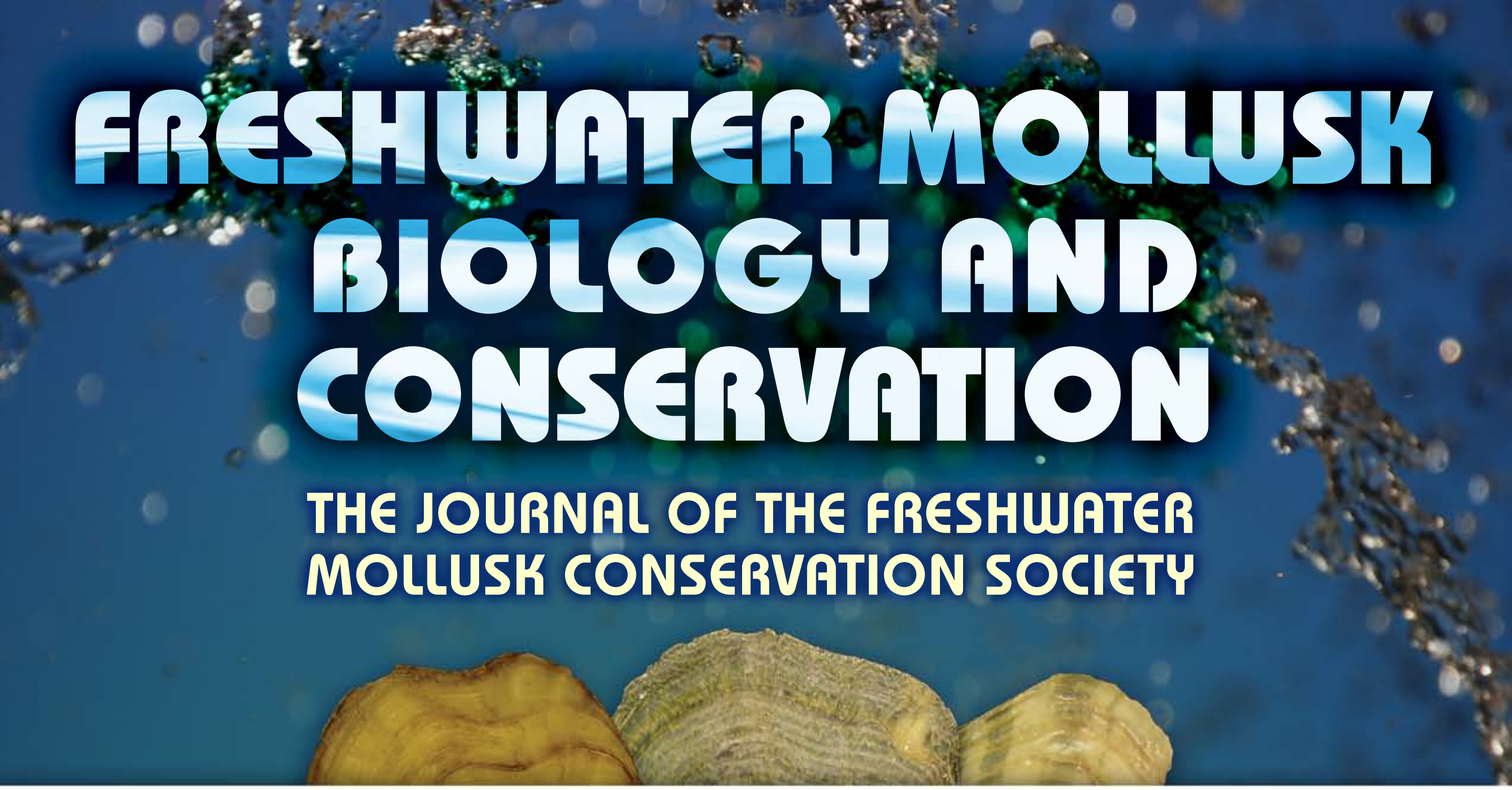

# FRESHWATER MOLLUSK BIOLOGY AND CONSERVATION

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ARTICLE

# A NATIONAL STRATEGY FOR THE CONSERVATION OF NATIVE FRESHWATER MOLLUSKS*

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*ABSTRACT*

**In 1998, a strategy document outlining the most pressing issues facing the conservation of freshwater mussels was published (NNMCC 1998). Beginning in 2011, the Freshwater Mollusk Conservation Society began updating that strategy, including broadening the scope to include freshwater snails. Although both strategy documents contained 10 issues that were deemed priorities for mollusk conservation, the identity of these issues has changed. For example, some issues (e.g., controlling dreissenid mussels, technology to propagate and reintroduce mussels, techniques to translocate adult mussels) were identified in the 1998 strategy, but are less prominent in the revised strategy, due to changing priorities and progress that has been made on these issues. In contrast, some issues (e.g., biology, ecology, habitat, funding) remain prominent concerns facing mollusk conservation in both strategies. In addition, the revised strategy contains a few issues (e.g., newly emerging stressors, education and training of the next generation of resource managers) that were not explicitly present in the 1998 strategy. The revised strategy states that to effectively conserve freshwater mollusks, we need to (1) increase knowledge of their distribution and taxonomy at multiple scales; (2) address the impacts of past, ongoing, and newly emerging stressors; (3) understand and conserve the quantity and quality of suitable habitat; (4) understand their ecology at the individual, population, and community levels; (5) restore abundant and diverse populations until they are self-sustaining; (6) identify the ecosystem services provided by mollusks and their habitats; (7) strengthen advocacy for mollusks and their habitats; (8) educate and train the conservation community and future generations of resource managers and researchers; (9) seek long-term funding to support conservation efforts; and (10) coordinate development of an updated and revised strategy every 15 years. Collectively addressing these issues should strengthen conservation efforts for North American freshwater mollusks.**

***KEY WORDS*** - freshwater mollusks, conservation, management, strategy, snails, mussels

## INTRODUCTION

In 1995, federal, state, academic, and private sector managers and researchers concerned with the decline of freshwater mussels (order Unionoida, families Unionidae and Margaritiferidae) in North America formed the National Native Mollusk Conservation Committee (NNMCC) to discuss the conservation status of this imperiled fauna. Realizing the scope and immediacy of the issues associated with mussel imperilment, the NNMCC decided that a nationwide coordinated effort was needed to stem population declines and extinctions. They produced a document to address the conservation needs of mussels and drafts of that document—the National Strategy for the Conservation of Native Freshwater Mussels (hereafter referred to as the National Strategy)—were presented at two North American

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mussel symposia (in 1995 and 1997). In 1998, the National Strategy was published in the *Journal of Shellfish Research* (NNMCC 1998). This document has been used to prioritize research and management actions related to mussel conservation. In addition, the National Strategy has helped organize activities conducted by the Freshwater Mollusk Conservation Society (FMCS)—the organization that evolved from the NNMCC—that is dedicated to the conservation and advocacy of freshwater mollusks. For brevity, we refer to native freshwater mussels (mussels) and gastropods (snails) simply as mollusks.

The FMCS was initiated as a grass-roots effort by dedicated individuals among agencies and organizations, academia and private citizens, and across states and countries. The work of this society has resulted in the recruitment and training of many scientists and managers who work at local, state, regional, national, and international levels on mollusk conservation. As a consequence, many published papers, agency reports, and government documents have been generated by this growing community. Our understanding of mollusks and the ecological role they play in aquatic ecosystems has grown exponentially as reflected in the peer-reviewed literature (Figure 1). Communication and outreach on mollusks has also increased the scope and breadth of awareness of the conservation challenges facing managers and researchers to wider audiences, including decision and policy makers. As a result of enhanced partnerships, funding to protect mollusks and their habitats has increased.

Haag and Williams (2014) assessed the state of progress made under each of the 10 “problems” outlined in the 1998 National Strategy and suggested ways to improve conservation implementation. After nearly two decades of progress and change, it was clear that an updated document was needed to address evolving conservation challenges, research needs, and emerging threats for mollusks. In 2011, the FMCS formed a committee to revise the National Strategy, and, in 2013, a list of overarching issues in mollusk conservation and strategies to address them was approved by the FMCS membership. Here, we present those issues as an updated National Strategy intended to identify research, monitoring, management, and conservation actions needed to sustain and recover mollusks. The issues are presented without prioritization because the strategies within the issues are independent and may vary due to numerous factors. This manuscript intends to give guidance to our conservation partners, but is not intended to prioritize action—that is a decision that should be made by local, state, or regional partners based on regional and taxonomic priorities, funding, expertise, etc.

The 1998 National Strategy was restricted to the conservation of mussels; however, many of the same factors that led to the imperilment of mussels has also affected snails. Recent compilations indicate that both groups have a similar high level of continental imperilment (~74%, J.D. Williams, Florida Museum of Natural History, personal communication; Johnson et al. 2013). In addition, snails are less studied than mussels, with most species defined only in terms of their taxonomy (see Graf 2001; Perez and Minton 2008). However, there is a growing body of knowledge regarding their distribution, ecology, and conservation status (Brown et al. 2008; Lysne et al. 2008; Johnson et al. 2013). We know that both mussels and snails require healthy aquatic ecosystems, but their specific life histories and ecological requirements can be quite different. For example, snails often occur in different habitats than mussels, have fundamentally different life histories, have higher rates of endemism, often have highly restricted distributions, and have dispersal mechanisms not dependent on hosts. The diversity of the snail fauna in the U.S. and Canada (16 families) implies a wide range of life history traits and needs (Johnson et al. 2013). These requirements will need to be considered, in addition to those for mussels, for effective conservation of the mollusk fauna.

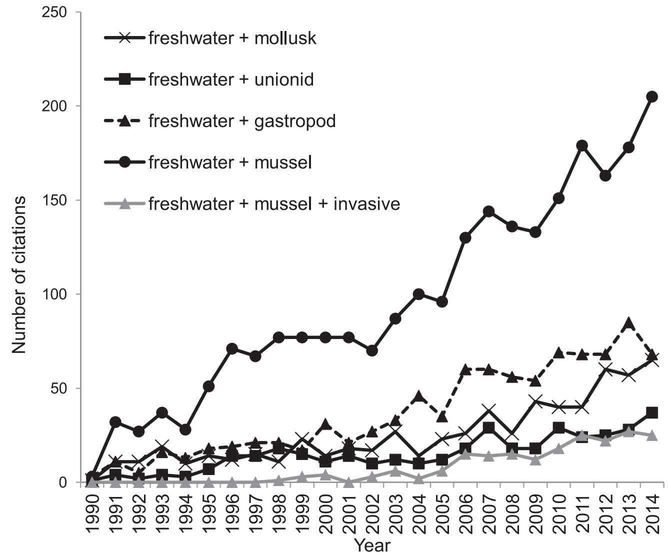


Figure 1. Number of peer-reviewed scientific articles during 1990–2014 that referenced freshwater mollusks as their topic. Data from Web of Science (February 2015) and are per year (i.e., not cumulative).

