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MEASURING MUSSEL BEHAVIOR AND ANALYZING HIGH FREQUENCY NITRATE DATA TO EXPLORE NEW PHENOMENA IN DYNAMIC NUTRIENT CYCLING

by

Jeremy Bril

A thesis submitted in partial fulfillment
of the requirements for the Master of
Science degree in Civil and Environmental Engineering
in the Graduate College of
The University of Iowa

May 2010

Thesis Supervisor: Professor Gene F. Parkin

Graduate College
The University of Iowa
Iowa City, Iowa

CERTIFICATE OF APPROVAL

____

MASTER'S THESIS

____

This is to certify that the Master's thesis of

Jeremy Bril

has been approved by the Examining Committee for the
thesis requirement for the Master of Science degree in
Civil and Environmental Engineering at the May 2010 graduation.

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Jerald L. Schnoor

To my amazing family

The ultimate test of a man's conscience may be his willingness to sacrifice something today for future generations whose words of thanks will not be heard.

- Gaylord Nelson

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First of all I would like to thank my incredible wife who has given me the love and support I have certainly needed throughout my work on this project. Secondly, I would like to thank my advisor Craig Just. Without your guidance and encouragement this project never would have been so enjoyable and successful. I have truly enjoyed working with you over the past two years and I am grateful for the friendship we have developed.

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TABLE OF CONTENTS

LIST OF TABLES......vii

LIST OF FIGURES.... viii

CHAPTER 1 INTRODUCTION AND OBJECTIVES 1

CHAPTER 2 LITERATURE REVIEW 7

2.1 Introduction....7
2.2 The Role of Bacteria....7
2.2.1 Denitrification 7
2.2.2 Denitrification in the Mississippi River 8
2.2.3 The Impact of Flooding on Denitrification 9
2.2.4 Anammox Bacteria 10
2.2.5 Microbial Community Fingerprint. 11
2.3 The Role of Freshwater Mussels 12
2.3.1 Background 12
2.3.2 Mussels in the Mississippi River....13
2.3.3 Ecosystem Impacts....15
2.3.4 Impacts of Mussels on Nitrogen Dynamics 16
2.4 Mussels as Biological Early Warning Systems 19
2.4.1 Monitoring Mussel Behavior 19
2.4.2 Remote-Sensing Mussels 21

CHAPTER 3 MATERIALS AND METHODS 29

3.1 Introduction....29
3.2 Bathymetric Survey 29
3.2.1 Data Collection 29
3.2.2 Data Filtering 30
3.2.3 ArcGIS 30
3.3 Grain Size Distribution Analysis 30
3.3.1 Sample Preparation 30
3.3.2 Wet Sieving....31
3.3.3 Dry Sieving 32
3.4 Serum Bottle Nitrate Reduction....32
3.5 Nitrate Reduction Beaker Experiments 33
3.6 Ion Chromatograph Nitrate Testing....34
3.7 Anammox Analysis 34
3.8 Terminal Restriction Fragment Length Polymorphism (T-RFLP)....35
3.8.1 DNA Extraction 35
3.8.2 PCR and Purification 35
3.8.3 Restriction Enzyme Digestion....36
3.8.4 Analysis....36
3.9 Total Organic Carbon Analysis 37
3.9.1 Calibration Curves 37

3.9.2 TOC Measurement 37
3.10 Sensor Cluster Buoys..38

CHAPTER 4 CHARACTERIZATION OF MUSSEL BED 40

4.1 Introduction..40
4.2 Study Site Background 40
4.3 Physical Characterization 41
4.3.1 Riverbed Bathymetric Survey 41
4.3.2 Mussel Density and Diversity 42
4.3.3 Grain Size Distribution 43
4.4 Biological Characterization 45
4.4.1 Presence of Denitrifying Bacteria 45
4.4.2 Presence of Anammox Bacteria 46
4.4.3 Microbial Community Fingerprint............................47
4.5 Chemical Characterization...48
4.5.1 Total Organic Carbon..48
4.5.2 Water Quality 50

CHAPTER 5 MUSSEL LABORATORY MICROCOSM...68

5.1 Introduction..68
5.2 Configuration and Setup 68
5.3 Survivability Studies..70
5.4 Gape Sensor Data 70

CHAPTER 6 DIURNAL NITROGEN DYNAMICS...79

6.1 Introduction..79
6.2 Boundary Conditions 79
6.3 Nitrate-N Mass Calculations...80
6.4 Mussel Ammonia-N Contribution 81
6.5 Impact of Flow Rate on Nitrate-N Mass Calculations...........82
6.6 Diurnal Processes Contributing to Nitrogen Dynamics.........84
6.7 Diurnal Process Modeling 86

CHAPTER 7 CONCLUSIONS AND FUTURE WORK...96

7.1 Conclusions..96
7.1.1 Mussel Bed and Main Channel Hydrodynamics........96
7.1.2 Mussel Characteristics 97
7.1.3 Sunlight, Temperature, and Phytoplankton...............99
7.1.4 Summary 99
7.2 Future Work...100

REFERENCES.......... 102

LIST OF TABLES

Table 2.1: Detection limits (mg/L) for two zebra mussel biological early warning systems (Musselmonitor® and Dreissena-Monitor) for different toxins . . 27

Table 5.1: Survival rates of mussels in laboratory batch microcosm habitat. 75

Table 6.1: Estimated area of each peak/valley used to calculate total nitrate-N mass for Day 1 (8/5/2009) and Day 2 (8/6/2009). 91

Table 6.2: Using observed mussel densities from mussel survey to calculate overall average mussel density in control volume. 92

Table 6.3: Estimated mussel excreted mass flux of ammonia-N based on different literature excretion rates. 92

Table 6.4: Comparison of estimated mussel nitrogen contribution based on measured nitrate data and calculated mussel nitrogen contribution based on rate of ammonia excretion. 92

Table 6.5: Estimated area of each peak/valley used to calculate total nitrate-N mass based on flow rates for Day 1 (8/5/2009) and Day 2 (8/6/2009). 94

Table 6.6: Contribution of mussel ammonia-N excretion to mass of nitrogen under Day 1 and Day 2 peaks for both the control volume and flow rate analysis. 95

LIST OF FIGURES

Figure 1.1: Total nitrogen yield delivered to the Gulf of Mexico from the incremental drainage reaches within the basin of the Mississippi and Atchafalaya Rivers ..5

Figure 1.2: Bottom dissolved oxygen levels (mg/L) in Gulf of Mexico "dead zone" measured July 21-27, 2008. ..5

Figure 1.3: Hydrograph displaying Mississippi River stage height (in feet) above Lock and Dam 16 in Muscatine, Iowa during 2008 ..6

Figure 2.1: Pathways of nitrogen processing in riverine sediments. 24

Figure 2.2: The role of anammox in the oceanic nitrogen cycle. 24

Figure 2.3: Typical anatomy (side view) of a freshwater mussel. 25

Figure 2.4: Life cycle diagram of freshwater mussel 25

Figure 2.5: Fish "lure" containing glochidia created by female mussel to attract host fish 26

Figure 2.6: Distribution of zebra mussel sightings in 1988 and 2004. 26

Figure 2.7: Schematic diagram showing the attachment of the Hall sensor system used for determining mussel gape angle. 28

Figure 4.1: Location of mussel bed study site within Navigation Pool 16 of the Upper Mississippi River Basin (UMRB). 52

Figure 4.2: Multibeam bathymetric survey for Pool 16 mussel bed completed August 2008. 52

Figure 4.3: Estimated circulation of flow occurring within mussel bed causing decreased velocities in upstream section of bed. 53

Figure 4.4: Density of mussels (mussels per square meter) located in Pool 16 mussel bed in 2003 53

Figure 4.5: Diversity of mussels (species per square meter) located in Pool 16 mussel bed in 2003 54

Figure 4.6: Diversity of mussels found during mussel bed site exploration trip conducted in 2008. 54

Figure 4.7: Grain size distribution for sediments located within low mussel density sections of mussel bed. 55

Figure 4.8: Grain size distribution for sediments located within medium mussel density areas of the mussel bed. 56

Figure 4.9: Grain size distribution for sediments located within high mussel density areas of the mussel bed. 57

Figure 4.10: Comparison of grain size distribution for post-flood (2009) and pre-flood (2004) sediments located in low mussel density areas of the bed. ... 58

Figure 4.11: Comparison of grain size distribution for post-flood (2009) and pre-flood (2004) sediments located in medium mussel density areas of the bed. 59

Figure 4.12: Comparison of grain size distribution for post-flood (2009) and pre-flood (2004) sediments located in high mussel density areas of the bed. .. 60

Figure 4.13: Serum bottle nitrate reduction in mildly organic (medium mussel density) and richly organic (low mussel density) sediments compared to a sand control........ 61

Figure 4.14: Reduction of nitrate over time in low mussel density sediments (standard deviations based on samples analyzed in triplicate)........ 61

Figure 4.15: Microbial community fingerprint for high mussel density sediment (all families consisting of less than 1 percent of sample population not labeled). 62

Figure 4.16: Microbial community fingerprint for low mussel density sediment (all families consisting of less than 1 percent of sample population not labeled). 63

Figure 4.17: Locations of samples obtained for total organic carbon (TOC) analysis relative to mussel density and bathymetric survey data. 64

Figure 4.18: Variation of total organic carbon (TOC) content throughout mussel bed relative to mussel density and bathymetric survey data. 64

Figure 4.19: Average percent of total organic carbon (TOC) for each sediment sample obtained from mussel bed (standard deviations based on samples analyzed in triplicate). 65

Figure 4.20: Example of sensor cluster buoy used to measure nitrate concentration located in mussel bed. 65

Figure 4.21: Nitrate concentrations over time for three month period during 2009... 66

Figure 4.22: Evidence of diurnal pattern differences in nitrate concentrations between water column above mussel bed and in main channel near LACMRERS over a three day period in August 2009. 67

Figure 5.1: Example of changes in mussel microcosm documented when water was drained and replenished........ 73

Figure 5.2: Initial aerated batch system mussel laboratory microcosm. 73

Figure 5.3: Modified microcosm habitat to incorporate continuous flow of water through system. 74

Figure 5.4: Mussel microcosm flow-through system equipped with influent and effluent pipe, flow control valve, and aeration hose. 75

Figure 5.5: Stress expressed by mussels in microcosm evidenced by discharge of conglutinates (packets of larvae). 76

Figure 5.6: Schematic of mussel equipped with gape sensors (left) and actual mussel placed in microcosm habitat equipped with sensors (right). 76

Figure 5.7: Mussel tank covered in opaque paper to represent altered light cycle. 77

Figure 5.8: Time-series of raw data with the maximum output from the Hall sensor being 2.5 volts and the large amplitude spikes being noise from an unknown external source. 77

Figure 5.9: Raw data after application of a median filter to remove impulse noise (blue), followed by a 5 minute zero-phase moving average-type digital filter (red) (low voltages indicate mussel valve is open). 78

Figure 5.10: Phase comparison of mussel gape in natural light diurnal cycle (red) with mussel gape in shifted (approximately 10 hours) diurnal light cycle (green). 78

Figure 6.1: Locations of two sensor clusters (LACMRERS and Mussel Bed) used in determining daily fluxes of nitrate. 87

Figure 6.2: Evidence of diurnal nitrate concentrations existing throughout August 2009. 87

Figure 6.3: Two days worth of data analyzed to quantify contribution of mussels to daily nitrate dynamics. 88

Figure 6.4: Mussel bed defined control volume used to calculate mass of nitrate present. 88

Figure 6.5: Nitrate-N mass for the analyzed days with baselines established for observed peaks and valleys. 89

Figure 6.6: Example of trapezoidal rule used to compute total nitrate-N mass under peak. 90

Figure 6.7: Potential contribution of total nitrate-N mass to water column by mussels for the two days analyzed. 91

Figure 6.8: Sensor locations used to determine flow rates in mussel bed (1 = Mississippi River at Clinton, Iowa, 2 = Rock River at Joslin, Illinois, and 3 = Green River at Geneseo, Illinois) (Background image courtesy of MapsofWorld.com). 93

Figure 6.9: Estimated cross-section of Mississippi River in main channel near LACMRERS used to fractionate flow rate..93

Figure 6.10: Nitrate-N mass fluxes for the analyzed days with baselines established for observed peaks and valleys (LACMRERS is on the primary y-axis and the mussel bed is on the secondary y-axis). 94

Figure 6.11: Schematic of mass-balance model being developed to assist in determining the processes affecting diurnal nitrogen dynamics (layers not drawn to scale). 95

CHAPTER 1

INTRODUCTION AND OBJECTIVES

Labeled by the National Academy of Engineering (NAE) as one of fourteen Grand Challenges for Engineering, the management of the nitrogen cycle has become an increasingly difficult obstacle for sustainable development. Before nitrogen fertilizer use increased dramatically in the decades following 1950 (Mitsch et al., 2001), nitrogen fixation by microorganisms (with a small amount attributed to lightning strikes) was the only path through which nitrogen made its way from the environment into living organisms. However, human production of additional nitrogen nutrients has disrupted the natural nitrogen cycle as fertilizer use accounts for more than half of the annual amount of nitrogen fixation attributed to human activity (NAE, 2008). Although it may not offer as flashy a label as "global warming," human-induced changes in the global nitrogen cycle pose engineering challenges just as critical as coping with the environmental consequences of burning fossil fuels for energy (NAE, 2008).

One of the most widespread manifestations of anthropogenic mismanagement of nitrogen is eutrophication on the continental shelf of the northern Gulf of Mexico causing a hypoxic "dead zone." Excess nitrogen delivery to the Gulf from the extensively row-cropped Midwest via the Mississippi River (Figure 1.1) has decreased dissolved oxygen concentrations to levels below which aquatic life can survive, creating a "dead zone" ranging in size from 13,000 to 20,000 square kilometers (km^2) annually (Mitsch, et al., 2001). In 2008, the "dead zone" was determined to be over 20,720 km^2, the second largest area ever reported since mapping began in 1985 (LUMCON, 2008) (Figure 1.2). Additionally, climate-induced changes, such as the devastating floods that occurred in Eastern Iowa and nearly the entire Upper Mississippi River Basin during May-July 2008 (Figure 1.3), are further increasing the challenges faced by scientists.

In an effort to help overcome these challenges, the goal of our study is to expand on the limited scientific understanding of how the nitrogen cycle within aquatic environments may be affected by increasing human- and climate-induced changes. To this end, we are using freshwater mussels as a sentinel species to better understand the impacts of ecosystem perturbation on nitrogen processing in large river systems.

Freshwater mussels have been referred to as "ecosystem engineers" because they exert control over food resources and alter habitats for other organisms (Spooner & Vaughn, 2006). Also, mussels play a major role in nitrogen cycling in large river systems as studies suggest mussel-associated nitrogen cycling largely results from direct nutrient uptake by phytoplankton and zooplankton followed by subsequent uptake of these organisms by mussels (Gardner et al., 1995). Under 'normal' environmental conditions, mussels appear to process nitrogen more rapidly than denitrifying bacteria (Gardner, et al., 1995). However, due to the Midwest flooding of 2008, scientists have predicted an almost unprecedented delivery of sediment and nutrients to the Mississippi River and Gulf of Mexico (USGS, 2008b). The substantial deposition of carbon-rich sediment resulting from the extreme flooding may potentially increase bacterial denitrification and subsequently alter overall nitrogen processing rates.

Our study will also examine the suitability of using real-time biological responses from freshwater mussels to further parameterize a nutrient model to more fully describe the impacts of extreme events on nutrient dynamics. The specific behavioral response measured will be the rhythmic opening and closing of the mussels bivalve shells (i.e. gape responses). Studying the mussels' gape response to different environmental conditions will allow us to better understand how the rate at which mussels process nitrogen will be affected by dynamic conditions.

Nutrient processing rates and concentrations combined with physical characteristics (e.g. light availability, temperature) are influential factors controlling the lower food web dynamics in large aquatic ecosystems such as the Mississippi River. To

help develop mass-balance box models for these complex systems, nutrient concentration data are commonly used to determine both the status and trends of nutrients. However, nutrient cycling rates are often not measured and can therefore only be estimated in ecosystem models (Gardner, et al., 1995). Our study will examine the initial development of a mass-balance model that will be used to further refine these rates by monitoring mussels' behavioral responses to variable environmental conditions. Current dead zone mitigation plans call for coupling management actions with enhanced monitoring, modeling, and research on nitrogen delivery to, and processing within, the Mississippi River (Turner, Rabalais, & Justic, 2008). Thus, development of a dynamic mass-balance model, sensitive to diurnal mussel behavior, can help to determine how nitrogen processing rates within the lower food web are impacted by freshwater mussels.

Additionally, studying mussel behaviors and incorporating these behaviors into an ecosystem model will help to develop a better understanding for what makes mussel habitats sustainable. Recent accounts of mussels in freshwater rivers across the Midwest indicate that their numbers are plummeting (Vaughn & Hakenkamp, 2001). Approximately 300 types of mussels are known to exist in North America with over 40 percent of the species in danger of extinction (USFWS, 2006c).

The reduction of mussel habitat viability appears to be related to ecosystem scale which leaves critically endangered species, such as the Higgins eye (*Lampsilis higginsii*), little opportunity to thrive in large systems such as the Mississippi River. A paucity of information to institute the successful design of mussel retention and re-establishment programs leave fisheries biologists and environmental engineers at a loss to provide any sort of calculated ecosystem management or engineering. Coupled with this current shortfall of scientific understanding of mussel habitat threats in the Midwest is the increased uncertainty of the future viability resulting from ever more frequent and more extreme precipitation patterns that come with human-induced climate change (Hegerl, Zwiers, Stott, & Kharin, 2004). Ironically, mussels may influence emissions of the

powerful greenhouse gas, nitrous oxide, by removing large quantities of nitrogen-rich phytoplankton from the water column through filter-feeding (W. F. James et al., 2000). The positive role played by mussels in a healthy ecosystem should not be underestimated and the increasingly acute nature of threats to mussel habitat provides grounds for further study.

Thus, the specific objectives of our study were as follows:

- Determine the physical, biological, and chemical characteristics of a mussel bed located within the Mississippi River
- Evaluate the impact of the floods of 2008 on the mussel bed and how the floods may have altered the ecosystem’s nitrogen processing capabilities
- Establish a well-equipped mussel laboratory habitat to permit examination of mussel responses to different environmental conditions
- Investigate the mussel behavioral response to light intensity using gape sensors
- Analyze highly time resolved nitrate data collected from the mussel bed to examine potential mussel influence on diurnal nitrogen dynamics
- Begin initial development of dynamic mass-balance nutrient model that incorporates mussel behavioral responses

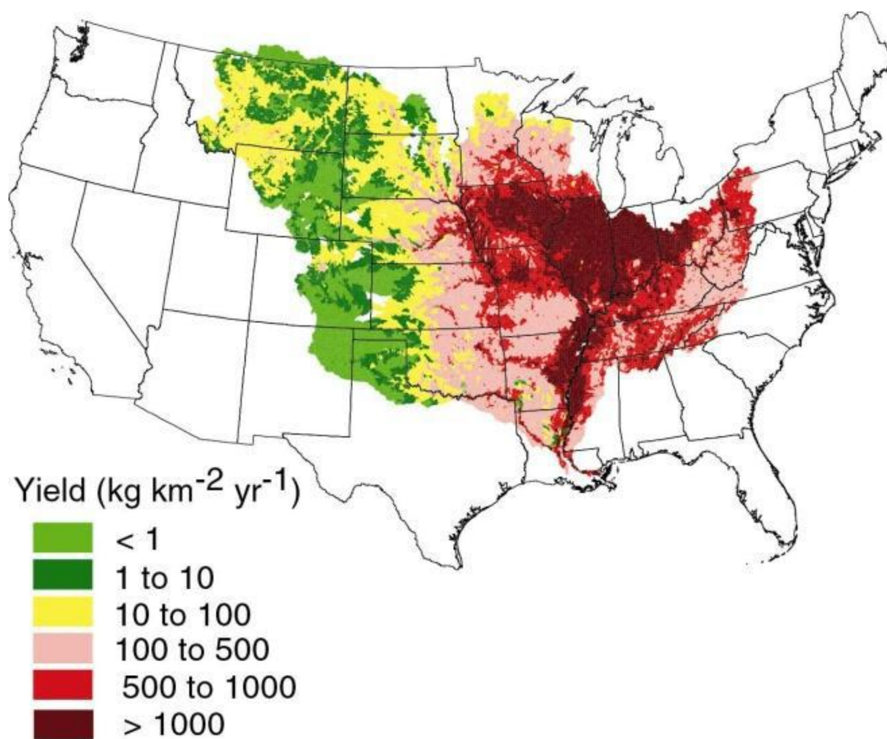

Figure 1.1: Total nitrogen yield delivered to the Gulf of Mexico from the incremental drainage reaches within the basin of the Mississippi and Atchafalaya Rivers (Alexander et al., 2008).

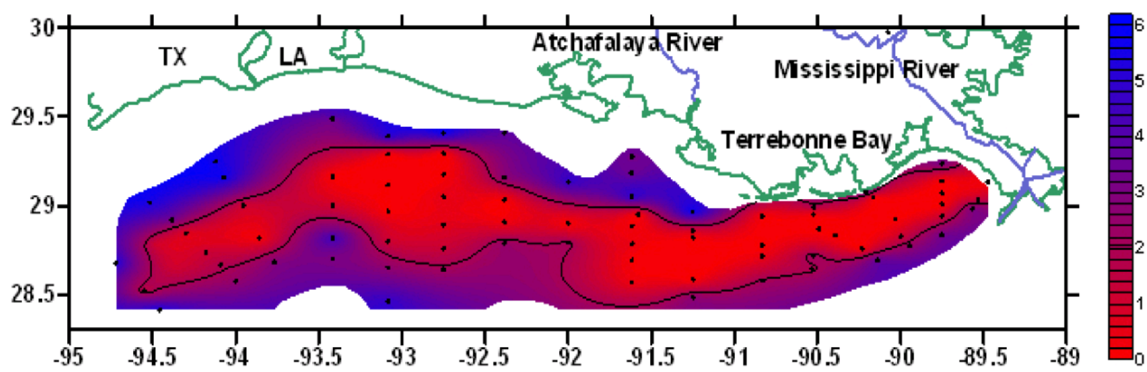

Figure 1.2: Bottom dissolved oxygen levels (mg/L) in Gulf of Mexico “dead zone” measured July 21-27, 2008 (NOAA, 2008).

Figure 1.3: Hydrograph displaying Mississippi River stage height (in feet) above Lock and Dam 16 in Muscatine, Iowa during 2008 (USACE, 2008).

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The purpose of this literature review is to give an overview of the important components analyzed in our study to determine how ecosystem perturbation impacts nitrogen processing within large river systems. This chapter will first discuss the role of bacteria in the nitrogen cycle in the Upper Mississippi River Basin. The chapter will then describe the impacts freshwater mussels have on aquatic ecosystems and their role in nitrogen processing. Finally, the chapter will examine recent research utilizing freshwater mussels as dynamic ecosystem indicators.

2.2 The Role of Bacteria

Substantial amounts of excess nitrogen, primarily as nitrate (NO_3^-), are a major pollutant of rivers, lakes, and estuaries and can often adversely affect aquatic life (Parry, 1998). While we have limited knowledge about how exactly nitrogen in rivers such as the Mississippi is processed, stored, or biologically removed (USGS, 2008a), certain bacteria play a potentially prominent role in reducing nitrogen loads due to their ability to remove nitrate from riverine systems through the denitrification process.

2.2.1 Denitrification

Denitrification, which is an anaerobic microbially mediated process (Figure 2.1), is limited by carbon availability, nitrate delivery rate, the presence of oxygen, and sediment moisture (W. R. Richardson et al., 2008). In respiratory denitrification, nitrate acts as the terminal electron acceptor for the oxidation of organic matter under anaerobic conditions. When denitrification occurs in riverine sediments, nearly all the nitrate is converted into nitrogen gas (N_2) with a small amount escaping as nitrous oxide (N_2O).

Because nitrogen gas is not readily available for use by living organisms, denitrification is considered to be a permanent removal pathway of nitrogen from the ecosystem. While many estimates for denitrification rates in soils, wetlands, and surface waters have been derived, values vary greatly within and among environments as well as between different measurement techniques (Burgin & Hamilton, 2007).

2.2.2 Denitrification in the Mississippi River

The Mississippi River watershed drains nearly 40 percent of the continental United States (Turner & Rabalais, 1991) and delivers an average of $1x10^6$ tonnes of nitrate per year to the Gulf of Mexico (Goolsby & Battaglin, 2001). A modeling study has shown that greater than 90 percent of the nitrate that enters the Mississippi River will be transported to the Gulf of Mexico (Alexander, Smith, & Schwarz, 2000), implying that the River is essentially a nonreactive transport conduit with little nitrogen processing occurring in transit (W. B. Richardson et al., 2004). This is not surprising as nitrogen loss in streams has been shown to decline rapidly with increasing channel size (Alexander, et al., 2000). Research analyzing the water chemistry and stable isotopes of nitrogen in water from the middle and lower Mississippi River also indicates that very little nitrogen is lost in transit and that transformations of nitrogen are due primarily to assimilation and not denitrification (Battaglin, Kendall, Chang, Silva, & Campbell, 2001). However, the backwater lakes and riparian wetlands within the *Upper* Mississippi River (UMR) contain highly organic, anoxic sediments and abundant rooted macropyhtes, creating optimal conditions for removal of nitrate through microbial denitrification (W. B. Richardson, et al., 2004).

Measured denitrification rates within the UMR were found to be similar to or higher than rates found in other sediment systems (W. B. Richardson, et al., 2004). Rates of denitrification were determined to be highest in the winter and lowest in the fall (Strauss et al., 2006), and average denitrification rates in the main channel (0.14 μg

$N \cdot cm^{-2} \cdot h^{-1}$) were significantly less than the rates found in the backwaters (1.97 µg $N \cdot cm^{-2} \cdot h^{-1}$) (W. B. Richardson, et al., 2004). Studies also showed that anywhere from 0 to 55 percent of the original nitrate source had been denitrified within the drainage basin of the River (Panno, Hackley, Kelly, & Hwang, 2006).

While the backwaters of the UMR contain favorable conditions for denitrification, they are often limited by nitrate delivery (W. B. Richardson, et al., 2004). Due to the backwaters' low hydrological connectivity with the nitrate-rich main channel (Soballe, Fischer, Hodge-Richardson, & Clemment, 2002), denitrification rates are most likely much lower than they could be in these aquatic habitats (Strauss, et al., 2006).

2.2.3 The Impact of Flooding on Denitrification

Floods occurring within the UMR may be able to further increase denitrification rates if they are able to reconnect backwater areas of the UMR with the nitrate-laden water of the main channel (W. R. Richardson, et al., 2008). However, a study by Richardson et al. (2004) examining seasonal UMR denitrification rates following a record flood in 2001 indicates that floods appeared to have negative affects both in the short and long term. With the record flooding, spring denitrification rates were extremely low even though nitrate concentrations were nearly double those measured in the preceding winter. Additionally, the flood appeared to have a lasting effect on denitrification as rates in months following the flood were significantly lower than those recorded in the previous year (W. B. Richardson, et al., 2004). As water temperatures and dissolved oxygen concentrations were not significantly different between these two time periods, Richardson et al. (2004) concluded that physical disturbances and erosion of the sediment surface resulted in reduced populations of denitrifying bacteria and thus decreased denitrification rates.

