) by causing physical and chemical changes to the water and (b) by altering the bottom conditions due to subsequent settling out of silt load [84]. Particularly, the settling of silt could blanket the stream bottom with layers of silt, which may consequently smother out the existing fauna including freshwater mussels. Based on the experiments results, although different mussel species showed different resistance, a layer of silt from one-fourth of an inch to one inch (about 0.6-2.5 cm) on either sand or gravel bottoms could result in high mortality (≥ 90%) for all species once the silt layer covered the sand or gravel bottom permanently [84].

In 1972, Imlay examined the responses of four mussel species (*Pyganodon grandis*, *Ligumia recta*, *Fusconaia flava*, and *Flasmigona costata*) to smothering in aerated jars by burying the mussels (all adults) with different materials (detritus, river sand, lake sand, sand/clay mixture, silt and grit) to various depths [85]. For each species, mussels showed different reemergence rates from different burial materials. Meanwhile, for the same burial material, different species also presented different reemergence rates. Take Giant Floater (*Pyganodon grandis*) as an example, (a) 100% of Giant Floater mussels resurfaced from 7.62 cm detritus in 5 days, while only 25% of them reemerged from 11.43 cm sand/clay mixture within 6 days; and (b) none of them recovered from 19.05 cm of river sand within 7 days but 88.9% of them successfully resurfaced from lake sand with the same depth within 4 days. Compared to Giant Floater mussels, the other three species performed worse at unburying themselves from various materials. For example, under 7.62 cm detritus, only 37.5% of Black Sandshell mussels (*Ligumia recta*) and 12.5% of Wabash Pigtoe mussels (*Fusconaia flava*) reemerged within 4 and 7 days, respectively.

In 1977, Marking and Bills observed the reemergence rates of adult Wabash Pigtoe (*Fusconaia flava*), Fatmucket (*Lampsilis siliquoidea*), and Plain Pocketbook (*Lampsilis cardium*) mussels from sand (all three species tested) and silt (Wabash Pigtoe not tested) burial layers of different depths (0, 5, 10, 15, 20 and 25 cm) for 96 h [86]. For mussels positioned upright, for all three species, the reemergence rates decline with increased depths. Mussels easily resurfaced from 5 cm depths (90-100%) while drastically declined numbers of mussels reemerged from 25 cm depth (0-10%, except Fatmucket with 70%), and for mussels successfully resurfaced, they usually made it quickly within first several hours. Differences among species were noted, and Wabash Pigtoe was the most vulnerable one when buried by silt. By sitting Plain Pocketbook mussels horizontally rather than uprightly, they found that only 30% of them could resurface from 15 cm silt within 96 h, while the reemergence rate was 90% when mussels were naturally positioned (upright), indicating the importance of mussel positioning when an acute burial event occurs. Moreover, by extending the burial duration to 14 days, they pointed out that only those that reemerged could survive as those remained buried were found dead after extended burial duration. In their opinion, the observed differences in escaping from sand or silt among mussel species may be due to the variations in their physical characteristics. Thus, Plain Pocketbook with a large body size and broad, strong foot may benefit from these traits when it comes to their movement in sand or silt layers, while Pigtoe, with a smaller size, were less capable of escaping from the burial layers.

In a more recent study, Rumbelow studied the effects of several environmental stress factors on Texas Pigtoe (*Fusconaia askewi*) including sedimentation. Texas Pigtoe buried by 50 cm sand showed notably increased mortality (35%) compared to those buried by 25 cm (15%) after 96 h [87]. It was stated that in the preliminary trial, only 1 out of 20 Texas Pigtoe mussels were found alive after 10 days, indicating that with increased buried time, higher mortality might occur. More importantly, none of the mussels was able to unbury themselves on their own in the tests, and no vertical or horizontal movement of Texas Pigtoe was observed under 50 cm of sand, while a mean vertical movement around 10.5 ± 0.5 cm was observed among 15% of mussels buried by 20 cm sands. Interestingly, for those that migrated vertically, no specific direction was observed.

In brief, the current understanding of the impacts of sediment deposition on freshwater mussel decline remain poor. Nonetheless, studies working on marine mussels may provide useful information to better understand the observed responses of freshwater mussels upon sediment deposition.

2.2.3 Impacts of Sediment Deposition on Marine Mussels

In 1979, Jackson et al. buried three different sizes of *Cerastoderma* with three depths (0, 5 or 10 cm) of sand, finding that 100% survival was achieved within 72 h tests, and most *Cerastoderma* returned to the surface within one or two days at 5 cm depth but few resurfaced when buried at 10 cm deep [88]. In 1981, Maurer *et al.* tested the vertical migration and mortality of three mollusks, including two bivalve (*Mercenaria mercenaria* and *Nucula Proxima*) and one gastropod (*Ilyanassa obsoleta*), when buried by dredged materials [89]. By burying *Mercenaria mercenaria* with different depths of sand (1 to 16 cm) at 22 to 25 ℃ for 2 h and 24 h, they found that the vertical migration distance of *Mercenaria mercenaria* increased with the increased burial depths. Although the mean vertical migration distance was only 3.6-4.2 cm at the highest burial depths (14-16 cm), some of them successfully resurfaced within a short period of time. In another series of tests, remarkably increased mortality of *Mercenaria mercenaria* was recorded with increased burial sand depths (up to 85 cm) and extended burial duration (up to 18 days) at 19 to 22 ℃. Moreover, they also buried *Mercenaria mercenaria* with different substrates (mix of sand and silt-clay at various ratios) up to 32 cm at two different temperatures (winter *vs* summer) for different lengths of time (up to 15 days). Results showed the percent migration of *Mercenaria mercenaria* increased with time at both temperatures. However, compared to the summer temperature, both the percent of migration and the percent of *Mercenaria mercenaria* that reached upper layers were lower under winter conditions. *Nucula proxima* were also tested under summer conditions with a silt-clay (51-56%) mixture and sand mixture with depths from 4 to 32 cm and a duration of up to 8 days. Significant vertical migration of *Nucula proxima* were observed for all depths. Overall increased mortalities were noticed with increased depths and duration, and interestingly only those buried shallowly (8 and 16 cm) were able to reach the top layer. Subsequently, they concluded that the increased mortalities of the tested species were associated with increased burial depth, time and amounts of exotic sediments as well as summer temperatures [89].

In a similar study, Maurer *et al.* examined the vertical migration and mortality of several species, including *Mercenria mercenaira*, of marine benthos in dredged materials. Similar results were reported, with sediment depth and type, burial time, and temperature affecting the vertical migration and mortality of the tested species [90]. More importantly, they pointed out that the water chemistry and sediment pore water chemistry changed significantly within a 15-day period, with decreased dissolved oxygen (DO) and increased ammonia and sulfide, between the surface and below 2 cm of the burial layer, detected. To further explore the possible causes of marine benthic migration and mortality buried by dredged material, Maurer *et al*. analyzed the changes of overlying water and sediment pore water chemistry [91]. Particularly, they found that compared to the overlying water, several water quality parameters of the sediment pore water sampled at different depths changed significantly, with declined dissolved oxygen, increase dissolved ammonia, and increased dissolved sulfide. Declined dissolved oxygen might be due to organic decomposition via oxic respiration, while increased ammonia and sulfide might originate from remineralization of organic matter by bacteria in the sediment via sulfate reduction [92]. Although no mussels or other benthos were buried during these tests, Maurer *et al.* suggested that during the burial events, in addition to the synergistic effects of sediment type, burial load (depth), burial duration, temperature, and overburden stress [93], changes of sediment geochemistry also should be considered.

Several studies have focused on the effects of harvesting-related substrate disturbance on marine bivalves. In 1995, Bellchambers *et al.* explored the mortality of remaining unharvested *Katelysia scalarina* due to substrate disturbance after applying different harvesting methods, including finger ploughing and digging implements such as spade or pitchfork. They reported that substrate disturbance due to digging implements resulted in much higher mortality (30-40%) of *Katelysia scalarina* than finger ploughing, and burial of the animals beneath the tailings might be the cause of high mortality [94]. Following this assumption, they accordingly buried *Katelysia scalarina* (adults and juveniles) in two field sites with sand to different depths from 0 cm to 30 cm. Although the two sites showed different mortality rates, it is clear that notable increased mortality was spotted with increased burial depths (> 10 cm) and adults (size 3 – 4.5 cm) were more vulnerable compared to juveniles (size < 3 cm) after three weeks [94].

In 2016, Hendrick and Last *et al.* studied responses of various marine species, including queen scallops (*Aequipecten opercularis*) to sudden burial, and also reported that increased mortality for all tested species resulted from increased burial duration (up to 32 days) and depth (2 cm, 5 cm, and 7 cm) with finer sediment fractions [95]. In the case of *Aequipecten opercularis*, only a small portion (25.9%) were able to emerge from 2 cm burial but none from 5 cm and 7 cm burial depths. All those that remained buried were found dead after 32 days. Meanwhile, mortality increased quickly with increased burial depths and days, and more mussel died when buried by fine sediments (0.1-0.25 mm) than coarse (1.0-2.0 mm) and medium (0.25-0.95 mm) sediment fractions. Thus, it was determined that the tolerance of *Aequipecten opercularis* to burial is weak.

The Hendrick and Last team also evaluated the responses of two marine mussels, *Modiolus modiolus* and *Mytilus edulis*, to different burial events, including three burial depths (2 cm, 5 cm, and 7 cm), three sediment fractions (fine, medium-fine and coarse), five burial durations (up to 32 days), and three temperatures (8℃, 14.5℃, and 20℃) [96]. The two species performed differently under different burial events. In the case of *Modiolus modiolus*, they were not able to resurface once covered, and the mortality rate went up with increased burial duration (increased from around 0% on Day 8 to 50% on Day 16) and sediment coarseness (0.1-0.25 mm), but not affected by the burial depth. In the case of *Mytilus edulis*, much smaller in size compared to *Modiolus modiolus*, a similar mortality trend was observed, with a higher mortality occurring with extended burial duration (increased from 4% on Day 2 to 44% on Day 32) and fine sediment fraction. Additionally, temperature affected the mortality of *Mytilus edulis*, with higher temperatures leading to increased mortality under the test conditions. However, unlike *Modiolus modiolus*, which failed to unbury themselves, *Mytilus edulis* showed the capability to emerge from burial, especially from shallow burials (29%) and coarse sediment (19%). It was suggested that the enhanced production of total byssus might facilitate their vertical migration and subsequently their emergence, which might result in the better tolerance of *Mytilus edulis* towards long term burial.

Using *Mytilus edulis*, Last and team further investigated the impacts of organic material and temperature on mussels' tolerance to burial [97]. They stated that (a) organically loaded fine sediments caused significantly increased mortality compared to control coarse sediments after 2 days at a burial depth of 5 cm, and (b) higher summer temperature (20℃) led to higher mortality compared to the ambient group (15℃). They suggested that the reactive organic matter in the burial medium may benefit the growth of bacteria and thus subsequently result in higher mortality of *Mytilus edulis* via pathogenic infection. Moreover, the higher temperature and the stable interstitial conditions in the fine sediments may worsen the scenario by enhancing bacterial metabolism.

In a more recent study focusing on New Zealand cockle, *Austrovenus stutchburyi*, Anderson *et al.* thoroughly evaluated the impacts caused by native sandy marine sediment deposition under different conditions, including different deposition depths (up to 25 cm), frequent deposition (2 cm daily for 5 days), cockle size (adult ≥ 20 mm *vs* subadult < 15 mm) as well as cockle orientation (natural *vs* disturbed) [98]. Tested cockles, adults and subadults, placed in a natural upright orientation, quickly (> 70% within one day) unburied themselves from up to 10 cm of sediment. However, for those placed in a disturbed (inverted) orientation, their speed to resurface was significantly slower than those in an upright orientation, where the resurfacing rated changed with mussel sizes and burial depths. When the burial depth was increased to 10 cm, subadults performed better at resurfacing, with almost all of them reaching the surface after 3 days. On the contrary, 62% of adults failed to unbury themselves within 7 days with 75% of them still in their original inverted positions. Natural upright oriented cockles were further buried to a depth of 25 cm for 7 days, and >50% of them resurfaced within 2 days and >70% resurfaced within 1 week, with no clear differences found between adults and subadults. For those that remained buried, most were found at depths between 10 to 18 cm with only three cockles (including one dead) staying in their original places. In the case of the cockles repeatedly reburied by 2 cm of sediment for 5 days, although fewer cockles (upright or inverted) resurfaced in the later days, no notable difference was observed compared to the single 10 cm deposition conditions. By comparing with other studies, they concluded that the direct impacts on *Austrovenus stutchburyi* buried by sediment with similar grain sizes to their native habitat would be limited but increased mortality would happen with increased depths of the sediment. Moreover, they highlighted that some mortality would be predicated when the cockles are physically disturbed with changed orientation.

In summation, effects of sediment deposition on freshwater mussels may be remarkably different due to different species, different sediment types (such as source and particle size distribution), variation in burial depths, and experimental setting (such as water circulation, container, temperature). As very limited data is available, it is challenging to conclude the underlying mechanisms leading to the adverse effects on freshwater mussels. Therefore, it is critical to devote more efforts to investigate (a) responses of more mussel species, (b) sediment deposition under different conditions and (c) possible mechanisms to achieve better understanding towards how sediment deposition would impact survival of freshwater mussels.

Chapter 3. Impacts of High Suspended Solid Concentration on Freshwater Mussels

3.1 Study Methodologies

3.1.1 Test Organisms

The three mussel species tested in this study were Fatmucket, Arkansas Brokenray, and Washboard. Specifically, Fatmucket and Arkansas Brokenray were propagated at U.S. Geological Survey Columbia Environmental Research Center (CERC), while Washboard was provided by Genoa National Fish Hatchery (Megan Bradley, U.S. Fish and Wildlife Service). For Fatmucket and Arkansas Brokenray, newly metamorphosed juveniles were collected during the 2-day peak of the drop-off period from the host fish, and juveniles were quickly and carefully transferred to a flow-through autofeeding system at 25 °C with diluted CERC well water with a hardness 100 mg/L as $CaCO_3$ (named as 100 hardness water) [41]. An algal mixture of *Nannochloropsis* concentrate (Nanno 3600, algal size ~1-2 µm, Reed Mariculture, Campbell, CA) and Shellfish Diet (Shellfish Diet 1800, a unique mix of four microalgae, *Isochrysis lutea*, *Pavlova sp*., *Tetraselmis sp*., and *Thalassiosira weissflogii*, ~4-20 µm, Reed Mariculture, Campbell, CA USA) were used to feed juveniles with a constant algal density of 5 -10 nl cell volume/ml in the culture water. Ambient laboratory light of 500 lx with 16:8 h light:dark photoperiod was applied through the mussel culture and exposure test periods. For Washboard, newly metamorphosed juveniles (< 5 days old) were collected during the 2-day peak of the drop-off period and shipped overnight to CERC. Once received, the Washboard juveniles were cultured with the same method described for the other two species. For the following acute exposure tests, ~2-month-old juveniles of all three species (Fatmucket: 1.5 - 3 mm; Arkansas Brokenray: 1 - 2 mm; Washboard < 1mm), ~ 1-week-old Fatmucket (< 500 µm) and Arkansas Brokenray (< 500 µm), and ~2-week-old Washboard (~500 µm) were used. For the 28-day (28-d) chronic experiment, ~2-month-old Fatmucket (1.5 - 3.5 mm) were examined. For the Fatmucket tests, juveniles of ~1-week-old for the acute test and ~2-month-old for the chronic test were used and were from one cohort. However, the juveniles (~2-month-old) used for the acute test were from another cohort. For the other two species, the tested juveniles originated from the same cohort.

3.1.2 Sample Collection, Characterization, and Suspension Preparation

Three local sediment/soil samples were selected for the suspension exposure tests, including Spring River, MO, sediment (SRS), (approximately at 37.11867, -94.2586, collected in September 2018), Osage River, MO, clay soil (ORC) from the bank (approximately at 37.11194, -94.2233, collected in November 2021), and fine limestone particles from Columbia, MO, (LMT) (approximately at 39.00692, -92.2452, collected in September 2021). Spring River sediment was chosen as it has been commonly used as control sediment in toxicity testing with benthic macroinvertebrates including freshwater mussels [99-102]. Here, the SRS was used to represent turbid conditions caused by disturbance of river sediment due to various conditions, such as construction activities and flood events. The ORC was selected to represent soils that may enter the water column because of terrestrial erosion during the construction events. LMT was tested because crushed limestones are frequently applied as a construction material including road base and cofferdam construction, and thus may be delivered to the riverbank and/or enter the water during construction [103-105].

The collected samples were quickly sealed in 20-L buckets and stored at 4 °C. Before further use, samples were passed through a 2-mm sieve, and the sieved samples were named as bulk samples. To determine the potential chemical contaminants of the bulk samples, analysis including metals (n = 26), polycyclic aromatic hydrocarbons (PAHs, n = 18), and *n*-alkanes (C9-C40) were carried out (details in **Appendix C, Table C1-4**). The results showed that background levels of metals, and relatively low levels of PAHs and *n*-alkanes were detected in the bulk samples. Total organic carbon (TOC), total inorganic carbon (TIC), total carbon (TC), and total nitrogen (TN) of the bulk samples were also analyzed (**Figure 3-1 (a)**). Moreover, particle size distribution (PSD) expressed as percent of dry weight were also measured (details in **Appendix C and Figure C-1**).

Before preparing test suspensions of different TSS levels, stock suspensions were first prepared by mixing the 2-mm sieved bulk sample with 100 hardness water using an agitator. Detailed stock suspension preparation methods are described in **Appendix C** and **Table C-5**. TSS levels of stock suspension were determined following the Method 2540 D (2015). Properties of the stock suspension including TOC, TIC, TC, TN, and PSD were examined (**Figure 3-1 (b)**). In addition to PSD, particle shape can also influence the response of aquatic biota to suspended solids [70]. Here, a scanning electron microscope (SEM, Phenom ProX, Thermo Fisher Scientific, Waltham, MA) took results of all three stock suspensions and illustrated that the particle shapes were determined as irregular (see **Figure C-2**). Results showed that the TOC and TN of stock suspensions were similar to the bulk samples, while their PSD patterns were significantly different. Coarser particles were removed after the sedimentation process, leading to largely increased percentages of clay- and silt-sized (2-50 μm) particles. Specifically, particles smaller than 50 μm accounted for 92.7% to 100% of all three stock suspensions. Both SRS and ORC were dominated by clay- and fine silt-sized particles, and the most abundant fraction of LMT stock suspension was determined as silt-sized (fine and coarse) particles.

Test suspensions of different TSS levels (0, 250, 500, 1000, 2500, and 5000 mg/L) were prepared by diluting the stock suspensions using 100 hardness water. The detected actual TSS levels of test suspensions were regularly measured (Acute experiments: measured on Day 0 and Day 4; Chronic experiment: measured on days suspensions were made and changed, see **Table C6-8**) throughout the study to observe the changes of TSS levels. Once stock and test suspensions were made, they were generally used within 5 days and stored in the cold room at 4 °C, if not immediately used. All suspensions were stirred at 1000 - 1200 rpm for 3-10 min prior to use.

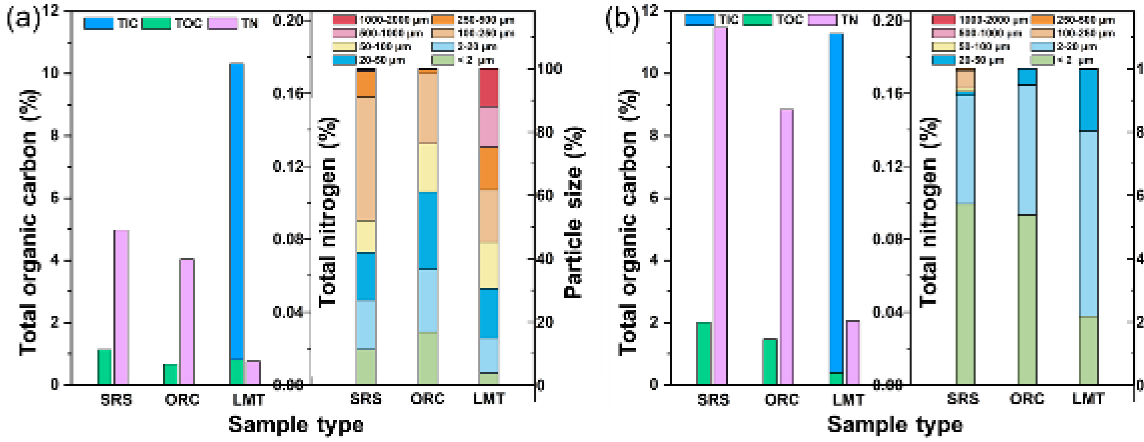


Figure 3-1. Percentage (dry weight) of total organic carbon (TOC), total inorganic carbon (TIC), total nitrogen (TN), and particle size distribution (PSD) of (a) the bulk samples and (b) the suspension stocks (b). SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone. Classification of particles: very coarse sand (1000-2000 μm), coarse sand (500-1000 μm) and, medium sand (250-500 μm), find sand (100-250 μm), very fine sand (50-100 μm), coarse silt (20-50 μm), fine silt (2-20 μm), and clay (< 2 μm).

3.1.3 Acute and Chronic Suspended Solids Exposure Tests

Acute 96 h and chronic 28 d exposure tests were carried out referring to American Society for Testing and Materials (ASTM) standard guide for conducting laboratory toxicity tests with freshwater mussels [106]. An 'exposure unit' was specifically designed for the tests. The exposure unit consisted of a 600 mL beaker, in which a screen-bottom inner chamber was suspended in a glass frame (**Figure 3-2**). When used, the juvenile mussels rested on the screen and a magnetic stir bar underneath the inner chamber circulated the suspension or 100 hardness water as control (~500 mL) and created a downwelling flow.

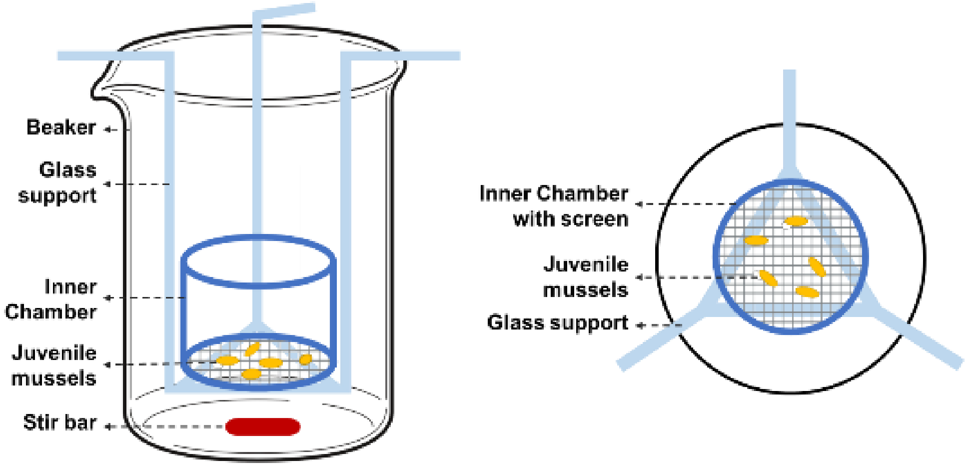


Figure 3-2. Front view and top view of the recirculating exposure unit with juvenile mussels. Image is not to scale.

All 96-h acute exposure tests were carried out at 22 ± 2 °C without feeding from October 2021 to April 2022. For 2-month-old Fatmucket and Arkansas Brokenray, six TSS concentrations (0, 250, 500, 1000, 2500, and 5000 mg/L) were tested, while for 2-month-old Washboard only three concentrations (0, 1000, and 5000 mg/L) were tested due to limited juvenile numbers. For 1-week-old Fatmucket, Arkansas Brokenray and 2-week-old Washboard, four TSS concentrations (0, 250, 1000, and 5000 mg/L) were examined as a result of limited juvenile numbers. Typically, each tested condition was done with four replicates. Before the tests, juvenile mussels were first examined for foot movement and then were impartially assigned to replicates. Exposure started once juvenile mussels were placed into each exposure unit and the stirrer was turned on. The survival rate was checked twice during the exposure test at 48 h when suspension was refreshed and at 96 h when exposure test ended, respectively. To determine the survival rate, juvenile mussels were carefully observed under a dissecting microscope and those with an empty shell or with a gaped shell containing swollen or decomposed tissue were classified as dead.

Approximately 2-month-old Fatmucket juvenile mussels were tested for the 28-d chronic exposure study. Before the test, juvenile mussels were sieved from the culture substrate, rinsed into glass dishes, and carefully observed under a dissecting microscope. Ten juvenile mussels with observed active foot movement were transferred into each replicate exposure unit. To record the initial length and dry weight, another 40 juvenile mussels were sampled and preserved in 70% ethanol. The chronic test began once all mussels were transferred to the exposure unit and the stirrers were turned on. Manual feeding was applied twice daily (in the morning and late afternoon) through the experiment with 3 mL of algal mixture [107]. The 28-d test was carried out in a room with temperature control at 21 ± 1 °C. Suspensions in each exposure unit were regularly replaced on each Monday, Wednesday, and Friday. On Day 28, juvenile mussels were observed microscopically, cleaned, and preserved in 70% ethanol for future growth analysis (more details in **Appendix C**).

3.1.4 Water Quality Characterization

For both acute exposure and chronic exposure tests, reconstituted water with 100 mg/L hardness as $CaCO_3$ was used as the control water as well as to prepare suspensions. The measured pH, conductivity, DO, alkalinity, and hardness of the 100 hardness water (expressed as mean with standard deviation) were 8.27 ± 0.11, 250.4 ± 5.47 µS/cm, 8.72 ± 0.20, 94.7 ± 4.79 mg/L as $CaCO_3$, 101.3 ± 4.27 mg/L as $CaCO_3$, respectively. Parameters including DO, pH, turbidity, hardness, alkalinity, and total ammonia nitrogen (Total NH_3) were regularly tested following standard methods to record changes of water quality due to addition of sediment/soil samples. For acute tests, water quality was analyzed before and after the tests, while water quality was measured when suspensions were replaced during the 28-d study. Typically, suspension samples were first centrifuged at 5000 rpm for 5 min, and then the upper supernatant water were analyzed. Results showed that the addition of the sediment/soil samples at high TSS levels slightly altered these water parameters (**Table C6-11 and Figure C3-4**): high concentrations of SRS and ORC led to slightly decreased pH, conductivity, alkalinity, and hardness, while high concentrations of LMT resulted in increased conductivity and hardness. Nevertheless, these changes were not expected to adversely affect juvenile mussels. As expected, suspension turbidity was positively correlated with TSS concentrations, and SRS and

ORC were positively correlated with larger proportions of very fine particles, and also presented higher turbidity levels compared to LMT.

3.1.5 Data Analysis

For chronic tests, dry weight was measured to evaluate effects of increased TSS levels on growth of juvenile mussels. Statistical differences in growth and percent changes were assessed by one-way analysis of variance (ANOVA, Originpro 2020b). A Tukey's *post-hoc* test (ANOVA, Originpro 2020b) was performed to determine impact of SRS, ORC and LMT on juvenile growth among different TSS levels of SRS, ORC and LMT, respectively. A Dunnett's *post-ho*c test (Graphpad Prism v.3.02, Graphpad Software) was applied to identify concentrations of sediment/soil samples with percent of dry weight changes significantly differed from the control groups. Significance was accepted at $p < 0.05$. For acute tests, lack of mortality prevented calculation of effect concentrations. For chronic test, EC20 (20% effective concentration) for dry weight was calculated for each soil/sediment sample. Here, the EC20s were calculated using different methods, including TRAP (the Toxicity Relationship Analysis Program, Ver 1.30a) and log-linear regression with and without Ctrl (**Table C-21**) [108].

3.2 Study Results

3.2.1 Acute 96-h Exposure

Acute 96-h exposure was designed to evaluate the capability of young juvenile mussels to endure short term disruption events. For 2-month-old juvenile mussels, the survival results were summarized in **Table C12-14**, where neither a dose-response nor lethal effect was noticed across various treatments regardless of mussel species and sediment/soil types. Briefly, the survival of Ctrls (controls) was 100%. For Fatmucket and Washboard, each species had one juvenile die in the treatment groups. In the case of 2-month-old Washboard juveniles, with remarkably smaller sizes compared to the other two species due to their lower growth rate, loss of mussels probably occurred due to the renewal of suspension or the washing process before final observation, even though inner chambers with smaller screen size were utilized to hold juveniles. After exposure, surviving juvenile mussels remained active with frequent foot movement (**Figure 3-3**), suggesting that these 2-month-old juvenile mussels could tolerate short-term exposure of up to 5000 mg/L of these three sediment/soil samples.

Juvenile mussels were not fed during the acute exposure tests, and the green color of their guts (See Figure C-6) faded because of the lack of algal food or diluted food because of existence of the non-food particles. Nonetheless, unfed juvenile mussels exposed to suspensions produced more feces and pseudofeces than Ctrls, revealing that juvenile mussels actively filtered their surrounding water even at TSS level up to 5000 mg/L. Meanwhile, the introduction of suspended solids also resulted in deposition of sediment particles on the mussel shells (in **Figure 3-3**). Juvenile mussels of Ctrls had clean shells while those exposed to suspended sediments/soils were at least partially covered by the deposited particles. It is challenging to assess whether such deposition on shells may negatively affect the juvenile mussels' activities, such as valve gaping behavior and mobility. However, considering their burrowing behavior and the foot movement observed immediately after they were transferred to the clear water, it is reasonable to deduce that such deposition on shells is not expected to cause adverse effects.

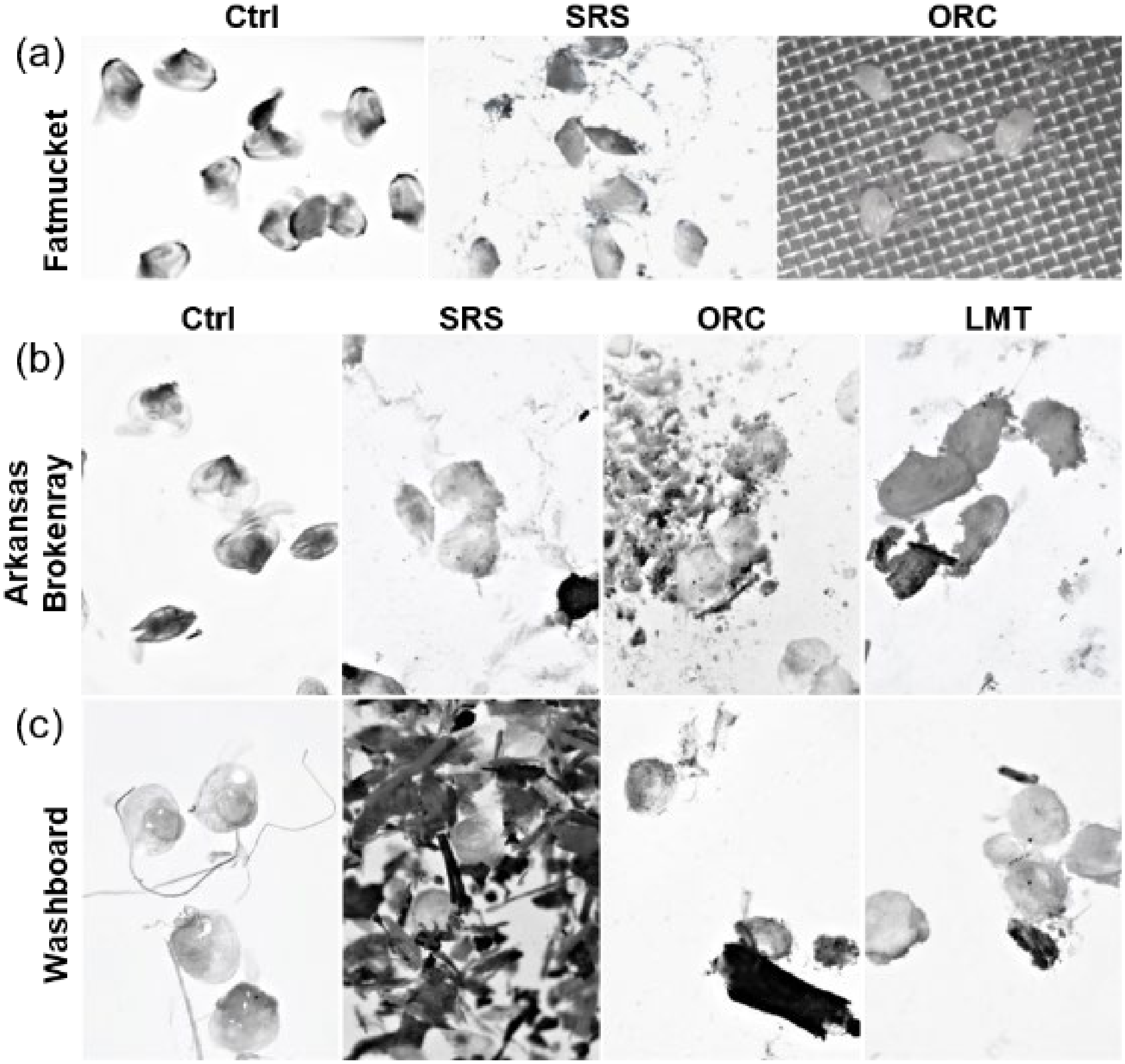


Figure 3-3. Images of 2-month-old mussels, including (a) Fatmucket (Lampsilis siliquoidea), (b) Arkansas Brokenray (Lampsilis reeveiana), and (c) Washboard (Megalonaias nervosa) (c), after 96-h exposure to 5000 mg/L of SRS, ORC and LMT. Images of the same species were taken under the same magnification. For each species, the images from left to right represented juvenile mussels exposed to Ctrl, SRS, LMT and ORC, respectively. The image of Fatmucket exposed to 96-h LMT was not available. Ctrl = Control water; SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

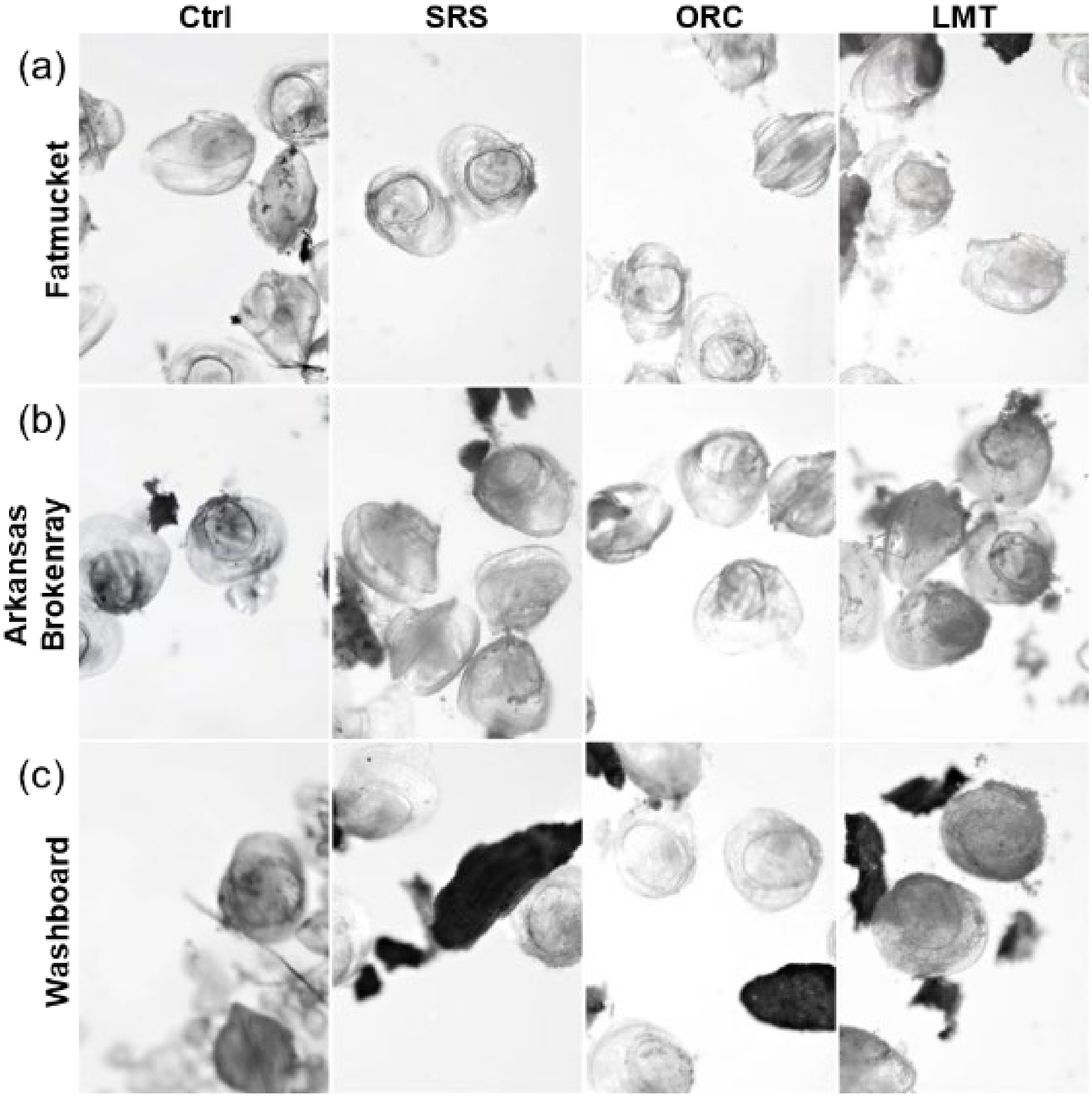


Figure 3-4. Images of (a) 1-week-old Fatmucket (Lampsilis siliquoidea), (b) 1-week-old Arkansas Brokenray (Lampsilis reeveiana), and (c) 2-week-old Washboard (Megalonaias nervosa) after 96-h exposure to 5000 mg/L SRS, ORC and LMT. Images of the same species were taken under the same magnification. For each species, the images from left to right represented juvenile mussels exposed to Ctrl, SRS, ORC and LMT, respectively. Ctrl = Control water; SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

Similarly, no lethal impact was observed among newly transformed 1- or 2-week-old juvenile mussels tested (**Table C15-17**). Because of the small sizes of these younger juvenile mussels, some were missed after exposure. Moreover, though one or two dead juvenile mussels could be occasionally observed, no correlation to TSS levels was noticed. Unexpectedly, in one replicate of 2-week-old Washboard exposed to 250 mg/L ORC, all four juvenile mussels found in the beaker were dead. This replicate was identified as an outlier as it only occurred in this beaker where floating red particles were witnessed after 96-h exposure (**Figure C-5**). When being observed after the first 48-h exposure period, these juvenile mussels were still alive without red particles spotted in the beaker, implying that such particles might have entered the beaker when the suspension was renewed. As ORC soil were sampled from the fields, it is possible that a small portion of the sample was contaminated, possibly by a manmade product which was accidentally discarded at that location. However, it is impossible to determine the composition or source of these contaminants due to the very limited number of red particles that were found after this test. Generally, young juvenile mussels of all three tested species were very active after the 96-h exposure (**Figure 3-4**) with production of feces and pseudofeces and pale guts. Therefore, like the older mussels, it was determined that they are also capable of tolerating short exposure to the three selected sediments/soils, of concentration up to 5000 mg/L. Nonetheless, the death observed in the beaker with red particles suggested that even though natural sediments/soils themselves may not pose notable negative impacts on the survival of juvenile mussels over the short term, at certain concentrations, contaminants associated with sediments/soils may affect the survival of the early-stage mussels [100, 109].

3.2.2 Chronic 28-d Exposure

Fine-grained particles such as clay and silt can remain suspended for days to weeks in surface waters, depending on local catchment features [110, 111]. Therefore, it is necessary to be cautious about relatively long-term impacts of suspended solids on mussels. In this study, approximately 2-month-old Fatmucket mussels were chosen for a chronic 28-d exposure test. An overall mussel recovery of 98.7% was recorded on Day 28 (**Table C-18**), with several mussels lost, presumably, during suspension changes. An experiment-wide survival rate of 99.6% (missing mussels were not included for calculation) was determined, with three dead juvenile mussels found in different treatments, suggesting that exposure to suspended solids up to 5000 mg/L of the three tested sediment/soil samples showed no clear effect on the survival of these juvenile mussels. Nonetheless, strong effects on juvenile growth were observed after 28-d exposure. Variations of shell length and dry weight of juvenile mussels among different treatments after 28-d exposure is illustrated in **Figure 3-5**. Significant differences in dry weight were observed among the mussels at different TSS levels of each sediment/soil sample by ANOVA (SRS: $F_{6,25} = 26.44$, $p < 0.0001$; ORC: $F_{6,25} = 17.45$, $p < 0.0001$; LMT: $F_{6,25} = 6.53$, $p = 0.0003$, **Table C-19**).

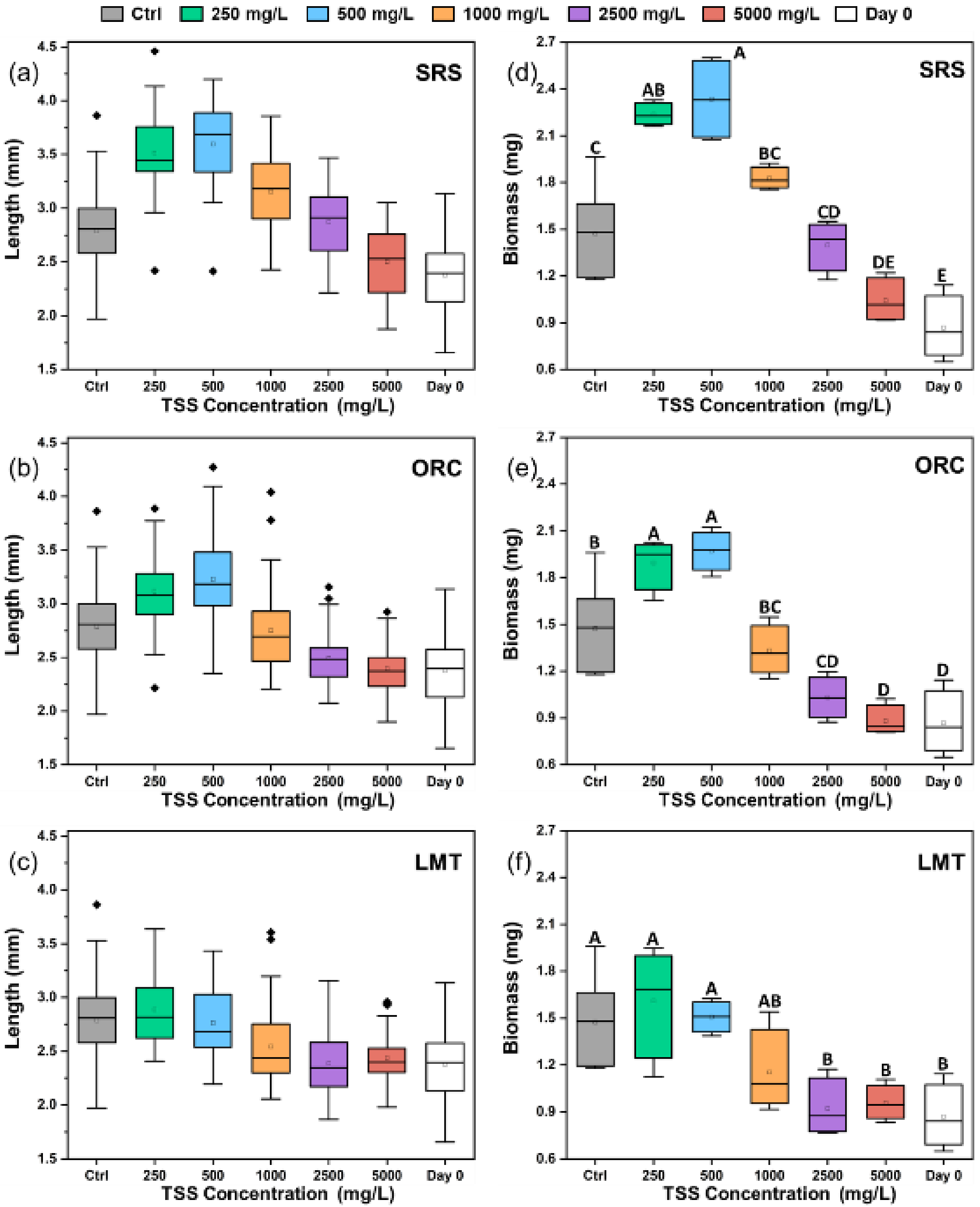


Figure 3-5. Box plots of shell length of Fatmucket (Lampsilis siliquoidea) juveniles after 28-d exposure to different concentrations of (a) SRS, (b) ORC, and (c) LMT; box plots of biomass after 28-d exposure to different concentrations of (d) SRS, (e) ORC and (f) LMT. The upper and lower portions of the bar are the upper and lower quartiles, the line in the middle of the box represents the median, the dots are outliers, and the lower and upper lines represent the lowest and greatest values excluding outliers. Letters above the bars represent level of significance among treatments (*p* < 0.05). Ctrl = Control water; Day 0 = juvenile mussels kept on Day 0; SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

Compared to those preserved on Day 0 (shell length: 2.38 ± 0.35 mm; dry weight: 0.87 ± 0.20 mg, see **Table C-18** and **Figure 3-5**), the shell length and dry wight of Ctrl after 28-d growth significantly increased, by ~17% and ~70% growing to 2.79 ± 0.19 mm and 1.45 ± 0.32 mg, respectively. **Figure 3-6** shows images of juvenile mussels after the chronic study. In this study Ctrl measures were used as the baseline to evaluate the relative growth changes of tested juvenile mussels, and the percent changes of length and dry weight are displayed in **Figure 3-7**. Overall, ANOVA revealed significant differences in percent change of dry weight ($F_{16,55} = 22.70$, $p < 0.0001$, **Table C-20**) among the Ctrl and the treatment groups. Interestingly, two different growth patterns were noticed. A significant boost to the growth of juvenile mussels was found at the lower range of TSS concentrations of SRS (up to 1000 mg/L, $p < 0.01$) and ORC (up to 500 mg/L, $p < 0.05$). For example, the shell length and dry weight after 28-d exposure to 500 mg/L SRS were up to 1.3 times and 1.6 times larger than Ctrl and were approximately 1.5 times and 2.7 times larger than those of Day 0 (**Figure 3-7**). A distinctive pattern was noted for LMT, where no increased growth was detected among juvenile mussels exposed to 250 and 500 mg/L of LMT after 28-d exposure with their final shell lengths and dry weights close to those of Ctrl. At 1000 mg/L LMT, juvenile mussels tended to grow slower, remaining the sizes and weights close to those of Day 0 after 28-d exposure. It is worth noting that for all three tested sediment/soil samples, an increase in TSS levels began to inhibit the growth of the juvenile mussels, and remarkably smaller shell lengths and less dry weight were recorded compared to those that grew in low TSS levels. Moreover, among the three tested sediment/soil samples, exposure to LMT suspensions had the strongest effect on the juvenile mussels.

In summation, although no clear impact on survival was found after the 28-d exposure for all three sediment/soil samples, high TSS concentrations clearly hinder the growth of Fatmucket juveniles, which may subsequently affect their survival in the long term. Therefore, it is still critical to estimate thresholds such as EC20s of these sediment/soil samples to facilitate making recommendations to support mussel conservation. Compared to Ctrl, significantly Fatmucket juvenile growth ($p < 0.05$) was observed for the lower two TSS concentrations (250 and 500 mg/L) of SRS and ORC, while these two lower LMT TSS levels resulted in growth close to the Ctrl. This made Ctrl (water-only treatment) not suitable for modelling response curves to generate effect concentrations or for comparing differences in the mean growth among different treatments. Instead, the 250 mg/L treatment for each sample was selected as reference data for comparison to higher TSS concentrations and for modelling EC20s using a variety of methods (Table C-21). In this study, EC20s, calculated by log-linear regression using the lowest test concentration (250 mg/L) as reference data, were used. The EC20s were 1227, 969, and 839 mg/L for SRS, ORC and LMT, respectively.

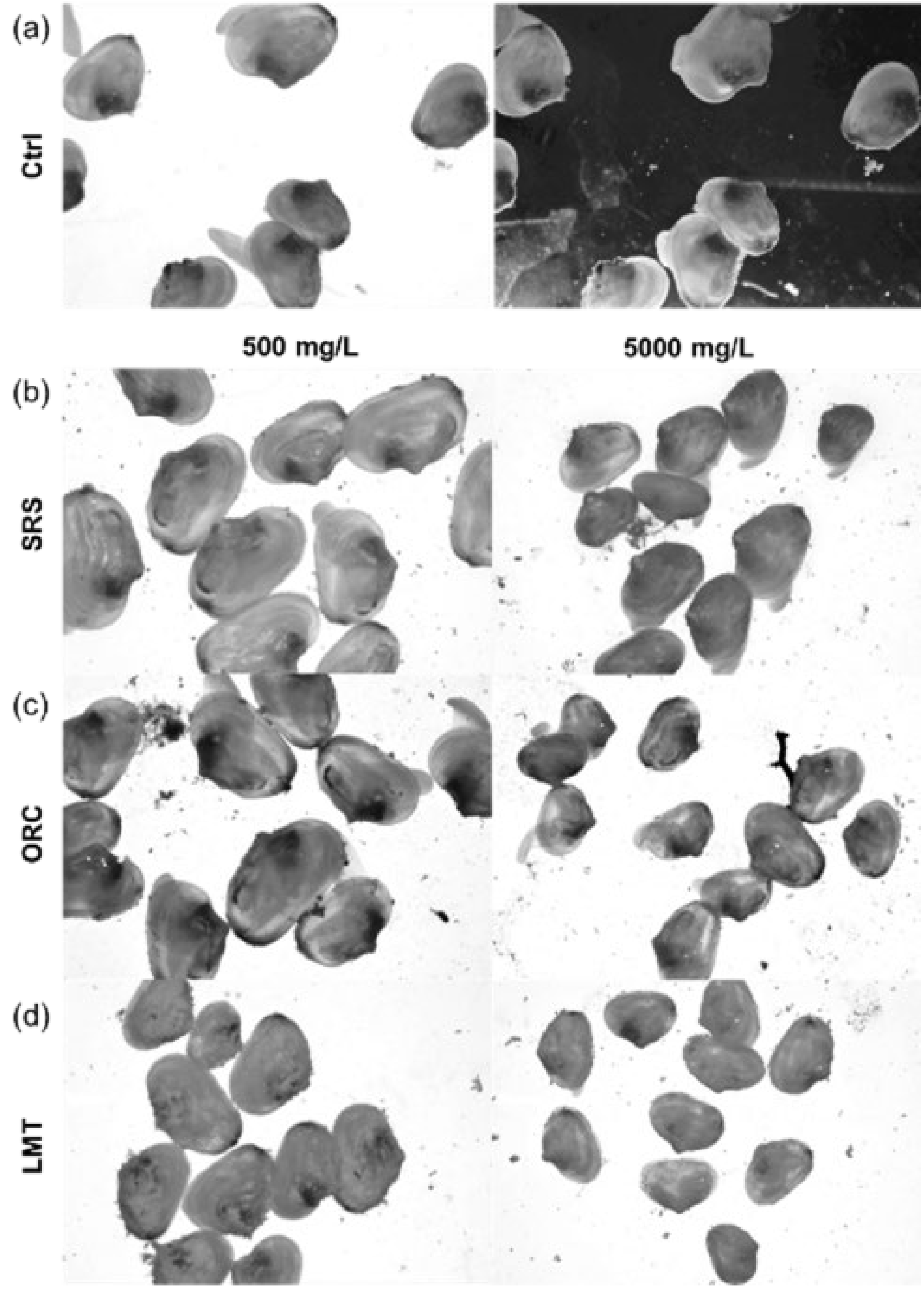


Figure 3-6. Images of Fatmucket (Lampsilis siliquoidea; starting age: 2-month-old) after 28-d exposure to (a) Ctrl (a), (b) SRS (b), (c) ORC, and (d) LMT at 500 mg/L (b), and 5000 mg/L (left to right). Juvenile mussels were rinsed before imaging. Ctrl = Control water; SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

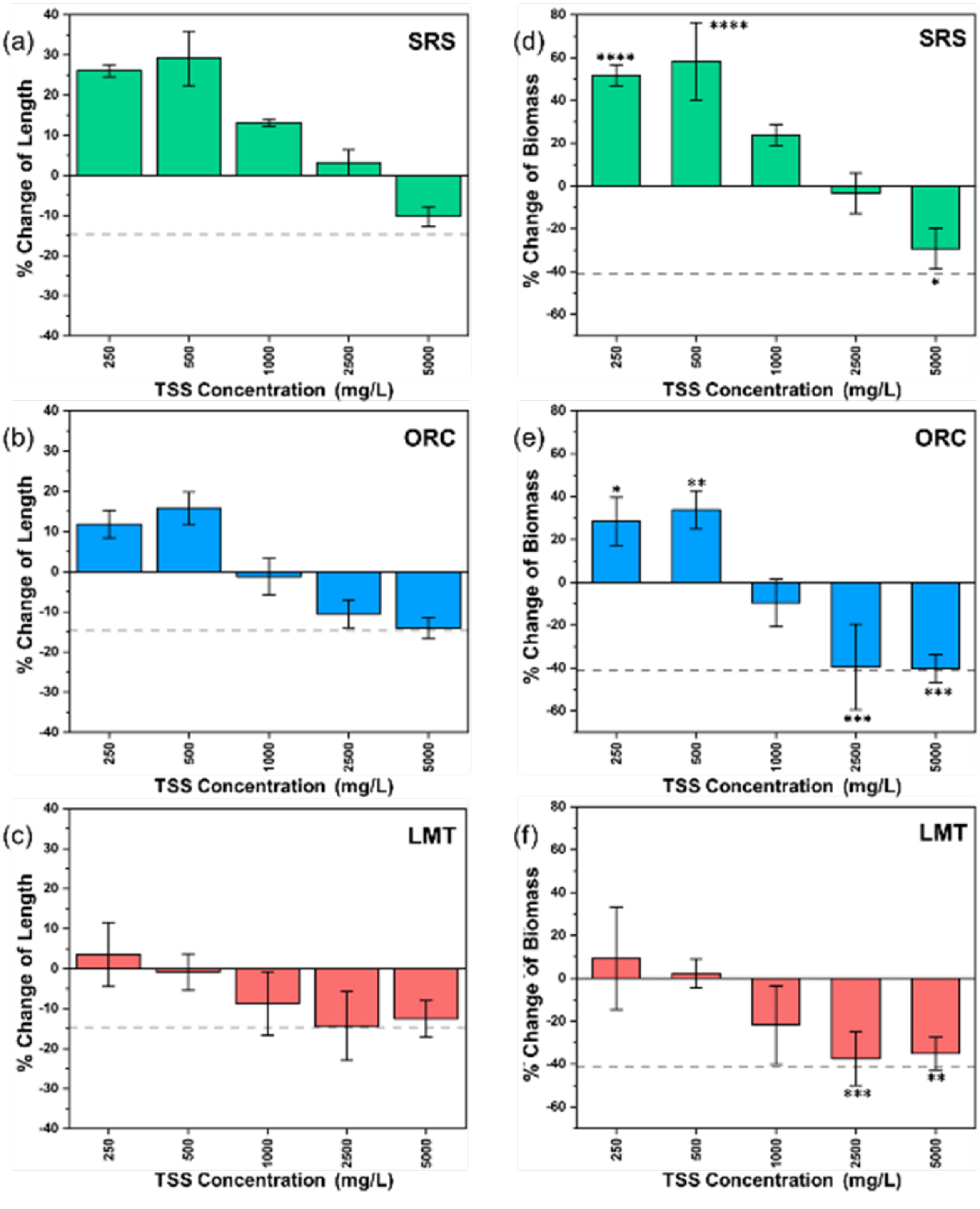


Figure 3-7. Percent changes of growth in shell length (a-c) and biomass (d-f) of Fatmucket (Lampsilis siliquoidea) juvenile mussels exposed to different levels of SRS, ORC, and LMT, respectively. Error bars represent standard deviation (SD) of means. Ctrl = Control water; Day 0 = juvenile mussels kept on Day 0; SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone. Dashed lined represent the shell length and biomass of juvenile mussels of Day 0 when compared to those of Ctrl. * = *p* < 0.05, ** = *p* < 0.005, * = *p* < 0.001, **** = *p* < 0.0001 when compared with Ctrl.**

3.3 Discussion

To date, studies reporting the acute effects of suspended sediments on juvenile freshwater mussels are limited. In the case of survival rate, the high survival rate reached in this study is similar to the study conducted by Buczek *et al*. In that study, ~17-month-old Fatmucket juveniles (shell length of 5.34 ± 0.80 mm) were exposed to relatively high concentrations (up to 3500 NTU) of suspended sediments (particle size 12.2 ± 11.8 μm) sampled from a road construction site, and 100% survival in all treatments after 96-h were determined [112], which also illustrated that juvenile mussels are tolerant of acute high suspended solid exposure. Additional studies focusing on effects of suspended solid on marine mussels also reported no lethal effect noted after days of exposure, though at lower concentrations [113, 114].

Many studies utilized clearance rate (CR) to characterize the response of mussels to suspended sediment, with reduced CR potentially indicating adverse impacts. However, both increased and decreased CR have been reported by previous studies, and impacts of suspended solids on CR may be complicated and vary due to many factors, including mussel species [49, 50], mussel age [49], habitat condition [115], sediment types, sources particle size distribution, and suspended sediment concentrations. Valve gaping behavior, which is related to CR, has also been used as a factor to evaluate responses of mussels to suspended sediment at relatively low TSS levels. Similar to CR, valve gaping behavior results were not consistent among studies, owing to various factors, such as mussel species, particle size, turbidity level, and duration [116-119]. Though mussels are suggested to close their valves when facing harsh conditions, valve gaping activity, as well as CR, observed in very short time periods may not be sufficient to reveal a negative effect on mussels' response to suspended solids. The response of adult *Unio pictorum* (mean length of 8.6 ± 0.9 cm) to suspended sediments of three particle size ranges (< 45 μm, 45-63 μm, 63-125 μm) up to 10 g/L was studied by Lummer *et al.*, and results suggested that (a) the duration of gaping activity was not related to either suspended sediment concentrations or particle sizes, and (b) the mussels cleared 35% of the suspended sediments independent of the concentration or particle size tested [118]. That study accordingly concluded that mussels could not recognize the difference between food and non-food particles and likely unselectively filter the particles in the surrounding environment. In this study, young juvenile mussels with much smaller sizes were investigated, and the difference, both physiological and behavioral, between adults and small juvenile mussels (such as those used in this study) makes it difficult to simply assume that juvenile mussels may behave in similar ways to adults, which were investigated in other studies. Though available data remain limited, no clear correlation between short-term exposure to suspended solids and juvenile mussel survival rate has been revealed, indicating their short-term tolerance to worsened surrounding conditions due to elevated suspended solid levels. Nonetheless, more data is desired to achieve a better understanding of responses of juvenile mussels to increased suspended solid levels.

Studies focused on marine mussels have reported both positive and negative effects of suspended sediment on growth [113, 114, 120, 121]. However, studies investigating the influence of suspended solids on juvenile freshwater mussels are rare, and most used much lower TSS levels. To date, this study is the first chronic study focusing on impacts of high suspended solid concentration exposure on the growth of freshwater mussel juveniles. Some research on the survival rate of juvenile mussels has been conducted, with high survival rates in the range of 80% to 98% by Day 20 reported by Buczek *et al.*', but growth of juvenile mussels was not reported [112]. In another study, Foe *et al.* investigated the influence of suspended sediment on the growth of clam (*Corbicula fluminea*) juveniles. The suspended sediment was collected from subtidal mudflat at Sherman Island, and the clam juveniles were exposed to suspended sediment, up to 150 mg/L, at two different temperatures, 15.3 ºC and 24 ºC, for 30-d [122]. Although growth was detected, less tissue growth (not statistically significant) was obtained at higher concentrations (50 and 150 mg/L). They concluded that the growth of clam tissue was independent of suspended sediment concentration within their tested concentration range, while high chlorophyll α levels promote tissue growth ($p < 0.05$). Thus, their results suggested that food limitation may be a more important factor affecting growth under their experiment conditions. Though using different bivalves, these findings are somewhat consistent with results of the current study, as significant growth of juvenile Fatmucket was determined at low levels of SRS, ORC, and even LMT in 28-d, suggesting that the tested juvenile Fatmucket were able to access enough food to not only overcome the energetic costs of sorting food from nonfood particles but also to support their growth.