This National Strategy focuses largely on conservation issues in the U.S. and Canada, although similar threats to mollusks and their habitats are occurring worldwide (Lydeard et al. 2004). We acknowledge that the factors affecting mollusk communities vary in scope and intensity across regions. In the interest of brevity, we do not address region-specific issues or provide an exhaustive literature review for the broad issues we do cover.

The goal of this National Strategy is to identify and clarify issues and actions that are essential to conserving our nation’s mollusk fauna and ensure that their ecological, social, and economic values to society are maintained at sustainable levels. This revised strategy does not address fingernail clams (Sphaeriidae), largely because not enough data or expertise is available to include them. This document presents the 10 issues that are considered priorities for the conservation of mollusks. For each issue, we state the overall goal, list strategies needed to address the issue, provide an overview of the topic, offer examples of successes since the 1998 National Strategy, identify research needs, and recognize opportunities that will aid the conservation and management of mollusks.

## Issue 1 – Increase knowledge of the distribution and taxonomy of mollusks at multiple scales over time and make that information available.

Goal: Understand the status and trends of mollusk populations to better manage and conserve species.

Strategies:
1. Continue to refine knowledge of systematics, taxonomy, and genetic structure of species.
2. Update and maintain a database of the accepted scientific nomenclature.
3. Use survey methods that provide data needed for trend analyses.
4. Identify uniform data collection and reporting standards that will support periodic status assessments.
5. Encourage reporting of distribution data.
6. Assess and publish the conservation status of mollusks every 10 years.

### Overview

Knowledge of the distribution of mollusks is lacking, which hinders our ability to manage their populations. A key difficulty in understanding the distribution of mollusks has been poor taxonomy. Although considerable progress has been made in taxonomy since 1998 (Bogan and Roe 2008; Johnson et al. 2013), most species have not been studied using modern techniques. Many waterbodies are virtually unsampled, and even in well-studied regions, many drainages have not been adequately surveyed in half a century or more. An update of our approaches to taxonomy and generating and managing distribution data is needed to make this information more accurate and widely available.

### Success stories in mollusk distributions

Numerous stream and lake surveys have been published since 1998. Many of these studies have resulted in range extensions, new drainage records, population trends, and demographic data (e.g., Evans and Ray 2010; Haag and Warren 2010; Zanatta et al. 2015). Compilation of distributional information for species status assessments, recovery plans listed under the Endangered Species Act (ESA), and other studies have contributed to a greater understanding of mollusk distributions, which are critical for conservation efforts (e.g., U.S. Fish and Wildlife Service [USFWS] 2004; Butler 2007; Crabtree and Smith 2009). One example of a broad-scale survey was conducted in Texas rivers in 2010 (Burlakova and Karatayev 2010; Burlakova et al. 2011). New populations of endangered species and fewer populations of other species were documented, leading to a more accurate assessment of the global status of these species, including a recommendation that they be listed as endangered by Texas.

Some advances have been made in applying molecular and other data to the phylogenies of mollusks, thus facilitating a better understanding of their distribution. A review of the systematics of mussels was published in 2007 (Graf and Cummings 2007) and a number of papers describing new species or evolutionarily significant units have contributed to their conservation (Serb et al. 2003; Bogan and Roe 2008; Chong et al. 2008). Despite these efforts, the population genetics of most mussel species remain completely unexplored. Molecular phylogenies of some Physidae and Pleuroceridae snails have been published (Lydeard et al. 1997; Holznagel and Lydeard 2000; Minton and Lydeard 2003; Wethington and Lydeard 2007), but recent data suggest mitochondrial molecular data are unreliable for Pleuroceridae (Whelan and Strong 2016). Dozens of species of Hydrobiidae and Lithoglyphidae snails have been described from the Southwestern and Southeastern U.S. through the use of molecular data, electron microscopy, and detailed anatomical review (Hershler et al. 2008; Hershler and Liu 2009, 2010). However, most snail families remain unexamined using modern techniques. Data from molecular studies have been used to strengthen conservation efforts for some mussel (Jones et al. 2006; Jones and Neves 2010; Jones et al. 2015) and snail (Wethington and Guralnick 2004; Morningstar et al. 2014) species.

Several efforts to make mollusk information publicly available have gained momentum. For example, papers updating the continental status and summarizing the known distributions of mussels and snails have been published (Williams et al. 1993; Johnson et al. 2013). The Freshwater Gastropods of North America (Dillon et al. 2013) contains distribution and ecological information organized by geographic region. Keys to the snails of some U.S. states have become available online (e.g., Thompson 2004; Perez and Sandland 2015). NatureServe and the American Fisheries Society databases remain the primary sources for distribution information, though both have significant limitations (Williams et al. 1993; Johnson et al. 2013; NatureServe 2015).

### Research to aid conservation and management

In addition to the basic need for alpha taxonomy, detailed phylogenetic and population genetic studies are also needed. Analyzing datasets across multiple sources (e.g., multiple genes, microsatellites, morphology, ecological relationships) will ensure that these data are accurate and representative of natural populations. Collectively, these data may be important in recognizing patterns of imperilment of species, species groups, and genera. Determining the genetic structure of healthy populations will serve as a benchmark for the levels of genetic diversity and composition necessary for sustainability.

The last assessment on the names of mollusks was conducted by Turgeon et al. (1998); this list needs immediate revision. Taxonomic changes that have occurred among mollusks need to be updated and these findings need to be published every 10 years. Maintaining an ongoing, publicly-available record of accepted names would be ideal (e.g., fishbase.org). Similarly, comprehensive conservation status assessments for mussels and snails should be conducted every 10 years. The first snail assessment was published in 2013 (Johnson et al. 2013), and the second mussel assessment is in progress (J.D. Williams, Florida Museum of Natural History, personal communication). Ideally, future assessments will have more spatially-explicit distribution data, such as major river drainages, rather than simply states and provinces of occurrence. Further, efforts should transition to up-to-date, accessible online databases, rather than solely periodic publications. Online museum databases are useful for distribution records in some groups, (e.g., taxa with unique shell morphologies), but are questionable for other mollusks (especially snails), as no museum has comprehensively revisited the current identifications of mollusk holdings. Therefore, online distribution records are often incorrect, compromising their usefulness (Graf 2013; Johnson et al. 2013). Obtaining funding for maintaining taxonomic and distributional databases is a critical conservation need.

Standardized sampling methods should be used to assess mollusks over space and time. Although guidelines for standardized sampling methods exist for mussels (e.g., Strayer and Smith 2003), a companion work for snails has not been developed. Sampling methods will vary by objective and system type (e.g., wadeable streams, large rivers, lakes), and ideally contain a quantitative component so that results can be statistically evaluated across species, localities, watersheds, and time. In addition to the number of mollusks sampled alive, data such as shell-only individuals, shell condition (i.e., fresh dead or relic), sex ratio, length, and age may be useful for assessing status and trends. Also, data on habitat conditions, potential stressors, and characteristics of aquatic communities (e.g., host presence) may improve understanding of status and trend information on mollusks and inform conservation.

### Opportunities for conservation and management

Inadequate taxonomy and poor understanding of species distributions make species extinctions more likely and challenge effective conservation and management (May 1990; Perez and Minton 2008). For example, a rare species that is superficially similar to a more common relative may be overlooked and conservation measures not taken because it is unobserved when the rare species declines in abundance. If species distributions are not well-documented, then we cannot tell when populations are lost to human actions. The decreasing costs and increasing availability of genomic tools such as microsatellites and next-generation sequencing offer managers and researchers an opportunity to capture needed data on systematic relationships, taxonomy, and ultimately species distributions by identifying all ecologically significant units within a species. These new techniques offer great promise for the conservation of mollusks.

Our understanding of the conservation status of mollusks at the state level has increased substantially due to databases maintained under their Heritage programs and Comprehensive Wildlife Action Plans (CWAP), in addition to the publication of faunal books (e.g., Williams et al. 2008; Watters et al. 2009). These efforts have facilitated more accurate continental scale faunal assessments. Unlike the first mussel assessment, where nearly 5% of the fauna were of undetermined status (Williams et al. 1993), the forthcoming mussel update assesses status for all species (J.D. Williams, Florida Museum of Natural History, personal communication). In contrast, a recent assessment of the conservation status for snails indicated that the status of 4% of species was undetermined (Johnson et al. 2013). We need to update the status of the 4% of the snail taxa that are still undetermined and develop conservation plans that cover entire faunal regions (e.g., Mobile, Ohio, Mississippi). Improving our knowledge of taxonomy, life history traits, distribution, dispersal routes, and population connectivity will help managers prioritize imperiled species for conservation and increase the likelihood that more faunas can be conserved. This could lay the foundation for the partial restoration of ecosystem services that mollusks provide to aquatic and terrestrial organisms, ultimately including mankind (see Issue 6).

### Issue 2 – Address the impacts of past, ongoing, and newly emerging stressors on mollusks and their habitats.

Goal: Minimize threats to mollusks and their habitats.

Strategies:

1. Prepare white papers on known stressors (e.g., impoundments, dredging, contaminants, runoff, invasive species, disease) describing the risk and magnitude of effects on mollusks.
2. Describe the impacts of emerging stressors (e.g., climate change, increased energy development, water use conflicts, unregulated contaminants, hormone disruptors) and possible synergistic effects on mollusk populations.
3. Compile comprehensive threats assessments on community, river, watershed, and faunal province spatial scales.
4. Predict how species and communities will change in response to threats.
5. Work with the states and the U.S. Environmental Protection Agency (USEPA) to modify water quality criteria, develop new standards, and modify total maximum daily loads to protect mollusk populations.
6. Advocate for consistent and effective enforcement of environmental protection laws and regulations and evaluate whether existing regulations adequately protect mollusks and their habitats.
7. Develop an early detection and rapid response system for new aquatic invaders.
8. Develop and publish protocols to avoid the spread of disease and invasive species.
9. Describe the ecological and economic impact of mollusks

as vectors of parasites on fish, wildlife, livestock, and human health.