2.2.4 Anammox Bacteria

Nitrate removal in soils and riverine ecosystems is typically assumed to be contributed largely to denitrification (Burgin & Hamilton, 2007). However, estimates of denitrification based on direct assays (e.g. acetylene blockage techniques) often account for less than half of the total nitrate removed (Seitzinger, 1988). While adequate methods to extrapolate from site-specific rates to whole ecosystems may not yet have been developed (Cornwell, Kemp, & Kana, 1999), much of the nitrate removed in sediment and riverine systems may be attributed to process other than denitrification or assimilation (Burgin & Hamilton, 2007). One such method of nitrate removal is anaerobic ammonium oxidation (i.e. anammox).

The anammox process, which involves bacteria reacting ammonium with nitrite to form nitrogen gas under anoxic conditions (Figure 2.2), was first described in bioreactors of wastewater treatment plants (Schubert et al., 2006). The nitrite is made available from the reduction of nitrate, possibly by denitrifying bacteria (Burgin & Hamilton, 2007). Very little is known about the specific bacteria that perform the anammox process and no pure cultures currently exist (Strous et al., 2006). The anammox process requires anoxic waters that contain sufficient concentrations of both nitrate and ammonium, but the process can be inhibited by simple organic compounds such as pyruvate, ethanol, and glucose (Jetten et al., 1998).

In addition to wastewater treatment plants, the presence of anammox bacteria has been found in oxygen-depleted zones of the ocean, temperate shelf sediments, sea ice, and cold Arctic shelf sediments (Jetten, et al., 1998; M. M. M. Kuypers et al., 2003; Rysgaard & Glud, 2004; Rysgaard, Glud, Risgaard-Petersen, & Dalsgaard, 2004). The extent of the anammox process occurring within freshwater ecosystems is largely unknown as there have been very few studies conducted (Burgin & Hamilton, 2007). The only study revealing evidence of anammox bacteria in freshwater systems (Lake Tanganyika) found that between 7-13 percent of the nitrogen gas production was derived

from anammox processing (Schubert, et al., 2006). This significant contribution to nitrogen removal in freshwater systems could play an important role if anammox bacteria are also found in riverine systems. Based on research examining shallow marine and estuarine waters, the anammox process appears to be less important to overall nitrate removal. However, it has been determined that these areas have higher absolute rates of anammox processing (Dalsgaard, Thamdrup, & Canfield, 2005), indicating that shallow freshwater systems may also contain the same potential for nitrogen processing due to anammox bacteria.

2.2.5 Microbial Community Fingerprint

Microbial communities can be extremely complex assemblages containing diverse phylogenies and physiologies (Marsh, 1999). The number of bacterial species and the sizes of species populations within a community are important parameters when determining community structure and diversity (Liu, Marsh, Cheng, & Forney, 1997). Conventional culture-dependent analysis methods limit the quantification of these two parameters due to a large majority of bacterial species being refractory to cultivation (Amann, Ludwig, & Schleifer, 1995). Thus, a variety of culture-independent methods have been developed (Kent & Triplett, 2002) to compare microbial communities and to relate community composition to environmental parameters (Kent, Smith, Benson, & Triplett, 2003).

Terminal restriction fragment length polymorphism (T-RFLP) is a culture independent method used to determine the fingerprint of a microbial community. T-RFLP has been used to study bacterial, archaeal, and eukaryal populations in their natural habitats (Osborn, Moore, & Timmis, 2000) and can potentially identify specific organisms within a microbial community based on their terminal restriction fragment (T-RF) (Kent, et al., 2003). The benefit of T-RFLP over other culture-independent techniques is the ability to use the determined T-RF sizes to obtain direct taxonomic

information. Methods such as denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), and single-strand conformation polymorphism (SSCP) all permit the analysis of multiple samples but do not translate directly into taxonomic information (Osborn, et al., 2000). Web-based tools have been created to rapidly generate phylogenetic assignments from the submitted T-RFLP profiles through the use of a database of fragments produced by the 16S rRNA gene sequences of known bacteria (Kent, et al., 2003). In addition to the web-based tools allowing for rapid analysis of samples, T-RFLP is very sensitive (Marsh, 1999) and easily reproducible (Smalla et al., 2007), making it an ideal method for comparing microbial communities.

2.3 The Role of Freshwater Mussels

Freshwater mussels are believed to strongly influence ecosystem processes within freshwater systems through their ability to exert control over ecosystem function and structure (Vaughn & Hakenkamp, 2001). As rapid rate filter feeders, mussels are able to transfer nutrients and energy from the water column to the sediments and thus stimulate production across multiple trophic levels (Vaughn, Nichols, & Spooner, 2008). Freshwater mussels have also been distinguished as ecosystem engineers because they modify habitat, making it more suitable for other organisms (Vaughn, et al., 2008).

2.3.1 Background

Freshwater mussels (Family: Unionidae) are large (2- to 30-centimeter) bivalved (two-shelled) mollusks that live in the sediments of rivers, streams, and lakes worldwide (Bauer & Wachtler, 2000). A total of about 1,000 species of freshwater mussels are known, 300 of which live in North America. Mussels are long-lived creatures that can have life spans ranging from several decades up to over a century (Strayer et al., 2004). Unlike marine bivalves and zebra mussels, which typically occur as aggregations of only a few species, freshwater mussels usually occur as diverse, multispecies assemblages (Vaughn, et al., 2008).

These soft-bodied animals are enclosed by two hard shells made mostly of calcium carbonate and connected by a hinge (USFWS, 2006a). The shell of the mussel is formed largely out of calcium carbonate that has been extracted from the water where the mussels live and then deposited in successive layers. The hard shell provides some protection for the inner animal. The mussels' body consists of gills for breathing, a digestive tract for food processing, a large muscular foot used for movement, and a mantle tissue that produces the shell (Figure 2.3) (USGS, 2006).

With perhaps one of the most unique life styles of any aquatic organism, freshwater mussels require a fish to successfully reproduce (Figure 2.4). Some freshwater mussels even require a specific species of host fish to complete their life cycle (USGS, 2006). Male mussels release their sperm into the water column and the downstream female draws the sperm in as she filters water for food. After a period of days to months, fertilized eggs develop into larvae known as glochidia (IDNR, 2002). In most species, the female mussel creates a "lure" to attract a host fish (Figure 2.5). As the fish bites the "lure," the glochidia are released and clamp down on tissue (usually gills) of the host fish (IDNR, 2002). The glochidia then live as parasites on the host fish for a period of hours to weeks. After a suitable amount of time, the glochidia become juvenile mussels and drop from the host fish to the river bed. After anywhere from 2-9 years (depending on species) the juveniles mature into adult mussels capable of reproduction (IDNR, 2002).

2.3.2 Mussels in the Mississippi River

Historically, 51 species of freshwater mussels have been found to live in the Upper Mississippi River Basin (UMRB) with 44 of these species being found in recently conducted mussel surveys (USFWS, 2006b). Four species in the UMRB are listed as federally endangered in the Endangered Species Act of 1973 and over half of the species residing within the UMRB are locally endangered, threatened, or requiring special

concern (USFWS, 2006c). The 29 locks and dams in the UMRB that were created for commercial navigation have altered mussel habitats. The locks and dams have changed water depths, water currents, water temperatures, and restructured fish and algal communities, all of which can negatively impact freshwater mussels (USFWS, 2006a). Also, due to the flow current velocity caused by the dams, increased amounts of suspended sediments are allowed to drop out of the water column and can literally bury existing mussel beds (USFWS, 2006a).

Mussels in the UMRB are also threatened by invasive species such as the zebra mussel. Zebra mussels, which have almost eliminated most native freshwater mussels in portions of the Great Lakes, were first found in the UMRB in 1991 (USFWS, 2006a). Since then, they have spread throughout the UMRB as well as many of its tributaries and inland lakes (Figure 2.6). Zebra mussels attach to hard surfaces, including the shells of native freshwater mussels, and do not require a fish host, allowing them to reproduce several times per year. The number of zebra mussels rapidly spreading throughout the UMRB has been detrimental to the native mussels as zebra mussels compete with native mussels for food and also can interfere with successful reproduction (USFWS, 2006a). Other invasive species currently threatening native populations include the Quagga mussel, Golden mussel, and black carp (USFWS, 2006a).

Mussels have also been threatened due to their value as a commercial product. In the early 1900's, the button industry thrived on mussel shells with sixty button factories located in the Mississippi River Valley (USFWS, 2006a). More recently, mussels have been exploited for their use in cultured pearls. Mussel shells have been determined to be excellent seed material for culturing pearls in pearl oysters. When spherical beads created from the shells of freshwater mussels are placed into marine pearl oysters, they serve as excellent nuclei for the creation of pearls (USFWS, 2006a). With about 90 percent of the weight of a cultured pearl being the shell of a freshwater mussel, mussels

are still threatened by this industry as 2,000 pounds of shell only produces about 40 to 60 pounds of usable nuclei (USFWS, 2006a).

2.3.3 Ecosystem Impacts

The impacts that mussels have on their ecosystem varies largely with species abundance, community composition, environmental factors such as hydrologic residence time and temperature, and spatial and temporal scales (Vaughn, et al., 2008). Most of these impacts mussels exert on ecosystem processes are thought to be directly related to mussel feeding behavior. Originally assumed to be suspension feeders, recent research demonstrates that mussels can also access benthic and planktonic food supplies (Nichols, Silverman, Dietz, Lynn, & Garling, 2005). In large productive rivers such as the Mississippi, mussels have been shown to feed almost exclusively on phytoplankton (Thorp, Delong, Greenwood, & Casper, 1998).

The mussels' ability to filter feed on suspended material such as phytoplankton and algae greatly stimulates primary production as mussels are able to convert the filtered material to dissolved nutrients (Vaughn, Spooner, & Galbraith, 2007). Mussels provide nutrients to other organisms by excreting ammonia (NH_3) and phosphorus through biodeposition of feces and pseudofeces (Vaughn, et al., 2008) as well as the release of stored nutrients when dead mussels decay (Vaughn & Hakenkamp, 2001). Transfer of suspended materials to dissolved nutrients is especially important in areas where nutrients are limited as a 61-mm-long mussel can filter an estimated 0.5 to 1 liter/hour (L/h) of river water (Kryger & Riisgard, 1988). If mussel biomass is sufficient, research has shown that the volume of water filtered by freshwater mussels can equal or exceed daily stream discharge (Vaughn & Hakenkamp, 2001). Mussels have even been shown to cause "biological oligotrophication" by significantly decreasing phytoplankton biomass and total phosphorus and thus increasing water clarity (Welker & Walz, 1998). Mussels may also be able to utilize dissolved organic matter (DOM) as a food source and some

species may even rely on DOM as a significant source of nutrition (Roditi, Fisher, & Sanudo-Wilhelmy, 2000).

In addition to providing nutrients for other organisms, buried mussels have also been shown to provide substrate stabilization of stream bed sediments (Strayer, et al., 2004). Studies have indicated that these dense mussel beds occur in stream areas that remain stable during peak flood events (Morales, Weber, Mynett, & Newton, 2006a; Strayer, 1999). The stabilization of sediments by mussels may provide refuge for macroinvertebrates and their food resources during flooding events (Vaughn, et al., 2008). Besides being able to stabilize sediments, the burrowing activities of mussels often cause bioturbation of the sediment. This increases sediment water, nutrient, and dissolved oxygen (D.O.) content, all of which help improve invertebrate habitat (Vaughn, et al., 2008). Bioturbation in marine ecosystems has been shown to stimulate microbial metabolism (Dame, 1996). If bioturbation has a similar impact in freshwater systems, it may also be able to increase bacterial food resources for both mussels and invertebrates (Vaughn, et al., 2008).

Beyond providing nutrients and substrate stabilization, mussels also influence freshwater ecosystems through the generation of habitat. The spent shells of mussels are an important habitat use for aquatic organisms especially in places where other hard substrata are scarce (Gutierrez, Jones, Strayer, & Iribarne, 2003). Living mussels also impact habitat generation as macroinvertebrate densities within mussels beds are higher than densities found outside of the beds (Vaughn & Spooner, 2006). Macroinvertebrates also tend to accumulate more readily on sediments containing mussel biodeposits (Howard & Cuffey, 2006; Spooner & Vaughn, 2006).

2.3.4 Impacts of Mussels on Nitrogen Dynamics

In marine systems, mussels have been shown to be important cyclers of nitrogen by releasing ammonium (NH_4^+) and dissolved organic nitrogen that can be consumed

directly by phytoplankton (Dame, 1996). Studies on freshwater mussels indicate that the feces and pseudofeces excreted by mussels could be an important and readily usable resource for both phytoplankton and the benthic algal community (M. R. James, 1987; Lauritsen & Mozley, 1989). However, to our knowledge, there is very limited literature on the impact of native freshwater mussel species on nitrogen dynamics as the majority of recent research focuses primarily on the effects of zebra mussels (Bruesewitz, Tank, Bernot, Richardson, & Strauss, 2006; Gardner, et al., 1995; Heath, Fahnenstiel, Gardner, Cavaletto, & Hwang, 1995; Lavrentyev, Gardner, & Yang, 2000). Although zebra mussels are a constant threat to native mussels, their ability to process nitrogen and influence community nitrogen dynamics may be similar to those expressed by native species.

Research implies that the most important effect of zebra mussels on community nitrogen dynamics appears to be the direct excretion of NH_4^+ (Gardner, et al., 1995). Having one of the highest nitrogen excretion rates of any animal (Vanni, 2002), research shows that the NH_4^+ excreted by zebra mussels enhances NH_4^+ mineralization (Gardner, et al., 1995; Gardner, Yang, Cotner, Johengen, & Lavrentyev, 2001) and increases porewater NH_4^+ concentrations (Effler et al., 1996). Increasing NH_4^+ concentrations may increase the potential for nitrification (i.e. the microbial oxidation of NH_4^+ to nitrate) (Lavrentyev, et al., 2000). Zebra mussels may also increase nitrification by filtering out bacterivorous protozoa that would otherwise feed on nitrifying bacteria (Lavrentyev, et al., 2000).

Under certain conditions, zebra mussels may increase rates of denitrification by increasing nitrate availability, decreasing D.O., and excreting carbon-rich pseudofeces (Seitzinger, 1988). In a study conducted by Bruesewitz et al. (2006) in the Mississippi River, zebra mussels were found to influence sediment denitrification rates primarily in the winter, when water temperatures and discharge were low. During periods of high discharge and high hydraulic conductivity, it is suspected that increased nitrate delivery

confounds any impact the zebra mussels may have on denitrification (Bruesewitz, et al., 2006). Similarly, denitrification enhanced by the presence of zebra mussels may not surpass the nitrogen loads in ecosystems containing anthropogenically elevated concentrations of nitrogen (Bruesewitz, et al., 2006).

Bruesewitz et al. (2006) did determine that the zebra mussels' influence on denitrification was most likely not due to increasing carbon availability or by creating anoxic conditions by lowering D.O. concentrations. In fact, the study determined that sites containing zebra mussels typically had lower sediment organic carbon than sites without zebra mussels (Bruesewitz, et al., 2006). This is largely due to zebra mussels preferring rocky areas for colonization and the accumulation of spent shells, which are largely inorganic carbon, replacing the organic sediments as the upper layer of the riverbed (Bruesewitz, et al., 2006). Although Bruesewitz et al. (2006) were not able to measure D.O. in their study, other research performed on similar sediments showed that while sediments inhabited by zebra mussels had decreased D.O. concentrations compared to bare sediments, the sediments containing zebra mussels were not anoxic (Beekey, McCabe, & Marsden, 2004).

Research shows that the most likely mechanisms through which zebra mussels increase denitrification rates are increased nitrification rates and increased delivery of nutrients to the benthos (Bruesewitz, et al., 2006). Both of these methods increase denitrification rates by alleviating the nitrogen limitation commonly found in Mississippi sediments. This is done by increasing porewater nitrate concentration through increased nitrification rates (if oxygen is present) and increasing the amount of nitrogen available to the benthos through filtering (Bruesewitz, et al., 2006). However, zebra mussels can also have a negative impact on ecosystem nutrient uptake rates by directly removing phytoplankton and bacteria from the water column (Gardner, et al., 1995).

2.4 Mussels as Biological Early Warning Systems

Biological early warning systems (BEWS) for monitoring issues such as water pollution involve using organisms as sentinels for the overall quality of the environment. Typically, the chosen organisms are fast in their response time (minutes) to a variety of pollutants (Dezwart, Kramer, & Jenner, 1995). BEWS were initially developed to build upon the monitoring of the quality status of natural waters traditionally completed with physico-chemical techniques (Kramer, Jenner, & Dezwart, 1989). The main goal of BEWS is to trigger the start of an extensive analytical program for the identification of a toxicant (Gruber, Frago, & Rasnake, 1994). Mussels have been established as excellent candidates for BEWS as they are sedentary, abundant, available throughout the year, of a manageable size, and resilient enough to be handled in the laboratory (Kramer, et al., 1989; Phillips, 1977).

2.4.1 Monitoring Mussel Behavior

In conditions of clean water, mussels move the two halves of their shells according to a characteristic pattern (Musselmonitor, 2005). This rhythmical valve movement has been well described in the lab (Kramer, et al., 1989) as well as in the field (V. Englund & Heino, 1994). Studies using sensors to examine mussel behavior in natural habitats indicate that mussels tend to open their valves wider and more often at night than during the day (V. Englund & Heino, 1994; R. Wilson, Reuter, & Wahl, 2005). Research has also shown that time spent with the valves open can be impacted by ambient temperature, pH, and food particle concentration (V. P. M. Englund & Pynnonen, 1996).

When mussels detect contamination in the water, they often signify its presence by displaying a variation in normal valve behavior (Musselmonitor, 2005). When mussels sense contamination such as toxins, they close their shells to reduce exposure to the toxin (EPA, 2005). Other mussel responses include increasing their activity level by opening and closing more frequently than normal (i.e. flapping) or reducing their average

value of opening over a certain period of time (Musselmonitor, 2005). If the mussels cease all movement or their shell remains open for longer than the normal maximum open position (i.e. gaping), this usually indicates the mussel is no longer living (Musselmonitor, 2005).

BEWS utilizing mussels (both freshwater and marine) have been introduced as mussels were found to respond to a variety of toxins found in aquatic systems (Table 2.1) (Borcherding, 2006). In freshwater systems, zebra mussels are the species of mussel typically implemented into the BEWS. Several of these systems, including the Dreissena-Monitor and the MosselMoniter®, have been applied in routine monitoring networks and are even available to be purchased commercially (Borcherding, 2006; Musselmonitor, 2005).

While BEWS employing the use of mussels have been proven effective in some systems, the complexities of the mussels often proves problematic under different water quality conditions. Research has shown that natural water parameters such as pH, temperature, conductivity, and amount of suspended particles may alter the reaction of the mussels to a certain target concentration of a toxicant (Borcherding & Wolf, 2001). Mussels have also been shown to react differently during cold temperatures. A study conducted by Borcherding (2006) determined that mussels had significantly fewer valve movements in cold temperatures (5-7°C) than during the warmer temperatures (20°C) in the summer. Furthermore, mussel valve movements have also been shown to vary drastically between rivers and lakes (V. P. M. Englund & Heino, 1996). Thus, knowledge of the mussels' normal behavior in the aquatic system of interest is important to establish prior to implementation of a BEWS (Borcherding, 1992).

The use of mussels for BEWS has been established due to the amount of work completed in identifying the rhythmical valve movements of different species. However, most of the experiments determining the normal behavior of mussels have been carried out under laboratory conditions (V. Englund & Heino, 1994). Research examining valve

movements in the lab and in the mussels' native habitat found that valve rhythms are much more regular under lab conditions and that the mussels have their valves closed most of the time (V. Englund & Heino, 1994). This is thought to be due either to the stress caused by the method recording valve movements or by the overly sterile conditions of a laboratory environment (V. Englund & Heino, 1994). Mussels have also been shown to have decreased growth rates in the lab as compared to in the wild (Famme, Riisgard, & Jorgensen, 1986; Jorgensen, Larsen, & Riisgard, 1990).

Wilson et al. (2005) studied the gape angle differences between marine mussels in the lab and the wild and discovered the absence of a well-defined diurnal rhythm in the lab mussels compared to those in the wild. Additionally, the study revealed that when mussels used in lab experiments were returned to the wild, they had a very slow return (more than 2 days) to the more obvious diurnal pattern expressed by mussels in the wild (R. Wilson, et al., 2005). Wilson et al. (2005) concluded that the lab mussels were substantially disturbed even though standard aquaculture protocols were followed to maximize well-being.

2.4.2 Remote-Sensing Mussels

Using mussels for biomonitoring presumes an understanding of valve movement under natural conditions (V. Englund & Heino, 1994). If mussels housed in laboratory conditions behave considerably different than those in the wild, the 'natural' valve movements may be incorrectly implemented into BEWS. To better clarify the natural valve movements, remote-sensing monitoring techniques have been established. With only a handful of studies having examined mussels' behavior through remote-sensing (Curtis, Williamson, & Depledge, 2000; Dezwart, et al., 1995; Redpath & Davenport, 1988; R. Wilson, et al., 2005), the approach is thought to have the potential to significantly enhance understanding of marine and freshwater invertebrates such as mussels (R. Wilson, et al., 2005).

Although the technology and sensors used in remote-sensing vary, the goal of the technique is to develop animal-attached systems that automatically record activity at all times, even if the animal is far from land or in deep water (Naito, 2004). For mussels, remote-sensing is especially valuable in areas where direct observation is difficult because of turbid water or the mussels burying themselves in the sediment (R. Wilson, et al., 2005). Remote-sensing also eliminates unnatural mussel valve closures due to procedures involving divers entering the habitat or workers entering the lab (if remote-sensing was utilized in lab experiments) (R. Wilson, et al., 2005).

Wilson et al. (2005) developed a remote-sensing technique using a magnet and a magnetic-field strength Hall sensor to study mussel gape angle (Figure 2.7). Originally proposed to study the feeding behavior of penguins (R. P. Wilson, Steinfurth, Ropert-Coudert, Kato, & Kurita, 2002), the system relies on a small magnet being attached to a moving element on the animal and the Hall sensor being attached to a non-moving element (R. Wilson, et al., 2005). In the case of the mussels, the magnet and Hall sensor were each attached to one of the mussel's shells. This approach has been shown to be advantageous over other existing remote-system technologies due largely to sand and mud not affecting magnetic fields (R. Wilson, et al., 2005). Also, unlike strain gauges (Redpath & Davenport, 1988), attachment of the sensors is not inhibiting and unlike coil-based systems (Dezwart, et al., 1995), the Hall sensor approach does not require a high voltage source or for power to be delivered to both sides of the shell (R. Wilson, et al., 2005).

The elevated sensitivity of the Hall sensor/magnet technology provides a solution for a variety of issues related to mussel monitoring and the system can also gather data at highly variable frequencies (R. Wilson, et al., 2005). Coupling the high frequency recordings with heart beat frequency can be useful for monitoring pollutants (Curtis, et al., 2000) if the system were to be implemented into a BEWS. Additionally, the technology may also allow researchers to determine the growth rate of mussels in long-

term studies as the oppositely mounted magnets and sensors will tend to move farther apart as the animal grows (R. Wilson, et al., 2005).

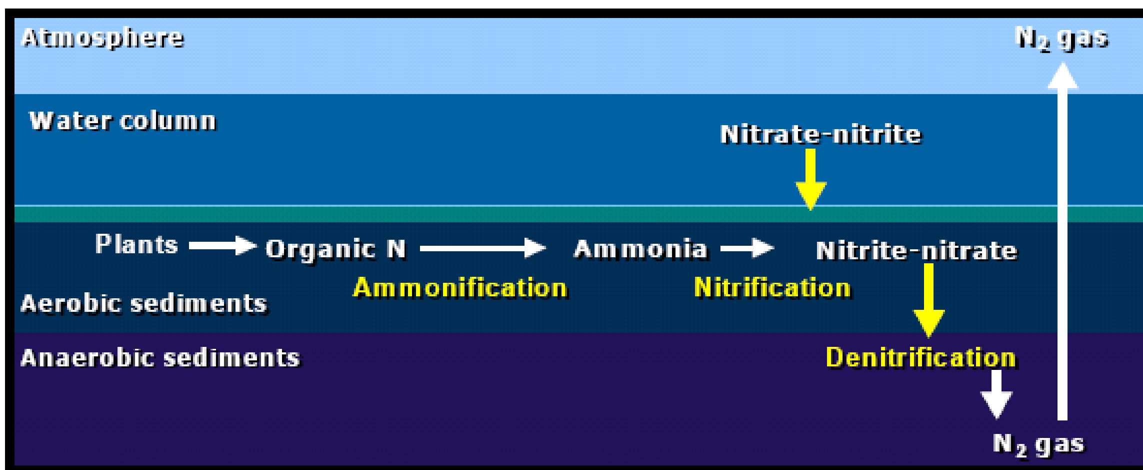

Figure 2.1: Pathways of nitrogen processing in riverine sediments (W. R. Richardson, et al., 2008).

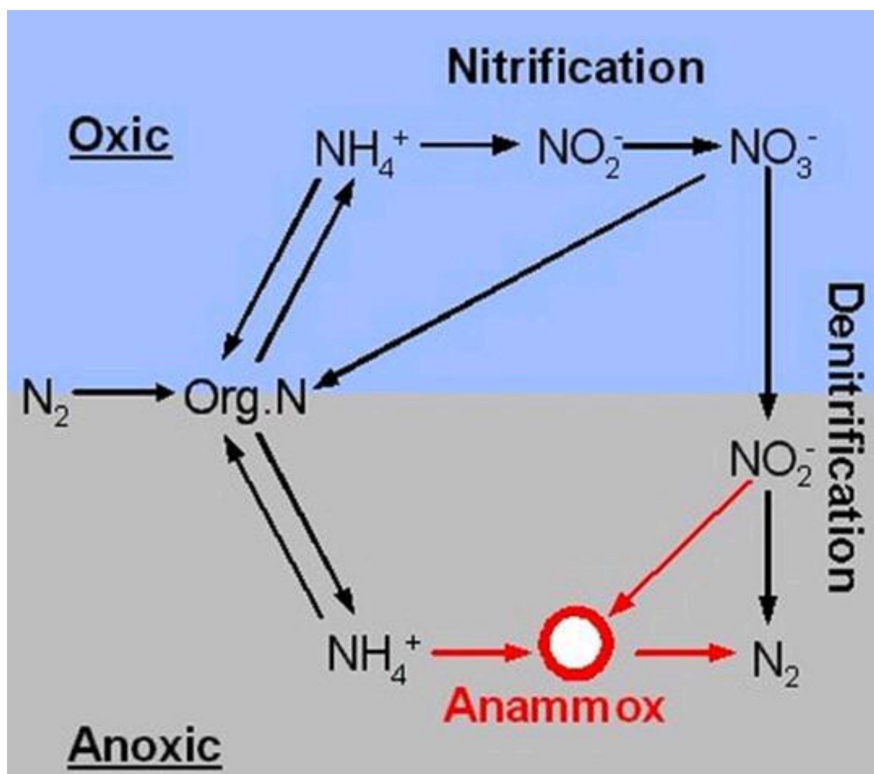

Figure 2.2: The role of anammox in the oceanic nitrogen cycle (M. Kuypers, 2010).