Turbidity and sedimentation have been suggested to be possible causes of production and recruitment failure of freshwater mussels [46, 47, 51]. However, both the current study and some previous studies illustrated that their influence on freshwater mussels can be quite complicated. The discovery of an enhanced growth effect at low TSS levels of SRS and ORC was unexpected. Nonetheless, deposited sediment has been previously reported to enhance mussel growth [123]. In 1996, Gatenby *et al.* reported that fine river sediment prompted the growth of juvenile rainbow mussels (*Villosa iris*) compared to those grown without sediment [123]. It was suggested that the resident bacteria in the aquatic sediments might benefit juvenile mussels by enhancing enzymatic activity or digestion [124, 125]. However, by adding bacteria commonly associated with riverine systems, neither enhanced growth nor survival rate compared to those reared on sediment was observed by Gatenby *et al.*, which implied that sediment itself may play a different role benefiting the mussels [123]. They proposed that sediment could (a) provide a substratum or a place to facilitate juvenile mussels' access to food, or (b) work as an internal grinding substrate to assist juvenile mussels in digesting algal cells [123]. In another study, Cumberlandian combshell (*Epioblasma brevidens*) juveniles were reared on 11 river sediments sampled from different locations along Clinch River (in Virginia and Tennessee), together with three control samples including Spring River sediment, West Bearskin sediment, and sand. Results found that juvenile mussels reared on Spring River sediment with low TOC (0.70%) grew faster than those on West Bearskin sediment with high TOC (8.80%), while one sediment sample (Indian Creek, TOC = 5.18%) from Clinch River showed even better growth than that of Spring River sediment [100]. These findings are consistent with results of Gatenby *et al.* and observations of the current study that some sediments may benefit juvenile growth, but via undefined ways.

It is difficult to conclude what factor(s) resulted in the observed growth differences when juvenile mussels are exposed to suspended solids. Source and composition of suspended solids may be critical factors influencing their growth, especially at relatively lower TSS concentrations.

Lower TSS levels may not impair juvenile mussels' filtering ability, and as Lummer *et al.*'s deduced, juvenile mussels may, whether voluntarily or forced, uptake and process the surrounding particles unselectively [118]. One possibility is that, similar to the idea of Kiørboe *et al*., SRS as river sediment and ORC as riverbank soil with complex composition profiles may provide extra food and/or nutrients to benefit juvenile mussels' growth. Though LMT with the lowest level of TN led to the smallest effects on growth, no significant correlation was determined among growth and TOC, TIC, TC or TN levels (Spearman correlation, significance was accepted at $p < 0.05$, data not shown). Thus, the composition of particular nutrients in the sediments/soils and their potential roles in facilitating growth of juvenile mussels remain unknown, and further investigations are thereafter required.

With increased TSS levels, the existence of suspended solids around the juvenile mussels may gradually cause negative impacts on them. Particularly, although live juvenile mussels are still processing particles and accessing food (food in gut as shown in Figure C-8), the food particles (algal cells) become progressively diluted by the non-food particles with increased TSS concentrations [78]. Once the value of the nutrients from the suspended solids is outweighed by the maintenance cost, such as sorting and rejecting filtered particles by pseudofeces production and/or the cost of passing particles through the digestive tract [126, 127], the negative effects of increased TSS levels will occur. Moreover, though not observed in this study, juvenile mussels might have longer closed valve time to avoid long-term exposure to high levels of suspended solids, which may shorten the time available to capture and sort food. Consequently, under such harsh conditions, the energy obtained from food may be adequate to cover maintenance costs, such as to rejecting or excluding non-food particles, but not sufficient to support growth [78].

Another important factor affecting juvenile mussels' growth may be PSD of the suspended solids. SRS and ORC were rich in clay-sized particles, with sizes closer and even smaller than that of algae as food. Their smaller size may make them easier for juvenile mussels to process. It is suggested that particle size is a critical parameter affecting bivalves' particle capture efficiency [128]. Beck *et al.* found that Rainbow mussels (*Villosa iris*) of all three ages tested (2-3-day-old, 50-53-day-old and 3-6-year-old) tended to reject the algae species *Scenedesmus quadricauda*, which is larger (22.3-44.5 µm) than two other algae species (2.8 - 8.5 µm), preferred by the mussels [129]. This may indicate that when surrounded by larger particles, even as food, juvenile mussels may find it more challenging to ingest them as well as to eliminate them. Moreover, larger particles and higher TSS levels may cause gill damage which may result in reduced respiration and feeding activities [130]. By observing the gill damage in 60 and 80 mm Green-Lipped mussels (*Perna viridis*) exposed to < 63 µm (small), > 125-< 250 µm (medium), and > 250-< 500 µm (large) sediment particles for 14-d, Cheung *et al.* stated that mussels exposed to small particles had less damage on the frontal cilia and reduced depletion of abfrontal cilia than mussels exposed to larger particles [130]. However, they used mussels 30 to 40 times larger than the juvenile mussels tested in the current study, and thus smaller particles used here, like LMT that dominated by silt-sized particles, may still damage the gills of the smaller juvenile mussels. However, the possible damage to the gills could not be examined in this study, and future studies are needed to better explain the observed growth difference.

3.4 Summary

This study aimed to explore how elevated suspended solids associated with construction and maintenance activities may affect the survival and growth of freshwater mussels. Though no clear impact on survival was noted, effects on growth were observed, not only among different sediment/soil types, but also among different TSS concentrations from the chronic study. Unexpected but significant growth enhancement effects were observed at lower concentrations of SRS and ORC, possibly attributed to their complex composition profiles, which may benefit mussel growth. This growth effect was not observed in LMT treatment. It may be due to its simple composition profile and higher proportion of larger particles. On the contrary, high levels of all three sediment/soil samples could become a stressor inhibiting or stopping the growth of juvenile mussels.

It is also important to point out the limitations of this study: (a) it only tested the response of the selected juvenile mussels at two age groups, to three sediments; (b) it focused on the effects of the suspended solids and thus other important factors, such as DO, temperature, and food, were optimized for the survival and growth of juvenile mussels. More studies are needed to achieve better understanding of how mussels may respond to a complex and degraded environment. Even with these limitations, the current study indicates that suspended solids may not always be a stressor to juvenile mussels. Juvenile mussels may benefit from lower levels of suspended solids originating from some types of sediments and soils. Thus, this study provides important information to policy makers to facilitate the preparation of guidelines and policies associated with construction and maintenance activities for mussel conservation purposes. The EC20 values estimated here may be useful thresholds for resource managers. Moreover, it is suggested that although in this study the relative lower levels of suspended solids themselves may not be a threat to the juvenile mussels, it is important to consider other factors that may affect responses in natural systems.

Chapter 4. Impacts of Deposited Sediment on Freshwater Mussels

4.1 Study Methodologies

4.1.1 Test Organisms

Mussel species including Arkansas Brokenray (*Lampsilis reeveiana*), Fatmucket (*Lampsilis powellii*), Butterfly (*Ellipsaria lineolata*), Pink Mucket (*Lampsilis abrupta*), Giant Floater (*Pygandon grandis*), Washboard (*Megalonaias nervosa*), and Deertoe (*Truncilla truncata*) were selected for the sediment bury tests. Adult mussels were used for all species selected. For Fatmucket, two different sources (sizes) of mussels were examined, including some reared at CERC with a size range of 5 - 8 cm at age of ~3-year-old, and another collected from the Kansas Zoo in DEC 2022 with a size range of 10 - 15 cm at age of 15-20-years-old. Arkansas Brokenray with size of 4-8 cm were cultured at CERC at age of ~3-year-old. Deertoes were around 2–6 year-old, and Washboard around 12 - 15 cm in the range of 14-19-years-old. Pink mucket (10-13 cm, age 7-9-year-old) and Butterfly (10 - 13 cm, age 8-10-year-old) were also collected from Kansas Zoo in DEC 2022. Giant floater from CERC around 12 - 15 cm were in the range of 7-11-year-old. Mucket from CERC were about 12 - 15 cm with age around 15-20-year-old. Abbreviated names for the mussel types are defined in **Table 4-1** and are used in the rest of the report.

Table 4-1. Summary of short name, and source of different species.

Short name	Common name	Source
AB	Arkansas Brokenray	CERC
cFT	Fatmucket	CERC
zFT	Fatmucket	Kansas Zoo
BF	Butterfly	Kansas Zoo
PM	Pink Mucket	Kansas Zoo
MK	Mucket	CERC
GF	Giant Floater	CERC
WB	Washboard	CERC
DT	Deertoe	CERC
mDT	Deertoe	CERC
sDT	Deertoe	CERC

Before tests, mussels from CERC were reared in a 550-L flow-through tank with CERC well water (hardness ~300 mg/L as $CaCO_3$, alkalinity ~250 mg/L as $CaCO_3$, pH ~7.8) at a flow rate of 2 L/min. Specifically, each species were placed into plastic containers (56 × 40 × 14 cm; without lids) with a 3-cm layer of commercial creek gravel (~0.2 - 1.5 cm diameter) submerged in the tank. The number of mussels in each container was adjusted based on the size of the mussels to avoid crowding. Water in the mussel holding tanks was continuously aerated. Part of the food source for the mussels came directly from the pond at CERC, and extra food was prepared by mixing 20 mL of a commercial nonviable microalgal *Nannochloropsi*s concentrate (Nanno 3600; Reed

Mariculture) and 20 mL of a unique mix of six microalgae (Shellfish Diet 1800; Reed Mariculture) in a 20 L container kept at 4 °C. The food was renewed daily and was continuously pumped to each mussel container.

For mussels collected from other facilities, a minimum 14-d quarantine was required to avoid spreading diseases and parasites before they were transferred to the room where other mussels were cultured. Typically, once they were transferred to CERC, they were sent to a specific quarantine room and quarantined for at least two weeks. During the quarantine period, water was continuously aerated, and mussels were fed ad libitum by adding approximately 20 mL of a commercial nonviable microalgal *Nannochloropsis* concentrate (Nanno 3600; Reed Mariculture) and 20 mL of a unique mix of six microalgae (Shellfish Diet 1800; Reed Mariculture) once daily. After quarantine, mussels were transferred to the room where other mussel species were reared and were treated the same way as previously described.

All research complied with the requirements of the US Geological Survey Columbia Environmental Research Center (USGS CERC; Columbia, MO) Institutional Animal Care and Use Committee, with all applicable sections of the Final Rules of the Animal Welfare Act regulations, and with all CERC standard operating procedures for the humane treatment of test organisms during culture and experimentation.

4.1.2 Sediment Collection and Characterization

During construction, river sediment may be disturbed. Small sediment particles may remain suspended for a long period of time, while larger and heavier particles may settle down quickly and form a deposition layer, which may consequently bury the mussels and other benthic organisms in that area. Moreover, some construction material, such as limestone, may enter the water and form a burial layer. Thus, to evaluate the potential impacts of the deposition layer on freshwater mussels, one local sediment and one local limestone powder were collected for the following burial studies. Local sediment was collected from the Bourbeuse River in Missouri. Sediment samples were collected from the river bottom in a shallow area. Samples were dug out using a shovel and quickly passed through a 5-mm sieve to collect sediment particles < 5 mm in size (named as BBS < 5). The samples were sealed in 20-L buckets and stored at 4 °C. Test sediments were analyzed for chemical contaminants, including metals (n = 26), polycyclic aromatic hydrocarbons (PAHs, n = 18), and *n*-alkanes (C9-C40) (details in **Appendix D, Table D-1 to 3**). Generally, background levels of metals, and relatively low levels of PAHs and *n*-alkanes were detected, and therefore they were not considered to be possible factors that might lead to adverse effects, if any were to be observed, later in the tests.

4.1.3 Experimental Design and Set-up

A flow-through box containing two segments, for water in and water out, was designed as illustrated in Figure 4-1. Specifically, the box was made in glass with a length of ~45 cm, a width of ~30 cm and a height of ~30 cm. A "hanging" glass separated the box into the two segments, with the narrow and back part (B part) accounting ~15 cm of the total length and the wider and front part (F part) accounting for the rest of the length, ~30 cm. The "hanging" glass was designed to have a distance of 5 cm from the bottom, which would allow water to go through the box. There were three holes (diameter = 2.5 cm) in the front of the box, with a distance of 5 cm between each, which allowed for adjusting the surface water depth and how water went through the box, as well as avoiding potential overflow. Similarly, there was one hole (diameter = 2.5 cm) in the back of the box, which allowed water to flow in or out of the box and avoided potential overflow. Glass bending tubes and stoppers were made to fit the size of holes when the tests were set up.

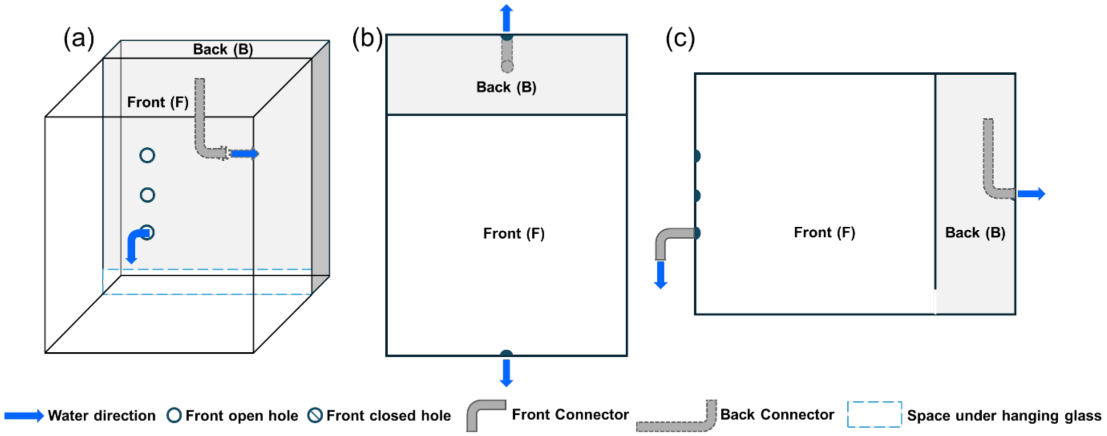


Figure 4-1. Images of the flow-through box: (a) overall view, (b) top view and (c) side view. Image is not to scale.

Using boxes designed as above, water flow through the sediment layer could occur in two directions, as shown in **Figure 4-2**. When water was added to B (Back) part, an upwelling condition through the sediment layer was created **Figure 4-2 (a and b)**. On the contrary, if water was added to F (Front) part, a downwelling situation through the sediment layer was established **Figure 4-2 (c and d).** During tests, a substrate layer of 10-14 cm (± 1 cm) was prepared for mussels to rest on at the bottom of the box. Specifically, a 6-8 cm layer of pea gravel was first put on the bottom of the entire box, then another 2-3 cm of small gravel layer was put onto the course gravel layer, followed by a 2-3 cm sand layer placed on top. Water flow through (upwelling or downwelling) the boxes was achieved by supplying fresh 100 hardness water hourly via an auto water suppling system. The water volume in each box could be controlled between 0 L and 1 L. Before the burial tests, mussels were taken out from their culture tank and acclimated to test water (100 hardness). Then, mussels were carefully transferred to the boxes with a total number of three mussels (four mussels when GF and WB were tested, details in **Table 4-3**) per box. To help the mussels to better settle down, the mussels (at least ½ of the body length) were gently insert into the constructed substrate in an upright orientation with their siphons facing up. After that, mussels were allowed to sit and adjust their position in the boxes for at least 24 h. During this period, the surface water maintained a depth of ~15 cm.

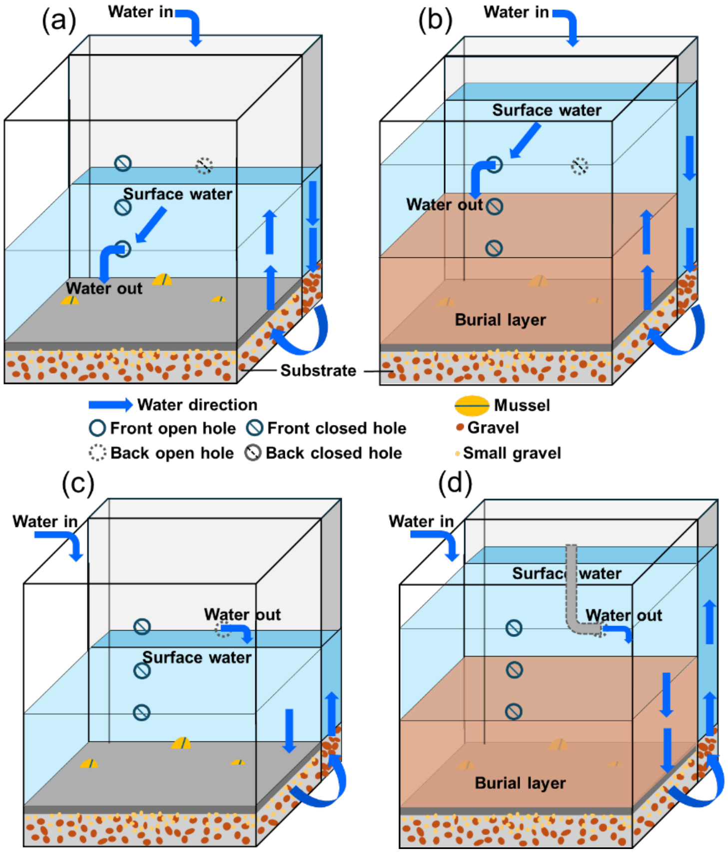


Figure 4-2. Images of flow-through boxes under different conditions: (a) upwelling water control; (b) upwelling water with burial layer; (c) downwelling water control; and (d) downwelling water with burial layer.

Typically, each tested condition was done in triplicate (named as R1, R2 and R3, respectively) and mussels were buried manually. First, about 2/3 of the volume of the surface water in each box was gently removed to make the following bury process easier. Then, sediment samples prewarmed to near room temperature were first well mixed, and then a small steel shovel was used to apply the sediment onto the mussels until the designed sediment burial depth was reached. Lastly, each box was flushed with 100 hardness water to reduce the turbidity of the surface water. For the whole process, working in a gentle way to avoid disturbance to the mussels, substrate and the burial layer created, was important.

Starting from the next day, the number and species of mussels that appeared on the surface were recorded and the water quality parameters including surface water temperature, conductivity, dissolve oxygen (DO) level, pH, alkalinity, hardness, and total ammonia level (TAN) were analyzed. Moreover, starting from the fourth day, water samples were also collected from the sediment burial layer, using a long pipette reconstructed from a turkey baster, and parameters including conductivity, DO, pH and TAN were measured. Tests ended at the end of Day 7, and mussels that were unburied were collected and transferred to clean culture water for further observation (> 2 weeks). Those mussels that remained buried (alive or dead) were carefully dug out and their location in the burial layer was recorded.

A series of burial tests on different species were conducted, utilizing the system discussed above that allows for control of both water flow direction and water volume, refreshed hourly, to each box. **Tables 4-2** and **4-3** summarize and name the tests. Arkansas Brokenray (AB) was used as a model animal to examine the response to burial events at various burial depths, water refreshing volumes per hour, and water flow directions. For each box, three AB were placed into the substrate as described earlier. Burial tests (**Table 4-2**) using AB included (a) Variations of burial depth from 5 - 20 cm (5 cm, 7.5 cm, 10 cm, 15 cm, and 20 cm) using BBS < 5 sediment and upwelling refreshing water volume of 1 L/h across the burial layer; (b) Alterations of upwelling refreshing water volume across the burial layer from 0 - 1 L/h (0 L/h, 0.25 L/h, 0.375 L/h, 0.5 L/h, 0.75 L/h and 1 L/h) at the burial depth of 15 cm; and (c) Changes of water flow directions across the burial layer, upwelling *vs* downwelling at different burial depth of 5 - 15 cm using BBS < 5 with the refreshing water volume of 1 L/h across the burial layer. Additionally, based on the results of the above conditions, another two tests were conducted to assess the possible impacts of sediment particle sizes at the burial depth of 20 cm. For all test conditions, mussels not buried were used as controls (C).

Table 4-2. List of short names of different tests at various conditions using Arkansas Brokenray (AB).

Test short name	Depth (cm)	Sediment type	Refreshing Water Volume (L/h)	Water flow direction
AB-5 cm	5	BBS<5	1.0	Upwelling
AB-7.5 cm	7.5			
AB-10 cm	10			
AB-15 cm	15			
AB-20 cm	20			
AB-15 cm-0.75	15		0.75	Upwelling
AB-15 cm-0.5	15		0.5	
AB-15 cm-0.375	15		0.375	
AB-15 cm-0.25	15		0.25	
AB-15 cm-0	15		0	Horizontal
AB-5 cm-D	5		1.0	Downwelling
AB-10 cm-D	10			
AB-15 cm-D	15			

Other species' ability to unbury themselves from the burial layer was also investigated using AB as a reference (**Table 4-3**). Particularly, as two sizes of FT were available originally from two different sources, the large ones from the Kansas Zoo were named zFT, while the smaller ones cultured at CERC were named cFT. Similarly, three different sizes of DT were tested, and based on their sizes they were named as DT (large Deertoe), mDT (middle sized Deertoe), and sDT (small sized Deertoe). As previous tests using adult AB have already revealed that high volume of upwelling water flow through the burial layer would benefit mussels' escape and survival, upwelling refreshing water of 1 L/h was applied through all the rest of tests. To mimic a mixed mussel bed situation and to compare the possible differences among various species, three mussels of different species were generally buried at the same time. For most tests, one adult AK used as a reference was buried with one cFT and one zFT, BF, PM, or MK, resulting in three mussels per box. In the case of WB and GF, one AK working as the reference was buried simultaneously with one DT, one sDT or mDT, and one large WB or GF mussel. As other species with large sizes were the main concern here, the tests were named generally based on the large mussels, for example, PM buried with AB and cFT with a burial depth of 5 cm was named as PM-5 cm, with a specific focus on whether PM with a much larger size compared to AB and cFT could escape from this depth of burial layer (see details in **Table 4-3**). The burial depth varied according to the preliminary results (data not shown). Fewer alternate conditions were tested due to the limited number of mussels. Similarly, mussels not buried were used as controls (C).

Table 4-3. List of short names of different tests at various conditions using multiple species.

Short Name	Species 1	Species 2	Species 3	Depth (cm)	Sediment type	Refreshing Water Volume (L/h)	Water flow direction
BF-7.5 cm	BF	AB	cFT	7.5	BBS<5	1.0	Upwelling
BF-12.5 cm				12.5			
zFT-7.5 cm	zFT			7.5			
zFT-12.5 cm				12.5			
PM-5 cm	PM			5			
PM-7.5 cm				7.5			
PM-12.5 cm				12.5			
MK-7.5 cm	MK			7.5			
GF-10 cm	GF		DT	10			
WB-7.5 cm	WB			7.5			

4.1.4 Data Analysis

For mussels of other species, due to the limited numbers and test conditions conducted, correlations among various factors were not analyzed, and their responses against burial events were mainly focused on their ability to resurface from the tested burial depth. For AB mussels, at the end of the 7-day test, numbers of mussels resurfaced, dead, and still buried but alive were counted. To determine whether there was any correlation between the percentage of mussel that reemerged and various tested factors (burial depth, vertical water volume, and vertical water direction), Spearman's rank correlation analysis using Originpro 2020 b was applied. Due to the lack of normality (determined by Shapiro-Wilk test) of the unburied mussel data across all tests, a Kruskal-Wallis non-parametric Analysis of Variance (ANOVA) was used (Originpro 2020 b) to test whether the number of mussels unburied was affected by burial depth, vertical water volume, and vertical water direction. In all cases, differences were accepted as significant at $p < 0.05$. For the changes of water quality including DO, TAN and conductivity in the overlying surface water and pore water in the burial layers, Spearman's rank correlation analysis was applied to determine whether they were correlated to factors such as burial depth, vertical water volume, and vertical water direction.

4.2 Study Results

4.2.1 Responses of Arkansas Brokenray to Various Burial Events

4.2.1.1 Impacts of Burial Depth

When mussels are buried, the depth of the burial layer is critical to both their ability to their recovery from the burial layer and their final survival. To determine the impacts of increased burial depth on mussels, the AB adults were buried using BBS < 5 at 5 different depths (5 cm, 7.5 cm, 10 cm, 15 cm, and 20 cm, respectively). Meanwhile, upwelling water across each box was set to 1.0 L/h for all treatments and controls. **Figure 4-3 (a) and (b)** display the results as percentage of mussels that reemerged from the burial layer (Unburied), percentage of mussels found alive (Survival), percentage of mussels that remained buried but were found alive (Buried alive), and the percentage of mussels found dead (Mortality), at the end of 7-d tests.

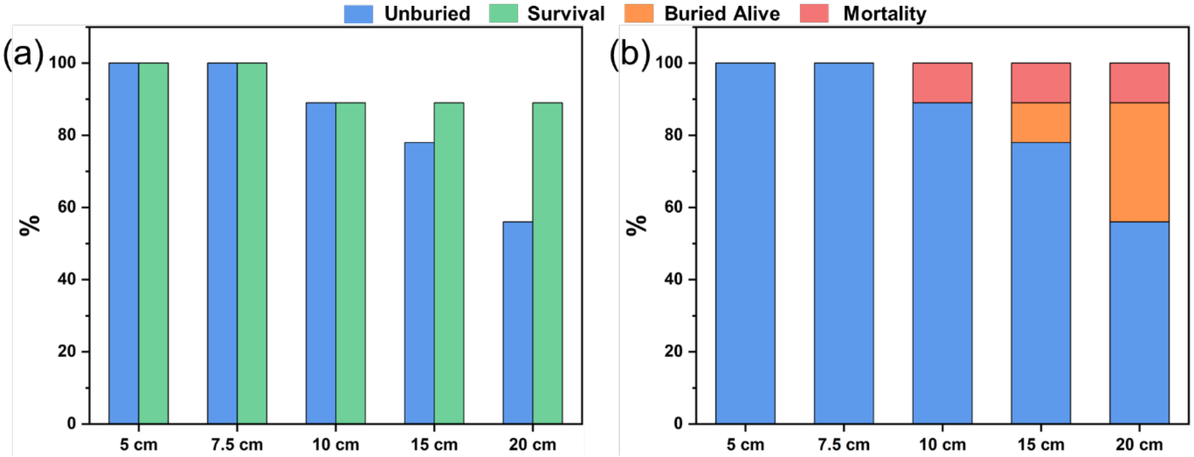


Figure 4-3. (a) % of AB unburied from the burial layer and % of AB survival at different burial depths; (b) Stacking column % of AB unburied, % AB Buried Alive, and % Mortality. AB: Arkansas Brokenray. Vertical water flow direction: upwelling.

Not surprisingly, results revealed that increased burial layer depth led to more mussel death. With a shallower burial depth, such as 5 cm and 7.5 cm, all mussels unburied themselves, with many reaching to the surface just overnight and the majority resurfaced within the first two days (**Table D-4**). However, once the burial depth was further increased, the number of mussels resurfaced started to decline, and only 89%, 78%, and 56% of mussels successfully unburied themselves within 7-d from 10 cm, 15 cm, and 20 cm burial layers, respectively. The resurface success was negatively correlated to the burial depth (Spearman Correlation Coefficient = -0.563, *p* = 0.0287). For those that failed to emerge, most were found dead at different depths in the burial layer. Nonetheless, remaining buried but alive mussels were occasionally observed (one at depth of 15 cm and another one at depth of 20 cm, see **Table D-4**). It is important to point out that for mussels buried deeper, time is essential, as the majority resurfaced within the first two days (**Table D-4**), and only a small portion of mussels successfully returned to the surface in the later periods of the 7-d tests. This indicates that once the burial event occurred, mussels might quickly lose their ability and chance to emerge, if they failed to immediately and quickly respond to the environment change and decide to move up. Thus, for those alive but remaining buried at the end of the 7-d tests, they might eventually die if the burial duration extended further.

Water quality parameters, such as temperature, DO, conductivity, pH, alkalinity, hardness, and TAN level, are important indicators to evaluate the surrounding environmental conditions of freshwater mussels. Surface water samples above the burial layers and the water samples directly collected from different depths of the burial layers were analyzed to assess the possible changes to water quality during the designed burial events.

Specifically, for upwelling conditions with different burial depths, water in the B part where fresh water was added (named as Water-I) and the surface water in the F part representing water exiting the burial layer (named as Water-O) were sampled and examined. Boxes with free mussels (no burial layer) were used as controls (C for short). Generally, Water-I samples had DO levels in the range of 7.5 - 8.5 mg/L and TAN concentrations < 0.05 mg/L (**Figure 4-4 (a)** and **(b)**) with no clear difference among boxes with or without a burial layer. For Water-O samples, controls (C1 and C2) showed no notable difference with Water-I samples because of the good and efficient water exchange. Nonetheless, the addition of a burial layer would evidently affect the quality of the surface water above the burial layers. Compared to the controls, the Water-O samples collected above the burial layer usually had decreased temperature (Data not shown) and slightly decreased pH (**Figure D-1 (f)**). More importantly, significantly declined DO (**Figure 4-4 (c)**) was detected with increased burial depths. Most detected DO fell into the range of 4.5 - 7.0 mg/L, while even lower DO levels were also observed in some cases. For example, the lowest DO concentration of 3.13 mg/L was detected in sample from R3 of AB-7.5 cm on Day 5, followed by a DO level of 3.49 mg/L in sample from R1 of AB-10 cm on Day 4, and 3.85 mg/L in R2 of AB-15 cm on Day 3. The lowered DO may result from (a) the consumption of mussels reemerged to the surface, (b) the consumption of alive mussels remaining in the burial layer, and (c) consumption during the decomposition process of dead mussels. Meanwhile, considerably raised TAN concentrations were occasionally recorded (**Figure 4-4 (d))**. For example, the highest TAN concentration of 1.482 mg/L was recorded in R1 of AB-10 cm on Day 4. Though the TAN levels in the same box started to decline in the rest of the days, a relatively high TAN of 0.381 mg/L were still observed on Day 7. Specifically, the daily changes of DO levels in Water-O were found negatively correlated to burial depths ($p < 0.05$), while such correlation was not observed in the case of TAN. It is worth noting that the dropped DO and increased TAN were more frequently detected in the boxes that ended up with dead mussels. For example, dead mussels existed in R1 of AB-10 cm and R1 of AB-20 cm, and both presented lower DO and higher TAN compared to others, indicating that such changes should be closely related to the mussel death.

The water quality of pore water samples collected directly from the burial layer may be a straightforward way to visualize the ongoing changes during the burial period. Thus, water samples sampled from different depths of the burial layer were analyzed, including DO, TAN, conductivity, and pH (**Figure 4-4** and **Figure D-1**). Compared to Water-I and Water-O, water samples collected from the burial layers, in general, presented degraded water quality. DO concentrations (**Figure 4-4 (e)**) were detected in a wider range of 4.5 - 7.5 mg/L, and elevated TAN concentrations (**Figure 4-4 (f)**) were sometimes recorded. Particularly, the detected highest

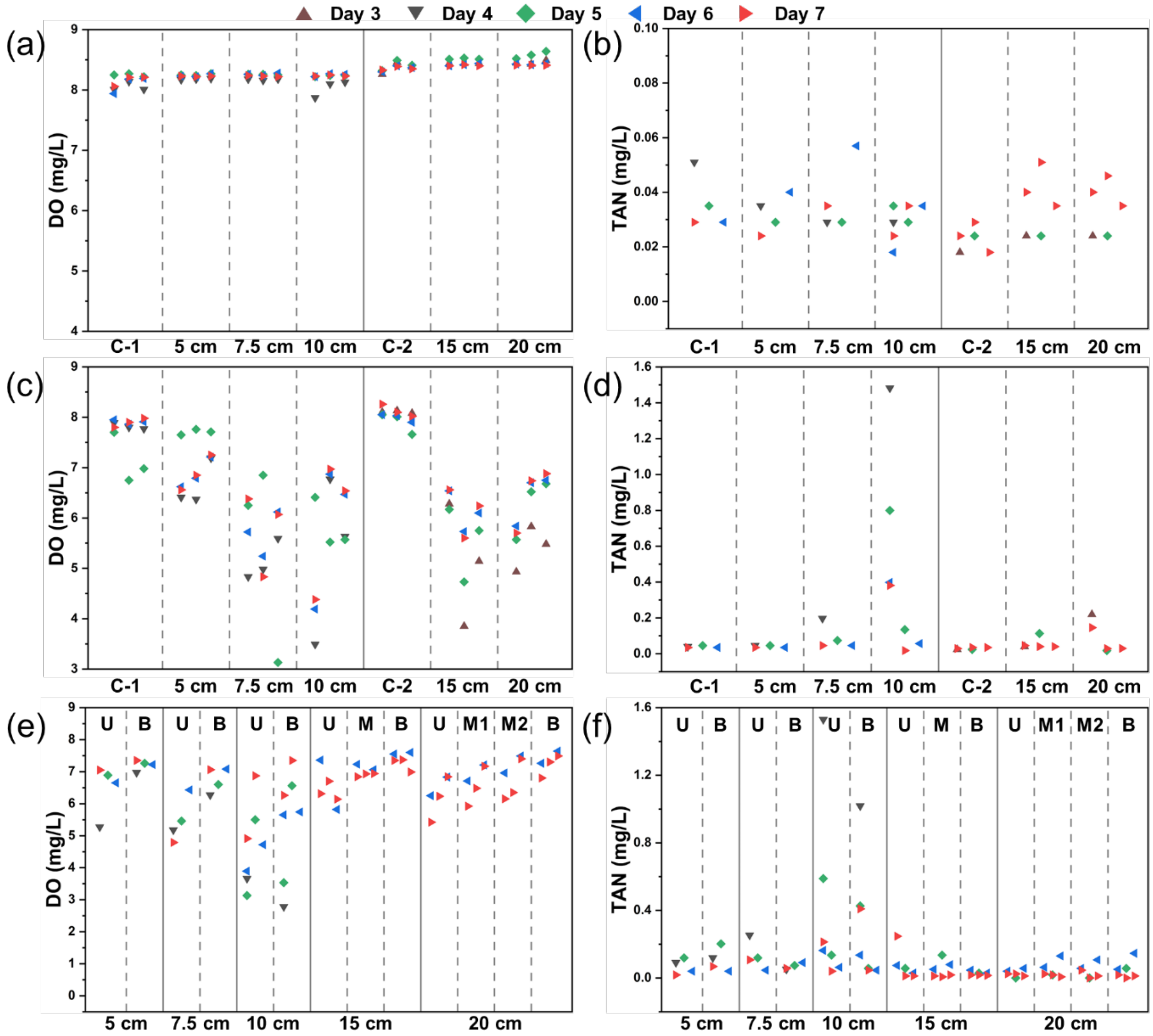


Figure 4-4. Impacts of increased burial depths on water quality changes when AB were buried, including: changes of surface water (a) DO and (b) TAN in Water-O; changes of surface water (c) DO and (d) TAN in Water Water-I; and changes of pore water (e) DO (e) and (f) TAN in the burial layer at different depths. U: Upper part of the burial layer; M: Middle part of the burial layer; and B: bottom part of the burial layer. AB: Arkansas Brokenray. Vertical water flow direction: upwelling.

TAN (1.529 mg/L) and lowest DO (2.78 mg/L) were from the same sample: water collected at around 5 cm depth from the burial layer surface of R1 of AB-10 cm on Day 4.

Moreover, DO and TAN in the burial layer were found to vary with several factors: (a) sampling time, (b) sampling location, and (c) mussel death. First, DO and TAN could change with time, and the different days could get different results even in the same box and at around the same sampling depth, indicating that the water quality changes were dynamic during the burial events. Second, one interesting finding is that for the same burial layer, water samples collected from different locations (various depths of the burial layer and different distance to a mussel) might have noticeably distinct DO and TAN concentrations. Overall, water samples from the bottom part of the burial layer had higher DO compared to those from the upper part. Take R1 of AB-7.5 cm on Day 7 as an example, the upper part had a detected DO of 4.79 mg/L while the bottom part had a significantly higher DO of 7.06 mg/L. Sampling depths also affected the TAN concentrations. Take R1 of AB-15 cm on Day 7 as an example, the TAN was 0.247 mg/L in the upper layer, while it decreased to 0.12 mg/L and 0.18 mg/L when went deeper. It is possible that the bottom layer had more efficient water exchange with the B part of the boxes where fresh water was supplied, and accordingly the water quality was improved compared to the higher sections. Finally, another noteworthy finding is that boxes with dead mussels were more likely to have low DO and high TAN concentrations (such as R1 of AB-7.5 cm and R1 of AB-10 cm) compared to those without dead ones. In addition to DO and TAN, another parameter closely related to dead mussels is conductivity (**Figure D-2 (a)**). For instance, significantly elevated conductivity (314 µS/cm) was observed in R1 of AB-10 cm in which a dead mussel was observed. Mussel death may be the main contributor to the declined DO, increased TAN and conductivity in the burial layer as well as in the Water-O surface water. Such changes may result from the decomposition process of their soft tissue, which would largely consume DO while also releasing ammonia and salts, resulting in increased conductivity. Consequently, the mussel death may strongly degrade the nearby water quality and worsen the living conditions of the mussels in the burial layers, which, in return, may lead to more mussel death.

4.2.1.2 Impacts of Upwelling Water Supply through the Burial Layer

The AB survival rates at different depths showed that most mussels could successfully escape from the burial layers up to 15 cm in depth with upwelling water of 1.0 L/h. However, little is known about whether mussels may still be capable of climbing up and reaching the burial layer surface if less fresh water across the burial layer is available. A sufficient water supply across the burial layer may be critical to mussels' survival after a burial event has occurred, as it may bring DO to the mussels and dilute as well as wash out accumulated toxicants (including ammonia). To verify this presumption, six different upwelling refreshing water volumes varied from 0 - 1 L/h (1.0 L/h, 0.75 L/h, 0.5 L/h, 0.375 L/h, 0.25 L/h, and 0 L/h) were deployed at the same burial depth of 15 cm using BBS < 5. As expected, fewer mussels resurfaced with the reduced volume of refreshing water supplied (**Figure 4-5** and **Table D-5**). At higher water volume (≥ 0.375 L/h), more than 80% of AB mussels could successfully recover. In the case of 0.5 L/h and 0.375 L/h, 89% of mussels returned to the surface. However, when water volume was reduced to 0.25 L/h and lower (0 L/h), the number of mussels that eventually appeared on the surface dropped sharply to only 44%. The number of mussels resurfaced was determined to be positively correlated to the water volume hourly supplied through the burial layer (Spearman Correlation Coefficient = 0.521, $p = 0.0267$), implying the importance of water supply to mussels' ability to unbury themselves and eventually to survive. For mussels that failed to escape when less water was supplied (0 - 0.5 L/h), all were found dead at different locations in the burial layer. It is difficult to determine when a mussel died but depending on the soft tissue decomposition conditions (most soft tissue degraded or nearly empty shells), they might have died at a very earlier stage after being buried, which in return, might have further worsened the surrounding water quality.

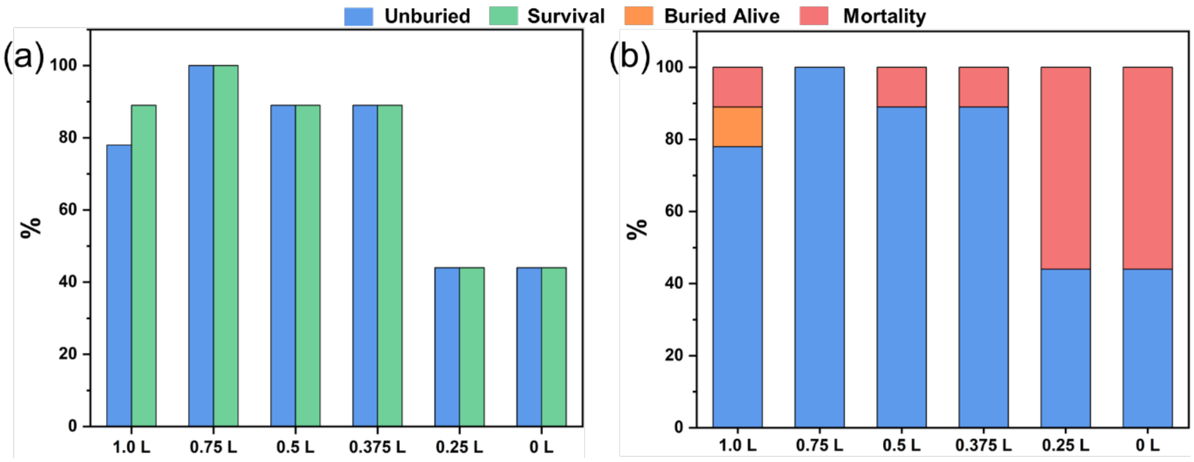


Figure 4-5. Impacts of refreshing water volume on (a) % of AB unburied from the burial layer and % of AB survival at different burial depths; (b) Stacking column % of AB unburied, % AB Buried Alive, and % Mortality. AB: Arkansas Brokenray. Vertical water flow direction: upwelling.

Figure 4-6 displayed the changes to DO and TAN during the 7-d tests with different upwelling refreshing water volumes. Similar to the results of various depths, for upwelling water volumes from 0.25 L/h to 1.0 L/h, the Water-I samples had trace amount of TAN, around 7.5 - 8.5 mg/L DO. For the Water-O samples, DO and TAN of controls (C1-C4) showed no clear difference with those Water-I samples. However, drastically decreased DO concentrations were noticed in boxes with a bury layer. Moreover, with the decline of upwelling water volume, high levels of TAN (**Figure 4-6 (d)**) as well as conductivity (**Figure D-3 (e)**) were more frequently observed in Water-O samples. For example, the lowest detected DO of 3.17 mg/L was recorded in R2 of AB-15 cm-0.75, the highest TAN of 1.862 mg/L was documented in R1 of AB-15cm-0.5. However, the changes to DO, TAN and conductivity were not clearly correlated to the variation of water volume hourly supplied, suggesting that their changes might be attributed to multiple factors. As discussed earlier, decreased DO in the Water-O samples may be due to the consumption of alive mussels and the decomposition of dead ones. The decomposition process of dead mussels may be the main contributor to the raised TAN and conductivity. Especially, when less water is supplied through the burial layer, ammonia and salts contributing to high conductivity would not be effectively diluted and washed out, which subsequently would lead to accumulation of TAN and increased conductivity.

Unlike the other treatments, the changes of TAN and DO of AB-15 cm-0 showed a different trend with high DO and low TAN levels in the F parts of the boxes. This is because AB-15 cm-0 was designed to represent a condition where fresh water would not go through the burial layer, and instead, water was directly added to the top of the burial layer in the F part of the box and quickly exited from the front outlet. Consequently, the hourly refreshed water improved the surface water over the burial layer with overall high DO and Low TAN detected. The B part of the box showed notably declined DO, raised TAN concentrations, and significantly increased conductivity, which should have originated from the burial layer with dead mussels. Such results indicate that the mussel activity and death in the burial layer might adversely affect the

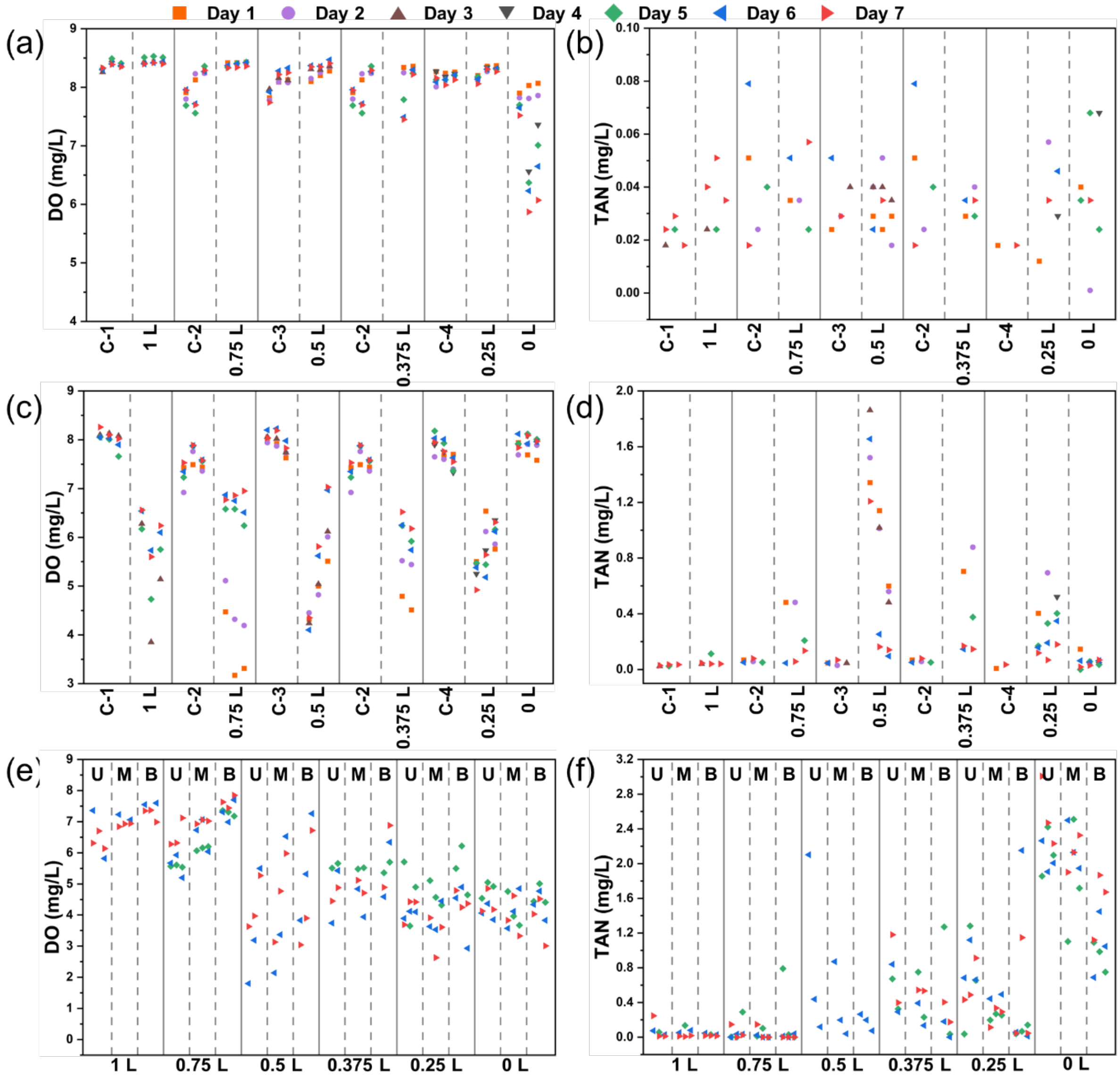


Figure 4-6. Impacts of decreased vertical water flow through the burial layers on water quality changes when AB were buried, including: changes of surface water (a) DO and (b) TAN in Water-O; changes of surface water (c) DO and (d) TAN in Water-I; and changes of pore water (e) DO and (f) TAN in the burial layer at different depths. U: Upper part of the burial layer; M: Middle part of the burial layer; and B: bottom part of the burial layer. AB: Arkansas Brokenray. Vertical water flow direction: upwelling.

surrounding water quality. With reduced water supplied, the water exchange might be lacking, but the diffusion process may still be the dominant mechanism resulting in changes DO and TAN in the overlying water.

The reduced upwelling water volumes strongly affected the pore water quality (DO and TAN) of the burial layers as illustrated in **Figure 4-6 (e and f)**. Compared to tests using higher volumes of upwelling water (0.75 - 1.0 L/h), reduced water volume (0-0.5 L/h) led to overall decreased DO

(**Figure 4-6 (e)**) and increased TAN (**Figure 4-6 (f)**) and conductivity (**Figure D-4 (a)**). For the worst condition at 0 L/h, high TAN concentrations in the range of 0.6 - 3.0 mg/L and high conductivity in the range of 320 - 450 µS/cm were determined, which is mainly attributed to the dead mussels in the burial layers and the reduced water exchange through the burial layers. Generally, the changes to DO, TAN, and conductivity were found to be strongly and negatively correlated to the upwelling water volume ($p < 0.0001$), illustrating the importance of good water supply to the water quality of pore water in the burial layers. Moreover, as pointed out earlier, samples from different sampling locations could have remarkably different DO, TAN, and conductivity. For upwelling water volumes from 0.5 L/h to 1.0 L/h, the lower parts of the burial layer usually had higher DO than the upper ones. However, this trend started to disappear with the further reduction of upwelling water volume, and for samples from 0.25 L/h and 0 L/h, the DO levels showed no clear differences at different sampling depths. In the case of TAN and conductivity, no clear differences were noticed among samples collected from different depths when water volumes were high at 0.75 L/h and 1.0 L/h. However, when the upwelling water volume started to decline (0 L/h to 0.5 L/h), water samples collected from the upper part of the burial layers started to have higher TAN and conductivity than the lower ones.

Such results highlight the importance of upwelling water supply through the burial layer. In summation, higher upwelling refreshing water volumes periodically supplied through the burial layer could more effectively dilute the water in the burial layer, which subsequently improves the water quality (supply of DO and dilution of TAN and conductivity) of the burial layer and accordingly benefits mussels' survival and resurfacing capability. However, this effect gradually diminishes if water volume through the burial layer is reduced. As a result, the water quality would become worse with dropping DO and rising TAN and conductivity. This may cause more mussel death and hinder their recovery process from the burial layer, and once more mussels died, their decomposition would further degrade the water quality and make the situation worse resulting in probably more death.

4.2.1.3 Impacts of Water Direction through the Burial Layer

Under natural conditions, mussel beds may be in areas where water goes through the sediment layer from various directions. Though relative studies remain rare, Klos *et al.* reported that increased mussel population density was found in areas with upwelling water [131]. Similarly, Norbury *et al.* pointed out that riffle-tails with upwelling water are the prime sites for reintroduction of juvenile pearl mussels (*Margaritifera margaritifera*) [132]. Therefore, water direction may also be a factor affecting mussels' survival once buried. In the last two sections, upwelling water was applied for all tests, which then raised the question of whether the alteration of water direction would subsequently change the response of AB when buried. To test this, downwelling refreshing water was added to the F part of the box and exited from the B part. BBS < 5 at three depths of 5 cm, 10 cm, and 15 cm was used in this test. The volume of refreshing water was still set at 1.0 L/h as earlier tests showed that this volume could sufficiently support the mussels' survival at upwelling conditions.

As illustrated in **Figure 4-7**, significantly fewer mussels reemerged from the burial layers when a thicker burial layer was applied with downwelling conditions compared with upwelling ones. Although all AB successfully returned to the surface at a burial depth of 5 cm, only 22% and 55%

of mussels unburied themselves when the burial depth increased to 10 cm and 15 cm, respectively. By comparing the number of mussels resurfaced from different depths and water directions, it was noticed that not only burial depth but also water direction was correlated to the ability of mussels to reemerge ($p = 0.0115$), and upwelling water direction might be more beneficial to mussels when buried. In addition to remarkably fewer unburied mussels at the end of the 7-d tests (**Table D-6**), mussels that remained buried were found dead near the interface of the substrate and the burial layer with notable decomposition of soft tissue, implying that they possibly died shortly after they were buried. For dead mussels discovered near where they were originally located before burial, it is difficult to explain why they failed to move up, but such failure eventually led to their death, and the downwelling water might have contributed to their failure. Thus, when buried by the same depth, downwelling conditions may be more challenging situations for AB mussels to escape and survive compared to upwelling ones.

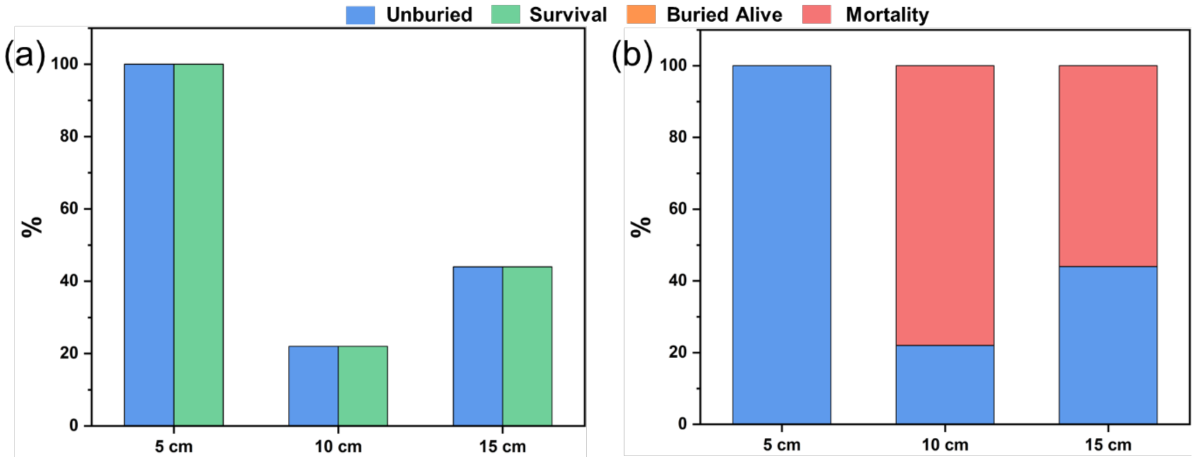


Figure 4-7. Impacts of water direction on (a) % of AB unburied from the burial layer and % of AB survival at different burial depths; (b) Stacking column % of AB unburied, % AB Buried Alive, and % Mortality. AB: Arkansas Brokenray. Vertical water flow direction: downwelling.

For downwelling conditions, Water-I surface water samples were redefined as the surface water above the burial layer, and accordingly Water-O samples were redefined as the surface water in the B part of the boxes where water left the box. **Figure 4-8** displayed the changes of DO and TAN of Water-O (**Figure 4-8 (a-b)**) and Water-I (**Figure 4-8 (c-d)**) samples. Though the refreshing water direction was reversed, the trends of DO and TAN changes of the surface water remained similar to those of the upwelling ones. Particularly, compared to the Water-I samples and controls, Water-O samples had lower DO and higher TAN levels with the increase of depth, indicating that water quality was degraded with thicker burial layer. The lowest DO of 3.77 mg/L was observed in R2 of AB-10 cm-D on Day 6, while the highest TAN of 0.378 mg/L was detected in R2 of AB-15 cm-D on Day 7. Moreover, Spearman rank correlation analysis showed that water directions were significantly correlated to daily changes of DO ($p < 0.04$) and conductivity ($p < 0.04$) but not TAN. Compared to those upwelling conditions, decreased DO and increased conductivity (**Figure D-5 (a)**) were more significant when the downwelling conditions were applied. This is because at the same burial depth of 10 cm and 15 cm, the downwelling conditions had higher mussel mortality compared to the upwelling ones, and the decomposition

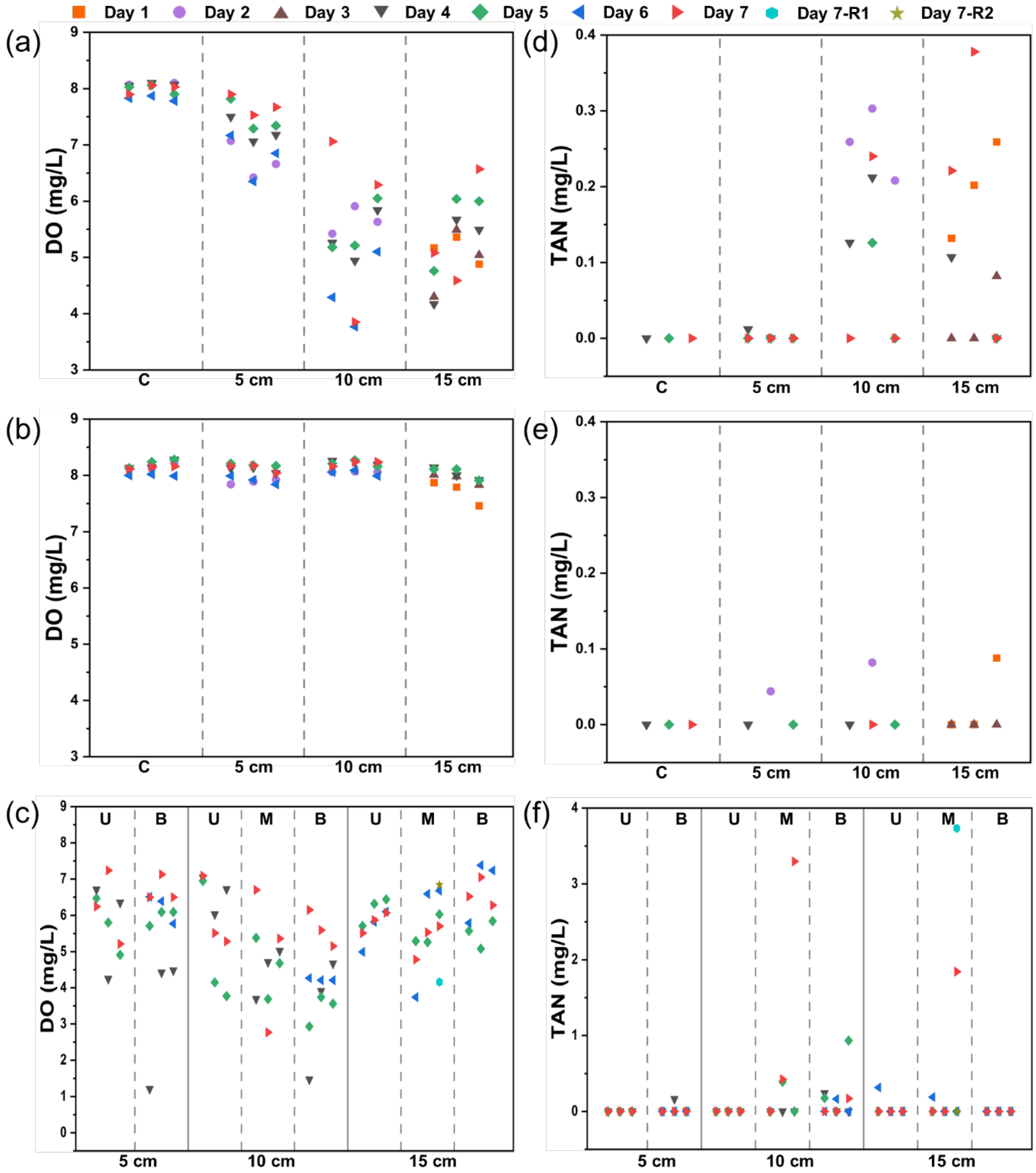


Figure 4-8. Impacts of downwelling vertical water flow direction through the burial layers at different depths on water quality changes when AB were buried, including: changes of surface water (a) DO and (b) TAN in Water-O; changes of surface water (c) DO and (d) TAN in Water Water-I; and changes of pore water (e) DO and (f) TAN in the burial layer at different depths. U: Upper part of the burial layer; M: Middle part of the burial layer; and B: bottom part of the burial layer. AB: Arkansas Brokenray.

process of dead mussels caused the increased conductivity together with dropped DO and raised TAN.

In the case of the water quality of the burial layers, as illustrated in **Figure 4-8 (e-f)**, the DO levels observed from different depths generally fell into the range of 2.5 - 7.5 mg/L, which, compared to upwelling conditions, shows low DO concentrations were more frequently detected in downwelling conditions. Extremely low DO of 1.21 mg/L was documented in the sample collected from the bottom part of the burial layer of R2 of AB-5 cm-D, followed by a 1.47 mg/L DO level also detected from the bottom part of the burial layer of R1 of AB-10 cm-D. Higher TAN concentrations and conductivities were sometime recorded. Specifically, vertical water directions were found to be negatively correlated to TAN ($p < 0.004$) and conductivity ($p < 0.02$) of the collected pore water, where at the same burial depth downwelling conditions might result in lower DO and higher conductivity compared to the upwelling ones. Here, sampling depths did not show a clear effect on the changes of DO, TAN and conductivity (**Figure D-6 (a)**). Nonetheless, mussel death may still be the main cause of the degraded water quality in the burial layer, and the more frequently detected low DO was because there were more dead mussels in the boxes compared to those of the upwelling ones with the same burial depth. Considering that the same burial depth and same refreshing water volumes were used, it is difficult to explain why more mussel died when water went through the burial layer in downwelling conditions compared to upwelling conditions.

4.2.2 Responses of Different Species to Various Burial Events

Tests using AB mussels illustrate that (a) burial layer depth, (b) refreshing water volume, (c) water direction through the burial layer, and (d) timing are critical factors affecting mussels' response and mortality once buried. However, little is known whether other species may act just like AB when facing a burial event. It is highly likely that mussels of different species may behave differently due to various factors including their sizes, living habits, and habitat preferences. For a mussel bed, mussels of multiple species with various sizes and ages may exist at the same time. Therefore, it is of great importance to evaluate the ability of mussels of different species to "escape" from a burial event. Specifically, another seven available species including FT, BF, PM, MK, GF, WB, and DT were selected and tested, with AB being used as a reference. Mussels were still buried with BBS < 5 with an upwelling refreshing water volume of 1 L/h at different burial depths (details in **Table 4-3**).

4.2.2.1 Responses of BF, zFT and PM to Various Burial Depths

BF, zFT and PM were buried with AB and cFT at different depths with BBS < 5, and results are summarized in **Table D-4** and **Figure 4-9**. Though multiple species were examined, it is notable that mussels with relatively smaller sizes (AB and cFT) showed a better ability to get out of the burial layer. At a burial depth up to 12.5 cm, almost all AB and cFT quickly resurfaced within the first two days. Across different tests, only three AB were found remaining buried with two dead (R1 of BF-12.5 cm and R3 of zFT-12.5 cm) and one alive (R2 of zFT-12.5 cm). Overall, the results of AB were consistent with previous tests focusing on AB mussels. Here, cFT emerged a bit quicker than AB and none of them remained buried, suggesting that cFT may have a slightly better capability of unburying themselves once buried. It is hard to explain why cFT performed better, it is possible that their slightly larger size than AB and/or better mobility than AB may help them more successfully and efficiently resurface after being buried.