## Overview

Due to their varied life histories, sedentary nature, and relatively poor dispersal mechanisms, mollusk populations are susceptible to numerous biotic and abiotic stressors. For example, understanding how contaminants affect mollusk populations is complicated by residual contamination from pollution and the lack of data on synergistic effects of multiple contaminants on mollusks. Thus, even though an initial stressor may be gone, other stressors may continue to adversely affect mollusks and their habitats.

The tissues and shells of mollusks provide a long-term record of past environmental conditions. For example, tissues bioaccumulate contaminants (Naimo 1995; Coogan and La Point 2008) and shells have been used to identify a suite of past environmental events, such as climate and water quality (Dunca et al. 2005; Rypel et al. 2008). Stressors on mollusks are continually changing and research will be required to identify and address the effects of new and existing stressors. Examples of new stressors fall into categories of biotic introductions (e.g., invasive snails, fishes) and abiotic modifications (e.g., climate change, emerging contaminants) of aquatic ecosystems. Since mollusks play an important role in nutrient cycling and structuring food webs in aquatic ecosystems (Atkinson et al. 2013, 2014a), clarification and quantification of how mollusks respond to these stressors may help develop criteria for healthy aquatic systems.

## Success stories in emerging stressors

Because stressors are ubiquitous in aquatic systems, there have been many opportunities to study their effects on mollusk populations. In the 1990s and 2000s, considerable research was completed on the effects of dreissenid mussels on native mussels (e.g., Nalepa and Schloesser 2014). This has resulted in several positive outcomes; for example, scientists now have new tools to predict and perhaps prevent the spread of dreissenids into new areas (Nalepa and Schloesser 2014; Karatayev et al. 2015). These studies have also identified refugia for mollusks in several systems where populations are surviving despite the presence of dreissenids (Nichols and Wilcox 1997; Crail et al. 2011; Zanatta et al. 2015). To our knowledge, little research has been done on the effects of dreissenids on snails. However, research has documented the effects of invasive snails on native snails (e.g., Brown et al. 2008). We are unaware of research on the effects of invasive snails on mussels.

Considerable progress has been made in understanding the effects of abiotic stressors on mollusk populations. First, mussels are now recognized as the most sensitive organisms ever tested to the effects of ammonia (Augspurger et al. 2007; Newton and Bartsch 2007; Wang et al. 2007a, 2007b). This research prompted a revision of water quality criteria for ammonia to be protective of mollusks (USEPA 2013). Second, the impoundment of rivers with dams has often been cited as a primary reason why many mollusk populations are fragmented (Downing et al. 2010). Dams and channelization are understood to be a leading cause for the extinction of at least 12 species of mussels and 45 species of snails (Haag 2009; Johnson et al. 2013). Consequently, there is substantial interest in removing dams to increase connectivity and improve mollusk populations (Sethi et al. 2004; Nijhuis 2009; Haag and Williams 2014). However, reproducing populations of mussels have been documented downstream of barriers, suggesting that river modifications should be considered on case-by-case basis because dams may have a protective role for some populations (Gangloff et al. 2011). Third, due to certain life history traits (e.g., patchy distribution, limited mobility, larval dependence on hosts, range fragmentation), mussels may be especially susceptible to the effects of climate change. Many of these life history traits may also adversely affect snail populations (Johnson et al. 2013). In fact, research has shown that many species of mussels are already living close to their upper thermal limits and that there are physiological and behavioral effects of climate change on mussels (Pandolfo et al. 2010; Archambault et al. 2013; Ganser et al. 2013). Advances in modeling suggest dire consequences for mussel populations as a result of climate change due to co-extirpation of mussels and their hosts (Spooner et al. 2011).

## Research to aid conservation and management

To assess and monitor the threats of stressors on mollusk populations, data on tolerance limits (e.g., temperature, dissolved oxygen, conductivity), sublethal effects, and habitat delineation are urgently needed (e.g., International Union for Conservation of Nature threats classification scheme). In addition, there are limited data on the synergistic effects of stressors on mollusks and on sensitivity of different taxa. Because the responses of mollusks to stressors may vary by watershed, climate, habitat type, taxa, and physiological state of mollusks, future studies should evaluate the synergistic effects of multiple stressors across species, life stages, spatial scales, and over varied environmental conditions. Mollusks require an abundant supply of clean water for physiological processes. Unfortunately, the quality and quantity of water in many mollusk habitats is in jeopardy (Poff et al. 1997; Arthington et al. 2006). Research is needed to understand how demands on water supply will affect mollusk communities and to develop instream flow criteria. We have evidence that low flow periods—due to drought conditions in Southern rivers—reduced species richness and relative abundance of mussels (Haag and Warren 2008; Galbraith et al. 2010). Scientists need to educate local water management districts on how their practices (e.g., water extraction, diversions) may adversely affect mollusk assemblages. Without this collaboration, conflict between stakeholders can occur (Buck et al. 2012). Endemic species (e.g., those limited to certain river reaches or springs) are at risk of extirpation with even one major climate event. Understanding the thermal limits of mollusks (and its interactions with flow) across species and life stages will allow us to forecast potential changes in mollusk assemblages due to climate change. There also is a need to understand the direct physical effects that biologists may have on mollusk assemblages and the potential for introducing pathogens into established populations.

### Opportunities for conservation and management

Advances in new technologies can help researchers and managers understand the effects of stressors on mollusk populations. For example, environmental DNA (eDNA) is currently being used to track the spread of invasive species and could be used to detect mollusk populations in the future (Olson et al. 2012; Pilliod et al. 2013). In a 5$^{th}$ order river, New Zealand mudsnails (*Potamopyrgus antipodarum*) were successfully detected using eDNA at densities as low as 11 individuals $m^{-2}$ (Goldberg et al. 2013). This technology could also be used to identify locations of at-risk populations, allowing mangers to focus their conservation efforts. In addition, the introduction of passive integrated transponder (PIT) tags has allowed researchers to non-invasively monitor the growth, survival, and movement of individual mussels (Kurth et al. 2007; Newton et al. 2015). These techniques allow scientists to identify and develop sensitive response metrics from exposure to environmental stressors. Finally, advances in testing methods and analytical techniques should result in improvements across a range of state and national water quality standards that could protect mollusk populations, similar to improvements made for ammonia.

Given that mollusks influence the ecology and economy of fish, wildlife, and ultimately human health, scientists need to provide managers with the tools (e.g., protocols for mollusk management) to make decisions about how future and current stressors and their interactions might affect the conservation of mollusks. Efforts should also be made to support research on the long-term effects of stressors because many are likely surrogates for other emerging stressors.

### Issue 3 – Understand and conserve the quantity and quality of suitable habitat for mollusks over time.

Goal: Increase understanding of physical, chemical, and biological characteristics of habitat to support sustainable assemblages of mollusks.

Strategies:
1. Define habitat requirements at multiple spatial scales (e.g., organismal, population, community, river, watershed, faunal province).
2. Define habitat requirements at multiple temporal scales (e.g., seasonal, annual, long-term), including the quality, quantity, and timing of ecological flows.
3. Quantify the amount of occupied and unoccupied habitat.
4. Identify and conserve habitats that will be resilient to changing climates.
5. Reduce habitat fragmentation and increase connectivity of historical habitats.
6. Conserve and restore habitat through land protection actions such as easements, acquisitions, and landowner agreements along riparian and upland areas within watersheds.
7. Identify best habitat management and restoration practices through evaluation, monitoring, and modeling.
8. Develop and test effective mitigation alternatives for activities that affect habitat.

### Overview

Habitat degradation and loss is a common threat to mollusks, and often a major concern for sustainability of at-risk mollusk populations (Lydeard et al. 2004; Strayer et al. 2004). Widespread construction of dams has resulted in vast changes to habitats, flow and temperature regimes, and ecological functions (Poff et al. 2007). Development of restoration and mitigation tools for mollusk habitat, therefore, is critical. For example, habitat loss is often listed as the most significant threat in recovery plans for endangered mollusks, but few plans provide guidance on habitat restoration and instead focus on the nonetheless important protection of diminishing and rare inhabited areas (e.g., USFWS 2004). Compared to other charismatic or commercially-important species (e.g., mammals, gamefish), the state of knowledge needed to address conservation and management of habitats for mollusks is poorly developed; yet this knowledge could help explain other aspects of the ecosystem. Management of mollusk habitat is further challenged by the complexity of variables involved, regulatory constraints, and by multiple and increasingly severe stressors on freshwater ecosystems (see Issue 2) that vary across and within ecoregions.

### Success stories in mollusk habitat

Although progress has been made toward understanding habitat requirements of some mollusks—particularly mussels—habitat needs of most species and life stages are not well understood and remain a critical bottleneck for conservation efforts (Johnson et al. 2013; Haag and Williams 2014). Assessment and quantification of snail habitat has been limited, but advances have been made toward understanding habitat requirements in several aquatic systems (e.g., Evans and Ray 2010; Calabro et al. 2013; Johnson et al. 2013). Such understanding has enabled conservation gains for some species. For example, the range of the endangered *Tulotoma magnifica,* a snail in the Coosa River, Alabama, had been reduced by 99% (Hershler et al. 1990). Removal of dams to restore riffle habitat, coupled with improved water releases at hydroelectric dams and habitat protection, resulted in recovery to 10% of its historical range and subsequent reclassification from endangered to threatened (USFWS 2011).

For mussels, past research using traditional habitat descriptors (e.g., velocity, particle size) was largely unsuccessful at predicting occurrence or density (Strayer and Ralley 1993; Brim Box et al. 2002). More recent studies across a variety of systems have suggested that certain complex hydraulic variables (e.g., shear stress, Reynolds number) are more predictive (Gangloff and Feminella 2007; Steuer et al. 2008; Zigler et al. 2008). While this has been a significant accomplishment in quantifying physical habitat, mollusks require more than physical habitat. Suitable patches of quality habitat also require availability of mussel hosts, limited predation, suitable water quality, and adequate food resources (Newton et al. 2008).