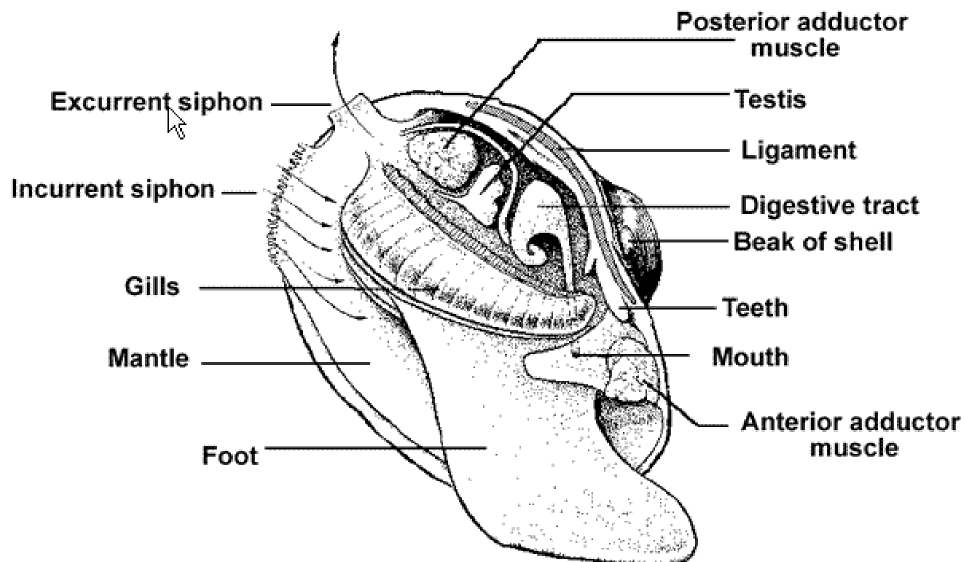

Figure 2.3: Typical anatomy (side view) of a freshwater mussel (Minnesota, 2006).

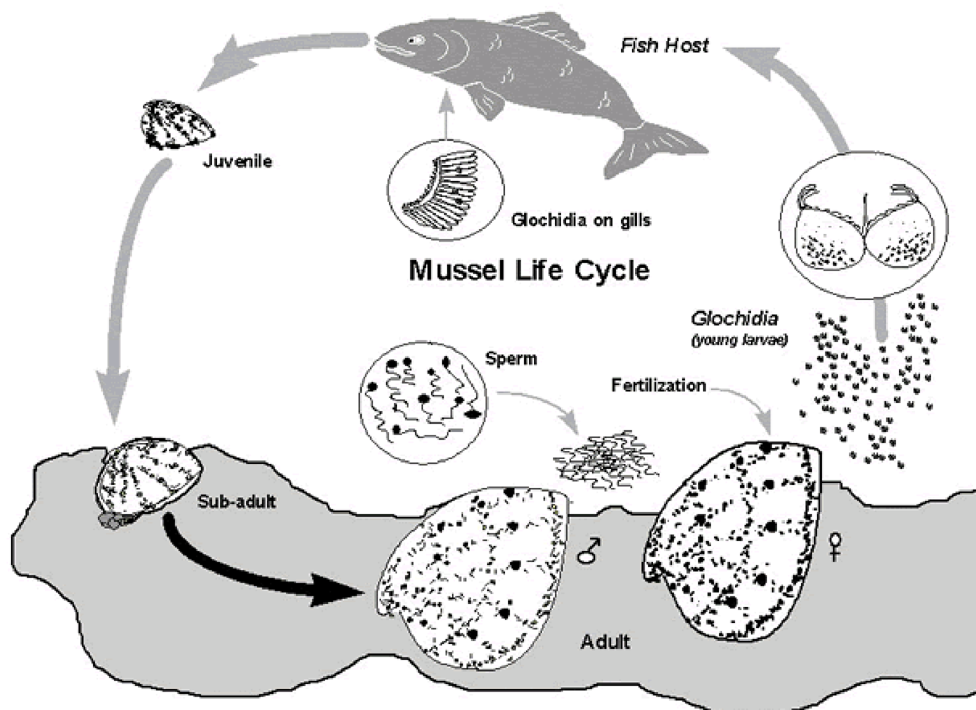

Figure 2.4: Life cycle diagram of freshwater mussel (IDNR, 2002).

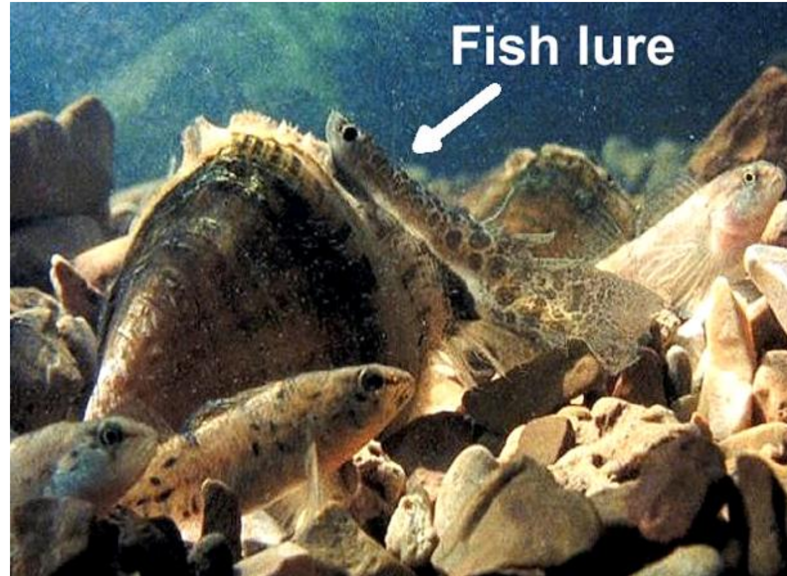

Figure 2.5: Fish "lure" containing glochidia created by female mussel to attract host fish (Barnhart, 2006).

Figure 2.6: Distribution of zebra mussel sightings in 1988 and 2004 (USFWS, 2006a).

Table 2.1: Detection limits (mg/L) for two zebra mussel biological early warning systems (Musselmonitor® and Dreissena-Monitor) for different toxins (Borcherding, 2006; Musselmonitor, 2005).

Component	Detection Limit (mg/L) Musselmonitor®	Dreissena-Monitor
Ammonium	-	37
Atrazine	0.5	2
Bentazone	0.75	-
Cadmium	0.15	1
Chloroform	43	33
2-chloro-4-	-	0.25
Chloropyriphos	0.05	-
Copper ($CuSO_4$)	0.01	0.03-1
Cyanide (KCN)	0.4	-
1.3 Dichlorobezene	1.4	-
Ethylparathion	-	0.8-0.9
Hexachlorbutadiene	0.15	-
Y-hexachlorcyclohexane	0.06	-
Hipochlorite (chlorine)	0.037	-
Lead	0.25	-
Lindane	0.11	0.15
2-nitrophenol	-	0.18
Pentachlorophenol (PCP)	0.01	0.02
Phenol	14	-
Selenium (selenite)	0.1	-
Toluene	6	-
Tributyltinoxide (TBTO)	0.006	0.04
Trichlorethylene	8	7.5-8.8
Xylene	16	-
Zinc	0.5	-

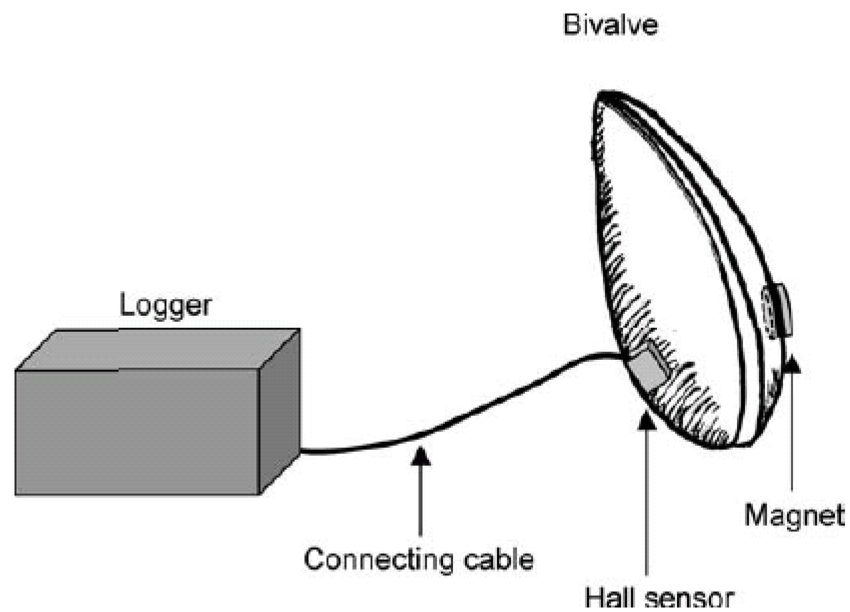

Figure 2.7: Schematic diagram showing the attachment of the Hall sensor system used for determining mussel gape angle (R. Wilson, et al., 2005).

CHAPTER 3
MATERIALS AND METHODS

3.1 Introduction

The purpose of this chapter is to describe the materials and methodology used in analyzing the different components of our study. Unless otherwise noted, all laboratory experiments were completed in the Environmental Engineering and Science Laboratories at the University of Iowa.

3.2 Bathymetric Survey

A bathymetric survey was completed for our study to define the bed geometry of the mussel bed. The survey was completed in August of 2008 by University of Iowa IIHR – Hydroscience & Engineering staff. Data analysis was completed at the C. Maxwell Stanley Hydraulics Laboratory at the University of Iowa.

3.2.1 Data Collection

Bathymetric data was collected using a state-of-the-art RESON SeaBat 7125 multi-beam echosounder which simultaneously samples 512 locations on the river bed in a 120-degree swath perpendicular to the direction of travel (Piotrowski, 2010). An Applanix POS-MV inertial motion detection system was used to track heading, pitch, roll, and yaw of the survey vessel. Bed soundings were synchronized with a RTK GNSS. Latitude, longitude, and depth were sampled at a rate of approximately 2 hertz (Hz). HYPACK was used to convert the latitude and longitude to a projected geographic coordinate system and to record the coordinates and soundings. Vertical and horizontal survey control was established using the Johnson County, Iowa GNSS control network. Elevation data from the RTK GNSS were time averaged using a moving 30 second

window. HYPACK automatically calculated bed elevation for each depth sounding by applying the corresponding time averaged elevation data and pitch, roll, and yaw values.

3.2.2 Data Filtering

Multi-beam data were filtered using HYPACK. Automatic filters were used to remove bed elevations that deviated from the adjacent soundings. Mean elevation values were used where data overlap occurred. Multi-beam data density was reduced in HYPACK and interpolated to a grid with a constant horizontal cell spacing of 20 cm. The mean elevation within each cell was applied at the cell center.

3.2.3 ArcGIS

The collected data set was exported from HYPACK as discrete points with X, Y, Z coordinates. These points were imported into ArcGIS for analysis. The multi-beam data, exported as a grid of points with a constant horizontal cell spacing of 20 cm, was converted to a raster with a cell size of 20 cm. The raster pixel values represented the Z values of the multi-beam data.

3.3 Grain Size Distribution Analysis

A total of 12 sediment samples were collected from the mussel bed in July of 2009 for grain size distribution analysis. Samples were collected using a Wildco petite PONAR dredge and stored in 3-gallon plastic bins. The horizontal position of each collected sample was recorded using latitude and longitude coordinates obtained from a GARMIN GPS 12 MAP handheld receiver. Each sample collected was prepped and wet sieved prior to the dry sieving process.

3.3.1 Sample Preparation

Field samples were allowed to settle once they were brought back to the lab. After settling, the surface water was decanted off of each sample. Samples were then divided into equivalent sections by the method of quartering and 100 grams (g) was taken

from each sample. The 100 g of each sample was then divided into subsample thirds with approximately 30 g being placed into appropriately labeled 125-milliliter (mL) Nalgene bottles. After recording the exact weight of sample added to each bottle, a deflocculating agent (sodium metaphosphate – $Na(PO_6)_3$) was introduced to ensure an accurate grain size distribution was obtained. The deflocculating agent was added in volumes of 30 mL containing a concentration of 35.7 grams/liter (g/L). Approximately 30 mL of deionized water was then added to each bottle. Exact amounts of water added to each bottle recorded to make sure that the bottles were at least half full. Each Nalgene bottle (three for each field sample) was then placed on a 225 revolutions per minute (rpm) shaker table overnight to enhance disaggregation.

3.3.2 Wet Sieving

After deflocculating, each subsample was washed through a 63 micrometer (µm) sieve (No. 230) with deionized water. The three subsamples representing one of the 12 field samples were all washed over the same collection basin to be recombined as one sample. The bottles were thoroughly washed with deionized water to ensure all particles were sieved and a rubber brush was used to assist in washing the samples through the sieve.

The sediment retained on the sieve for each of the 12 samples was transferred to an oven-safe beaker and placed in an oven at 110 ±5 °C for a period of 24 hours to completely dry the sample. Each sample was then removed from the oven and allowed to cool to room temperature. This was done to make sure that the particles would not still be expanded from the heat and thus potentially provide inaccurate sieve results.

The solution collected in the collection basin during wet sieving was placed aside for suspended particles to settle. To assist in the settling of the fine particles, 25 mL of 30.22 g/L aluminum sulfate ($Al_2(SO_4)_3$) was added to the solution for each sample. The solutions were then allowed to sit overnight. If suspended particles still remained,

additional aluminum sulfate was added and the process was repeated until all particles were settled. The sample was then decanted and placed in an oven at 110 ±5 °C for 24 hours to completely dry the sample. After drying, the sample was allowed to cool to room temperature. Once sufficiently cooled, each sample was weighed and added to the net weight of the pan weight from the dry sieve analysis.

3.3.3 Dry Sieving

Each sieve used in the analysis was weighed to establish initial weights prior to adding sediment samples. Sieves used in the dry sieving analysis were as follows: 4.75 millimeter (mm) (No. 2), 2.00 mm (No. 10), 1.00 mm (No. 18), 420 µm (No. 40), 250 µm (No. 60), and 125 µm (No. 120). The sieves were assembled in order with the coarsest (No. 4) sieve at the top of the stack and the finest sieve (No. 120) at the bottom of the stack. After each sample was allowed sufficient time to cool, it was weighed and placed in the sieve stack. The stack was then placed in the shaker device for a period of 20 minutes. After the shaking process was completed, each sieve was weighed separately to determine the amount retained on each sieve.

3.4 Serum Bottle Nitrate Reduction

The serum bottle experiment was completed to determine if denitrification was occurring within mussel bed sediment samples. A sample estimated to contain high organic content (upstream) and a sample estimated to contain mild amounts of organics (downstream) were compared to a sand control sample. Completed in triplicate, about 10 g of each sediment sample were placed in 160 mL serum bottles. The bottles were then spiked with 40 mL of 10 mg/L nitrate solution prepared using 1,000 mg/L ion chromatography nitrate standard (Fisher Scientific) and deionized water. After sealing the bottles and purging out the oxygen, a 1 mL water sample was taken (time 0 measurement). Water samples were filtered through a 0.45 µm nylon syringe filter (Tisch Environmental) and placed in an amber crimp-top 2 mL autosampler vial

(Fisherbrand), and stored inside a 4 °C refrigerator. All serum bottles were then placed on a 225 rpm shaker table to maintain a well-mixed environment for the sediment and water/nitrate solution. This test ran for 72 hours with 1 mL samples taken at the 24 hour and 72 hour time periods. Upon test completion each of the 1 mL samples taken at the three time periods was analyzed for nitrate content using ion chromatography.

3.5 Nitrate Reduction Beaker Experiments

The purpose of the nitrate reduction beaker experiment was to follow up on the serum bottle experiment by determining the presence of denitrification in the mussel bed sediments under more realistic habitat conditions. Another purpose of this experiment was to measure nitrate levels on a more frequent basis to capture a better denitrification rate curve. The beaker experiment was completed over a period of 24 hours with measurements obtained every 4 hours as opposed to the serum bottle experiment which was completed over 72 hours with samples obtained at the 0, 24, and 72 hour time periods. Three samples were measured during the serum bottle experiment (sand control, mildly organic, highly organic) but only two were measured for this experiment (sand control, highly organic).

The experiment was completed in triplicate using six 800 mL Pyrex beakers (3 for sand control, 3 for sediment sample). Approximately 200 mL of sediment/sand was added to each individual beaker. Sample mass was recorded to determine exactly how much sediment had been added to the beaker. Each beaker was then spiked with 500 mL of 10 mg/L nitrate solution prepared using 1,000 mg/L ion chromatography nitrate standard (Fisher Scientific) and deionized water. Using six 8-inch side port syringe needles (Hamilton Company), a 1 mL sample was taken from the pore water (defined as below the sediment) and the water column above the sediment. Porewater and water column temperatures were recorded upon sample collection. Collected water samples were filtered through a 0.45 μm nylon syringe filter (Tisch Environmental) and placed in

an amber crimp-top 2 mL autosampler vial (Fisherbrand), and stored inside a 4 °C refrigerator. Starting at time 0, samples were taken every 4 hours for 24 hours. After the 24 hour time period, all samples were measured for nitrate concentration using ion chromatography.

3.6 Ion Chromatograph Nitrate Testing

A Dionex BioLC ion chromatograph was used to analyze samples for nitrate concentrations. Analyte separation was accomplished using an IonPac AS18 anion-exchange column (Dionex) with chemical suppression and conductivity detection. A 39 millimolar (mM) potassium hydroxide (KOH) eluent was pumped at 1.0 mL/min. Chemstation software (Hewlett Packard) performed all data collection and processing. Seven aqueous standards were used to make each standard curve. Nitrogen standards were made with a 1,000 mg/L ion chromatography nitrate standard (Fisher Scientific) and deionized water. Standard concentrations used for each analysis were as follows: blank (deionized water), 0.5 mg/L, 1.0 mg/L, 2.5 mg/L, 5.0 mg/L, 10 mg/L, and 20 mg/L. Linear regression was employed to develop concentration as a function of instrument response. The method detection limit was 0.1 mg/L NO_3^- as N.

3.7 Anammox Analysis

The presence of anammox bacteria was analyzed on sediment samples collected from the mussel bed in 2008. After collection, samples were stored in a -80 °C freezer to preserve the bacterial populations. Samples were then sent to Temple University in Philadelphia, Pennsylvania and analyzed by Dr. Benoit Van Aiken and his colleagues. The presence of anammox bacteria was completed by designing primers for the specific amplification of the anammox markers. Once the primers were designed, DNA was extracted from the sediment samples and amplified through polymerase chain reaction (PCR) using the marker genes. The different signals obtained from PCR were then

examined to determine if the sample contained bacteria capable of performing the anammox process.

3.8 Terminal Restriction Fragment Length Polymorphism (T-RFLP)

Microbial community fingerprints were identified using Terminal Restriction Fragment Length Polymorphism (T-RFLP). Sediment samples analyzed were collected from the Mississippi mussel bed in December of 2008. A sample from the low mussel density sediment (Sample ID #5) and a sample from the high mussel density sediment (Sample ID #14) were analyzed using T-RFLP. Other than the DNA fragments sequencing which was performed by the University of Iowa DNA Sequencing Facility, all analyses were completed in the Environmental Engineering and Science Laboratories at the University of Iowa.

3.8.1 DNA Extraction

Sediment DNA was extracted from each of the two analyzed samples using the UltraClean Soil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, California). After weighing out 0.5 grams of each sample, extraction was completed according to the kit instructions.

3.8.2 PCR and Purification

The polymerase chain reaction (PCR) technique used was similar to the protocol described in previous research (Kent, et al., 2003). PCR amplification of extracted sediment sample DNA was completed using the HotStar Taq® Master Mix Kit (Qiagen) on a Mastercycler Gradient® thermocycler (Eppendorf, Hamburg, Germany). The thermocycler program used for this technique was as follows: a 15 minute start at 94 °C, followed by 35 cycles consisting of denaturation (35 seconds at 94 °C), annealing (45 seconds at 55 °C), and extension (90 seconds at 72 °C), and a final extension for 2 minutes at 72 °C. Reaction mixtures for PCR contained 50 µl HotStar Taq® Master Mix,

1 µl of each primer, 1 µl of DNA extract, and 2 µl of BSA buffer in a final volume of 100 µl. The primers used were 8F and 1492R. PCR products were then purified using a QIAquick® PCR Purification Kit (Qiagen) according to kit instructions.

3.8.3 Restriction Enzyme Digestion

PCR products were digested separately using the restriction enzymes Hha1, Msp1, and Rsa1. Multiple digests with the three restriction enzymes were carried out to increase the specificity of the phylogenetic assignments. The lengths of the terminal restriction fragments (T-RFs) were determined by electrophoresis through use of a Model 3730 DNA Analyzer (Applied Biosystems, Inc.). The analyzed mixture contained 1 µl of digested PCR product, 9.5 µl of HiDi Formamide, and 0.5 µl of DNA fragment length standard (GeneScan 1200 LIZ). Negative controls (blank samples not containing DNA) were processed through the entire method and showed that there was no significant contamination during any of the procedures.

3.8.4 Analysis

Data from the DNA fragment sequencing were analyzed using PeakScanner 1.0 software (Applied Biosystems, Inc.). Data tables containing the fragment size and abundance data for each digest were exported from PeakScanner as text files. The resulting text files were then uploaded to the T-RFLP phylogenetic assignment tool (PAT) provided by the University of Wisconsin Center for Limnology (Kent, et al., 2003). The PAT output data were then analyzed to determine the relative abundance for each phylogenetic assignment. The phylum, order, class, and family for each phylogenetic assignment were determined using the National Center for Biotechnology Information (NCBI) Nucleotide database.

3.9 Total Organic Carbon Analysis

Total organic carbon (TOC) analysis was completed using a Shimadzu Solids Sampler Module (SSM-5000) instrument coupled with a Shimadzu TOC-V CSH Total Organic Carbon Analyzer. Sediment samples analyzed were collected from the Mississippi mussel bed in December of 2008. TOC was determined through the difference method by determining total carbon (TC) and inorganic carbon (IC) and calculating the difference between the two values.

3.9.1 Calibration Curves

Calibration curves for both TC and IC had to be established prior to analysis. The TC calibration curve was determined using glucose monohydrate which had a known carbon content of 40 percent. Five glucose monohydrate samples of known mass were used as standards to develop the curve. The masses of samples used included: 1.0 mg, 3.0 mg, 5.0 mg, 10 mg, and 20 mg. The established curve was stored as TC Calibration Curve #4 (SSM-TC4) on the instrument.

A similar approach was used to determine the IC calibration curve. Sodium carbonate (11.3 percent carbon) was used as the standard and five samples of known mass were again used to develop the curve. The masses of samples used included: 1.0 mg, 5.0 mg, 10 mg, 15 mg, and 25 mg. An 85 percent (by weight) phosphoric acid solution was diluted to a 1:1 ratio with deionized water to serve as the reagent necessary for the IC analysis. The established curve was stored as IC Calibration Curve #4 (SSM-IC4) on the instrument.

3.9.2 TOC Measurement

Each sample studied for TOC was divided into six subsamples so triplicates could be run for both the TC and IC analysis. Subsample masses ranged from 100-200 mg with exact masses recorded prior to measurement.

For the TC analysis, each subsample was placed in a ceramic weigh boat and then inserted into the sample port of the TC sampler. The correct calibration curve was then selected (SSM-TC4) and the sample was entered into the 900 °C TC combustion oven. Due to the limitations of the instrument only being able to measure one sample at a time and a limited number of ceramic weigh boats, the weigh boats were reused for multiple samples. After measurement of a sample was completed, the used weigh boats were washed and then placed back into the 900 °C TC combustion oven to burn off any remaining organics. Areas of the measured peaks and calculated masses of carbon for each subsample were then recorded to determine percent of TC present.

Similar to the TC analysis, the IC analysis involved placing each subsample into a ceramic weigh boat and then inserting the boat into the sample port of the IC sampler. The correct IC calibration curve (SSM-IC4) was then selected. The IC reagent (phosphoric acid solution) was then added to the sample boat through use of an auto-pipette. The volume added to each boat was 0.5 mL. Each sample was then entered into the 150 °C IC combustion oven for measurement. Again, due to the limitations of the instrument to measure one sample at a time and a limited number of weigh boats, used boats were washed and placed in the 900 °C TC combustion oven to burn off any remaining organics. Areas of the measured peaks and calculated masses of carbon for each subsample were then recorded to determine percent of IC present. Percentage of IC present for each subsample was then subtracted from determined TC percentages to obtain percentage of TOC. The three subsamples were then combined to determine an average and standard deviation for each collected field sample.

3.10 Sensor Cluster Buoys

The sensor cluster buoys were designed and constructed by IIHR – Hydroscience & Engineering staff. Each buoy was data logging and telemetry-enabled via a Campbell Scientific CR1000 datalogger, an AirLink Communications Raven CDMA cellular

modem, two rechargeable marine batteries and charge controllers, and two BP SX20U solar panels. The networking equipment was coupled with a Hach DS5X water quality sonde, a Forestry Technology Systems DTS-12 turbidity probe, and a Hach Nitratax UV sc plus nitrate sensor. The three sensors installed on each buoy allowed for the following real-time (every 15 minutes) parameters to be measured: dissolved oxygen, pH, conductivity, temperature, turbidity, and nitrate-N.

CHAPTER 4
CHARACTERIZATION OF MUSSEL BED

4.1 Introduction

To determine the potential impact of human- and climate-induced changes on nitrogen processing, we studied the different characteristics of a mussel habitat located in the Mississippi River. The purpose of this chapter is to give background on the study site and to discuss the physical, biological, and chemical characteristics we examined.

4.2 Study Site Background

The focus area of our study was a mussel bed located in Navigation Pool 16 of the Mississippi River. The UMRB was determined to be an appropriate study site as it is already known to inadequately process nitrogen to the degree required to protect the Gulf of Mexico from hypoxic episodes. Navigation Pool 16 of the UMRB extends 41 kilometers from Lock and Dam 15 near Rock Island, Illinois, to Lock and Dam 16 near Muscatine, Iowa (Figure 4.1). The only major tributary to this reach, the Rock River, enters just south of Rock Island, and the Andalusia Island Complex dominates a significant stretch of the pool.

Specifically, the focus area of this project is a 1200-meter (m) long mussel bed in Pool 16 near Buffalo, Iowa. A substantial amount of adult and juvenile mussels, including the federally endangered Higgins' eye mussels, were observed at the site in 2003 (Helms, 2003) and again in 2008. University of Iowa's IIHR – Hydroscience & Engineering Lucille A. Carver Mississippi Riverside Environmental Research Station (LACMRERS) is located adjacent to Pool 16 in Fairport, Iowa.

The mussel bed was analyzed in terms of its physical, biological, and chemical characteristics. These characteristics were compared spatially and temporally to

determine the most significant factors impacting the nitrogen processing capabilities of mussels and bacteria within the ecosystem.

4.3 Physical Characterization

Our study examined the physical characteristics of the mussel bed to establish how alterations in physical habitat potentially impact ecosystem nitrogen processing. Physical characterization was conducted through riverbed bathymetric surveys, mussel density and diversity studies, and pre- and post-flood grain size distribution analyses.

4.3.1 Riverbed Bathymetric Survey

A multibeam hydrographic survey of the mussel bed was completed in August of 2008 to obtain a detailed representation of the elevation and contours of the riverbed. The bathymetric survey displayed both the natural and man-made (wing dams) characteristics of the riverbed located within the mussel bed (Figure 4.2). Based on the results of the survey, it was hypothesized that the upstream (east) section of the mussel bed experiences lower velocities than those in the middle and downstream (west) sections of the bed. This hypothesis was based on the presence of thin, tightly spaced contour lines for the upstream section and wider, less tightly spaced contours for the middle and downstream sections.