Compared to AB and cFT, large mussels performed very differently among species when buried. Particularly, BF demonstrated the best ability at unburying themselves, and all of them emerged from up to the 12.5 cm burial layer of BBS < 5. On the contrary, PM only showed the ability to escape from the 5 cm burial layer of BBS < 5. When the depth was increased to 7.5 cm, none of the buried PM successfully reached to the surface with two found dead with significant soft tissue decomposition and one found alive (**Table D-7**). For the depth of 12.5 cm, one PM reached the surface, while the rest were discovered buried near their original locations but alive. In the case of zFT, they performed better than PM as all buried mussels resurface from the 7.5 cm burial layers, however only one of three reemerged when the burial depth raised to 12.5 cm, with two remaining buried but alive (one died one day after it was transferred to the clean water). Although several mussels including some zFT and PM were found buried alive, they failed to climb up and stayed where they located before buried. Therefore, it is rational to predict that they would have died if they were buried longer.

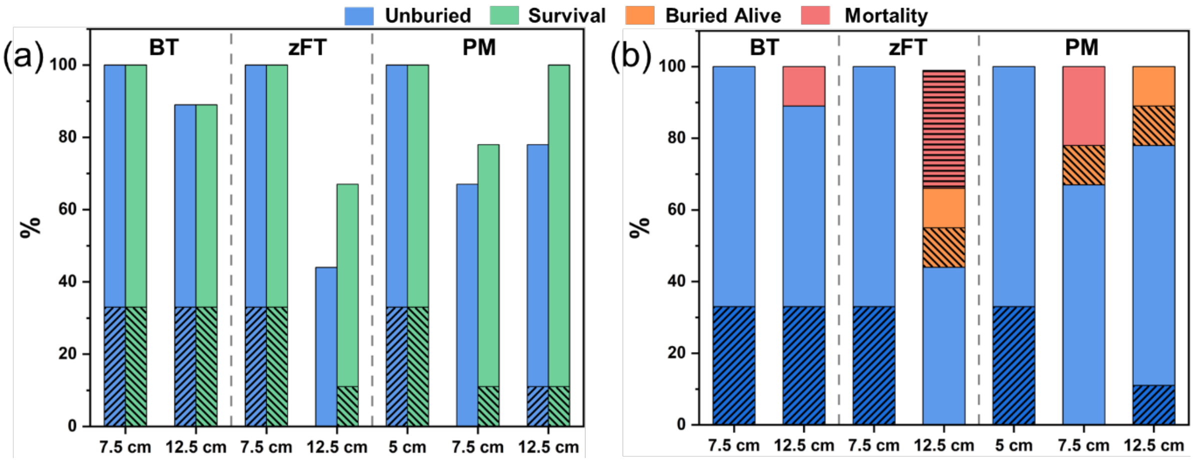


Figure 4-9. Reponses of BT, zFT, and PM towards certain burial depth: (a) % of mussels unburied from the burial layer and % of mussels' survival at different burial depths; (b) Stacking column % of mussels unburied, % mussels Buried Alive, and % Mortality. Shadows represent % of large mussels, including BT, zFT and PM.

Changes of surface water quality were recorded as depicted in **Figure 4-10, Figure D-7** and **Figure D-8**. Similar to results of tests using AB, Water-O samples generally had declined DO and increased TAN with increased burial depth compared to Water-I samples and their controls. Mussel death would worsen the water quality of the surface water above the burial layer (Water-O), where lower DO and higher TAN would be spotted (such as R1 of BT-12.5 cm and R1 of zFT-12.5 cm). Moreover, buried but alive large mussels also could contribute to declined DO and increased TAN, because they would consume DO in the burial layer and release toxicants including ammonia.

Results of pore water quality of the burial layers are summarized in **Figure 4-11** and **Figure D-9**. Changes to DO, TAN and conductivity were closely related to (a) burial depth, (b) sampling location (especially in relation to dead mussels), and (c) buried alive mussels. Specifically, decreased DO and increased TAN occurred more frequently detected with increased burial depths. This is because increased burial depths could result in more mussel death, which the decomposition process would strongly affect DO and TAN levels. For example, water collected from the middle part of the burial layer of R1 of zFT-12.5 cm with a dead zFT had an extremely high TAN of 16.392 mg/L on Day 7, while water from the top parts of R1 and R3 of PM-7.5 cm with dead PMs presented DO < 1.0 mg/L on Day 7. As mentioned earlier, mussel death would also result in sharply increased conductivity. High conductivity around 400 was sometimes recorded in samples collected from a burial layer with dead mussels. Moreover, buried alive mussels could also lower the DO levels as they would consume DO to survive. This could explain why some samples had low DO levels but not necessarily high TAN. For example, relatively low levels of TAN were detected in samples from R1 and R2 of PM-12.5 cm with buried alive PM. It is clear that the main source of TAN is from the decomposition process of dead mussels, while consumption of DO could be attributed to both dead and alive mussels. Meanwhile, this could explain why extremely high TAN concentrations were observed in tests with large mussels when compared to results of tests using AB mussels. The degradation of a mussel with a much larger body size would, no doubt, produce more toxicants including TAN. Thus, the death of large mussels may more strongly affect their surrounding conditions when a burial event occurred.

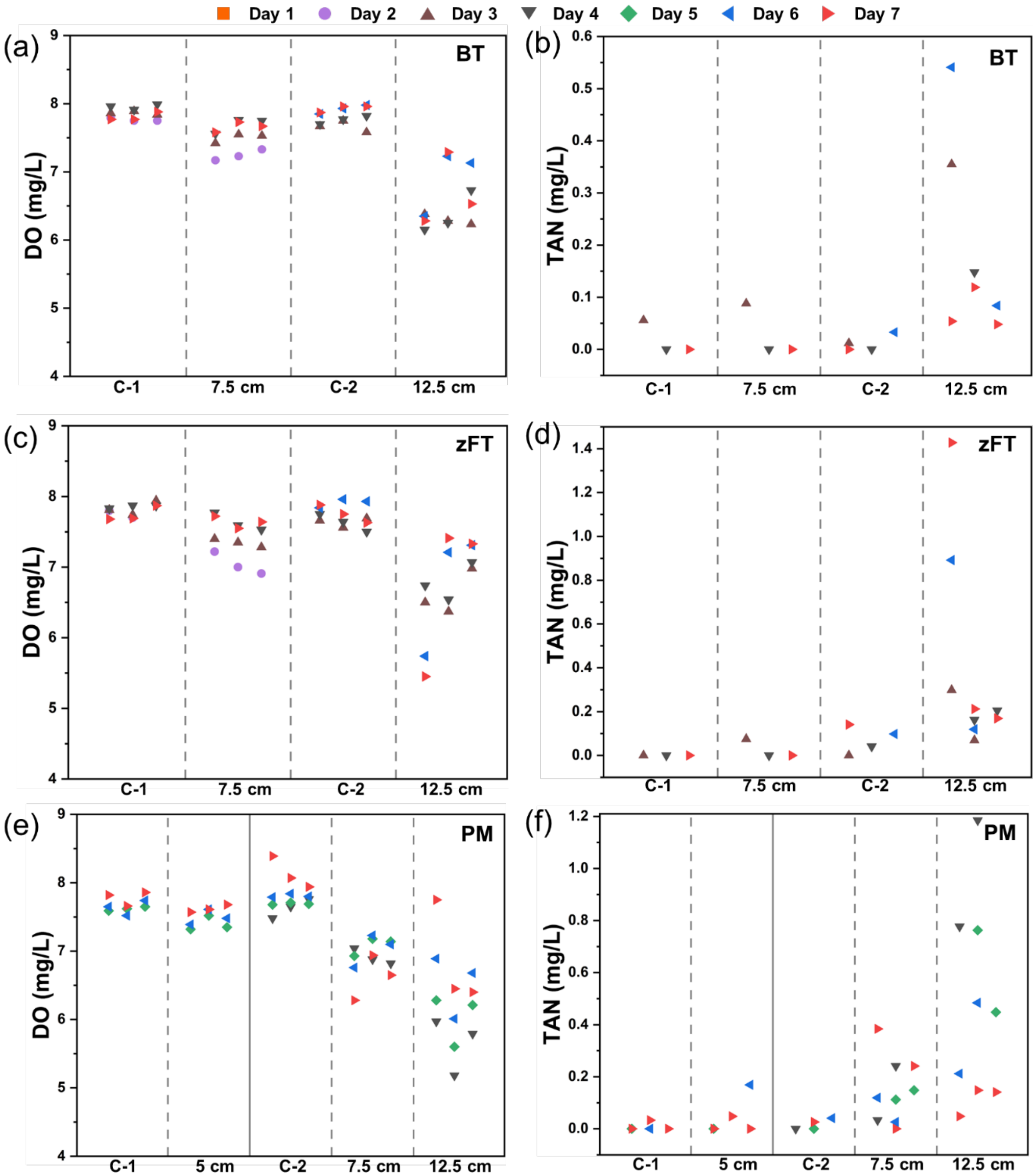


Figure 4-10. Changes of surface water quality when different mussel species were buried, including: changes of surface water (a) DO and (b) TAN in Water-O when BT mussels were buried with AB and cFT; changes of surface water (c) DO and (d) TAN in Water Water-O when zFT mussels were buried with AB and cFT; and changes of pore water (e) DO (e) and (f) TAN in Water-O when PM mussels were buried with AB and cFT. BT: Butterfly; zFT: large Fatmucket from Kansas Zoo; and PM: Pink Mucket; AB: Arkansas Brokenray; cFT: small Fatmucket from CERC.

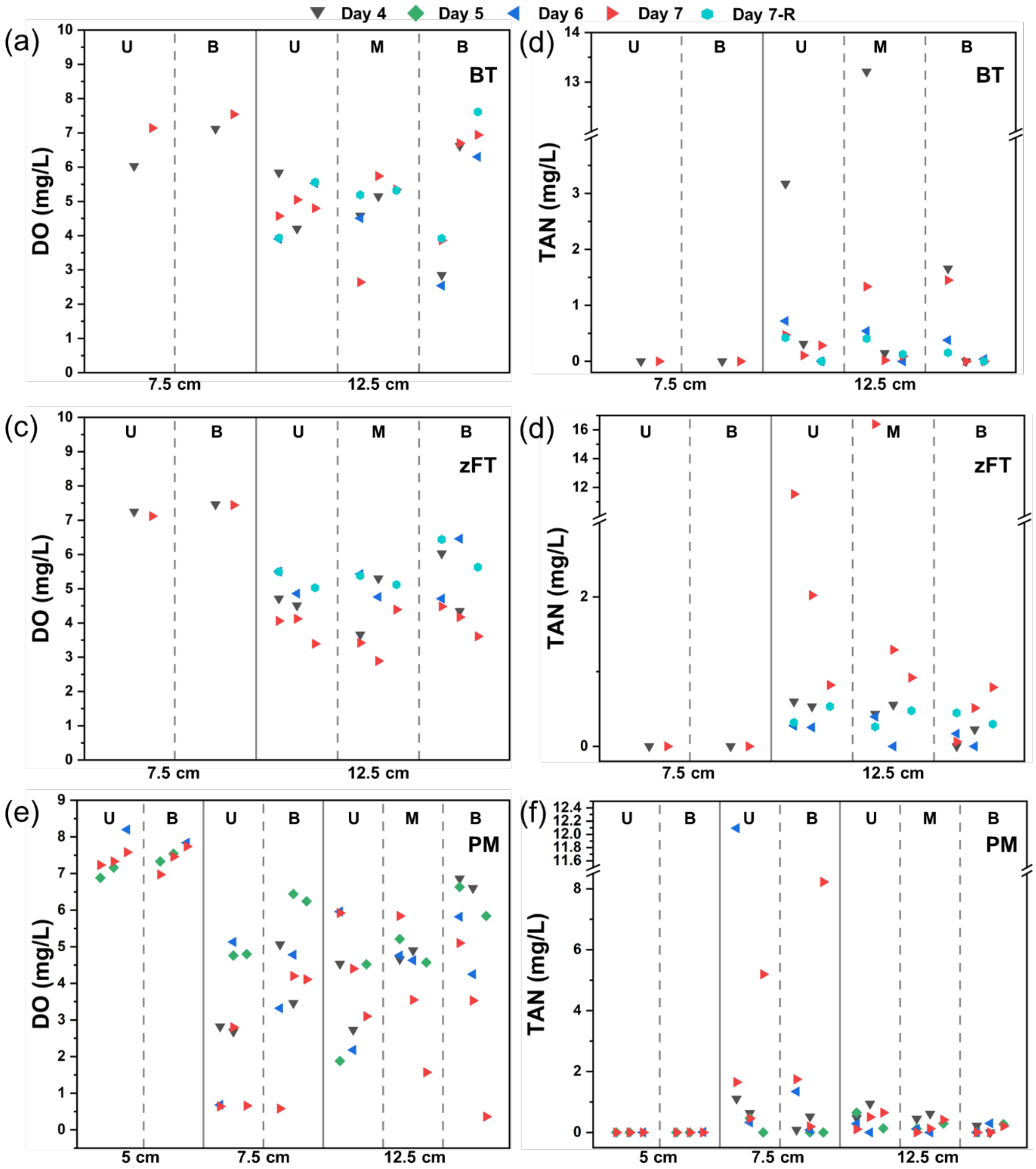


Figure 4-11. Changes of pore water quality in the burial layer at different depths when different mussels were buried, including: Change of DO (a) and TAN (b) when BT mussels were buried; Change of DO (c) and TAN (d) when zFT were buried; and Changes of DO (e) and TAN (f) when PM were buried. BT: Butterfly; zFT: large Fatmucket from Kansas Zoo; and PM: Pink Mucket. U: Upper part of the burial layer; M: Middle part of the burial layer; and B: bottom part of the burial layer.

4.2.2.2 Responses of MK, WB, DT and GF to Various Burial Depths

The ability of four more species, including MK, WB, DT and GF, to recover from a burial event was also studied. As tests using BF, zFT and PM implied that a burial layer of 7.5 cm BBS < 5 may be a challenging depth for some large mussels to unbury themselves, here 7.5 cm was chosen for the tests using MK and WB. GF were buried with 10 cm depth as GF presented better activity with frequent movement when raised in clean water. AB mussels were still used as references and buried together with the large mussels, while DT of different sizes were buried with WB and GF (details in **Table 4-3**).

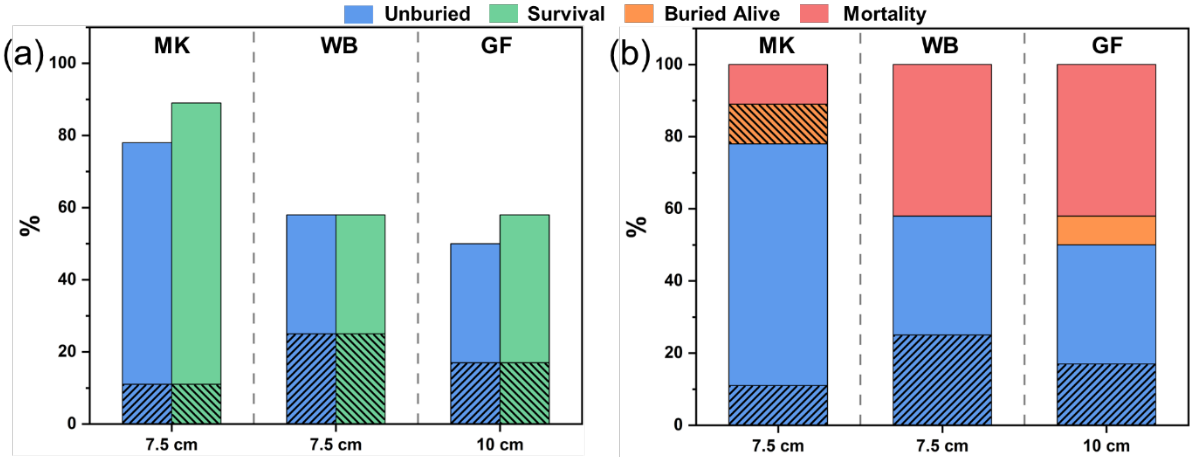


Figure 4-12. Reponses of MK, WB and GF buried with AB, cFT or DT towards certain burial depth: (a) % of mussels unburied from the burial layer and % of mussels' survival at different burial depths; (b) Stacking column % of mussels unburied, % mussels Buried Alive, and % Mortality. Shadows represent % of large mussels, including MK, WB, and GF. MK: Mucket; WB: Washboard; GF: Giant floater; AB: Arkansas Brokenray; cFT: small Fatmucket from CERC; DT: Deertoe.

As summarized in **Figure 4-12** and **Table D-8**, not surprisingly, different mussels performed differently after being buried. For MK, only one of the three successfully reached the surface of the burial layer while the other two remained buried with one alive and another one dead. All buried WB quickly unburied themselves within 24-h. On the contrary, although two of the three GF eventually resurfaced, they appeared much later with one arriving at the surface on Day 2 and the other on Day 4. For smaller mussels buried together with MK, all AB and cFT quickly unburied themselves within 24-h.

However, for those buried with WB and GF, including AB and different sizes of DT, the results were quite unexpected (**Table D-8**). First, although most AB tried to move up, as they were discovered close to the surface of the burial layer, only one of six survived and successfully appeared on the surface (R3 of GF-10 cm). Second, the different sized DT performed differently, with the smaller sizes tending to be more capable of emerging from the burial layer. Two of three mDT (buried with WB) and all three sDT (buried with GF) successfully unburied themselves (most within the first two days, details in **Table D-8**). On the contrary, only one large DT resurfaced (R1 of WB-7.5 cm) with one buried alive (R1 of GF-10 cm, almost recovered with 1-2 cm distance to the burial layer surface). The rest of the large DT were found at different depths of the burial layer, with some almost reaching the surface but eventually dying. Interestingly, the soft tissue of most dead AB and DT had been degraded when they were dug out. This indicates that they died at an earlier stage after they were buried. It appears that they attempted to climb up, but the worsened water quality led to their death.

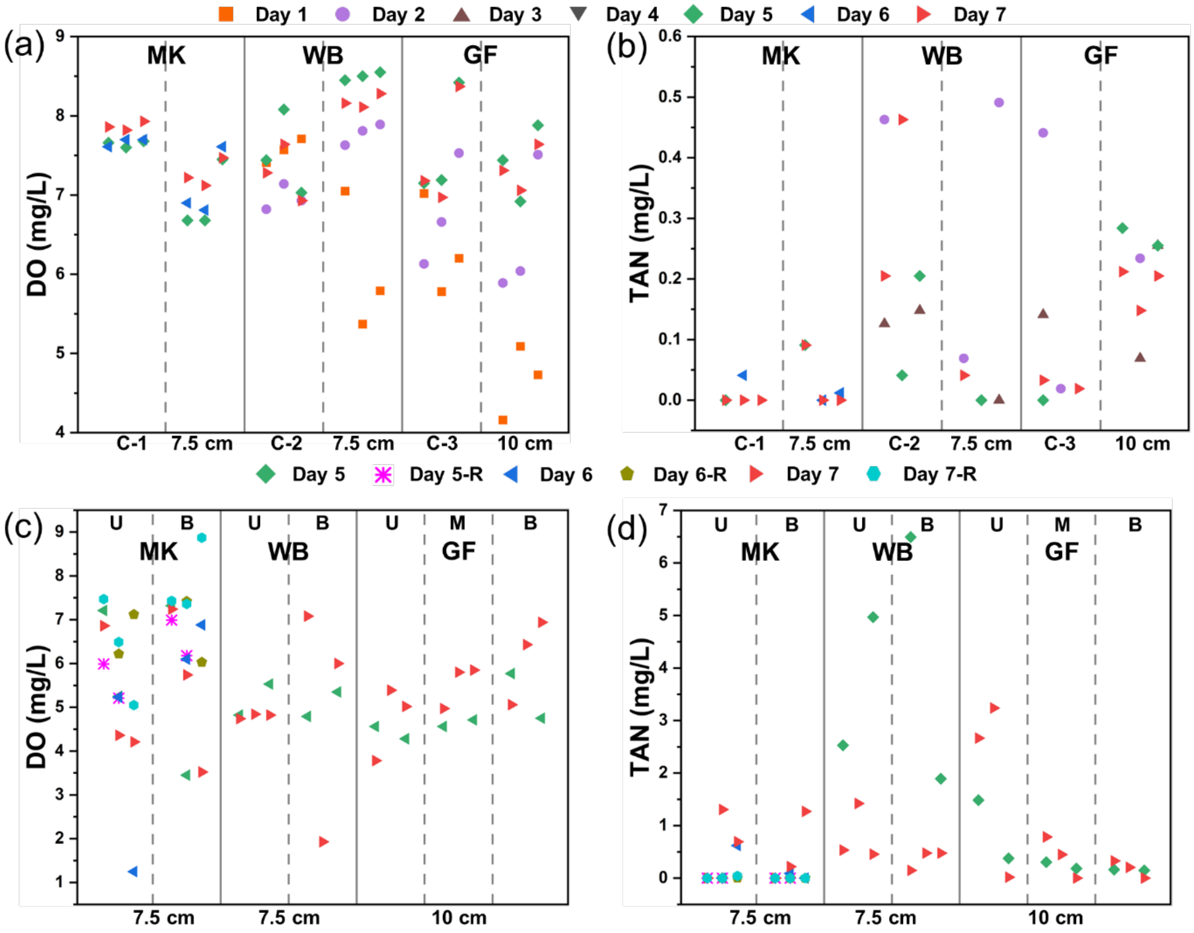


Figure 4-13. Changes of water quality including (a) DO and (b) TAN in Water-O when MK, WB, and GF mussels were buried with AB, cFT or DT; and Changes of pore water quality including (c) DO and (d) TAN in the burial layer at different depth when MK, WB, and GF mussels were buried with AB, cFT or DT. Mucket; WB: Washboard; GF: Giant floater; AB: Arkansas Brokenray; cFT: small Fatmucket from CERC; DT: Deertoe. U: Upper part of the burial layer; M: Middle part of the burial layer; and B: bottom part of the burial layer.

The death of small mussels, especially AB, was not expected as previous results showed that they had the ability to escape from 15 cm of BBS < 5. Here, their death, together with DT, may be partially explained by the water quality changes in Water-O samples and sediment water samples. Compared to other large mussels, even Water-O samples of WB and GF controls (mussels not buried) presented slightly lower DO levels (**Figure 4-13 (a)**), indicating that WB and GF may consume more DO due to their larger body sizes and activity. Similarly, slightly higher accumulated TAN levels (**Figure 4-13 (b)**) were also detected in Water-O samples of their controls, suggesting that their existence could add more ammonia to the environment. Such slightly degraded water quality could adversely affect the smaller mussels, and conditions could become worse once they were buried, with activity of WB and GF deteriorating their capabilities to quickly move up. As discussed earlier, timing is essential for a mussel to unbury itself. If it fails to respond quickly, it might soon lose its chance to successfully escape from the worsened environment. Subsequently, dead mussels would be decomposed, which would, in turn, degrade the water quality around it with further dropped DO and raised TAN (**Figure 4-13 (c)** and **(d)**) in the burial layers, hindering the activity of other mussels. Meanwhile, as pointed out earlier, the detected water quality of the burial layers was related to the sampling depth and location, with water collected near dead mussels more likely to have low DO, high TAN, and increased conductivity (Figure D-12). Mussel death should be the main cause for the locally degraded water quality.

4.3 Discussion

By burying adult AB with BBS<5 at various conditions, impacts of several critical parameters including burial depth, refreshing water volume supplied hourly, and the water flow direction across the burial layer on mussels' response to the burial events were systematically studied with a burial duration of 7-Day.

Burial depth is critical to the survival of AB mussels. Similar to previous work studying freshwater and marine mussels, results of this study demonstrate that for the same type of sediment, the deeper mussels are buried, the lower the possibility that they can reemerge. For example, Marking and Bills found that 90-100% of the three tested species, including Wabash Pigtoe (*Fusconaia flava*), Fatmucket (*Lampsilis siliquoidea*), and Plain Pocketbook (*Lampsilis cardium*), quickly unburied themselves from 5 cm of sand and silt, but the percentages of mussels resurfaced from 20 cm dropped sharply (0-10%, except Fatmucket with 70%) [86]. By burying marine mussel *Cerastoderm* with sand, Jackson et al. found that most mussels successfully resurfaced from 5 cm depth but only a small proportion reemerged from the burial layer of 10 cm [88]. In another study, Hendrick and Last *et al.* observed that around 25.9% of queen scallops (*Aequipecten opercularis*) resurfaced from 2 cm of sediment (commercial), while when the burial depths increased to 5 cm and 7 cm, all tested scallops failed to emerge [95].

Among studies, one common finding is that for mussels capable of resurfacing, they usually reach to the surface quickly after being buried, and the majority do so within the first 2 days [86, 88, 98]. With the extension of burial time, those remaining buried quickly lose their ability to reemerge from the burial. The consequence of failing to reemerge from the burial layer is lethal. Although some mussels were still alive after being buried for some time, many were found dead, especially when the burial duration was longer [89, 95, 96]. Therefore, survival of buried mussels is highly dependent on their ability to emerge from the burial layers. With increased burial depth and burial duration, the opportunity for mussels to resurface becomes lower and lower, which will ultimately result in mussel death. The increased burial depth creates increased overburden pressure, which can result in a decrease in porosity and void ratio that affects the permeability due to the increased consolidation of burial layers [133]. Increased depth with declined porosity and permeability would lead to reduction of available oxygen-saturated porewater around buried mussels, which subsequently increase the probability of suffocation before they make their way to the surface [134]. Meanwhile, the diffusion of toxic molecules, such as ammonia, would also be limited, and mussels may be exposed to locally high levels of toxics. Thus, with increased depth, mussels buried may face not only increased overburden stress, the direct mechanical stress, hindering their ability to move vertically, but also degraded water quality, and indirect respiratory stress. Those that failed to "overcome" such stresses would then die and their death would, in return, further degrade the water quality, leading to more death.

For mussels living in rivers and streams, mussel distribution and abundance may be strongly affected by the properties of the sediment-water interface, especially, hyporheic exchange. Hyporheic exchange is defined as the volumetric flow of water across the sediment-water interface either in an upward or downward way due to the hydraulic gradients resulting from the interaction of in-stream flow and the topography of the riverbed [131]. Although it remains largely poorly understood, there have been studies focusing on the complex relationships between benthic fauna and the hyporheic conditions of difference species [135, 136].However, few involve freshwater mussels [131, 132, 137]. In 2007, by working on 26 streams in seven European countries, Geist *et al.* observed that the recruitment of freshwater pearl mussels (*Margaritifera margaritifera*) would improve with increased hyporheic exchange [137]. Similarly, Norbury stated that riffle tails, where water ejects in an upwelling way, are the prime sites for reintroduction of freshwater pearl mussels. In a more thorough study done by Klos *et al.*, the impacts of various hydrogeomorphic variables, including direction and range of hyporheic exchange on population distributions of adult mussels of the middle Allegheny River at five reaches, USA, were evaluated. Interestingly, they found that the density of mussel population increased with increased upward hyporheic exchange but decreased hyporheic exchange variability [131]. Several mechanisms were accordingly proposed: (a) increased hyporheic exchange would enhance circulation of interstitial waters around mussels which would result in higher levels of DO; (b) upward hyporheic exchange with input of regional-scale groundwater will contribute to the upward hyporheic exchange and thus change the water chemistry or temperature regime which may be beneficial to mussels; (c) increased groundwater input may increase levels of beneficial nutrients like Calcium while dilute levels of toxic chemicals like ammonia [131].

Therefore, though rarely studied so far, water supply through the burial layer is another essential factor affecting mussels' survival once buried. Most previous experimental designs ignored the fact that under real scenarios, water may flow through the burial layer from multiple directions, not only horizontally but also vertically. In previous studies, some attempted to create turbulence of the overlay water, but none of them considered the water vertically through the burial layer. For the first time, to investigate the potential effluence of vertical water across the burial layer on mussels, a design that allows water to move vertically (upwelling or downwelling) was proposed in this study. As expected, water volume vertically through the burial layer is important to mussels' survival, as with declined water volume supplied, remarkably fewer AB mussels could resurface. However, it is surprising to note that water direction also affects AB mussels' ability to reemerge once buried. Specifically, results here suggested that for buried AB musses, it was easier to escape from the burial layer with upwelling vertical water going through than the downwelling conditions.

Such results, to some extent, are consistent with the discoveries of some previous studies. For example, Klos *et al.* found areas with upwelling water through the substrate may be a more beneficial choice for mussels [131], while by linking existence of Clubshell mussels (*Pleurobema clava*) with groundwater movement, Roley *et al.* concluded that Clubshell mussels presently and historically inhabited sites with upwelling groundwater [138]. However, it is difficult to determine how upwelling water assists AB mussels during their journey to reemerge, as degraded water quality in the burial layer with reduced DO and increase TAN was still observed just like observed in the downwelling ones. It is possible that water flow downward might be extra pressure in addition to overburden stress due to the burial layer itself that mussels need to endure. Another possibility is that, as illustrated by the changes of DO levels in the burial layers, the bottom part of burial layers with upwelling water usually had higher DO concentrations probably because freshwater water would first encounter the bottom when supplied, providing mussels buried at bottom with more oxygen and diluted toxics. Thus, mussels might be less stressed and could thereby respond quickly and migrate vertically. Once they started the vertical migration, the pressure due to the burial would decrease due to the declined burial burden, which, accordingly, gave them a higher chance to resurface. On the contrary, when water was supplied the other way (downwelling), the supplied water would reach to the top layer first and the bottom layer last, where DO might have been partially depleted, and thus the bottom part of the burial layers remained low while the dilution effect also became less significant. Thus, mussels might be more stressed even when just buried due to the burial burden and the degraded water quality, which limited their ability to move up. Nonetheless, although both water volume supplied and water direction were shown to be critical to mussels' ability to reemerge and survival, this study was not able to verify these assumptions and more future research is desired.

By comparing the responses of various species tested in this study, it is worth noting that mussels of different species might react differently towards burial events, and here Pink mucket (the endangered species) turned out to be the most vulnerable species when under burial layers of BBS < 5. Similar results showing that different spices may perform very differently after being buried have been previously reported for both freshwater mussels and marine mussels. Among the four species tested (*Pyganodon grandis*, *Ligumia recta*, *Fusconaia flava*, and *Flasmigona costata*), Imlay observed that Giant Floater (*Pyganodon grandis*) overall presented the best ability to resurface from the various materials (detritus, river sand, lake sand, sand/clay mixture, silt and grit) tested than the other three [85]. When burying Wabash Pigtoe (*Fusconaia flava*), Fatmucket (*Lampsilis siliquoidea*), and Plain Pocketbook (*Lampsilis cardium*) with 25 cm of sand and silt, Marking and Bills noticed that around 70% of Fatmucket mussels could successfully and quickly reemerge but only 0-10% of the other two species made it [86]. In the case of marine mussels, Hendrick and Last found that *Mytilus edulis*, with a smaller size, were observed to have a superior ability to unbury themselves, compared to larger *Modiolus modiolus*, from shallow burial layers and coarse sediments [139]. The differences among species may be due to various factors, including the behavioral and morphological features of each species (such as shell thickness, body size, shape, and mobile characteristics) [83, 86, 134, 140], different physical habitat requirements (such as tolerance to substrate stability and water flow) [83, 140], and tolerance to degraded physiological conditions (such as degraded water quality) [134]. It was found that species living in a habitat where sediment movement is naturally frequent may have a better ability to reemerge from the burial events [134, 139]. Particularly, species with good mobility might migrate up quickly to reach the new sediment-water interface, and thus have the higher chance to survive after being buried [134]. When both *Modiolus modiolus* and *Mytilus edulis* were buried under the same condition, Hutchison *et al.* pointed out that only *Mytilus edulis* reemerged with help of the increased production of byssus which could attach on vertical surface and sediment particles [139]. Meanwhile, species with better tolerance to degraded water quality, such as low oxygen stress and high ammonia level, may also have a higher chance of survival. However, with limited data available about the differences among various species, it is challenging to deduce which characteristics may play more essential roles in deciding mussels' ability to respond to burial events and to resurface from the burials. Because a mussel bed is usually composed of multiple species, it is important to attempt to document the responses of as many species as possible towards the burial events.

So far, some possible mechanisms have been proposed to explain the mussels' mortality when buried. First of all, mechanical stress due to the deposition layer (overburden stress) is one cause of mortality, as the burden may be too great for mussels to migrate vertically [93]. Another mechanism that has been discussed is that sediment deposition layers may smother mussels once buried. As early as the 1930s, Ellis stated that the layers of silt that rapidly blanket the stream bottoms would smother out the existing fauna leading to mortality [84]. Moreover, many other studies pointed out that sediment burial, especially fine sediment, would result in smothering of mussels [65, 85, 141]. Fine particle deposition can clog the interstitial space within the sediment of a river or stream which reduces water circulation and accordingly affects the chemical conditions in the sediment, including reduce the oxygen levels [67, 138, 142-144]. Once the reduced oxygen levels become too low for mussels' survival, mortality would occur [67]. Reduced DO levels in the burial layer were clearly observed in this study as well as in other studies working on both freshwater mussels and marine mussels, highlighting that it is a critical stress factor affecting mussels' survival when buried. The reduced water circulation due to sedimentation also results in the accumulation of toxicants, such as ammonia, in the sediment [138]. Ammonia is a contaminant of special concern for freshwater mussels, especially juveniles, as they are sensitive to ammonia toxicity [107, 145]. Increased sedimentation can inhibit the diffusion of ammonia to the overlying surface water and provide more binding surface area for particles [146, 147]. Thus, pore-water ammonia levels tend to be higher than the levels in the overlying surface water [67, 148]. Therefore, in addition to the declined DO levels, elevated ammonia levels may be another important stressor affecting mussels' survival [138].

Unfortunately, most studies on the impacts of sediment deposition on freshwater mussels did not evaluate the changes of sediment water quality, making available data very rare. In the case of marine mussels, Maurer *et al.* analyzed the changes of pore water chemistry including DO, ammonia and sulfide in the simulated dredge, where significant changes were observed between overlying water and the pore water within 15-days [91]. Specifically, they observed that the chemistry of overlying water was overall very constant over time, while decreased DO, increased ammonia, and increased sulfide in pore water in any sediment type tested compared to the overlying water. The changes of pore water DO was suggested to be caused by the organic decomposition via oxic respiration, while ammonia and sulfide increases were due to remineralization of organic matter by bacteria within the sediment via sulfate reduction [92]. Though during these tests, no animals were buried in the sediments and their contribution to the changes of pore water chemistry were not evaluated, the authors proposed that the changes of pore water chemistry may lead to vertical migration of the benthic communities through avoidance or deleterious responses. Different from those tests, in this study, the chemistry changes of both pore water and overlying surface water were analyzed while mussels were buried. Here, although extremely high ammonia levels were frequently detected in the burial layers, they were found to strongly be related to the occurrence of mussel death, indicating that the decomposition process of mussels may be the main source of ammonia. On the contrary, when pore water was sampled far from dead mussels, the ammonia level would be much lower. Such differences between this study and less effective diffusion to the surface water in the Maurer *et al.* study could probably be caused by (a) the different experiment design where vertical water flow through the burial layer was supplied in this study to mimic the hyporheic flow of real conditions; (b) the organic content was much higher in their study which could largely deplete oxygen and release ammonia. Nonetheless, as shown by both studies, water chemistry of the burial layer would become worse and worse within increased burial depth and extended burial duration, while mussel (as well as other organism) death would further worsen the situation. The degraded water quality thereby strongly impairs mussels' ability to resurface, especially for those that failed to respond quickly and migrate immediately.

In addition to the mechanical stress and degraded water quality, another factor that strongly affects mussels' capability to reemerge from burial events is the differences among species. Mussels may be different in habitat preference, tolerance for the low DO, shell thickness, body shape, and mobility, and all those may influence their response to burial events. Mussels may take different strategies once buried. Some may quickly respond and migrate vertically, while others may decide to reduce activity to attempt to overcome the worsening environment. This explains why for the same species, some quickly escaped while others stayed where they originally located. It has been widely known that different mussel species have different tolerance for low DO, and the most tolerant species generally occur in lentic or other low-oxygen habitats [149]. However, such database towards each species tolerance for DO, as well as ammonia and other toxicants, has not been established, making the comparison among species challenging.

In short, the response of freshwater mussels to burial events may be a synergistic combination of various mechanical, chemical, and biological factors. Burial depth and duration, water supply through the burial layer, changes of water quality in the burial layer, and characteristics of mussels may work together affecting their ability to withstand and survive the deteriorating environment. However, the limited data available makes it difficult to determine which factor(s) may be the most essential to mussels' capabilities to survive and to successfully resurface, and thus more relevant studies are needed.

4.4 Summary

In this study, the responses of various mussel species were examined using sediment (particle size < 5 mm, BBS < 5) collected from Boubous River, MO. Different from all previous studies, for the first time, a new design which allowed the vertical water flow, either upwelling or downwelling, through the burial layers were proposed. Using this flow-through design, the potential effects of vertical water flow, which mimic the hyporheic water flow connecting the underground water and surface water through the sediment in the real-life scenario, were investigated. Using Arkansas Brokenray as a model animal, critical factors, including burial layer depth, vertical water flow volume, and vertical water flow direction, that affect mussels' ability to resurface and survive were examined. Not surprisingly, with increased burial depth and decreased vertical water supplied, fewer mussels were capable of reemerging from the burial layers and thereby increased mortality was observed. However, it was not expected that vertical water flow direction could also affect the responses of mussels to the burial event, however, downwelling water seems to be a worse condition for mussels to survive in once buried than upwelling water. By testing the ability of different mussel species to unbury themselves from BBS < 5, clear differences among species were discovered. Pink Mucket was found to be the most vulnerable species to a burial event, and a layer of 5 cm BBS < 5 could inhibit their capability to resurface and result in significant mortality.

It is critical to point out that the observed mortality of mussels may be synergistically caused by several stress factors, including the overburden stress due to the burial layer, the degraded water condition in the burial layer (lower DO and higher ammonia), and the ability of different mussels to overcome different stresses. More importantly, mussels themselves could be another factor threatening the survival of others once they die. This is because their decomposition could strongly worsen their surrounding environment by causing extremely low DO and high ammonia. Therefore, when the burial layer is thinner or more vertical water is supplied, the sufficient exchange of water between the burial layer and the overlying surface water could benefit mussels' survival and probably provide a better chance of them resurfacing. On the contrary, a thick burial and low water supply may strongly inhibit mussels' reemergence and accordingly result in mussel death, which further worsens the scenario.

There are several limitations in this study, which require additional work to be done to better reveal the relationship between burial events and mussels' survival. First, a limited number of mussel species and a limited number of experimental conditions were tested due to mussel availability. Future studies with more mussel species under more conditions are desired. Second, although a novel design was proposed, it mainly focused on the effects of vertical water flow through the burial layer but not horizontal water flow through the burial layer or in the surface water. Moreover, here water is periodically supplied rather than continuously, which may only represent certain conditions. However, the water flow through the mussel bed/habitat can be very complex, and a better design may be a system that not only provides continuous vertical water flow but also horizontal surface water flow, which mimics more complex conditions. Third, only relatively clean sediments from the Boubous River were tested, however the properties of sediments would also affect mussels' response once buried. Sediment with high organic content may be a more challenging scenario for mussels once buried, and particle size distribution can also impact mussels' response, with very fine and sticky particles strongly hindering water exchange potentially being more lethal than those sediments with coarse particles. Fourth, mussel density may be a parameter affect mussels' survival once buried as the denser mussels are buried together, the quicker the oxygen depletion and ammonia accumulation would occur, which may more quickly lead to mussed death and worsen the water quality condition. Here only one mussel density (except when Deertoe was used) was tested, and it is necessary to consider a situation when a high mussel density exists.

Although with many limitations, results of this study are still able to provide essential information towards (a) understanding of mussels' response once buried, and (b) conservation purposes. Specifically, it is rational to conclude that sediment deposition on a mussel bed/habitat may be lethal depending on the thickness (and composition) of the deposition layer, the hydraulic conditions of the location, and the composition (and density) of the mussel bed. It is thus important to avoid sediment deposition on mussel bed/habitat during construction as well as during some other natural processes. If deposition is not avoidable, it is critical to make sure that the deposition layer would be thin enough to allow most mussels to quickly resurface, or to relocate the mussel bed to another location that would not be impacted by the sediment deposition.

Chapter 5. Evaluating the Exposure and Burial Zone for Freshwater Mussels from Point-Source Sedimentations

5.1 Model Development

5.1.1 Lagrangian Particle Tracking Model

To understand the impacts of an acute sedimentation event on freshwater mussels, as could occur during a construction project, a Lagrangian particle tracking (LPT) model was applied to predict the deposition of sediments in streams and rivers. The tracking equation for individual sediment particles are:

$$x(t+\Delta t) = x(t) + (U(t,x_i) + u'(t,x_i))\Delta t \tag{5-1}$$

$$y(t+\Delta t) = y(t) + (V(t,x_i) + v'(t,x_i))\Delta t \tag{5-2}$$

$$z(t+\Delta t) = z(t) + (W(t,x_i) + w'(t,x_i))\Delta t \tag{5-3}$$

where x_i is the location of the sediment particle with the subscript i = 1, 2, 3 representing the three directions in the Cartesian coordinate system (i.e., $x = x_1$, $y = x_2$, $z = x_3$), tracked for each time step Δt; U, V, and W (collectively U_i) are the time-averaged velocities in three dimensional space; u', v', and w' (collectively u_i) are turbulent velocities; Vs is the terminal settling velocity of the sediment, which will be discussed in **Chapter 5.1.2**.

Typically, U_i can be obtained from measurements in the streams or using hydrodynamic models. Turbulent velocities u_i are instantaneous fluctuations around U_i, which are determined using a Markov-chain continuous random walk (CRW) model [150-152]:

$$u_i'(t+\Delta t) = u_i'(t)\exp\{(-\Delta t/\tau_i)\} + \sigma_i(1-\exp\{(-2\Delta t/\tau_i)\})^{1/2}\xi_i \tag{5-4}$$

where ξ_i is a standard Gaussian white noise, which is used to model the velocity fluctuations with the mean of zero. The Markov-chain CRW model is the explicit solution for the stochastic differential equation that governs the turbulent velocities, known as Langevin equation:

$$\frac{du_i'}{dt} = -c_1 u_i' + c_2 \xi_i \tag{5-5}$$

where the coefficients c_1 and c_2 are given by the Lagrangian time scale of turbulent eddies (τi) and the root mean square (RMS) of the instantaneous velocity in each direction (σi): $c_1 = 1/\tau i$ and $c_2 = \sigma i\ (2/\tau i\ )^{0.5}$ [153].

5.1.2 Sediment Settling Velocity

The terminal settling velocity of sediment in stationary water is determined from the balance of buoyancy, gravitational forces, and the drag force:

$$(\rho_p - \rho)g\frac{\pi d^3}{6} = \frac{1}{2}C_D\rho V_s^2\frac{\pi d^2}{4} \tag{5-6}$$

Where ρ and ρ_p are density of water and sediment particle, respectively, g is gravitational acceleration, d is equivalent spherical diameter, C_D is drag coefficient, and Vs is the terminal settling velocity of the sediment. The drag coefficient for natural sediment particles is determined [154]:

$$C_D = [(\frac{32}{Re_p})^{1/1.5} + 1]^{1.5} \tag{5-7}$$

where particle Reynolds number is defined as $Rep=Vs\, d/\nu$ with ν being the kinematic viscosity of water. Combining **Equations 5-6** and **5-7** the terminal settling velocity of sediment particles can be computed. **Figure 5-1** illustrates the validation of the settling velocity calculation using literature reported data synthesis [154], and experimental data [155, 156] which reproduced and summarized additional experimental data [157-160]. The validation shows satisfactory results that cover the sediment diameter over four orders of magnitude (10^{-6}~10^{-2} m).

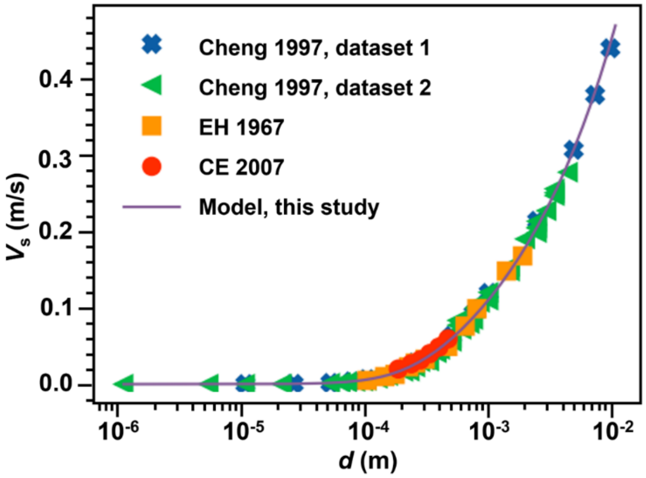


Figure 5-1. Comparison between the measured sediment settling velocity with the calculated value in the model as a function of diameter. The data include those reported in Cheng (1997) [161] (dataset 1: [158]; dataset 2: [157, 159, 160], EH1967 in the legend[155], and CE2007 in the legend [156]).

5.1.3 Model Validation

5.1.3.1 Experiment Condition

The sedimentation model in stream flows is validated using the experimental data collected by Cuthbertson and Ervine (2007) [156] in an open channel flume. Cuthbertson and Ervine (2007) [156] released two grades of fine sediment (median diameters d_{50} = 250 and 97 μm) at the water surface with water depth of 0.093 - 0.143 m and the flow velocity within 0.34-0.68 m/s. Several flow velocities were carried out to test the mean flow profile and turbulence over three different bed materials (uniform glass spheres, coarse river gravel, and finer crushed gravel), resulting in the Froude number (F_r) ranging from 0.36-0.60 and shear velocity (u_*) from 0.034 - 0.05 m/s. The deposited sediments were collected from the bed traps so that the distribution of sediment deposition along the longitudinal direction was obtained. For the model application, the sediment deposition data reported in Cuthbertson and Ervine (2007) [156] was used, where the flow depth was kept at 0.111 m and mean flow at 0.603 m/s (personal communication, Cuthbertson).

5.1.3.2 Flow Characteristics

The model uses a log-law expression for the mean flow velocity over a rough wall [156, 162]

$$\frac{U}{u_*} = \frac{1}{\kappa}\ln\left(\frac{z}{k_S}\right) + B_r \tag{5-8}$$

where z is distance from the bed, κ=0.41 is the von Kárman constant, Br is a coefficient and kS is Nikuradse roughness height of sand. Cuthbertson and Ervine (2007) [156] reported k_s = 0.8 - 1.5 cm for uniform glass sphere bed, and 3.9-4.3 cm for coarse gravel bed. Kironoto and Graf (1994) suggest B_r = 8.5 ± 15% [162].

The comparison of the mean velocity profile between the equation used in this model and the measured data for two reported uniform glass sphere bed cases in Cuthbertson and Ervine (2007) [156] is given in **Figure 5-2**. The experimental conditions are as follows: (1) Case 1, water depth H = 0.143 m, flow rate Q = 0.029 m^3/s; (2) Case 2, H = 0.093 m, Q = 0.016 m^3/s. The comparison shows a satisfactory agreement with a root-mean-square-error (RMSE) of 3.6 and 5.9 cm/s in cases 1 and 2, corresponding to 5.3% and 10.4% of mean flow velocity, respectively. The vertical profiles of turbulent velocity scales are calculated using open channel theory in the model:

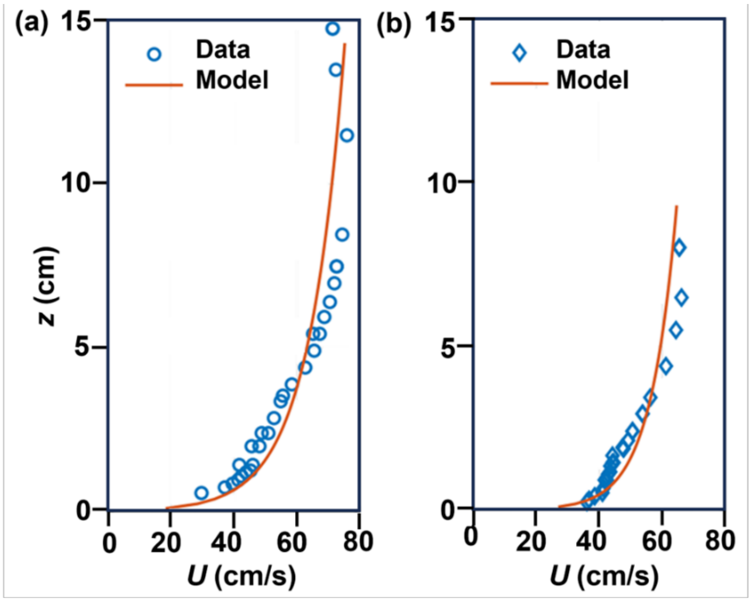


Figure 5-2. Comparison of mean flow velocity measured in the experiment and the equation used in the model: (a) case 1: water depth H = 0.143 m, flow rate Q = 0.029 m^3/s, Froude number Fr = 0.57. ks = 0.015 m, u_* = 0.047 m/s, and Br = 10.5 are used in the model; (b) case 2: H = 0.093 m, Q = 0.016 m^3/s, Fr = 0.60. ks = 0.008 m, u_*= 0.034 m/s, and Br = 13 are used in the model.

$$u'_{rms}/u_* = D_u \exp\left(-C_k z/H\right) \qquad (5\text{-}9)$$

$$v'_{rms}/u_* = D_v \exp\left(-C_k z/H\right) \qquad (5\text{-}10)$$

$$w'_{rms}/u_* = D_w \exp\left(-C_k z/H\right) \qquad (5\text{-}11)$$

where u'_{rms}, v'_{rms}, and w'_{rms} are the root-mean-square of turbulent velocity fluctuations in x, y, and z directions, known as turbulence intensities. Coefficients C_k = 1.0, D_u = 2.30, D_v = 1.27, and D_w = 1.63 [163]. The turbulence intensities represent the characteristic velocity scales of turbulence in each direction.

Turbulent intensities of two cases (Case 1: uniform glass sphere bed, water depth H = 0.143 m, flow rate Q = 0.029 m^3/s; Case 4, coarse gravel bed, H = 0.143 m, Q = 0.022 m^3/s) were reported in Cuthbertson and Ervine [156], which are compared with the model calculation in **Figure 5-3.** The comparison shows satisfactory results with the calculated streamwise turbulent intensities agreeing better to the measured data, while the calculated vertical turbulent intensities slightly overestimate the measurement.

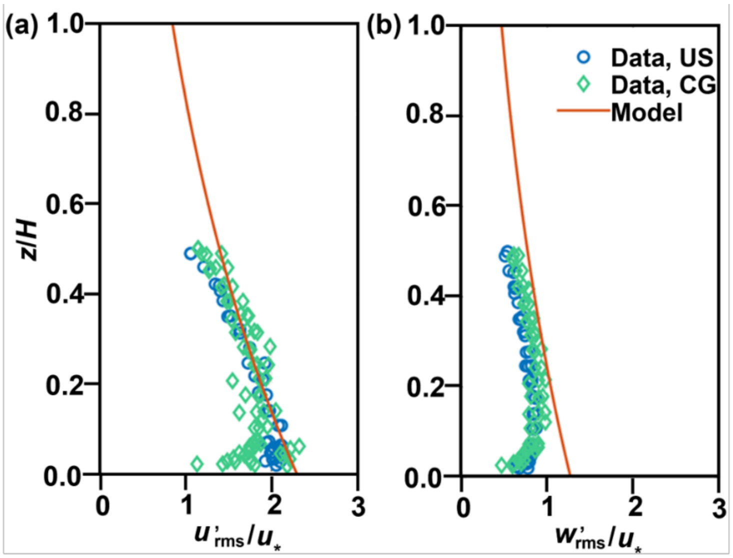


Figure 5-3. Comparison of turbulent intensities measured in the experiment and the equation used in the model: (a) streamwise component (b) vertical component. Both velocity scales are normalized using the shear velocity, and height above bed is normalized using the flow depth. The legend of 'US' and 'CG' represents uniform sphere (case 1) and coarse gravel (case 4) in Cuthbertson and Ervine (2007) [156].

5.1.3.3 Sediment Deposition Distribution

In **Figure 5-4**, the model predicted deposition distribution of unsieved Loch Aline grade sands (diameters of d = 125 - 625 µm, d_{50} = 250 µm) with that measured along the streamwise direction in the flume (Cuthbertson and Ervine, 2007) were compared [156]. In this model, 150,000 individual sand particles were generated using a log-normal distribution (µ = 5.52, σ = 0.4, in µm), which gives the median diameter as 249.6 µm. Particles that are not in the range 125 µm ≤ d ≤ 625 µm are removed from the modeling. The modeling results show a reasonable agreement to the measured data in predicting the probability density function (PDF) of the sediment deposition (RMSE = 0.026 m^{-1}).

Cuthbertson and Ervine [156] also dried and sieved the sediments, which yields a plot of PDF of six individual sieved sand fractions (**Figure 5-5**). The results indicate that larger sediments settle closer to the source with a higher settling velocity, whereas smaller sediments have longer residence time in the water column and therefore are advected further and spread out in a wider distribution because of dispersion process. Despite some deviation, the model predicted PDFs are in a reasonably good agreement to the measured data with RMSE being 0.076, 0.077, 0.089, 0.083, 0.133, 0.207 m^{-1} for six size ranges with increasing diameter, respectively.

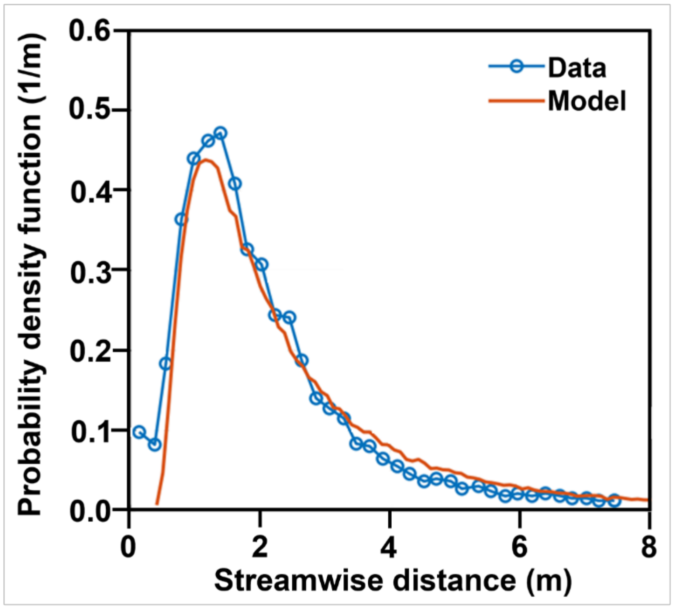


Figure 5-4. Probability density function of sand deposition along the streamwise direction for unsieved sands (125 μm ≤ d ≤ 625 μm).

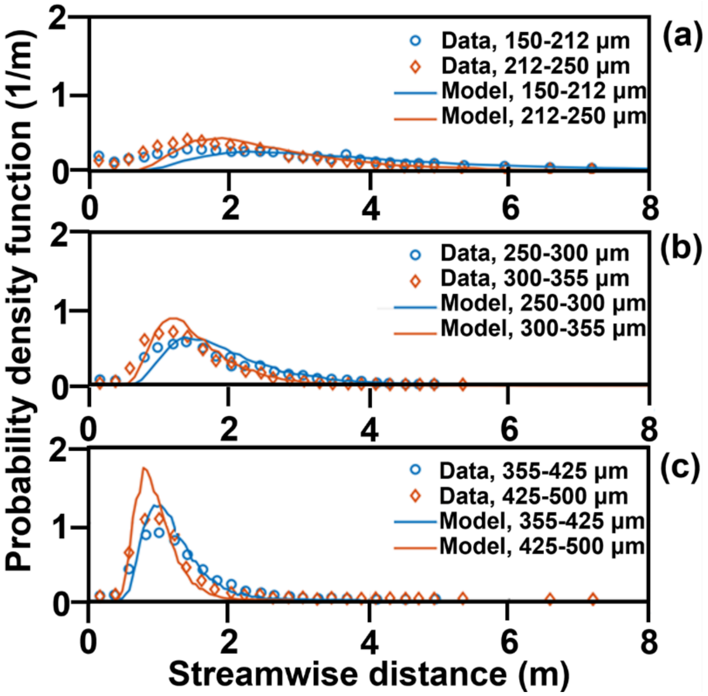


Figure 5-5. Probability density function of sand deposition along the streamwise direction in each sediment class of sieved sands.

5.2 Model Application in Missouri Sensitive Aquatic Streams Reaches

5.2.1 Study Streams

This research focuses on the stream reaches in Missouri where sensitive aquatic species are affected [164]. These species include all mussel and hellbender species with a federal status (endangered, threatened, and proposed endangered). Hydraulic data from 49 out of 62 USGS gaging stations located on these streams was analyzed (**Figure 5-6**). These stations were selected because field data of velocity, flow depth, and channel width and cross-sectional area were routinely collected. Because these velocity and stream geometry data are temporally discrete, these data were analyzed by combining the continuously recorded gaging station data to extract input parameters (i.e., mean flow velocity and depth) for the modeling analysis.

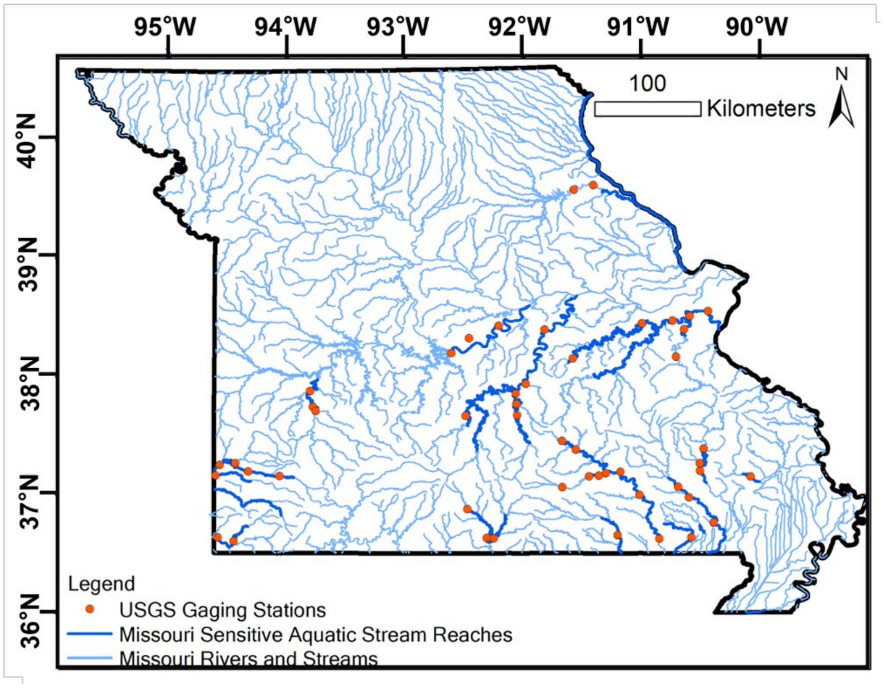


Figure 5-6. The selected 49 USGS gaging stations on the Missouri Sensitive Aquatic Stream Reaches, Missouri, USA

5.2.2 Measured Hydraulic Data

The field data were examined for several purposes. First, the measured mean flow velocity, discharge, and channel geometry (width and cross-sectional area) was used to calculate the average flow depth for each stream. This allowed for investigation of the quantitative relationships between flow velocity and depth, as well as discharge and depth. Second, the field-measured discharge data with that determined from USGS gauges were compared to assess if field measurements could capture the entire range of stream hydraulics over long-term monitoring.

5.2.3 Determining Model Parameters from Hydraulic Data

Time-averaged flow velocity for uniform equilibrium flows in open channels can be approximated using a power-law relation [161]

$$\frac{u}{u_{max}} = (\frac{z}{H})^{1/m} \tag{5-12}$$

where u is time-averaged flow velocity, $u_{\max}$ is the maximal velocity, z is the distance from the stream bed, and H is the flow depth. m=6 is used in this study, which is implied from Manning equation [161], although values between 4 and 12 have been reported in the literature [165]. The law-of-the-wall relation:

$$\frac{u}{u_*} = \frac{1}{\kappa} \ln (\frac{z}{z_0}) \tag{5-13}$$

where u_* is friction velocity (or shear velocity), z_0 is hydraulic roughness length, defined by the distance from the stream bed at which the velocity given by the law-of-the-wall goes to zero, $\kappa = 0.41$ is the Von Kármán constant.

To relate Eqs. 5-12 and 5-13, natural log on both side of Eq. 5-12:

$$\ln\left(\frac{u}{u_{max}}\right) = \frac{1}{6}\ln\left(\frac{z}{H}\right) \tag{5-14}$$

Considering the Taylor series expansion on the left hand side for $0<u/u_{max}<1$:

$$\ln\left(1-\left(1-\frac{u}{u_{max}}\right)\right) = -\left(1-\frac{u}{u_{max}}\right) + high\ order\ terms \tag{5-15}$$

By neglecting high-order terms and taking the right-hand side of Eq. 5-14:

$$-\left(1-\frac{u}{u_{max}}\right) = \frac{1}{6}\ln\left(\frac{z}{H}\right) \tag{5-16}$$

Therefore,

$$u = u_{max} + \frac{u_{max}}{6}\ln\left(\frac{z}{H}\right) \tag{5-17}$$

Examining Eqs. 5-13 and 5-17, the shear velocity was estimate using the maximal velocity of power-law approximation:

$$u_* = \frac{\kappa u_{max}}{6} \tag{5-18}$$

Using $\kappa = 0.41$, u_* is estimated to be $0.068u_{max}$, within the general range of 5 - 10% of the flow velocity.