### Research to aid conservation and management

Future strategies to conserve or enhance habitat for mollusks should focus on two areas. First, understanding temporal and spatial patterns in mollusk habitat across multiple scales and life stages will be critical in developing effective conservation strategies. Predictive models that quantify mollusk habitat and clarify functional habitat attributes limiting mollusks are urgently needed. However, development of habitat models is often challenged by a lack of biological and environmental data, but recent advances in technology (e.g., remote sensing, low cost hydraulic models) and techniques for statistical and geospatial modeling of habitat will facilitate future efforts. Depending on the factors limiting mollusk habitat, these models may need to be scale-specific (e.g., site, river, watershed, faunal province). Models are also needed to understand the effects of dams, flow alteration, and other anthropogenic stressors on habitat and to forecast likely outcomes under differing management scenarios and emerging issues such as competition for water resources and climate change (Spooner et al. 2011). Additional research is needed to describe the importance of spatial arrangement and connectivity of quality inhabited and uninhabited habitat patches to persistence of mollusk assemblages.

Second, research is needed to develop tools for implementing and assessing conservation and restoration projects which increase the amount of quality habitat for mollusks. Partners may lack expertise, and often face difficult decisions regarding efficient allocation of limited resources. Management efforts such as artificial propagation may be futile if the habitat is unsuitable due to other factors such as degraded watersheds, and altered hydrology or hydraulic conditions. Evaluation and monitoring to determine the effectiveness of management actions to protect or mitigate lost or impaired habitats is essential for improving methods in an adaptive management framework. Improvements in methods for restoration or mitigation are likely to be incremental and require long-term commitments to fully understand ecological outcomes.

### Opportunities for conservation and management

Conservation of mollusks depends greatly on continued advances in conservation of their habitats. Opportunities for improving mollusk habitat will range from local to watershed-level efforts. In some systems such as the Upper Mississippi River, scientists, managers, and engineers are in the planning stages of constructing habitat restoration projects to benefit mussels by altering local physical and hydraulic conditions. Such projects present substantial opportunities for understanding the important features of mussel habitat and developing restoration and mitigation tools. Larger scale projects that might target mollusk habitat include improving land-use in the watershed (e.g., controlling sediment and contaminant inputs) and restoring a more natural hydrograph and ecological flows in rivers. Targeted dam removal, practices that improve water and sediment quality, and repairing altered or channelized streams are other actions that may improve mollusk habitat. Habitat restoration projects for many drainages are underway through partnerships such as the National Fish Habitat Action Plan and National Fish Passage programs of the USFWS and state and non-governmental initiatives. Biologists and managers should engage and partner with these organizations to include objectives for restoring mollusk habitat. Because mollusks can provide beneficial ecological services (see Issue 6), and can function as keystone species (Geist 2010), there can be substantial synergy between efforts to restore habitat and conservation goals for other ecosystem components.

## Issue 4 – Understand the ecology of mollusks at the individual, population, and community levels.

Goal: Increase fundamental knowledge of the biology of mollusks so managers can more effectively conserve them.

Strategies:
1. Describe life history and host-species relationships at the appropriate scale.
2. Define environmental and nutritional requirements necessary for physiological maintenance, reproduction, and persistence of all life stages.
3. Describe the ecological functions of mollusks in the environment.
4. Increase knowledge of negative and positive interactions among mollusk species.
5. Increase understanding of demographic, genetic, and physiological characteristics that influence long-term population viability.
6. Encourage development of population viability analyses to better predict species' persistence.
7. Develop population goals for managing rare and common species.

### Overview

Although we have learned a great deal about the ecology of mollusks since the 1998 National Strategy, we still have much to learn. Most work has concentrated on mussels, but we need more information on their demography, host-use and movement, nutritional needs, habitat requirements for juveniles, specific water quality limits, and sensitivity of all life stages to multiple stressors. Snails have received considerably less attention and there are significant data gaps in many areas including life history, population demography, habitat requirements, species interactions, and ecological function. Understanding the ecology of mollusks at the individual, population, and community levels is needed to design effective conservation and restoration strategies.

### Success stories in mollusk ecology

Summaries of the ecology of mussels are contained in two books (Strayer 2008; Haag 2012) and several review articles (Vaughn and Hakenkamp 2001; Vaughn et al. 2008; Haag and Williams 2014). Studies have begun to reveal the unique traits that mollusks possess that enable them to cope with variable environments. For example, research suggests that mussels can change their feeding strategy in response to varied food sources by feeding across trophic levels (e.g., bacteria vs. phytoplankton) or mechanistically (e.g., pedal vs. filter feeding) (Vaughn et al. 2008; Newton et al. 2013). Mussels also have a variety of responses to cope with environmental contaminants, such as varied detoxification capabilities (Newton and Cope 2007). Improved understanding of the strengths and weaknesses of survey methods has expanded our understanding of populations and population processes (Strayer and Smith 2003). For example, increased use of excavation techniques that enhance detection of smaller mussels can improve demographic information. Demographic models have been developed for some rare species (Crabtree and Smith 2009; Jones et al. 2012) leading to better understanding of their ecology. Use of conservation genetics can help determine long-term viability of populations (Jones et al. 2006). We have also developed temperature criteria for many species and life stages of mussels (Spooner and Vaughn 2008; Pandolfo et al. 2010, 2012).

### Research to aid conservation and management

We have made substantial progress in understanding mussel life histories and host-species relationships over the past two decades (e.g., Barnhart et al. 2008). However, there are still substantial data gaps. We have host-use data for only about a third of the North American mussel fauna, and much of that information is incomplete (Haag and Williams 2014). Replicated, robust studies that document the breadth of host-use across co-occurring fishes are needed (Haag and Williams 2014). Once potential hosts are known, there is also the challenge of determining which hosts are most important in natural systems. We also need to understand patterns in juvenile mussel dispersal that host movements provide. Studies using PIT tags and other novel techniques will increase knowledge in this area. We also need data on sperm dispersal in the field and reproductive success at low mussel densities, which may be a bottleneck for the conservation of small and isolated populations. In snails, representative pleurocerid and pulmonate species have been the subject of life history studies (e.g., Huryn et al. 1994; reviewed in Brown et al. 2008), but we need more information on reproductive biology of most species.

While vast progress has been made understanding habitat requirements of mussels in the last decade (see Issue 3), more work is required. In particular, we have limited knowledge of the habitat needs of juveniles. We also need to examine sensitivities to habitat perturbation in terms of multiple stressors, rather than single stressors (Shea et al. 2013). There is evidence that the early life stages of mussels are particularly sensitive to contaminants, such as un-ionized ammonia and road salt (Augspurger et al. 2003; Newton et al. 2003; Gillis 2011), but there are thousands of contaminants that have not been evaluated (see Issue 2). Sensitivities of mussels to the effects of climate change are just becoming apparent, but there is much more to learn (Ganser et al. 2013; Archambault et al. 2014). We also have inadequate knowledge of nutritional requirements of mollusks across life stages (Haag 2012).

Because we cannot determine the ecological requirements and sensitivities for all mollusk species, one approach is to look at requirements and tolerances of guilds or suites of species with similar traits (Statzner and Beche 2010; Lange et al. 2014). For example, groups of mussel species with similar thermal traits respond differently to drought-driven thermal stress (Atkinson et al. 2014b). Thermal and life history traits of species guilds have been combined to make flow recommendations for mussels, based on the traits and criteria for the most sensitive species (Gates et al. 2015). Comparative life history studies across populations should be performed on representatives from the major snail families to see if ecological requirements and sensitivities are similar across species or genera (e.g., Huryn et al. 1994).

Mussels are largely filter-feeding omnivores that feed across trophic levels on bacteria, algae, and other suspended material (Vaughn et al. 2008). Many mussels live in multispecies aggregations (i.e., mussel beds); thus, species interactions should be important in their communities. Most studies have concentrated on negative species interactions (competition) with varied results (Haag 2012), and more work on interactions between and within mussel species is needed. In particular, studies are needed on when and where food and space may be limiting. In addition, positive (facilitative) interactions may also be important in mussel communities, similar to marine bivalves and other groups of freshwater filter feeders (Cardinale et al. 2002; Spooner and Vaughn 2009; Angelini et al. 2011). As with the ecological requirements, the largest data gap is for early life stages. For example, juvenile survival rates and the effect of critical factors such as parasitism and predation rates are mostly unknown. Research is also needed to answer questions about the ecological interactions of adult and juvenile mussels (e.g., Do adults facilitate juvenile survival by providing a protective environment? Does the community of adults and different species create micro- or macro-environments of suitable habitat for juveniles? Do adults compete with juveniles for limited food resources, or do adults provide organic substrates that help build food resources for juveniles?).

Similar to mussels, research on snails is needed to better understand species and ecosystem interactions. Snails can be the most abundant invertebrate grazers in streams in the Southeastern U.S. with large ecosystem effects (Richardson et al. 1988; Hill 1992). Snails can reduce periphyton biomass and alter algal community composition, which can lead to changes in primary production (Brown et al. 2008). For example, the invasive New Zealand mud snail consumes up to 93% of primary production in streams (Hall et al. 2003), and the invasive Chinese mystery snail, *Cipangopaludina chinensis malleata*, can influence algal community structure (Johnson et al. 2009). Aside from a few representative pleurocerids, much of the work on species interactions in snails has been on interactions with invasive snail species. For example, the Chinese mystery snail affects native snails by reducing their populations, modifying their habitats, and increasing parasite pressure (Harried et al. 2015).

We know little about how to measure the relative health of mollusk populations, or what constitutes a self-sustaining population. Traditional measures of mussel populations (e.g., density, species richness) may not be sensitive enough due to long response times and life spans (Newton et al. 2008). Lack of demographic data at the species and community levels complicates resource management. Few studies describe demographics (e.g., recruitment, age structure), species richness, and species evenness—metrics that might be used to evaluate mussel community health (but see Villella et al. 2004; Haag and Warren 2010; Newton et al. 2011). Long-term monitoring studies that describe how demographics vary over space and time are also lacking. Multi-metric indices have been developed for sensitive fish and invertebrate communities to assess the health of these communities in rivers and lakes. However, investigations into the sensitivity of mollusks to contaminants and habitat perturbations are relatively recent. Experimental multi-metric indices have been developed for mussel communities in several states and regions, but have not been rigorously tested. Unfortunately, we know little about the physiological or genetic characteristics that promote long-term population viability (but see Gough et al. 2012; Archambault et al. 2013; Gray and Kreeger 2014). The lack of these data limits our ability to assess how human activities might adversely affect mollusk populations.