The lower flow velocities likely create less bed shear and promote increased sediment deposition. Previous research on the bed indicates that the decreased velocities may be caused by circulating of flow occurring within the bed (Figure 4.3) (Young, 2006). The increased velocities from the main channel are diverted away from the mussel bed by an outcrop of land at the most upstream end. This outcrop appears to initially deflect the higher velocities from the main channel, causing a small eddy-like circulation to occur within the bed. Deflecting the flow from the main channel likely provides protection to the mussel bed and also increases sediment deposition throughout the bed due to decreased velocities.

4.3.2 Mussel Density and Diversity

Physical characterizations of the mussels within the bed were determined through a mussel density and diversity survey as well as several site visits. The diversity and density survey was conducted in 2003 by Helms and Associates. In the survey, 1244 individual mussels were found at the sampled sites and 26 different species were represented (Helms, 2003). Additionally, several federally endangered Higgins' eye mussels were found in the bed. By sampling 78 1-m^2 quadrats throughout the bed, the survey concluded that the highest density of mussels was located near the middle section of the bed (Figure 4.4). The survey determined the downstream section of the bed to contain medium mussel densities and the upstream section of the bed to have very low densities present.

Similar results were obtained for the diversity of mussels throughout the bed. Again analyzing 78 1-m^2 quadrats, the highest diversity of mussels was found in the middle and downstream sections of the bed (Figure 4.5). The upstream section of the bed contained much lower diversities and many sample sites were completely absent of mussels altogether. The survey also showed that the highest amounts of mussel density and diversity existed nearest to the bank of the river. Sample sites located nearer to the main channel often lacked mussels, even in areas of the bed which contained high density and diversity of mussels near the bank.

Combining the mussel density and diversity survey with the bathymetric survey completed in 2008 (Figure 4.4, Figure 4.5), it was determined that mussels tended to prefer areas of the mussel bed that experienced higher velocities. Due to the high density of mussels located in the middle and downstream sections of the bed; where velocities were determined to be higher than the upstream section, it was concluded that mussels favored the areas of the bed that were less prone to siltation. This observation is not surprising as mussels likely prefer sufficient velocities to enhance their ability to filter-feed on passing particulates.

A site exploration trip we conducted in October 2008 confirmed that mussel populations within the mussel bed remained substantial (Figure 4.6). Based on visual observations, we speculated that the highest density and diversity of mussels still existed in the middle and downstream portions of the bed. We also determined that the greatest densities of mussels were located in the areas of the bed that contained more sandy sediments compared to areas containing silty sediments. Assuming the silty sediments exist in areas of increased deposition and decreased velocities within the bed, this observation supports the conclusions derived from combining the bathymetric and mussel density/diversity surveys.

4.3.3 Grain Size Distribution

The physical characteristics of the mussel bed were further defined through grain size distribution analyses. Grain size distribution was completed for 12 sediment samples taken from throughout the mussel bed in July 2009. Sediment samples were compared based on their location within the high, medium, or low mussel density areas of the bed as previously determined by the mussel density survey. In the low mussel density sections (upstream), the sediments appeared to be very uniform and thus well-sorted and poorly-graded (Figure 4.7). Areas of the bed containing medium mussel densities (downstream) contained sediments with much more variance in size (Figure 4.8). These areas of the bed were comprised of sediments that were poorly-sorted and well-graded. Similarly, areas of high mussel density (middle of the bed) contained sediments that varied in size and were thus poorly-sorted and well-graded (Figure 4.9).

To determine the physical disturbance in the mussel bed caused by the extreme floods of 2008, grain size distribution analyses from before and after the flood were evaluated. Researchers investigating the physical characterization of mussel habitats in Pool 16 gathered and analyzed sediments in 2004 as part of their study (Young, 2006). These pre-flood sediments were compared to the post-flood sediments we collected from

the bed in 2009. Post-flood sediment samples were collected from locations very similar to the pre-flood samples collected in 2004. The analysis was again divided into areas of the bed containing low, medium, and high mussel densities.

Comparing these pre- and post-flood sediments indicated that flood-related sediment deposition was unevenly distributed throughout the mussel bed. The areas of the bed containing low mussel densities expressed minimal change in sediment grain size distribution caused by the flood as both were well-sorted and poorly-graded (Figure 4.10). These areas of the bed may have been less vulnerable to changes caused by the flood due to the protective outcrop of land present at the most upstream edge of the bed. Other than a slight increase in the percentage of fines (by weight) present at small particle sizes, the grain size distribution for sediments in the low mussel density areas was very similar between pre- and post-flood sediments.

The flood appeared to cause much more significant changes to occur in the medium and high mussel density areas of the bed. Sediments in the medium mussel density areas changed from a pre-flood distribution of well-sorted and poorly-graded sediments to a post-flood distribution that was more poorly-sorted and well-graded (Figure 4.11). The post-flood distribution also contained a much higher percentage of fines than were present in the pre-flood sediments.

Sediments in the high mussel density areas also experienced significant changes due primarily to a much higher percentage of fines present in the post-flood sediments (Figure 4.12). Both the pre- and post-flood distributions represented poorly-sorted and well-graded sediments, but the increase in the percentage of fines was much greater than any other areas of the mussel bed. This observation led us to conclude that the middle and downstream sections of the bed appear to be the most vulnerable to changes caused by the flooding. Since these areas contain the highest populations of mussels, further research will be necessary to determine how these changes in physical habitat may impact the mussels.

4.4 Biological Characterization

The biological characteristics of the mussel bed were defined in terms of the bacteria present at the site. The presence of denitrifying bacteria was determined through laboratory experiments and the presence of anammox bacteria was evaluated using the polymerase chain reaction technique. Diversity of microbial communities in two areas of the bed was compared using terminal restriction fragment length polymorphism (T-RFLP).

4.4.1 Presence of Denitrifying Bacteria

Samples obtained from the low and medium mussel density areas of the mussel bed were analyzed to determine if denitrifying bacteria were present in the bed. The low mussel density sediments were chosen based on the decreased velocities and the assumption that more organic carbon would be present in these areas due to increased deposition. Since denitrifying bacteria require carbon as an energy source to drive denitrification, we assumed that denitrifying bacteria would be located in areas that are most likely to contain the greatest amounts of organic carbon. The medium density sediments were also analyzed as they were assumed to also contain organic carbon.

Anaerobic serum bottle tests completed in the lab compared a sand control to the mildly organic (medium mussel density) sediment and highly organic (low mussel density) sediment. The serum bottle tests demonstrated that the presence of denitrifying bacteria was most evident in the low mussel density sediments (Figure 4.13). Within the first 24 hours of the 72 hour test, nearly all of the nitrate introduced to the low mussel density sediments had been reduced, presumably through denitrification, by bacteria. Nitrate concentrations in the medium mussel density sediments did not noticeably decrease throughout the duration of the test, indicating that there was an insufficient amount of bacteria present to reduce the nitrate. Thus, under entirely anaerobic conditions, the low mussel density sediments contain the greatest capacity for nitrate transformation.

To further investigate the presence of denitrifying bacteria and rates of denitrification within the bed, nitrate reduction beaker experiments were completed in the lab. The beakers were batch systems left open to the atmosphere to provide a better representation of the mussel bed habitat. Since the serum bottle tests indicated that denitrifying bacteria were only present in the low mussel density sediments, these were the only sediments analyzed in the beaker experiments. Also, due to the majority of nitrate disappearing within the first 24 hours of the serum bottle test, the beaker experiments were only run for a period of 24 hours.

The results of the beaker experiments further supported the serum bottle tests in revealing that reduction of nitrate was occurring in the bed and that denitrifying bacteria were present (Figure 4.14). However, the rate at which nitrate was reduced was much slower in the beakers than in the serum bottles. This can most likely be explained by the lack of entirely anaerobic conditions within the system. The reduction of nitrate over time fit fairly well (R^2=0.8511) to an exponential equation, indicating that nitrate was experiencing a first-order transformation rate. Further research will be necessary to determine more accurate denitrification rates as well as the quantity and speciation of denitrifiers present within the sediments.

4.4.2 Presence of Anammox Bacteria

Due to the potential of anammox bacteria as a pathway for nitrate reduction, sediment throughout the mussel bed was analyzed for the presence of these specialized bacteria. Sediment samples were obtained from the low, medium, and high mussel density areas of the bed to test for anammox presence. A signal for 16S rDNA was observed, indicating that bacteria were indeed present in each of the samples. However, a signal for the anammox marker was not detected, revealing that anammox bacteria were not present within the mussel bed sediments.

Examining sediments for anammox bacteria can be difficult due to the specific genes occasionally being present at very low concentrations and thus not easily identified. Since anammox bacteria have primarily been located in marine environments, it was not unexpected that they were absent in the analyzed Mississippi River sediments.

4.4.3 Microbial Community Fingerprint

The microbial community fingerprint of mussel bed sediments was assessed using terminal restriction fragment length polymorphism (T-RFLP). The T-RFLP technique does not quantify the amount of bacteria present, but rather distinguishes the diversity of microbial phylogenetic families present in the sample. T-RFLP also expresses the percentage of each family as it relates to the entire microbial population being analyzed.

Microbial community fingerprints were determined for the low and high mussel density sediments as these were assumed to contain the most significant differences in community dynamics. The high mussel density sediment was found to contain 34 different families of bacteria (Figure 4.15). For the samples analyzed, 49 percent of the microbial population belonged to the unclassified burkholderiales family. The next most prominent family contained only 6 percent of the entire population, indicating that the unclassified burkholderiales were clearly the most dominant family in the high mussel density sediments.

The low mussel density sediment contained increased microbial community diversity as 48 different families of bacteria were found (Figure 4.16). No single family appeared to dominant the population as the most prominent family, which was again unclassified burkholderiales, consisted of only 13 percent of the entire community. The distribution of families was much more uniform in the low mussel density sediments as the second most prominent family consisted of 12 percent of the entire population. This trend continued throughout the distribution of families as the third and fourth most

prominent bacterial families consisted of 10 percent and 9 percent of the total population, respectively.

Comparing the T-RFLP analysis with the grain size distribution analysis, it was evident that the post-flood, low mussel density areas support a more diverse microbial community. Examining the pre-and post-flood grain size distributions for the low and high mussel density sediments shows that the distributions were much more comparable in the pre-flood sediments than in the post-flood (Figure 4.10, Figure 4.12). Since the T-RFLP samples were obtained from post-flood sediments, it is likely that the extreme flooding caused a change in microbial community dynamics similar to the change it caused in the high mussel density grain size distributions. Based on the assumption that significant changes in physical habitat would alter microbial community dynamics, we concluded that prior to the flood, the microbial diversity in the low and high mussel density areas was likely more comparable. Since the high mussel density areas of the bed experienced the greatest change in grain size distribution and the low mussel density areas experienced minimal changes, we inferred that the pre-flood microbial fingerprint for both areas was more similar to the post-flood low mussel density sediments.

4.5 Chemical Characterization

The final characterization of the mussel bed involved analyzing certain chemical components of the bed. Chemical characterization involved studying the variation in sediment total organic carbon that existed within the bed as well as nitrate concentrations present in the water column above the mussel bed.

4.5.1 Total Organic Carbon

Total organic carbon (TOC) content was examined in 14 post-flood sediments collected in 2008 from throughout the entire mussel bed (Figure 4.17). Samples were collected in locations corresponding to where transects from the mussel density surveys were completed so comparisons could be made between TOC content and density of

mussels present. Testing the different sediments for TOC content revealed that the greatest percentages of TOC were present at the most upstream end of the bed in areas of low mussel density (Figure 4.18). The lowest percentage of TOC appeared to be present in the downstream (medium mussel density) areas of the bed as the middle of the bed (high mussel density) areas contained TOC values that ranged in between the high and low percentages. In general, the trend of TOC values decreased from the upstream end of the bed to the downstream end of the bed. Percentage of TOC throughout the bed ranged from 3.1 percent in the upstream sediments to about 0.3 percent in the downstream sediments (Figure 4.19).

For pre-flood sediments, we were hoping to infer TOC content from the pre-flood grain size distribution analysis as organic content has been found to be generally proportional to greater percent finer grain size (Thomas, 1969). However, comparing the post-flood TOC results to the post-flood grain size distribution analyses, our data indicated that this proportionality may not apply to our study site. While the greatest percentage of TOC was determined to be present in the upstream low mussel density sediments, the grain size distribution analysis indicates that the greatest percent finer grain size is located in the high mussel density sediments. Thus, if the proportionality were true for our site, the greatest TOC percentages should exist within the high mussel density sediments and the low mussel density sediments should theoretically contain the lowest TOC percentages. Since this relationship was not observed in the mussel bed, we were unable to infer pre-flood organic carbon content using percent finer grain size.

The TOC analysis did confirm a relationship between organic carbon content and microbial community diversity as research has shown that large amounts of heterogeneous organic carbon play a significant role in structuring microbial community dynamics (Zhou et al., 2002). Our study, which determined that the greatest TOC content and microbial diversity was present in the low mussel density sediments, supported the claim that sediments containing higher percentages of TOC are expected to contain the

greatest microbial diversity (assuming our organic carbon was heterogenous). Our results also supported the observation that increased carbon resources often result in more uniform diversity patterns (Zhou, et al., 2002) as the low mussel density microbial diversity distribution was much more uniform that the diversity analyzed in the high mussel density sediments.

4.5.2 Water Quality

The presence of nitrate in the overlying water was an important component to consider when determining the chemical (and biological) characteristics of the mussel bed. Mussel bed in-situ nitrate concentrations were measured by employing the use of the University of Iowa's Upper Mississippi River Envirohydrologic Observatory. The Observatory operates six sensor clusters throughout Pool 16 to measure highly time resolved data over geospatial and temporal scales to accurately predict or simulate the movement of water, nutrients, or sediments. One of these six sensor clusters is located directly in the mussel bed (Figure 4.20). Networking equipment in each sensor cluster allows for real-time data collection (every 15 minutes) to be sent back to the University via cellular modem and collected for analysis.

For our study, we focused on the nitrate-N data obtained from the sensor clusters during the three month period of June – August 2009. Plotting the entire data set (Figure 4.21) for the three month period indicated that nitrate-N concentration was highly variable, ranging from values of 5 mg/L to over 22 mg/L. The majority of the data points fell within the range of 5 mg/L to 13 mg/L, which was determined to be relatively reasonable (although a little high) for the Mississippi River. It should be noted that the primary purpose for examining the water quality data was to investigate trends, not concentrations, as grab samples were not obtained to support the collected sensor data.

During our analysis of the data, we observed an apparent diurnal pattern in nitrate-N concentrations (Figure 4.22). Our initial assumption is that this diurnal trend is

caused by the mussels excreting ammonia which is then nitrified to nitrate in the water column. This assumption is based on the absence of a diurnal cycle observed at another one of the sensor clusters located in the main channel near the mussel bed at LACMRERS (Figure 4.22). The hydrologic characteristics of the mussel bed (decreased velocities, circulation of flow) may increase the phytoplankton concentrations present in the bed which could also cause a diurnal nitrate-N pattern.

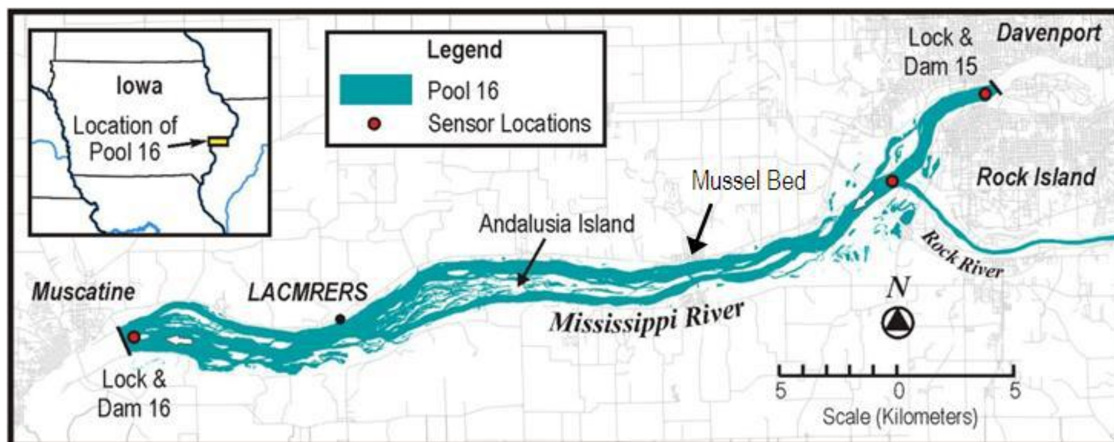

Figure 4.1: Location of mussel bed study site within Navigation Pool 16 of the Upper Mississippi River Basin (UMRB).

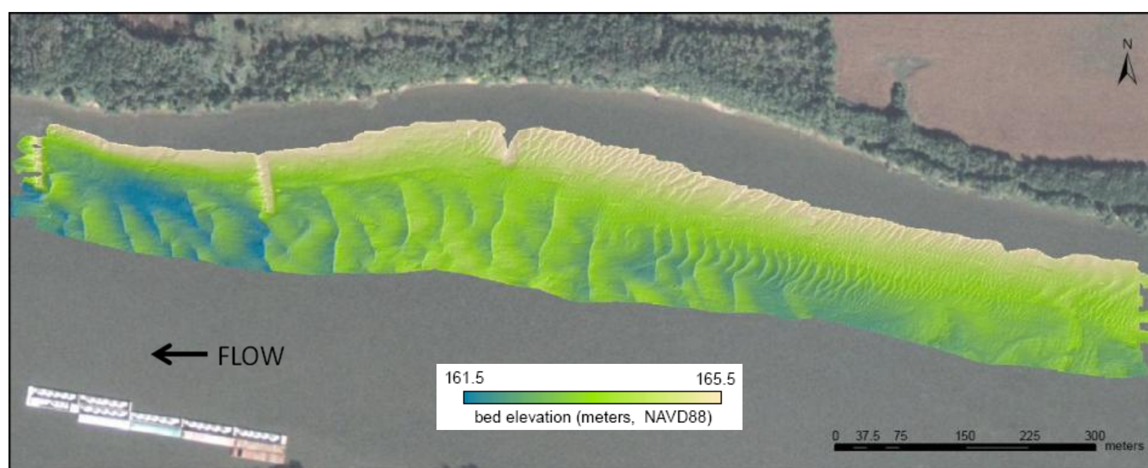

Figure 4.2: Multibeam bathymetric survey for Pool 16 mussel bed completed August 2008.

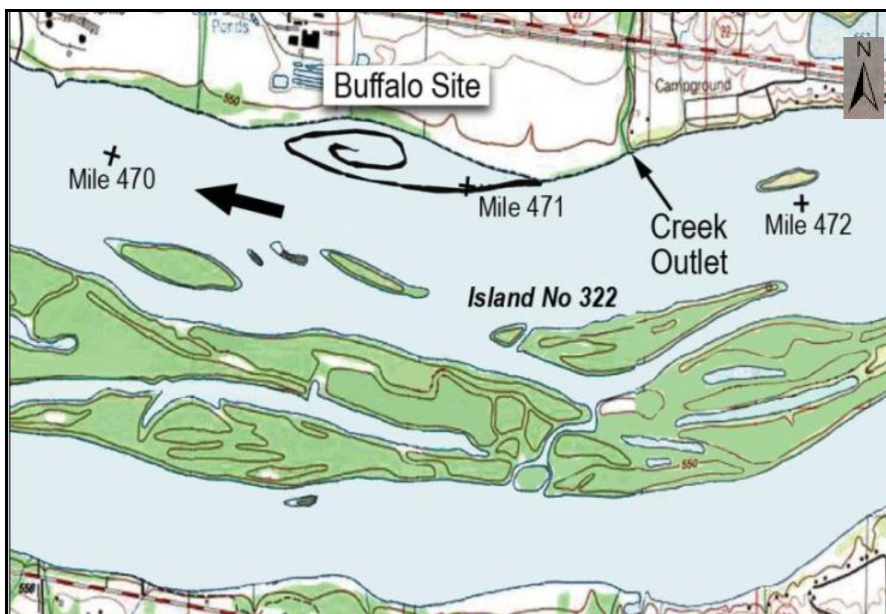

Figure 4.3: Estimated circulation of flow occurring within mussel bed causing decreased velocities in upstream section of bed.

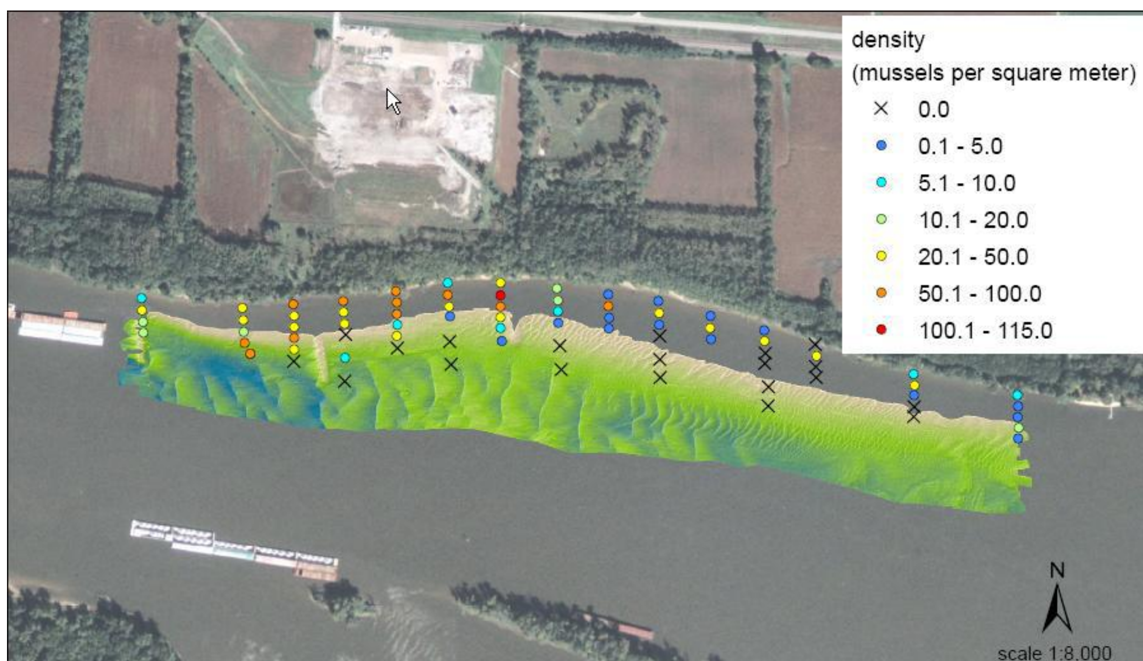

Figure 4.4: Density of mussels (mussels per square meter) located in Pool 16 mussel bed in 2003 (Helms, 2003).

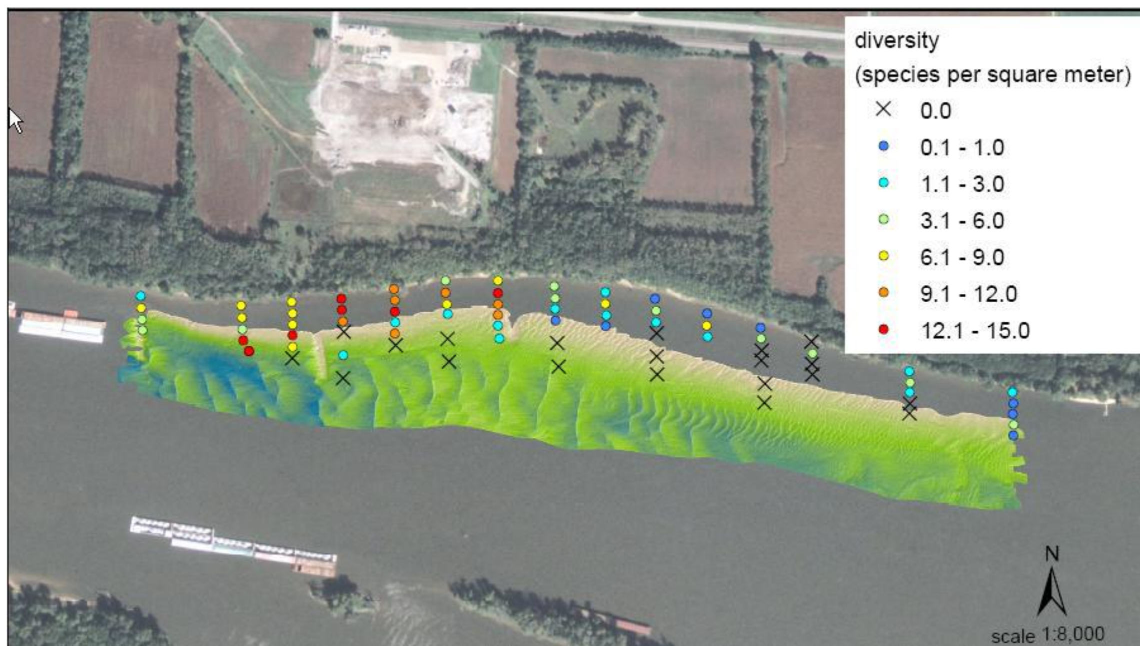

Figure 4.5: Diversity of mussels (species per square meter) located in Pool 16 mussel bed in 2003 (Helms, 2003).

Figure 4.6: Diversity of mussels found during mussel bed site exploration trip conducted in 2008.

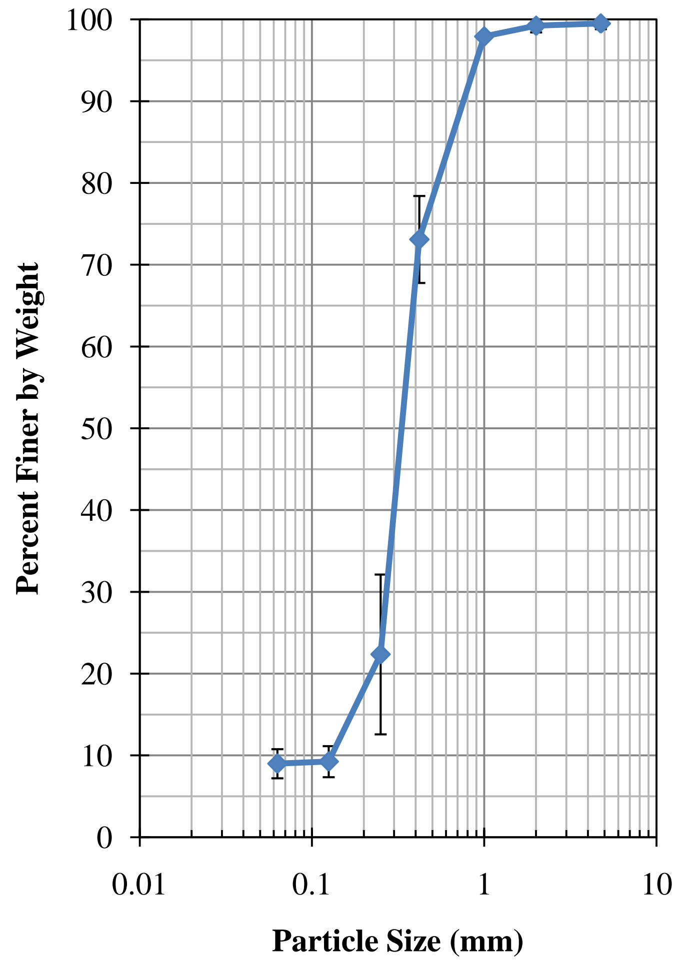

Figure 4.7: Grain size distribution for sediments located within low mussel density sections of mussel bed.