Although Eq. 5-18 is an approximation, it provides an explicit relationship to determine shear velocity from the flow characteristics, which can be linked to the suspension of sediments in streams.

5.2.4 Model Setup

150,000 sediment particles in two size ranges are released from water surface and transported downstream in generic flow conditions of the 49 Missouri streams with sensitive aquatic species. Diameters of 0.01 - 1 mm with median size $d_{50} = 0.1$ mm represent very fine to coarse sands (size range A). Diameters of 0.1-20 mm with median size $d_{50} = 1.5$ mm represent medium to very coarse sands and small gravels (size range B). The distributions of particle diameters were generated following a log-normal distribution for both size ranges [166].

This study focuses on the settling of the heavy particles that would potentially bury downstream mussels in the streams. Therefore, a simple Rouse number criterion was applied to determine the critical diameter for the sediments that are unable to suspend in the water column [167, 168]. Rouse number is defined as:

$$R_0 = \frac{V_S}{\beta \kappa u_*} \qquad (5\text{-}19)$$

where V_S is sediment terminal settling velocity, $\beta = \max(1 + 2(V_S/U_*)^2, 3)$ is the factor that accounts for the response of sediment diffusion to turbulent eddies [168]. The range of Ro can be used to classify the type of sediment transport: sediment transport is classified as bed load for $Ro \geq 2.5$, sediments are partially suspended for $1.2 \leq Ro < 2.5$, sediments are fully suspended for $0.8 \leq Ro < 1.2$, and sediment transport is classified as wash load for $Ro < 0.8$ [167]. The simple *Ro* criterion has been used to identify suspension of invasive carp eggs in North America rivers and streams [169, 170]. Here, the critical Rouse number $Ro = 2.5$ was used as the criterion to determine sedimentation, i.e., where the sediments would be deposited onto the riverbed, which could potentially bury the mussels.

5.3 Results

5.3.1 Stream Hydraulics

An example of mean flow velocity versus depth and discharge versus depth relationships is illustrated in **Figure 5-7** for two rivers. In Sac River near Hwy J below Stockton, MO, where the measured maximal discharge is less than 450 m^3/s, the flow velocity is near-linearly correlated to the flow depth. In Spring River near Waco, MO, where the measured maximal discharge was approximately 1600 m^3/s, the flow velocity exhibits anon-linear relation with flow depth, with considerable scatter. Good power-law relationships were observed between discharge and flow depth for both rivers.

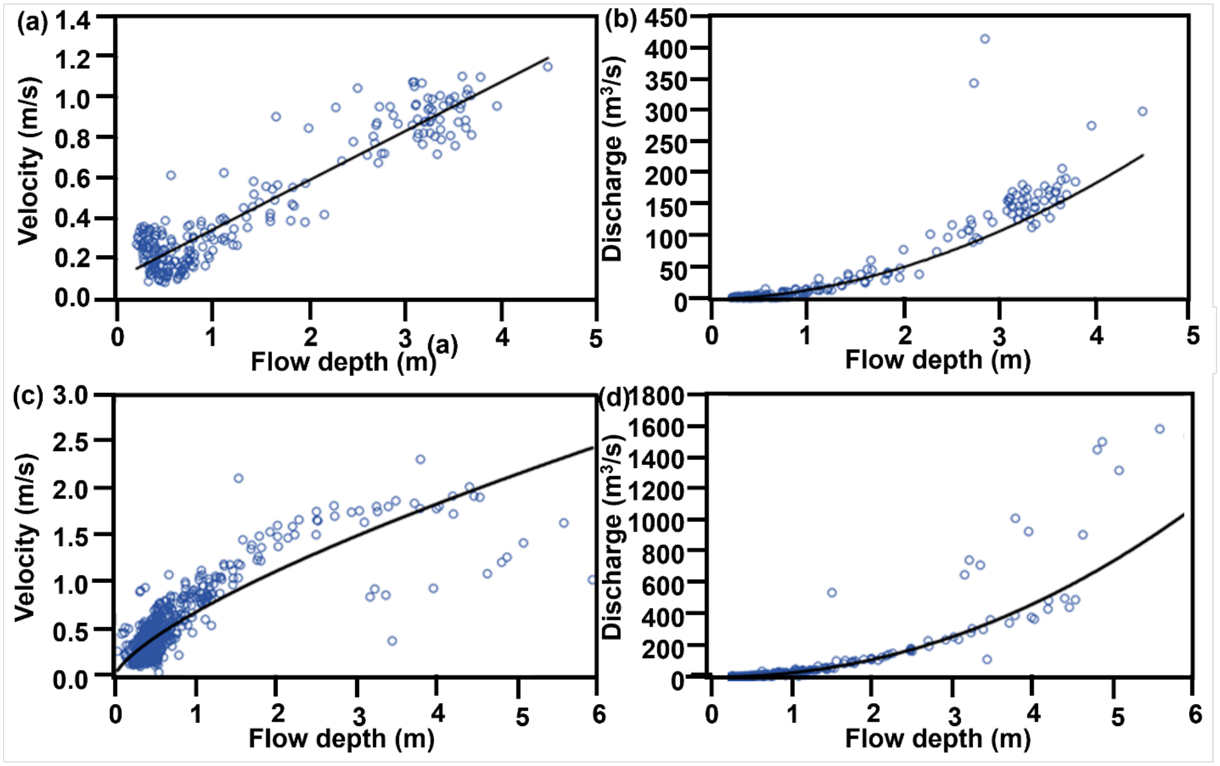


Figure 5-7. Relationships between measured mean flow velocity, discharge, and flow depth in two Missouri rivers. (a) Sac River at Hwy J below Stockton (USGS gage 06919020, data range 1972 - 2023) - flow velocity and depth. The fitted linear relation is $U = 0.24H + 0.10$ with U and H being flow velocity and depth, respectively, n = 259, $R^2 = 0.88$. (b) Discharge and depth. The fitted power-law relation is $Q = 13.87H^{1.87}$ with Q being discharge, n = 259, $R^2 = 0.86$. (c) Spring River near Waco (USGS gage 07186000, data range 1924 - 2023) - flow velocity and depth. The fitted power-law relation is $U = 0.68H^{0.72}$, n = 116, $R^2 = 0.71$. (d) Discharge and depth. The fitted relation is $Q = 25.31H^{2.09}$, n = 568, $R^2 = 0.86$.

For each river or stream, this study applied either linear or power-law equations to relate flow velocity to depth, while a power-law equation was used to model the discharge-depth relationship. Details on curve fitting to the field survey data from each station are provided in the supplementary file. Thereafter, these best-fit equations were applied to the continuously recorded gage data to obtain the discharge and flow velocity at each site. The results for all 49 USGS field monitoring stations are illustrated in **Figure 5-8**.

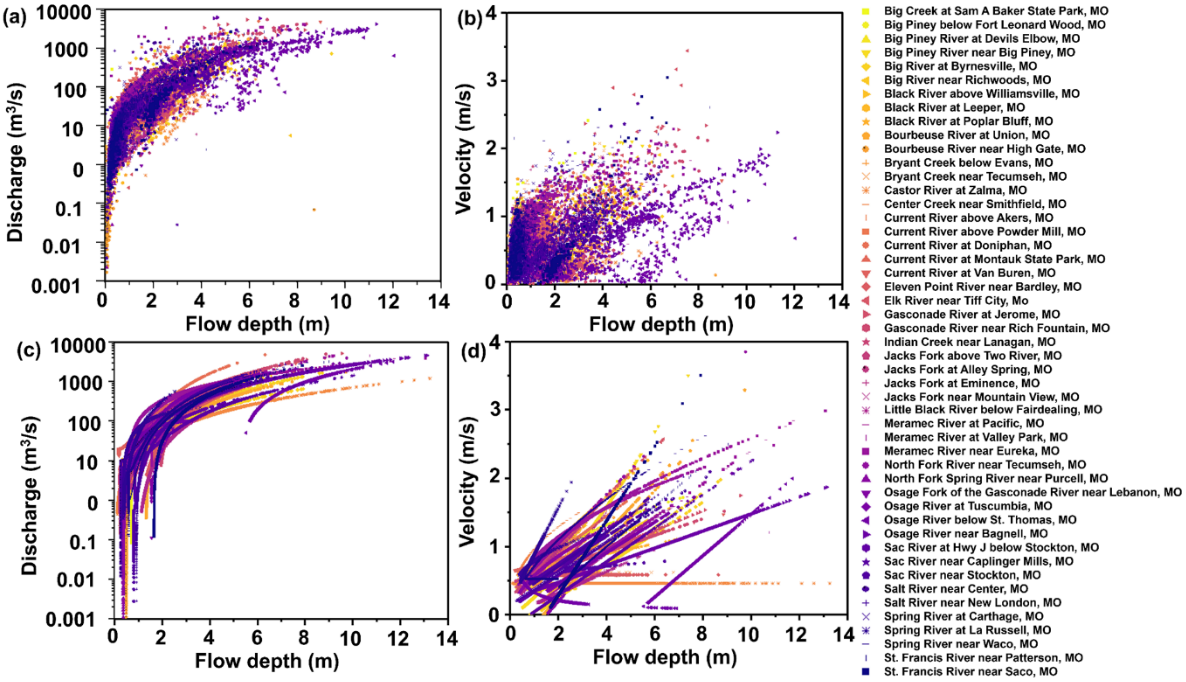


Figure 5-8. Scatter plots of discharge-depth relation and velocity-depth relation (16290 data points) for 49 USGS monitoring sites and gaging stations: (a-b) field-measured discharge and velocity at the monitoring sites; (c-d) calculated discharge and velocity from the continuously recorded gage data using the best-fit equations to the field data.

Before proceeding with sedimentation modeling, whether the discrete field measurements capture the full range of variability observed in the continuously recorded gaging station data was assessed. Inspection of the box plots (**Figure 5-9**) and the scatter plot (**Figure 5-10**) suggests a good agreement between the measured and calculated discharge values. The ranges of measured and calculated discharge appear similar, indicating that the field measurements capture the essential variability observed in the continuous gaging station data. Therefore, the field data and the analysis can be used to represent the hydraulics in these 40 rivers and streams.

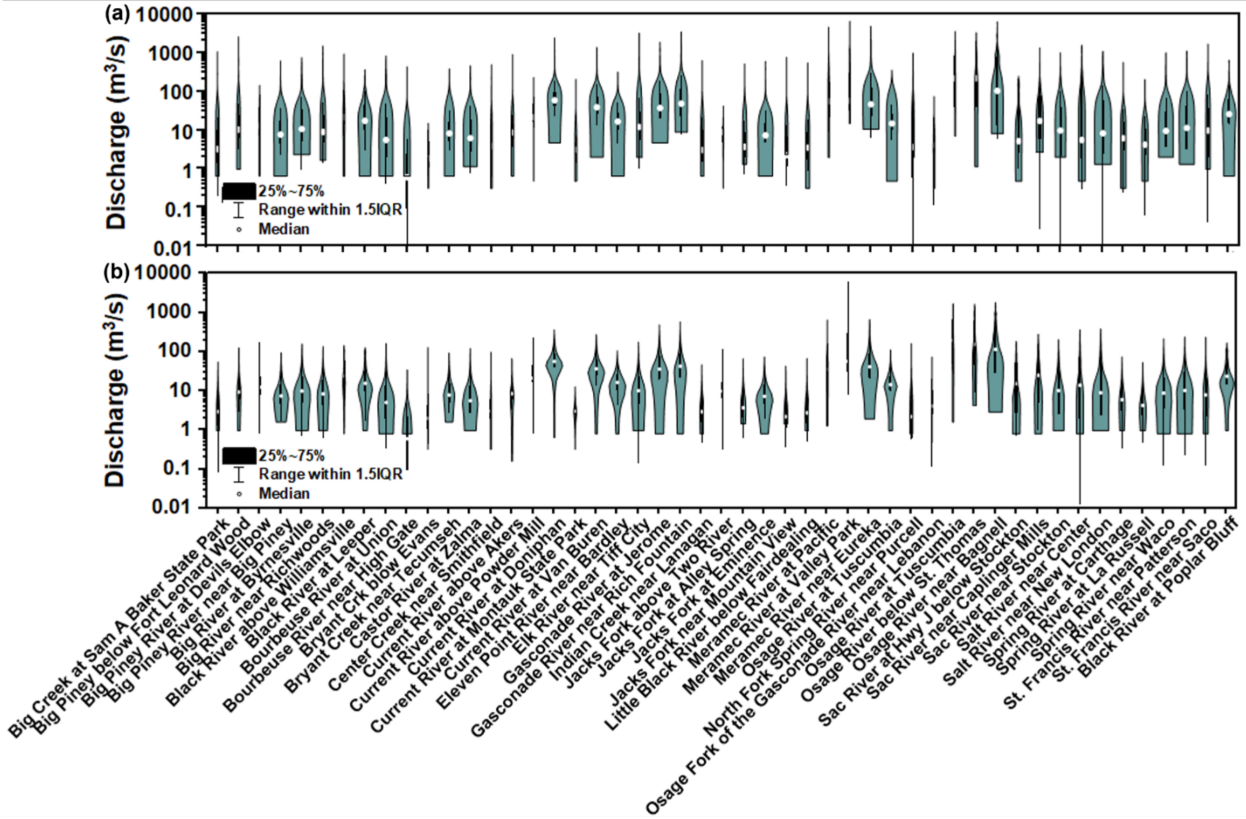


Figure 5-9. Box diagram for measured and calculated discharges in the 49 streams of concerns. The data of median, 25-75th percentile, and 1.5 times interquartile rang (IQR) are presented. (a) Field measured discharge, (b) calculated discharge from gage data.

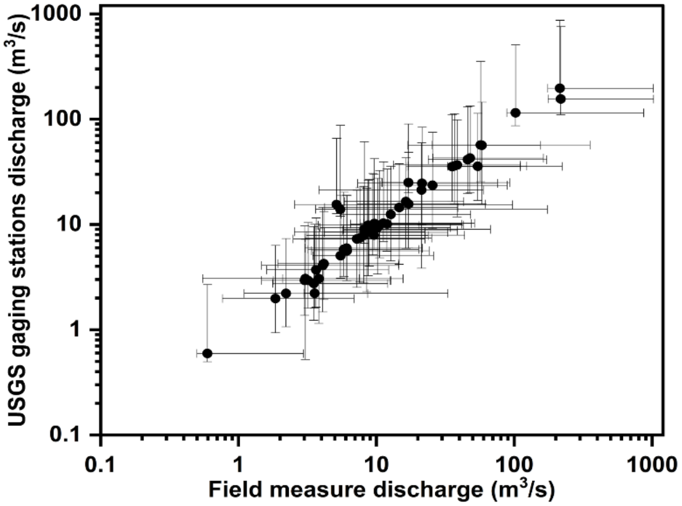


Figure 5-10. Comparison between measured and calculated river and stream discharges. The black dots represent the median. Error bars represent the range of first to third quarters in each stream. The solid 1:1 line represents the perfect match between measured and calculated discharge data.

5.3.2 Modeling Parameters

From all the field data, the 5th, 50th, and 95th percentile of velocity distribution at 0.1-m segment of flow depth ranging from 1 to 12 m were calculated, to represent the slow, normal, and fast flow conditions, respectively, for all 49 rivers and streams. By fitting a power-law relation to each condition, the following formula was obtained:

$$U = \begin{cases} 0.1236H^{0.6245} & \text{slow flow} \\ 0.5074H^{0.4084} & \text{normal flow} \\ 1.1217H^{02695} & \text{fast flow} \end{cases} \qquad (5\text{-}20)$$

where the R-square values of curve fitting are 0.5285, 0.7183, and 0.6113, respectively.

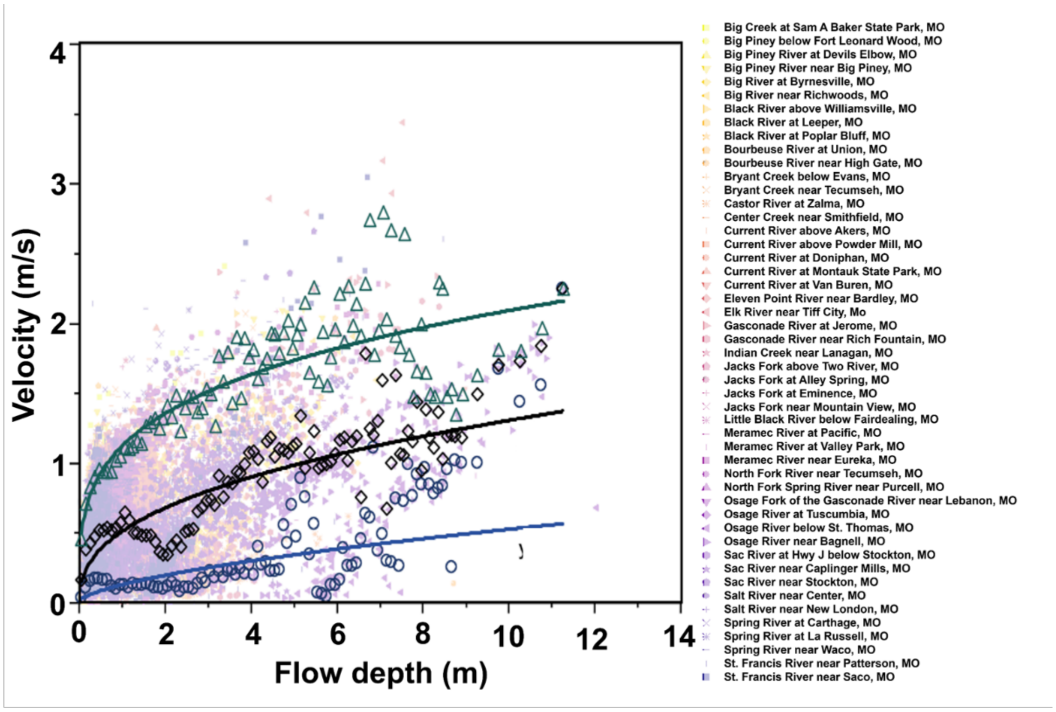


Figure 5-11. Representative flow characteristics of all 49 streams. Symbols with reduced opacity represent the field measured data. Blue circles, black diamonds, and green triangles represent the 5th, 50th, and 95th percentile of the velocity distribution at different flow depths, calculated at 0.1-m segment. Power-law relations were then fitted to these three datasets (Eq. 5-20).

The mean flow velocity (*U*) for three general flow conditions was calculated based on Eq. 5-19 in the depth range of 1-12 m with the interval of 1 m. The shear velocity (u_*) can then be calculated based on Eqs. 5-12 and 5-18 and the flow velocity *U*. With R_{oc} = 2.5 and u_*, the critical settling velocity ($V_{s,c}$) and diameter (d_c) for the deposited sediments can be obtained for each condition. The results of *U*, u_*, $V_{s,c}$, and d_c are summarized in **Table 5-1**.

Table 5-1. Calculated hydraulic parameters (flow velocity U and shear velocity u_*) and critical sediment settling velocity ($V_{s,c}$) and diameter (dc) for the analyzed generic conditions in the 49 streams of concerns in Missouri, United States.

Flow depth (m)	Slow flow				Normal flow				Fast flow			
	U (m/s)	u_* (m/s)	$V_{s,c}$ (m/s)	d_c (mm)	U (m/s)	u_* (m/s)	$V_{s,c}$ (m/s)	d_c (mm)	U (m/s)	u_* (m/s)	$V_{s,c}$ (m/s)	d_c (mm)
1	0.124	0.010	0.010	0.041	0.507	0.040	0.041	0.276	1.122	0.089	0.092	0.802
2	0.191	0.015	0.016	0.074	0.673	0.054	0.055	0.403	1.352	0.108	0.110	1.031
3	0.245	0.020	0.020	0.104	0.795	0.063	0.065	0.504	1.508	0.120	0.123	1.194
4	0.294	0.023	0.024	0.132	0.894	0.071	0.073	0.591	1.630	0.130	0.133	1.326
5	0.338	0.027	0.028	0.159	0.979	0.078	0.080	0.668	1.731	0.138	0.141	1.437
6	0.378	0.030	0.031	0.186	1.055	0.084	0.086	0.738	1.818	0.145	0.149	1.536
7	0.417	0.033	0.034	0.211	1.123	0.090	0.092	0.803	1.895	0.151	0.155	1.624
8	0.453	0.036	0.037	0.236	1.186	0.095	0.097	0.864	1.965	0.157	0.161	1.705
9	0.487	0.039	0.040	0.261	1.245	0.099	0.102	0.922	2.028	0.162	0.166	1.779
10	0.521	0.042	0.043	0.285	1.299	0.104	0.106	0.977	2.086	0.166	0.170	1.848
11	0.553	0.044	0.045	0.309	1.351	0.108	0.110	1.030	2.141	0.171	0.175	1.913
12	0.583	0.047	0.048	0.333	1.400	0.112	0.114	1.080	2.191	0.175	0.179	1.975

The data analysis revealed that the critical sediment settling velocities are slightly larger than the shear velocities, aligning well with the established practice of using this comparison as a criterion for sediment and particle transport [168, 169]. The critical sediment diameters range from 0.041 to 0.333 mm, 0.276 to 1.080 mm, and 0.802 to 1.975 mm for slow, normal, and fast flow conditions, respectively. Smaller sediments are mobile and less likely to bury mussels on the downstream streambed.

5.3.3 Downstream Distance of Sediment Deposition

Figure 5-12 illustrates the modeled sediment settling locations under slow-flow conditions within the range of 1 - 12 m of flow depth in Missouri streams of sensitive species for size range A particles (very fine to coarse sands, 0.01 - 1 mm, d_{50} = 0.1 mm). The results indicate that sediment settling is predominantly concentrated within tens of meters from the release point in shallow waters, with distribution expanding downstream as flow depth increases. For example, at a water depth of 12 m, the highest particle count is observed approximately 140 m downstream from the release point, with a significant amount of sediment extending beyond 200 m. As water depth increases from 1 to 12 meters, the critical sediment diameter also increases from 0.041 to 0.333 mm.

Consequently, the proportion of settled sediments decreases from 96.2% to 0.8%. Under the normal-flow and fast-flow conditions, very few sediments are able to deposit in the streams, therefore, the size range A sediment was not modeled.

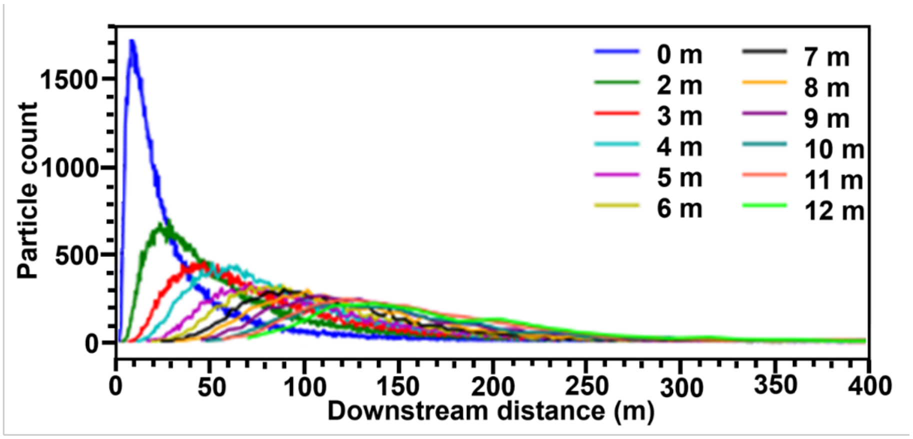


Figure 5-12. Sediment settling location of size range A particles (very fine to coarse sands) in the water depths of 1 - 12 m in the slow-flow condition. 0 m on the x-axis indicates the location of sediment release.

The distributions of particle counts for size range B particles (coarse sands to small gravels, 0.1 - 20 mm, with a median diameter of d_{50} = 1.5 mm) under slow, normal, and fast flow conditions are illustrated in **Figure 5-13**. As expected, the sedimentation distance increased with the rising flow depth under similar flow conditions and from slow to fast flows at the same water depth. Under the slow-flow condition, the majority of deposited sediments were found within tens of meters from the release point, extending to approximately 100 m for the deepest water at 12 m (**Figure 5-13(a)**). A similar trend was observed under normal-flow conditions, with deposited sediments reaching approximately 200 m at the water depth of 12 m (**Figure 5-13(b)**). Under the fast-flow condition, particles settled farther downstream (**Figure 5-13(c)**), with the maximum particle count at each flow depth showing relative consistency compared to the pronounced decrease in maximum particle count from shallow to deep waters in the slow-flow condition. Meanwhile, in the normal-flow condition, a decreasing but not as pronounced trend was observed. The reduction in particle count near the release point under faster flows can be partially attributed to the heightened advection of heavier particles downstream. Moreover, the stronger turbulence present in faster flows enhances the streamwise spreading of sediment transport.

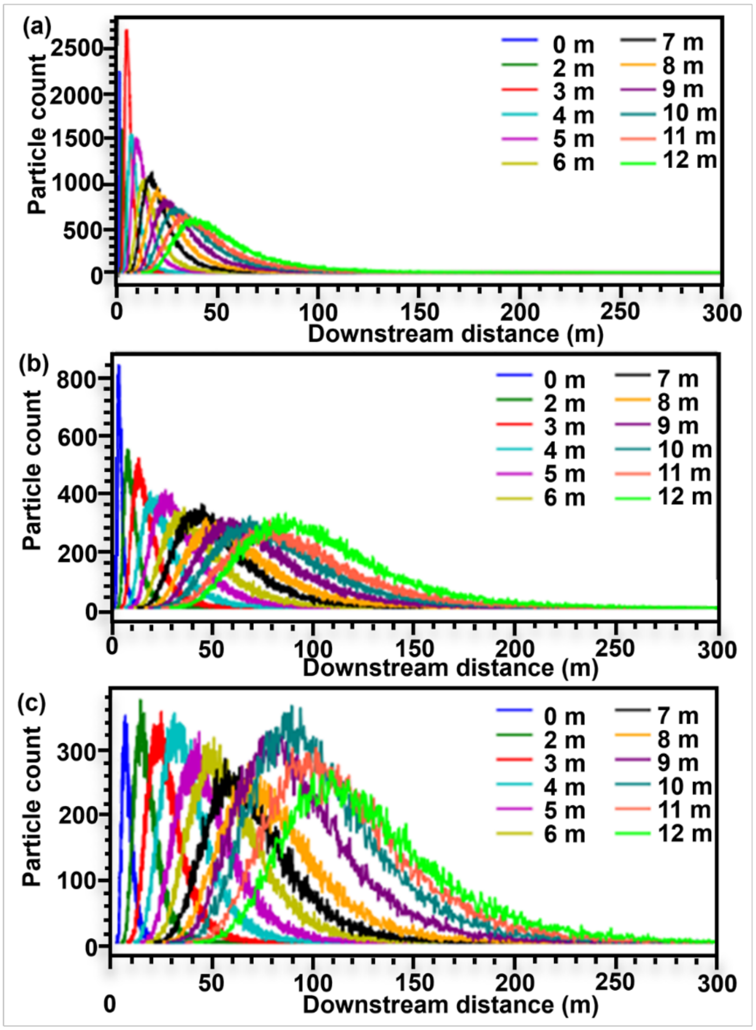


Figure 5-13. Sediment settling locations of size range B particles (medium to very coarse sands and small gravels) in the flow depths of 1 - 12 m: (a) slow flow; (b) normal flow; (c) fast flow.

A log-normal distribution to the particle count distribution of each condition was fitted and summarized the mode, median, and mean to illustrate the key distance of sediment deposition (**Figure 5-14**). Under the slow-flow condition, the mode of size A particles settling ranged from 8.1 to 140.6 m across flow depths of 1 to 12 m. The median values ranged from 21.7 to 159.2 m, while the mean values ranged from 35.6 m to 169.4 m. For size B particles, the mode values of the log-normal distribution of settlement distance under the slow-flow condition ranged from 0.7 to 38.1 m, with median values spanning from 0.9 to 48.6 m, and mean values ranging from 1.0 to 54.9 m. Under the normal-flow condition, mode values ranged from 2.7 to 86.6 m, median values from 3.4 to 98.3 m, and mean values from 3.9 to 104.8 m. In fast-flow conditions, mode values ranged from 6.1 to 110.3 m, median values from 7.0 to 122.1 m, and mean values from 7.6 to 128.6 m.

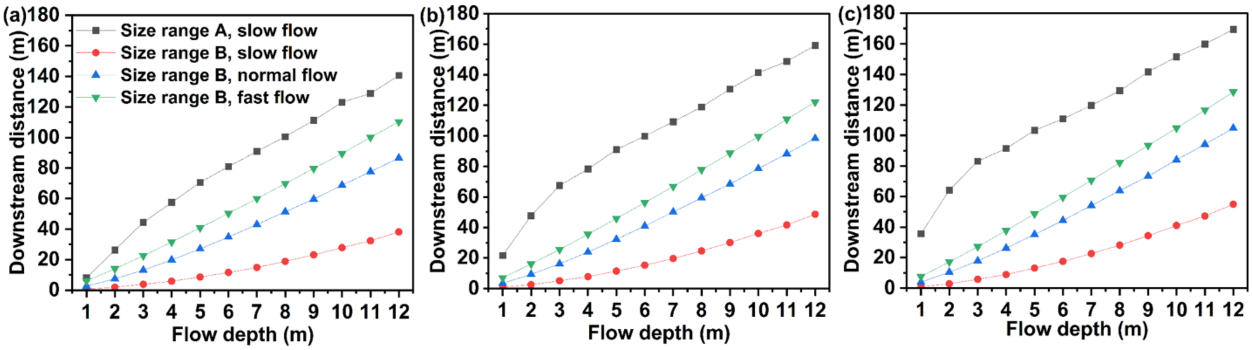


Figure 5-14. Key parameters in the fitted log-normal distribution to the modeled distribution of deposited sediment based on particle count: (a) mode; (b) median; (c) mean.

While the mean particle deposition distance can reach nearly 200 meters based on particle count, this distance may not represent the most impactful distance to the mussel bed. Therefore, the volume of each particle and plot the distribution of deposited particle mass normalized by the total sediment mass was calculate, assuming a constant density across all sediments (**Figure 5-15**). The results indicate that a significant portion of mass is deposited near the release point, especially in shallow waters. However, as the mass distribution extends downstream in deeper waters, reaching distances of hundreds of meters, the mass ratio diminishes considerably due to higher spreading.

A log-normal distribution was also fitted to the mass distribution of deposited sediment, illustrating varying key distances as a function of flow depth for different sediment size ranges and flow conditions (**Figure 5-16**). For size range A particles in slow flows, the mode ranged from 2.6 to 24.8 m, with median and mean values spanning from 6.8 to 55.8 m and 11.0 to 83.6 m, respectively. For size B particles in slow flows, the mode, median, and mean distances ranged from 1.0 to 19.9 m, 2.5 to 53.1 m, and 4.0 to 86.9 m, respectively. In normal flows, these distances were 2.2 to 24.9 m (mode), 6.1 to 62.9 m (median), and 10.2 to 99.8 m (mean). In fast flows, the distances were 2.4 to 27.1 m (mode), 6.1 to 66.4 m (median), and 9.8 to 104.0 m (mean).

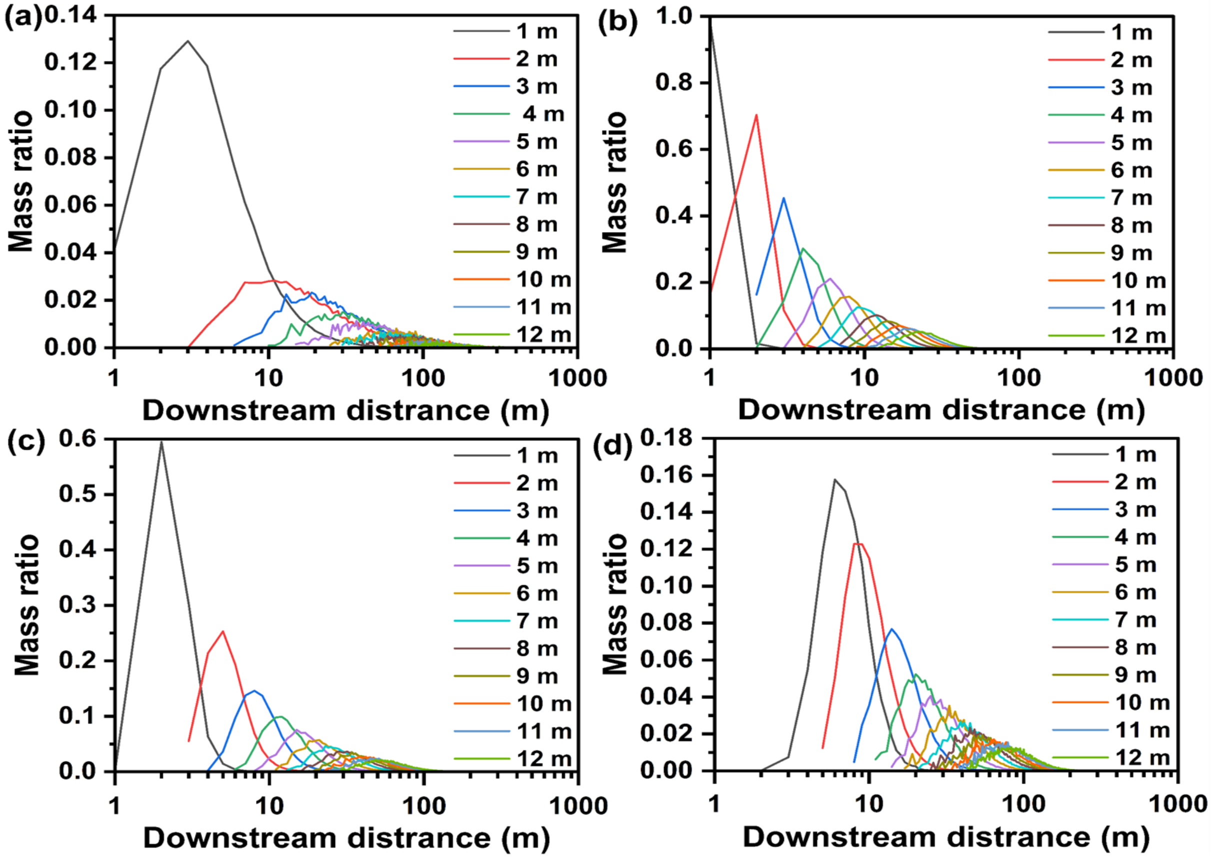


Figure 5-15. Distribution of mass ratio for deposited sediments: (a) size range A particles in the slow-flow condition; (b) size range B particles in the slow-flow condition; (c) size range B particles in the normal-flow condition; (d) size range B particles in the fast-flow condition.

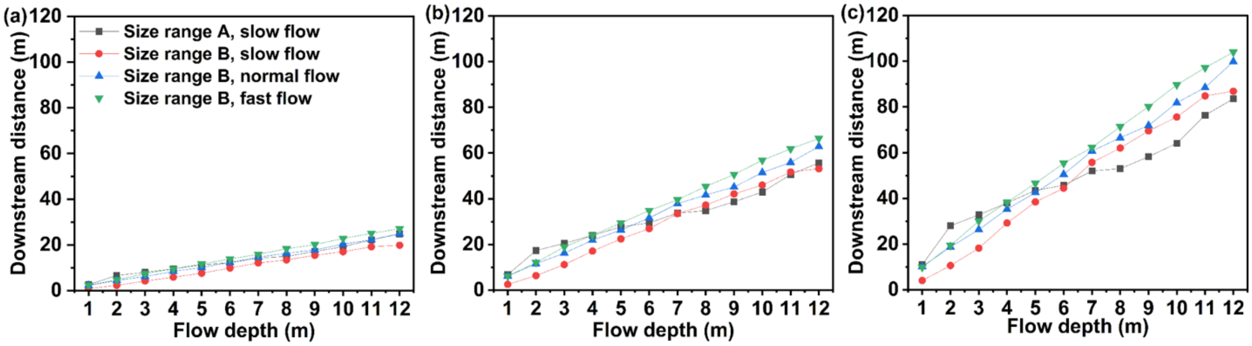


Figure 5-16. Key parameters in the fitted log-normal distribution to the modeled distribution of deposited sediment based on mass: (a) mode; (b) median; (c) mean.

In addition to analyzing sediment deposition locations, the proportion of deposited sediments for each condition was computed based on both particle count and mass (**Figure 5-17**). In the case of size range A under slow-flow conditions, an increase in flow depth led to a significant decrease in the proportion of deposited particles, declining from 96.2% at a 1 m depth to 0.8% at a 12 m depth (**Figure 5-17(a)**). The proportion of deposited particles based on mass ranged from 99.9% at 1 m depth to 18.1% at 12 m depth (**Figure 5-17(b)**). For size range B, nearly all sediment (> 99.4%) settled under slow-flow conditions. The particle count decreased from 99.8% at a 1 m depth to 70.4% at a 12 m depth under normal-flow conditions, and from 84.3% at a 1 m depth to 31.0% at a 12 m depth under fast-flow conditions (**Figure 5-17(a)**). Consequently, the proportion of deposited sediment mass ranged from nearly 100.0% at a 1 m depth to 98.9% at a 12 m depth under normal-flow conditions, and from 99.7% at a 1 m depth to 89.3% at a 12 m depth under fast-flow conditions (**Figure 5-17(b)**).

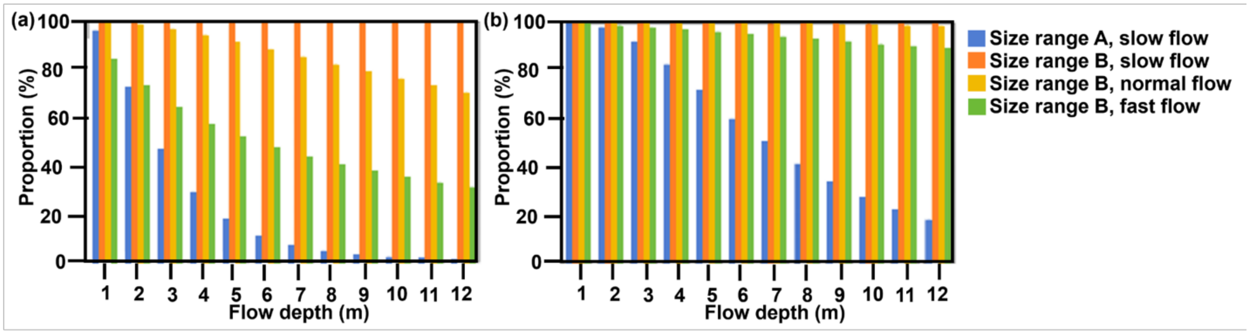


Figure 5-17. The proportion of deposited sediments based on (a) particle count; (b) particle mass.

5.4 Discussion

This chapter has demonstrated that sediment settling locations can extend tens to hundreds of meters downstream from the release point in Missouri streams, depending on particle size and flow conditions (see **Figure 5-14**). While the mode, median, and mean values of settling locations in particle count distribution are relatively close (similar plots in three subplots of **Figure 5-14**), significant variability exists in the sediment mass distribution. The modeling findings reveal that the peaks of sediment mass are consistently located within approximately 20 m from the release point across all flow conditions and particle sizes (see **Figure 5-15(a)**). Median mass locations remain consistent across various particle sizes and flow conditions, typically within 40-50 m from the release point (see **Figure 5-16(b)**). Similarly, mean mass locations fall within the range of approximately 80-100 meters and exhibit consistency across particle sizes and flow conditions (see **Figure 5-16(c)**).

This study neglect particle-particle interactions and the impact of sedimentation on water flows. During construction-related sedimentation events, a sediment plume may form, entraining water to co-flow with settling particles. This could substantially reduce the downstream distance of potential mussel burials compared to the predicted values. To address this effect, it is crucial to consider both the quantity of sediment and its release rate into the stream to determine the dynamics of the sediment plume.

In this study, stream flow was treated as a one-dimensional channel, and simple empirical power-law equations were applied to simulate water velocity as a function of water depth. However, in natural streams and rivers, flow structures are highly localized, with significant spatial variations in both longitudinal and transverse directions. To address these complexities, three-dimensional computational fluid dynamics (CFD) models offer a more detailed analysis. Nonetheless, CFD requires significantly more input parameters such as bathymetry and necessitates dedicated calibration and validation for each stream, along with a substantial increase in computational cost. Instead of providing a detailed, stream-specific analysis, this study examined generic flow conditions across 49 Missouri streams to provide general insights into quantitative measures of downstream locations regarding potential risks of sediment burial to mussel habitats during construction-related events.

5.5 Summary

To provide a quantitative tool in evaluating the impact of construction-relevant sedimentation on freshwater mussel habitats in streams, this chapter presents the development, validation, and applications of a Lagrangian particle tracking model. The model utilizes the canonical mean velocity and turbulence profiles in open channels, and tracks individual sediment particles using well accepted drag equations for non-cohesive sediment within the diameter range of 100 – 104 µm.

Using the laboratory data reported in Cuthbertson and Ervine (2007) [156], this chapter analyzed the vertical profiles of velocity and turbulent intensities, and particle deposition distance for sands with diameters of 125 - 625 µm. The satisfactory results in the comparison between the model and measured data validated the model in predicting the sedimentation process.

This chapter explored the model applications in evaluating the impact of sedimentation on freshwater mussels. Using field data collected at the Osage River, Missouri, this chapter modeled two hypothetical sedimentation processes at two discharges, representing both low and high flow conditions in the river (**Appendix E**). **Appendix E** simulated flow characteristics using computational fluid dynamics and compared the modeled velocities with the field data, obtaining reliable flow conditions to drive the particle tracking model. To provide insights on the impact of sedimentation on freshwater mussels, **Appendix E** analyzed two scenarios: the effect of sediment exposure and sediment burial using three classes of sediment sizes.

The effect of sediment exposure was modeled for all sediment sizes, which indicates profound downstream distances can be affected by sediments, i.e., the mussels would be exposed to sediment clouds. For example, in the low flow condition, exposed mussels would be affected by large particles tens of meters downstream from a point source, and medium to fine particles tens to hundreds of meters downstream from a point source. Similarly, in the high flow condition, large particles would affect mussels about a hundred meters downstream, and medium and fine particles could affect mussels in the kilometer range downstream from a point source. This chapter notes that the affected distance is strongly determined by the particle diameter, flow velocity, depth, and turbulence in the stream.

This chapter noted that the current model simplifies the sedimentation process by neglecting particle-particle interactions and the effect of sedimentation on water flows. With significant sedimentation in the water column, the water flow induced by sedimentation should be considered, which enhances the settling velocity of sediments. As such, the sediments would settle closer to the source. In addition, the processes of sediment resuspension and deposition are far more complicated than the probability implemented in the current model. To address the impacts of specific sedimentation events on freshwater mussels in a reach, sophisticated computational fluid dynamics models would provide better insights on sedimentation, resuspension, and deposition.

This chapter aims to provide general and quantitative insights into potential locations for mussel burials within the mussel habitats of Missouri streams during episodic construction-related sedimentation events. By examining the field measurements and continuously monitored USGS gaging data, this chapter investigated 49 streams and rivers and classified three general flow conditions (slow, normal, and fast) for possible flow depth ranging from 1 to 12 m. The empirical equations of velocity versus flow depth were derived for each flow condition, resulting in a total of 36 flow conditions, which was used to drive sediment transport in the streams using an LPT model.

Two log-normally distributed particle size ranges were considered, representing very fine to coarse sands (size range A: 0.01-1 mm) and coarse sands to small gravels (size range B: 0.1-20 mm). Sediments from each size range were released from the water surface for each flow condition, resulting in a total of 72 model cases. A Rouse number criterion was applied in each model case to determine the critical sediment diameter that distinguishes between suspension and settling of sediments. The distribution of sediment settling locations was analyzed based on particle count and mass, with mode, median, and mean values provided.

This analysis indicates that the mean flow velocity exhibited a range from 0.124 m/s to 2.191 m/s in Missouri mussel habituated rivers and streams, with shear velocity ranging from 0.010 m/s to 0.175 m/s. The flow conditions correspond to the critical sediment diameters spanning from 0.041 to 1.975 mm. The model results indicate that settling of Size A sediments was exclusively observed in slow-flow conditions, whereas the normal and fast flows would keep the majority if not all of the sediment suspended. Size B sediments settle across all three flow conditions. An increase in flow velocity and depth corresponded with a decrease in sedimentation proportion and an increase in downstream distance. According to particle count, the downstream affected distance spans from tens to hundreds of meters, depending on flow depth, flow velocity, and particle sizes. In contrast, the distribution based on sediment mass exhibits different shape and values. For instance, for fast-flow in deepest water (12 m), the peak of particle count distribution for size B sediments (i.e., mode) occurs at 110.3 m from the release point, while this distance is 27.1 m based on sediment mass distribution in the stream. In this paper, this chapter also noted several limitations and the simplicity of the model, given the aim in offering generic insights of sedimentation for mussel burials. This study provides quantitative measures on the locations for sediments to settle, but many other factors should be considered for risk analysis of mussel burials and smothering, such as mussel tolerance, and actual conditions of sediments and flows. When bathymetry data is available, a detailed CFD model is desirable to provide more realistic simulations of hydraulics and sediment transport.

Chapter 6. Recommendations for MoDOT's Engineering Policy Guide

6.1 Guiding Principles for Engineering Policy Guide Recommendations

This chapter provides a process for incorporating all of the data and information collected during this study into a set of actions to mitigate the potential impacts of transportation projects on freshwater mussels that may be incorporated into the MoDOT Engineering Policy Guide (EPG).

Both public and private organizations have responsibilities with respect to the protection of mussel species that may be impacted by transportation construction activities. Among the essential responsibilities of state and federal agencies are those related to the determination of whether there are impacted species in proximity to a project. This task is undertaken through mussel surveys. The survey of state departments of transportation that was undertaken as a part of this research project provides important insights into the practices that various agencies have developed and refined over time. Because the conduct of mussel surveys is beyond the scope of the EPG, no recommendations to that end are provided. The private organizations with responsibilities for the protection of mussel species are engineering contractors and subcontractors. Their actions are dictated by the EPG, so the focus of this chapter is on the establishment of requirements and guidelines for those undertaking construction activities.

In addition to the survey of state departments of transportation, these recommendations are also informed by the mussel experiments and modeling exercises described in this document.

The intention is to be proactive in limiting harm to all mussel species whatever their status with respect to conservation concern. An overall process is outlined, recognizing that some species are of particular conservation concern and may warrant a more in-depth treatment.

Mussels may be impacted at different life stages and seasons: juvenile, reproductive and adults between reproductive periods. Juvenile and reproductive impacts may be due to water quality impacts, as from suspended sediment washing off of the landscape in the vicinity of the construction site or from sediment loads from overtopping of causeways or sediment suspension from high-velocity jets from culverts. The impacts may be failure to thrive (juveniles) or reduced reproductive success of females. Adults may be impacted by sediment deposition that limits their ability to access sufficient oxygen or food, or even crushes their shells. Many regulatory strategies focus on the most vulnerable life stage for protection. In addition to juveniles and reproductive females, the adult life stage is also critical and requires protection—while the loss of juveniles is a significant setback in a given reproductive season, the loss of adults means the loss of juveniles for years to come, until surviving animals reach reproductive age. Thus, these recommendations address both juvenile and adult protections.

Water quality impacts to mussels may come from local runoff into the waterway that carries sediment with it. Extreme precipitation events or failure of stormwater BMPs may produce sediment in sufficient quantities that burial of mussel beds is possible. Physical impacts to mussels may also come from watershed-based flows that impact causeway performance and produce sediment in quantities sufficient to bury mussel beds. To address the dual sources of impacts, the recommendations address streamside and instream locations of construction activities, including initial implementation and maintenance. Further, because of the sensitivity of the species to water quality conditions, the preference will be for nature-based strategies, rather than through the additional of chemicals, although chemical stormwater additives may be necessary in some circumstances.

The process is divided into individual components based on the type of information collected or the required planning, calculations, or assessments. The steps are provided in bullet form, suitable for reformatting for inclusion in the EPG. All of the steps associated with the management of stormwater runoff must be undertaken in consultation with personnel from multiple state and federal agencies, including the Missouri Department of Transportation, the Missouri Department of Conservation and the U.S. Fish and Wildlife Service. Conversations with state and federal agency personnel will be necessary to ensure that the policy recommendations discussed here are consistent with all other policies, procedures, and intentions.

6.2 Engineering Policy Guide Recommendations

6.2.1 Streamside Locations

- Employ passive strategies before employing active strategies.
- Manage runoff in upslope locations before managing runoff in downslope locations.
- Utilize natural-based products (e.g., seed and mulch) before engineered products (e.g., flocculants).
- Enhance sustainability by considering the cost of products and waste that may be generated through the use of various processes (e.g., the purchase of flocculant and then the disposal of the flocculant/sediment mixture).
- Consider all animals. As an example, if a mesh is needed to stabilize the seed and mulch, use products where the intersections are not welded but are loose, to allow snakes to move through easily and not get caught.

6.2.1.1 Site Assessment

- Examine topography
    - Identify locations of overland flow.
        - Where possible, employ BMPs in areas of overland flow to reduce the volume of water flowing to and through drainage pathways.
    - Identify water drainage pathways.
    - Examine drainage pathways to determine locations where stormwater runoff concentrates
        - BMPs should be placed along drainage paths to manage runoff as it concentrates. Managing the runoff as it concentrates may allow for smaller BMPs that may fit better within a confined right-of-way.

        - Identify and calculate the contributing area for these locations of concentration in order to design the BMPs.
- Identify soil texture.
    - Silty soils are more erosive and may require additional BMPs in order to control erosion and the transport of sediment to the waterway.

6.2.1.2 Planning for Runoff Management

- Require contractor to develop a plan outlining the deployment of various stormwater BMPs (what, where, and when).
- Require contractor to develop a plan outlining the preventative actions (what, where, and when) that will be taken when a large precipitation event is anticipated/has begun.
- Establish a process to recognize contractors employing innovative BMPs or demonstrating exceptional utilization and maintenance of stormwater BMPs.

6.2.1.3 Passive Mitigation Strategies/Best Management Practices

- Perform activities during typically low flow seasons.
- Perform activities when mussels are not spawning.
- Prohibit equipment below ordinary highwater mark (OHWM)
- Limit vegetation removal to only that required for construction activities and only clear vegetation in the locations and only when needed for specific construction activity (i.e., do not clear the entire site at the beginning of the project if a portion will not be needed until later in the project).

6.2.1.4 Active Mitigation Strategies/Best Management Practices

- Build a work pad to localize construction traffic and limit the area of active disturbance. Give consideration to using a tracking pad.
- Employ barriers to sediment movement around the perimeter of the site and between active and non-active locations.
    - Silt fences
    - Silt bags
- Seed and mulch areas once they are no longer being actively used for construction or will not be used again until later in the project.
- Employ ditch checks to slow down the flow of runoff and allow sediment to settle out.
- Employ best practices in the use of standard engineering designs to enhance performance (e.g., use serpentine flow paths and baffles to prevent the short circuiting of flow in a sedimentation basin).
- For those locations where landscape-based BMPs have not been completely effective in controlling sediment, employ flocculants or in-water turbidity barriers.

6.2.1.5 Maintenance

- Require contractor to develop a plan for ongoing maintenance (e.g., removing sediment from the ditch check to ensure continued functionality).
- Require contractor to establish a maintenance plan for recovering BMP functionality after large precipitation events (greater than a 2-yr storm)

6.2.2 Instream Locations

6.2.2.1 Mussel-Specific Information

- Determine whether mussel beds of any species exist in the vicinity (upstream or downstream) of the project.
  - Backwater calculations provide context for the consideration of upstream distance.
  - Modeling results provide context for the consideration of downstream distance.
- If mussel beds are present, specify the specific species.
- Use mussel experimental results to identify, where possible:
  - The ease or difficulty with which various species were able to dig themselves out from burial.
  - Identify the sediment depths from which the mussels were not able to dig themselves out.
- Identify the season of the year of active reproduction, when project activities may need to be halted.

6.2.2.2 Watershed-Specific Information

- Identify the contributing watershed, considering that the project site is the watershed outlet—the location through which all of the runoff from the entire watershed will pass. The interest is in the magnitude of the flows that may be expected with various return period storms.
- Using a topographic map or a digital elevation model (DEM), identify the watershed boundary specifying the physical area from which stormwater runoff will collect and pass through the project site.
- Calculate the watershed area.
- If flow records are available (e.g., from the USGS):
  - Identify peak discharges representing flows from more rare and more frequent precipitation events.
  - Identify the precipitation events producing the peak discharges above.
  - Utilize NOAA 14 Precipitation Atlas [186] to approximate the return period of the various peak discharges.
  - Identify non-storm flows throughout the year to assess seasonal baseflows
  - For flows that are measured at locations other than the project site, perform a contributing area-based interpolation to approximate the flow at the project site.
- If flow records are not available, use the USGS regression equations [187] to calculate peak discharges for 2- to 500-year precipitation events.
  - Determine in which region the project site is located:
    - I. Central Lowlands (area and slope are the parameters used).
    - II. Ozark Plateau (area and slope are the parameters used).
    - III. Mississippi Alluvial Plain (area is the parameter used).
  - Determine watershed contributing area (mi^2) from a topographic map or a DEM.
  - Determine watershed slope (ft/mi) from a topographic map or a DEM.

    - Determine the difference in elevations at points 10% and 85% of the distance along the main channel from gage location to basin divide (ft).
    - Determine the distance between the two points (mi).
    - Slope is calculated as the elevation difference divided by the distance between the two points (ft/mi).
- If flow records are not available investigate flow records that may be available for nearby waterways flowing through comparable watersheds (i.e., with respect to contributing area and slope).

6.2.2.3 Pre-Project Conditions

- Determine the approximate stream baseflow.
  - From above where there are records.
  - From 1-yr flow calculations if no records are available (a conservatism).
- Determine cross-section geometry of stream at project location.
- Calculate uniform depth of flow from Manning's equation.
- Calculate approximate stream velocity from Manning's equation.
  - Recognizing that flow velocity will be greater in the central portion of the stream and slower along the shallower stream banks and all along the wetted perimeter.

6.2.2.4 Culvert Principles

- Culverts are used within causeways to maintain stream flows during construction.
- Culverts have a smaller cross-section than the pre-construction stream cross-section geometry.
- Forcing the flow through the smaller cross-sectional culverts causes energy losses as the water flows into, through, and out of the culverts
- In order to compensate for the energy losses, the flow will backup upstream of the culverts (as a headwater) in order to create additional energy represented as head (flow depth).
  - Backup results in a greater depth of flow upstream (headwater) than would be calculated from Manning's equation.
  - The distance upstream with the depth of flow greater than uniform depth will vary with the flow, slope, channel geometry, and channel roughness.
  - The upstream distance for backwater effects can be calculated using commercial software or estimated using a spreadsheet.
- Relationship between culvert cross-sectional area, headwater depth, and culvert exit velocity.
  - The greater the storm flow, the larger the depth of the headwater and the greater the velocity of the flow exiting the culverts.
  - The larger the cross-sectional area of the culverts, the smaller the headwater depth and the smaller the velocity exiting the culverts.
- The lower momentum (the product of the mass and velocity) of the slower downstream flow will work to attenuate the impact of the greater momentum of the flow exiting the culverts.

6.2.2.5 Potential Causeway Impacts to Mussel Beds

- If the causeway elevation is not sufficiently high, the passage of the rarer events (i.e., greater streamflows) may cause the water to back up to an elevation such that the water would flow over the causeway, washing out any fines contained within the causeway material.
- The smaller the cross-sectional area of the culverts, the greater the velocity of the water exiting the culverts that may suspend sediments in the stream.

6.2.2.6 Culvert Design

- Establish the uniform depth of baseflow.
  - Baseflow is the flow that exists when there is no impact from a precipitation event (see steps above).
  - Uniform depth is the depth of the baseflow as calculated by Manning's equation for equilibrium flow.
- Establish the uniform depth of flow for the storm event of interest (e.g., 100-yr event).
- Use commercially available software (e.g., CulvertMaster) to perform multiple culvert calculations that balance the impacts and costs of causeway elevation and total cross-sectional area of culverts to determine the final design (number and size of culverts) to manage the storm event of interest.
  - Consider various maximum headwater depths.
  - Consider various culvert numbers and sizes.
- Establish the causeway elevation as the maximum headwater elevation produced from the final design plus a factor of safety (e.g., 1').

6.2.2.7 Specifications

- Specify clean aggregate for use in the construction of the causeway.
- Estimate the distribution of the sizes of fines from the clean aggregate being used for causeway construction.

6.2.2.8 Modeling Results

- The modeling effort produced the distribution of settled particles with distance from a release point (i.e., the project site) for multiple particles sizes, for multiple depths of flow, and for slow, normal, and fast flow velocities.
- Because of the design criteria outlined above, absent a failure of the causeway itself, there will be no expectation that the causeway will be overtopped and release sediment with low or normal flows.
- Perform assessments for the storm event of interest (rarer event with greater flows and higher velocities).
- For the storm event of interest, use the above steps to characterize:
  - Particle size distribution expected to be released at a project site.
  - Approximate flow depths and velocities.
- Use the modeling results to assess the longitudinal distribution of sediment that would be released with an overtopping event at the project site.

6.2.2.9 Maintenance Requirements

- After every rain event that is greater than a 2-yr storm, contractors will be required to inspect the causeway and its elevation and condition to ensure that that no subsidence or overtopping has taken place.
- After every rain event that is greater than a 2-yr storm, contractors will be required to inspect the culverts and remove debris as necessary to ensure the full functionality of the culverts.
- After every rain event that is greater than a 2-yr storm (and once the water level has returned to the baseflow elevation), contractors will be required to inspect the downstream end of the culverts to assess whether any suspension of bed materials has occurred.
- Remedial activities may be required depending on the extent of the changes and the proximity of the project site to mussel beds.

6.3 Summary of Recommendations for MoDOT Engineering Policy Guide

Engineering resources and practice have been utilized to develop recommendations for revisions to the MoDOT Engineering Policy Guide to mitigate the potential impacts of construction projects on freshwater mussels in Missouri. The recommendations incorporate lessons learned from other state departments of transportation and provide a link between both the mussel experiments and the modeling exercises and project site engineering. An overall strategy guides the development and implementation of stormwater management BMPs, for both streamside and instream locations. Conversations with state and federal agency personnel will be necessary to ensure that the policy recommendations discussed here are consistent with all other policies, procedures, and intentions.

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Appendix A: DOT Survey

MoDOT Project TR202109

EVALUATING SEDIMENTATION IMPACTS TO FRESHWATER MUSSELS

Survey

Letter to the Respondent

Dear Participant,

The Missouri Department of Transportation (MoDOT) is sponsoring a research study titled "Evaluating Sedimentation Impacts to Freshwater Mussels." The research is being performed by the University of Missouri in collaboration with the United States Geological Survey and Missouri State University. The objective of the research is to evaluate the acute sediment impacts of transportation-sector construction activities on freshwater mussels as well as new and existing approaches that could mitigate the impact of various sediments from construction activities to mussels.

Your cooperation in completing this survey will help to ensure the success of this research study and better protect mussels and potentially be used to inform management of other endangered species such as fish. This survey is being sent to one person from each state DOT. You have been identified as the appropriate person at your DOT to complete this survey. The survey link that you received is unique for your DOT. If it would be more appropriate for someone else at your DOT to take this survey, please forward the email with the survey link to them or send their name and email address to Henry Brown (brownhen@missouri.edu). Additional instructions are provided at the beginning of the survey. If you would like to download a PDF version of the survey for informational purposes, please click here.

Please complete this survey by September 30, 2021. The survey includes 13 questions, and we estimate that the survey will take approximately 15 to 30 minutes to complete, depending on the level of detail you provide in the comments. If you have any questions, please contact Henry Brown, at (573) 882-0832 or brownhen@missouri.edu. Any supporting materials may be sent by email to Henry in lieu of providing URLs. Thank you for participating in this survey!

Survey Instructions

1. To begin the survey, click the forward arrow at the bottom of this page.
2. To view and print the entire survey for informational purposes, click on this survey link and download and print the document.
3. To save your partial answers and complete the survey later, close the survey. Answers are automatically saved upon closing the browser window. To return to the survey later, open the original email from Henry Brown and click on the survey link.
4. To pass a partially completed survey to a colleague, close the survey and forward the original email from Henry Brown to a colleague. Note that only one person may work on the survey at a time; the survey response should only be active on one computer at a time.
5. To view and print your answers after completing the survey, submit the survey by clicking "Submit" on the final page. Download and print the PDF on the following page which contains a summary of your responses.
6. To submit the survey, click on "Submit" on the last page.

Survey Tips

1. Survey navigation is conducted by selecting the forward and back arrows at the bottom of each page.
2. If you are unable to complete the survey, you can return to the survey at any time by reentering through the survey link.