### Opportunities for conservation and management

Robust data on mussel abundance, distribution, and status are starting to be obtained in many drainages, providing critical data for conservation efforts. Meta-analyses of these data within and across drainages should uncover interesting demographics and inform management strategies. However, life history data for many imperiled species (e.g., Hydrobiidae) are lacking and should be addressed. Techniques for documenting host-use by mussels are expanding, including the use of advanced microscopy and genetics. Advances in analytical chemistry now make it possible to measure environmental contaminants that are biologically active at low concentrations (e.g., pharmaceutical products, nanoparticles, hormones). Coupled with an increased awareness of environmental contaminants in waterbodies, there are many opportunities to understand the effects of contaminants on mollusks and their hosts. The success of propagation efforts has made multiple species and life stages available, greatly expanding the potential for toxicological testing with mollusks. Advances in population and environmental modeling tools provides an opportunity to understand and predict the effects of multiple, interacting stressors on mollusk populations as well as developing effective and cost-efficient management strategies.

### Issue 5 – Restore abundant and diverse mollusk populations until they are self-sustaining.

Goal: Conserve and restore viable populations and communities of mollusks.

Strategies:

1. Develop population and community indices to monitor and evaluate sustainability over time.
2. Develop conservation and restoration plans (e.g., reintroduction or augmentation) at the river, watershed, and faunal province levels.
3. Implement restoration that results in self-sustaining populations and does not adversely affect resident fish and mollusk populations and their habitats.
4. Maintain a database of translocation, propagation, and stocking events.
5. Identify uniform methods and metrics for monitoring outcomes of augmentations and reintroductions.

### Overview

Mollusks have a 74% imperilment rate, with many species that rank among the most imperiled animals on the continent (Johnson et al. 2013; J.D. Williams, Florida Museum of Natural History, personal communication). In addition to species considered imperiled and federally endangered, many wide-ranging and common species also are no longer prevalent. This loss diminishes biodiversity and affects the ecosystem services mollusks provide in functional aquatic systems (Spooner and Vaughn 2008). Moreover, diverse and abundant mollusk communities are more robust and resistant to invasion by non-native species (Strayer and Malcolm 2007). High mussel species diversity is found in communities with rare species and good water quality, so community data would be required for understanding the sustainability of all populations. Population trend analyses would also give biologists essential data to understand the ecological causes of these declines.

To increase the abundance and distribution of mussel populations, more needs to be known about demographics, viability, and genetics of fragmented populations (Haag and Williams 2014). Indeed, the role of population fragmentation on species and community declines must be addressed. Mollusk restoration efforts have largely focused at the site scale for individual species; rarely have these efforts been addressed at the population, much less community, level. It is imperative that defined population goals are established and that methods are developed to monitor populations and communities over time, given the pervasiveness of disjunct and isolated populations with limited recruitment. Conservation strategies should maximize the benefits of disjunct populations to the overall species conservation (i.e., tracking genetic diversity of animals used for propagation, mixing of animals from adjacent shoals). This includes establishing accessible databases to hold unpublished community data.

## Success stories in mollusk restoration

With the passage of the ESA in 1973 and the current listing of 91 mussel and 31 snail species in North America, research facilities began to investigate the life histories of individual species. The goal of these investigations was the development of propagation techniques for restoring imperiled species through stocking of cultured progeny. Since the late 1990's, several propagation programs throughout the U.S. have been successful in propagating and stocking mollusks. Research efforts for mussels have identified suitable diets, feeding regimes for adult broodstock and juveniles, host-species, and numerous culture and grow-out systems (e.g., Barnhart 2006; Gatenby et al. 2013; Mair 2013). These efforts were often associated with stocking programs aimed at restoring mussel beds adversely affected by damages from chemical spills or other instream activities such as bridge replacements (Morrison 2012; Morrison et al. 2013; Lane et al. 2014). Propagation technology has improved greatly over the past 20 years and some mussel species have been propagated at a production scale (Neves 2004; Haag and Williams 2014). Over a dozen federal and state culture facilities now exist in the Midwest, mid-Atlantic, and Southeast regions of the U.S. Examples of culture and population restoration efforts include several imperiled mussel species in the Tennessee River drainage, the Upper Mississippi River drainage, and several drainages in Missouri (Barnhart 2002; Davis 2005; Carey et al. 2015). Culture facilities and population restoration programs are, however, absent from other parts of North America, and some highly imperiled taxa are virtually unstudied (e.g., the endemic springsnails, Order Rissooidea). Evidence of self-sustaining populations of mussels from propagation efforts is generally lacking due to their longevity and slow population response times. Propagation of snails is fairly recent, with only a few programs available. For instance, Alabama has been culturing several species of pleurocerid snails and is conducting population restoration activities in several streams (P.D. Johnson, Alabama Aquatic Biodiversity Center, personal communication).

## Research to aid conservation and management

The focus of most mollusk restoration programs has been on recovery of endangered species, primarily due to limited funding. Managers must strive to obtain the resources necessary for community restoration. Community restoration—including rare and common species alike—will restore the broad range of ecosystem services mussels contribute to aquatic systems (Spooner and Vaughn 2008; Vaughn et al. 2008). Further, restoration of healthy populations increases the resiliency of populations and enhances the probability for successful restoration efforts (Sethi et al. 2004; Zanatta et al. 2015). Learning how to propagate mollusks across taxonomic groups will allow managers to increase abundance and distribution of populations, contributing directly to recovery of imperiled species which are usually found with common species. Coupled with efforts towards defragmenting habitats and populations, the status of some imperiled species may improve to the point of precluding their listing under the ESA while enhancing the probability of recovering federally listed species.

Stressors that affect entire assemblages of mollusks are often habitat alteration-based, and include population fragmentation, non-point and point source runoff, environmental (e.g., chemical spills, extreme droughts) and demographic (e.g., altered fertilization and recruitment rates) stochasticity, and altered flow regimes (Lande et al. 2003; Haag and Williams 2014). Contaminants may affect mollusks differently depending on species, life stage, and habitat conditions. Since mollusks appear to be some of the most sensitive of all aquatic organisms to some stressors, entire communities are vulnerable (see Issue 2). The effects of physical habitat changes on mollusk communities are needed at a scale suitable for management (see Issue 3). Maintenance of adequate flow regimes is important for managing mollusk populations. There are also numerous unexplained community-level "enigmatic declines" in mollusk populations that need to be addressed (Haag 2012).

Many aquatic ecosystems rely on the index of biotic integrity (IBI) for understanding biotic and abiotic interactions. Mollusk data are often lacking in IBIs or at best, are included only at the taxonomic class level (e.g., Calabro et al. 2013). Incorporating mollusk biodiversity into IBIs and/or developing new IBIs would create stronger predictions of aquatic communities and foster tracking the status of mollusks over time. Mollusk-specific IBIs might be developed to provide a quantitative means of evaluating the relative health or value of mollusk assemblages, as a tool for conserving mollusk resources including measuring impacts, assessing the efficacy of restoration techniques, and informing regulatory tasks. For example, scientists in the Upper Mississippi River have created a mussel community assessment tool to explore metrics to assess mussel community health (Dunn et al. 2012).

Basic propagation technology (e.g., culturing, feeding, growing out) and protocols for determining genetics, handling, and quarantining mussels are generally available, but may need to be revised to meet species-specific needs (Gatenby et al. 2000; Cope et al. 2003; Jones et al. 2006). However, there is a need to improve grow-out technology, in addition to identifying the best host species and understanding the physiological requirements to improve growth and survival. Most mussel propagation research has been conducted on Lampsilines, but efforts need to be expanded to include Anodontines and Amblemines, which include many imperiled species. Snail propagation research is still in the early stages of development, partly due to a paucity of ecological and basic life history data (Johnson et al. 2013). The diversity and environmental sensitivity of some snails (e.g., Rissooidea, Pleuroceridae) also pose challenges for developing propagation methods. In addition, obtaining broodstock is often a limiting factor in culture efforts. Research to facilitate natural reproduction in the laboratory would allow culturists to circumvent the need for wild broodstock. In vitro culture of mollusks could reduce labor and space costs by eliminating the need for mussel host species and may reduce the risk of disease in hatchery or culture settings.

Minimizing risks to aquatic systems from population reintroduction efforts must become a priority (Olden et al. 2010). We should aim to reduce risks to resident populations of mollusks and host-species and carefully consider genetic diversity of both resident and stocked mollusks. Choosing taxonomically well-studied populations for activities (Jones et al. 2006; Hoftyzer et al. 2008) should lessen the threat from inbreeding and outbreeding depression. Restored populations should be closely monitored to detect any potential negative interactions that may occur. Because restoration of natural populations is a primary management goal, it is important to identify and publish metrics for monitoring outcomes of management actions to promote development of uniform, repeatable, and successful methods.

### Opportunities for managing and restoring populations and communities of mollusks

Coupled with further advances in the biology and ecology of mollusks, malacologists are calling for comprehensive and universal protocols by resource agencies engaged in restoring mollusks through stocking programs (Jones et al. 2006; Hoftyzer et al. 2008; McMurray 2015). For example, the development of a comprehensive course on propagation of mussels—held at the National Conservation Training Center (NCTC) of the USFWS—has trained biologists to develop propagation programs for mussels. We have a few examples of restoration aimed at the community-level; however, more restoration efforts should target mollusk communities. Indeed, several recovery plans, strategies, and restoration plans approach restoration through methodical and coordinated planning across geographic scales (e.g., Hartfield 2003; USFWS 2004, 2010, 2014; Cumberlandian Region Mollusk Restoration Committee [CRMRC] 2010). These plans prioritize species, streams, and activities for conservation and management efforts, which will aid managers in restoring populations and communities at the watershed level. Restoration plans typically stress reintroducing extirpated populations over augmenting extant ones for two reasons: 1) recovery can only be achieved by creating additional populations and 2) augmentations have unique inherent risks to existing populations (CRMRC 2010; Haag and Williams 2014). Stream reaches and lakes affected by legacy environmental or human development issues can be restored to promote connectivity of mollusk populations (Newton et al. 2008).