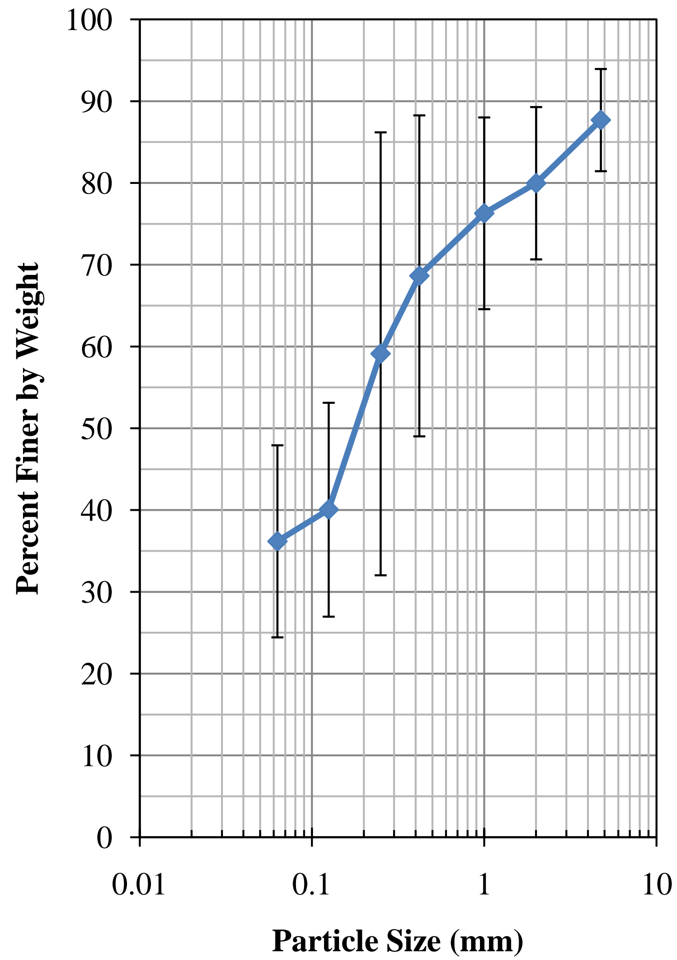

Figure 4.8: Grain size distribution for sediments located within medium mussel density areas of the mussel bed.

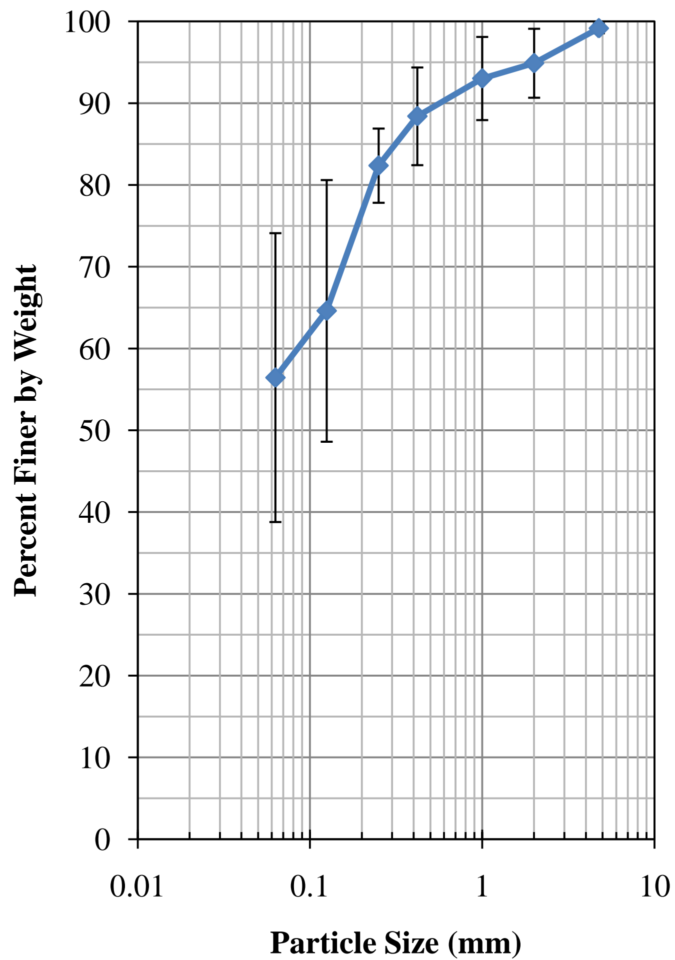

Figure 4.9: Grain size distribution for sediments located within high mussel density areas of the mussel bed.

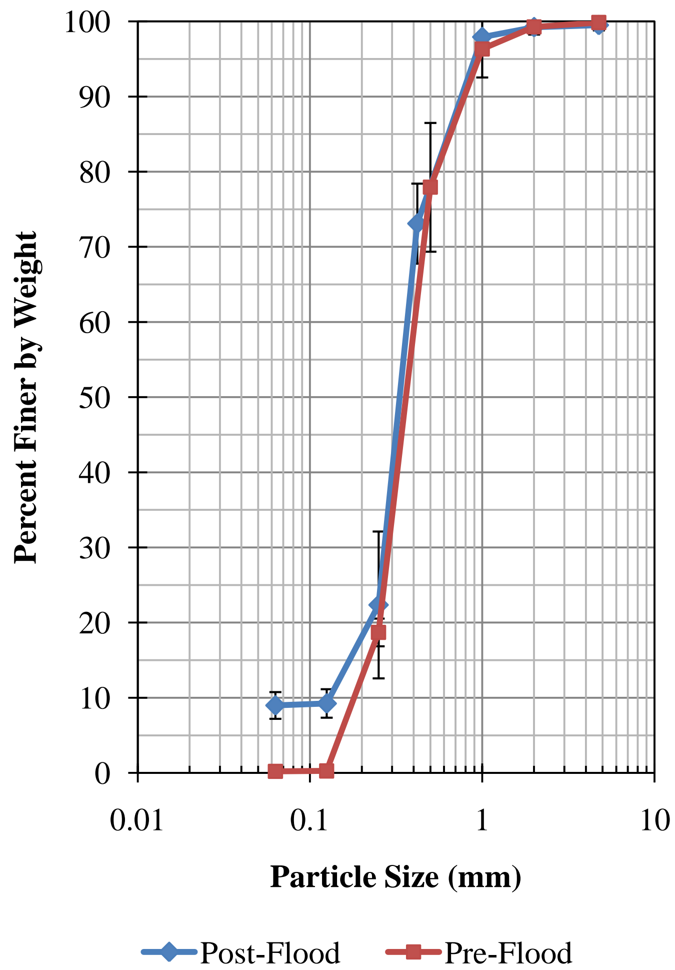

Figure 4.10: Comparison of grain size distribution for post-flood (2009) and pre-flood (2004) sediments located in low mussel density areas of the bed.

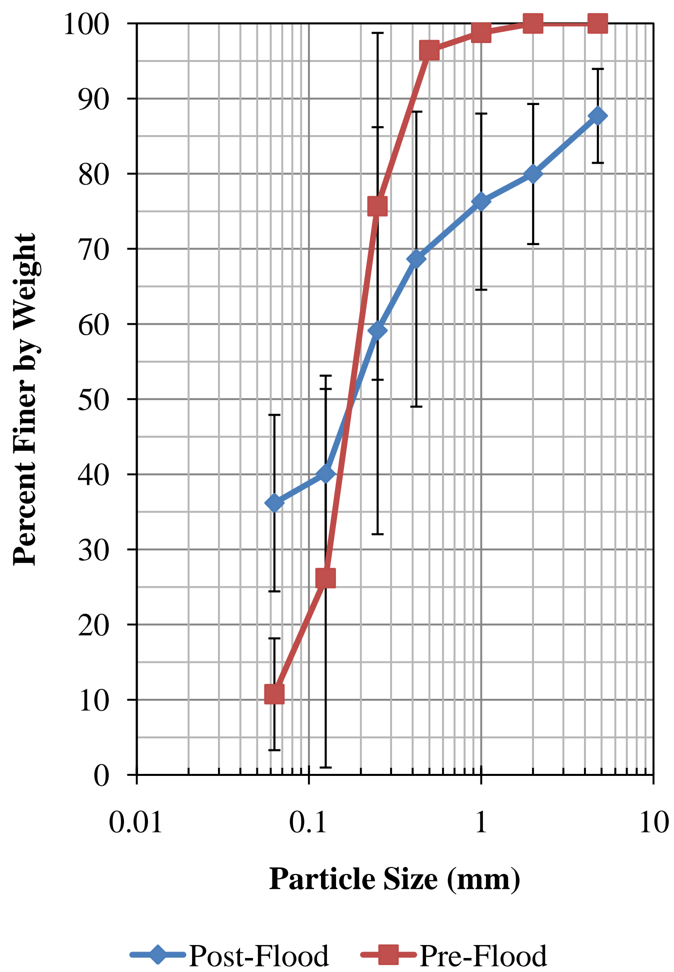

Figure 4.11: Comparison of grain size distribution for post-flood (2009) and pre-flood (2004) sediments located in medium mussel density areas of the bed.

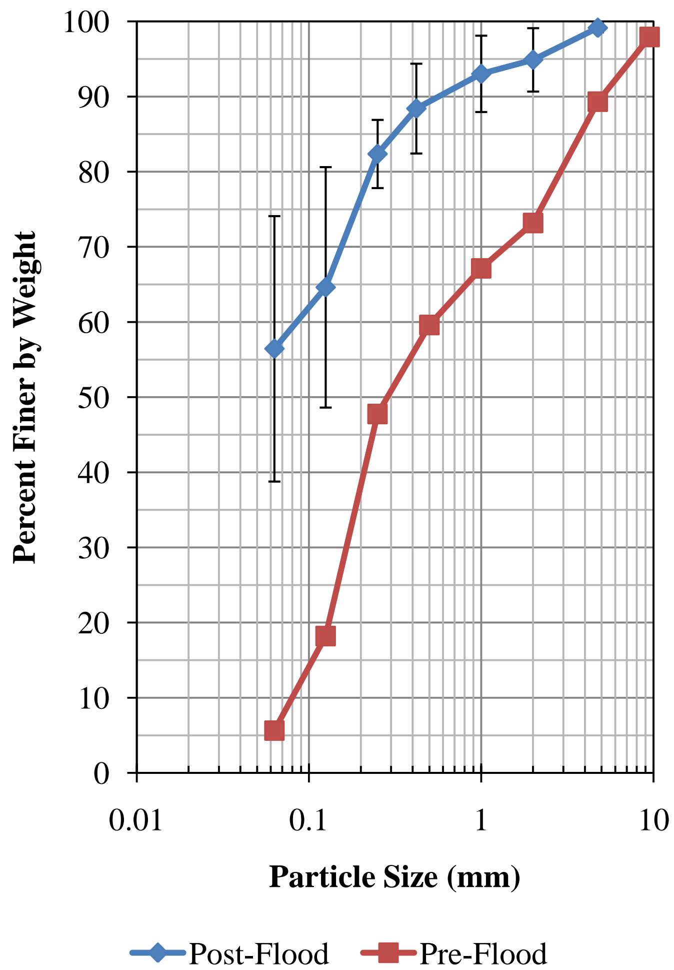

Figure 4.12: Comparison of grain size distribution for post-flood (2009) and pre-flood (2004) sediments located in high mussel density areas of the bed.

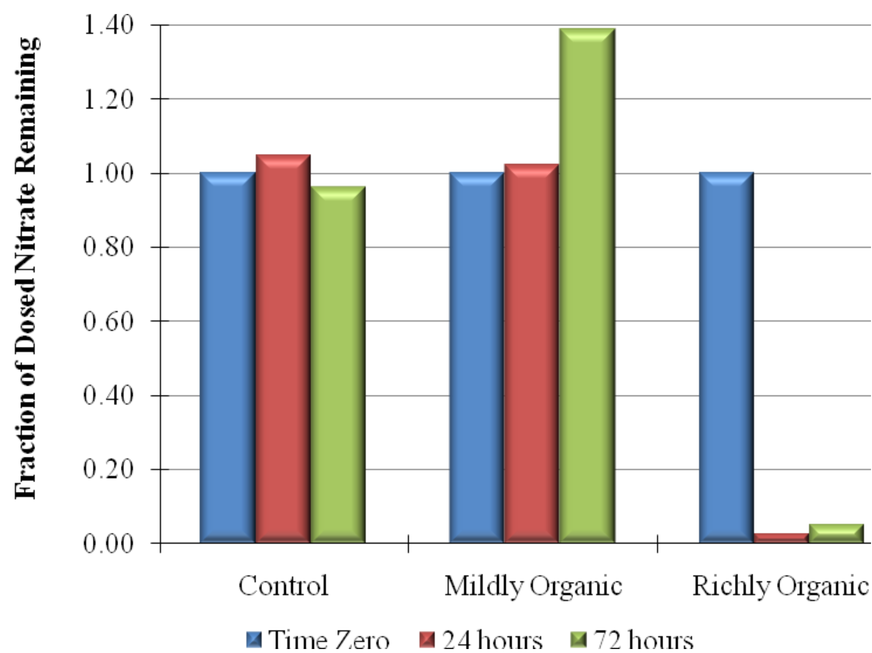

Figure 4.13: Serum bottle nitrate reduction in mildly organic (medium mussel density) and richly organic (low mussel density) sediments compared to a sand control.

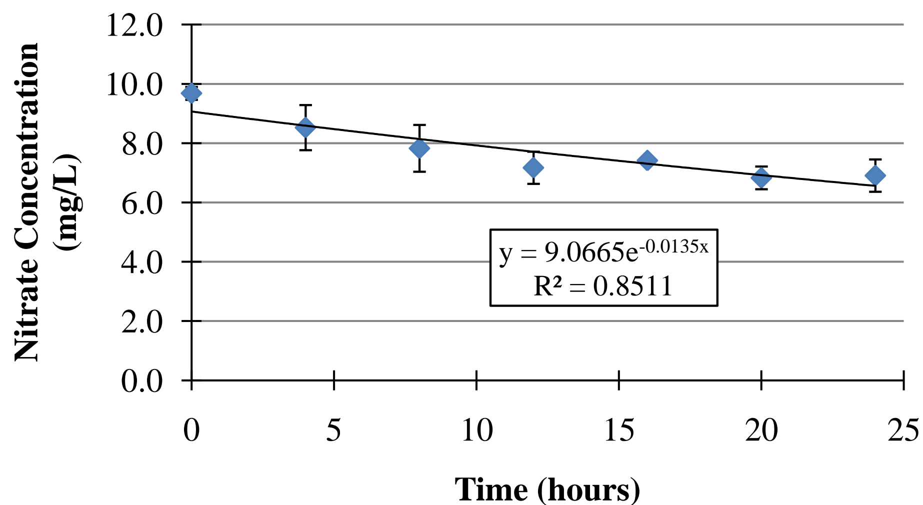

Figure 4.14: Reduction of nitrate over time in low mussel density sediments (standard deviations based on samples analyzed in triplicate).

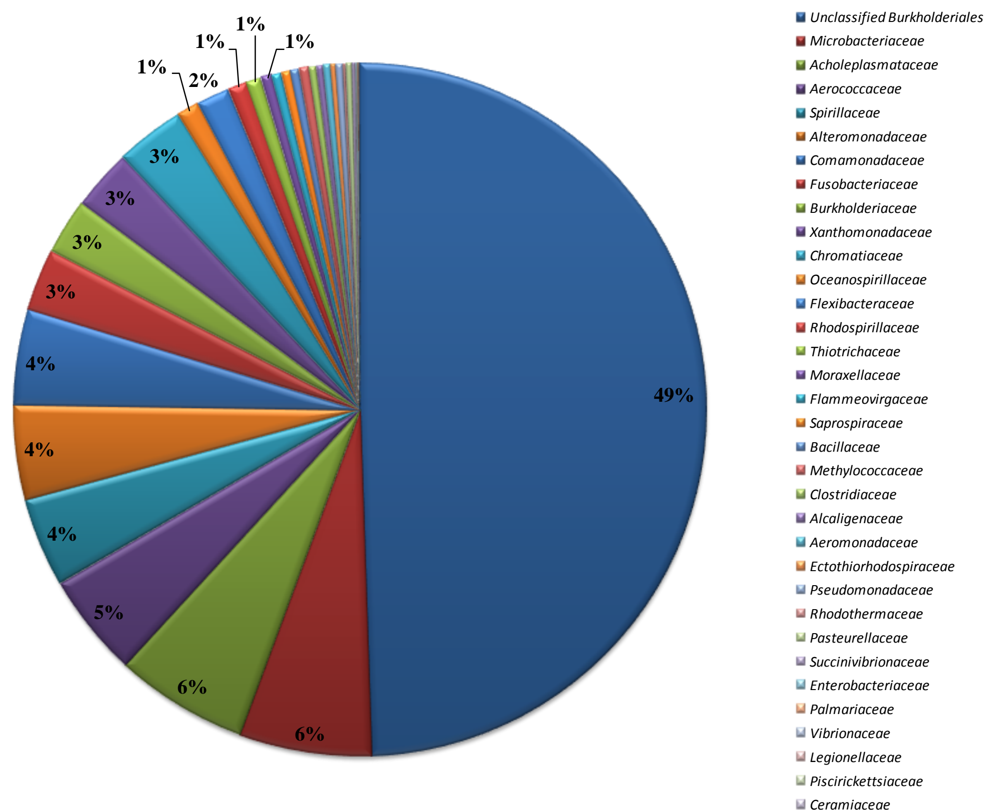

Figure 4.15: Microbial community fingerprint for high mussel density sediment (all families consisting of less than 1 percent of sample population not labeled).

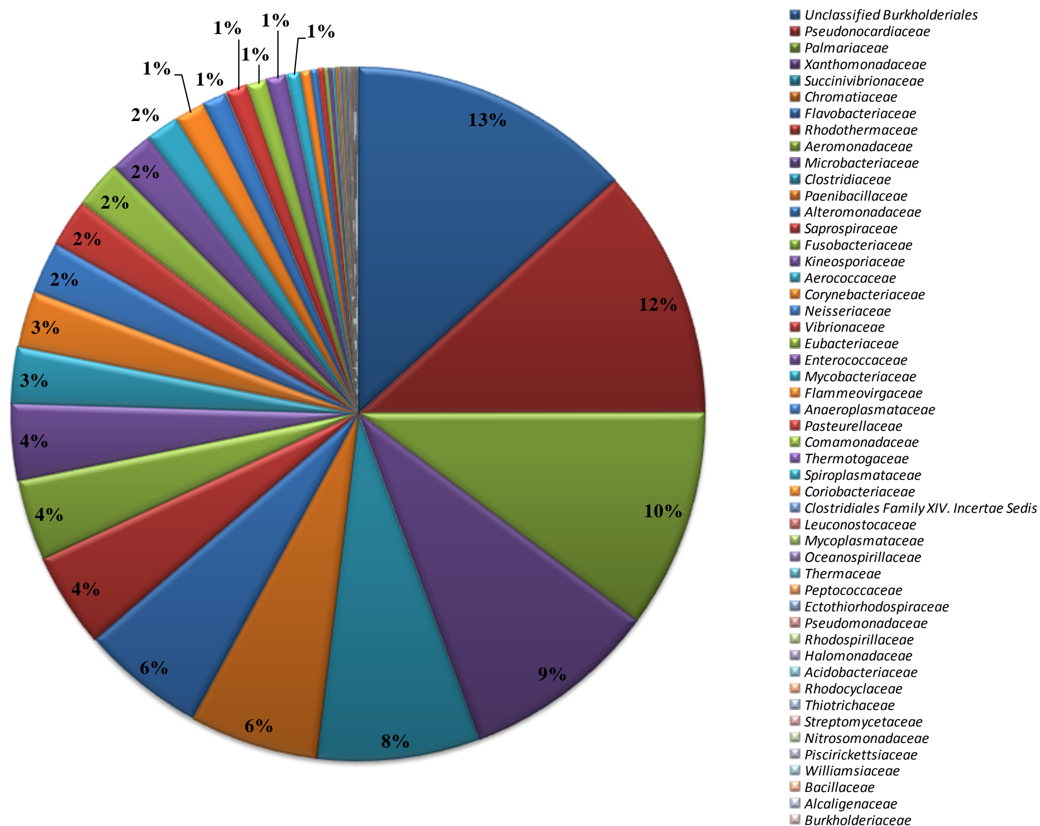

Figure 4.16: Microbial community fingerprint for low mussel density sediment (all families consisting of less than 1 percent of sample population not labeled).

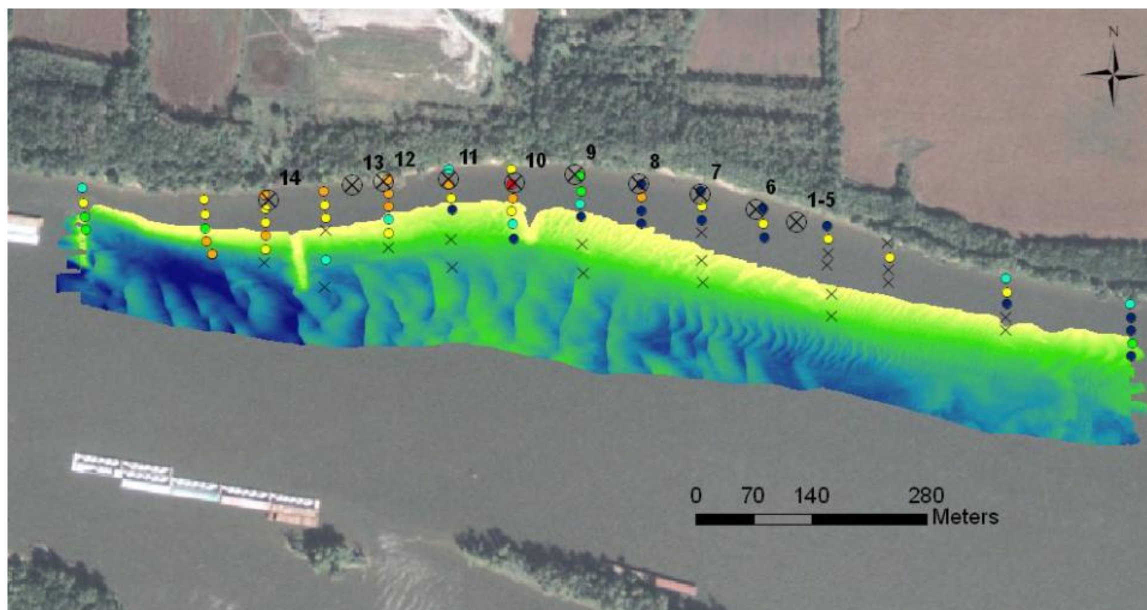

Figure 4.17: Locations of samples obtained for total organic carbon (TOC) analysis relative to mussel density and bathymetric survey data.

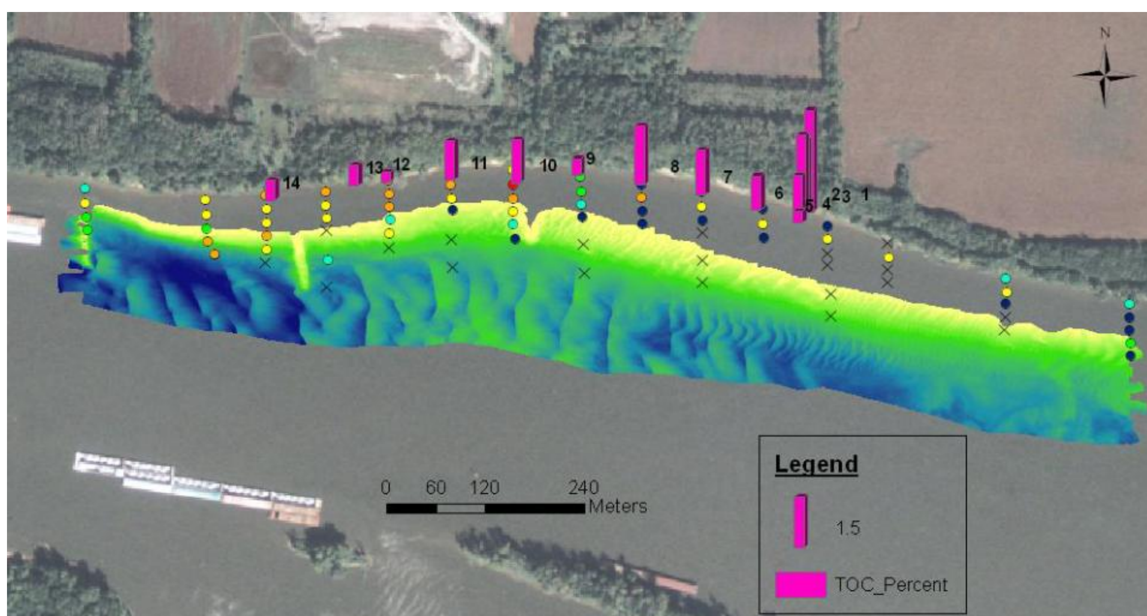

Figure 4.18: Variation of total organic carbon (TOC) content throughout mussel bed relative to mussel density and bathymetric survey data.

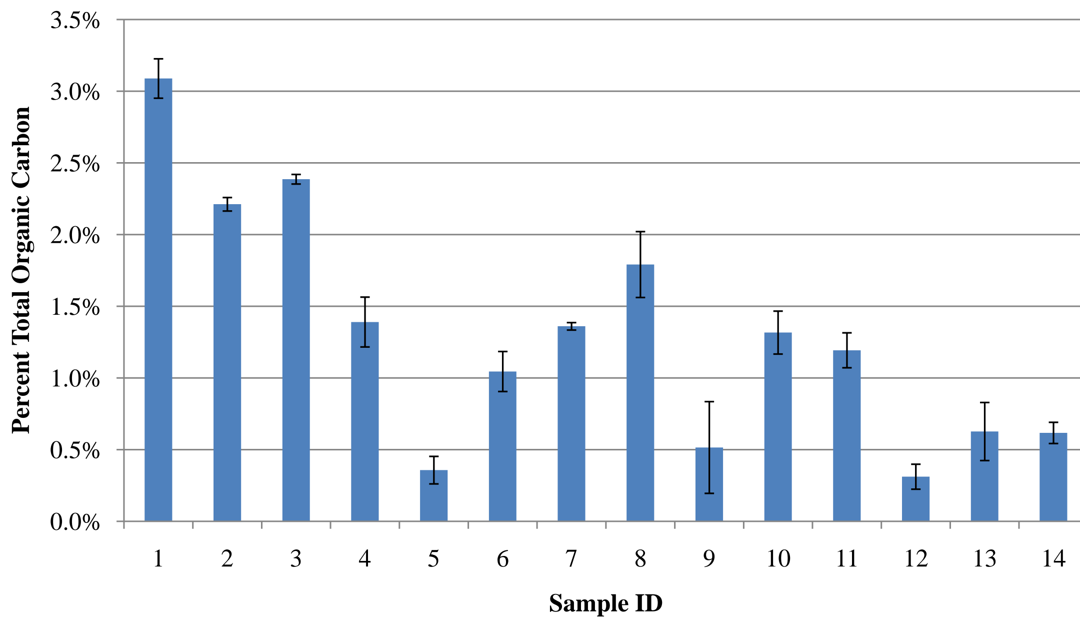

Figure 4.19: Average percent of total organic carbon (TOC) for each sediment sample obtained from mussel bed (standard deviations based on samples analyzed in triplicate).