Questions

Contact Information

Name ____________________
State ____________________
Job Title ____________________
Phone Number ______________________
Email Address _______________________

1. Approximately how many construction projects per year does your agency identify as having the potential to cause sedimentation impacts to freshwater mussels?

   - ☐ 0
   - ☐ 1 to 5
   - ☐ 6 to 10
   - ☐ 11 to 25
   - ☐ 26 to 50
   - ☐ More than 50

Comments:

____

2. How frequently does your agency use each of the following mitigation strategies or Best Management Practices (BMPs) on projects identified as having potential sedimentation impacts to freshwater mussels during construction?

BMP	**Always**	**Almost Always**	**Sometimes**	**Rarely**	**Never**
Brush Barriers	☐	☐	☐	☐	☐
Design Causeways	☐	☐	☐	☐	☐
Ditch Checks	☐	☐	☐	☐	☐
Flocculants	☐	☐	☐	☐	☐
Gabion Baskets	☐	☐	☐	☐	☐
In-Water Turbidity Barriers	☐	☐	☐	☐	☐
Limit Vegetation Removal	☐	☐	☐	☐	☐
Monetary Compensation	☐	☐	☐	☐	☐
No Equipment Below Ordinary High Water Mark (OHWM)	☐	☐	☐	☐	☐
Relocate Mussel Beds	☐	☐	☐	☐	☐
Seed and Mulch	☐	☐	☐	☐	☐
Silt Bags	☐	☐	☐	☐	☐
Silt Fence	☐	☐	☐	☐	☐
Triangular Silt Dikes	☐	☐	☐	☐	☐

BMP	Always	Almost Always	Sometimes	Rarely	Never
Work During Specific Times of Year (e.g., No/Low Flow, No Spawning)	☐	☐	☐	☐	☐
Work Pads	☐	☐	☐	☐	☐
Other (Please describe) _____	☐	☐	☐	☐	☐

Comments:

____

3. Based on your response to the previous question, the mitigation strategies or Best Management Practices (BMPs) utilized by your agency are listed below. On a scale of 1 to 5 (1 = Poor, 5 = Outstanding), how would you rate the effectiveness of each of the following mitigation strategies or Best Management Practices (BMPs) in reducing sedimentation impacts to freshwater mussels during construction?

BMP	Performance Rating
Brush Barriers	☐
Design Causeways	☐
Ditch Checks	☐
Flocculants	☐
Gabion Baskets	☐
In-Water Turbidity Barriers	☐
Limit Vegetation Removal	☐
Monetary Compensation	☐
No Equipment Below Ordinary High Water Mark (OHWM)	☐
Relocate Mussel Beds	☐
Seed and Mulch	☐
Silt Bags	☐
Silt Fence	☐
Triangular Silt Dikes	☐

BMP	Performance Rating
Work During Specific Times of Year (e.g., No/Low Flow, No Spawning)	☐
Work Pads	☐
Other (Please describe) _____	☐

Comments:

____

4. Which of the following types of mussel surveys does your agency conduct? Please select all that apply.

   ☐ Cells, a.k.a. quadrats (quantitative)
   ☐ Transect
   ☐ Moving transect
   ☐ Timed search (qualitative)
   ☐ eDNA
   ☐ Other (Please describe) _____

Comments:

____

5. How likely do you think that each of the following construction activities will cause sedimentation impacts to freshwater mussels at a specific site?

Construction Activity	**Very Likely**	**Somewhat Likely**	**Neutral**	**Somewhat Unlikely**	**Very Unlikely**
Bridge Construction	☐	☐	☐	☐	☐
Bridge Rehabilitation	☐	☐	☐	☐	☐
Bridge Removal	☐	☐	☐	☐	☐
Cofferdam Construction	☐	☐	☐	☐	☐
Cofferdam Removal	☐	☐	☐	☐	☐
Culvert Replacement	☐	☐	☐	☐	☐
Drilled Shafts	☐	☐	☐	☐	☐
General Soil Disturbance of Overall Construction Site	☐	☐	☐	☐	☐
Grading	☐	☐	☐	☐	☐
Pile Driving	☐	☐	☐	☐	☐
Riprap Placement	☐	☐	☐	☐	☐
Temporary Causeway Construction	☐	☐	☐	☐	☐
Temporary Sheet Piling Installation	☐	☐	☐	☐	☐
Other (Please describe) ______	☐	☐	☐	☐	☐

Comments:

____

6. How strongly do you agree or disagree that the following concerns have hindered your agency's efforts to reduce sedimentation impacts to freshwater mussels during construction?

Concern	Strongly Agree	Somewhat Agree	Neither Agree Nor Disagree	Somewhat Disagree	Strongly Disagree
Agency Understaffed	☐	☐	☐	☐	☐
Coordination with Other Agencies	☐	☐	☐	☐	☐
Cost	☐	☐	☐	☐	☐
Lack of Agency Buy-In	☐	☐	☐	☐	☐
Lack of Available Data	☐	☐	☐	☐	☐
Lack of Available Guidance	☐	☐	☐	☐	☐
Lack of Contractor Buy-In	☐	☐	☐	☐	☐
Need for Ground-Truthing for Assessment of Impacts	☐	☐	☐	☐	☐
Proper Expertise for Evaluation and Mitigation	☐	☐	☐	☐	☐
Public Awareness	☐	☐	☐	☐	☐
Staff Awareness	☐	☐	☐	☐	☐
Other (Please describe) _____	☐	☐	☐	☐	☐

Comments:

____

7. Does your agency have access to data (e.g. locations, composition, density) regarding mussel beds?

   ☐ Yes, my agency maintains a database with this information
   ☐ Yes, my agency has access to an external database with this information
   ☐ No

Comments:

____

8. Has your agency performed any post-construction monitoring to assess construction impacts to freshwater mussels?

   ☐ Yes
   ☐ No

Comments:

____

9. Which of the following resources related to minimizing sedimentation impacts to freshwater mussels has your agency developed? Please select all that apply.

☐ Survey protocol
☐ BMP guidelines
☐ Specifications
☐ Special provisions
☐ Evaluation studies
☐ Other (please describe) _____________

If you selected any resources in Question 9, please provide URL(s) for resources in the box below or email files to brownhen@missouri.edu:

____

Comments:

____

10. Which of the following organizations does your agency collaborate with to evaluate and minimize sedimentation impacts to freshwater mussels? Please select all that apply.

☐ Consultants
☐ Non-profit organizations
☐ Other state agencies
☐ Universities
☐ U.S. Department of Agriculture
☐ U.S. Environmental Protection Agency
☐ U.S. Fish and Wildlife Service
☐ U.S. Forest Service
☐ U.S. Geological Survey
☐ U.S. Army Corps of Engineers
☐ Other (please describe) _____________
☐ None

Comments:

____

11. Would you be willing to participate in a follow-up interview to discuss in greater detail your agency's practices for setting work zone speed limits and speed limit compliance?

☐ Yes
☐ No

Comments:

____

12. Would the staff at your agency (including construction staff) be interested in learning more about why reducing sediment is important for the stream ecosystem and freshwater communities including freshwater mussels?

☐ Yes
☐ No

Comments:

____

13. Please provide any additional comments that you may have regarding sedimentation impacts to freshwater mussels.

____
____
____

Submittal Instructions

To complete the survey and record your answers, please click the "Submit" button.

Please note that once you click the "Submit" button, you will not be able to modify your answers. To save your partial answers and complete the survey later, close the survey. Answers are automatically saved upon closing the browser window. To return to the survey later, open the original email from Henry Brown and click on the survey link. To pass a partially completed survey to a colleague, close the survey and forward the original email from Henry Brown to a colleague. Note that only one person may work on the survey at a time; the survey response should only be active on one computer at a time. To review your answers before submitting, please select the forward and back arrows at the bottom of each page.

End of Survey

Thank you for completing this survey. Your efforts are greatly appreciated. Your responses are very important, and your feedback is welcome. For your information, a copy of your responses is provided below. You may download your responses in pdf format using the "Download pdf" link shown below. If you have any questions or comments, please contact Henry Brown:

Henry Brown, P.E.
E2509 Lafferre Hall
University of Missouri
Columbia, MO 65211
(573) 882-0832
brownhen@missouri.edu

Your responses have been recorded, and you may now close your browser.

Appendix B: Survey Responses by DOT

Table B-1. Individual responses to Question 1 (number of construction projects per year identified as having the potential to cause sedimentation impacts to freshwater mussels).

Respondent	Response
Alabama	1 to 5
Alaska	0
Arizona	0
Arkansas	6 to 10
California	-
Colorado	11 to 25
Connecticut	1 to 5
Delaware	0
District of Columbia	-
Florida	26 to 50
Georgia	11 to 25
Hawaii	-
Idaho	0
Illinois	26 to 50
Indiana	1 to 5
Iowa	1 to 5
Kansas	1 to 5
Kentucky	6 to 10
Louisiana	11 to 25
Maine	1 to 5
Maryland	-
Massachusetts	-
Michigan	11 to 25
Minnesota	26 to 50
Mississippi	1 to 5
Missouri	6 to 10
Montana	0
Nebraska	0
Nevada	0
New Hampshire	6 to 10
New Jersey	-
New Mexico	-
New York	-

Respondent	Response
North Carolina	-
North Dakota	1 to 5
Ohio	More than 50
Oklahoma	6 to 10
Oregon	0
Pennsylvania	More than 50
Rhode Island	1 to 5
South Carolina	1 to 5
South Dakota	0
Tennessee	11 to 25
Texas	More than 50
Utah	0
Vermont	1 to 5
Virginia	26 to 50
Washington	0
West Virginia	6 to 10
Wisconsin	-
Wyoming	0
Number of Responses	**41**

Table B-2. Comments for Question 1 (number of construction projects per year identified as having the potential to cause sedimentation impacts to freshwater mussels).

Comment
The impacts to freshwater mussels are assessed as part of the project review for Threatened and Endangered species and sent to our department of wildlife & parks for review. If a project will impact a Threatened and Endangered (T&E) mussel species, they will require an Action Permit with special conditions to help mitigate impacts to the species. Projects that take place on streams that do not have identified T&E mussel species will still follow BMPs to reduce sedimentation in the stream channel.
Almost every bridge replacement and some bridge rehabilitation projects impact areas known to contain freshwater mussels.
Including routine facility/structure maintenance projects in addition to construction projects.
This is for both local road projects and state led projects.
1 project every 2-3 years or more.
It is an uncommon occurrence for our state. There have perhaps been 3 projects in the last 15 years.
Rough count of transportation projects immediately adjacent to streams known to harbor mussels.
The majority of our projects that cause sedimentation impact marine mussels, about 2 projects per year. We usually mitigate the impacts to "no effect" using various methods.
This response is based on potential impacts to state or federal listed mussel species. The number of projects would likely be significantly higher if the question intends to include potential impacts to non-listed mussel species ("More than 50").
Our DOT surveys and relocates mussels from perennial waters to avoid impacts to mussels.
Our state has a significant amount of stream miles and associated road crossings scattered throughout the commonwealth. A big portion of our workload focuses on bridge structures and reducing the amount of deficient crossings within our transportation network. Bridge replacements, super structure replacements, and other forms of bridge preservation or maintenance projects are programmed on an annual basis.
I can only recall one river bridge replacement project in the past 18 years where resource agencies indicated a concern for freshwater mussels.
We handle mussels about once every 10-15 years.
Our DOT manages sedimentation on construction projects but there is only one species of freshwater mussel known in our state and they remaining populations are in a relatively undeveloped area.
Our DOT does not flag projects specifically for impacts to freshwater mussels.

Table B-3. Individual responses to Question 2 (frequency of use of mitigation strategies or BMPs).

3	Brush Barriers	Design Causeways	Ditch Checks	Flocculants	Gabion Baskets	In-Water Turbidity	Limit Vegetation Removal	Monetary Compensation	No Equipment Below Ordinary High-Water	Relocate Mussel Beds	Seed and Mulch	Silt Bags	Silt Fence	Triangular Silt Dikes	Work During Specific Times of Year (e.g., No/Low Flow, No	Work Pads	Other (Please describe)
Alabama	3	1	5	4	1	4	5	1	5	5	5	3	5	1	3	3	-
Alaska	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Arizona	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-
Arkansas	1	3	5	2	2	2	5	3	4	3	5	5	5	4	3	4	-
California	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Colorado	2	2	4	2	2	3	4	3	4	1	5	3	4	2	3	3	-
Connecticut	1	5	4	1	1	4	5	1	4	5	5	3	5	1	5	5	-
Delaware	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
District of Columbia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Florida	3	2	3	2	3	4	4	1	3	3	3	3	4	3	3	3	-
Georgia	1	3	5	3	2	3	3	1	3	3	5	3	5	1	3	3	-
Hawaii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Idaho	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Illinois	1	1	5	1	1	1	5	1	1	2	5	1	5	1	2	3	-
Indiana	1	5	5	1	1	2	4	1	4	3	5	-	5	1	5	5	-

3	**Brush Barriers**	**Design Causeways**	**Ditch Checks**	**Flocculants**	**Gabion Baskets**	**In-Water Turbidity**	**Limit Vegetation Removal**	**Monetary Compensation**	**No Equipment Below Ordinary High-Water**	**Relocate Mussel Beds**	**Seed and Mulch**	**Silt Bags**	**Silt Fence**	**Triangular Silt Dikes**	**Work During Specific Times of Year (e.g., No/Low Flow, No**	**Work Pads**	**Other (Please describe)**
Iowa	-	3	-	-	-	-	-	-	3	3	-	-	-	-	3	-	-
Kansas	1	2	2	1	1	1	1	2	2	2	2	1	2	1	2	2	-
Kentucky	2	3	3	2	3	2	4	4	4	2	5	4	5	2	2	5	1
Louisiana	2	3	4	1	3	3	2	3	3	4	4	3	4	2	3	3	-
Maine	-	-	3	-	-	4	4	-	-	-	4	-	4	-	4	-	-
Maryland	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Massachusetts	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Michigan	1	2	4	2	2	3	3	1	1	4	5	1	4	2	2	2	-
Minnesota	3	3	3	3	3	4	4	1	4	1	5	4	4	4	4	4	-
Mississippi	-	-	-	-	-	-	-	-	3	-	-	-	-	-	5	-	-
Missouri	1	4	4	1	3	2	4	2	3	3	5	3	4	3	4	4	-
Montana	1	1	1	1	1	1	1	1	2	2	1	1	2	1	2	1	-
Nebraska	1	2	4	2	1	3	4	1	4	1	5	2	5	4	4	3	-
Nevada	-	-	-	-	-	-	-	-	4	-	-	4	4	-	4	4	-
New Hampshire	2	3	2	3	3	5	5	2	4	4	4	3	5	2	3	3	-

3	Brush Barriers	Design Causeways	Ditch Checks	Flocculants	Gabion Baskets	In-Water Turbidity	Limit Vegetation Removal	Monetary Compensation	No Equipment Below Ordinary High-Water	Relocate Mussel Beds	Seed and Mulch	Silt Bags	Silt Fence	Triangular Silt Dikes	Work During Specific Times of Year (e.g., No/Low Flow, No	Work Pads	Other (Please describe)
New Jersey	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
New Mexico	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
New York	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
North Carolina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
North Dakota	1	3	3	1	1	5	1	1	1	3	5	1	5	1	5	5	-
Ohio	1	2	3	1	1	3	3	2	3	5	5	3	5	1	4	5	3
Oklahoma	1	1	3	1	1	2	5	1	3	4	5	1	5	5	4	4	-
Oregon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pennsylvania	4	5	4	1	3	2	5	3	3	3	5	5	5	2	4	5	5
Rhode Island	1	1	1	1	3	3	3	1	2	1	3	4	3	1	4	2	-
South Carolina	2	1	1	1	1	1	1	1	4	1	1	2	5	1	1	1	-
South Dakota	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

3	**Brush Barriers**	**Design Causeways**	**Ditch Checks**	**Flocculants**	**Gabion Baskets**	**In-Water Turbidity**	**Limit Vegetation Removal**	**Monetary Compensation**	**No Equipment Below Ordinary High-Water**	**Relocate Mussel Beds**	**Seed and Mulch**	**Silt Bags**	**Silt Fence**	**Triangular Silt Dikes**	**Work During Specific Times of Year (e.g., No/Low Flow, No**	**Work Pads**	**Other (Please describe)**
Tennessee	1	1	4	1	1	2	3	1	3	3	5	4	5	1	3	3	-
Texas	-	-	3	2	3	3	3	1	2	5	5	3	5	1	2	-	-
Utah	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-
Vermont	1	1	1	1	1	4	4	1	2	3	4	4	4	2	3	1	-
Virginia	2	3	3	1	2	4	3	1	3	4	3	4	4	2	4	2	-
Washington	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
West Virginia	3	4	4	3	3	3	4	3	3	4	5	3	4	3	4	4	-
Wisconsin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wyoming	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Average	1.5	2.3	3.0	1.5	1.8	2.6	3.1	1.5	2.8	2.7	3.8	2.6	3.9	1.8	3.0	2.9	2.0
Standard Deviation	0.8	1.3	1.4	0.8	0.9	1.3	1.5	0.9	1.2	1.4	1.6	1.3	1.4	1.1	1.3	1.4	1.7
Number of Responses	32	33	34	33	33	34	34	33	36	34	34	33	35	33	37	33	6

NOTE: 5 = Always, 4 = Almost Always, 3 = Sometimes, 2 = Rarely, 1 = Never

Table B-4. Text responses for "Other" for Question 2 (frequency of use of mitigation strategies or BMPs).

Other – Text Response
We have a mussel Programmatic Biological Opinion (PBO) with USFWS for federally listed species in the state's river basin. With that we have several other standard BMPs not listed here. Some deal with limiting risk associated with refueling equipment, spill response plans and other pollution control measures.
Filtering dewatering water through vegetated areas.
The stormwater construction general permit, SP3, is the primary sediment management process.
Design modification to avoid beds, protective resource fence to keep work away from beds, coffer dams.

Table B-5. Comments for Question 2 (frequency of use of mitigation strategies or BMPs).

Comment
I answered this question from the perspective of controlling project sedimentation for our state's T&E minnow species that are affected by sedimentation.
Again, our DOT doesn't flag projects for freshwater mussels. Responses above are in relation to BMPs in general on our DOT projects.
Appropriate Erosion Prevention and Sediment Control (EPSC) measures like silt fence, erosion logs, and seed and mulch are used on all projects regardless of presence or absence of freshwater mussels. As specified by permit, if we have greater than 10, (typically) mussel relocation is completed up to a year in advance of construction. There is follow up monitoring of the relocation site.
Our Department of Natural Resources (DNR) and the United States Fish and Wildlife Services (USFWS) offices in our state are generally more concerned about direct in-stream impacts to mussels rather than sedimentation effects.
Our DOT is transitioning towards new BMPs based on recently completed research by the state university for freshwater mussels. I will share the final report so you can access their recommendations for BMPs.
I don't work specifically with the construction side of our agency, but if any restrictions or methods are specifically required by T&E agencies, then we comply with the request.
Our wildlife department will request specific BMP's that can be utilized when needed to reduce in stream impacts for aquatic species. BMPs are used as part of the Stormwater Pollution Prevention Plan (SWPPP) for our DOT's projects.
We have no projects identified as having potential sedimentation impacts to freshwater mussels during construction.

Comment
We use many of these treatments often on projects that do not cause sedimentation to freshwater mussels; however, I have limited my answers to projects involving freshwater mussels.
If impacts to federally listed mussels are anticipated, we usually just try and stay out of the water completely. If we can't do that, generally we will use enhanced sedimentation measures and pay a mitigation fee.
The specialty silt mitigation measures (beyond work pads and silt fence) are usually used only on Scenic Rivers and rivers with federal mussels. We avoid in-stream work more often, especially on small stream with no piers on the old bridge
The work activity greatly influences the type of BMP used - sealing joints, resurfacing only cause very little overspray or spread outside of the footprint of work. My answers reflect the various types and take into consideration how often the in-water type of project might occur. Our DOT does a lot of maintenance projects to keep what is there usable. At least 75% of projects are this type.
Again, most of our projects (usually bridge construction/rehabilitation) are in intertidal zones or brackish water zones. I chose "never" for all the options: however, we regularly use in-water turbidity barriers, silt bags, silt fences, limit vegetation removal, work during specific times of year, and sometimes monetary compensation.
Work below Ordinary High Water Mark (OHW) is driven by the project description and related to the type of water quality permit. The types of erosion and sediment controls are project specific and typically conditions of a project's Water Quality (WQ) permit.
We do not have projects identified as having impacts to mussels. There may be some in our state, but this issue has not come up.
I answered this question from the perspective of controlling project sedimentation for our state’s T&E minnow species that are affected by sedimentation.

Table B-6. Individual responses to Question 3 (performance ratings of mitigation strategies or BMPs).

Respondent	Brush Barriers	Design Causeways	Ditch Checks	Flocculants	Gabion Baskets	In-Water Turbidity Barriers	Limit Vegetation	Monetary	No Equipment Below Ordinary High Water Mark (OHWM)	Relocate Mussel Beds	Seed and Mulch	Silt Bags	Silt Fence	Triangular Silt Dikes	Work During Specific Times of Year (e.g., No/Low Flow, No	Work Pads	Other (Please describe)
Alabama	3	-	4	4	-	2	4	-	5	5	4	3	4	-	4	3	-
Alaska	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arizona	-	-	3	-	3	-	4	-	3	-	3	-	3	-	-	3	-
Arkansas	-	3	4	2	4	3	3	1	4	3	3	3	3	3	4	3	-
California	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Colorado	1	1	3	2	2	2	3	1	4	-	4	3	3	2	4	3	-
Connecticut	-	4	4	-	-	4	5	-	5	5	4	4	5	-	5	5	-
Delaware	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
District of Columbia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Florida	4	1	4	4	4	3	5	-	3	2	2	3	4	4	4	4	-
Georgia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hawaii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Idaho	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Illinois	-	-	4	-	-	-	4	-	-	3	5	-	5	-	5	5	-
Indiana	-	5	5	-	-	4	5	-	5	4	5	4	5	-	4	4	5
Iowa	-	-	-	-	-	-	-	-	5	5	-	-	-	-	5	-	-
Kansas	-	4	4	-	-	-	5	5	5	4	3	-	4	-	5	4	-
Kentucky	1	1	1	1	2	3	4	3	3	3	3	3	3	1	1	4	-

Respondent	Brush Barriers	Design Causeways	Ditch Checks	Flocculants	Gabion Baskets	In-Water Turbidity Barriers	Limit Vegetation	Monetary	No Equipment Below Ordinary High Water Mark (OHWM)	Relocate Mussel Beds	Seed and Mulch	Silt Bags	Silt Fence	Triangular Silt Dikes	Work During Specific Times of Year (e.g., No/Low Flow, No	Work Pads	Other (Please describe)
Louisiana	1	3	4	-	4	4	3	2	4	4	2	3	3	3	4	2	-
Maine	-	-	3	-	-	5	4	-	-	-	4	-	3	-	5	-	-
Maryland	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Massachusetts	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Michigan	-	1	2	-	2	3	2	-	-	3	4	-	3	-	3	-	-
Minnesota	2	3	2	2	3	4	5	-	4	-	4	4	4	4	5	4	-
Mississippi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Missouri	-	4	3	-	2	3	2	1	3	4	2	2	1	1	4	4	-
Montana	-	-	-	-	-	-	-	-	5	3	-	-	3	-	4	-	-
Nebraska	-	4	3	1	-	2	5	-	3	-	4	1	3	3	4	3	-
Nevada	-	-	-	-	-	-	-	-	5	-	-	3	3	-	4	4	-
New Hampshire	-	3	-	3	-	4	4	-	3	4	-	-	3	-	4	-	-
New Jersey	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
New Mexico	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
New York	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
North Carolina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
North Dakota	-	5	1	-	-	5	-	-	-	3	5	-	5	-	5	3	-
Ohio	-	2	3	-	-	5	4	1	4	4	4	2	4	-	3	3	3
Oklahoma	-	-	3	-	-	4	5	-	5	2	4	-	3	3	4	4	-
Oregon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Respondent	Brush Barriers	Design Causeways	Ditch Checks	Flocculants	Gabion Baskets	In-Water Turbidity Barriers	Limit Vegetation	Monetary	No Equipment Below Ordinary High Water Mark (OHWM)	Relocate Mussel Beds	Seed and Mulch	Silt Bags	Silt Fence	Triangular Silt Dikes	Work During Specific Times of Year (e.g., No/Low Flow, No	Work Pads	Other (Please describe)
Pennsylvania	4	4	4	-	2	3	5	1	4	4	5	5	5	3	4	3	-
Rhode Island	-	-	-	-	4	4	4	-	-	-	4	4	2	-	4	-	-
South Carolina	3	-	-	-	-	-	-	-	4	-	-	4	5	-	-	-	-
South Dakota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tennessee	-	-	4	-	-	3	5	-	5	5	4	4	4	-	5	4	-
Texas	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Utah	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vermont	-	-	-	-	-	4	2	-	4	4	3	4	4	3	4	-	-
Virginia	-	3	3	-	-	3	3	-	3	4	3	3	3	-	4	3	-
Washington	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
West Virginia	3	4	3	4	4	4	4	4	5	4	4	4	4	3	4	3	-
Wisconsin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wyoming	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Average	2.4	3.1	3.2	2.6	3.0	3.5	4.0	2.1	4.1	3.7	3.7	3.3	3.6	2.8	4.1	3.5	4.0
Standard Deviation	1.2	1.3	1.0	1.2	1.0	0.9	1.0	1.5	0.8	0.9	0.9	0.9	1.0	1.0	0.8	0.7	1.4
Number of Responses	9	18	23	9	12	23	25	9	25	22	25	20	29	12	28	22	2

NOTE: 5 = Outstanding, 1 = Poor

Table B-7. Comments for Question 3 (performance ratings of mitigation strategies or BMPs).

Comment
We do abide by water turbidity requirements and do stormwater control (seed and mulch). Nothing specific for mussels.
Minimizing sedimentation is included in comments/recommendations from the Army Corps of Engineers (ACOE) or the Department of Environmental Quality (DEQ) (fed/state permitting agencies) and are incorporated into water quality permits as a required permit condition. Our DOT also has internal environmental commitments for larger projects that include sediment controls/BMPs, and often include relocation of mussels from the project impact zone prior to start of instream work.
Proper installation, routine maintenance/replacement are key to BMPs working correctly; some items scored as a 4 would have received a 5 if consistent maintenance/repairs occurred in the field.
I put a 1 star on things we do not often do. Also, we use work pads all the time. I feel that appropriate placement, management, and removal of these work pads can go a long way in reducing the sediment into the stream.
Our DOT is in the process of making this evaluation. Results pending.
Again....from the state’s minnow perspective.
We have no projects identified as having potential sedimentation impacts to freshwater mussels during construction.
We strongly believe in maintaining as much of the existing vegetation/land cover as possible. What we do not maintain, we try to replace with native seeding/fast growing cover/etc., depending on the project.
Relating to reducing sedimentation in general. Silt fencing rarely installed properly.
Certain physical BMPs have been certified to be effective for sediment control for the 5-year 24-hr storm event. Physical BMPs are often specified for use in series or with other BMPs. At times, agencies have requested redundancy in a single BMP, e.g. more than one row of silt fence.
I would rate our mitigation strategies/ BMPs as a 4. We value our natural resources and take environmental impacts seriously.
A university study analyzed effectiveness of different BMPs, as well as the risk of various BMPs being overwhelmed by large/extended storm events or risk of non-maintenance. Any values I add for BMPs would be purely anecdotal, so recommend referencing the report.
I don't think I could answer, as we've never done post-work surveys.
For work pads, they minimize silt in streams with loose substrate but probably cause more siltation in areas that are mostly bedrock bottom.
BMP to reduce sedimentation are not specific to freshwater mussels but are consistent with maintaining water quality standards.

Comment
It is difficult to assess the effectiveness as related to freshwater mussels since we have very few projects that have documented impacts. The assessment for the above BMPs was given in relation to impacts to aquatic species.

Table B-8. Individual responses to Question 4 (types of mussel surveys).

Respondent	Cells, a.k.a. quadrats (quantitative)	Transect	Moving transect	Timed search	eDNA	Other (Please describe)	Other (please describe)
Alabama	-	-	-	-	-	-	-
Alaska	-	-	-	-	-	-	Yes
Arizona	-	-	-	-	-	-	-
Arkansas	-	Yes	-	Yes	-	-	-
California	-	-	-	-	-	-	-
Colorado	-	-	-	-	-	-	-
Connecticut	-	Yes	-	-	-	-	-
Delaware	-	-	-	-	-	-	-
District of Columbia	-	-	-	-	-	-	-
Florida	-	Yes	-	-	-	-	-
Georgia	-	Yes	-	Yes	-	-	-
Hawaii	-	-	-	-	-	-	-
Idaho	-	-	-	-	-	-	Yes
Illinois	-	Yes	-	Yes	-	-	-
Indiana	-	Yes	-	-	-	-	-
Iowa	-	Yes	-	Yes	-	-	-
Kansas	-	-	-	-	-	-	-
Kentucky	-	Yes	-	Yes	-	-	Yes
Louisiana	-	Yes	-	Yes	-	-	-
Maine	-	-	-	Yes	Yes	-	Yes
Maryland	-	-	-	-	-	-	-
Massachusetts	-	-	-	-	-	-	-
Michigan	-	-	Yes	Yes	-	-	-
Minnesota	-	-	-	Yes	-	-	Yes
Mississippi	-	-	-	-	-	-	Yes
Missouri	Yes	Yes	-	Yes	-	-	-

Respondent	**Cells, a.k.a. quadrats (quantitative)**	**Transect**	**Moving transect**	**Timed search**	**eDNA**	**Other (Please describe)**	**Other (please describe)**
Montana	-	-	-	-	-	-	Yes
Nebraska	-	-	-	-	-	-	Yes
Nevada	-	-	-	Yes	-	-	-
New Hampshire	-	-	-	-	-	-	-
New Jersey	-	-	-	-	-	-	-
New Mexico	-	-	-	-	-	-	-
New York	-	-	-	-	-	-	-
North Carolina	-	-	-	-	-	-	-
North Dakota	-	Yes	-	-	-	-	-
Ohio	-	Yes	Yes	Yes	Yes	-	-
Oklahoma	-	Yes	Yes	Yes	-	-	-
Oregon	-	-	-	-	-	-	-
Pennsylvania	-	Yes	-	Yes	-	-	-
Rhode Island	-	-	-	-	-	-	-
South Carolina	-	-	-	-	-	-	-
South Dakota	-	-	-	-	-	-	Yes
Tennessee	-	Yes	-	Yes	-	-	-
Texas	-	Yes	-	Yes	-	-	-
Utah	-	-	-	-	-	-	Yes
Vermont	-	Yes	-	-	-	-	Yes
Virginia	-	-	-	Yes	-	-	-
Washington	-	-	-	-	-	-	Yes
West Virginia	-	Yes	Yes	Yes	-	-	-
Wisconsin	-	-	-	-	-	-	-
Wyoming	-	-	-	-	-	-	Yes
Total Yes	1	18	4	18	2	0	13

Table B-9. Text responses for "Other" for Question 4 (types of mussel surveys).

Other – Text Response
The only mussel survey that I can recall was a simple presence absence survey. When determined to be present, we instituted sedimentation controls.
None
Information from other agencies, field investigations and personal knowledge.
We've never had to do a survey.
None
Relocation - diminishing returns
None
All mussel surveys are completed by consultants. I have looked into eDNA but have not conducted any studies with this.
Typically, we perform transects surveys with times searches in between the transects.
We are currently partnering with state agencies and UMO to develop eDNA test.
None

Table B-10. Comments for Question 4 (types of mussel surveys).

Comment
We set transects across the stream every 50 meters up- and downstream, then specify a person-hour value for each transect based on stream width. So, a single transect with 1 person hour would take 30 minutes with 2 surveyors, 20 minutes for 3 surveyors, or 15 minutes for 4 surveyors. A copy of our aquatic survey protocols can be found here: http://www.dot.ga.gov/PartnerSmart/EnvironmentalProcedures/Ecology1/References/Aquatic%20Survey%20Protocols%20-%20GDOT-OES.pdf
Not sure.
None
One of our river bridges had an extensive survey, and I know of one smaller one on the Wabash River, but I don't know the method. Other projects are known areas with appropriate bed materials but do not require surveys.
For eDNA, we are just now starting some pilot studies comparing results to traditional mussel surveys. It is not yet being used for regulatory purposes.
Mussel surveys conducted for our DOT projects needing approval from the state’s wildlife department often require that survey be completed by an approved mussel survey expert from the agency. Our DOT has not gotten approval to complete mussel surveys with existing staff. Most locations that have required mussel surveys needed additional equipment & certifications (scuba, cold water scuba).
We have no projects currently surveying for impacts to freshwater mussels during construction.
We have the capabilities to do transect/moving transect surveys, but our state’s mussel survey protocol only requires quantitative and qualitative sampling.
On one occasion a mussel relocation for a bridge construction required transects due to the density of mussels along the impact zone.
With the recent USFWS proposal to list six species, the USFWS and our state’s parks and wildlife are developing joint protocols which will define the survey methods.
We do not conduct mussel surveys.

Table B-11. Individual responses to Question 5 (likelihood of construction activities causing sedimentation impacts to freshwater mussels).

Respondent	Bridge Construction	Bridge Rehabilitation	Bridge Removal	Cofferdam Construction	Cofferdam Removal	Culvert Replacement	Drilled Shafts	General Soil Disturbance of Overall Construction Site	Grading	Pile Driving	Riprap Placement	Temporary Causeway Construction	Temporary Sheet Piling Installation	Other (Please describe)
Alabama	4	4	4	5	5	5	4	4	4	4	4	3	4	-
Alaska	4	2	4	5	5	5	4	4	4	3	4	4	4	-
Arizona	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arkansas	4	1	5	1	4	4	4	5	4	2	4	2	2	-
California	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Colorado	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Connecticut	4	3	4	2	4	2	4	5	5	2	2	2	2	-
Delaware	5	4	5	4	4	4	4	5	5	5	4	4	4	-
District of Columbia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Florida	5	4	5	3	3	5	4	4	5	4	5	5	4	-
Georgia	5	3	5	4	4	5	1	5	4	1	2	5	4	-
Hawaii	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Idaho	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Illinois	4	2	4	2	2	4	3	2	2	2	2	2	2	-
Indiana	4	2	4	4	5	4	4	4	4	3	4	4	4	-
Iowa	4	-	4	4	-	4	-	-	-	4	-	-	-	-
Kansas	4	2	4	5	5	4	3	4	4	3	3	4	4	-
Kentucky	5	4	5	5	5	5	3	3	3	3	4	5	3	-

Respondent	Bridge Construction	Bridge Rehabilitation	Bridge Removal	Cofferdam Construction	Cofferdam Removal	Culvert Replacement	Drilled Shafts	General Soil Disturbance of Overall Construction Site	Grading	Pile Driving	Riprap Placement	Temporary Causeway Construction	Temporary Sheet Piling Installation	Other (Please describe)
Louisiana	5	4	5	5	5	3	4	3	3	4	3	4	3	-
Maine	5	-	5	5	5	5	3	2	2	2	4	5	3	-
Maryland	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mass.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Michigan	4	2	4	3	3	4	3	5	4	2	4	5	3	-
Minnesota	5	4	5	3	4	4	3	4	4	3	4	5	3	5
Mississippi	5	1	5	-	-	5	-	2	2	-	4	-	4	-
Missouri	5	4	5	4	4	4	4	4	4	3	4	5	4	-
Montana	4	1	4	4	4	4	4	2	2	4	5	3	4	-
Nebraska	4	3	5	5	5	2	2	4	3	1	2	5	3	-
Nevada	5	4	5	5	5	3	2	2	2	3	4	4	3	-
New Hampshire	3	1	4	3	4	2	3	4	2	3	3	4	3	-
New Jersey	-	-	-	-	-	-	-	-	-	-	-	-	-	-
New Mexico	-	-	-	-	-	-	-	-	-	-	-	-	-	-
New York	-	-	-	-	-	-	-	-	-	-	-	-	-	-
North Carolina	-	-	-	-	-	-	-	-	-	-	-	-	-	-
North Dakota	5	5	5	5	5	4	3	4	3	3	5	4	3	-

Respondent	Bridge Construction	Bridge Rehabilitation	Bridge Removal	Cofferdam Construction	Cofferdam Removal	Culvert Replacement	Drilled Shafts	General Soil Disturbance of Overall Construction Site	Grading	Pile Driving	Riprap Placement	Temporary Causeway Construction	Temporary Sheet Piling Installation	Other (Please describe)
Ohio	4	4	4	3	4	4	4	5	5	3	3	3	4	-
Oklahoma	5	4	5	4	4	4	5	5	5	4	4	4	4	-
Oregon	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pennsylvania	4	4	2	2	2	2	2	2	2	2	2	4	2	-
Rhode Island	5	2	5	5	5	5	4	4	4	4	2	3	4	-
South Carolina	4	4	4	3	3	4	3	3	3	3	3	3	3	-
South Dakota	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tennessee	5	2	5	2	4	5	2	2	2	2	1	4	2	-
Texas	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Utah	1	1	1	1	1	1	1	1	1	1	1	1	1	-
Vermont	2	2	2	4	4	4	4	2	2	2	4	5	2	-
Virginia	4	4	4	5	5	4	2	4	3	2	4	5	3	-
Washington	-	-	-	-	-	-	-	-	-	-	-	-	-	-
West Virginia	5	4	5	4	4	3	4	4	4	3	4	4	3	-
Wisconsin	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wyoming	5	-	5	4	4	4	-	4	-	4	4	-	-	-

Respondent	Bridge Construction	Bridge Rehabilitation	Bridge Removal	Cofferdam Construction	Cofferdam Removal	Culvert Replacement	Drilled Shafts	General Soil Disturbance of Overall Construction Site	Grading	Pile Driving	Riprap Placement	Temporary Causeway Construction	Temporary Sheet Piling Installation	Other (Please describe)
Average	4.3	2.9	4.3	3.7	4.1	3.9	3.2	3.5	3.3	2.8	3.4	3.9	3.2	5.0
Standard Deviation	0.9	1.2	1.0	1.2	1.0	1.0	1.0	1.2	1.1	1.0	1.1	1.1	0.8	-
Number of Responses	34	31	34	33	32	34	31	33	32	33	33	31	32	1

NOTE:

5 = Very Likely, 4 = Somewhat Likely, 3 = Neutral, 2 = Somewhat Unlikely, 1 = Very Unlikely

Table B-12. Text responses for "Other" for Question 5 (likelihood of construction activities causing sedimentation impacts to freshwater mussels).

Other – Text Response
Causeway Removal
This question complicated by when/if BMPs are implemented. Our DOT's expectation is that proper implementation of BMPs will minimize the sediment impacts to freshwater mussels.

Table B-13. Comments for Question 5 (likelihood of construction activities causing sedimentation impacts to freshwater mussels).

Comment
Bridge construction/demolition is the big one for me. Causeway removal (and I suppose all removals) to me are more likely to release sediments vs. installation.
The impacts depend on construction methods or project activities and whether there will be instream work or work below OHW. Erosion and Settlement (E&S) controls are installed prior to construction to reduce sedimentation. If work is below OHW & mussel are present, relocation surveys are conducted prior to commencement of construction.
Again, recommend referencing the report, though I have more experience to base responses on this question.
Each of these really depends on the size of the bridge and length of time with disturbed earth during construction. Bridge rehab is too broad of a category to answer accurately. Pier and deck patching isn't going to impact mussels, but a full superstructure replacement could.
Above answers reflect an activity occurring within close proximity to a known downstream mussel population. Bridge removal answer was based on the requirement to either not have any debris fall into the stream during demolition and removal or the use of a rock causeway beneath the structure to capture the bridge in pieces as it's being demolished. The use of the causeway would include a preconstruction mussel survey and salvage relocation if present. The natural sediment transport in our stream systems due to land use practices and development has been observed to be much higher than what is typical of a bridge construction project utilizing proper E&S BMPs.
On-land ground disturbance depends on how well the erosion & sediment controls were installed and maintained. In-water disturbance depends on stream bottom characteristics (i.e. silty vs bedrock).
Most anything that can disturb a streambed or adjacent bank has potential to cause runoff putting some sediment in the stream.
We have no projects identified as having potential sedimentation impacts to freshwater mussels during construction.

Table B-14. Individual responses to Question 6 (concerns that hinder efforts to reduce sedimentation impacts to freshwater mussels during construction).

Respondent	Bridge Construction	Bridge Rehabilitation	Bridge Removal	Cofferdam Construction	Cofferdam Removal	Culvert Replacement	Drilled Shafts	General Soil Disturbance of Overall Construction Site	Grading	Pile Driving	Riprap Placement	Temporary Causeway Construction
Alabama	2	2	4	4	3	2	5	3	2	3	4	-
Alaska	3	4	3	3	3	3	3	3	3	3	3	-
Arizona	-	-	-	-	-	-	-	-	-	-	-	-
Arkansas	2	2	4	2	4	4	5	4	2	4	4	-
California	-	-	-	-	-	-	-	-	-	-	-	-
Colorado	-	-	-	-	-	-	-	-	-	-	-	-
Connecticut	3	1	1	1	1	2	2	2	2	4	4	-
Delaware	3	2	4	3	3	2	3	4	4	4	4	-
District of Columbia	-	-	-	-	-	-	-	-	-	-	-	-
Florida	2	2	2	2	4	4	3	4	4	3	3	-
Georgia	4	2	5	2	4	5	5	3	4	1	4	-
Hawaii	-	-	-	-	-	-	-	-	-	-	-	-
Idaho	-	-	-	-	-	-	-	-	-	-	-	-
Illinois	2	3	5	3	4	4	3	3	3	3	4	3
Indiana	4	3	3	4	4	4	4	4	4	3	4	-
Iowa	-	-	-	-	-	-	-	-	-	-	-	-
Kansas	3	3	3	4	4	4	5	3	4	5	3	-
Kentucky	4	3	3	3	4	3	3	4	3	3	3	-
Louisiana	2	2	2	3	3	4	3	3	4	4	4	-
Maine	1	1	1	1	1	1	1	1	1	1	1	1
Maryland	-	-	-	-	-	-	-	-	-	-	-	-
Massachusetts	-	-	-	-	-	-	-	-	-	-	-	-
Michigan	3	2	2	4	4	4	4	4	3	4	4	-
Minnesota	2	2	2	3	4	5	3	5	4	3	3	-
Mississippi	1	1	1	1	1	1	1	3	1	1	1	-
Missouri	3	1	4	4	5	5	5	4	4	3	3	-
Montana	3	2	3	4	2	2	1	2	1	3	2	5

Respondent	**Bridge Construction**	**Bridge Rehabilitation**	**Bridge Removal**	**Cofferdam Construction**	**Cofferdam Removal**	**Culvert Replacement**	**Drilled Shafts**	**General Soil Disturbance of Overall Construction Site**	**Grading**	**Pile Driving**	**Riprap Placement**	**Temporary Causeway Construction**
Nebraska	3	3	3	3	3	3	3	3	3	3	3	-
Nevada	4	4	4	4	4	4	4	4	5	3	4	-
New Hampshire	1	3	3	4	3	3	3	3	3	3	3	-
New Jersey	-	-	-	-	-	-	-	-	-	-	-	-
New Mexico	-	-	-	-	-	-	-	-	-	-	-	-
New York	-	-	-	-	-	-	-	-	-	-	-	-
North Carolina	-	-	-	-	-	-	-	-	-	-	-	-
North Dakota	3	1	4	1	4	1	4	1	3	4	4	-
Ohio	4	1	4	4	5	5	5	5	2	4	1	-
Oklahoma	1	1	1	5	5	5	5	5	3	5	5	-
Oregon	-	-	-	-	-	-	-	-	-	-	-	-
Pennsylvania	4	2	2	2	3	2	4	5	4	4	4	-
Rhode Island	4	4	5	4	3	3	5	4	4	5	5	-
South Carolina	2	1	2	2	2	1	1	2	2	2	2	-
South Dakota	-	-	-	-	-	-	-	-	-	-	-	-
Tennessee	2	2	3	3	2	2	4	3	4	3	4	-
Texas	-	-	-	-	-	-	-	-	-	-	-	-
Utah	1	1	1	1	1	1	1	1	1	2	2	-
Vermont	2	2	2	2	2	2	4	2	1	3	3	-
Virginia	3	3	4	3	5	4	5	5	4	3	4	-
Washington	-	-	-	-	-	-	-	-	-	-	-	-
West Virginia	3	3	4	4	3	3	4	3	3	3	4	-
Wisconsin	-	-	-	-	-	-	-	-	-	-	-	-
Wyoming	3	3	3	3	4	3	3	3	4	4	4	-
Average	2.6	2.2	2.9	2.9	3.2	3.1	3.5	3.3	3.0	3.2	3.3	3.0
Standard Deviation	1.0	1.0	1.2	1.1	1.2	1.3	1.3	1.2	1.1	1.0	1.1	2.0
Number of Responses	33	33	33	33	33	33	33	33	33	33	33	3

NOTE: 5 = Strongly Agree, 4 = Somewhat Agree, 3 = Neither Agree Nor Disagree, 2 = Somewhat Disagree, 1 = Strongly Disagree

Table B-15. Text responses for "Other" Question 6 (concerns that hinder efforts to reduce sedimentation impacts to freshwater mussels during construction).

Other – Text Response
Regulatory drivers and overall concern for mussels.
Proper implementation can be a challenge on a project specific basis. However, in general our DOT has good support at all levels for implementation of necessary measures to minimize impacts from sediment to freshwater mussels.

Table B-16. Comments for Question 6 (concerns that hinder efforts to reduce sedimentation impacts to freshwater mussels during construction).

Comment
I answered these questions referring to marine mussels.
I believe sometimes there is an overall lack of understanding about specific species, and also the whole stream ecosystem appears to get over simplified.
Your average DOT staff or contractor is not going to know the life cycle of a mussel and won't apply any BMPs and/or avoidance and minimization measures unless they're told they are required.
Every project we implement uses best management practices to reduce sedimentation across the board. When mussel-specific concerns are highlighted by USFWS or a state protection agency, we do what is advised to properly address those concerns.
I feel more research is needed to fully understand if sedimentation from a bridge construction project utilizing proper E&S and mussel related BMPs has an impact on freshwater mussel species.
Fish and Wildlife Services (FWS) Section 7 asking more stringent questions about long term sedimentation and impacts to mussels from construction/project activities. We often receive recommendations/requests for pre- and post-construction monitoring of mussels. Much of this is due to lack of data.
Our DOT successfully implements BMPs for minimizing sedimentation on projects that may result in effects. These BMPs are in place not only for mussels but other aquatic species and general water quality.
When required our DOT uses our protected species on-call to access appropriate resources for mussel identification (ID), relocation, etc. They use in-state specialists to do the work. The Department of Natural Resources (DNR) has also provided support to include relocation efforts. My staff currently includes an individual with sufficient experience to support outside efforts, but that effort would be ancillary to their primary job functions. Initial notice of potential impacts comes from the DNR Early Coordination or USFWS species list. Often, projects are not near where the species were identified - upstream, etc. I think there is a lot of extirpation from previously known reaches. I just finished reviewing the draft of the Endangered, Threatened, and Rare (ETR) chapter for the Mid-States Corridor project and that was frequently mentioned in the mussels section.
Our DOT has the capabilities and desire to limit sedimentation to mussels, but it is unclear what might be necessary beyond what is already required in our DNR/ United States Army Corps of Engineers (USACE)/NPDES permits. If there was a clearer quantitative evidence/thresholds of impacts, our DOT would follow that guidance.

Table B-17. Individual responses to Question 7 (access to data regarding mussel beds).

Respondent	Yes, my agency maintains a database with this information	Yes, my agency has access to an external database with this	No	No Response
Alabama	-	-	Yes	-
Alaska	-	-	Yes	-
Arizona	-	-	Yes	-
Arkansas	-	Yes	-	-
California	-	-	-	-
Colorado	-	-	-	-
Connecticut	-	Yes	-	-
Delaware	-	-	Yes	-
District of Columbia	-	-	-	-
Florida	-	Yes	-	-
Georgia	-	Yes	-	-
Hawaii	-	-	-	-
Idaho	-	-	Yes	-
Illinois	-	Yes	-	-
Indiana	-	Yes	-	-
Iowa	-	Yes	-	-
Kansas	-	-	Yes	-
Kentucky	Yes	-	-	-
Louisiana	-	-	Yes	-
Maine	-	Yes	-	-
Maryland	-	-	-	-
Massachusetts	-	-	-	-
Michigan	Yes	-	-	-
Minnesota	Yes	-	-	-
Mississippi	-	Yes	-	-
Missouri	Yes	-	-	-

Respondent	**Yes, my agency maintains a database with this information**	**Yes, my agency has access to an external database with this**	**No**	**No Response**
Montana	-	Yes	-	-
Nebraska	-	Yes	-	-
Nevada	-	Yes	-	-
New Hampshire	-	Yes	-	-
New Jersey	-	-	-	-
New Mexico	-	-	-	-
New York	-	-	-	-
North Carolina	-	-	-	-
North Dakota	-	Yes	-	-
Ohio	Yes	-	-	-
Oklahoma	-	Yes	-	-
Oregon	-	-	Yes	-
Pennsylvania	-	Yes	-	-
Rhode Island	-	-	Yes	-
South Carolina	-	Yes	-	-
South Dakota	-	-	Yes	-
Tennessee	-	Yes	-	-
Texas	-	Yes	-	-
Utah	-	-	Yes	-
Vermont	-	Yes	-	-
Virginia	-	Yes	-	-
Washington	-	-	Yes	-
West Virginia	-	Yes	-	-
Wisconsin	-	-	-	-
Wyoming	-	-	Yes	-
Total Yes	5	22	13	0

Table B-18. Comments for Question 7 (access to data regarding mussel beds).

Comment
Our state’s fish and wildlife department has an RTE database that can be searched.
We have access to known occurrence locations of protected mussel species, not non-listed species.
Our DOT has access to information data bases at our state’s game and parks commission.
We have limited access the State resource agency for information regarding past mussel surveys/collection records (approx. locations, dates). However, we do not have access to densities or population compositions data. This information is usually obtained through verbal (or electronic) communication with our state malacologist.
I only have access to critical habitat designations, but I can request information from the USFWS per project.
We hold the only collection database from mussel surveys performed under our state’s survey mussel protocol. We funded its creation and do all data entry.
We coordinate with our local Division of Natural Resources office, and they let us know if there are any documented mussel beds within our project areas.
We utilize a state-wide natural resource GIS (Geographic Information System) database for project screening. This inventory or database contains information specific to our state's game commission, fish commission, and conservation and natural resource dept. (plants and unique geological areas) areas of focus. The project screening analysis tool screens the project area for state and federally listed fish, mussel, mammal, plant, and invertebrate species. It also screens for species of local or state concern.
Our state’s natural resource agencies maintain a database of known mussel occurrences and we have an agreement with them to ensure it is shared information.
We would receive that information during our coordination with applicable regulatory agencies.
As mentioned previously this information would come from the DNR or USFWS. I'm not sure what type of information they maintain.
Our DOT does not have a database regarding mussel beds. I coordinate with the state’s natural resource/environmental control department on environmental impacts. From our coordination/conversations I don't believe they have a dedicated database for mussel beds either. However, they do have records of previous surveys, or will survey the site if mussel beds are a possibility.
A subset of our state’s office of environmental stewardship has license to access to our state DNR's Natural History Inventory database. Mussel data provided include species, number, disposition, date of survey, efforts, etc. Our state must provide DNR with mussel survey data (report and raw electronic data) if a mussel survey done in-house.
Our DOT invested in development of a mussels database maintained by the state university. This is the primary source of current mussel location data. Our DOT also uses the parks and wildlife department's data but finds the other database to be most up to date.

Comment
Our states Natural Heritage Database depicts some mussel beds (unusual concentrations of invertebrates). It does not track all, just some that are more diverse.
Both. We have an internal database as well as access to natural heritage database for the state.
Our DOT does not have access to any know mussel database information for the state. I believe that our state’s wildlife department would be the most likely source of this information, but only accessed during project consultation.
Any freshwater mussel data we collect is submitted to our State and Federal partner agencies.

Table B-19. Individual responses to Question 8 (use of post-construction monitoring).

Respondent	Response
Alabama	No
Alaska	No
Arizona	No
Arkansas	No
California	-
Colorado	-
Connecticut	Yes
Delaware	No
District of Columbia	-
Florida	No
Georgia	Yes
Hawaii	-
Idaho	No
Illinois	Yes
Indiana	No
Iowa	Yes
Kansas	No
Kentucky	No
Louisiana	Yes
Maine	No
Maryland	-
Massachusetts	-
Michigan	Yes
Minnesota	No
Mississippi	No
Missouri	Yes
Montana	No
Nebraska	No
Nevada	No
New Hampshire	Yes
New Jersey	-
New Mexico	-
New York	-
North Carolina	-
North Dakota	No

Respondent	Response
Ohio	Yes
Oklahoma	Yes
Oregon	No
Pennsylvania	Yes
Rhode Island	No
South Carolina	Yes
South Dakota	No
Tennessee	No
Texas	Yes
Utah	No
Vermont	Yes
Virginia	Yes
Washington	No
West Virginia	Yes
Wisconsin	-
Wyoming	No
Total Yes	16
Total No	24
Number of Responses	40

Table B-20. Comments for Question 8 (use of post-construction monitoring).

Comment
As part of our Mussel PBO with USFWS we often perform post construction monitoring of the project area and any projects that utilize mussel salvage and relocation.
We have completed one project where we were able to do a before, and then 1, 3, and 5 years post construction completion surveys. We had good results, and the mussels were coming back into the relocation area.
Typically, we only conduct post-construction monitoring when we do a mussel relocation for a project to assess the success of the effort and the effect on the population. We typically do not conduct surveys after most construction projects.
We are required to do post-construction monitoring anytime we are required to relocate listed mussels. When we relocate mussels, we have to relocate all mussels. The post-construction monitoring is really monitoring the listed species survival though.
I only know of one project that would have warranted this. I don't remember if it has post-construction monitoring requirements. I need to check.
Post-construction monitoring to evaluate relocation impacts to mussels, not necessarily construction impacts.
We are aware of only 1 or 2 projects where this was needed that was a permit conditions per a Biological Opinion issued for the project. However, we anticipate this will be requested by resource/regulatory agencies in the future.
We have done a post-construction field visit with the USFWS to evaluate site conditions and if any follow up was required. As a part of my first BO, we were required to do a one-year monitoring survey for where we relocated the freshwater mussels to (upstream of the construction site). But no monitoring or re-survey downstream of project or within project footprint.
We have conducted one study in which effects were monitored during and post construction.
We mostly did mark recapture studies in the early 2000s. Now we only do monitoring for federal species
On rare occasion DOT staff have performed post-construction surveys to assess relocated mussels.
DNR does not require any post-construction monitoring. This should probably be required, especially for projects involving a mussel relocation (monitor relocated individuals and monitor salvaged area/construction zone for mussel recolonization).

Table B-21. Individual responses to Question 9 (development of resources).

Respondent	Survey protocol	BMP guidelines	Specifications	Special provisions	Evaluation studies	Other (please describe)
Alabama	Yes	Yes	Yes	Yes	Yes	-
Alaska	-	-	-	-	-	Yes
Arizona	-	-	-	-	-	-
Arkansas	-	Yes	Yes	Yes	Yes	-
California	-	-	-	-	-	-
Colorado	-	-	-	-	-	-
Connecticut	-	Yes	Yes	Yes	Yes	-
Delaware	-	-	-	-	-	-
District of Columbia	-	-	-	-	-	-
Florida	-	-	-	-	-	Yes
Georgia	Yes	Yes	Yes	Yes	Yes	-
Hawaii	-	-	-	-	-	-
Idaho	-	-	-	-	-	Yes
Illinois	-	-	-	-	-	Yes
Indiana	-	-	-	Yes	-	-
Iowa	Yes	Yes	-	-	-	-
Kansas	-	-	-	-	-	-
Kentucky	Yes	-	-	-	-	-
Louisiana	Yes	-	-	-	-	-
Maine	Yes	Yes	-	-	-	-
Maryland	-	-	-	-	-	-
Massachusetts	-	-	-	-	-	-
Michigan	-	-	-	-	-	-
Minnesota	-	-	-	-	-	Yes
Mississippi	-	-	-	-	-	-
Missouri	Yes	Yes	Yes	Yes	Yes	-
Montana	-	-	-	-	-	Yes
Nebraska	-	-	-	-	-	Yes
Nevada	-	-	-	-	-	-
New Hampshire	-	-	-	-	-	-

Respondent	Survey protocol	BMP guidelines	Specifications	Special provisions	Evaluation studies	Other (please describe)
New Jersey	-	-	-	-	-	-
New Mexico	-	-	-	-	-	-
New York	-	-	-	-	-	-
North Carolina	-	-	-	-	-	-
North Dakota	-	-	-	-	-	-
Ohio	Yes	Yes	Yes	Yes	Yes	Yes
Oklahoma	Yes	Yes	-	-	-	-
Oregon	-	-	-	-	-	-
Pennsylvania	Yes	Yes	Yes	Yes	Yes	-
Rhode Island	-	-	-	-	-	-
South Carolina	-	Yes	-	Yes	-	-
South Dakota	-	-	-	-	-	Yes
Tennessee	Yes	Yes	-	-	-	-
Texas	Yes	Yes	Yes	Yes	Yes	-
Utah	-	-	-	-	-	Yes
Vermont	-	-	-	-	-	Yes
Virginia	-	-	-	-	-	Yes
Washington	-	-	-	-	-	-
West Virginia	-	Yes	Yes	Yes	-	-
Wisconsin	-	-	-	-	-	-
Wyoming	-	Yes	-	-	-	-
Total Yes	12	15	9	11	8	12

Table B-22. Text responses for “Other” for Question 9 (development of resources).

Other – Text Response
None....Mussel survey was a one-time event for our DOT to date.
None specifically for mussels.
We have not developed any resources in particular to freshwater mussels. Mostly permit conditions are followed.
We have nothing that is specific to sedimentation impacts to freshwater mussels. We do have guidance regarding Storm Water Pollution Prevention.
None
None
It is very project specific. We simply don't encounter this often in our state.
Our DOT developed a Programmatic Agreement with the USFWS in 2017.
Minimizing sedimentation is included in comments/recommendations from ACOE or DEQ (fed/state permitting agencies) and are incorporated into water quality permits as a required permit conditions. Our DOT also has internal environmental commitments for larger projects that include sediment controls/BMPs, and often include relocation of mussels from the project impact zone prior to start of instream work.
We contributed the reconnaissance protocol to the overall OMSP. I am also on the review team.

Table B-23. Resources submitted for Question 9 (development of resources).

Respondent	Resource Description
Arkansas	Standard Specifications for Highway Construction
Arkansas	Evaluation of Freshwater Mussel Conservation Strategies at Multiple Scales: Macro-molecules, Behavior, Habitat, and Policy.
Arkansas	Survival and Horizontal Movement of the Freshwater Mussel Potamilus capax (Green, 1832) Following Relocation within a Mississippi Delta Stream System
Arkansas	Storm Water Pollution Prevention Plan
Arkansas	Standard Specifications for Highway Construction Section 110 Amendment
Florida	Amendment to the Freshwater Mussel Phase 1 Programmatic Approach for Transportation Work Activities
Georgia	Aquatic Survey Protocols for Transportation Projects within the State of Georgia
Georgia	Environmental Procedures Guidebooks
Georgia	Review of Special Provisions and Other Conditions Placed on GDOT Projects for Imperiled Species Protection Volume I
Georgia	Review of Special Provisions and Other Conditions Placed on GDOT Projects for Imperiled Species Protection Volume II
Georgia	Review of Special Provisions and Other Conditions Placed on GDOT Projects for Imperiled Species Protection Volume III
Georgia	Review of Special Provisions and Other Conditions Placed on GDOT Projects for Imperiled Species Protection Volume IV
Tennessee	Tennessee Erosion and Sediment Control Handbook: A stormwater planning and Design Manual for Construction Activities
Tennessee	Standard Drawings Library

Table B-24. Individual responses to Question 10 (collaboration with other organizations).