In certain cases, federal listing under the ESA (and possibly even state listing) has been construed as a hindrance to recovery. This is despite the substantial benefits that Section 6 of the ESA and the State Wildlife Grants Program (SWG) have had on state funding for recovery actions for federally listed mollusks and the high profile that federal and state listing has provided. Regulatory burdens may be increased due to construction projects with a federal connection (under Section 7 of the ESA) or recovery activity implementation may have time constraints imposed due to permitting issues (Section 10). These issues can be ameliorated with better communication and relationships among USFWS, state, and other partners. The Section 7 process can and should be implemented to further recovery of species potentially affected by construction projects. Discussions with state biologists on how to implement recovery activities while reducing regulatory requirements will serve to expedite recovery of listed species.

### Issue 6 – Identify the ecosystem services provided by mollusks and their habitats.

Goal: Improve science-based consideration of the social and economic values of mollusk communities and functioning aquatic systems.

Strategies:

1. Describe ecosystem services provided by mollusks to humans and river ecosystems.
2. Develop and publish the social and economic values of healthy mollusk communities to society.
3. Update the values and replacement costs of mollusk communities.
4. Publish a comparison of mollusk replacement costs with other biologically engineered mitigation alternatives.

#### Overview

Ecosystem services are the benefits that humans derive from healthy ecosystems. These include provisioning services obtained directly from the ecosystem such as water, food, and timber; regulating services such as water purification, climate control, carbon storage, and pollination; and cultural services which are the benefits that people obtain through tourism and recreation, aesthetic experiences, or spiritual enrichment. Biologically complex freshwater systems provide many important ecosystem services that benefit society such as provisioning of clean water (water filtration and nutrient processing), recreation, and ecotourism (Brauman et al. 2007; Dodds et al. 2013). Mollusks provide all of these services for humans ranging from food, water filtration and nutrient processing, to use in a multi-billion dollar pearl jewelry industry. Mussels were also culturally important to Native Americans as a food source and for use in jewelry, pottery, and tools (Klippel and Morey 1986; Haag 2012). Harvest of mussels is a reserved treaty right in the U.S. for some Native American tribes (Brim Box et al. 2006). Thus, ecosystem services provided by mollusks are important for human wellbeing.

### Success stories in ecosystem services provided by mollusks

Researchers have learned a great deal about the services that mollusks provide to lakes and rivers over the past 20 years. Snails are important grazers and detritivores that support decomposition, influence algal and bacterial communities, and are an important food source for many fish, reptiles, and birds (Hall et al. 2006; Brown et al. 2008). Mussel shells provide habitat and refugia for other organisms, including benthic algae, macroinvertebrates, nesting fish, and other mussel species (Vaughn 2010). Mussels can aerate sediments and alter sediment stabilty and erosional processes, further improving habitat for mussels and other organisms (Zimmerman and de Szalay 2007; Allen and Vaughn 2011). Assemblages of common mussel species also improve conditions for rare mussel species that are typically found within larger assemblages (Spooner and Vaughn 2009). Filtering mussels remove seston from the water column, which can decrease water treatment costs and improve water quality (Newton et al. 2011). Mussel beds create biogeochemical hotspots via nutrient excretion and storage (Strayer 2014; Atkinson and Vaughn 2015). Where nutrients are limiting, fertilization by mussel excreta supports the rest of the food web, leading to increases in benthic algae, macroinvertebrates, fish, and even riparian spiders (Allen et al. 2012; Atkinson et al. 2014c). Mussel-provided nutrients can also alter algal composition, leading to decreasing blue-green algae populations and increasing water quality (Atkinson et al. 2013). There are few comparable data on ecosystem services provided by snails.

### Research to aid conservation and management

Although we have a better understanding of the ecological services that mollusks provide to aquatic systems, we now need to quantify how losing these services may affect aquatic systems, and the resulting economic and social consequences to humans. A good place to start would be to partner with scientists and managers who have conducted natural resource damage and restoration assessments for oyster beds (Beck et al. 2011). Discussions with those who have performed rare species valuations would be especially valuable (Richardson and Loomis 2009). These assessments should explicitly include the preferences and perceptions of major stakeholder groups (Menzel and Teng 2010). Considering data on economic values without also considering social values will underestimate mollusk ecosystem services (Seppelt et al. 2011). Gathering and analyzing these data will require that biologists collaborate with social scientists and economists skilled at these analyses.

### Opportunities for conservation and management

Nutrients stored in mussels (particularly in shells) are retained in the system long-term because most mussels are relatively long-lived. Thus, nutrients recycled by mussels are incorporated into the stream food web rather than being transported downstream (Atkinson et al. 2014c). This is also likely the case for snails, but research is needed to quantify their nutrient cycling and storage capabilities. Although nutrients retained in this manner in one river may seem insignificant, summed across multiple watersheds, this biological nutrient retention could help mitigate the effects of nutrient pollution. Large-scale production of mussels could be used to restore their biomass to enhance nutrient abatement. In addition, outreach and communication about the value of mollusks for providing important ecosysytem services should be developed to increase public support for protecting their populations (see Issue 7). Ongoing research in mollusk physiology (see Issue 4) will help quantify their contribution to improving water quality and reducing treatment costs.

Both marine bivalves and freshwater dreissenids can increase nitrification and denitrification in sediments by biodepositing organically rich feces and pseudofeces (undigested particles) on which bacteria feed (Bruesewitz et al. 2009; Kellogg et al. 2013; Smyth et al. 2013). Mollusks should also have strong effects on coupled nitrification-denitrification by biodepositing organic material, thus increasing rates of both processes, and by bioturbating sediments as they move (Vaughn and Hakenkamp 2001). However, the effects of mollusks on nitrification-denitrification need to be quantified.

### Issue 7 –Strengthen advocacy and build support for the conservation of mollusks and their habitats.

Goal: Increase information sharing and communication among citizens and decision-makers at multiple levels (e.g., local, state, regional, national, international) regarding conserving mollusk resources.

Strategies:

1. Develop a formal communication plan to guide conservation of mollusks into the future.
2. Develop science-based communication tools for local decision makers to build organizational and public support for land-use practices that support healthy aquatic systems including mollusks.

3. Develop communication and outreach materials targeting the general public, and to build awareness, appreciation, and support for conservation of mollusk resources.
4. Empower citizens with necessary outreach materials to advocate for consistent and effective enforcement of laws and regulations or to develop new regulations.
5. Recruit communication specialists to the mollusk conservation community.
6. Work with international partners to develop a global strategy for the conservation of mollusks.
7. Increase collaboration with other aquatic societies.
8. Increase collaboration with other resource agencies and resource groups.

### Overview

Knowledge of the current conservation status of mollusks is limited to experts and others that have been educated as a result of acute and chronic impacts to the environment. To conserve mollusks and their habitats, the risks and benefits outlined in this manuscript need to be effectively communicated to decision-makers, conservation groups, and the public. There is a growing need for information that will aid public advocacy for environmental legislation. Most of the communication by scientists is done formally through scientific presentations of research, technical reports, and peer-reviewed publications, or informally through face-to-face meetings, email, and social media. While sharing scientific knowledge and new discoveries is important, science writing is often directed at a small and specialized audience. To reverse declines in habitat quality, biodiversity, and budgets for mollusk conservation, we need to work with others who can affect change. Our communication skills and tools need to be broadened to convey information that will reach other conservation groups, the public, and policy makers.

### Success stories in advocacy and communication

Partnerships and communication have greatly increased support for mollusk conservation. Hundreds of papers, agency reports, and government documents on mollusks have been published (Figure 1). Publication and access to these data has resulted in many of the success stories highlighted throughout this manuscript. Advances in computer information transfer and online access to publications, unpublished reports, and other data have played a role in facilitating conservation and management efforts. For example, numerous websites for mollusks now exist that aid managers, researchers, and the public in accessing information and providing timely updates on imperiled species (Barnhart 2010; Dillon et al. 2013; Graf and Cummings 2015; Watters and Byrne 2015). Global partnerships can benefit this National Strategy through many mechanisms including external pressures on policy, increased knowledge, and new technologies.

In 2009, the FMCS organized a symposium with an international focus that brought together researchers and managers engaged in mollusk conservation. Several on-going international collaborations resulted from this effort. Other international meetings aimed at mollusk conservation have occurred since 2009, strengthening communication and collaboration among the international mollusk community. Likewise, scientists working with mollusks have had a marked presence at a variety of non-mollusk conferences (e.g., American Fisheries Society, Society for Freshwater Science, American Society of Limnology and Oceanography), raising awareness and advocacy for the conservation of mollusks and their habitats. These examples show that partnering with other natural resource or taxonomic groups can leverage resources and expertise in protecting aquatic resources.

The mollusk conservation community has also developed many outreach and communication tools to increase awareness and advocacy of the global plight of mollusks. For example, children's books and informational posters have been created on the biodiversity of mollusks, ecosystems, watersheds, and the history of pearl culture. A large floor-model display of the biology of mollusks has facilitated outreach at conferences and videos on the biology of mussels are available (Barnhart 2010). The quarterly online FMCS newsletter, *Ellipsaria* (http://molluskconservation.org/Ellipsaria-archive.html), contains outreach materials and is an important communication tool for researchers, managers, and the public.

### Communication to aid conservation and management

To have a greater effect on environmental and societal issues that threaten the health and resiliency of aquatic ecosystems, our passion for mollusks needs to reach those outside the mollusk conservation community. The public needs to understand how important mollusks are in creating healthy environments for a variety of other animals, including humans (see Issue 6). Recognizing that landowners often have direct influence on the management of riparian zones, outreach to farmers and urban planners should be prioritized. We should also increase collaboration with human health and industry groups to build partnerships to reduce environmental threats to humans and aquatic resources.

In today's ever-changing communication landscape, there is considerable competition for news and information. Compelling stories appropriate for varied media outlets are needed to ignite passion for conserving our limited freshwater resources. To ensure that information reaches the most influential audiences, we must develop strategic and creative ways to deliver our message. Specifically, a communication plan should be developed to address topics such as (1) training scientists and managers to effectively communicate with the public; (2) recruiting communication specialists as partners in creating effective communication that achieves conservation; (3) developing presentations and outreach materials aimed at public forums (e.g., video and film; outreach with zoos, aquaria, and museums), policy makers (e.g., graphics showing ecosystem services provided by mollusks, graphics highlighting cost-benefit analyses of restoring mollusk assemblages), and/or news media (e.g., blogs, news articles, press releases); (4) developing a more visible presence on social media; and (5) working with non-traditional groups outside the field of conservation.