Figure 4.20: Example of sensor cluster buoy used to measure nitrate concentration located in mussel bed.

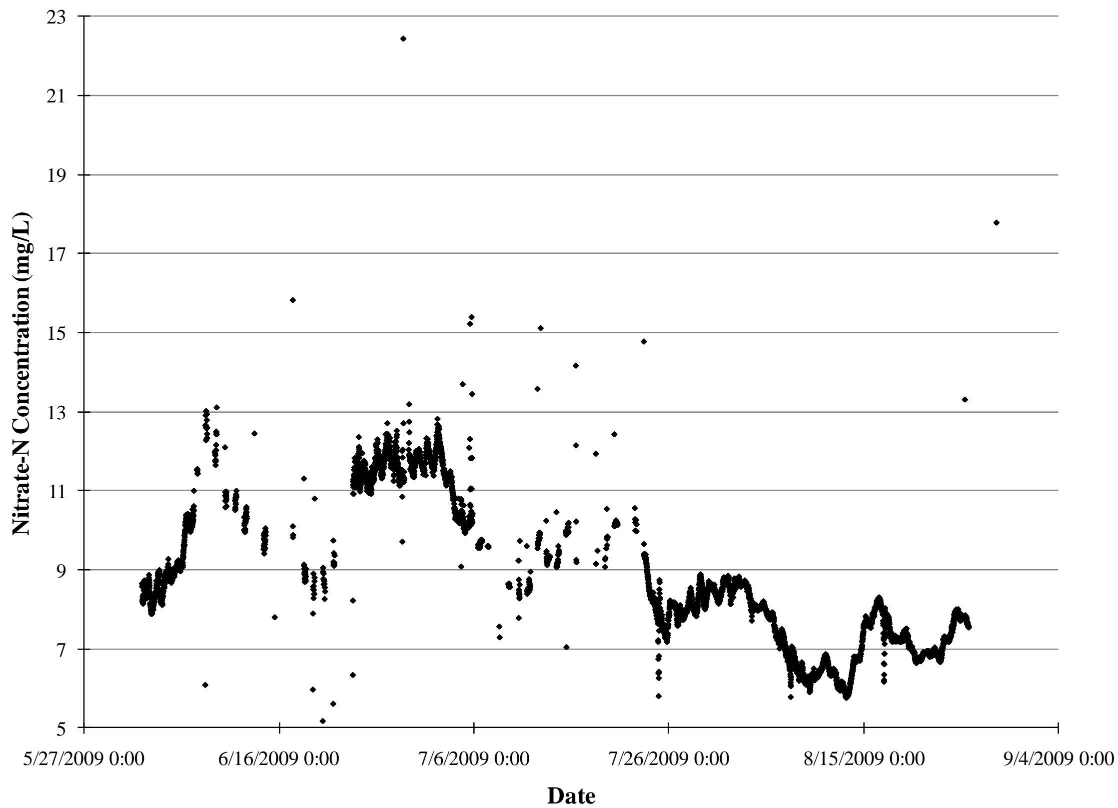

Figure 4.21: Nitrate concentrations over time for three month period during 2009.

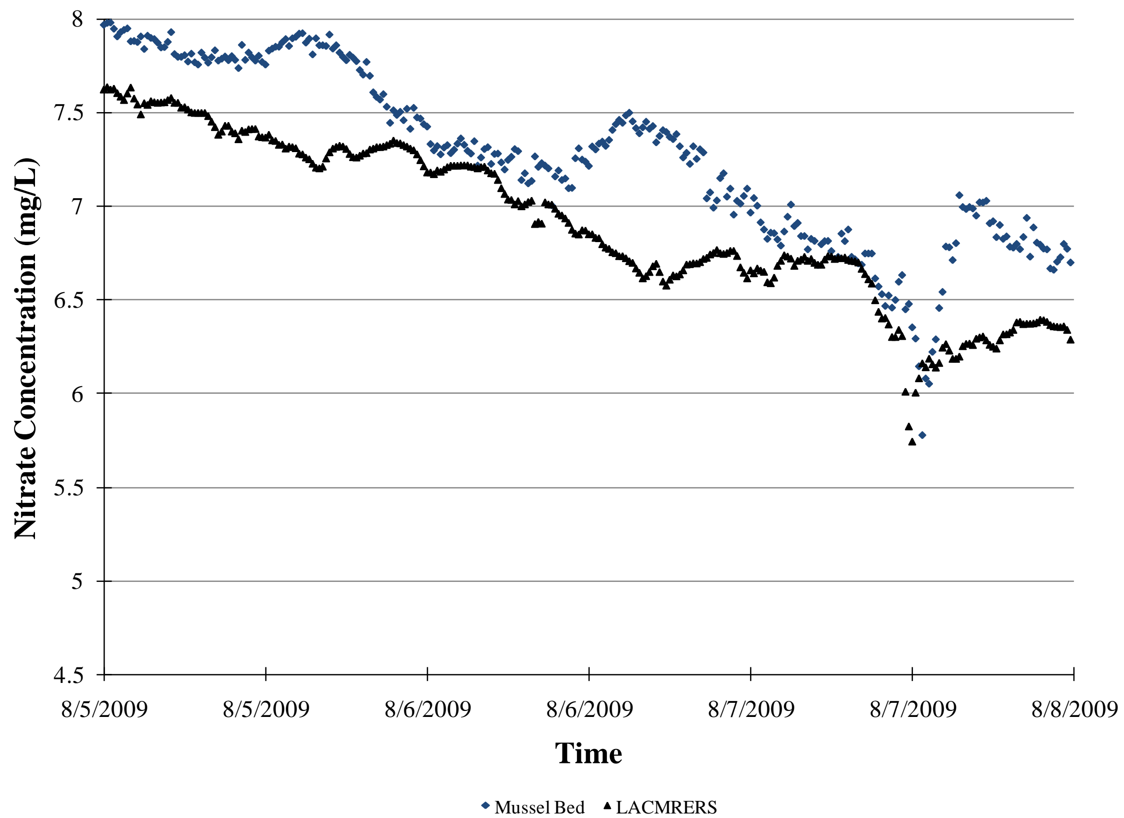

Figure 4.22: Evidence of diurnal pattern differences in nitrate concentrations between water column above mussel bed and in main channel near LACMRERS over a three day period in August 2009.

CHAPTER 5

MUSSEL LABORATORY MICROCOSM

5.1 Introduction

The purpose of this chapter is to discuss the development of a laboratory microcosm to house the mussels during our study. The chapter will first discuss how the microcosm was constructed and modified to best represent the mussels' natural habitat. It will then discuss survivability studies conducted in the lab and initial data from a gape response experiment.

5.2 Configuration and Setup

To enable the investigation of mussel responses to different environmental conditions, we built and maintained a fully-equipped mussel microcosm in a wet laboratory housed within the University of Iowa Drinking Water Treatment Plant facility. This facility is located to the adjacent to the Iowa River which served as a convenient, natural water source for mussel feeding operations.

Mussels were collected from the Pool 16 mussel bed through a number of sampling campaigns. We gathered mussels by wading into the water and using kick-nets to collect individual mussels (with care taken to reject any endangered species). Mussels were then transported to the lab via plastic containers full of river water. Once at the lab, mussels were placed in a large 20-gallon plastic tank. Mussels were placed on top of four inches of clean sand and then 10 gallons of raw untreated Iowa River water was poured into the tank. To provide sufficient oxygen for the mussels, an aeration block was placed in the tank. The river water in the tank was drained and refilled two-three times per week to provide the mussels with necessary nutrients and to remove any accumulated wastes. Each time the water was replenished we examined and documented any noticeable

changes that occurred in the microcosm habitat (e.g. mussel movement, burrowing, and death) (Figure 5.1).

Initially, the microcosm resembled an aerated batch system that required frequent replenishment of river water (Figure 5.2). After a year of operation, the system was modified to incorporate continuous flow of water through the microcosm to better represent the mussels' natural habitat. A head tank was constructed above the mussel tank so water could continuously flow by gravity to the mussels (Figure 5.3). The head tank was hooked up to a sink in the lab and equipped with a toilet bowl float to ensure the tank remained full and at a constant head at all times. Since the source for influent water was tap water, two de-chlorination tanks were hooked up to the system prior to water entering the head tank to prevent mussel mortality. PVC piping was attached to the mussel tank so that water would enter the tank just above the sand layer (Figure 5.4). The effluent pipe was connected at the top of the mussel tank on the opposite side of the influent pipe to prevent short-circuiting of fresh water that entered the system. A valve connected to the influent pipe allowed for control over amount of flow entering the system.

Equipping the laboratory microcosm system with a flow-through component produced numerous benefits for us as well as the mussels. As previously mentioned, the flow-through system was more representative of the mussels' natural habitat. Additionally, since the microcosm had continuous flow of water and no longer represented a batch system, the volume of water in the tank was increased to 20 gallons. Continuous flow also increased the amount of oxygen within the tank. However, since flow into the tank was kept fairly low, an aeration hose was placed in the tank to make certain oxygen levels remained sufficient. The mussels were fed by draining the head tank of the tap water and filling it with 5-10 gallons of raw Iowa River water. The river water was then mixed with the tap water and allowed to slowly flow from the head tank to the mussel tank. This allowed the suspended particulates which the mussels feed on to

remain suspended rather than settling quickly as they did in the batch system. Due to the river water being slowly fed into the system, the mussels were only fed 1-2 times per week instead of the previous 2-3 times per week.

5.3 Survivability Studies

The well-documented resilience of mussels (Kramer, et al., 1989) has made them popular test subjects in the laboratory. With limited knowledge in the area of establishing acceptable laboratory mussel habitats, we were pleased with the survivability rates we obtained during our study. In the initial batch microcosm habitat, which was determined to be the poorer of the two habitats, we obtained 87 percent survival rates for mussels a 3 month time period (Table 5.1). Although they may have survived, several mussels showed signs of stress while housed in the microcosm habitat. Such signs of stress included burrowing deeply into the sand or discharging conglutinates (packets of larvae) (Figure 5.5). Both of these behaviors were determined to be fairly common for mussels as they adjusted to a laboratory setting.

Survivability rates were well over 90 percent when the microcosm was modified to include continuous flow of water through the system. A population of 50 mussels was housed in the laboratory habitat for a period of 4 months, with only four deaths observed during that time. Mussels did not seem as stressed while in the flow-through microcosm as less burrowing was observed and no conglutinates were discharged. We also observed that more mussel movement occurred in the flow-through system and mussels tended to open their gapes more often. Increased rates of mussel survivability allowed us to perform studies with the mussels without having to make frequent trips to the mussel bed or worry about high mortality rates.

5.4 Gape Sensor Data

Using mussels housed in the laboratory microcosm habitat, a gape sensor study was completed by equipping mussels with two Hall-effect sensors and a magnet. The

two sensors were glued on one shell of the mussel and the magnet was glued on the other shell (Figure 5.6). A USB-based data acquisition module and Matlab-based acquisition software were developed by Anton Kruger and Nick Sitter to enable sampling of mussel responses at 10 second intervals.

Since mussels have often been used as biological sensors to monitor water quality, the purpose of this sensor study was to determine how the mussels' gape (rhythmic opening and closing of a mussel's valve) changed under externally-imposed light cycles. The gape response was measured for four weeks under two different light cycle scenarios. The first light cycle was a normal (unaltered) cycle defined by sunlight coming into the lab through windows. The second light cycle was an altered cycle facilitated by covering the entire mussel tank with opaque paper to prevent natural light from shining in the tank. A lamp was used as a controlled light source and provided light to the mussel tank from 2:30 AM to 10:30 AM (Figure 5.7).

Through the four-week test, the raw data revealed the maximum output from the Hall-effect sensor to be 2.5 volts (data provided by Nick Sitter and Anton Kruger). However, the data also contained significant noise from an unknown external source (Figure 5.8). The noise was eliminated and the data was smoothed out by applying a median filter followed by a 5-minute zero phase moving average digital filter (Figure 5.9). Based on the filtered data, mussels expressed a clear diurnal response to light intensity. Comparing the mussel gape response in the natural light diurnal cycle with the mussel gape response in the altered diurnal light cycle indicated that the light cycle appeared to have shifted by approximately 10 hours (Figure 5.10). This strongly suggests that the mussel adjusted its gape to the externally-imposed light cycle, revealing that mussels possess an apparent sensitivity to light. Additionally, our study showed that when new river water was introduced to the habitat, the mussels' diurnal cycle was disrupted and appeared to skip a cycle (Figure 5.9). Further research will be necessary to determine whether the disruption was caused by a change in environmental conditions

(e.g. influx of phytoplankton in the fresh river water) or a stress response due to the habitat being temporarily drained during water replacement.

Figure 5.1: Example of changes in mussel microcosm documented when water was drained and replenished.

Figure 5.2: Initial aerated batch system mussel laboratory microcosm.

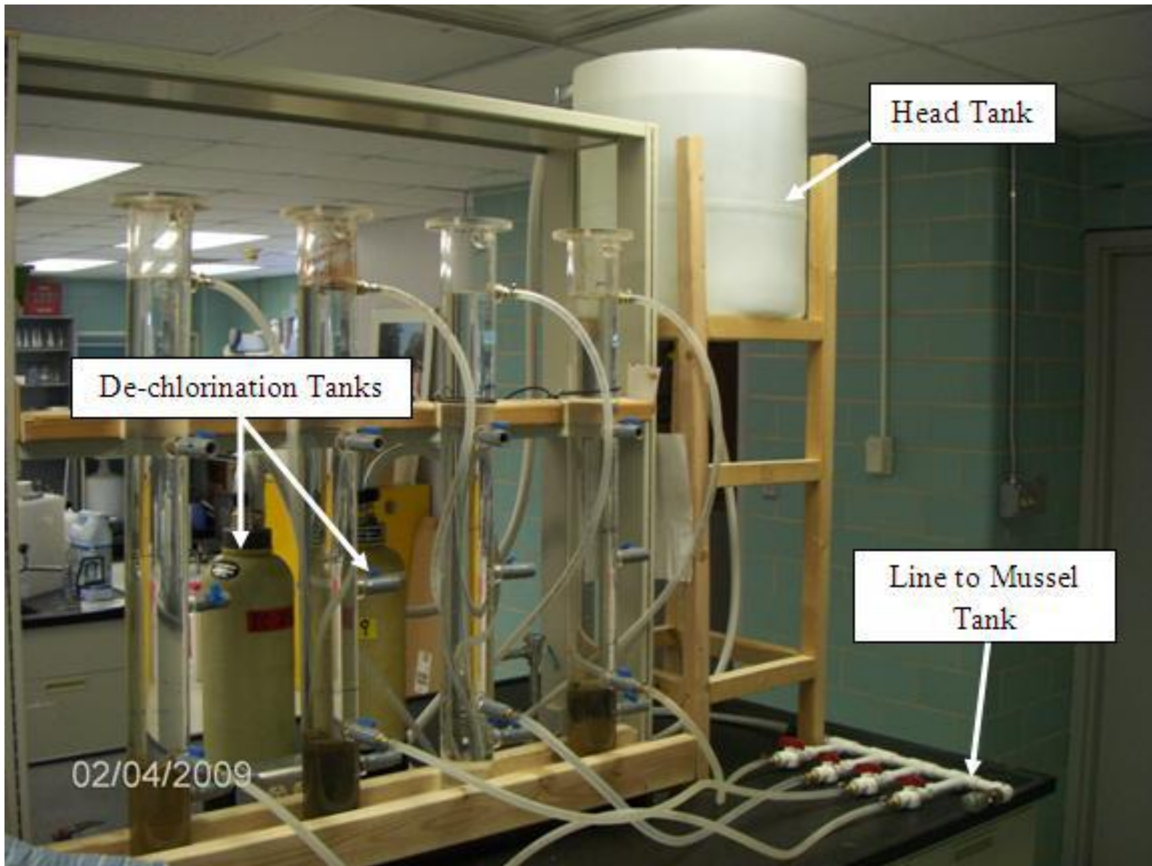

Figure 5.3: Modified microcosm habitat to incorporate continuous flow of water through system.

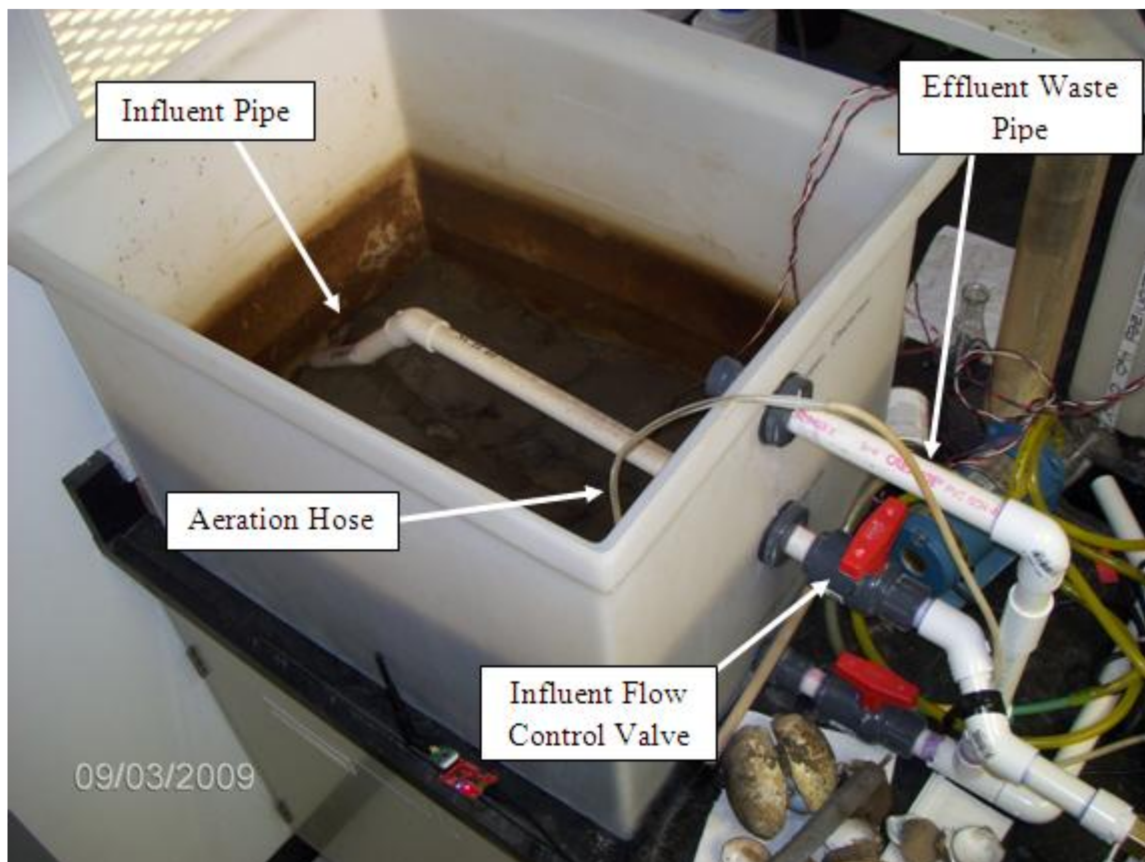

Figure 5.4: Mussel microcosm flow-through system equipped with influent and effluent pipe, flow control valve, and aeration hose.

Table 5.1: Survival rates of mussels in laboratory batch microcosm habitat.

Collection Date	No. of Mussels	No. of Deaths	Percent Survival
October 2008	17	3	82.4%
December 2008	20	2	90.0%
Total	**37**	**5**	**86.5%**

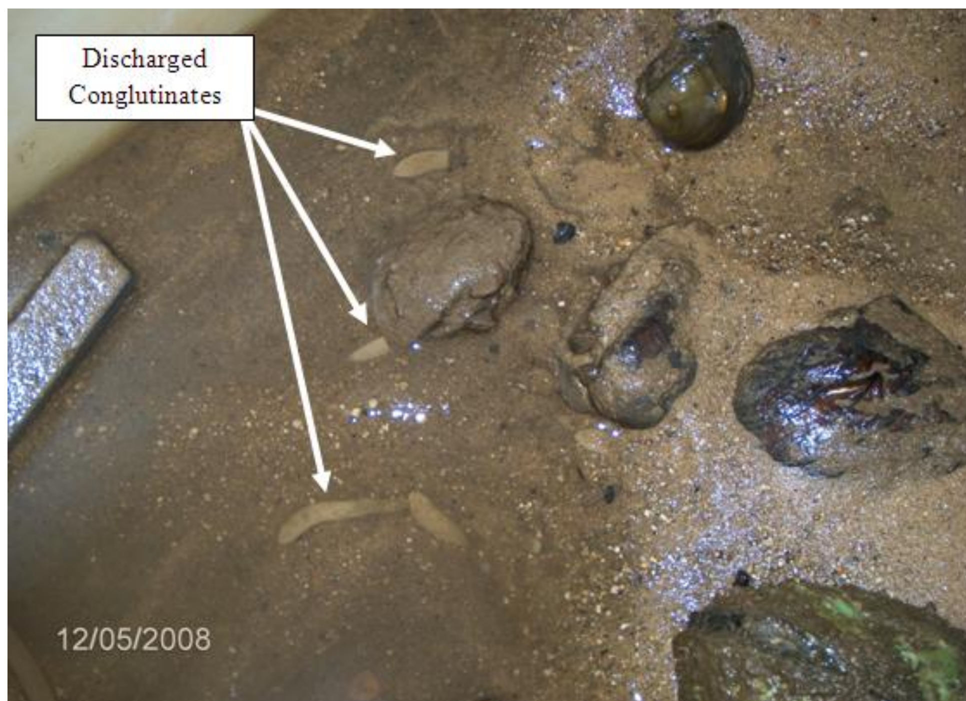

Figure 5.5: Stress expressed by mussels in microcosm evidenced by discharge of conglutinates (packets of larvae).

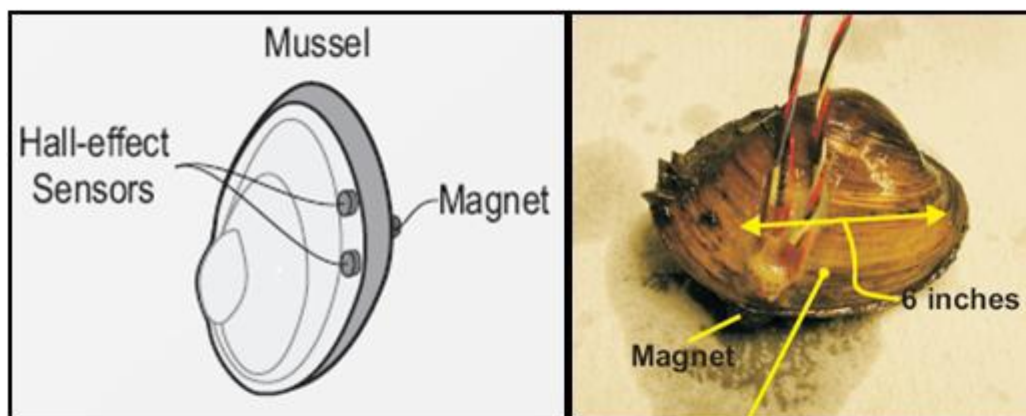

Figure 5.6: Schematic of mussel equipped with gape sensors (left) and actual mussel placed in microcosm habitat equipped with sensors (right).

Figure 5.7: Mussel tank covered in opaque paper to represent altered light cycle.

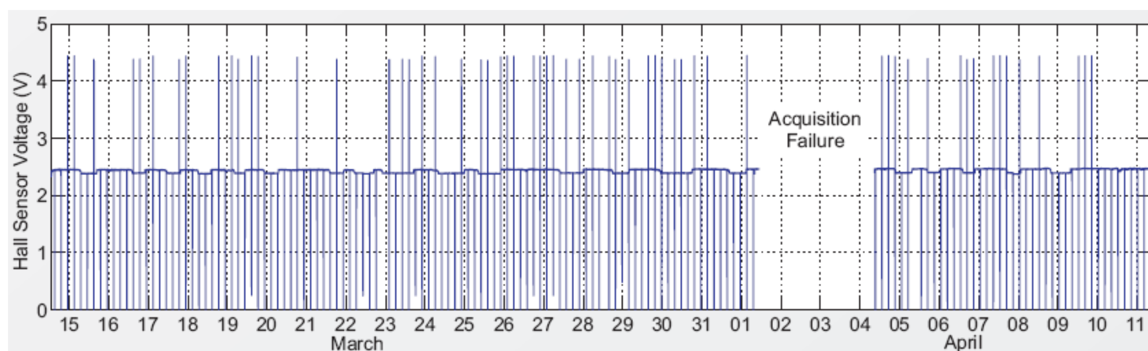

Figure 5.8: Time-series of raw data with the maximum output from the Hall sensor being 2.5 volts and the large amplitude spikes being noise from an unknown external source.

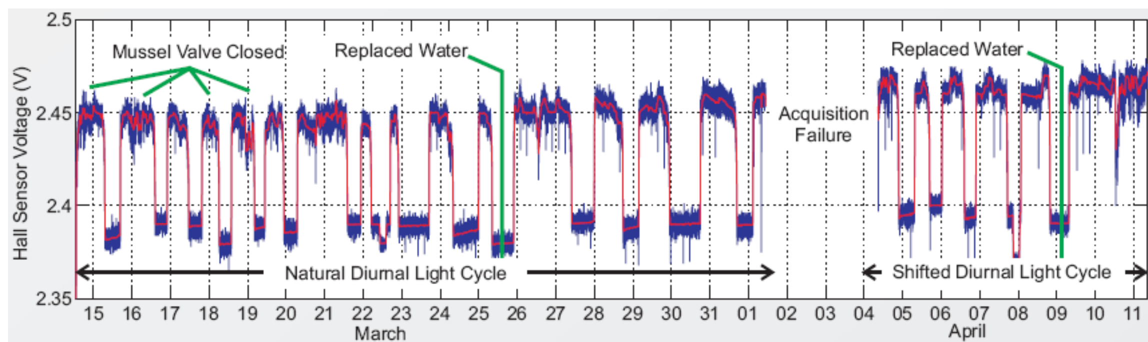

Figure 5.9: Raw data after application of a median filter to remove impulse noise (blue), followed by a 5 minute zero-phase moving average-type digital filter (red) (low voltages indicate mussel valve is open).