Respondent	Consultants	Non-profit organizations	Other state agencies	Universities	U.S. Department of Agriculture	U.S. Environmental Protection Agency	U.S. Fish and Wildlife Service	U.S. Geological Survey	U.S. Army Corps of Engineers	Other (please describe)	None
Alabama	Yes	-	-	Yes	-	-	Yes	-	Yes	-	-
Alaska	-	-	-	-	-	-	-	-	-	Yes	-
Arizona	-	-	-	-	-	-	-	-	-	-	Yes
Arkansas	-	-	Yes	-	-	-	Yes	-	-	-	-
California	-	-	-	-	-	-	-	-	-	-	-
Colorado	-	-	-	-	-	-	-	-	-	-	-
Connecticut	Yes	-	Yes	-	-	Yes	-	-	Yes	-	-
Delaware	Yes	-	Yes	-	-	-	-	-	Yes	Yes	-
District of Columbia	-	-	-	-	-	-	-	-	-	-	-
Florida	Yes	-	-	-	-	-	Yes	-	-	-	-
Georgia	Yes	-	Yes	Yes	-	-	Yes	-	Yes	-	-
Hawaii	-	-	-	-	-	-	-	-	-	-	-
Idaho	-	-	-	-	-	-	-	-	-	-	Yes
Illinois	-	-	Yes	-	-	-	Yes	-	-	Yes	-
Indiana	Yes	-	Yes	Yes	-	-	Yes	-	-	-	-
Iowa	Yes	-	Yes	-	-	-	Yes	-	-	-	-
Kansas	Yes	-	Yes	-	-	-	Yes	-	-	-	-
Kentucky	Yes	-	Yes	-	-	-	Yes	-	-	-	-
Louisiana	Yes	-	Yes	-	-	-	Yes	-	Yes	-	-
Maine	Yes	-	Yes	-	-	-	-	-	-	-	-
Maryland	-	-	-	-	-	-	-	-	-	-	-
Massachusetts	-	-	-	-	-	-	-	-	-	-	-
Michigan	-	-	Yes	-	-	-	Yes	-	-	-	-
Minnesota	-	-	-	-	-	-	-	-	-	-	Yes
Mississippi	-	-	-	-	-	-	-	-	-	-	-
Missouri	Yes	-	Yes	Yes	-	-	Yes	Yes	Yes	-	-
Montana	-	-	Yes	-	-	-	Yes	-	Yes	-	-

Respondent	Consultants	Non-profit organizations	Other state agencies	Universities	U.S. Department of Agriculture	U.S. Environmental Protection Agency	U.S. Fish and Wildlife Service	U.S. Geological Survey	U.S. Army Corps of Engineers	Other (please describe)	None
Nebraska	-	-	Yes	-	-	-	Yes	-	-	-	-
Nevada	-	-	Yes	-	-	-	-	-	Yes	-	-
New Hampshire	Yes	-	Yes	Yes	-	Yes	Yes	-	Yes	-	-
New Jersey	-	-	-	-	-	-	-	-	-	-	-
New Mexico	-	-	-	-	-	-	-	-	-	-	-
New York	-	-	-	-	-	-	-	-	-	-	-
North Carolina	-	-	-	-	-	-	-	-	-	-	-
North Dakota	Yes	-	Yes	Yes	-	-	Yes	-	Yes	-	-
Ohio	Yes	-	Yes	Yes	-	-	Yes	-	-	-	-
Oklahoma	Yes	-	Yes	-	-	-	Yes	-	-	-	-
Oregon	-	-	-	-	-	-	-	-	-	-	-
Pennsylvania	Yes	Yes	Yes	Yes	-	Yes	Yes	-	Yes	-	-
Rhode Island	-	-	-	-	-	-	-	-	-	-	Yes
South Carolina	-	-	-	-	-	-	Yes	-	-	-	-
South Dakota	-	-	-	-	-	-	-	-	-	-	Yes
Tennessee	-	-	Yes	Yes	-	-	Yes	-	-	-	-
Texas	Yes	-	Yes	Yes	-	-	Yes	-	-	-	-
Utah	-	-	-	-	-	-	-	-	-	Yes	-
Vermont	Yes	-	Yes	-	-	-	Yes	-	-	-	-
Virginia	-	-	Yes	Yes	-	-	Yes	-	Yes	-	-
Washington	-	-	-	-	-	-	-	-	-	-	-
West Virginia	-	-	Yes	-	-	-	Yes	-	-	-	-
Wisconsin	-	-	-	-	-	-	-	-	-	-	-
Wyoming	-	-	-	-	-	-	-	-	-	-	Yes
Total Yes	19	1	26	11	0	3	25	1	12	4	6

Table B-25. Text responses for “Other” for Question 10 (collaboration with other organizations).

Other – Text Response
Impacts not evaluated.
National Oceanic and Atmospheric Administration (NOAA) National Marine Fisheries Service (NMFS) in the Greater Atlantic Regional Fisheries Office (GARFO).
We are required to coordinate for any mussel bed that would be impacted that has either state or federally listed mussels.

Table B-26. Comments for Question 10 (collaboration with other organizations).

Comment
Both state (the state’s wildlife department) and Federal (USFWS) are consulted on projects that could potentially impact T&E species.
I coordinate with National Marine Fisheries Service (NMFS) and GARFO regularly on endangered species and essential fish habitat for projects that fall within their jurisdiction (they almost always do). Again, this is for marine mussels. I have yet coordinated with USACE. Their jurisdiction covers freshwater species, marshes, streams, rivers etc.
Our DOT has not specifically collaborated with other agencies with regards to sedimentation impacts to freshwater mussels. Of course, we coordinate with DNR, USACE, USFWS, National Park Service (NPS), with regards to impacts to mussels *themselves*.

Table B-27. Individual responses to Question 11 (willingness to participate in follow-up interview).

Respondent	Response Text
Alabama	No
Alaska	No
Arizona	No
Arkansas	Yes
California	-
Colorado	-
Connecticut	Yes
Delaware	No
District of Columbia	-
Florida	Yes
Georgia	Yes
Hawaii	-
Idaho	No
Illinois	Yes
Indiana	Yes
Iowa	No
Kansas	Yes
Kentucky	Yes
Louisiana	No
Maine	Yes
Maryland	-
Massachusetts	-
Michigan	Yes
Minnesota	Yes
Mississippi	-
Missouri	Yes
Montana	No
Nebraska	Yes
Nevada	No
New Hampshire	Yes
New Jersey	-
New Mexico	-
New York	-
North Carolina	-
North Dakota	No

Respondent	Response Text
Ohio	Yes
Oklahoma	Yes
Oregon	Yes
Pennsylvania	Yes
Rhode Island	No
South Carolina	No
South Dakota	No
Tennessee	Yes
Texas	Yes
Utah	Yes
Vermont	Yes
Virginia	Yes
Washington	-
West Virginia	Yes
Wisconsin	-
Wyoming	Yes
Total Yes	25
Total No	13
Number of Responses	38

Table B-28. Comments for Question 11 (willingness to participate in follow-up interview).

Comment
We're still working on protocols and assessment methods at this point.

Table B-29. Individual responses to Question 12 (interest in learning more about sedimentation impacts to freshwater communities).

Respondent	Response Text
Alabama	No
Alaska	No
Arizona	No
Arkansas	Yes
California	-
Colorado	-
Connecticut	Yes
Delaware	Yes
District of Columbia	-
Florida	No
Georgia	Yes
Hawaii	-
Idaho	No
Illinois	No
Indiana	Yes
Iowa	Yes
Kansas	No
Kentucky	Yes
Louisiana	No
Maine	No
Maryland	-
Massachusetts	-
Michigan	-
Minnesota	Yes
Mississippi	-
Missouri	Yes
Montana	No
Nebraska	No
Nevada	No
New Hampshire	Yes
New Jersey	-
New Mexico	-
New York	-
North Carolina	-
North Dakota	Yes

Respondent	Response Text
Ohio	Yes
Oklahoma	Yes
Oregon	No
Pennsylvania	Yes
Rhode Island	Yes
South Carolina	No
South Dakota	No
Tennessee	Yes
Texas	Yes
Utah	No
Vermont	Yes
Virginia	Yes
Washington	-
West Virginia	Yes
Wisconsin	-
Wyoming	No
Total Yes	20
Total No	17
Number of Responses	37

Table B-30. Comments for Question 12 (interest in learning more about sedimentation impacts to freshwater communities).

Comment
Not sure, I've not asked them this question.
Due to absence....this has not been an area of interest for our DOT.
We know why it is important. The things we are learning and studying is the best ways to prevent erosion.
I've done research on freshwater mussels, so I understand why it's important. Trying to get agency construction staff to set aside time to learn why isn't the best use of their time. They're going to respond with "just tell us what we need to do and we'll do it".
Some of our staff anyway :)
I say yes because I think they should know. I'm not sure how the effective the knowledge would be to change behavior beyond existing ESC practices.
As in any organization I'm sure some staff are interested in protecting mussels and other wildlife species where others may be less excited.
We have mechanisms in place to reduce sedimentation on our projects for many other reasons, not solely for mussels.

Table B-31. Comments for Question 13 (additional comments).

Comment
Additional focus could look at the use of BMPs to enhance connectivity within riverine systems such as removing unneeded low head dams or other instream obstructions. Look to establish programmatic interagency type habitat improvement efforts which aim to enhance mussel larvae host fish or eel species numbers and their habitat needs, along with other habitat related improvements to offset minor impacts associated with a DOT's annual bridge program.
I thought it was going to be basically a WQ questionnaire, but it is really specific to mussels, and we don't really do anything specifically for them in our state. I don't think our DOT has enough experience to be of much value to the study or at least this survey.
As I stated, we have not encountered issues with freshwater mussels. Could be due to lack of information and lack of prioritization by regulatory agencies.
Mussels are a natural filter mechanism. How much sedimentation effects a mussel would be of value in accessing ‘take’. Standard water quality requirements on projects are to not exceed 29 Nephelometric Turbidity Units (NTUs) above background (Nephelometric Turbidity Unit). I would think mussels are naturally capable of withstanding turbidity up to some level of NTU above background. Maybe this value could be determined in the research community.
We very, very rarely deal with mussels in particular. When we do, it is due to a USFWS concern which happens <<1/year. Aside from that, we always implement standard BMPs to reduce sediment/disturbance to waterways. Our DOT has not studied how our projects may impact mussels on the whole. We do implement whatever requests USFWS has if a project area has T&E mussel species.
We use erosion and sediment BMPs as per the General Permit for Construction and have plan notes regarding Aquatic Invasive Species (Administrative Rule 41:10:04:02) and projects that my need water extraction but no BMPs specific to freshwater mussels.
No Environmental Site Assessment (ESA) listed or state-listed mussels in our state though they seem to be declining. No state agency seems to care about them. We encounter mussels about once every 10-15 years and may or may not attempt to salvage. In the past, when we had time to coordinate, we reached out to our state’s department of fish and wildlife and Xerces Society. We don't have any details on this coordination/implementation.
The definition of BMPs was confusing and inconsistent with our DOT’s standard terminology. We feel those responses would most benefit from further discussion of the terms to best understand similarities and differences between MoDOT and our DOT’s practices.

Comment
This is a fairly significant issue for our state given that almost all of our waterways are extremely turbid. This puts our DOT at a disadvantage in that in-house staff don't have the resources/experience/time to conduct surveys in turbid waters, so consultants have to be procured...an unexpected expense for an already expensive roadway project. It would be helpful if there was a threshold for turbidity levels to know whether a survey would be beneficial. Meaning if a river has been experiencing severe bank erosion for multiple consecutive years, is it worth doing a survey? Are there other methods acceptable to regulatory agencies that doesn't involve putting staff in low-visibility waters where you need to pick up everything, view it 3 inches from your face, only to see that it's a rock? While our DOT would love to conserve our natural resources, financially and economically, it's not always feasible. Find a way to make it feasible from a business standpoint and you'll make strides to getting buy-in.
Looking forward to seeing your results and finding ways to improve our processes. Thanks for your thorough outreach on this research project.

Appendix C: Impact of Suspended Solids on Freshwater Mussels

C-1. Methods

C-1.1 Sediment/Soil Characterization

The three sediment/soil samples were sieved to 2-mm and air dried at room temperature. Total organic carbon (TOC), total inorganic carbon (TIC), total carbon (TC), and total nitrogen (TN) were analyzed using a combustion analyzer and particle size distribution (PSD) were analyzed by South Farm Research Center (Columbia, MO, USA). The particle size distribution (PSD) of the dry sediment/soil samples was classified according to the United States Department of Agriculture (USDA) soil texture classification standard with seven classes, including clay (< 2 µm), fine silt (2-20 µm), coarse silt (20-50 µm), very fine sand (50-100 µm), find sand (100-250 µm), medium sand (250-500 µm), coarse sand (500-1000 µm) and very coarse sand (1000-2000 µm). Contamination analysis, including metals (n = 26), PAHs (n = 18), *n*-alkanes (C9-C40) were conducted by ALS company, following USEPA methods 6020A, 6010C and 7471B for metals determination, method 8270D for PAHs analysis, and method 8015C for *n*-alkane analysis. Moreover, for PAHs and selected metals with available probably effect concentrations (PECs), the detected concentrations were normalized to their respective PEC as a measure of toxicity. Mean PEC quotient (PEC-Q) of each sample was also calculated to evaluate whether these contaminants may result in toxicity to mussels.

The largest proportion of 2-mm sieved bulk SRS was fine sand (39.0%), followed by 15.6% fine silt, 15.0% coarse silt, 11.3% clay, 1% very fine sand, and 8.2% medium sand. The proportions of coarse and very coarse sands only accounted 0.8%, indicating that SRS bulk contained more than 90% of particles < 250 µm and dominated by the 100-250 µm fraction. The bulk SRS used in this study had less sand fraction (total of 58.1%) compared to 75.9% reported by Archambault *et al.* [100]. Such differences may be caused by different collection times and locations. In the 2-mm sieved bulk ORC, like SRS, fractions larger than 250 µm only accounted a tiny proportion and very coarse sand was not detected. The rest of the classes spread from 15.3% (very find sand) to 22.4% (course silt). The 2-mm sieved bulk LMT showed a different PSD pattern, of which, most fractions but not clay, distributed quite evenly in the range of 11.1% (fine silt) and 16.5% (fine sand). On the contrary, clay only comprised 3.8% of bulk LMT.

Compared to bulk LMT and ORC, SRS had a higher TOC and TN concentrations, 1.13% and 0.085%, respectively. The detected TOC of SRS matches with previous studies which also found that the TOC level of the Spring River sediment was around 1% [99, 100]. LMT showed a slightly higher content of TOC (0.81%) but remarkably less TN (0.013%) compared to ORC (0.67% TOC and 0.069% TN). Only LMT had measurable TIC of 9.15%. TOC/TN ratios have been widely used to determine source of organic matters in the sediments and soils [171-174]. Here, bulk LMT had the highest TOC/TN ratio (62.3), implying that its primary organic matter source was from vascular plants [175]. SRS had a TOC/TN ratio of 13.3, indicating that a mixture of algal and land plant as the organic matter source [176]. ORC presented the lowest TOC/TN value of 9.74, which may reflect the main source of organic matters comes from alga production [176]. These results are consistent with the sample collection locations, where SRS was collected from the river in the summer, ORC was collected from a riverbank without much vegetation in the winter, and LMT collected from an area away from water sources and vegetation.

Table C-2 summarized the metal concentrations of each sample. Calcium (Ca) was the most abundant metal detected in SRS with a concentration of 9950 µg/g followed by iron (Fe) of 5750 µg/g and aluminum (Al) of 2190 µg/g. Not surprisingly, Ca was the dominant metal of LMT with a concentration of 301 mg/g and was almost 10 times of magnesium (Mg), the second abundant metal detected. The Fe was 3300 µg/g in LMT, but it became the most abundant metal in ORC of 13600 µg/g, followed by 7610 µg/g of Al, 2440 µg/g of Ca, and 1470 µg/g of Mg. By referring to available PECs provided by sediment quality guidelines [177], the individual PEC quotients (PEC-Q) of eight metals, including arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) were calculated (**Table C-3**). Overall, the individual PEC-Q of all three bulk samples were low (0.002 to 0.288), and the average PEC-Q was 0.095 for SRS, 0.040 for LMT, and 0.11 for ORC, respectively. Therefore, for all three bulk samples, PEC-Q of these metals were much lower than 1.0, the sediment toxicity benchmark [43, 100]. Subsequently, these metals of low concentrations should not be considered as the contributor to the potential adverse impact observed, if any, to the juvenile mussels during the following suspended sediment exposure experiments. Similarly, the detected PAHs of the bulk samples were analyzed (**Table C-4**) and all were at least one order of magnitude lower than their PECs [177], reflecting that PAHs, just like metals, would not be reasons leading to the potential toxicity to the juvenile mussels. Specifically, the total concentration of the tested PAHs (> MDL) was 42.75 ng/g for SRS, 42.29 ng/g for LMT, and 48.44 ng/g for ORC. Similarly, the detected concentrations of *n*-alkane were very low (**Table C-5**) and were then not further discussed.

C-1.2 Stock and Suspension Preparation Method and Characterization

Samples were suspended in excess in 100 hardness water and continuously stirred for approximately 1 h at 1000 - 1200 rpm using IK ARW 20 digital agitator (Wilmington, NC, USA) The suspension was then allowed to settle for up to 1 hour to remove larger and denser particles. The upper fraction was then carefully collected using decanting method avoiding disturbing the sediment on the bottom, and only the collected upper suspension was used as stock (Stock) for the following studies. The initial sample:water ratio and settling time of each sample were adjusted to obtain similar TSS concentrations in each of the three stock suspensions. TSS of the stock suspensions was measured following Method 2540 D (2015) method (**Table C-1**).

In the case of the PSD patterns of the suspensions, ORC suspension only contained clay (53.8%) and silt (41.0% fine silt and 5.2% coarse silt), and no other fraction > 50 µm was found. Similarly, SRS suspensions were dominated by clay (57.3%) and fine silt (34.2%). Different to SRS and ORC, the most abundant fraction of LMT suspension was silt-sized particles (77.9%, 58.2% fine silt and 19.4% coarse, respectively). Clay-sized particles still accounted 21.8% of LMT suspension. Once sediment is disturbed or soil enters the river, fine-grained particles, such as clay and silt, generally take longer time to deposit and are more possible to resuspend, making it possible for them remain suspended in the water for a longer time with good mobility and to become the primary contributors to water turbidity [110, 178-180]. Therefore, instead of focusing on coarse particles that can quickly settle, it is more crucial to study the impacts of suspended solids dominated by fine particles like clay and silt.

C-1.3 Chronic Exposure: Food and Post-exposure analysis

Algal Mixture was freshly prepared daily by mixing 0.5 mL of Nanno 3600 and 1 mL of Shellfish Diet 1800 into 0.9 L culture water with an algal concentration of ~510 nL cell volume/mL [107]. Algal Food was kept in the refrigerator (4 °C) if not used immediately. After 28-d exposure, mussels in each replicate were carefully examined for survival under a dissecting microscope. Mussels with an empty shell or with a gaped shell containing decomposed tissue were classified as dead. Surviving juvenile mussels in each replicate were rinsed to remove debris associated with mussel shells, and then counted and preserved in 70% ethanol for subsequent growth measurements including shell length and total dry weight of surviving mussels. Upon measurement, juvenile mussels in the 70% ethanol were first pooled, and any remaining debris was carefully removed with assistance of a microscope. Only when no debris as well as no other impurities (e.g., sands) were observed, could the juvenile mussels be measured. The maximum shell length of each juvenile per replicate was measured to the nearest 0.001 mm using a digitizing system with video micrometer software (Image Caliper; Resolution Technology). After measurement of length, the juvenile mussels per replicate were dried at 60 °C for 48 h, and then the total dry weight of each replicate was recorded to the nearest 0.001 mg using a Mettler Toledo Microbalance, the mean dry weight of the replicate was accordingly calculated.

C-1.4 Calculation of EC20s

When TRAP (the Toxicity Relationship Analysis Program,Ver 1.30a) was used to calculate EC20s, the TSS exposure concentrations were log-transformed, and the response of each replicate was used for the calculation. A threshold sigmoid model was adopted for growth and biomass data analyses. The level of statistical significance was set at $\alpha = 0.05$.

Table C-1. Individual metal concentrations (µg/kg, dw) detected in SRS, ORC, and LMT bulk samples. SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

		SRS	ORC	LMT	SRS duplicate	MDL	MRL
	Analyte Name	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Al	Aluminum	2190	7610	388	2340	0.500	1.80
Sb	Antimony	ND	0.069	ND	ND	0.018	0.045-0.046
Ar	Arsenic	1.87	5.25	1.25	1.96	0.050	0.45-0.46
Ba	Barium	39.0	142	10.1	42.6	0.018	0.045-0.046
Be	Beryllium	0.275	0.689	0.058	0.290	0.005	0.018
Bo	Boron	0.610	1.9500	2.72	0.470	0.180	0.45-0.46
Cd	Cadmium	0.643	0.308	0.34	0.524	0.006	0.018
Ca	Calcium	9950	2440	301000	10500	0.900	3.6-3.7
Cr	Chromium	14.0	10.8	2.79	14.0	0.050	0.18
Co	Cobalt	4.23	8.75	0.918	4.48	0.005	0.018
Cu	Copper	2.79	8.96	1.36	2.71	0.036	0.089-0.091
Fe	Iron	5750	13600	3330	5960	0.360	0.89-0.91
Pb	Lead	7.13	13.1	2.99	7.70	0.018	0.045-0.046
Mg	Magnesium	237	1470	34800	229	0.200	1.8
Mn	Manganese	139	971	299	154	0.018	0.089-0.091
Hg	Mercury	0.012*	0.016*	0.002*	ND	0.003	0.019-0.025
Mo	Molybdenum	0.305	0.415	0.659	0.284	0.018	0.045-0.046
Ni	Nickel	7.06	14	3.84	7.06	0.030	0.18
K	Potassium	228	861	249	229	9.00	36-37
Se	Selenium	0.16*	0.29*	0.25*	0.15*	0.080	0.89-0.91
Ag	Silver	0.037	0.066	0.025	0.037	0.004	0.018
Na	Sodium	23.0*	31.0*	221	23.0*	4.00	36-37
Sr	Strontium	6.61	11.8	130	7.29	0.018	0.089-0.91
Ti	Thallium	0.045	0.113	0.033	0.042	0.004	0.018
V	Vanadium	9.74	19.1	2.39	10.1	0.030	0.180
Zn	Zinc	100	38.3	35.1	71.8	0.180	0.460

NOTE:

MRL: method reporting limit; MDL: method detection limit; ND: Not detected (<MDL)

Number with*: the detected concentration was larger than the MDL but smaller than the MRL.

Table C-2. Individual metal PEC-Q for SRS, ORC and LMT bulk samples. SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

	SRS	**ORC**	**LMT**	**PEC**	**SRS**	**ORC**	**LMT**
Name	mg/kg	mg/kg	mg/kg	mg/kg	PEC-Q	PEC-Q	PEC-Q
Ar	1.87	5.25	1.25	33.0	0.057	0.159	0.038
Cd	0.643	0.308	0.34	4.98	0.129	0.062	0.068
Cr	14.0	10.8	2.79	111	0.126	0.097	0.025
Cu	2.79	8.96	1.36	149	0.019	0.060	0.009
Pb	7.13	13.1	2.99	128	0.056	0.102	0.023
Hg	0.012	0.016	0.002	1.06	0.011	0.015	0.002
Ni	7.06	14.0	3.84	48.6	0.145	0.288	0.079
Zn	100	38.3	35.1	459	0.218	0.083	0.076
				Mean PEC-Q	0.095	0.040	0.108

NOTE:

PEC: Possible effect concentrations; PEC-Q: PEC quotient

Table C-3. Individual PAHs concentrations (µg/kg, dw) detected in SRS, ORC and LMT bulk samples. SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

	SRS	**ORC**	**LMT**
Analyte Name	(µg/kg)	(µg/kg)	(µg/kg)
2-Methylnaphthalene	0.95*	0.86*	3.4*
Acenaphthene	ND	ND	1.1*
Acenaphthylene	ND	0.45*	ND
Anthracene	ND	0.52*	ND
Benz(a)anthracene	2.7*	4.0*	1.0*
Benzo(a)pyrene	4.5*	6.0*	2.7*
Benzo(b)fluoranthene	5.0*	5.3*	2.7*
Benzo(g,h,i)perylene	1.6*	2.1*	2.4*
Benzo(k)fluoranthene	1.6*	2.0*	0.65*
Chrysene	4.3*	3.8*	2.8*
Dibenz(a,h)anthracene	0.4*	0.39*	0.24*
Dibenzofuran	1.2*	0.82*	4.8
Fluoranthene	6.4	7.4	3.7*
Fluorene	ND	ND	1.2*
Indeno(1,2,3-cd)pyrene	1.8*	2.8*	1.3*
Naphthalene	1.2*	1.5*	6.9*
Phenanthrene	5.6*	2.8*	2.0*
Pyrene	5.5*	7.7	5.4
Total PAHs (ΣPAHs)	42.8	48.4	42.3
PEC of ΣPAHs (µg/kg, dw)	22800		
MRL (µg/kg)	6.1	4.8	6.2

NOTE:

MRL: method reporting limit; MDL: method detection limit;

Number with*: the detected concentration was larger than the MDL but smaller than the MRL.

ND: Not detected (<MDL)

ΣPAHs: included individual PAHs > MDLs

PEC: Possible effect concentrations

Table C-4. Summary of n-alkanes (C9-C40) concentrations (mg/kg dw) detected in SRS, ORC and LMT bulk samples. SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

	SRS	**ORC**	**LMT**
Analyte Name	(mg/kg)	(mg/kg)	(mg/kg)
n-Nonane	ND	ND	ND
n-Decane	0.022	0.024	ND
n-Undecane	0.096	0.094	0.021
n-Dodecane	0.17	0.12	0.040
n-Tridecane	0.16	0.076	0.032
n-Tetradecane	0.02	0.021	ND
n-Pentadecane	ND	ND	ND
n-Hexadecane	0.018	ND	ND
n-Heptadecane	ND	ND	ND
Pristane	ND	ND	ND
n-Octadecane	ND	ND	ND
Phytane	ND	ND	ND
n-Nonadecane	ND	ND	ND
n-Eicosane	ND	ND	ND
n-Heneicosane	ND	ND	ND
n-Docosane	ND	ND	ND
n-Tricosane	0.016	ND	ND
n-Tetracosane	ND	ND	0.019
n-Pentacosane	0.031	ND	0.019
n-Hexacosane	0.025	0.017	0.025
n-Heptacosane	0.066	0.045	0.053
n-Octacosane	0.018	ND	0.034
n-Nonacosane	0.19	0.13	0.16
n-Triacontane	0.024	ND	0.028
n-Hentriacontane	0.17	0.098	0.095
n-Dotriacontane	ND	ND	0.056
n-Tritriacontane	0.074	0.060	0.26
n-Tetratriacontane	ND	ND	0.045
n-Pentatriacontane	ND	0.029	0.13
n-Hexatriacontane	0.095	0.11	0.44
n-Heptatriacontane	ND	ND	0.082
n-Octatriacontane	ND	ND	ND
n-Nonatriacontane	ND	ND	ND
n-Tetracontane	ND	ND	ND
Total Extractable Matter (C9 - C44 TEM)	17	16	22

NOTE:

MRL: method reporting limit; MDL: method detection limit; ND: Not detected (<MDL);

Number with*: the detected concentration was larger than the MDL but smaller than the MRL.

Table C-5. Stock preparation formula. SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

	SRS	ORC	LMT
Sample:Water ratio	1:9 (v/v)	1 kg:6.5 L (w/v)	1 kg:8 L (w/v)
Sedimentation time (min)	25-30	60	2-3
Stock TSS (mg/L)	4600-7600	12000-17000	16000-21000

NOTE:

SRS was prepared using volume ratio as the Spring River sediment collected contains a large portion of water.

Table C-6. Summary of water quality of fresh prepared SRS suspensions of different TSS concentrations for acute study, data shown as mean ± standard deviation (SD). SRS = Spring River Sediment.

Nominal TSS (mg/L)	Turbidity (FTU)	pH	Conductivity (µS/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Dissolved Oxygen (mg/L)	Measured TSS (mg/L)
0	0	8.27 ± 0.13	249.4 ± 5.7	92.7 ± 5.75	100.0 ± 2.83	8.85 ± 0.68	NA
250	367.0 ± 134.2	8.26 ± 0.09	246.8 ± 6.38	93.2 ± 3.03	97.0 ± 3.83	8.5 ± 0.21	253.5 ± 62.8
500	710 ± 297.0	8.35 ± 0.08	243.8 ± 0.35	89.0 ± 1.41	96.0	8.28 ± 0.00	316 ± 223.5
1000	1544.2 ± 315.8	8.14 ± 0.15	246.5 ± 5.21	91.0 ± 2.10	97.2 ± 6.26	8.62 ± 0.73	1125.8 ± 369.6
2500	3590.0 ± 1569.8	8.12 ± 0.01	238.5 ± 0.71	89.0 ± 1.41	98.0 ± 2.83	7.97 ± 0.45	2785.0 ± 1067.7
5000	8050.0 ± 2703.3	7.87 ± 0.17	241.0 ± 4.83	82.8 ± 4.82	86 ± 10.39	7.63 ± 1.41	5087.5 ± 407.8

Table C-7. Summary of water quality of fresh prepared ORC suspensions of different TSS concentrations for acute study, data shown as mean ± standard deviation (SD). ORC = Osage River clay soil.

Nominal TSS (mg/L)	Turbidity (FTU)	pH	Conductivity (µS/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Dissolved Oxygen (mg/L)	Measured TSS (mg/L)
0	0	8.21 ± 0.07	251.4 ± 3.80	94.7 ± 3.93	100.8 ± 3.25	8.98 ± 0.61	NA
250	434.0 ± 136.3	8.23 ± 0.08	251.4 ± 6.39	93.2 ± 2.68	101.6 ± 2.61	8.54 ± 0.17	246.3 ± 34.1
500	770.0 ± 14.1	8.24 ± 0.06	251.0 ± 12.7	92.0 ± 2.83	102.0 ± 2.83	8.57 ± 0.11	467.0 ± 7.07
1000	1660.8 ± 390.3	8.20 ± 0.05	250.0 ± 6.60	92.3 ± 1.51	99.3 ± 3.01	8.79 ± 0.65	1105.1 ± 183.2
2500	3910.0 ± 268.7	8.12 ± 0.17	243.0 ± 15.6	91.0 ± 1.41	101.0 ± 1.41	8.55 ± 0.08	2537.5 ± 24.8
5000	7924.0 ± 1574.5	8.09 ± 0.13	236.8 ± 12.2	84.3 ± 9.67	99.7 ± 7.84	8.79 ± 0.62	5395.0 ± 541.6

NOTE:
NA: not applicable.

Table C-8. Summary of water quality of fresh prepared LMT suspensions of different TSS concentrations for acute study, data shown as mean ± standard deviation (SD). LMT = Columbia Limestone.

Nominal TSS (mg/L)	Turbidity (FTU)	pH	Conductivity (µS/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Dissolved Oxygen (mg/L)	Measured TSS (mg/L)
0	0	8.27 ± 0.16	253.3 ± 1.41	96.3 ± 3.67	101.7 ± 4.80	9.01 ± 0.59	NA
250	200.8 ± 39.6	8.31 ± 0.16	258.8 ± 7.01	97.5 ± 5.00	105.5 ± 9.98	8.67 ± 0.21	243.6 ± 19.2
500	370.0 ± 84.9	8.59 ± 0.44	273.5 ± 2.12	100.0	112	8.81 ± 0.01	502.9 ± 9.72
1000	934.0 ± 85.1	8.31 ± 0.13	270.5 ± 7.66	100.4 ± 4.56	114.0 ± 6.00	8.88 ± 0.53	1085.5 ± 65.4
2500	2200.0	8.39 ± 0.16	313.5 ± 61.5	104 .0	140	8.84 ± 0.01	2795.5 ± 184.6
5000	4539.0 ± 498.6	8.29 ± 0.09	319.3 ± 29.3	100.4 ± 4.56	122.0 ± 14.7	8.94 ± 0.68	5127.7 ± 500.2

NOTE:
NA: not applicable.

Table C-9. Summary of water quality of used SRS suspensions of different TSS concentrations for acute study, data shown as mean ± standard deviation (SD). SRS = Spring River Sediment.

Nominal TSS (mg/L)	Turbidity (FTU)	pH	Conductivity (µS/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Dissolved Oxygen (mg/L)	Measured TSS (mg/L)
0	0 ± 0	8.32 ± 0.07	264.0 ± 9.30	97.7 ± 3.44	105.7 ± 5.99	8.59 ± 0.20	0.02 ± 0.03
250	225.5 ± 36.8	8.28 ± 0.07	261.4 ± 7.02	95.6 ± 3.29	105.6 ± 6.69	8.4 ± 0.20	153.1 ± 25.8
500	510.0 ± 0.00	8.29 ± 0.08	253.0 ± 7.07	93.0 ± 4.24	108.0 ± 11.3	8.41 ± 0.30	438 ± 263.04
1000	1092.9 ± 94.4	8.28 ± 0.08	259.0 ± 8.12	92.7 ± 2.73	104.0 ± 3.58	8.34 ± 0.21	674.3 ± 121.4
2500	2400.0 ± 452.6	8.26 ± 0.06	245.0 ± 5.66	86.0 ± 2.83	100.0 ± 0.00	8.37 ± 0.36	1725.0 ± 403.1
5000	5325.0 ± 1160.1	8.17 ± 0.16	248.2 ± 9.26	82.7 ± 10.3	97.7 ± 6.98	7.95 ± 0.52	3250.8 ± 811.9

Table C-10. Summary of water quality of used ORC suspensions of different TSS concentrations for acute study, data shown as mean ± standard deviation (SD). ORC = Osage River clay soil.

Nominal TSS (mg/L)	Turbidity (FTU)	pH	Conductivity (µS/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Dissolved Oxygen (mg/L)	Measured TSS (mg/L)
0	0	8.32 ± 0.10	268.2 ± 5.49	99.7 ± 1.97	105.7 ± 5.99	8.47 ± 0.23	NA
250	385.0 ± 131.1	8.33 ± 0.05	268.6 ± 5.32	97.6 ± 2.61	105.2 ± 6.57	8.23 ± 0.08	210.8 ± 36.4
500	730.0 ± 70.7	8.39 ± 0.00	267.0 ± 2.83	99.0 ± 7.07	112.0 ± 0.00	8.54 ± 0.35	471.5 ± 27.6
1000	1619.6 ± 535.7	8.28 ± 0.12	262.4 ± 3.01	97.0 ± 5.62	105.7 ± 3.20	8.02 ± 0.65	954.6 ± 262.3
2500	3700.0 ± 282.8	8.35 ± 0.03	254.0 ± 5.66	94.0 ± 8.49	102.0 ± 2.83	8.15 ± 0.01	2417.5 ± 46.0
5000	7148.0 ± 1152.2	8.25 ± 0.07	244.3 ± 11.9	86.3 ± 8.98	100.7 ± 5.89	8.24 ± 0.06	4883.3 ± 699.1

NOTE:

NA: not applicable.

Table C-11. Summary of water quality of used LMT suspensions of different TSS concentrations for acute study, data shown as mean ± standard deviation (SD). LMT = Columbia Limestone.

Nominal TSS (mg/L)	Turbidity (FTU)	pH	Conductivity (µS/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Dissolved Oxygen (mg/L)	Measured TSS (mg/L)
0	0	8.34 ± 0.1	268.2 ± 3.25	100.0 ± 2.19	105.7 ± 5.99	8.56 ± 0.18	NA
250	84.4 ± 38	8.38 ± 0.08	288.9 ± 36.7	98.8 ± 1.79	109.6 ± 8.76	8.29 ± 0.26	93.2 ± 48.4
500	325.0 ± 21.2	8.43 ± 0.09	296.5 ± 19.1	104.0 ± 2.83	116.0 ± 5.66	8.41 ± 0.22	381.6 ± 64.5
1000	650.0 ± 179.2	8.38 ± 0.05	298.9 ± 44.7	101.3 ± 3.01	115.3 ± 4.68	8.38 ± 0.09	704.2 ± 168.3
2500	1680.0 ± 678.8	8.39 ± 0.07	279.5 ± 2.12	99.0 ± 1.41	128.0 ± 0.00	8.48 ± 0.23	1922.3 ± 753.4
5000	3413.3 ± 979.3	8.36 ± 0.04	332.7 ± 44.5	98.0 ± 5.06	130.0 ± 12.8	8.35 ± 0.07	4041.3 ± 909.3

NOTE:

NA: not applicable.

Table C-12. Summary of juvenile of 2-mo-old Fatmucket (Lampsilis siliquoidea) after 96 h acute exposure to SRS, ORC and LMT at different TSS concentrations. Ctrl = Control water, SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

		No. of Alive				No. of Missing	No. of Dead
Name	**Nominal TSS (mg/L)**	**R1**	**R2**	**R3**	**R4**		
Ctrl	0	5	5	5	5	0	0
SRS	250	5	5	5	5	0	0
	500	5	5	5	5	0	0
	1000	5	5	5	5	0	0
	2500	5	5	5	5	0	0
	5000	5	5	5	5	0	0
ORC	250	5	5	5	5	0	0
	500	5	5	5	5	0	0
	1000	5	5	5	5	0	0
	2500	5	5	5	5	0	0
	5000	5	4*	5	5	0	1
LMT	250	5	5	5	5	0	0
	500	5	5	5	5	0	0
	1000	5	5	5	5	0	0
	2500	5	5	5	5	0	0
	5000	5	5	5	5	0	0

NOTE:

Number with * means replicate with dead juvenile mussel observed. R1-R4: Replicate No. 1 to 4.

Table C-13. Summary of juvenile of 2-mo-old Arkansas Brokenray (Lampsilis reeveiana) after 96 h acute exposure to SRS, ORC and LMT at different TSS concentrations. Ctrl = Control water, SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

		No. of Alive				No. of Missing	No. of Dead
Name	**Nominal TSS (mg/L)**	**R1**	**R2**	**R3**	**R4**		
Ctrl	0	5	5	5	5	0	0
SRS	250	5	5	5	5	0	0
	500	5	5	5	5	0	0
	1000	5	5	5	5	0	0
	2500	5	5	5	5	0	0
	5000	5	5	5	5	0	0
ORC	250	5	5	5	5	0	0
	500	5	5	5	5	0	0
	1000	5	5	5	5	0	0
	2500	5	5	5	5	0	0
	5000	5	5	5	5	0	0
LMT	250	5	5	5	5	0	0
	500	5	5	5	5	0	0
	1000	5	5	5	5	0	0
	2500	5	5	5	5	0	0
	5000	5	5	5	5	0	0

NOTE:

R1-R4: Replicate No. 1 to 4.

Table C-14. Summary of juvenile of 2-mo-old Washboard (Megalonaias nervosa) after 96 h acute exposure to SRS, ORC and LMT at different TSS concentrations. Ctrl = Control water, SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

		No. of Alive				No. of Missing	No. of Dead
Name	**Nominal TSS (mg/L)**	**R1**	**R2**	**R3**	**R4**		
Ctrl	0	5	5	4	5	1	0
SRS	1000	5	5	5	4	1	0
	5000	4	5	5	5	1	0
ORC	1000	5	5*	5	5	0	1
	5000	4	5	4	5	2	0
LMT	1000	5	5	5	5	0	0
	5000	5	5	5	5	0	0

NOTE:

Number with * means replicate with dead juvenile mussel observed. R1-R4: Replicate No. 1 to 4.

Table C-15. Summary of survival of 1-week-old Fatmucket (Lampsilis siliquoidea) after 96 h acute exposure to SRS, ORC and LMT at different TSS concentrations. Ctrl = Control water, SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

		No. of Alive				No. of Missing	No. of Dead
Name	**Nominal TSS (mg/L)**	**R1**	**R2**	**R3**	**R4**		
Ctrl	0	5	5	4	5	1	0
SRS	250	5	5	4	4*	1	1
	1000	5	5	5	5	0	0
	5000	5	5	5	5	0	0
ORC	250	5	5	5	5	1	0
	1000	5	5	5	5	0	0
	5000	5	4	5	5	1	0
LMT	250	5	4	5	5	1	0
	1000	5	5	5	5	0	0
	5000	5	5	4	5	1	0

Note: Number with * means replicate with dead juvenile mussel observed. R1-R4: Replicate No. 1 to 4.

Table C-16. Summary of survival of 1-week-old Arkansas Brokenray (Lampsilis reeveiana) after 96 h acute exposure to SRS, ORC and LMT at different TSS concentrations. Ctrl = Control water, SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

		No. of Alive				No. of Missing	No. of Dead
Name	**Nominal TSS (mg/L)**	**R1**	**R2**	**R3**	**R4**		
Ctrl	0	5	5	5	5	0	0
SRS	250	4	5	5	5	1	0
	1000	5	5	5	5	0	0
	5000	5	5	5	5	0	0
ORC	250	5	5	0*	5	1	4
	1000	5	5	5	5	0	0
	5000	5	5	5	5	0	0
LMT	250	5	5	5	5	0	0
	1000	5	5	5	5	0	0
	5000	5	5	5	5	0	0

NOTE:

Number with * means replicate with dead juvenile mussel observed. R1-R4: Replicate No. 1 to 4.

Table C-17. Summary of survival of 2-week-old Washboard (Megalonaias nervosa) after 96 h acute exposure to SRS, LMT and ORC at different TSS concentrations. Ctrl = Control water, SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

		No. of Alive				No. of Missing	No. of Dead
Name	**Nominal TSS (mg/L)**	**R1**	**R2**	**R3**	**R4**		
Ctrl	0	5	5	5	5	0	0
SRS	250	5	5	5	5	0	0
	1000	5	5	5	5	0	0
	5000	4*	4	6	5	0	1
ORC	250	5	5	5	5	0	0
	1000	5	4*	5	5	0	1
	5000	5	5	5	5	0	0
LMT	250	5	5	5	5	0	0
	1000	5	4	5	5	1	0
	5000	4	5	5	5	1	0

NOTE:

Number with * means replicate with dead juvenile mussel observed. R1-R4: Replicate No. 1 to 4.

Table C-18. Summary of survival, length, and dry weight of 2-mo-old (starting age) Fatmucket (Lampsilis siliquoidea) after 28-d chronic exposure to SRS, LMT and ORC at different TSS concentrations. Ctrl = Control water, SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

Name	Nominal TSS (mg/L)	No. of Replicate	No. of found	No. of missing	No. of Dead	Survival[a] (%)	Length[b] (mm)	Dry weight[c] (mg)
Day 0[d]	NA	1	10	0	0	100	2.381 ± 0.232	0.818
		2	10	0	0		2.662 ± 0.178	1.143
		3	10	0	0		2.414 ± 0.380	0.865
		4	10	0	0		2.053 ± 0.303	0.649
Ctrl-1	0	1	10	0	0	100	2.866 ± 0.197	1.673
		2	10	0	0		2.906 ± 0.235	1.631
		3	10	0	0		3.138 ± 0.274	1.962
		4	10	0	0		2.732 ± 0.352	1.424
Ctrl-2	0	1	10	0	0	100	2.782 ± 0.505	1.536
		2	10	0	0		2.677 ± 0.294	1.178
		3	10	0	0		2.528 ± 0.295	0.990
		4	10	0	0		2.668 ± 0.448	1.199
SRS	250	1	10	0	0	100	3.526 ± 0.393	2.329
		2	10	0	0		3.566 ± 0.293	2.206
		3	9	1	0		3.475 ± 0.304	2.250
		4	10	0	0		3.485 ± 0.510	2.162
	500	1	10	0	0	100	3.646 ± 0.310	2.525
		2	10	0	0		3.519 ± 0.251	2.077
		3	10	0	0		3.395 ± 0.461	2.133
		4	10	0	0		3.836 ± 0.373	2.599
	1000	1	10	0	0	100	3.172 ± 0.311	1.799
		2	10	0	0		3.160 ± 0.461	1.825
		3	10	0	0		3.116 ± 0.323	1.754
		4	10	0	0		3.166 ± 0.382	1.922
	2500	1	10	0	0	100	2.935 ± 0.264	1.405

		2	10	0	0		2.746 ± 0.332	1.172
		3	10	0	0		2.896 ± 0.343	1.467
		4	9	1	0		2.928 ± 0.307	1.551
	5000	1	10	0	0	97.5	2.553 ± 0.303	1.220
		2	10	0	0		2.543 ± 0.449	1.091
		3	10	0	0		2.498 ± 0.190	0.942
		4	10*	0	1		2.402 ± 0.307	0.918
ORC	250	1	10	0	0	100	3.138 ± 0.353	1.968
		2	10	0	0		3.135 ± 0.304	1.930
		3	10	0	0		3.212 ± 0.432	2.025
		4	10	0	0		2.983 ± 0.278	1.654
	500	1	10	0	0	100	3.322 ± 0.385	1.980
		2	10	0	0		3.252 ± 0.423	2.126
		3	10	0	0		3.255 ± 0.269	1.982
		4	9	1	0		3.064 ± 0.452	1.695
	1000	1	10	0	0	97.5	2.910 ± 0.479	1.548
		2	10*	0	1		2.773 ± 0.503	1.326
		3	9	1	0		2.714 ± 0.277	1.307
		4	10	0	0		2.609 ± 0.215	1.151
	2500	1	10	0	0	100	2.359 ± 0.154	0.874
		2	9	1	0		2.561 ± 0.401	1.054
		3	10	0	0		2.498 ± 0.24	1.003
		4	10	0	0		2.569 ± 0.182	1.196
	5000	1	10	0	0	100	2.431 ± 0.279	0.83
		2	10	0	0		2.456 ± 0.298	1.023
		3	10	0	0		2.398 ± 0.203	0.863
		4	10	0	0		2.297 ± 0.128	0.810
LMT	250	1	10	0	0	100	3.060 ± 0.320	1.950
		2	10	0	0		3.016 ± 0.319	1.752
		3	10	0	0		2.900 ± 0.341	1.616

		4	10	0	0		2.566 ± 0.103	1.123
	500	1	10	0	0	97.4	2.947 ± 0.344	1.625
		2	10	0	0		2.669 ± 0.342	1.536
		3	9*	1	1		2.776 ± 0.230	1.484
		4	10	0	0		2.688 ± 0.300	1.388
	1000	1	10	0	0	100	2.854 ± 0.367	1.541
		2	9	1	0		2.521 ± 0.416	1.070
		3	10	0	0		2.468 ± 0.241	1.088
		4	10	0	0		2.332 ± 0.212	0.915
	2500	1	10	0	0	100	2.743 ± 0.218	1.172
		2	10	0	0		2.225 ± 0.198	0.945
		3	10	0	0		2.281 ± 0.157	0.766
		4	9	1	0		2.292 ± 0.265	0.804
	5000	1	10	0	0	100	2.361 ± 0.172	0.833
		2	10	0	0		2.626 ± 0.25	1.106
		3	10	0	0		2.370 ± 0.149	0.939
		4	9	1	0		2.393 ± 0.223	0.951
			SUM	**9**	**3**			

NOTE:

Number with * means replicate with dead juvenile mussel observed.

NA: Not applicable

a: Missing juvenile mussels were not included when calculating the percent of survival.

b: Mean shall length (standard deviation) of juvenile mussels in the same replicate, data shown as mean ± standard deviation (SD), and numbers were rounded to three digits after the decimal point.

c: Mean individual dry weight of juvenile mussels in the same replicate, and numbers were rounded to three digits after the decimal point.

d: Data of juvenile mussels kept at day 0 were also listed for cross comparison.

Table C-19. One way ANOVA results on variations in juvenile growth (dry weight). SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

	Source of Variation	SS	DF	MS	F	*p* value
SRS	Between groups	7.683	6	1.280	26.44	<0.0001
	Residual	1.211	25	0.0484		
	Total	8.893	31			
ORC	Between groups	4.892	6	0.815	17.45	<0.0001
	Residual	1.168	25	0.467		
	Total	6.060	31			
LMT	Between groups	2.493	6	0.415	6.54	0.0003
	Residual	1.587	25	0.0635		
	Total	4.080	31			

NOTE:

SS: Sum of square; DF: Degrees of freedom; MS: Mean square; F: F-value; *p*: Significance.

Table C-20. One way ANOVA results on variations in percent changes of dry weight.

Source of Variation	SS	DF	MS	F	*p* value
Between groups	69269	16	4329	21.64	<0.0001
Residual	11002	55	200.0		
Total	80271	71			

NOTE:

SS: Sum of square; DF: Degrees of freedom; MS: Mean square; F: F-value; *p*: Significance.

Table C-21. Summary of EC20s calculated by different methods. SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

	SRS	ORC	LMT
TRAP	2269 (1094-4703)	1609 (795 - 3254)	1363 (505-3682)
TRAP (Ctrl excluded)	1261 (972 - 1638)	941 (652 - 1360)	970 (517-1821)
Log-Linear regression (Ctrl excluded) *	1042 (no CL)	849 (no CL)	867 (no CL)
Log-Linear regression (Ctrl and 250 mg/L data excluded) **	1227 (no CL)	969 (no CL)	839 (no CL)

NOTE:

*Uncertain confidence interval

** Uncertain confidence interval, data of 250 mg/L used as references.

TRAP: the Toxicity Relationship Analysis Program,Ver 1.30a

CL: 95% confidence interval

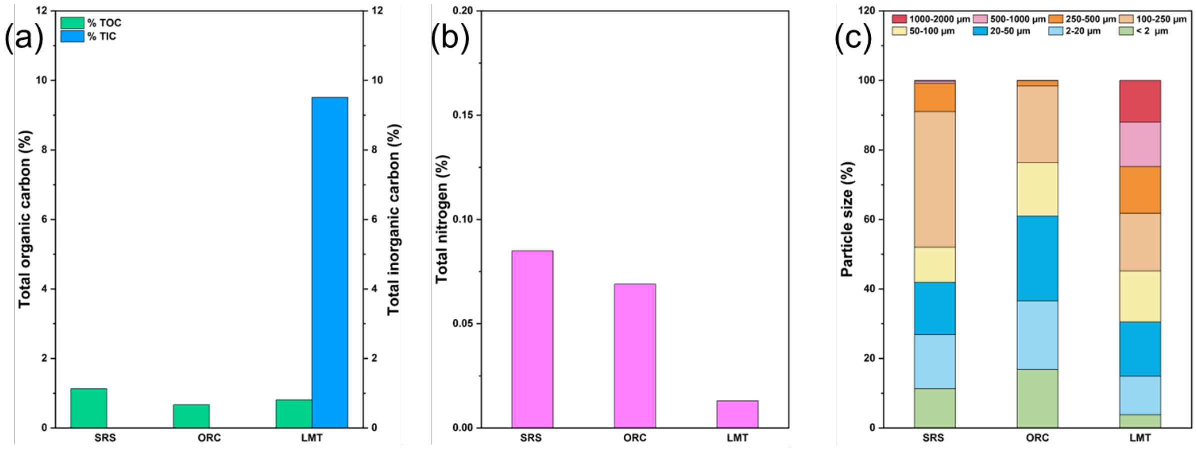

Figure C-1. Characterization of the bulk samples, including percentage (dry weight) of (a) total organic carbon (TOC) and total inorganic carbon (TIC), (b) total nitrogen (TN), and (c) particle size distribution (PSD). SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone. Classification of particles: very coarse sand (1000-2000 µm), coarse sand (5000-1000 µm) and, medium sand (250-500 µm), find sand (100-250 µm), very fine sand (50-100 µm), coarse silt (20-50 µm), fine silt (2-20 µm), and clay (< 2 µm).

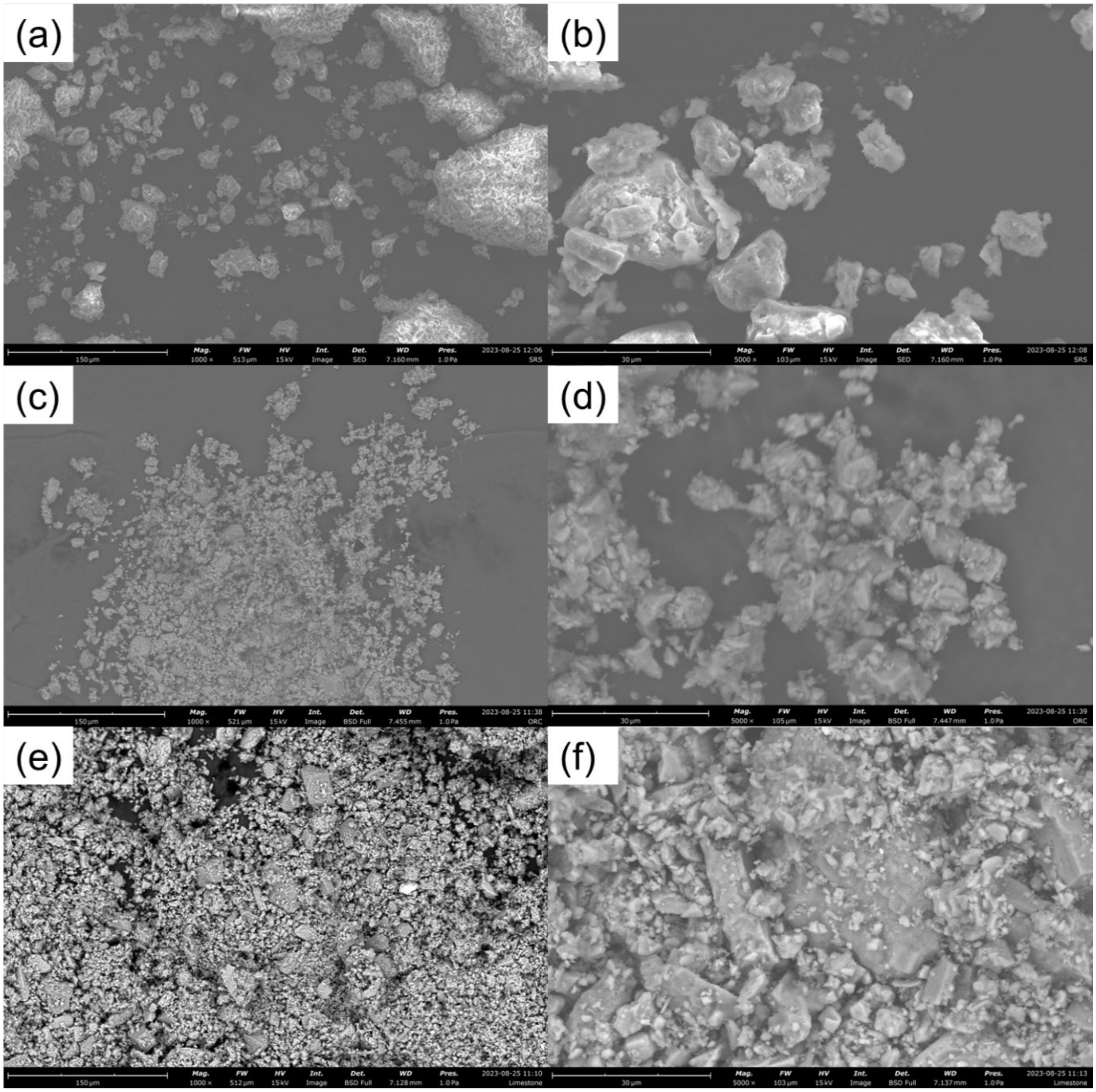

Figure C-2. SEM images of particles of SRS (a, b), ORC (c, d) and LMT (e,f) stock suspensions. Before taking images, the stock suspensions were air dried at room temperature. SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

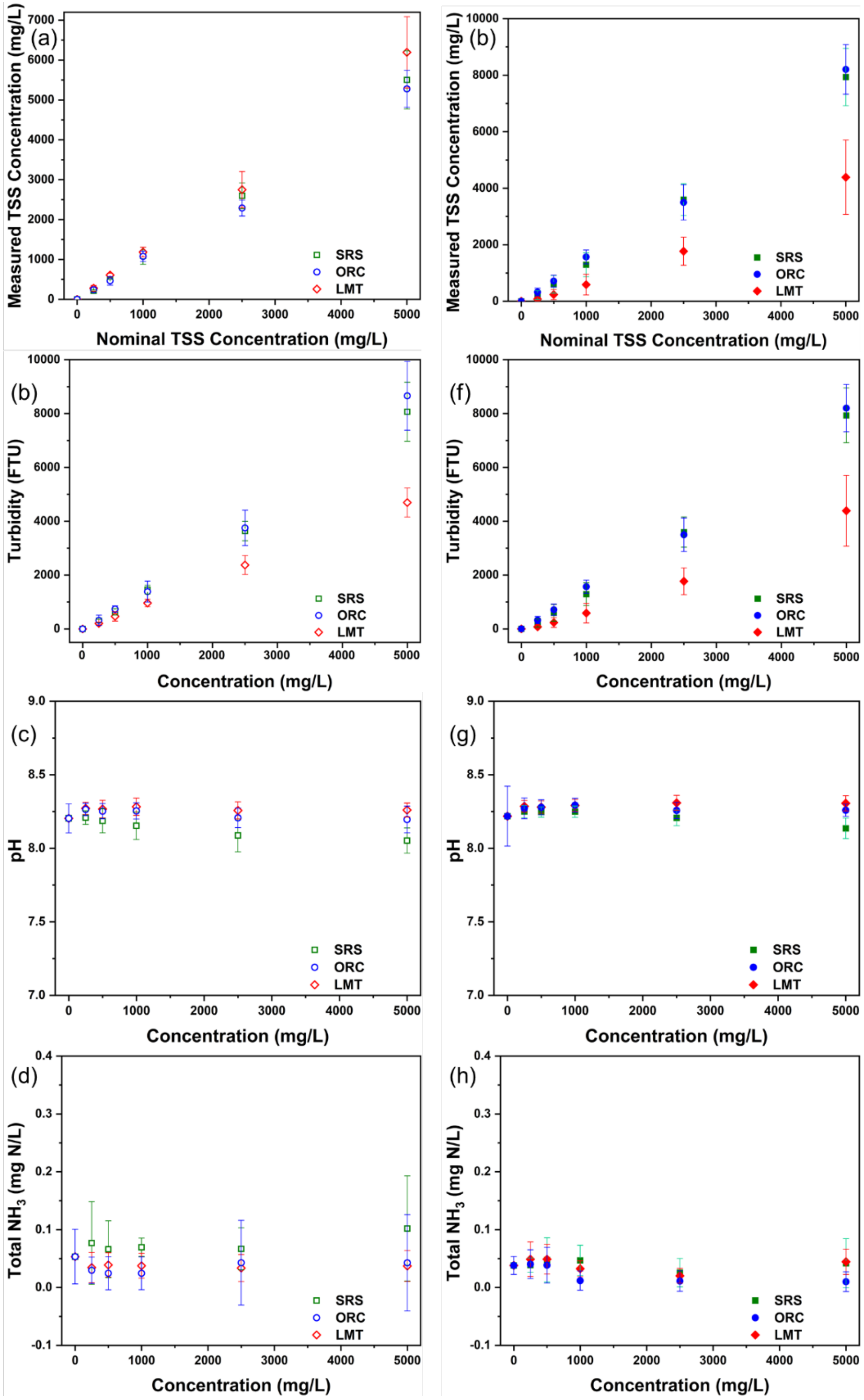

Figure C-3. Summary of water quality of fresh (left) and used (right) suspensions of SRS, ORC and LMT at different concentrations during the chronic study, including Measured TSS concentration (a and e), Turbidity (b and f), pH (c and g), and (d and h) Total NH3. Error bars represent standard deviation (SD) of means. SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

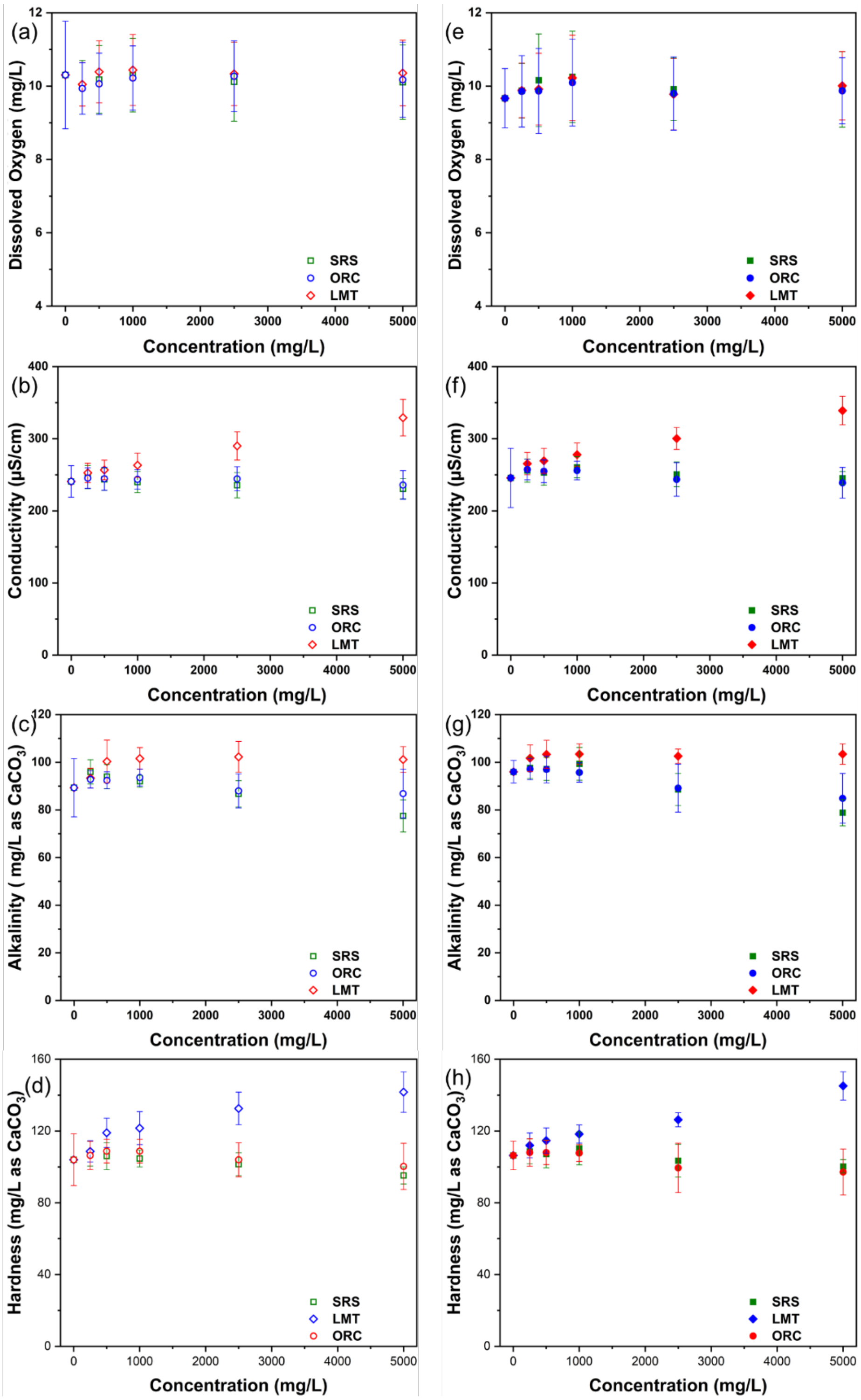

Figure C-4. Summary of water quality of fresh (left) and used (right) suspensions of SRS, ORC and LMT at different concentrations during the chronic study, including Dissolved Oxygen (a and e), conductivity (b and f), Alkalinity (c and g), and hardness (d and h). Error bars represent standard deviation (SD) of means. SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

Figure C-5. The replicate of 250 mg/L ORC with red impurities (red circles).