### Opportunities for communication to conserve mollusk assemblages

Partnerships offer opportunities to share resources and increase awareness across groups and individuals that otherwise would not be reached through the mollusk community alone. There are many opportunities to partner with others in the international community who are also working to reverse declines in mollusks and restore habitats. Joint research projects with international colleagues should result in enhanced opportunities to restore mollusks through sharing of knowledge and expertise. Collaboration on targeted communication campaigns can strengthen public advocacy and political support for conservation over the global landscape.

There are many ways to deliver a message, depending on the content of the message and the audience. Since the impact of major storms such as hurricanes Sandy and Katrina, there is growing awareness and support by the public, the media, and policymakers to restore landscapes that are resilient to rising water levels and flooding. The mollusk community should consider this enhanced awareness when promoting the ecosystem services provided by mollusks (see Issue 6) and to promote the benefits of intact, connected aquatic habitats (Issue 3) for protecting human lives and property, as well as the environment—facts that may be more compelling to audiences than protecting rare mollusks.

Developing a communication plan which addresses the issues outlined in this manuscript will make it easier to communicate the value of protecting and restoring mollusks and their habitats. There are many online examples of communication plans; however, it may be cost effective to enlist the help of professionals. In addition, science writing and environmental journalism programs often seek opportunities for students to work with scientists to communicate their message. Sharing our conservation message with the public, government representatives, the media, conservation groups, landowners, and landscape planners must be a priority to ensure conservation of mollusks is relevant to society.

### Issue 8 – Educate and train the conservation community and future generations about the importance of mollusks to ensure conservation efforts continue into the future.

Goal: Provide a suite of training opportunities to the greater conservation community, and inspire future generations to work on the conservation of mollusks.

Strategies:

1. Develop and recommend a list of key skills and competencies for mollusk conservation biologists and the supporting disciplines such as communication.
2. Develop and recommend new coursework for the study and conservation of mollusks based on the skills and competencies identified in Strategy 1.
3. Manage a database of training courses, internships, details, and other professional opportunities for students and practicing professionals to gain hands-on experience with mollusk conservation.
4. Provide travel funds for FMCS members to attend training courses, outreach events, educational institutions, college fairs, and job fairs to encourage students interested in biology and natural history to consider careers in mollusk conservation.
5. Develop a FMCS grant program for students and other conservation groups to advance the conservation of mollusks through research and management activities.

### Overview

Growing concern about the future of mollusk conservation has elevated education and training to important issues. Many long-time specialists in mollusk conservation are nearing retirement and retention of their expertise in the scientific community is needed for successful mollusk conservation. Efforts to conserve mollusks could be undermined by this loss of institutional knowledge and are further confounded by the fact that documenting the long-term success or failure of conservation actions may not be realized for decades or longer. Many species are long-lived, slow growing, and have low recruitment rates; knowledge gained from conservation efforts will require long-term monitoring beyond the time-frame of funded projects or careers.

Additionally, the science and technology underlying conservation actions are changing rapidly. Thus, it is imperative that we provide professional and academic training opportunities for the conservation community to inspire the next generation of scientists to pursue careers in mollusk conservation. Even given their high degree of imperilment, mollusks have been highly under-represented in publications compared to vertebrates (Lydeard et al. 2004). We also recognize that other areas of expertise and skills beyond the sciences are needed to ensure mollusks persist and to help conservationists deliver their message (see Issue 7).

### Success stories in mollusk education

Currently, there is no group of individuals charged with achieving these strategies. Rather, this issue largely overlaps with the activities of FMCS committees (e.g., outreach, symposium, information exchange, awards). The FMCS actively sponsors annual educational events including symposia and workshops that provide opportunities to share the current state of the science with the conservation community and provide a venue for students to share their research. Symposia and workshops have covered a range of themes, including outreach, propagation, snail ecology, regional mussel identification, environmental flows, climate change, and dam removals. The FMCS offers travel awards to support student attendance at symposia and provides monetary support to regional mollusk conservation groups.

Formal courses are now being offered by federal agencies on mussel conservation biology and propagation (e.g., NCTC). Many states and private conservation organizations also offer short courses, workshops, and training in mussel identification, sampling techniques and protocols, and methods for handling mollusks. A few states have developed guidelines, minimum standards, and tests that certify scientists before they receive a scientific collecting permit and conduct mollusk work within their boundaries (e.g., Ohio, Pennsylvania, West Virginia). Several universities offer graduate level classes and research opportunities in mollusk conservation. However, training opportunities for snail identification or biology remain infrequent and informal.

### Opportunities for education to enhance mollusk conservation

The FMCS should coordinate and act as a clearinghouse for relevant educational and training opportunities in mollusk conservation. The FMCS could set up a grant program to support conservation and communication projects by students and conservation groups, and develop new workshops that reflect the emerging science needed for effective conservation.

Few universities offer undergraduate classes on mollusks beyond a basic exposure in invertebrate zoology. There has been a gradual shift from courses on organismal biology, biodiversity, and taxonomy to courses on microbiology and theory at academic institutions (e.g., Greene 2005). This focus is needed to invigorate the study of mollusks and capture students' interest early in their educational career. In addition, the tools that will be required for effective conservation in the future extend well beyond traditional studies in identification, species biology, and ecology. Rather, they include areas such as conservation genetics, eDNA, landscape ecology, hydrology, and restoration engineering. Several conservation projects have already benefitted from the combined expertise of scientists from diverse backgrounds working together on mollusk conservation. Examples include key contributions of hydrologists and engineers in dam removal, water management, and riparian restoration projects; the critical work of chemists and toxicologists in deriving water quality standards that are protective of mollusks; and the foundational products of social scientists and climatologists in determining how climate-driven land-use changes may affect the distribution of mollusks on the landscape. We should continue to promote this multi-disciplinary approach to mollusk conservation.

### Issue 9 – Seek consistent, long-term funding to support mollusk conservation efforts.

Goal: Increase funding for mollusk conservation.

Strategies:
1. Identify and create a database of existing sources of funding for mollusk research and management activities.
2. Take advantage of existing local, state, national, and international grant programs wherever possible.
3. Advocate for prioritization of existing funding to mollusk research and management.
4. Develop guidelines for monetary mitigation banking.

### Overview

Most of the funding for mollusk conservation has come from federal resource agencies, state wildlife agencies, universities, regulatory agencies, private conservation organizations, mitigation funds, and damage assessments under the Clean Water Act and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Funding comes in many forms, including funding to support staff in organizations that are charged with restoration or protection of mollusk species and communities. However, federal and state budgets have been cut in recent years, and private funding for mollusk conservation and restoration by way of settlements is ironically driven by catastrophic events (e.g., chemical spills) rather than by strategic allocations of funds.

### Success stories in funding mollusk conservation

Some mitigation funds have targeted activities that benefit mussels, such as the Mussel Mitigation Trust Fund (established to mitigate for impacts from an Ohio River power plant) and the National Fish and Wildlife Foundation's Freshwater Mussel Conservation Fund (settlement with Tennessee Shell Company). Most of these funds are short-lived; when their intended purposes end and expenditures are depleted, there is little funding for long-term monitoring. Also, there have been millions of dollars in settlements or court awards from natural resource damage claims under CERCLA. Both the mitigation funds and settlement awards have supported key research and management activities in mollusk conservation far beyond the geographic scope of the aquatic systems damaged.

There are some examples of consistent and recurring sources of funding. Since 2000, the SWG program has provided federal funds to develop and implement programs that benefit wildlife and their habitats, especially non-game species. Priority is placed on projects that benefit species of greatest conservation need, and many states have identified mollusks as a focal group. These funds must be used to address conservation needs identified within a state's CWAP such as research, surveys, species and habitat management, or monitoring. In turn, the states administer funds to undertake work on their own or support cooperator projects. Since 2008, Congress has authorized funding for a competitive SWG program to encourage multi-partner projects that implement actions contained in the state CWAP. There is substantial funding for these programs; in 2014, there was \$45.7 million for all states and territories and another \$8.6 million through the competitive grants program. Mollusk conservation also gets a creative funding 'boost' from enlightened fish ecologists, whose fishery studies yield valuable information on mollusks.

Since so many mollusks are on the federal list of endangered or threatened species, the USFWS provides grants to states and territories for species and habitat conservation on private lands. This program is known as Section 6 of the ESA and four categories of grants are funded: Conservation Grants, Habitat Conservation Plan Land Acquisition, Habitat Conservation Planning Assistance, and Recovery Land Acquisition Grants. Across the country, this grant program provides a substantial amount of money (e.g., \$35 million in 2014) to support species conservation efforts. Although cost-sharing is required, the percentages are small, ranging from 10–25%.

## Opportunities for funding mollusk conservation

The need for long term, consistent funding overlays all other issues in mollusk conservation. Without stable, adequate funding, long-term research and management actions cannot be implemented and evaluated. Mitigation funding on larger geographic scales is emerging (e.g., NiSource Habitat Conservation Plan) and some of these projects have the potential to benefit mollusk conservation. We should evaluate the costs and benefits of managing a landscape-level mitigation bank and a mollusk conservation trust fund. States can increase their chances of securing competitive SWGs by collaborating and developing multi-state, multi-species, and cross-regional proposals as well as working across disciplines. States that do not currently consider mollusks should add them to their imperilment lists, where warranted.

There is also the option of advocating for re-programming of existing conservation funding to support mollusk activities; but with shrinking allocations in federal and state budgets, and strong competition for available funding, it may be a losing proposition. For example, there are not enough resources (e.g., funding, staff) currently allocated to recover all endangered mollusks, let alone keep common species common. There are numerous watershed groups operating on local and regional scales that can secure grants to work on stream restoration and concurrent mollusk recovery (e.g., Friends of the Clinch, Black Diamond, Upper Tennessee River Roundtable). If the conservation community can continue to convey the message that healthy mollusk populations are good for rivers and lakes, and therefore good for fishing, there is a large constituency of anglers and other outdoor enthusiasts who may advocate for funding. Additionally, states and non-profit conservation organizations are eligible for National Fish Passage and National Fish Habitat Partnership program grants. By strategically targeting habitat restoration (e.g., dam removal, culvert replacement) that benefits both fish and mollusks, these programs offer great opportunities to fund projects that benefit aquatic fauna over the long-term. Potential new sources of funding can be hard to predict, given the fast changing complexion of private and public funding mechanisms. The FMCS can play a key role in advocating for new and unique opportunities and centralizing information on potential sources of funding by creating and maintaining a database of those sources.