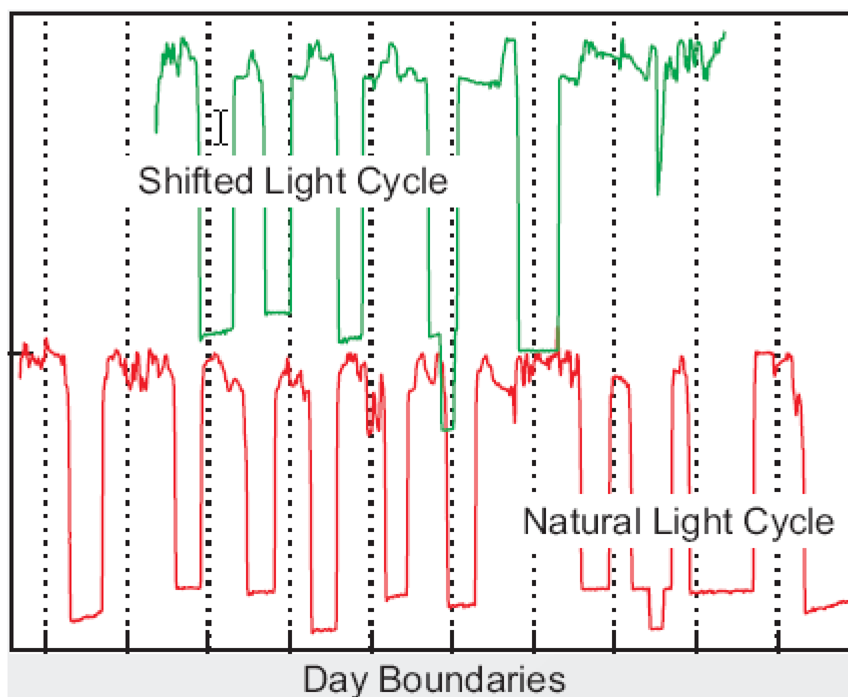

Figure 5.10: Phase comparison of mussel gape in natural light diurnal cycle (red) with mussel gape in shifted (approximately 10 hours) diurnal light cycle (green).

CHAPTER 6

DIURNAL NITROGEN DYNAMICS

6.1 Introduction

The discovery of distinct diurnal fluctuations in nitrate-N concentrations observed during the chemical characterization of the mussel bed (Chapter 4) prompted us to further investigate this phenomenon. The purpose of this chapter is to examine if the difference in diurnal fluctuations between the mussel bed and the main channel could be influenced by mussels. This was done by quantifying the mussels' potential influence on the diurnal patterns and examining other processes influencing the observed diurnal nitrogen dynamics.

6.2 Boundary Conditions

To quantify the potential impact of the mussels on the diurnal nitrate-N concentrations, data from the mussel bed was compared to data obtained from the main channel. The main channel location was defined as the sensor cluster located at LACMRERS while the mussel bed location was defined by the sensor cluster located directly in the middle of the mussel bed (Figure 6.1). The comparative analysis showed that nitrate-N concentrations collected from August of 2009 expressed distinct diurnal patterns throughout the entire month (Figure 6.2). For our study, two days worth of data were analyzed (8/5/2009 and 8/6/2009) as each expressed well-defined diurnal differences between the mussel bed nitrate-N concentrations and the LACMRERS nitrate-N concentrations (Figure 6.3).

Due to the unique hydrologic characteristics of the mussel bed (decreased velocities, circulation of flow), we assumed that modeling the mussel bed as a pool would be reasonable. Therefore, flows were assumed to be low compared to the main channel and the mussel bed was defined as a control volume. The control volume measured

1,000 m (length) by 170 m (width) by 1 m (depth) for a total volume of 170,000 m^3 ($1.7x10^8$ L) (Figure 6.4).

6.3 Nitrate-N Mass Calculations

Nitrate-N mass fluxes were calculated as the product of nitrate-N concentrations and the volume of the pool. The data of interest was collected from about 9:00 AM to 9:00 PM on both days. During this time, the mass of nitrate-N in the water column above the mussel bed started to increase around 9:00 AM, reached a maximum at 3:00 PM, and decreased from 3:00 PM to around 9:00 PM. Conversely, the mass of nitrate-N in the main channel near LACMRERS expressed a valley that decreased starting at 9:00 AM, reached a minimum at around 3:00 PM, and increased until 9:00 PM. If the mussel bed and main channel have similar hydrodynamic, physical, biological, and chemical characteristics, mass versus time plots would likely show similar diurnal patterns for nitrate-N. However, the observed patterns indicate that nitrate-N is being formed in the water column above the mussel bed while being removed from the main channel near LACMRERS.

A variation of the trapezoid rule was used to calculate the total mass of nitrate-N in the water column above the mussel bed and in the main channel near LACMRERS. First, a baseline, extending between the leading and ending edge of a peak or valley, was plotted (Figure 6.5). A series of rectangles were then used to estimate the total area of each peak or valley. The height of the rectangle at each time step was computed by averaging two adjacent measured points and subtracting the concurrent averaged baseline points. The area of the rectangle was obtained by multiplying the derived height by the 15 minute time step. This process was repeated until a rectangle was established for each time step during the evaluated period (Figure 6.6). The total mass was then estimated by summing the area of all the rectangles in each peak or valley (Figure 6.7) and dividing by the total time (in minutes). The resulting mass fluxes were determined to be the indirect

contribution of nitrate-N excreted by the mussels for each day. The first day evaluated (9/5/2009) was estimated to have a total nitrate-N mass of 40 kg while the second day (9/6/2009) was estimated to have a total nitrate-N mass of 60 kg (Table 6.1).

6.4 Mussel Ammonia-N Contribution

To determine if the mussels in the bed could indirectly form the amount of nitrate-N mass calculated for each day, we estimated the amount of ammonia-N excreted by the mussels in the bed. Mussel excretion of ammonia-N is most likely attributed to their production of a hypo-osmotic urine that consists primarily of ammonia (Burton, 1983). Determination of ammonia-N excretion rates in the mussel bed allowed us to examine if the diurnal fluctuation of nitrate-N in the water column above the mussel bed was indirectly influenced by the presence of mussels.

The ammonia-N excretion rates were obtained from a variety of literature sources and ranged from 0.003 to 0.042 mg NH_3/h/g dry weight (Baker & Hornbach, 2000, 2001; Naimo, Atchison, & Hollandbartels, 1992; Vaughn, et al., 2008). Mussel dry tissue biomass (dry weight) was estimated based on a mussel survey conducted in 2007 in Navigation Pool 18 of the Mississippi River. Since testing mussels for tissue dry mass is costly and requires the destruction of a large number of individuals (Golightly & Kosinski, 1981), the survey used length-mass regressions for mussels in the UMR (prior to the zebra mussel invasion). The average size across all mussel species was determined to be 45 mm (1.75 inches) and the corresponding dry tissue mass was calculated to be 1.4 g per mussel (Newton, 2010).

We estimated the total number of mussels present in the mussel bed by obtaining an overall average mussel density for the control volume and multiplying this density by the surface area of the control volume (170,000 m^2). Since the 2003 mussel survey reported the density observed at each site as a range, the median value was used to calculate the overall average density (Table 6.2). This technique resulted in an overall

average mussel density of 24 mussels/m^2 and an estimated mussel population of 4,080,000. Using the assumption that each mussel contained a dry tissue weight of 1.4 g, the total biomass for the mussel bed was calculated to be 5,700 kg.

Using the calculated total dry tissue biomass in the mussel bed (5,700 kg) and the estimated ammonia-N excretion rates (0.003-0.042 mg NH_3-N/h/g dry weight), the range for mass of ammonia-N excreted by the mussels in one day was computed to be 0.4 to 6 kg (Table 6.3). Comparing these values to the mass of nitrate-N measured in the water column above the mussel bed and in the main channel near LACMRERS (Table 6.4) showed that mussel ammonia-N excretion could potentially contribute 0.7 to 15 percent of the total daily nitrogen measured in the water column.

6.5 Impact of Flow Rate on Nitrate-N Mass Calculations

Mass of nitrate-N in the water column above the mussel bed and in the main channel near LACMRERS was also calculated using river flow rates. Flow rates were incorporated to obtain nitrate-N mass values to compare to the analysis which considered flow to be negligible (Section 6.3). Flow rates were obtained from three United States Geological Survey (USGS) WaterWatch gaging stations located upstream of the mussel bed (Figure 6.8). The product of flow rate and nitrate-N concentration resulted in a nitrate-N mass flux for each time step. It should be noted that time steps used in determining nitrate-N mass flux values were 30 minutes due to the lack of 15 minute flow data.

Flow rates obtained from USGS gaging stations were assumed to represent flows in the middle of the main channel. Based on the circulation of flow in the mussel bed, we assumed that the flow rate at the sensor location in the bed was equivalent to 1 percent of the determined main channel flow. Actual flow rate at the LACMRERS sensor location was estimated by fractionating the main channel flows based on the cross-sectional area of the river. The cross-sectional area of the river near LACMRERS (Figure 6.9) was

calculated using Google™ Earth and estimating depths based on a main channel depth of about 9 feet (NRC, 2005). Assuming a width of 170 m (to equate to mussel bed width), the cross-sectional area of the LACMRERS sensor location was determined to contain about 8 percent of the total cross-sectional area of the river. Flow rates in the main channel near LACMRERS were then found by assuming that 8 percent of the total cross-sectional area was equivalent to 8 percent of the total flow.

Nitrate-N mass fluxes at each location were calculated using the fractionated flow rate and corresponding nitrate-N concentrations (Figure 6.10). Incorporating flow into the analysis appeared to decrease the magnitude of the mussel bed peaks (especially on Day 1). However, the diurnal pattern remained evident as the mussel bed still expressed a slight peak when the main channel near LACMRERS demonstrated a valley. Flow rates also appeared to cause the Day 1 peak/valley to occur over a shorter period of time as the peak and valley were observed from 11:00 AM to 8:00 PM rather than the 9:00 AM to 9:00 PM observed on Day 2.

A variation of the trapezoid rule was again used to calculate the total mass of nitrate-N in the water column above the mussel bed and in the main channel near LACMRERS. Similar to the approach used in Section 6.3, a baseline extending between the leading and ending edge of a peak or valley was plotted. A series of rectangles were then used to estimate the total area of each peak or valley. Since the area of the rectangles was the product of mass flux and time, the resulting area was equivalent to mass (unlike Section 6.3 where the area had to be divided by total time). The sum of the rectangles for each peak and valley resulted in a total nitrate-N mass of 540 kg for Day 1 and 300 kg for Day 2 (Table 6.5). Comparing these values to the estimated mass of mussel ammonia-N excretion (0.4 to 6 kg) showed that mussel ammonia-N excretion could potentially contribute between 0.1 to 2 percent of the total daily nitrogen measured in the water column.

6.6 Diurnal Processes Contributing to Nitrogen Dynamics

Analysis of our hypothesis that the difference in diurnal patterns between the mussel bed and the main channel near LACMRERS was influenced by mussels showed that mussels could contribute a maximum of 15 percent of the total nitrate-N present in the water column. This indicates that other processes within the ecosystem are significant contributors to the observed diurnal fluctuations.

Based on our hypothesis, mussels would feed heavily during the night and excrete their hypo-osmotic urea primarily during the day to obtain the diurnal increase in nitrate-N. With the literature (V. Englund & Heino, 1994; R. Wilson, et al., 2005) as well as our laboratory studies (Section 5.4) indicating that mussels expressed sensitivity to light and a corresponding decrease in gap activity, nighttime feeding and daytime excretion is a reasonable assumption. However, mussels in the Mississippi are thought to be near-continuous filter feeders and thus would be assumed to continuously excrete ammonia (Newton, 2010). Also, mussels may not be exposed to a great deal of light as high levels of turbidity within the river may prevent light penetration to the river bottom. Therefore, processes correlated with phytoplankton, temperature, photolysis, bacteria, or hydrology are most likely significant contributors to the observed diurnal patterns.

Since the maxima of the nitrate-N peaks occurred during the middle of the day (about 3:00 PM), it is apparent that sunlight is an important factor influencing the diurnal nitrogen dynamics. Photolysis of nitrate is one process that could cause a difference in nitrate-N concentrations between the mussel bed and the main channel near LACMRERS. However, this process would most likely have a similar impact on the two locations as both sensors were installed on the north side of the river (Figure 6.1) under similar tree cover.

Sunlight also would impact phytoplankton growth as maximum growth rates occur during peak daylight hours likely causing a diurnal pattern similar to the nitrate-N concentrations. Assuming that increased phytoplankton would consume nitrate-N and

thus decrease the nitrate-N concentrations, mussels may impact the nitrogen dynamics by removing a large portion of the increased phytoplankton population from the water column. As mussels are continuous filter feeders and adult mussels can filter between 15-45 L/day (Newton, 2010), they may be able to remove significant quantities of phytoplankton which otherwise would be consuming nitrate-N. Phytoplankton consumption of nitrate-N would explain why nitrate-N concentrations express a valley in the main channel near LACMRERS; however, mussels removing phytoplankton from the water column above the mussel bed does not necessarily explain why nitrate-N concentrations increase. If the mussels do remove sufficient amounts of phytoplankton capable of impacting nitrate-N concentrations, we would expect that this process would simply remove the decrease in nitrate-N concentrations (valley), not cause an increase in nitrate-N concentrations (peak).

Temperature is another process impacted by sunlight that would exhibit a diurnal cycle similar to the observed nitrate-N concentrations. While exposure to sunlight is expected to be equivalent at both locations, increases in temperature could have a direct impact on other processes within the system. Mussels have been shown to increase their filtering rates as well as their ammonia-N excretion rates under increased temperatures (Vaughn, et al., 2008), which together could cause an increase in water column nitrate-N concentrations. If the increased filtering rates remove phytoplankton and thus remove the decrease in nitrate-N concentrations (valley), the increased excretion of ammonia-N could form the peak of nitrate-N concentrations. Based on this assumption, the mass of ammonia-N we calculated in our analysis could contribute anywhere from 0.4 to 20 percent of the total nitrogen found under the peaks (Table 6.6).

Additionally, increased temperature could enhance the processing abilities of bacteria present at both locations. While a comparison for microbial abundance and diversity between each location is currently unavailable, the hydrologic characteristics of the mussel bed likely provide increased amounts of total organic carbon (TOC) and

particulate organic matter (POM) which may fuel bacterial nutrient processing. Within the mussel bed we identified the lower velocity areas as containing increased TOC content and increased microbial diversity as compared to areas with higher velocities (Section 4.3.1), which could contribute to increased bacterial nutrient processing (e.g. nitrification). If temperature enhances the nutrient processing, bacteria would exhibit a diurnal pattern similar to that observed in the nitrate-N concentrations.

6.7 Diurnal Process Modeling

In an effort to capture some of these processes that may be impacting the nitrate-N diurnal pattern, we are currently working on developing a mass-balance model. The basic foundation for the model is based on the framework established in the Sediment Oxygen Demand (SOD)/Nutrient Flux Model utilized in the water quality model QUAL2K. The SOD/Nutrient Flux Model embedded within QUAL2K allows for oxygen and nutrient sediment-water fluxes to be computed based on the amount of POM existing in the overlying water (Chapra, Pelletier, & Tao, 2007). In addition to the overlying water, the model separates the sediment into an aerobic (≈10 mm) and anaerobic layer (≈10 cm).

We are currently working to modify the model to incorporate the impact of phytoplankton and mussels on the nitrogen fluxes at the sediment-water interface (Figure 6.11). Development of this model along with increased measurements of parameters such as phytoplankton concentrations, sunlight exposure, temperature, bacterial abundance/diversity, photolysis rates, turbidity (POM), and hydrologic characteristics will need to be incorporated into future research to identify the specific processes contributing to the diurnal nitrogen dynamics.

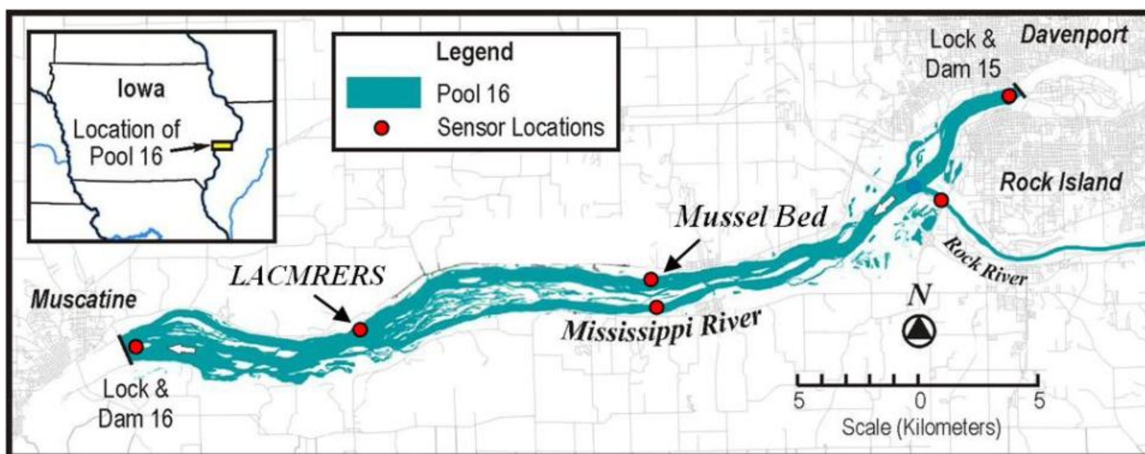

Figure 6.1: Locations of two sensor clusters (LACMRERS and Mussel Bed) used in determining daily fluxes of nitrate.

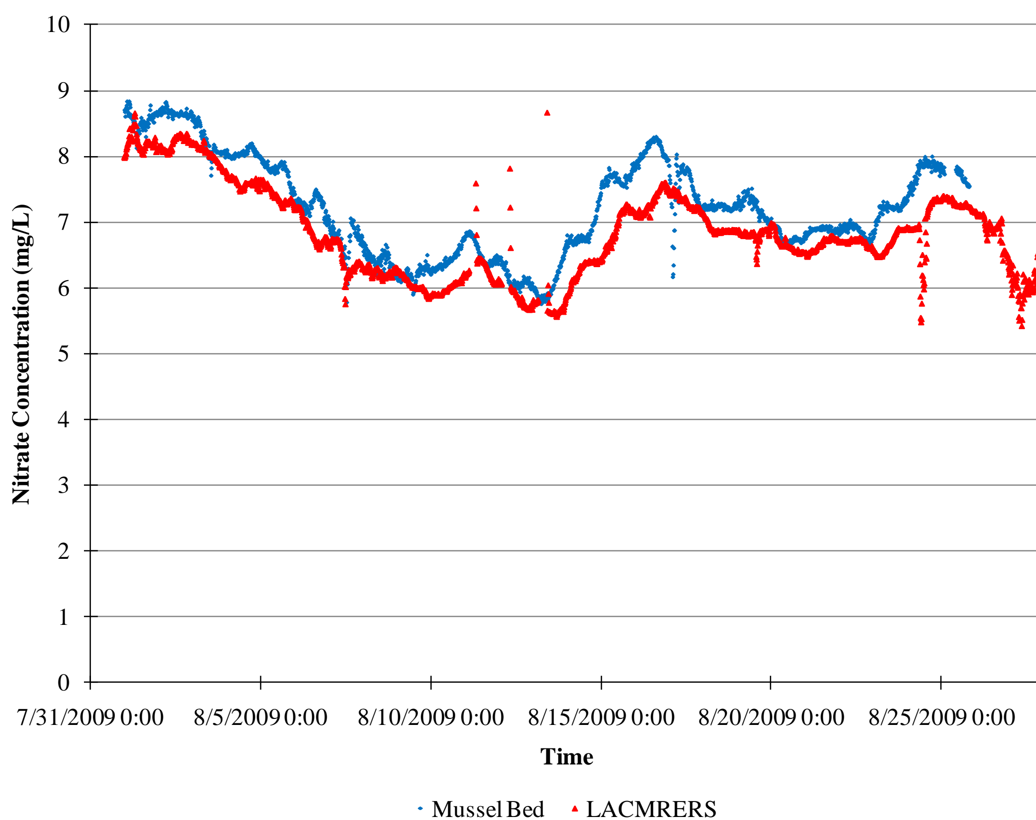

Figure 6.2: Evidence of diurnal nitrate concentrations existing throughout August 2009.

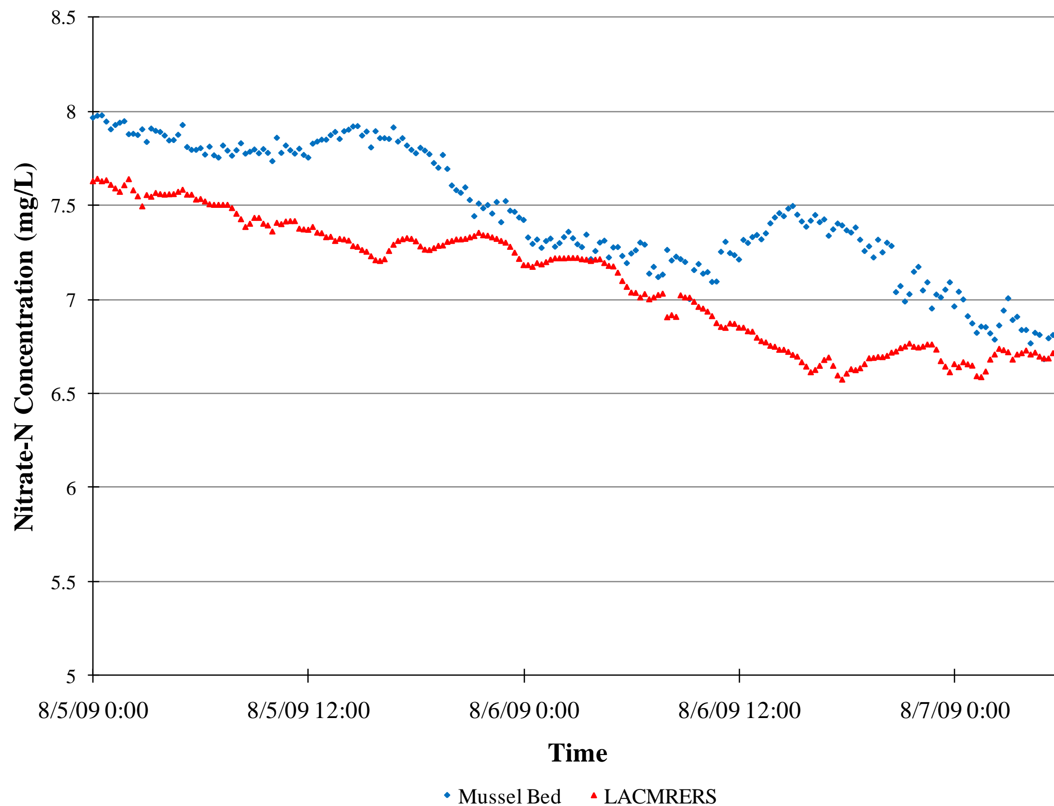

Figure 6.3: Two days worth of data analyzed to quantify contribution of mussels to daily nitrate dynamics.

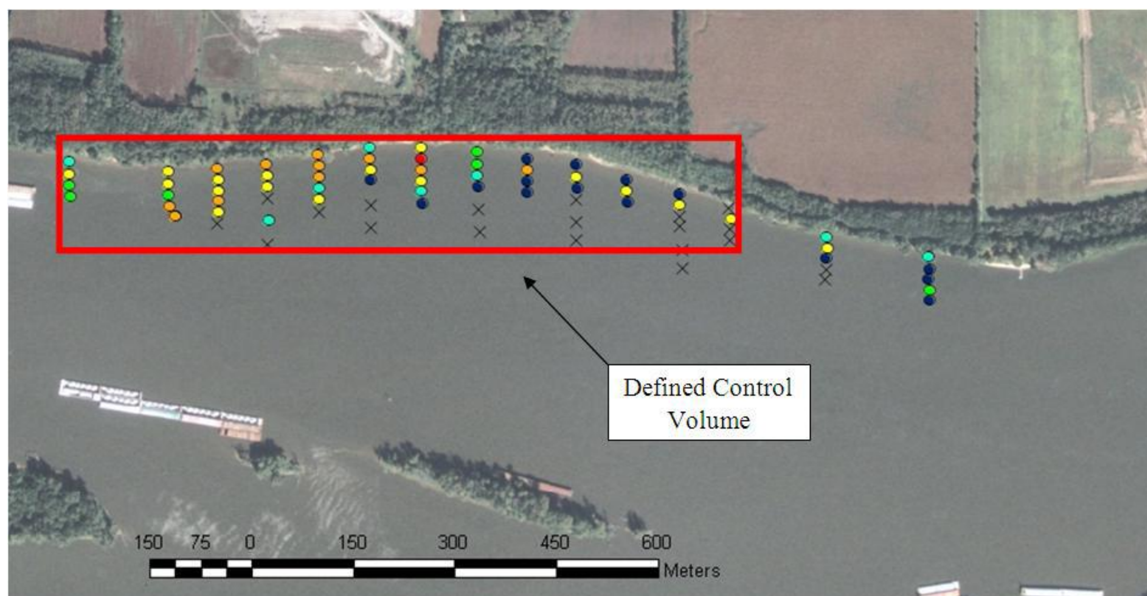

Figure 6.4: Mussel bed defined control volume used to calculate mass of nitrate present.

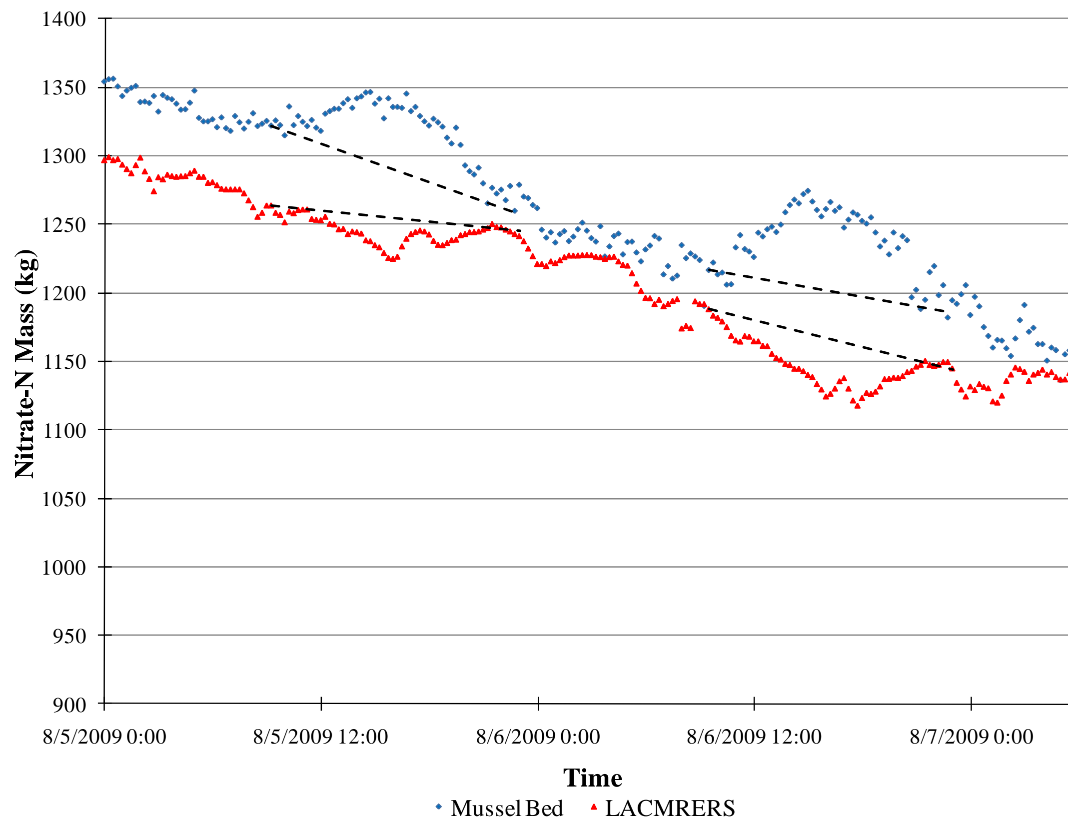

Figure 6.5: Nitrate-N mass for the analyzed days with baselines established for observed peaks and valleys.