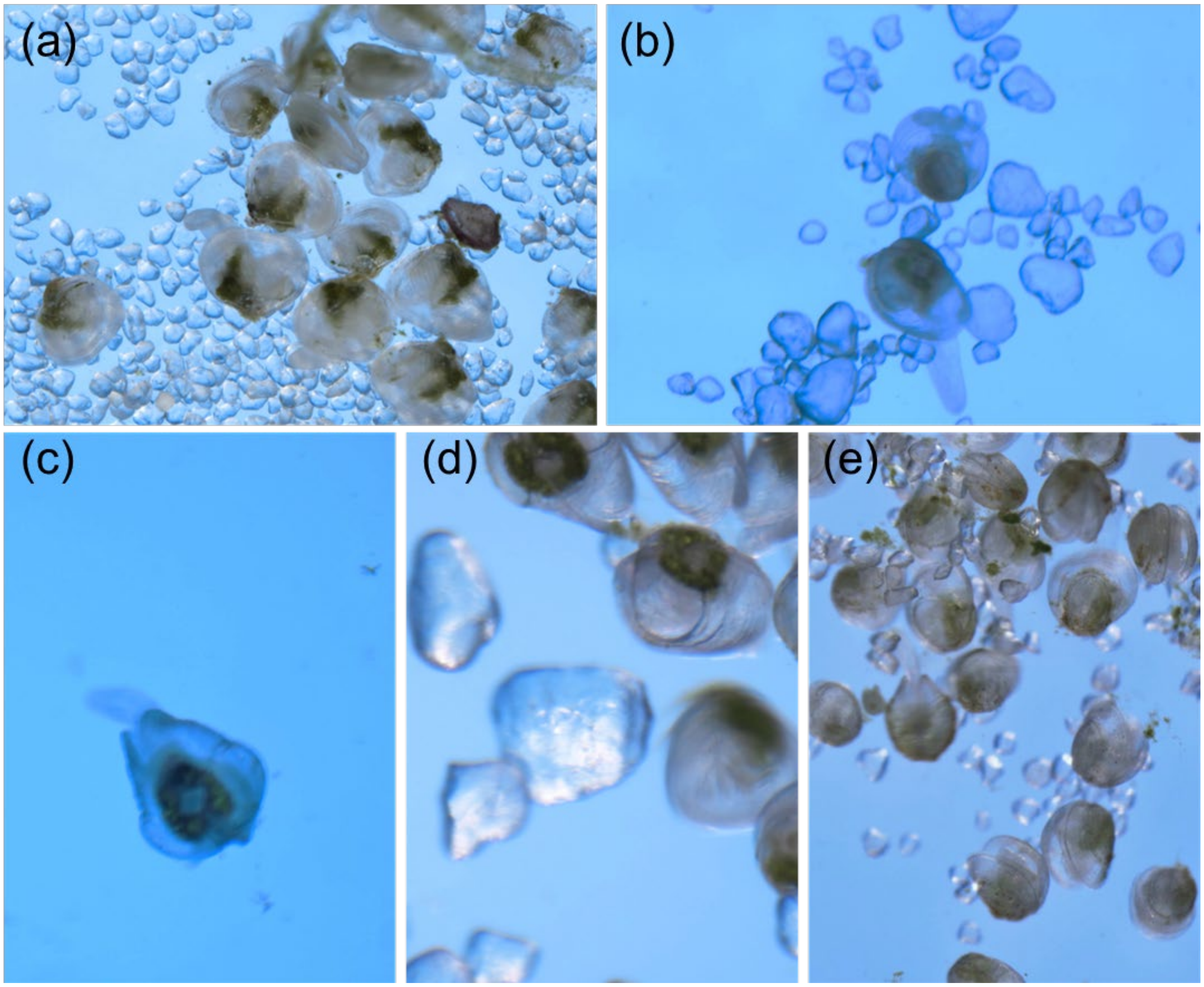

Figure C-6. Image of juvenile mussels on Day 0 before acute exposure test: (a) 2-month-old Arkansas Brokenray, (b) 2-month-old Washboard; (c) 1-week-old Fatmucket; (d) 1-week-old Arkansas Brokenray; and (e) 2-week-old Washboard.

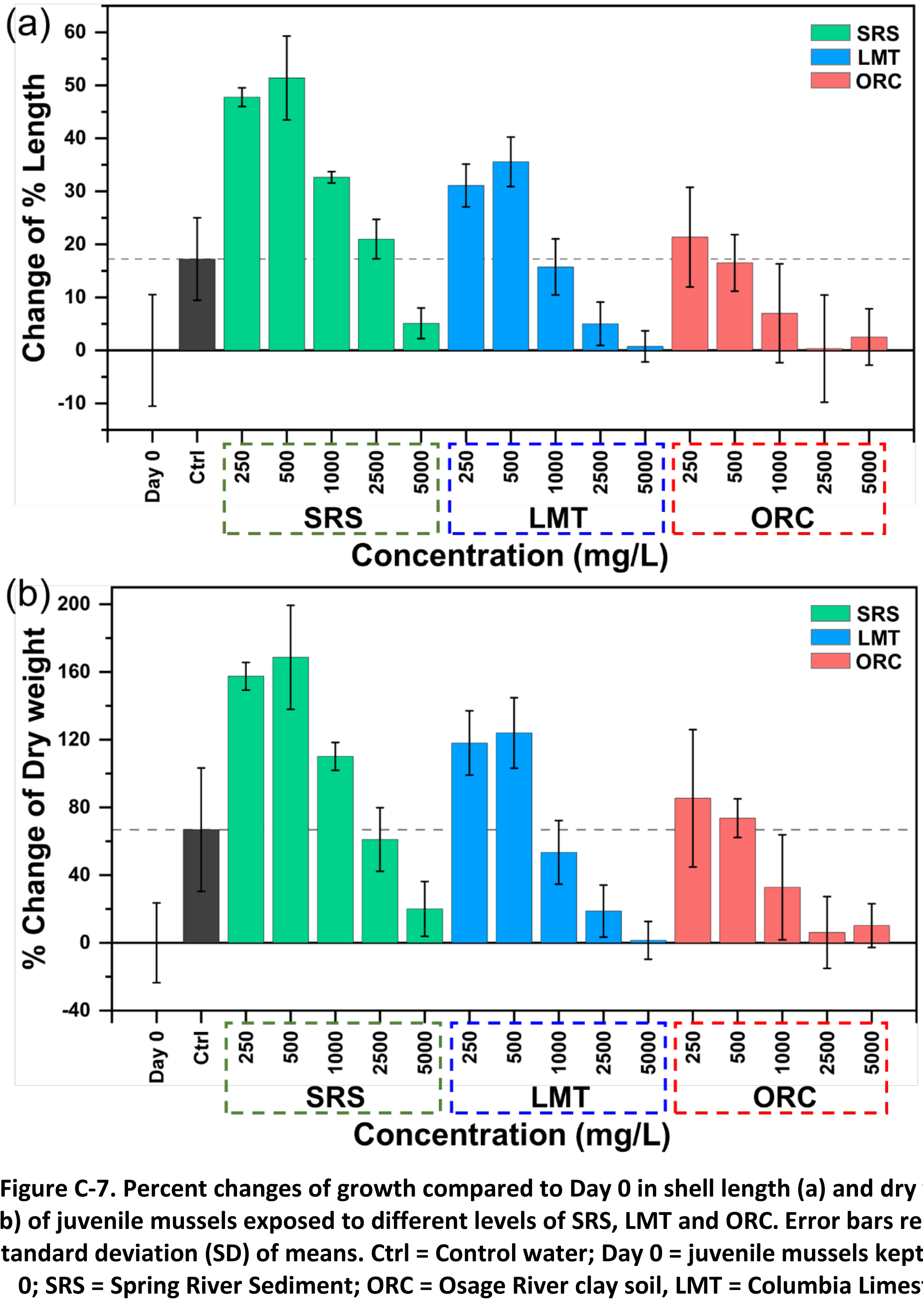

Figure C-7. Percent changes of growth compared to Day 0 in shell length (a) and dry weight (b) of juvenile mussels exposed to different levels of SRS, LMT and ORC. Error bars represent standard deviation (SD) of means. Ctrl = Control water; Day 0 = juvenile mussels kept on Day 0; SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

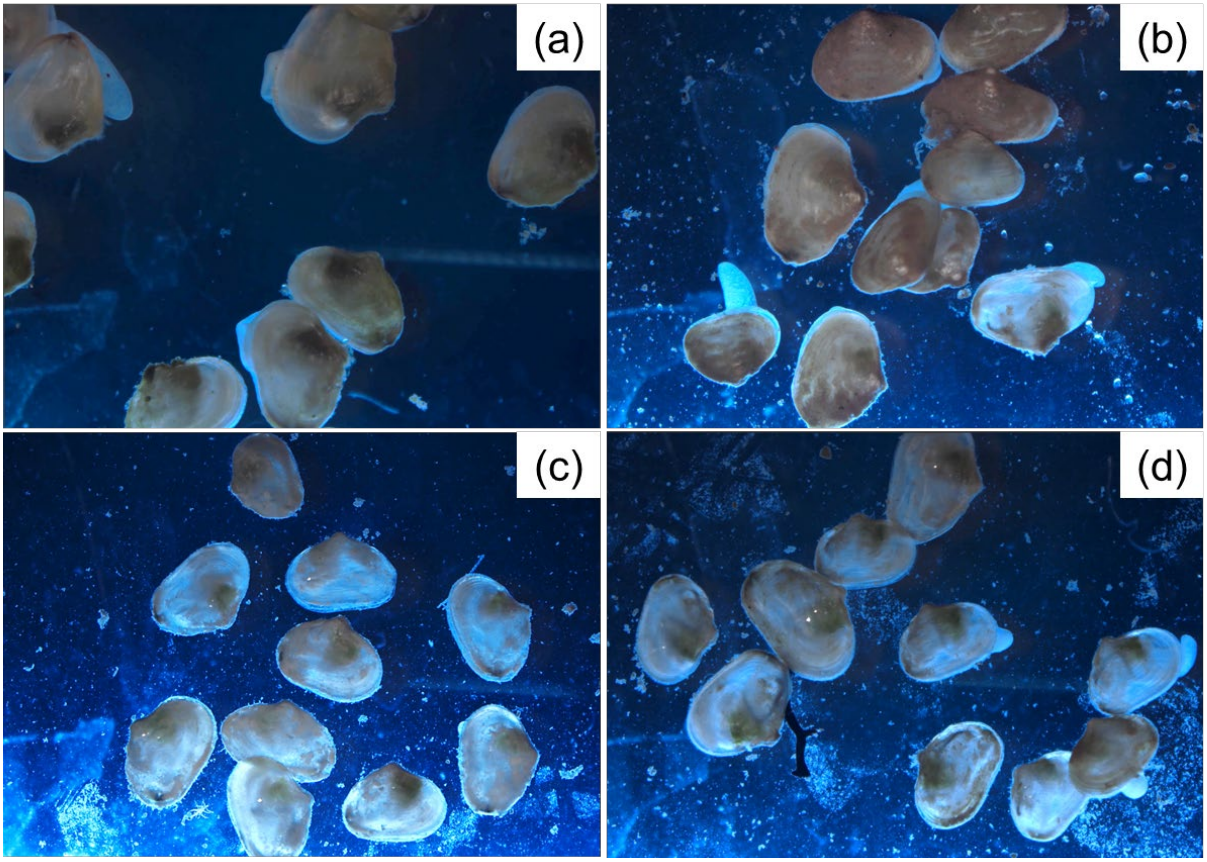

Figure C-8. Images of juvenile mussels after 28-d exposure to (a) the control water (Ctrl), 5000 mg/L of SRS (b), LMT (c) and (d) SRS. The green color indicated the food accumulation in the guts. SRS = Spring River Sediment; ORC = Osage River clay soil, LMT = Columbia Limestone.

1

Appendix D: Impact of Sediment Deposition on Freshwater Mussels

Table D-1. Individual metal concentrations (µg/kg, dw) detected in BBS < 5 bulk samples.

		BBS	**MDL**	**MRL**
	Analyte Name	mg/kg	mg/kg	mg/kg
Al	Aluminum	1120	0.500	1.80
Sb	Antimony	0.165	0.018	0.045-0.046
Ar	Arsenic	5.02	0.050	0.45-0.46
Ba	Barium	24	0.018	0.045-0.046
Be	Beryllium	0.589	0.005	0.018
Bo	Boron	0.27	0.180	0.45-0.46
Cd	Cadmium	0.031	0.006	0.018
Ca	Calcium	219	0.900	3.6-3.7
Cr	Chromium	21.9	0.050	0.18
Co	Cobalt	8.5	0.005	0.018
Cu	Copper	2.59	0.036	0.089-0.091
Fe	Iron	17500	0.360	0.89-0.91
Pb	Lead	6.6	0.018	0.045-0.046
Mg	Magnesium	138	0.200	1.8
Mn	Manganese	216	0.018	0.089-0.091
Hg	Mercury	0.006	0.003	0.019-0.025
Mo	Molybdenum	0.408	0.018	0.045-0.046
Ni	Nickel	10	0.030	0.18
K	Potassium	50	9.00	36-37
Se	Selenium	0	0.080	0.89-0.91
Ag	Silver	0.044	0.004	0.018
Na	Sodium	18	4.00	36-37
Sr	Strontium	0.834	0.018	0.089-0.91
Ti	Thallium	0.011	0.004	0.018
V	Vanadium	37	0.030	0.180
Zn	Zinc	11.5	0.180	0.460

NOTE:

MRL: method reporting limit; MDL: method detection limit; ND: Not detected (<MDL)
Number with*: the detected concentration was larger than the MDL but smaller than the MRL.

Table D-2. Individual PAHs concentrations (µg/kg, dw) detected in BBS < 5 bulk samples.

	BBS < 5
Analyte Name	(µg/kg)
2-Methylnaphthalene	0.52*
Acenaphthene	ND
Acenaphthylene	ND
Anthracene	ND
Benz(a)anthracene	0.46*
Benzo(a)pyrene	ND
Benzo(b)fluoranthene	ND
Benzo(g,h,i)perylene	ND
Benzo(k)fluoranthene	ND
Chrysene	ND
Dibenz(a,h)anthracene	ND
Dibenzofuran	ND
Fluoranthene	ND
Fluorene	ND
Indeno(1,2,3-cd)pyrene	ND
Naphthalene	0.74*
Phenanthrene	0.76*
Pyrene	0.6*
Total PAHs (ΣPAHs)	3.08

NOTE:

MRL: method reporting limit; MDL: method detection limit;

Number with*: the detected concentration was larger than the MDL but smaller than the MRL.

ND: Not detected (<MDL)

ΣPAHs: included individual PAHs > MDLs

Table D-3. Summary of n-alkanes (C9-C40) concentrations (mg/kg dw) detected in BBS < 5.

Analyte Name	BBS < 5 (mg/kg)
n-Nonane	ND
n-Decane	0.023*
n-Undecane	0.10*
n-Dodecane	0.12*
n-Tridecane	0.062*
n-Tetradecane	ND
n-Pentadecane	ND
n-Hexadecane	ND
n-Heptadecane	0.028*
Pristane	ND
n-Octadecane	ND
Phytane	ND
n-Nonadecane	ND
n-Eicosane	0.028*
n-Heneicosane	ND
n-Docosane	ND
n-Tricosane	ND
n-Tetracosane	ND
n-Pentacosane	ND
n-Hexacosane	ND
n-Heptacosane	ND
n-Octacosane	ND
n-Nonacosane	0.028*
n-Triacontane	ND
n-Hentriacontane	0.021*
n-Dotriacontane	ND
n-Tritriacontane	ND
n-Tetratriacontane	ND
n-Pentatriacontane	ND
n-Hexatriacontane	ND
n-Heptatriacontane	ND
n-Octatriacontane	ND
n-Nonatriacontane	ND
n-Tetracontane	ND
Total Extractable Matter (C9 - C44 TEM)	12

NOTE:

MRL: method reporting limit; MDL: method detection limit; ND: Not detected (<MDL);
Number with*: the detected concentration was larger than the MDL but smaller than the MRL.

Table D-4. Summary of number of AB mussels unburied daily and number of AB mussels remained buried on day 7 with increase of burial depth (upwelling].

Test condition		Number of Mussel Unburied							Number of Mussel Remain Buried	
Burial depth	**Vertical Water volume**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Alive**	**Dead**
5 cm	**1 L/h**	2	3	3	3	3	3	3	0	0
		3	3	3	3	3	3	3	0	0
		3	3	3	3	3	3	3	0	0
7.5 cm	**1 L/h**	1	2	2	3	3	3	3	0	0
		2	3	3	3	3	3	3	0	0
		2	2	2	2	2	3	3	0	0
10 cm	**1 L/h**	1	2	2	2	2	2	2	0	1
		3	3	3	3	3	3	3	0	0
		2	3	3	3	3	3	3	0	0
15 cm	**1 L/h**	1	1	1	1	1	1	1	1	1
		2	2	3	3	3	3	3	0	0
		2	2	2	3	3	3	3	0	0
20 cm	**1 L/h**	0	0	0	1	1	1	1	1	1
		1	1	1	1	1	1	1	2	0
		2	3	3	3	3	3	3	0	0

NOTE:

AB = Arkansas Brokenray

Table D-5. Summary of number of AB mussels unburied daily and number of AB mussels remained buried on day 7 with decrease of vertical water supply volume per hour (upwelling].

Test condition		Number of Mussel Unburied							Number of Mussel Remain Buried	
Burial depth	**Vertical Water volume**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**No. of Alive**	**No. of Dead**
15 cm	**1 L/h**	1	1	1	1	1	1	1	1	1
		2	2	3	3	3	3	3	0	0
		2	2	2	3	3	3	3	0	0
15 cm	**0.75 L/h**	2	3	3	3	3	3	3	0	0
		3	3	3	3	3	3	3	0	0
		3	3	3	3	3	3	3	0	0
15 cm	**0.5 L/h**	2	3	3	3	3	3	3	0	0
		1	2	2	2 (1)	3	3	3	0	0
		1	1	2	2	2	2	2	0	1
15 cm	**0.375/h**	2	3	3	3	3	3	3	0	0
		1(1)	2	2	2	2	2	2	0	1
		1(1)	2(1)	3	3	3	3	3	0	0
15 cm	**0.25 L/h**	0	1	1	1	1	1	1	0	2
		0	0	0	0	0	0	0	0	3
		1	3	3	3	3	3	3	0	0
15 cm	**0 L/h**	0	2	2	2	2	2	2	0	1
		0 (1)	1	1	1	1	1	1	0	2
		0 (1)	1	1	1	1	1	1	0	2

NOTE:

AB = Arkansas Brokenray

Number in brackets () means the mussel was coming out but was not seen in the surface when observed.

Table D-6. Summary of number of AB mussels unburied daily and number of AB mussels remained buried on day 7 with increase of burial depth when vertical water was supplied downward (downwelling].

Test condition		Number of Mussel Unburied							Number of Mussel Remain Buried	
Burial depth	Vertical Water volume	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	No. of Alive	No. of Dead
5 cm	1L/h	3	3	3	3	3	3	3	0	0
		2	2	2	2	2	2	2*	0	0
		3	3	3	3	3	3	3	0	0
10 cm	1L/h	1	1	1	1	1	1	1	0	2
		0	0	0	0	0	0	0	0	3
		1	1	1	1	1	1	1	0	2
15 cm	1L/h	0	0	0	0	1	1	1	0	2
		0	0	0	0	0	0	0	0	3
		1	1	1	2	2	2	2	0	1

NOTE:

AB = Arkansas Brokenray

* Only 2 AB mussels were buried at the beginning of the test.

Table D-7. Summary of number of mussels of different species unburied daily and number of mussels remained buried on day 7 at different depth (upwelling).

Test condition			Number of Mussel Unburied							Number of Mussel Remain Buried	
Mussel species	Burial depth	Vertical Water volume	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	No. of Alive	No. of Dead
BT	7.5 cm	1 L	3	3	3	3	3	3	3	0	0
			3	3	3	3	3	3	3	0	0
			3	3	3	3	3	3	3	0	0
BT	12.5 cm	1 L	2 [BT + cFT]	2 [BT + cFT]	2 [BT + cFT]	2 [BT + cFT]	2 [BT + cFT]	2 [BT + cFT]	2 [BT + cFT]	0	1 [AB]
			1 [BT]	3	3	3	3	3	3	0	0
			3	3	3	3	3	3	3	0	0
zFT	7.5 cm	1 L	2 [zFT + cFT]	3	3	3	3	3	3	0	0
			3	3	3	3	3	3	3	0	0
			3	3	3	3	3	3	3	0	0
zFT	12.5 cm	1 L	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	0	1 [zFT]
			1 [cFT]	1 [cFT]	1 [cFT]	1 [cFT]	1 [cFT]	1 [cFT]	1 [cFT]	2 [AB + zFT]	0
			1 [cFT]	1 [cFT]	1 [cFT]	1 [cFT]	1 [cFT]	1 [cFT]	1 [cFT]	0	2 [AB + zFT*]
PK	5 cm	1 L	3	3	3	3	3	3	3	0	0
			3	3	3	3	3	3	3	0	0
			3	3	3	3	3	3	3	0	0
PK	7.5 cm	1 L	2	2	2	2	2	2	2	0	1 [PK]
			1 [cFT] + (1 AB)	2	2	2	2	2	2	1 [PK]	0

			1 [cFT]	1 [cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	0	1 [PK]
PK	**12.5 cm**	**1 L**	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	1 [PK]	0
			2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	1 [PK]	0
			2 [AB + cFT] [1 PK]	3	3	3	3	3	3	0	0

NOTE:

BT = Butterfly; zFT = Large Fatmucket from Kansas Zoo

cFT = Small Fatmucket from CERC; PK = Pink Mucket

* The zFT was alive on Day 7 but died the next day

Number in brackets () means the mussel was coming out but was not seen in the surface when observed.

Number followed by Abbreviation in square brackets [] means the species of mussels.

Table D-8. Summary of number of mussels of different species unburied daily and number of mussels remained buried on day 7 at different depth (upwelling).

Test condition			Number of Mussel Unburied							Number of Mussel Remain Buried	
Mussel species	**Burial depth**	**Vertical Water volume**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**No. of Alive**	**No. of Dead**
MK	**7.5 cm**	**1 L**	2 [AB + cFT] + (1 MK)	3	3	3	3	3	3	0	0
			(1 AB)	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	1 [MK]	0
			2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	2 [AB + cFT]	0	1 [MK]
WB	**7.5 cm**	**1 L**	2 [WB + mDT]	2 [WB + mDT]	3 [WB + mDT + DT]	3 [WB + mDT + DT]	3 [WB + mDT + DT]	3 [WB + mDT + DT]	3 [WB + mDT + DT]	0	1 [AB]
				1 [WB]	1 (W)	2 [WB+DT]	2 [WB+DT]	2 [WB+DT]	2 [WB+DT]	0	2 [AB + m DT]
			2 [WB + mDT]	2 [WB + mDT]	2 [WB + mDT]	2 [WB + mDT]	2 [WB + mDT]	2 [WB + mDT]	2 [WB + mDT]	0	2 [AB + DT]
GF	**10 cm**	**1 L**	1 [GF]	1 [GF]	2 [GF + sDT]	2 [GF + sDT]	2 [GF + sDT]	2 [GF + sDT]	2 [GF + sDT]	1 [AB]	1 [DT]
			1 [sDT]	1 [sDT]	1 [sDT]	2 [GF + sDT]	2 [GF + sDT]	2 [GF + sDT]	2 [GF + sDT]	0	2 [AB + DT]
			0	(1 sDT)	2 [AB + sDT]	2 [AB + sDT]	2 [AB + sDT]	2 [AB + sDT]	2 [AB + sDT]	0	2 [GF + DT]

NOTE:

MK = Mucket; WB = Washboard; GF = Giant Floater; AB = Arkansas Brokenray

DT = large Deertoe; mDT = medium size Deertoe; sDT = small size Deertoe

Number in brackets () means the mussel was coming out but was not seen in the surface when observed.

Number followed by Abbreviation in square brackets [] means the species of mussels.

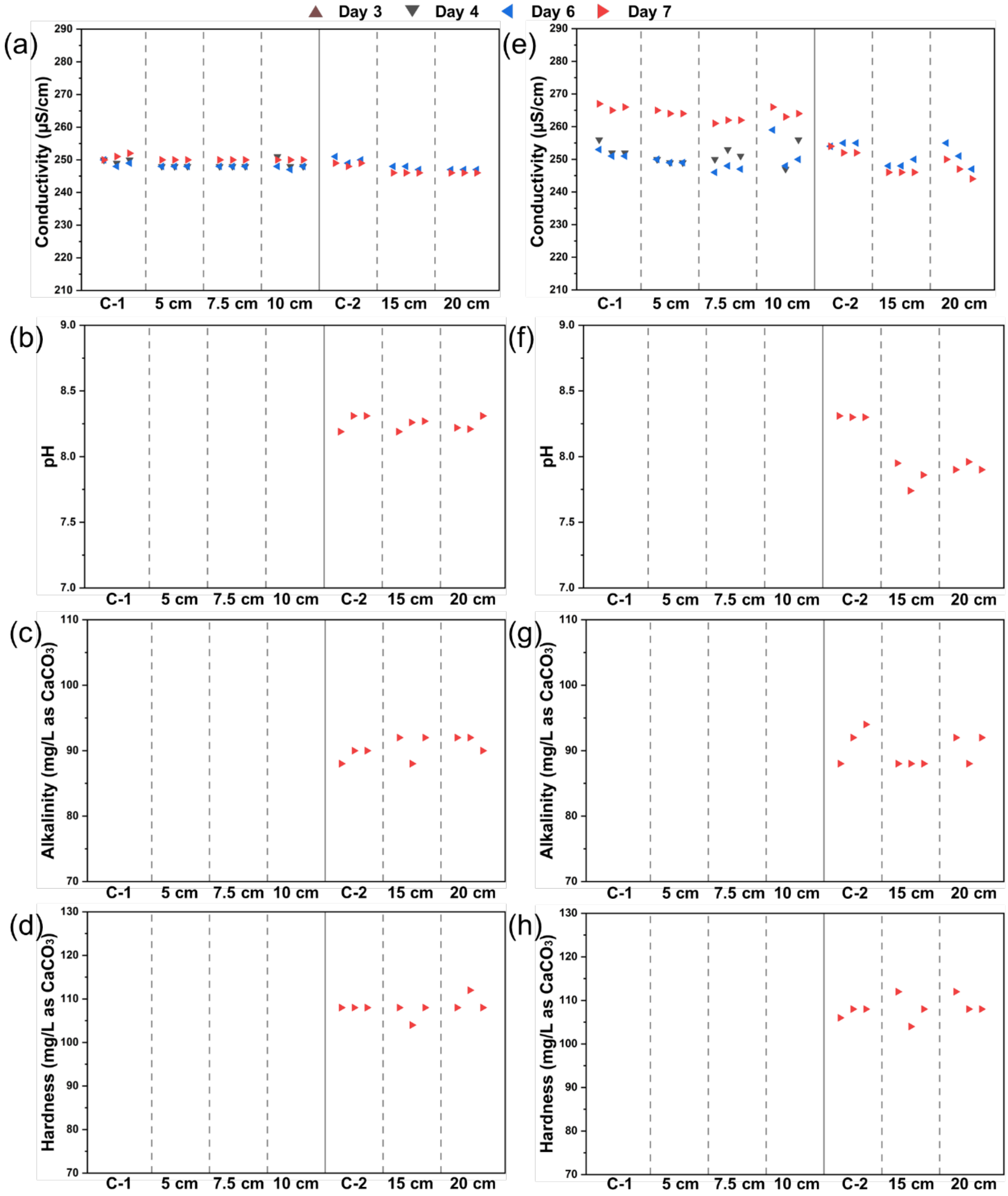

Figure D-1. Changes of water quality including conductivity, pH, alkalinity, and hardness of the surface water in Water-I (a-d) and Water-O (e-h) with increase of burial depths.

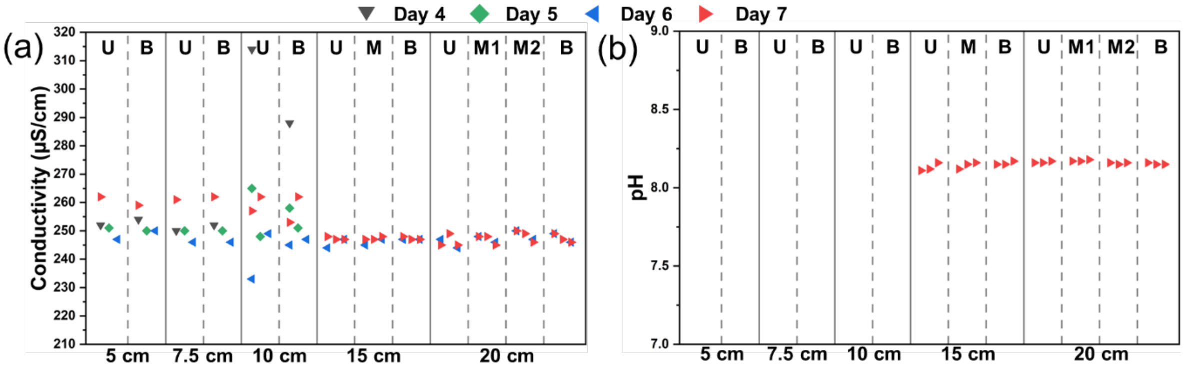

Figure D-2. Changes of pore water quality in the burial layer at different depths including (a) conductivity and (b) pH with increase of burial depths.

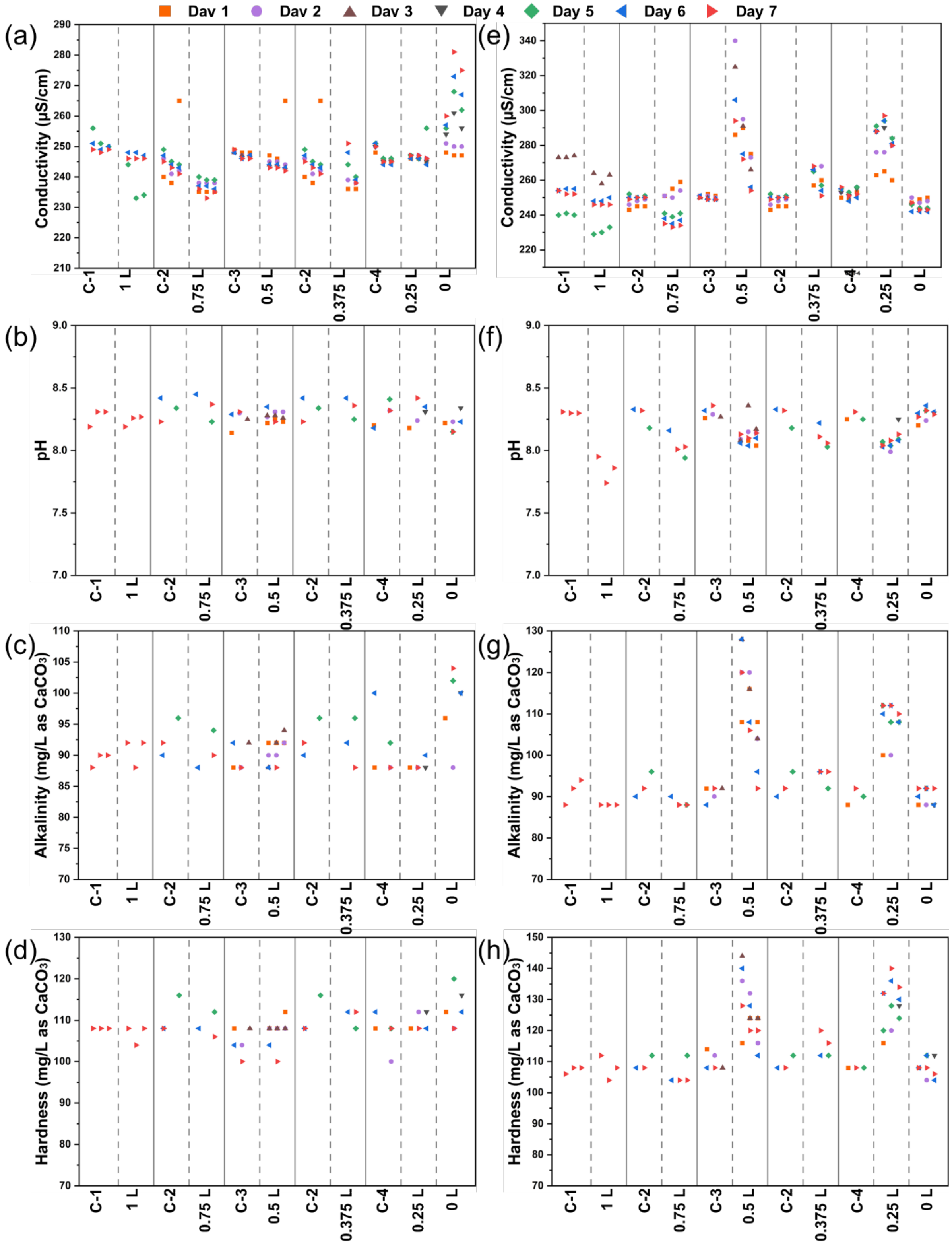

Figure D-3. Changes of water quality including conductivity, pH, alkalinity, and hardness of the surface water in Water-I (a-d) and Water-O (e-h) with decrease of vertical water flow volumes.

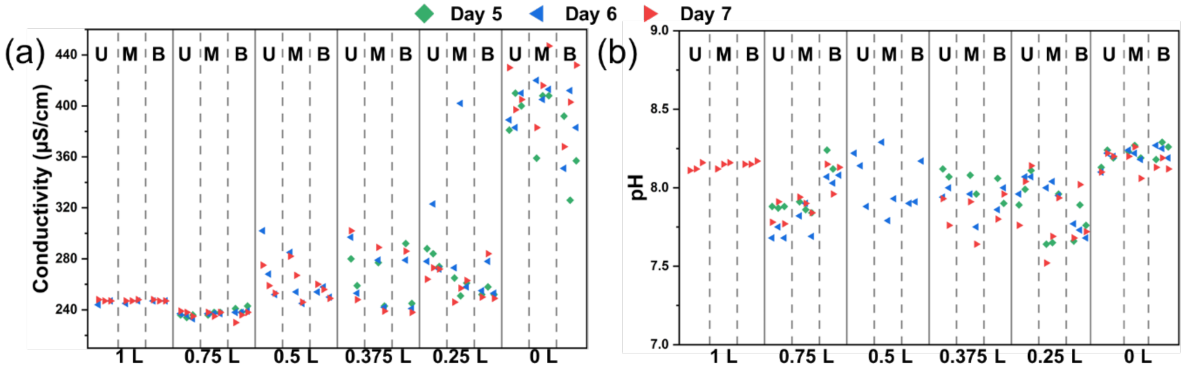

Figure D-4. Changes of pore water quality in the burial layer at different depths including (a) conductivity and (b) pH with decrease of vertical water flow volumes.

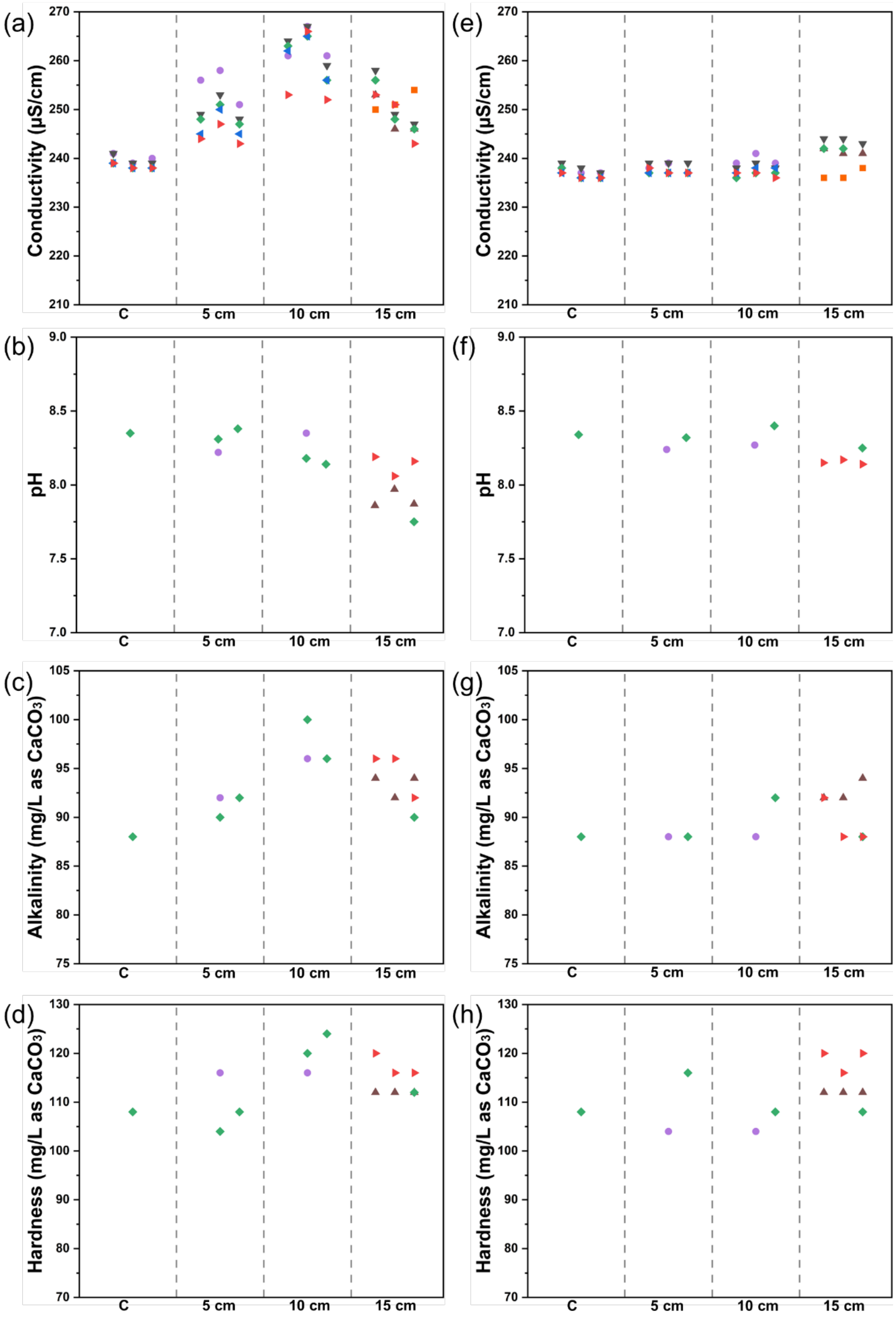

Figure D-5. Changes of water quality including conductivity, pH, alkalinity, and hardness of the surface water in Water-I (a-d) and Water-O (e-h) with upwelling vertical water flow and increased burial depths.

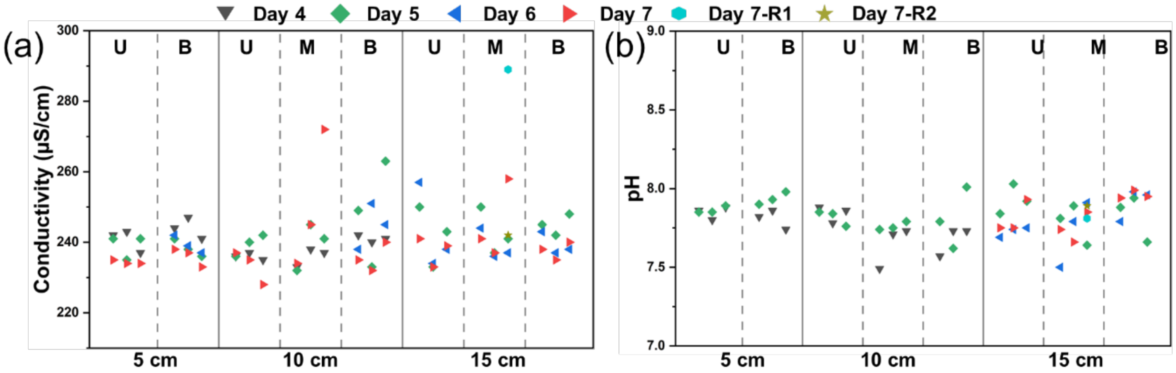

Figure D-6. Changes of pore water quality in the burial layer at different depths including (a) conductivity and (b) pH with upwelling vertical water flow and increased burial depths.

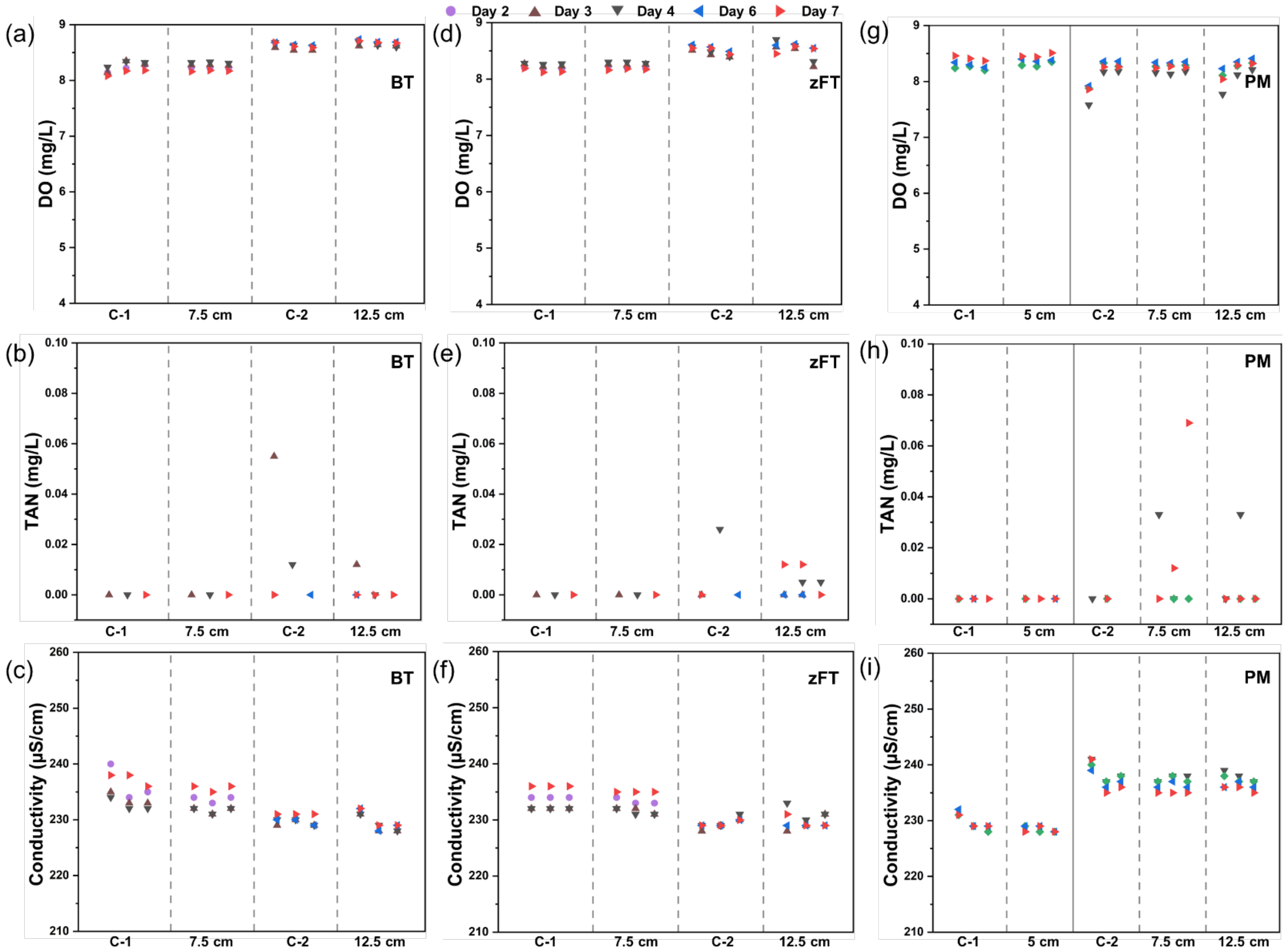

Figure D-7. Changes of water quality including DO, TAN, and conductivity of the surface water in Water-I: (a-c) when BT mussels were buried with AB and cFT; (d-f) when zFT mussels were buried with AB and cFT; and (g-i) when PM mussels were buried with AB and cFT.

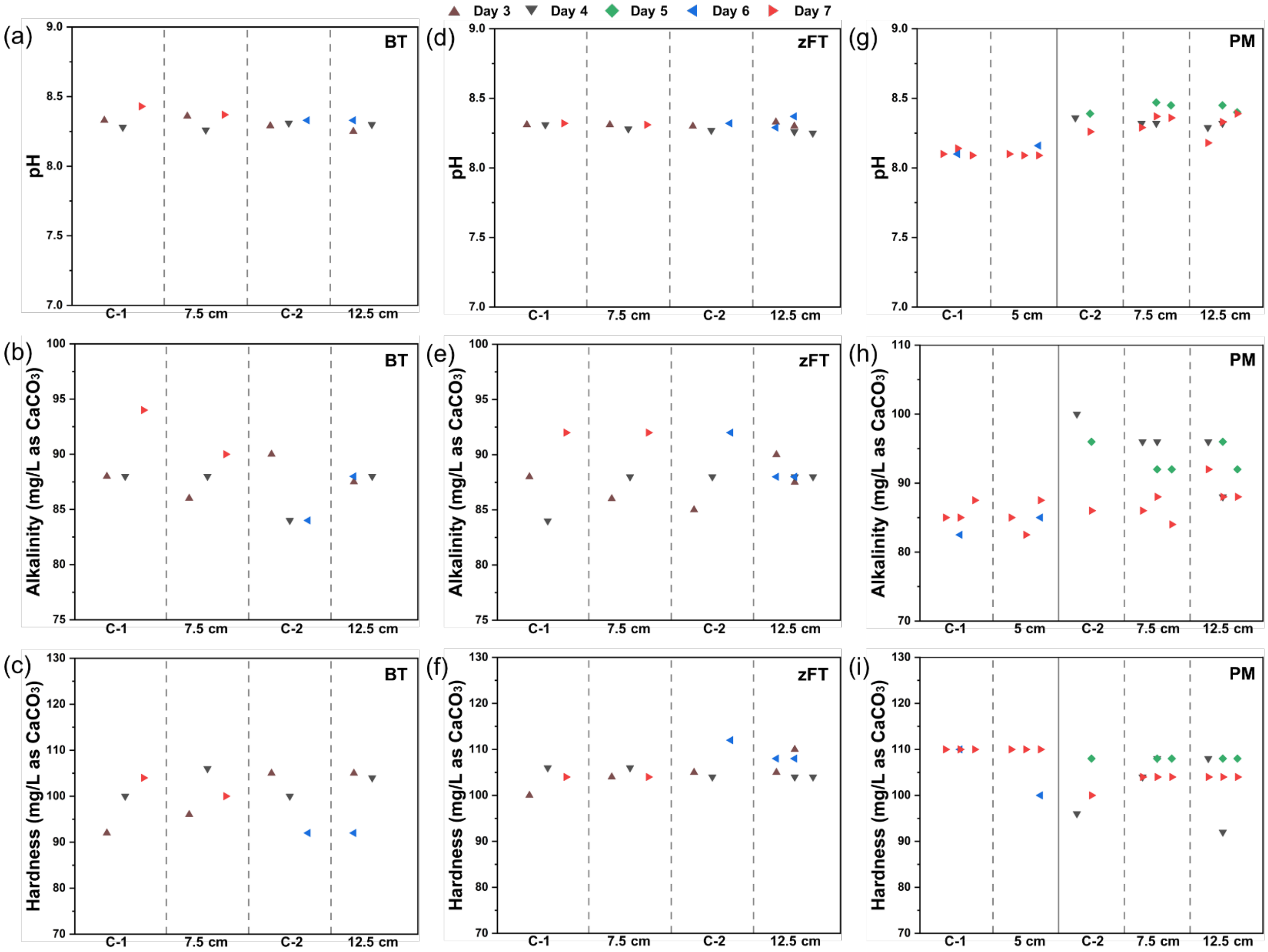

Figure D-8. Changes of water quality including pH, alkalinity, and hardness of the surface water in Water-I: (a-c) when BT mussels were buried with AB and cFT; (d-f) when zFT mussels were buried with AB and cFT; and (g-i) when PM mussels were buried with AB and cFT.

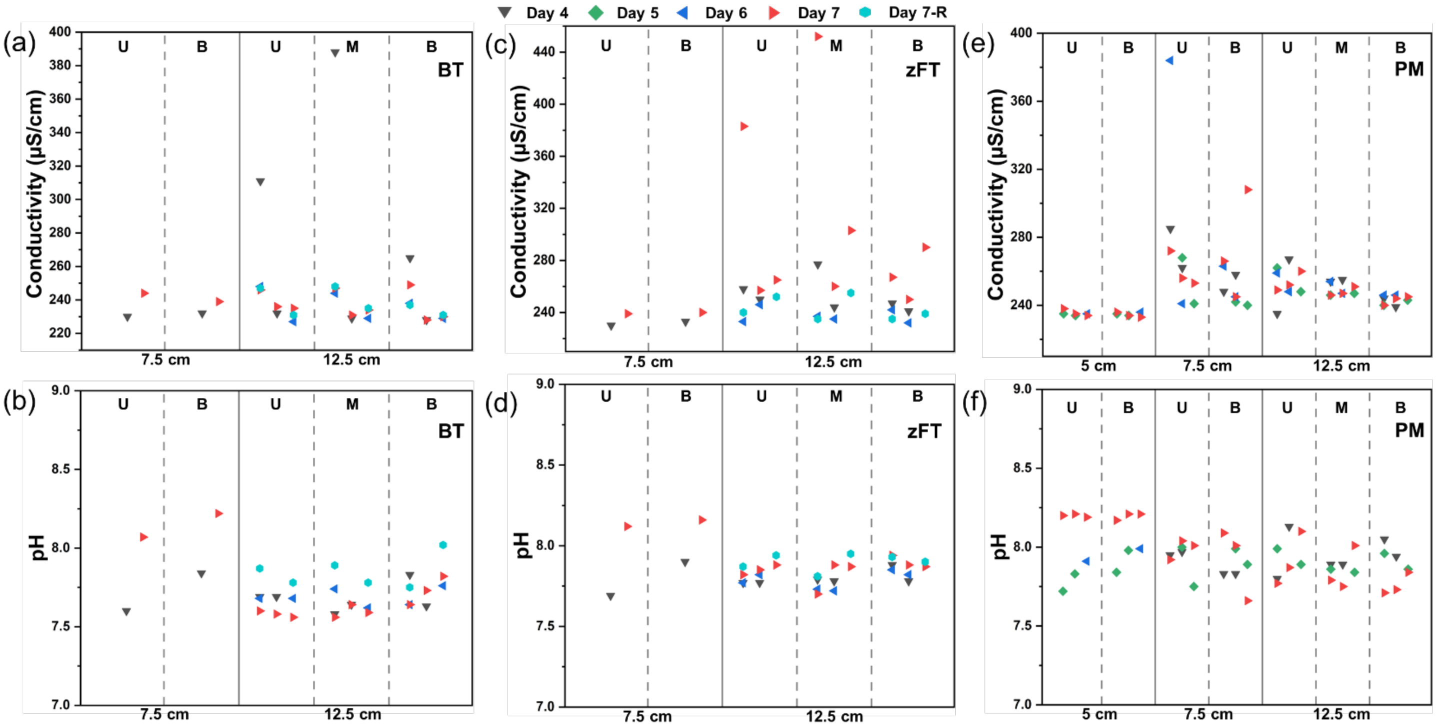

Figure D-9. Changes of pore water quality including conductivity and pH in the burial layers at different depths: (a-b) when BT mussels were buried with AB and cFT; (c-d) when zFT mussels were buried with AB and cFT; and (e-f) when PM mussels were buried with AB and cFT.

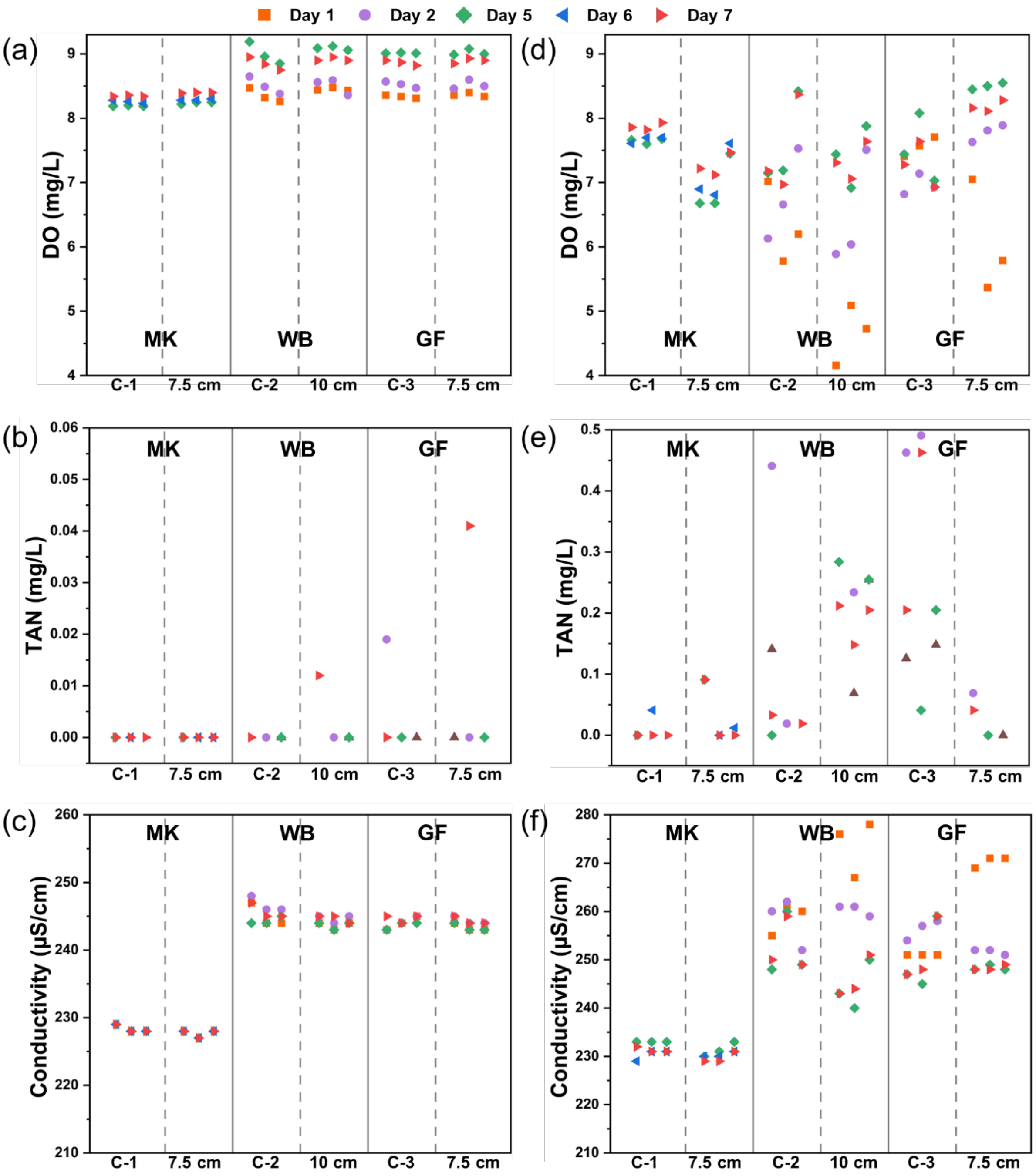

Figure D-10. Changes of pore water quality including DO, TAN, conductivity of the surface water in Water-I (Left) and Water-O (right): (a-b) when MK mussels were buried with AB and cFT; (c-d) when WB mussels were buried with AB, sDT and DT; and (e-f) when GF mussels were buried with AB, mDT and DT.

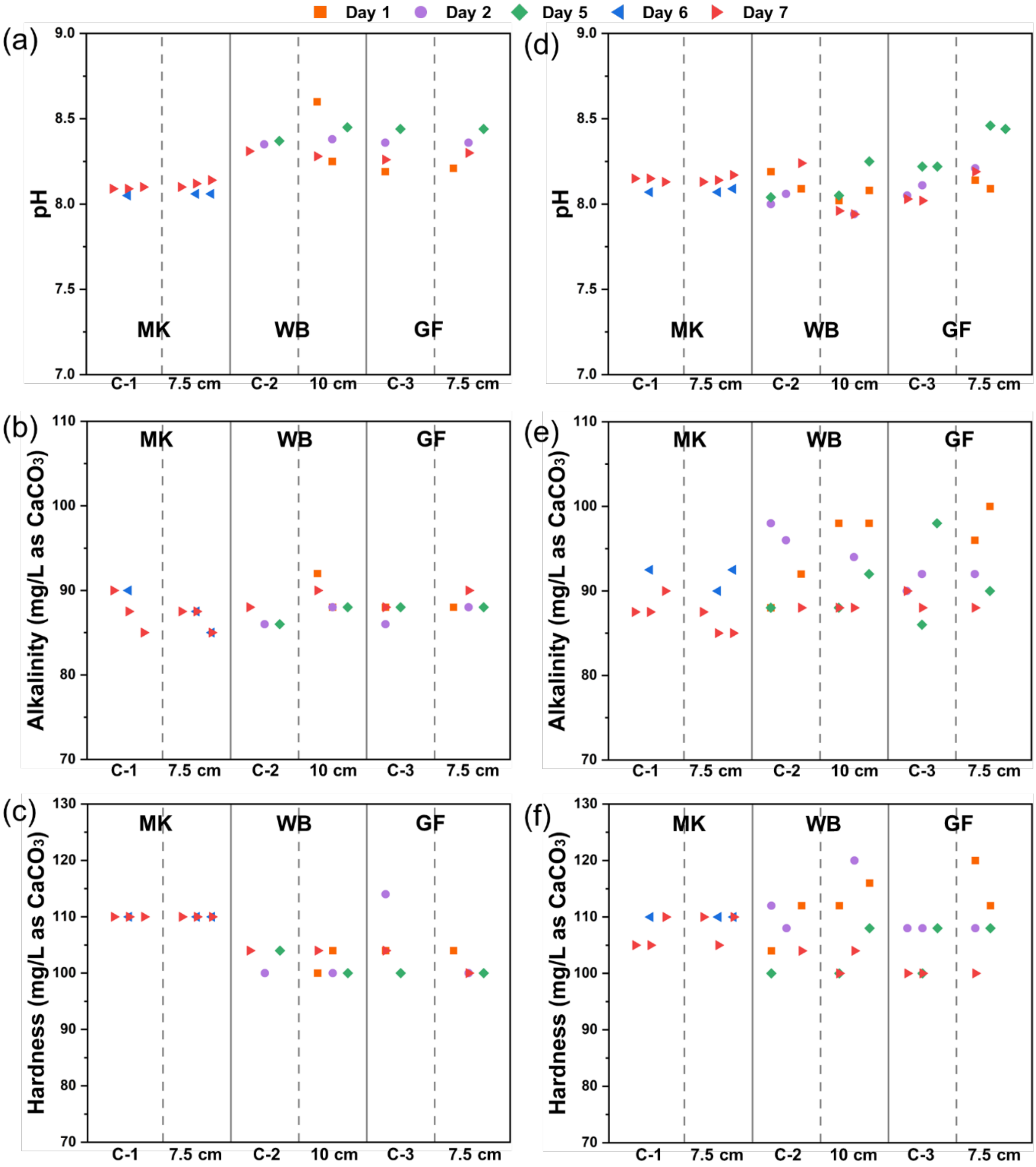

Figure D-11. Changes of pore water quality including pH, alkalinity, and hardness of the surface water in Water-I (Left) and Water-O (right): (a-b) when MK mussels were buried with AB and cFT; (c-d) when WB mussels were buried with AB, sDT and DT; and (e-f) when GF mussels were buried with AB, mDT and DT.