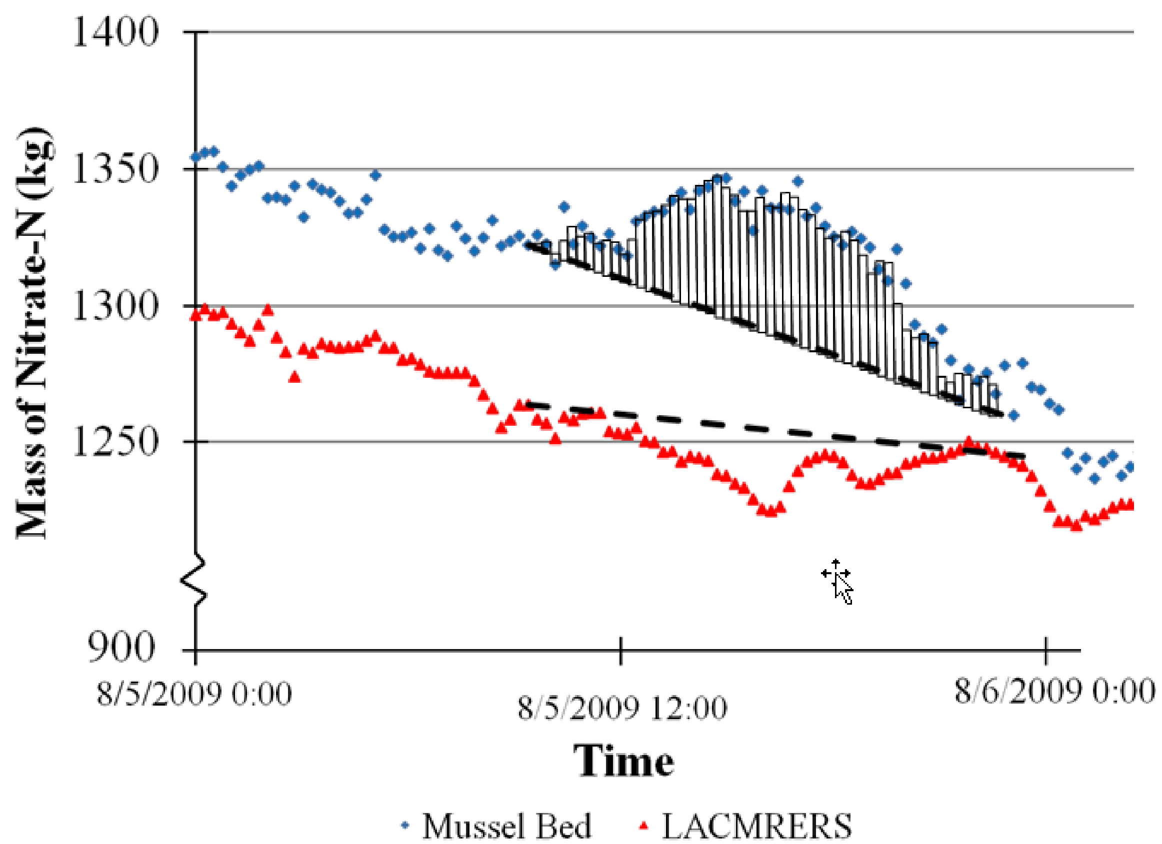

Figure 6.6: Example of trapezoidal rule used to compute total nitrate-N mass under peak.

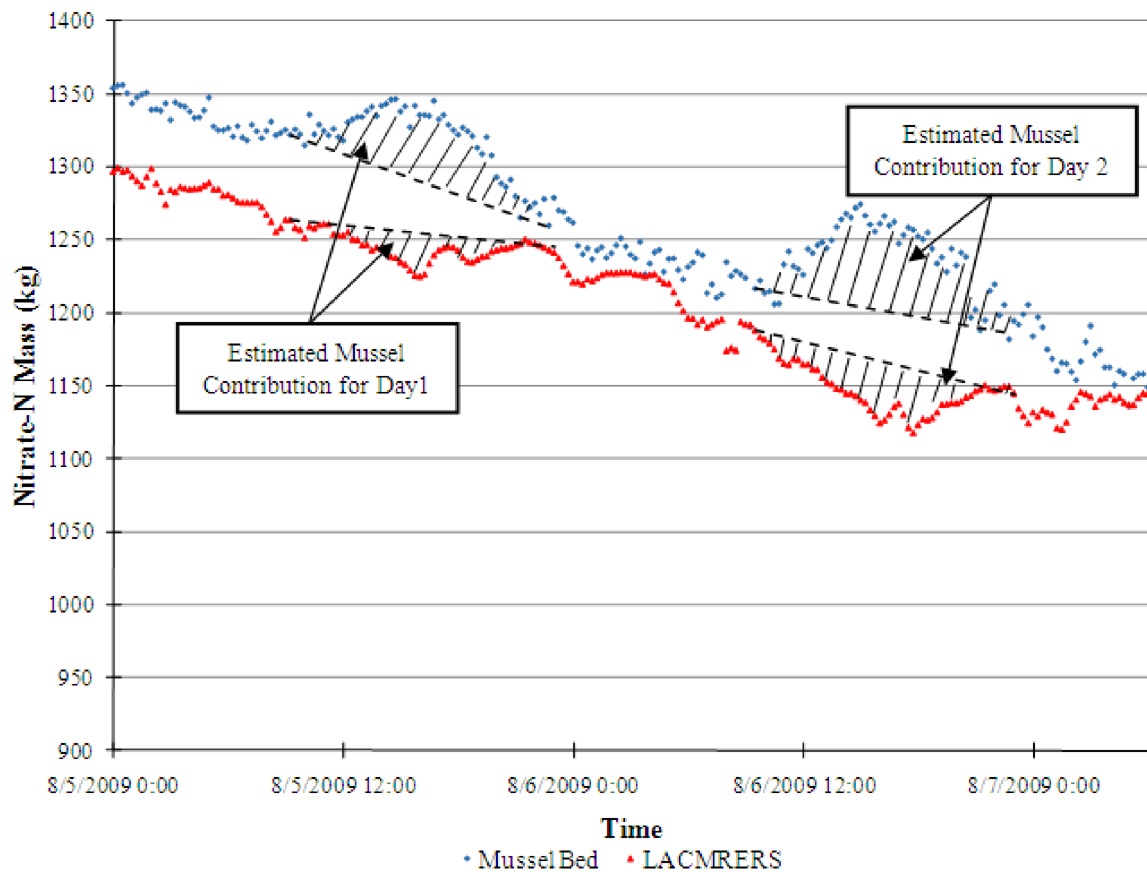

Figure 6.7: Potential contribution of total nitrate-N mass to water column by mussels for the two days analyzed.

Table 6.1: Estimated area of each peak/valley used to calculate total nitrate-N mass for Day 1 (8/5/2009) and Day 2 (8/6/2009).

	Day 1 Area (kg)	**Day 2 Area (kg)**
Mussel Bed Peak	30	40
Main Channel Valley	10	20
Total	**40**	**60**

Table 6.2: Using observed mussel densities from mussel survey to calculate overall average mussel density in control volume.

Mussel Density (mussels/m^2)	Number of Sample Sites	Total Density
0	17	0
3	11	28
8	6	45
15	5	75
35	16	560
75	11	825
108	1	108
Total	**67**	**1640**
Overall Average Density		**24**

Table 6.3: Estimated mussel excreted mass flux of ammonia-N based on different literature excretion rates.

No. of Mussels	Mussel Length (mm)	Dry Tissue Weight (g/mussel)	Total Mussel Biomass (g)	Excretion Rate (mg NH_3/h/g dry wt)	Mass Flux of NH_3 (kg/d)
4,080,000	45	1.4	5,712,000	0.003	0.4
4,080,000	45	1.4	5,712,000	0.020	2.7
4,080,000	45	1.4	5,712,000	0.023	3.2
4,080,000	45	1.4	5,712,000	0.040	5.5
4,080,000	45	1.4	5,712,000	0.042	5.8

Table 6.4: Comparison of estimated mussel nitrogen contribution based on measured nitrate data and calculated mussel nitrogen contribution based on rate of ammonia excretion.

Source	Mass of Nitrogen in Water Above Mussel Bed (kg)
Day 1 Estimated Mussel Contribution	40
Day 2 Estimated Mussel Contribution	60
Calculated Mussel Excretion	0.4 to 6

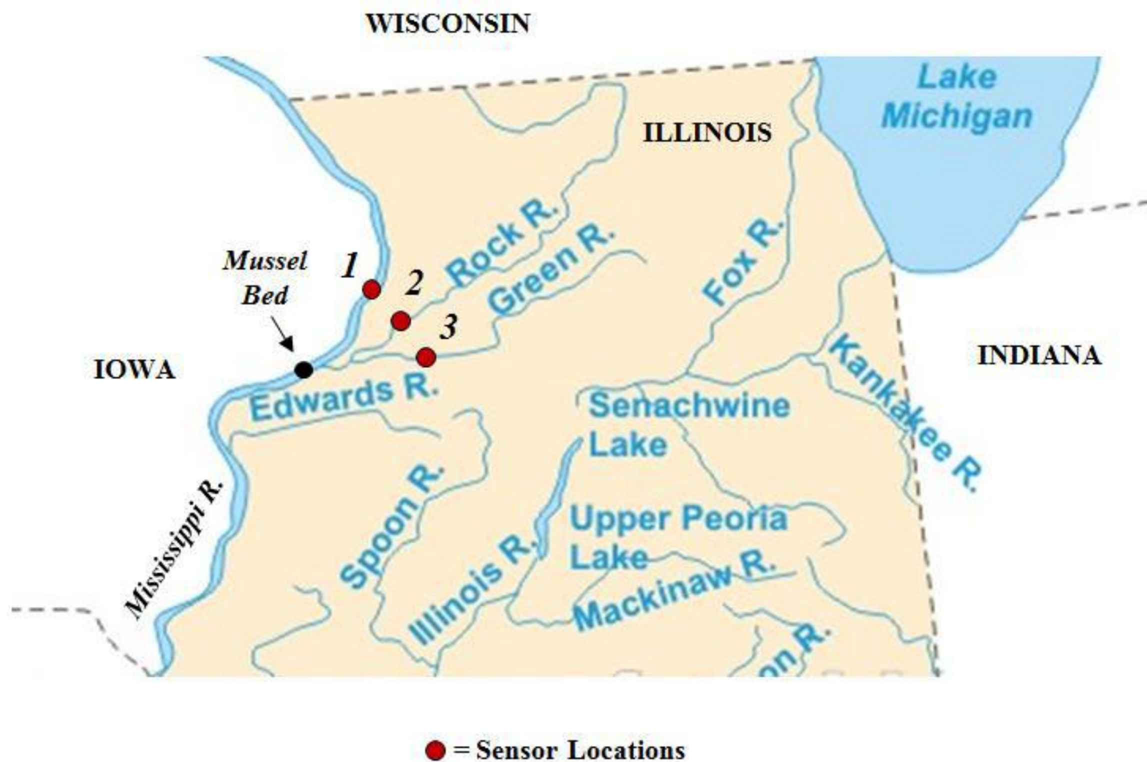

Figure 6.8: Sensor locations used to determine flow rates in mussel bed (1 = Mississippi River at Clinton, Iowa, 2 = Rock River at Joslin, Illinois, and 3 = Green River at Geneseo, Illinois) (Background image courtesy of MapsofWorld.com).

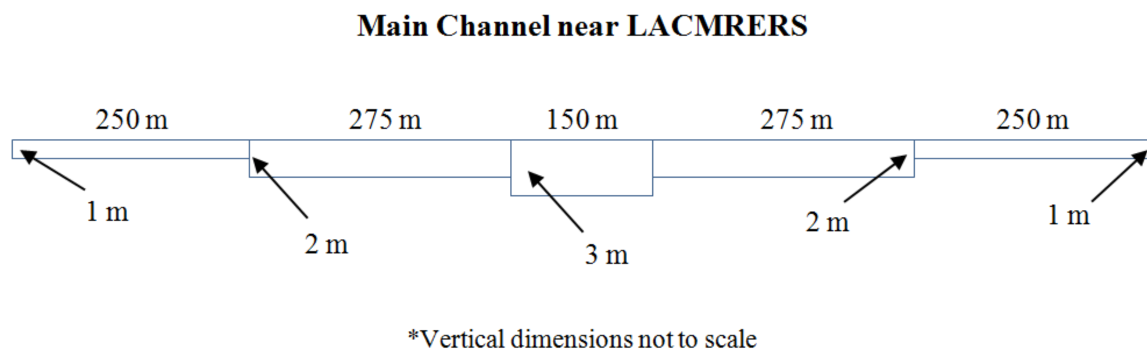

Figure 6.9: Estimated cross-section of Mississippi River in main channel near LACMRERS used to fractionate flow rate.

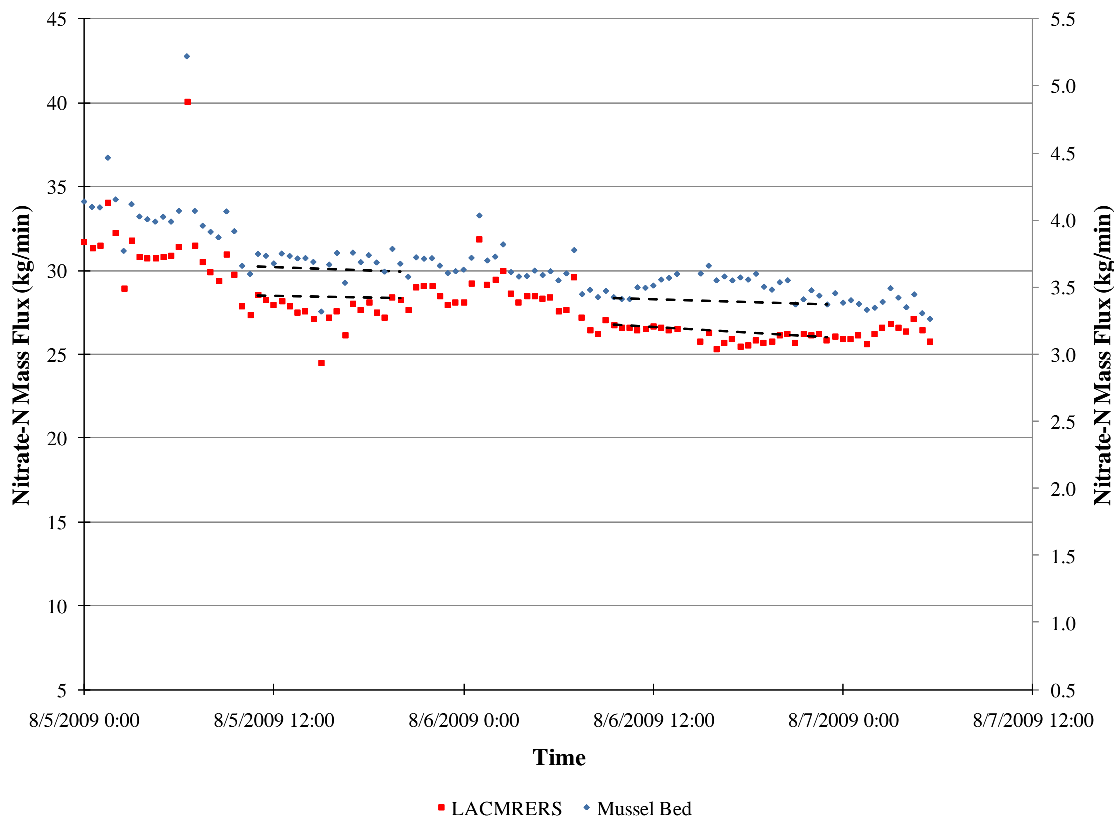

Figure 6.10: Nitrate-N mass fluxes for the analyzed days with baselines established for observed peaks and valleys (LACMRERS is on the primary y-axis and the mussel bed is on the secondary y-axis).

Table 6.5: Estimated area of each peak/valley used to calculate total nitrate-N mass based on flow rates for Day 1 (8/5/2009) and Day 2 (8/6/2009).

	Day 1 Area (kg)	Day 2 Area (kg)
Mussel Bed Peak	40	100
Main Channel Valley	500	200
Total	**540**	**300**

Table 6.6: Contribution of mussel ammonia-N excretion to mass of nitrogen under Day 1 and Day 2 peaks for both the control volume and flow rate analysis.

Day	Control Volume Mass (kg)	Flow Rate Mass (kg)	Mussel Excretion Mass (kg)	Percent Mussel Contribution
1	30	40	0.4 to 6	1 to 20
2	40	100	0.4 to 6	0.4 to 15

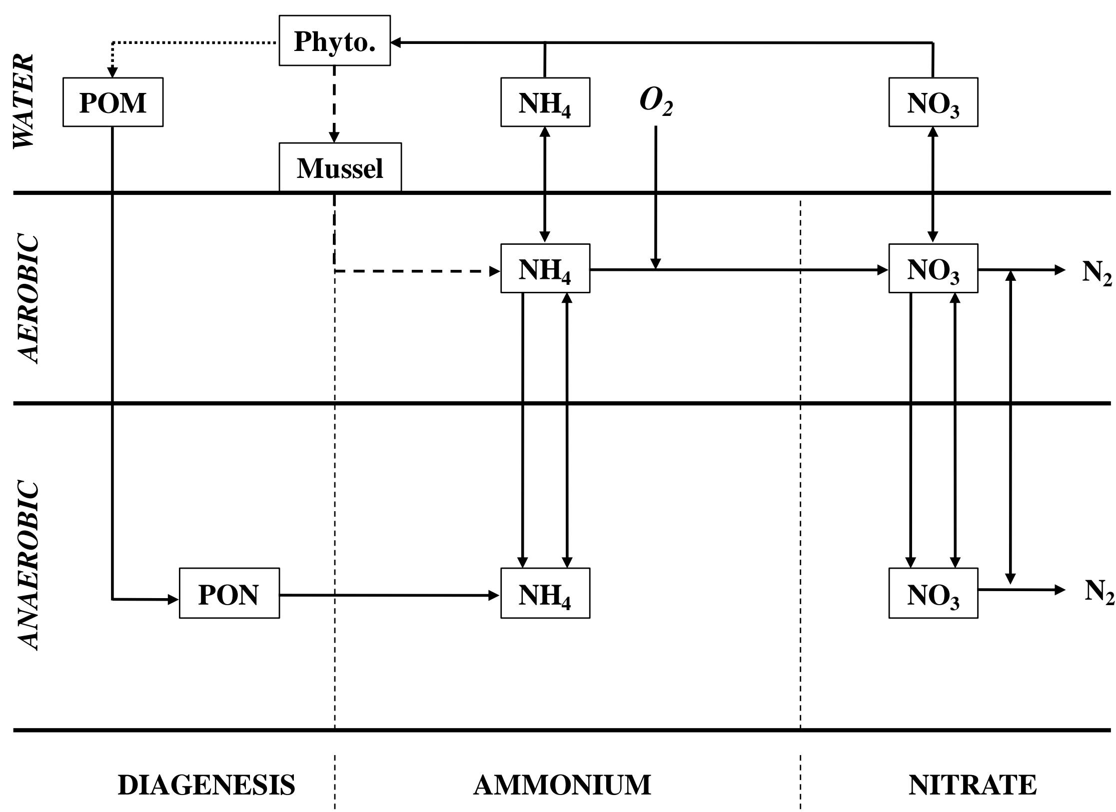

Figure 6.11: Schematic of mass-balance model being developed to assist in determining the processes affecting diurnal nitrogen dynamics (layers not drawn to scale).

CHAPTER 7
CONCLUSIONS AND FUTURE WORK

7.1 Conclusions

The primary goal of this research project was to investigate the potential impact of freshwater mussels on nitrogen cycling in the Mississippi River. This was completed by examining the physical, biological, chemical, and hydrodynamic characteristics of a mussel bed in the Mississippi River and evaluating the impact of the 2008 floods on the bed. We also established a well-equipped mussel laboratory habitat to investigate mussel behavioral responses and we analyzed highly time resolved nitrate data from the Mississippi River to examine the mussels' contribution to diurnal nitrogen dynamics.

Our analysis revealed that mussels may influence 0.1 to 15 percent of the diurnal nitrogen flux in the water column above the mussel bed as compared to the main channel. The diurnal nitrogen flux is thought to be dependent on the hydrodynamic characteristics, the mussel bed characteristics, and sunlight at the river location. The significance of these processes was evident when comparing the mussel bed and the main channel and also within the mussel bed itself.

7.1.1 Mussel Bed and Main Channel Hydrodynamics

Mussel bed and main channel hydrodynamics were thought to be an important process driver due to the measured impact on the physical, biological, and chemical characteristics of the bed. Within the mussel bed, we observed lower velocities at the upstream area of the bed and higher velocities at the downstream areas. This was caused by the unique flow circulation in the bed influenced by an outcrop of land present at the most upstream end of the bed. The resulting flow gradients led to increased deposition of silt fines in the upstream areas and less deposition of fines in the downstream areas. Additionally, these lower velocity, high deposition areas were likely more protected by

the submerged outcrop of land from the floods of 2008 than the higher velocity areas. Comparative grain size distribution showed that the downstream areas of the bed were more greatly impacted by flooding as evidenced by substantial increases in fine sediments. The upstream bed areas experienced minimal changes in grain size distribution.

Differential particle deposition also affected the microbial communities present throughout the bed. Microcosm tests completed in the laboratory demonstrated that nitrate reduction occurred readily in the silty upstream sediments but was minimal in the sandy downstream sediments. The microbial community in the post-flood, upstream sediments contained a more diverse population representing substantial numbers of at least 10 bacterial families. The downstream sediments contained fewer families of bacteria and were dominated largely by unclassified burkholderiales.

Increased diversity of bacteria in the upstream sediments was likely due to the increased deposition of total organic carbon (TOC) associated with lower velocities. Therefore, TOC was most abundant in the upstream sediments and increasingly less abundant downstream through the bed. High TOC content likely contributed to the differences in nitrate reduction capabilities between the two areas of the bed as denitrifying bacteria require organic carbon as an energy source.

7.1.2 Mussel Characteristics

The species and abundance of mussels present at the study site also contribute to variations in excretion rates and filtering capabilities. Mussel excretion rates are species and temperature dependent (Vaughn, et al., 2008) and the total mussel population greatly influences both the amount of ammonia excreted and amount of phytoplankton cleared from the water column.

Based on a mussel density study performed in 2003, we estimated the number of mussels in the bed to be about 4 million. Four million mussels contributing a maximum

of 15 percent to the daily nitrogen flux may seem insignificant at the bed scale, but extrapolating this relationship to the pool scale could potentially result in a prominent impact. Mussel population estimates for Navigation Pools 5, 6, and 18 of the Mississippi River were found to be 189, 61, and 212 million, respectively (Newton, 2010). Thus, the mussel bed likely represents a small fraction of the total mussel contribution to the Pool's nitrogen processing.

Correlating the mussel density data to grain size distribution revealed that the highest populations of mussels exist in areas of the bed most vulnerable to flood-induced sediment perturbations. This provides evidence that extreme flooding can potentially alter mussel nitrogen cycling capacity due to physical habitat alterations.

Linking mussel density and hydrodynamics showed that mussel densities are greatest in areas of higher water velocities. This preference for areas with lower sedimentation rates subsequently means that mussels are differentially located in areas with low TOC and low denitrifying capacity. Mussels appear to favor areas of the bed with the lowest microbial diversity and lowest TOC content, indicating that mussels tend to prefer areas with low populations of denitrifying bacteria.

We developed a hypothesis that diurnal increases in nitrate-N concentrations were related to nighttime feeding and subsequent daytime excretion. This hypothesis was supported by laboratory gape sensor experiments that indicated mussel gape closure upon exposure to light and gape opening in darkness. The open gape implies a filter feeding position. However, mussels in the Mississippi River are thought to be near-continuous filter feeders with excretion occurring more continuously than diurnally. To our knowledge, this assumption has not been thoroughly tested. Also, the Mississippi routinely displays high turbidity levels limiting mussel exposure to light and significantly reducing the likelihood of diurnal feeding behavior associated with light exposure.

7.1.3 Sunlight, Temperature, and Phytoplankton

Sunlight exposure processes potentially caused diurnal variations in mussel bed nitrogen cycling. The distinct diurnal fluctuations in nitrate-N concentrations were nearly identical to diurnal sunlight patterns. Therefore, processes impacted by diurnal sunlight, such as phytoplankton growth, photolysis, and temperature, play a likely role in the diurnal nitrogen dynamics. Mussels have been shown to increase both filtering capacity and excretion rates under increased temperatures (Vaughn, et al., 2008) and bacterial processing is also known to correlate with temperature. The diurnal increases in nitrate-N could be caused by the diurnal increase in phytoplankton filtering, which otherwise would be consuming nitrate-N. Also, rising temperatures would likely cause increased mussel excretion of ammonia and increased processing of ammonia to nitrate by nitrifying bacteria.

7.1.4 Summary

Quantification of the impact of mussels on diurnal nitrogen dynamics is a difficult process that represents an area of study that has not been sufficiently researched. Within the mussel bed alone (not comparing to the main channel), we identified several factors that could impact the influence mussels have on nitrogen cycling. While our study identified the most significant processes influencing the diurnal nitrogen dynamics as a combination of hydrodynamics, mussel bed characteristics, temperature, and sunlight, other important processes may not have been considered.

However, our study did show that human- and climate-induced changes such as increased intensity of flooding can have a significant impact on mussel habitat and likely cause a decrease in mussel nutrient processing capabilities. Also, the discovery of the diurnal nitrate dynamics provided validation for the use of sensors to measure highly time resolved data in large scale systems such as the Mississippi. Finally, our study provided evidence that mussels possess the necessary traits to influence ecosystem nitrogen cycling, both at the small and large scale.

7.2 Future Work

Obtaining measurements for the other processes thought to contribute to the diurnal nitrate pattern (e.g. hydrodynamics, temperature, phytoplankton) would be the first step in determining the primary process driving the diurnal nitrate cycle. Measurement of these processes would need to occur at both the mussel bed location and the main channel location. Sensors located at another mussel bed within Pool 16 would also be valuable in determining if the diurnal nitrate pattern is site specific or common in mussel beds. Similarly, establishing multiple main channel locations would also be recommended to develop a more accurate pool representation. Grab samples obtained from the sample locations would also assist in quality assurance/quality control.

Collecting measurements of mussel bed ammonia excretion rates would help in establishing reasonable mussel contributions for the site. Multiple sensor clusters located throughout the mussel bed would also assist in identifying how the diurnal nitrate pattern varies relative to different mussel densities. Additionally, equipping mussels with gape sensors in-situ and coupling their behavioral responses to the collected highly time resolved sensor data would allow for determination of how both the mussels and the diurnal processes change under different environmental conditions (e.g. flooding).

Coupling these dynamic mussel behavioral responses into the mass-balance model would allow for better parameterization of the model. Once the processes contributing to the diurnal nitrate cycle are identified, they also could be incorporated into the mass-balance model. Integrating diurnal mussel responses and the necessary diurnal processes into the model and completing its development would allow for the model to be dynamically coupled to an existing Mussel Dynamics Model (Morales, Weber, Mynett, & Newton, 2006b). This Mussel Dynamics Model, developed by Morales et al. (2006), has been used to investigate the spatial distribution of mussels and the effects of food competition. Using our nutrient mass-balance model to parameterize this Mussel Dynamics Model would allow us to develop a better understanding of the

ecological responses, as influenced by mussels, to both normal flow and extreme hydrologic events.

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