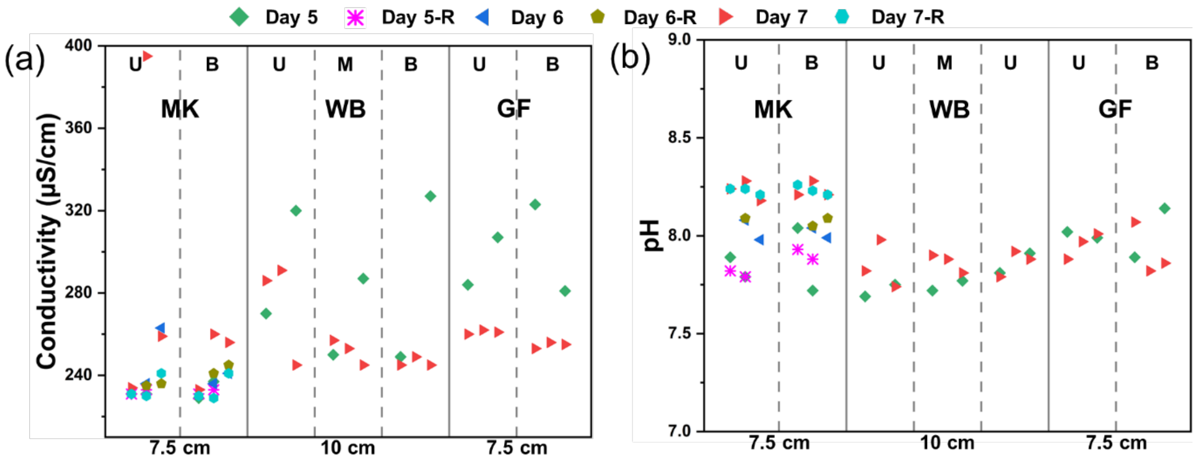

Figure D-12. Changes of pore water quality including conductivity (a) and pH (b) in the burial layers at different depths when MK mussels were buried with AB and cFT; (c-d) and when WB and GF mussels were buried with AB, sDT and DT.

Appendix E: Model Evaluation and Interpretation for Freshwater Mussels from Point-Source Sedimentations

E-1. Model Evaluation and Interpretation

E-1.1 Study Site and Measurements

To use the sediment deposition model in investigating sediment impacts on freshwater mussels, a study reaches in the Osage River near Bagnell, Missouri (**Figure E-1**) was select. The Osage River is a 444 - km long tributary of the Lower Missouri River and drains approximately 40,000 km^2 across east-central Kansas and west-central and central Missouri. The river is regulated for flood control and hydropower in central Missouri at Truman and Bagnell Dams. The selected study reach is about 4 km long and located 16 km downstream from Bagnell Dam in the lower Osage River. Bagnell Dam is a concrete gravity dam completed in 1931 that impounds the Osage River, creates the Lake of the Ozarks, and is used to generate hydroelectric power. The study reach has a robust mussel community with 19 live species, including one federally endangered species, that has been routinely monitored by the U.S. Fish and Wildlife Service (USFWS)[108]. Because the study reach is in close proximity and downstream from Bagnell Dam, the reach experiences frequent and abrupt changes in discharge, as well as extended period of high discharge events (**Figure E-2**).

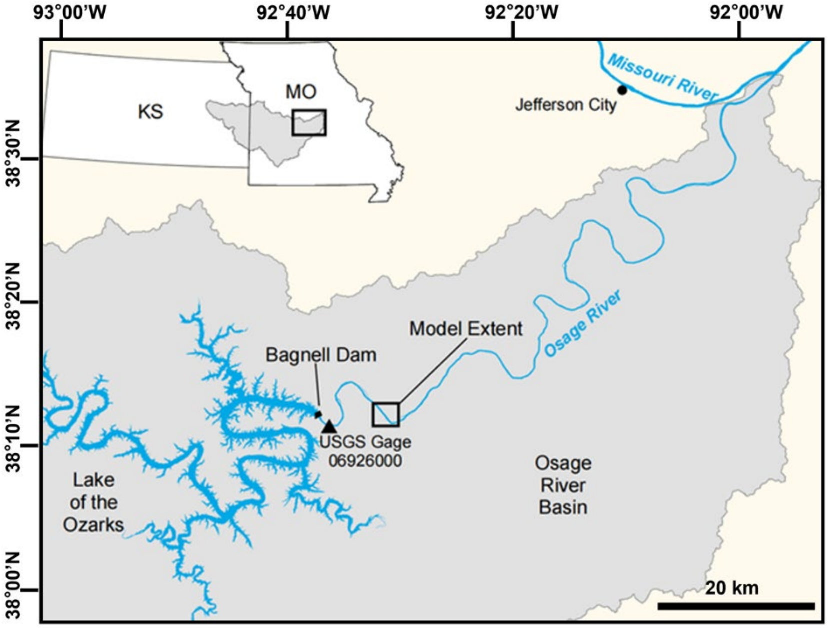

Figure E-1. Map showing the model extent for the study reach in the lower Osage River.

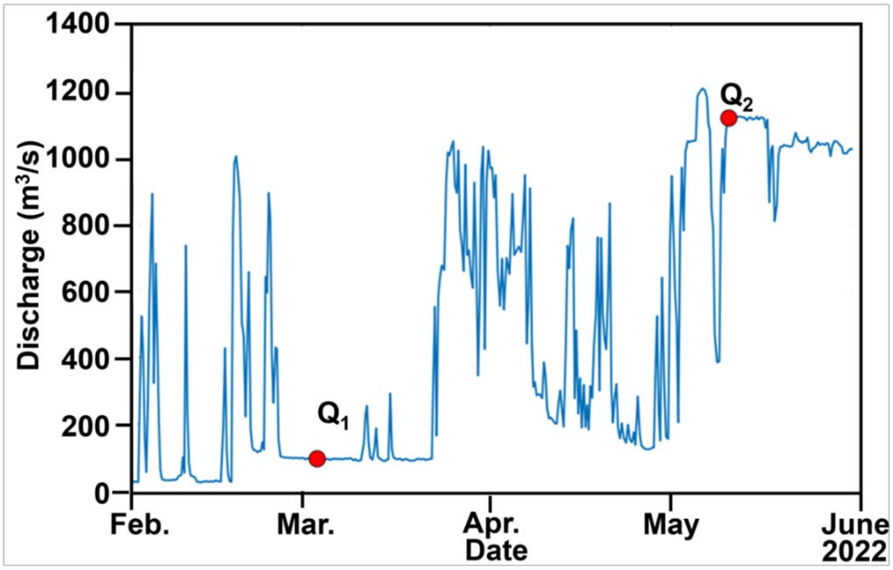

Figure E-2. River discharge at the USGS gaging station 06926000 during 02/01/2022-05/31/2022. Two red markers represent the two discharge conditions used in the modeling of sedimentation.

As part of a larger effort to document and characterize the mussel habitat at this study reach, site bathymetry and topography of exposed gravel bars and stream banks were measured and hydraulic data were collected across a range of discharge events. Bathymetry and topography were collected using Real-Time Kinematic Global Navigation Satellite Systems positioning systems, employing a single base station. Bathymetry data were collected using a single-beam echosounder (CEEPULSE 100 series, CEE Hydrosystems) by driving from bank to bank along planned transects spaced approximately 10 m apart and logged using Hypack Survey Software (Xylem Inc.). Exposed bars and banks were surveyed using a boat-mounted terrestrial lidar system (Velodyne LiDAR Puck LITE) with an Inertial Measurement Unit (IMU) (SBG Systems) while driving upstream and downstream through the reach. Terrestrial lidar data were logged in Hysweep survey software (Xylem, Inc). To supplement the field-collected bathymetry and topography data, aerial lidar for the surrounding floodplain were obtained from the Missouri Spatial Data Information Service (MSDIS) in Tag Image File format (TIFF). The bathymetry, terrestrial lidar, aerial lidar, and interpolated bathymetry shapefiles were combined to produce a triangulated irregular network (TIN) using Delaunay conforming triangulation and soft breaklines along the boundaries of each dataset to prevent artefacts produced by data gaps. The TIN was then converted to a 2 m Tag Image File Format (TIFF) Digital Elevation Model (DEM) using Natural Neighbors interpolation.

Discharge and velocity data were collected using a 600-kilohertz RiverRay acoustic Doppler current profiler (ADCP; Teledyne RD Instruments) mounted off the bow of a motorboat. Data were synchronized with positioning data from a Global Navigation Satellite Systems (GNSS) receiver mounted above the instrument and were recorded using WinRiver II software (Teledyne RD Instruments). Velocity and position data were collected while driving bank-to-bank transects until at least two transects in each direction were completed with a discharge error of less than 5 percent of the mean discharge. Water-surface elevation data were also collected for each discharge measurement by driving downstream through the reach, using a GNSS receiver mounted on a motorboat and recording water surface elevation data using Hypack survey software (Xylem Inc.) with a known offset to the water surface.

For this study, discharge data from two dates, March 3 and May 10, 2022 were used. These two measurements represent a low-flow (95 m^3/s) and high-flow (1140 m^3/s) condition, respectively.

Figure E-4 depicts the measured riverbed profile and the water surface elevation along the surveyed line shown in **Figure E-3** under two discharge conditions. Under the low-flow condition (Q_1), the majority of the stream within 3 km from the upstream survey point exhibited a flow depth of less than 2 m. The flow depth increased in the downstream segment due to the lower channel elevation. In the high-flow condition (Q_2), the flow depth was approximately 3.5 m higher than that observed in the Q_1 condition.

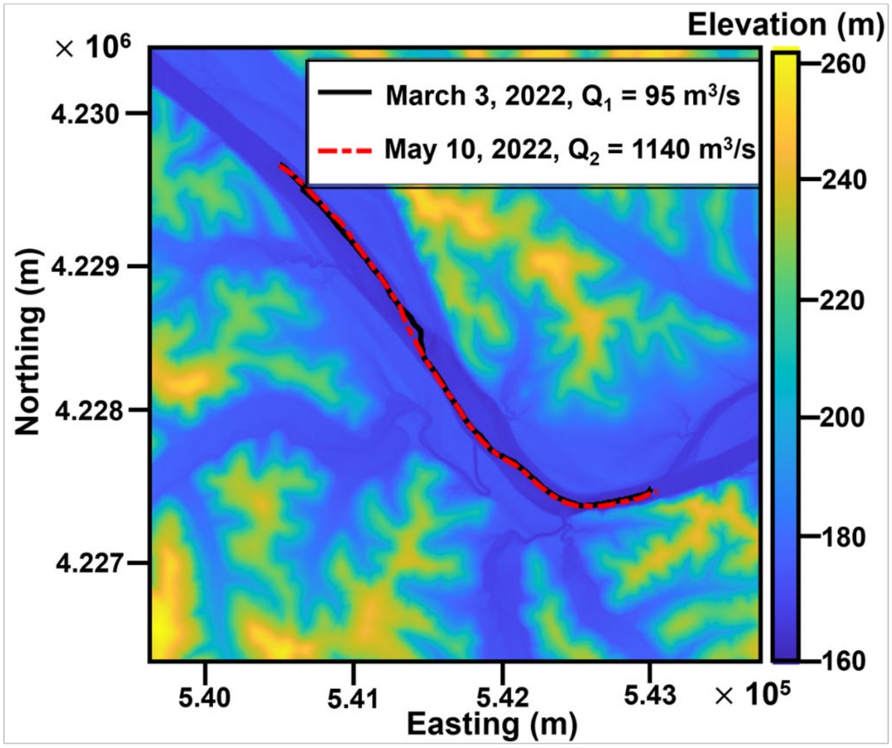

Figure E-3. The elevation map at the study site with superposition of in-stream paths for the water surface elevation measurements at two discharge conditions.

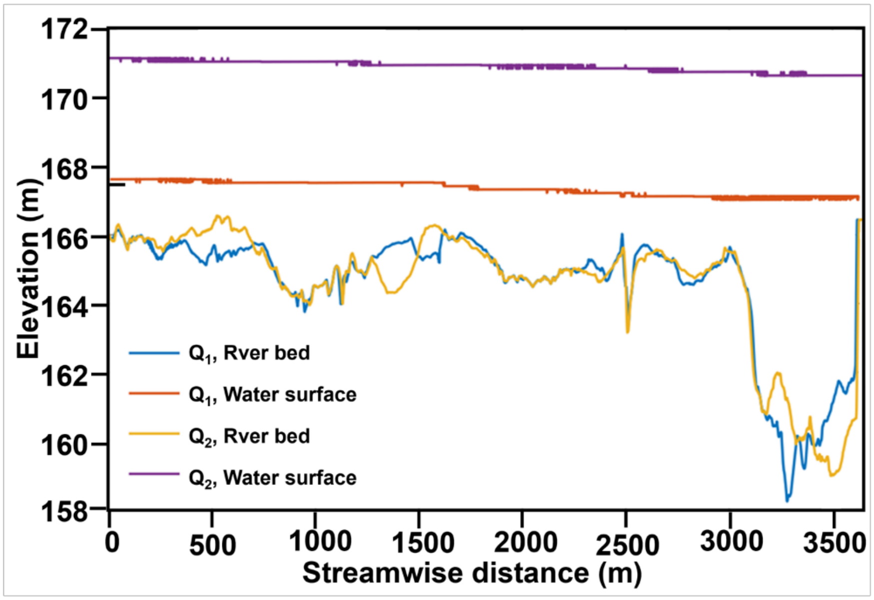

Figure E-4. Measured elevations of riverbed and water surface under two discharge conditions.

The transects for velocity measurements using Acoustic Doppler Current Profiler (ADCP) are shown in **Figure E-5,** Depth-average velocity ranged from 0.04 to 2.90 m/s at flow depths of 0.5 to 3 m at the three cross Chapters in the Q_1 condition, with the highest velocities occurring at a narrow segment of the channel. For the Q_2 condition, depth-averaged flow velocities ranged from 0.13 to 1.90 m/s at flow depths ranging from 2.5 to 6 m within two cross sections.

E-1.2 Hydrodynamic Modeling

The hydrodynamics in the stream was obtained using a three-dimensional computational fluid dynamics (CFD) model. FLOW3D-HYDRO was used to simulate the flow under the steady-state condition of Q_1 and Q_2, respectively. A Reynolds-averaged Navier-Stokes (RANS) solver was used with the Re-Normalization Group (RNG) modified k-ε turbulence closure model. The governing equations have been given elsewhere [181, 182] and therefore are not repeated here.

In the CFD modeling, the selected reach was reconstructed and meshed using the measured bathymetry data. After the mesh independence study, the final mesh of 5 m × 5m × 0.3 m (streamwise-spanwise-vertical) was chosen to run the simulation. The model was calibrated using the water surface elevation. Within approximately 3.5 km streamwise direction, the root-mean square-error of surface elevation between the CFD model and measured data is 0.14 and 0.12 m, corresponding to 5% and 2% of the mean flow depth for Q_1 and Q_2, respectively.

The model-reconstructed riverbed elevation, model-determined flow depth and velocity, with comparison to the measured data during the field survey, are illustrated in **Figure E-6** The comparison of riverbed elevation between the measured data and reconstructed data show an overall agreement, with deviation found between the single model-reconstructed riverbed with multiple individual single beam sonar surveyed data. Similarly, the modeled-determined flow depth and velocity are within general agreement with the measurement data. Although a considerable amount of scattering is evident between measured and modeled velocities, an overall agreement is observed, indicating the model reasonably represents the flow characteristics of the reach. The difference between the model-reconstructed riverbed and the measured riverbed is a contributing factor to the deviation of model-determined flow depth and velocity from the measured data. This is evidenced by the smaller errors after filtering out the elevation differences that are greater than 0.2 m (see filled markers in **Figure E-6**).

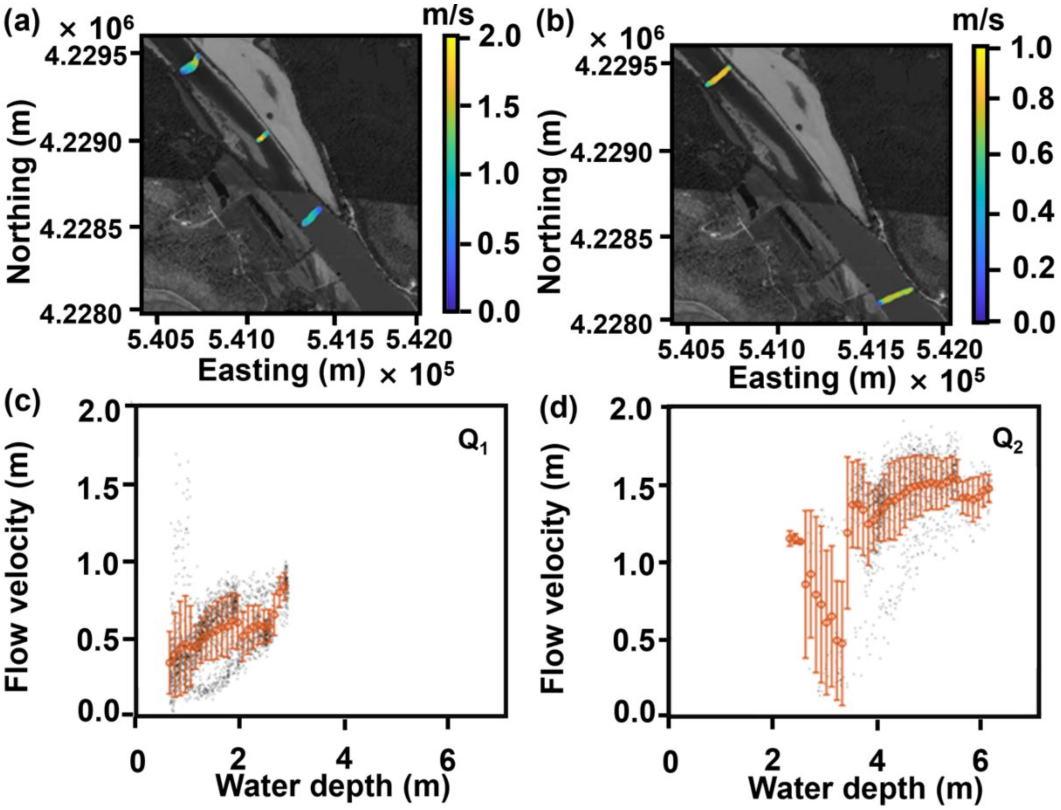

Figure E-5. Locations and the data for the acoustic Doppler velocity profiler (ADCP) measurements. The subplots (a) and (b) represent the cross section transects under condition Q_1 and Q_2, respectively. The subplots (c) and (d) display the measured depth-averaged flow velocities as a function of flow depth. Gray dots represent each individual data from all ADCP transects. Symbols and Error bars indicate the mean and standard deviation of the depth-averaged velocities averaged across 0.1 m depth intervals.

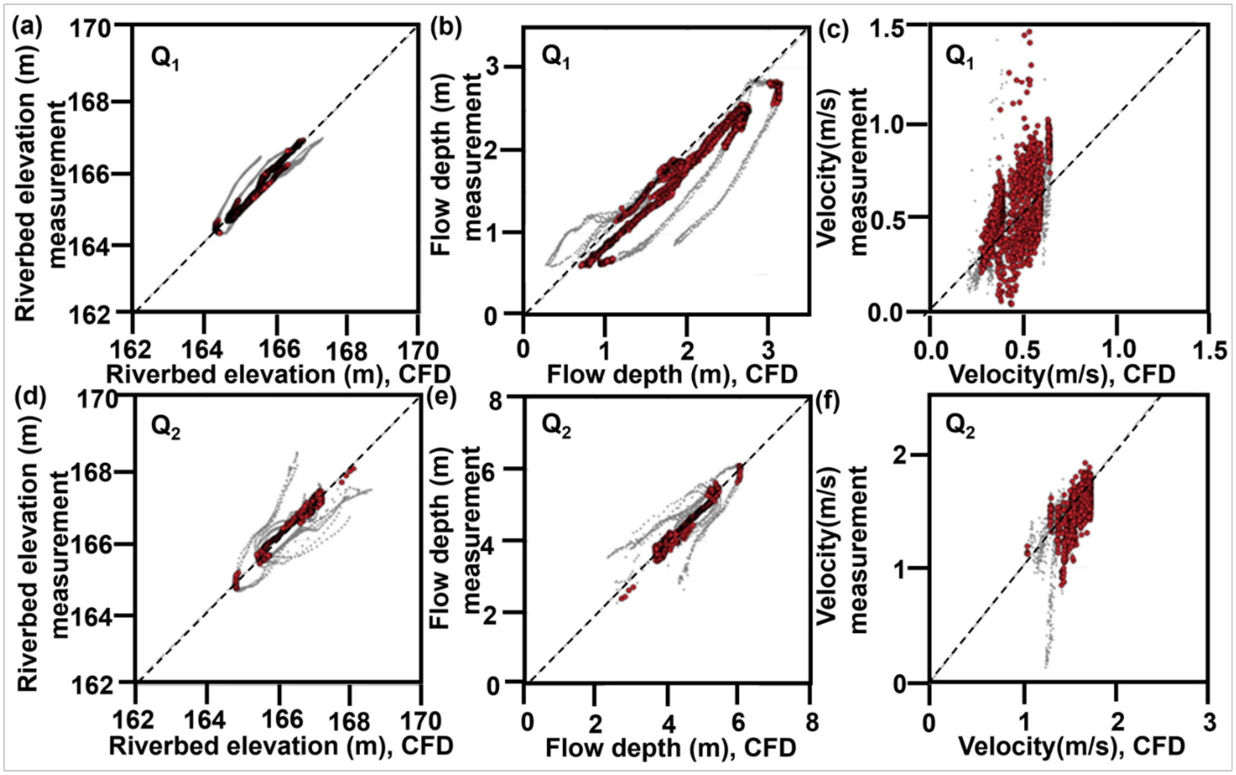

Figure E-6. Comparison between measurement data and computational fluid dynamics (CFD) reconstructed or modeled results. Flow condition Q_1: (a) riverbed elevation; (b) flow depth; (c) depth-averaged velocity; Flow condition Q_2: (d) riverbed elevation; (e) flow depth; (f) depth-averaged velocity. Colored symbols represent the location where the differences in riverbed elevation are less than 0.2 m between CFD and the measurement. The dashed lines represent 1:1 relationship.

The simulated depth-averaged flow velocity profile and flow depth along the main channel are plotted in **Figure E-7** for Q_1 and Q_2, respectively. For Q_1, the flow velocities vary around 0.6 m/s as a function of flow depth and decrease to approximately 0.3 m/s within 400 m at the downstream end. The mean flow velocity in the stream is 0.52 m/s and the standard deviation is 0.15 m/s. The maximal and minimal flow depth is 8 m and 0.6 m, respectively, with the mean flow depth of 2.6 m. For Q_2, the mean flow velocity is 1.5 m/s in the stream with the standard deviation of 0.2 m/s. The maximal, minimal, and mean flow depth is 11.3 m, 4 m, and 6 m, respectively.

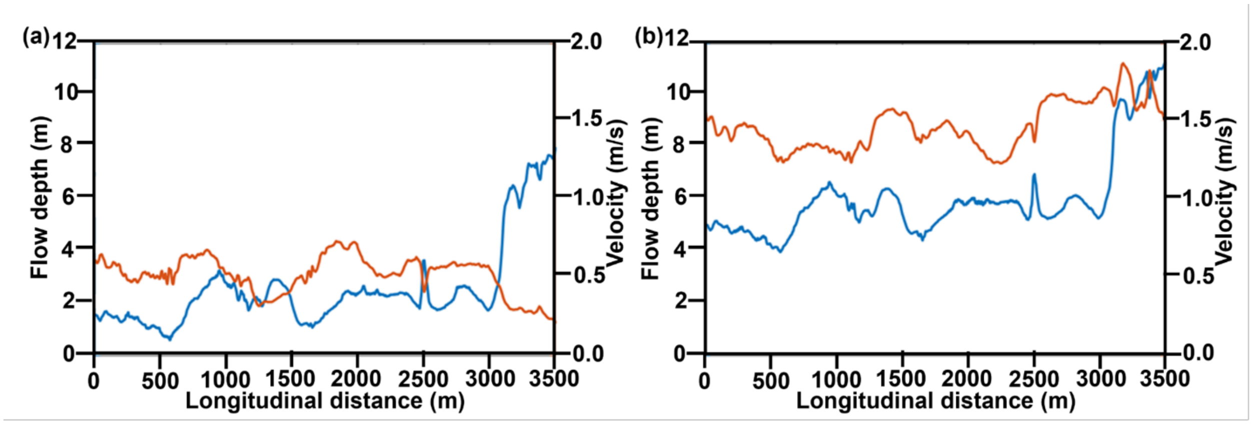

Figure E-7. Modeled water depth and flow velocity in the main channel along the streamwise direction for two discharge conditions: (a) Q_1; (b) Q_2.

E-1.3 Sediment Class for Sedimentation Modeling

Three different sediment classes based on the particle size distribution are used to model the deposition of sediments that may result from a construction project. Fine sediments are used to categorize the sediments within 0.001 - 0.1 mm, e.g., silts. Medium sediments are defined as those within 0.01 - 1 mm, representing very fine to coarse sands. Large sediments are defined within 0.1 - 20 mm, representing medium to very coarse sands and small gravels. In the sediment deposition modeling, log-normal distribution was used to generate the generic sediment particle size distributions for three sediment classes (**Figure E-8**). The resulted median sediment diameter is 0.01 mm, 0.1 mm, and 1.5 mm for the fine, medium, and large sediment class, respectively.

E-1.4 Modeling of Sediment Exposure Zone

Deposition of sediments in three classes were modeled using the Lagrangian particle tracking model (see **Chapter 5.1**) at two discharge conditions (see **Chapter E1.1**), where the hydrodynamics were provided by the CFD simulation results (see **Chapter E1.2**). The three-dimensional hydrodynamics data were converged in one dimension similar to the previous study for fish egg transport in rivers and streams to reduce computational cost [183]. For each sediment class, 150,000 particles are released onto the water surface at the upstream starting point of the survey line for water surface elevation measurement. All particles are tracked until they reach the riverbed.

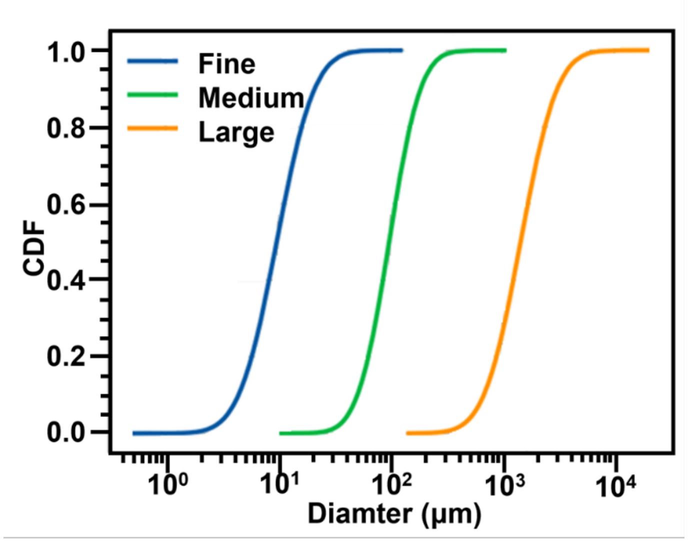

Figure E-8. Three different sediment classes used in this study. Fine particles: 0.001 - 0.1 mm, d_{50} = 0.01 mm, representing limestone and silts; medium particles: 0.01 - 1 mm, d_{50} = 0.1 mm, representing very fine to coarse sands; large particles: 0.1 - 20 mm d_{50} = 1.5 mm, representing medium to very coarse sands and small gravels. CDF means cumulative distribution function.

At first, the sediment exposure zone where freshwater mussel beds could be affected was examined. Once a particle reaches the riverbed, mussels would be affected. Therefore, re-suspension of sediments is out of the scope here and the modeling objective is to evaluate the initial settling location of all sediment particles.

Figure E-9 illustrates the probability density function for sediments to deposit onto the riverbed in Q_1 condition without consideration of particle-particle interaction and re-suspension. The results indicate that large, median, and fine particles deposit primarily within approximately 20, 400, and 600 m downstream from the source, respectively. For Q_2, the primary sediment deposition location is within 100, 1500, and 3000 m for large, medium, and fine particles, respectively (**Figure E-10**). The determined sediment deposition location in **Figures E-9** and **E-10** represents the initial touch down location of each sediment size resulting from sediment settling and turbulent mixing. They are the indicator of the location where freshwater mussels would be exposed to the sediment cloud. They do not represent the final deposition location of the sediment as some small particles would be re-suspended and transported downstream. The final sediment deposition location is determined by the flow condition and the particle diameter.

The results indicate that the sediment exposure to mussel beds is highly relevant to flow parameters and sediment diameters. Previous studies have shown that sediment exposure can pose risks to the growth of mussels [184, 185], therefore, necessary mitigation could be planned to reduce sedimentation load from a construction project. For example, implementing soil erosion control can significantly reduce the flux of fine sediments into streams, which can potentially mitigate the risks of sediment exposure for downstream mussels.

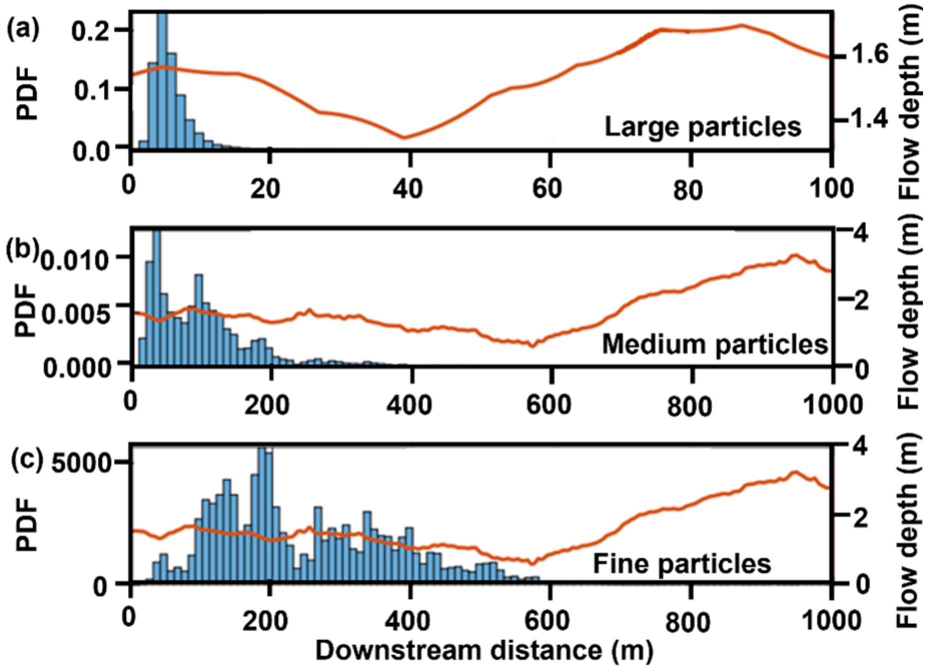

Figure E-9. The initial sediment deposition location in the stream under the condition of Q_1 for three different sediment classes.

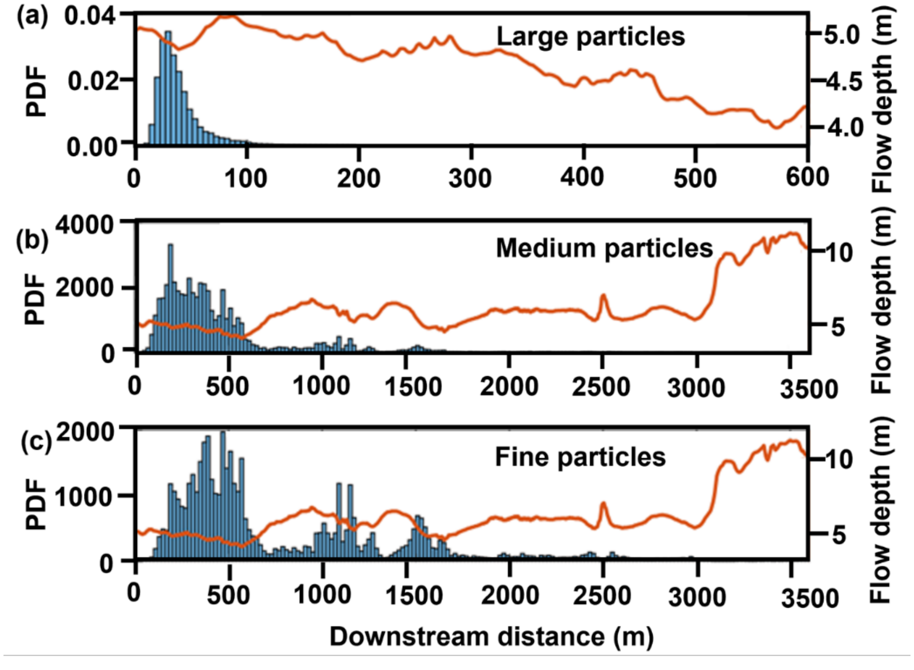

Figure E-10. The initial sediment touch-down location in the stream under the condition of Q_2 for three different sediment classes.

E-1.5 Modeling of Sediment Burial Zone

In this section, potential sediment burial of freshwater mussels from episodic erosion and deposition events was further examined. To account for suspension and re-suspension of small sediments in the flow, a simple Rouse number criterion was used to determine the classification of sediment transport [167, 168]. Rouse number is defined as $Ro = Vs/\beta\kappa u_*$, where $\beta = \max(1 + 2(Vs/u_*)^2, 3)$ is the factor that accounts for the response of sediment diffusion to the mixing of turbulent eddies [168]. If $Ro \geq 2.5$, sediment transport is classified as bed load; if $1.2 \leq Ro < 2.5$, sediments are partially suspended (i.e., 50% suspension); if $0.8 \leq Ro < 1.2$, sediments are fully suspended (i.e., 100% suspension); if $Ro < 0.8$, sediment transport is classified as wash load [167, 169].

To determine the critical sized sediment that would deposit onto the riverbed in the Osage River, averaged shear velocity was calculated u_*=2.0 and 6.6 cm/s for Q_1 and Q_2 from the hydrodynamic model output, respectively. Rouse number (Ro) is then determined as a function sediment diameter, which can be used to examine the critical sediment diameter for each sediment transport class (**Figure E-11**). Two critical sediment diameters were used in modeling sediment burial: (1) the sediment diameter corresponding to $R_{oc} = 2.5$ (d = 0.21 and 1.29 mm for Q_1 and Q_2, respectively) was used as the smallest "settling" particle diameter, i.e., all particles that are larger than 0.21 mm and 1.29 mm will be deposited in the stream without re-suspension in Q_1 and Q_2, respectively. Therefore, these particles will be placed at their first touch-down location in the modeling. (2) The sediments corresponding to $1.2 < Ro < 2.5$ are considered having 50% chance to be re-suspended once they touch-down on the riverbed. Therefore, in the modeling, each particle within 0.11 - 0.21 mm and 0.57 - 1.29 mm for Q_1 and Q_2, respectively, will be assigned with a 50% probability of re-suspension at each time the particle reaches the riverbed. If a particle is assigned to be re-suspended, the riverbed is used as a reflecting boundary to move the particle back into water column [164, 169].

Figure E-12 shows the spatial distribution of the final settling location of large sediments (greater than 0.11 and 0.57 mm for Q_1 and Q_2 respectively). The model results indicate that sediments are primarily settled within 10 m and 100 m downstream distance for Q_1 and Q_2, respectively. In an episodic sedimentation event with relevance to construction, the PDF of particles deposited (illustrated in **Figure E-12**) offers a valuable tool for estimating both the location and the amount of sediment deposition in a mussel burial event. Utilizing these quantitative predictions can serve as a useful guide in decision-making processes aimed at mitigating the impact of sedimentation on mussel habitat. For example, in the tested two discharge conditions, relocating mussels within 10 and 100 m downstream from the point-source sedimentation can be used to inform resource managers when mitigating effects of sediment deposition on mussel beds.

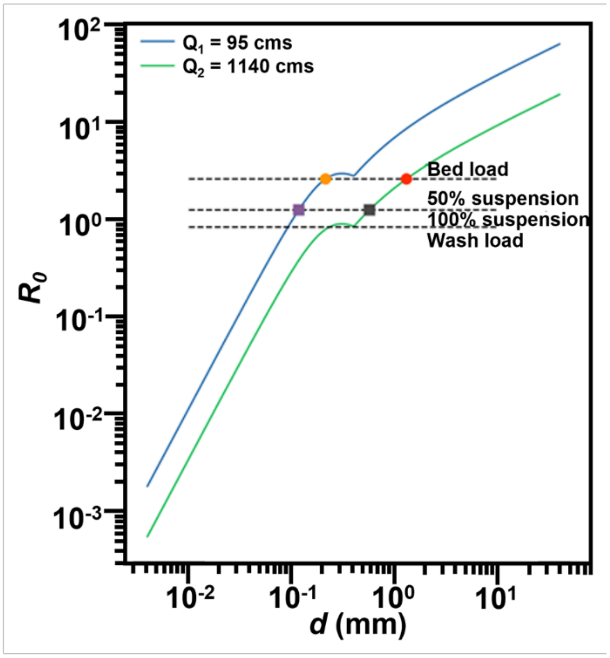

Figure E-11. Sediment transport classification in the Osage River under two discharge conditions. Two circles are (d = 0.21 mm, R_o = 2.5) and (d = 1.29 mm, R_o = 2.5) for Q_1 and Q_2, respectively. Two squares are (d = 0.11 mm, R_o = 1.2) and (d = 0.57 mm, R_o = 1.2) for Q_1 and Q_2, respectively.

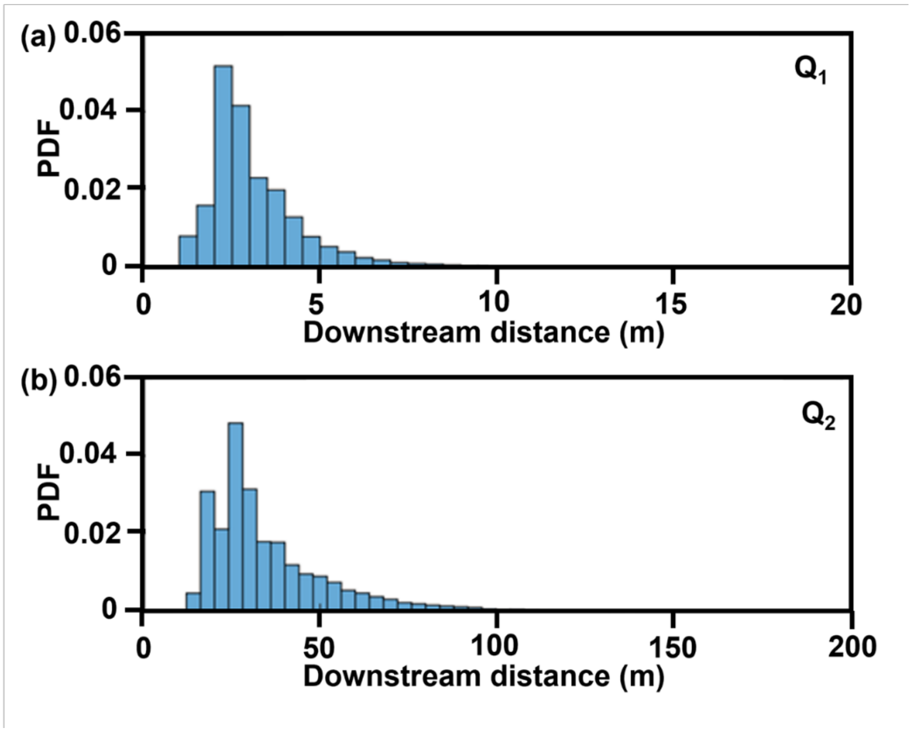

Figure E-12. The predicted mussel burial zone for the large particle class under two discharge conditions: (a) Q_1 (top) = 95 m^3/s and (b) Q_2 (bottom) = 1,140 m^3/s.

Appendix F: Potential Impacts of Sediment Deposition on Freshwater Juvenile Mussels

F-1. Research Objectives and Methods

F-1.1 Research Objective

Freshwater juvenile mussels can become buried during events including construction activities and flood events. Burial experiments were conducted in this study using various sediment/soil materials to investigate their ability to unbury themselves and survive after burial at different depths.

F-1.2 Research Methods

Juvenile Fatmucket (1 - 2 cm, and ~5 mm, respectively) and Arkansas Brokenray (1.5 - 2 cm) were selected for the burial experiments. Different materials including sand (~500 µm) (SND), crushed Columbia local limestone (LMT), Osage Riverbank clay soil (ORC), and different fraction of Bourbeuse River sediments including particle fractions at smaller than 2 mm (BBS<2), from 2 to 5 mm (BBS2-5), and larger than 5 mm (BBS<5). Experiments were conducted using 300 mL beaker with screen which allows water to overflow. Each test condition was carried out in four replicates. The bottom of the beaker was covered by 1 cm sand (~500 µm) working as substrate for juvenile mussels. Testing beakers were placed in a pulsed flow-through auto-feeding system capable of automatic mixing and delivery of pulses of water and food at timed intervals at the USGS Columbia Environmental Research Center (CERC) at the USGS Columbia Environmental Research Center (CERC) [186].

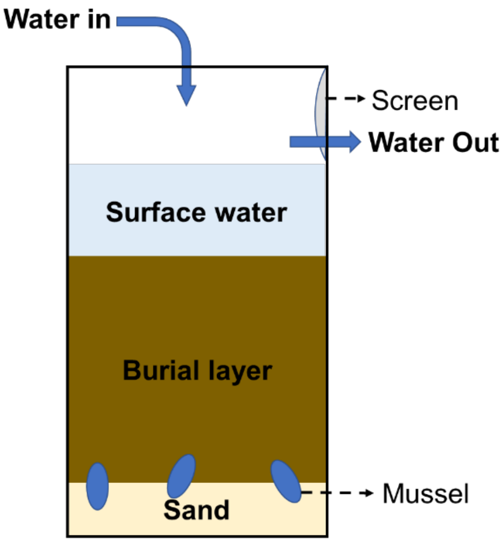

Figure F-1. Image of the flow-through beaker with juvenile mussel buried. Image is not to scale.

Typically, five juvenile mussels were randomly transferred into each beaker and were allowed to bury themselves in the sand overnight (16 - 24 h) before being further buried. The juvenile mussels were then carefully buried at various depths (up to 7 cm) using different material to initiate the burial experiments (**Figure F-1**). Beakers with juvenile mussels but not buried were used as controls (Ctrl). Juvenile mussels were buried for either 7 days or 14 days, and their appearance on the surface of the burial layer was observed and counted at least twice daily during the test period. At the end of the test, the juvenile mussels found on the surface were carefully removed and transferred to beakers with clean water for further observation. The burial layer was then carefully removed layer by layer using a small spoon to dig out and identify the location of the juvenile mussels remain buried. Live mussels were transferred to beakers with clean water, while dead ones were stored in zip bags.

Specifically, juvenile Fatmucket mussels (1 - 2 cm) were buried under 5 cm of each burial material, including SND, LMT, ORC, BBS<2, BBS2-5 and BBS<5. The burial duration was set to 14 days for all materials, with additional replicates of SND, BBS<2, BBS2-5 and BBS<5 set to 7 days to assess whether a shorter burial time might be lethal to the juvenile mussels. Similarly, Arkansas Brokenray juveniles (1.5 - 2 cm) were buried under 5 cm different material for 14 days. Another set of experiments was conducted to determine whether even smaller juveniles could escape from various burial materials, and thus ~5 mm Fatmucket juveniles were tested by burying them at different depths for 7 days.

F-2. Results

For slightly larger juvenile mussels of Fatmucket (1 - 2 cm) and Arkansas Brokenray (1.5 - 2 cm), the number of mussels resurfaced at the end of the test were recorded in **Table F-1 to F-3**.

Table F-1. The number of Fatmucket (1-2 cm) resurfaced on different days (Test duration = 7 days). SNA = Sand, ORC = Osage Riverbank soil, LMT = Crushed Columbia Limestone, BBS<2 = Bourbeuse River sediment fraction < 2 mm, BBS2-5, Bourbeuse River sediment fraction 2-5 mm, and BBS<5, Bourbeuse River sediment fraction < 5 mm.

Name	Replicate	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
SND	R1	4	4	5	5	5	5	5
	R2	3	4	4	4	4	4	4
	R3	3	4	4	4	4	4	4
	R4	4	4	4	4	4	4	4
BBS<2	R1	2	4	4	4	4	4	4
	R2	3	4	4	4	4	4	4
	R3	2	3	3	3	3	3	3
	R4	3	5	5	5	5	5	5
BBS2-5	R1	0	2	2	2	2	2	2
	R2	0	0	0	0	0	0	0
	R3	0	1	1	1	1	1	1
	R4	0	2	2	2	2	2	2
BBS<5	R1	0	1	1	1	1	1	1

	R2	0	1	1	1	1	1	1
	R3	0	0	1	1	1	1	1
	R4	0	1	1	1	1	1	1

Table F-2. The number of Fatmucket (1-2 cm) resurfaced on different days (Test duration = 14 days). SNA = Sand, ORC = Osage Riverbank soil, LMT = Crushed Columbia Limestone, BBS<2 = Bourbeuse River sediment fraction < 2 mm, BBS2-5, Bourbeuse River sediment fraction 2-5 mm, and BBS<5, Bourbeuse River sediment fraction < 5 mm.

Name	Replicate	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 14
SND	R1	5	5	5	5	5	5	5	5
	R2	4	4	4	4	4	4	4	4
	R3	1	1	1	1	1	1	1	1
	R4	4	4	4	4	4	4	4	4
ORC	R1	2	5	5	5	5	5	5	5
	R2	0	1	1	2	2	4	4	4
	R3	1	2	4	4	5	5	5	5
	R4	0	4	4	4	4	4	4	4
LMT	R1	3	3	3	3	3	3	3	3
	R2	3	3	3	3	3	3	3	3
	R3	1	1	1	1	1	1	1	1
	R4	4	4	5	5	5	5	5	5
BBS<2	R1	2	5	5	5	5	5	5	5
	R2	1	2	2	2	3	3	3	3
	R3	4	5	5	5	5	5	5	5
	R4	2	3	3	3	3	3	3	3
BBS2-5	R1	0	0	0	0	0	0	0	0
	R2	0	0	1	1	1	1	1	1
	R3	0	0	0	0	0	0	0	0
	R4	0	1	2	2	2	2	2	2
BBS<5	R1	0	0	0	0	0	0	0	0
	R2	0	1	1	1	1	1	1	1
	R3	0	2	2	2	2	2	2	2
	R4	0	2	2	2	2	2	2	2

As shown in **Table F-1 and F-2**, the number of juvenile Fatmucket mussels (1 - 2 cm) that surfaced after being buried at a depth of 5 cm in different materials varied significantly. Specifically, the majority (>70%) of Fatmucket juveniles quickly resurfaced from SND, ORC and BBS<2. In contrast, only ≤ 25% Fatmucket juveniles were able to unbury themselves from BBS2-5 and BBS<5. These differences may be related to the particle sizes of the burial materials, as BBS2-5 and BBS<5 contained larger particles which may be too heavy and rigid for juveniles to move through. Those remaining buried were mainly discovered at the bottom of the burial layer, indicating that they were unable to move up at all after being buried. In comparison, SND, ORC, and BBS<2 were mainly comprised of fine particles, so it might be easier for juveniles to push aside surrounding particles and climb to the surface. LMT, composed of fine particles but sticky in water, appeared to be slightly more challenging for juvenile Fatmucket mussels to climb through when compared to SND, ORC and BBS<2, with around 60% successfully resurfacing. Notably, regardless of burial material, those mussels that successfully resurfaced generally did so within the first two days. For those that remained buried, their chance of unburying themselves diminished over time. This trend was also evident when the burial time was extended to 14 days, as shown in **Table F-2**, the number of mussels on the surface remained unchanged until the end of the 14-day period.

All mussels that resurfaced were found to be alive. They responded quickly to disturbance and exhibited good mobility. For example, when touched with forceps, they would immediately attempt to re-burrow. Additionally, they were observed in clean water for several days, during which none died, indicating that they were still alert and healthy. Juvenile mussels that remained buried, however, were found dead, with some located in the middle of the burial layers with finer particles (SND, ORC and BBS<2). This suggests that they attempted to move upward but failed, likely due to deteriorating water quality in their surroundings. It is important to note that to avoid disturbing the juvenile mussels during the tests, the water quality in the burial layer was not tested.

For the Arkansas Brokenray juveniles (1.5 - 2 cm), the observed results were similar to those of Fatmucket (1 - 2 cm), though their ability to resurface was slightly less effective (**Table F-3**). Nevertheless, it was still easier (>60%) for Arkansas Brokenray juveniles to unbury themselves from burial materials with finer particles, such as SND, ORC and BBS<2, while unbury from BBS2-5 and BBS<5 was extremely difficult (only 10% for both materials). However, unlike Fatmucket juveniles, only 1 out of 20 Arkansas Brokenray juvenile successfully escaped from LMT, suggesting a reduced ability to resurface from this material.

Table F-3. The number of Arkansas Brokenray (1.5 - 2 cm) resurfaced on different days (Test duration = 14 days). SNA = Sand, ORC = Osage Riverbank soil, LMT = Crushed Columbia Limestone, BBS<2 = Bourbeuse River sediment fraction < 2 mm, BBS2-5, Bourbeuse River sediment fraction 2-5 mm, and BBS<5, Bourbeuse River sediment fraction < 5 mm.

Name	Replicate	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 14
SND	R1	4	4	5	5	5	5	5	5
	R2	3	4	4	4	4	4	4	4
	R3	5	5	5	5	5	5	5	5
	R4	2	3	3	3	3	3	3	3
ORC	R1	1	3	3	3	4	4	4	4
	R2	1	2	3	2	3	3	3	3
	R3	0	2	2	2	2	3	3	3
	R4	1	1	1	2	3	3	3	3
LMT	R1	0	0	0	0	0	0	0	0
	R2	0	0	0	0	0	0	0	0
	R3	0	1	1	1	1	1	1	1
	R4	0	0	0	0	0	0	0	0
BBS<2	R1	2	3	3	3	3	3	3	3

	R2	3	4	4	4	3	4	4	4
	R3	2	4	4	4	5	5	5	5
	R4	2	3	3	3	3	3	3	3
BBS2-5	R1	0	0	1	1	1	1	1	1
	R2	0	0	0	0	0	0	0	0
	R3	0	0	0	0	0	0	0	0
	R4	1	1	1	1	1	1	1	1
BBS<5	R1	1	1	2	2	2	2	2	2
	R2	0	0	0	0	0	0	0	0
	R3	0	0	0	0	0	0	0	0
	R4	0	0	0	0	0	0	0	0

As with the Fatmucket, most Arkansas Brokenray juveniles that resurfaced did so within the first two days, with very few able to resurface after that period. The number of mussels on the surface on Day 14 remained the same as on Day 7, indicating that extended burial time did not benefit the juveniles and might increase the likelihood of death, as it may become increasingly more difficult for them to climb to the surface over time. Similarly, all juveniles that resurfaced were found to be alive and active. In contrast, those that remained buried were all found dead. Most of them were located at the bottom of the burial layer, though some were found in the middle, suggesting that they attempted but failed to unbury themselves.

Based on the above two tests using Fatmucket (1 - 2 cm) and Arkansas Brokenray (1.5 - 2 cm), a 7-day burial duration may be sufficient to result in death of juvenile mussels. Additionally, for burial layers composed mainly fine particles (such as SND, ORC and BBS<2), 5 cm may be still manageable for juvenile mussels to escape. In contrast, a 5 cm of course particles may be too difficult for them to climb through. Therefore, in the following test using smaller Fatmucket (~ 5 mm), the burial duration was set to 7 days, and different burial depths up to 7 cm were applied to various materials (see details in **Table F-4 to F-9**). The results were summarized in **Table F-4 to F-9**.

As shown in **Table F-4 and F-5**, all Fatmucket juveniles (~5 mm) were able to quickly unbury themselves from SND and ORC at depths up to 7 cm. A high percentage (85 - 100%) of juvenile mussels resurfaced from various depths in BBS<2, suggesting that it is still relatively easy for them to quickly climb to the surface when buried in BBS<2 up to 7 cm deep. For the other three materials (LMT, BBS2-5 and BBS<5), a clear trend was observed: as the burial depth increased, significantly fewer juveniles resurfaced. Notably, no juvenile mussel escaped from 3 cm depth in BBS<5, and only 1 out of 20 reached to the surface from 5 cm depth in BBS2-5. Juvenile mussels that failed to resurface from BBS2-5 were found to be at the bottom of the burial layer, with some still alive. Similarly, living but buried juvenile mussels were also observed in LMT. These results imply that those small juvenile mussels could withstand the burial condition for a long time, although they might lose their ability to resurface. Compared to larger mussels tested earlier (Fatmucket 1.5 - 2 cm), the smaller juveniles could survive longer. Nevertheless, it may be unavoidable that they will eventually die with further extension of the burial duration.

Table F-4. The number of Fatmucket (~5 mm) resurfaced from SND on different days (Test duration = 7 days). SND = Sand.

Depth	Replicate	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
2 cm	R1	4	5	5	5	5	5	5
	R2	5	5	5	5	5	5	5
	R3	5	5	5	5	5	5	5
	R4	5	5	5	5	5	5	5
3 cm	R1	5	5	5	5	5	5	5
	R2	5	5	5	5	5	5	5
	R3	5	5	5	5	5	5	5
	R4	5	5	5	5	5	5	5
4 cm	R1	5	5	5	5	5	5	5
	R2	5	5	5	5	5	5	5
	R3	5	5	5	5	5	5	5
	R4	5	5	5	5	5	5	5
5 cm	R1	4	5	5	5	5	5	5
	R2	5	5	5	5	5	5	5
	R3	5	5	5	5	5	5	5
	R4	1	5	5	5	5	5	5
7 cm	R1	5	5	5	5	5	5	5
	R2	5	5	5	5	5	5	5
	R3	5	5	5	5	5	5	5
	R4	3	5	5	5	5	5	5

Table F-5. The number of Fatmucket (~5 mm) resurfaced from ORC on different days (Test duration = 7 days). ORC = Osage Riverbank soil.

Depth	Replicate	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
2 cm	R1	4	5	5	5	5	5	5
	R2	5	5	5	5	5	5	5
	R3	4	5	5	5	5	5	5
	R4	5	5	5	5	5	5	5
3 cm	R1	5	5	5	5	5	5	5
	R2	4	5	5	5	5	5	5
	R3	4	5	5	5	5	5	5
	R4	5	5	5	5	5	5	5
4 cm	R1	3	5	3	5	5	5	5
	R2	5	5	5	5	5	5	5
	R3	5	5	5	5	5	5	5
	R4	3	5	3	5	5	5	5
5 cm	R1	5	5	5	5	5	5	5
	R2	5	5	5	5	5	5	5
	R3	3	5	5	5	5	5	5
	R4	5	5	5	5	5	5	5
6 cm	R1	2	4	5	5	5	5	5
	R2	3	5	5	5	5	5	5
	R3	1	4	5	5	5	5	5
	R4	4	5	5	5	5	5	5
7 cm	R1	2	5	5	5	5	5	5
	R2	2	5	5	5	5	5	5
	R3	3	5	5	5	5	5	5
	R4	3	4	5	5	5	5	5

Table F-6. The number of Fatmucket (~5 mm) resurfaced from LMT on different days (Test duration = 7 days). LMT = Crushed Columbia limestone.

Depth	Duration	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1 cm	R1	4	5	5	5	5	5	5
	R2	4	5	5	5	5	5	5
	R3	5	5	5	5	5	5	5
	R4	2	4	3	3	4	4	4
2 cm	R1	3	3	3	4	4	4	4
	R2	4	5	5	5	5	5	5
	R3	4	4	4	4	4	5	5
	R4	5	5	5	5	5	5	5
3 cm	R1	3	4	3	4	4	4	4
	R2	2	3	2	3	3	3	3
	R3	0	3	3	3	4	4	4
	R4	2	3	2	3	3	3	3
4 cm	R1	2	2	2	2	2	2	2
	R2	4	4	4	4	4	4	4
	R3	0	2	2	2	3	3	3
	R4	1	1	1	1	1	1	1
5 cm	R1	0	0	0	0	0	0	0
	R2	1	2	2	2	2	2	2
	R3	1	1	1	2	2	2	2
	R4	2	2	2	3	3	3	3
6 cm	R1	0	0	0	2	2	2	2
	R2	0	0	0	1	1	1	1
	R3	0	0	0	1	1	1	1
	R4	1	1	1	1	1	1	1

Table F-7. The number of Fatmucket (~5 mm) resurfaced from BBS<2 on different days (Test duration = 7 days). BBS<2 = Bourbeuse River sediment fraction < 2 mm.

Depth	Replicate	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
2 cm	R1	4	4	4	4	4	5	5
	R2	4	5	5	5	5	5	5
	R3	3	3	3	4	4	4	4
	R4	4	5	5	5	5	5	5
3 cm	R1	4	5	5	5	5	5	5
	R2	4	4	4	4	5	5	5
	R3	1	1	1	1	1	1	1
	R4	5	4	5	5	5	5	5
4 cm	R1	4	5	5	5	4	5	5
	R2	5	5	5	5	4	5	5
	R3	5	5	5	5	4	4	5
	R4	3	5	5	5	3	4	5
5 cm	R1	2	2	2	2	2	2	2
	R2	4	4	4	4	4	5	5
	R3	2	3	4	4	4	4	5
	R4	5	5	5	5	5	5	5
6 cm	R1	4	5	5	5	5	5	5
	R2	3	5	5	5	5	5	5
	R3	4	5	5	5	5	5	5
	R4	4	5	5	5	5	5	5
7 cm	R1	4	5	5	5	5	5	5
	R2	4	4	5	5	5	5	5
	R3	5	5	5	5	5	5	5
	R4	5	5	5	5	5	5	5

Table F-8. The number of Fatmucket (~5 mm) resurfaced from BBS2-5 on different days (Test duration = 7 days). BBS2-5= Bourbeuse River sediment fraction 2-5 mm.

Depth	Replicate	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1 cm	R1	2	2	4	4	4	3	4
	R2	2	2	3	3	3	3	4
	R3	3	4	4	4	4	4	4
	R4	3	4	3	3	4	4	5
2 cm	R1	2	2	2	2	2	2	2
	R2	4	5	5	5	5	5	5
	R3	1	1	1	1	2	2	2
	R4	2	2	3	3	2	2	2
3 cm	R1	0	0	0	0	0	0	0
	R2	0	1	2	2	2	2	2
	R3	0	0	0	0	0	0	0
	R4	0	0	0	0	0	0	0
4 cm	R1	0	1	1	1	1	1	1
	R2	0	0	0	0	0	0	0
	R3	0	2	2	2	2	2	2
	R4	1	1	1	1	1	1	1
5 cm	R1	0	0	0	0	0	0	0
	R2	0	0	0	0	0	0	0
	R3	0	0	0	0	0	0	0
	R4	0	1	1	1	1	1	1

Table F-9. The number of Fatmucket (~5 mm) resurfaced from BBS<5 on different days (Test duration = 7 days). BBS<5 = Bourbeuse River sediment fraction < 5 mm.

Depth	Replicate	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1 cm	R1	1	2	3	3	3	4	4
	R2	2	4	4	4	4	4	4
	R3	2	5	5	5	5	5	5
	R4	1	5	5	5	5	5	5
2cm	R1	0	0	0	0	0	0	0
	R2	0	1	1	1	1	1	1
	R3	1	3	3	3	3	3	3
	R4	0	2	2	2	2	2	2
3 cm	R1	0	0	0	0	0	0	0
	R2	0	0	0	0	0	0	0
	R3	0	0	0	0	0	0	0
	R4	0	0	0	0	0	0	0

F-3. Research Limitations and Inspirations

In this set of tests, the ability of small Fatmucket and Arkansas Brokenray juvenile mussels to resurface from various burial materials at different depths and durations was explored. The results suggest that these juvenile mussels tended to unbury themselves more quickly from burial layers made of finer particles such as SND, ORC and BBS<2 when compared to burial layers of larger particles at the same burial depths. However, these tests may not fully represent the real field situation during a bury event. Specifically, the studies were conducted using flow-through beakers, during which the surface water might be refreshed hourly, but likely less water within the burial layer was refreshed. As a result, the water quality inside the burial layer may have deteriorated quickly over time. In contrast, in the natural environment, water exchange may occur between surface water and underground water, which could effectively change the water quality in the sediment/burial layer. The experimental system could not replicate such water exchange.

Nonetheless, the results obtained from tests using juvenile mussels are still valuable, as they demonstrate that at different conditions, juvenile mussels may exhibit varying abilities to withstand burial events. Specifically, an increased depth of deposition with larger particles may lead to fewer juvenile mussels resurfacing, thus leading to higher mortality as the burial depth increases. Therefore, it is important to avoid burying juvenile mussels with thick burial layers, in particular, when the particle size is relatively large, and the duration is long.