## Issue 10 – Coordinate a national strategy for the conservation of mollusk resources.

Goal: Increase coordination and information sharing among local, state, regional, national, and international partners in conserving mollusk resources.

Strategies:
1. Establish an ad hoc committee every 12 years to review and update the National Strategy.
2. Revise the National Strategy document every 15 years and implement it at multiple scales.
3. Help integrate the national strategies into regional, ecosystem, and state action plans.
4. Increase collaboration with international partners.
5. Encourage publication of research and management actions.

As the human population continues to increase and threats affecting mollusks and their habitats continue to evolve, researchers and managers will be challenged to meet all of the conservation needs of mollusks. Therefore, effective conservation of mollusks in the future will require coordinated efforts from a larger community of partners and stakeholders. Our recommendations for the future include suggested areas of urgently needed research, improved taxonomic inclusiveness, global representation, and regular updates of the National Strategy. The current topics that need research to conserve and restore mollusks have been described in Issues 1 to 9. Coordination of research information and activities will be imperative for the conservation of mollusks. The information in this National Strategy should be integrated into ecosystem and state, regional, and international action plans.

This National Strategy, while greater in breadth taxonomically than the 1998 Strategy, excludes a group of bivalve mollusks of concern, the Sphaeriidae. This group remains too poorly known for inclusion at this time. We hope this document spurs research on this group of mollusks to allow their inclusion in the next National Strategy.

The 1998 National Strategy greatly improved coordination across the mollusk community, agencies and organizations, academia, and private citizens. We anticipate that this document will be similarly used at local, regional, national, and international levels to help researchers and managers prioritize the needed activities to conserve and restore mollusk assemblages. A regularly revised National Strategy will serve as guide for decision-makers that prioritize research funding and activities, especially as new information becomes available and new stressors emerge.

Because declines in mollusk faunas are worldwide, research collaboration across aquatic systems, both nationally and internationally, will provide insights into mollusk biology and ecology that might not be obvious when addressed at only a local or regional level. Outreach and coordination activities among continental-based groups and the global community is, however, in its infancy and we encourage the mollusk community to look globally in their conservation efforts. Future revisions to the National Strategy should broaden its geographic scope, encourage international collaboration, and broaden the taxonomic range of mollusks considered.

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ARTICLE

# INVESTIGATION OF FRESHWATER MUSSEL GLOCHIDIA PRESENCE ON ASIAN CARP AND NATIVE FISHES OF THE ILLINOIS RIVER

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*ABSTRACT*

**Densities of introduced Asian carp (Silver Carp and Bighead Carp) in the Illinois River Basin are among the highest in the world. Asian carp have been reported to serve as hosts for *Sinanodonta woodiana* in their native territories, but no research has been conducted on the potential for Silver or Bighead Carp to host North American freshwater mussels. Our objectives were 1) to examine the presence of glochidia on native and non-native fishes from the Illinois River Basin, 2) to determine an optimal concentration and duration of potassium hydroxide (KOH) exposure for increasing transparency of preserved fish gills to more effectively detect the presence of glochidia and parasites, and 3) identify parasite burdens. Fifteen fish species (12 native and 3 non-native) were collected from the Illinois River Basin during summer of 2014. Preserved fins and gills of native and non-native fishes were examined for glochidia and parasite infections. We determined that a 20 min 5% KOH bath was optimal for increasing gill transparency. We recovered 242 glochidia from 5 native fish species: Bluegill, Largemouth Bass, Smallmouth Bass, Freshwater Drum, and Sauger. Based upon morphometric data, we were able to identify the glochidial larval stage of 5 groups of freshwater mussels: Group A-Lilliput, Group B- Threeridge, Group C- Deertoe or Fawnsfoot, Group D- Threehorn Wartyback, and Group E-Fragile Papershell. We did not locate glochidia on any of the non-native fish species. Future research should include the use of laboratory host trials to elucidate if Asian carp could serve as successful host fishes for native mussels or if they are recruitment sinks, a possibility that could have a major impact on the future stocks of currently imperiled freshwater mussels.**

***KEY WORDS*** - Unionoida, parasites, *Hypophthalmichthys molitrix*, *Hypophthalmichthys nobilis*, *Cyprinus carpio*

## INTRODUCTION

Freshwater mussels have experienced substantial declines in their populations worldwide over the past century (Starrett 1971; Lydeard et al. 2004). However, there have been positive developments in some locations where mussel populations have been able to recolonize areas from which they had previously been extirpated (Sietman et al. 2001). Unfortunately, the success of some of these recolonizations may be undermined by emerging threats. The introduction of Silver Carp (*Hypophthalmichthys molitrix*) and Bighead Carp (*Hypophthalmichthys nobilis*), hereafter referred to collectively as Asian carp, into North America has had a substantial negative impact on native fish species, including declines in condition indices and population size (Irons et al. 2007; Crimmins et al. 2015). However, little research has been conducted to evaluate how Asian carp are affecting other aquatic organisms.

The life cycle of freshwater mussels is complex and unique among bivalves. Larval mussels (glochidia) are released by the adult female and must attach to gills or fins of a suitable host fish (Kat 1984; Barnhart et al. 2008). If it is an appropriate host, glochidia remain attached to the fish for several days to several weeks and metamorphose into juvenile mussels. Juveniles are released from the host and fall to the benthic substrates to continue their life cycle as free-living organisms. Glochidia can also attach to non-suitable hosts but the fishes' immune systems eventually reject the glochidia, which fall from the fish and perish (Kat 1984).

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Asian carp have been reported to serve as fish hosts to freshwater mussels in their native range (Djajasasmita 1982; Girardi and Ledoux 1989; Domagala et al. 2007). However, no research has been conducted on the potential for Silver Carp and Bighead Carp to host North American freshwater mussels or to determine if they serve as recruitment "sinks." Asian carp densities in Illinois River Basin are among the highest in the world (~2,500 per river km; Sass et al. 2010), and glochidia may inadvertently attach to carp. If glochidia do not metamorphose, Asian carp may be reproductive sinks that prevent glochidia from attaching to suitable native host fishes.

We conducted a study to evaluate the potential effects of invasive Asian carp on native freshwater mussels. Our primary objective was to investigate the presence of glochidia on native and non-native fishes throughout the Illinois River Basin. Special emphasis was placed on examining three species of carp (Silver, Bighead, and Common Carp (*Cyprinus carpio*)), as these species may have the potential to intercept glochidia and interfere with glochidial attachment to suitable native fish hosts. Secondary objectives were to identify other parasite burdens carried by native and non-native fishes, as well as determining an optimal concentration and exposure time for potassium hydroxide (KOH) solution to increase clarity of preserved fish gills to more effectively locate and identify encysted glochidia.

## METHODS

Fishes were collected during May, June, and July of 2014, which coincides with peak glochidial release for most freshwater mussel species in the Illinois River Basin (Watters et al. 2009). We targeted native fishes that coincidentally occur in microhabitat with Asian carp, as well as natives that are proven hosts for a variety of common mussels in the basin. We also collected Common Carp, a non-native species that has existed in the river since the 1800's and has been reported to be a marginal host for three North American freshwater mussel species (Lefevre and Curtis 1910; Hove et al. 2011a; Hove et al. 2014). Fishes were collected from the Upper Illinois River (Dresden and Marseilles reaches), the Middle Illinois River (La Grange Reach), as well as from several major tributaries to the Illinois River (Kankakee, Spoon, Mackinaw, and Sangamon rivers, and Salt Creek of the Sangamon).

Fishes were collected using a combination of pulsed-DC boat electrofishing, hoop netting, and fyke netting and were then returned to the laboratory for analysis. Smaller fish were preserved whole in 95% ethanol, while gills and fins were removed from larger fish (e.g., invasive carp) and were either preserved in 95% EtOH or were frozen. All Animal Care and Use protocols for fish collection, anesthetization, and euthanasia were followed (University of Illinois IACUC #14023). Freezing gills is not believed to influence detection of glochidia (Cunjak and McGladdery 1991). Fish were identified to species and then gill and fin tissues were examined for glochidia and other parasites under a Leica S8 APO stereomicroscope (Leica Microsystems, Wetzlar, Germany). When glochidia were found, they were counted, photographed with a Leica DMC 2900 digital camera (Leica Microsystems, Wetzlar, Germany), and measured for their length (parallel to hinge), height (perpendicular to hinge), and hinge length using Leica scale bar measurements in ImageJ (version 1.46r). Glochidia were left in the gill tissue unless they released from the tissue during processing. Efforts were made to orient all of the glochidia onto a level plane prior to being photographed, and only glochidia with suitable orientations were used for measurements. To ensure accuracy of our measurements, a subset of glochidia were measured by using three different techniques (Leica scale bar measurements in ImageJ, micrometer photograph measurements in ImageJ, and Leica measurements using the Leica measurement software). Efforts were made to identify the glochidia using morphological features to the lowest taxonomic level possible. Based upon morphometric data and recent mussel community information from the Illinois River, we used a bivariate plot and simple qualitative overlap to determine the most-likely identity of each glochidium, as our sample size was small (Kennedy and Haag 2005). Morphometric data were derived from Waller (1987), Hoggarth (1999), Williams et al. (2008) and references therein, Watters et al. (2009), Hove et al. (2012), Hove et al. (2015) and M.C. Hove (Macalester College, personal communication). When other types of parasites were encountered, the parasite type and infection intensity was also recorded for all of the native and non-native fishes. We also documented the occurrence of telangiectasia, a condition in which the blood vessels within the lamellae of the gill filaments burst and blood pools in the lamellae tips, causing the gills to have cyst-like structures along the gill margins that superficially resemble parasitic infections. We recorded the occurrence of these structures and compared the probability of occurrence in native versus non-native fishes using logistic regression (R Core Package 2015).

### Potassium hydroxide study for gill clarification optimization

We completed an experiment to determine the most effective processing treatment for detection of gill parasites. Gill size was determined by measuring from dorsal to ventral margin of an entire gill arch removed from the fish; small, medium, and large size classes were approximately 10mm (i.e., Spotfin Shiner, *Cyprinella spiloptera*), 10-50mm (i.e., Smallmouth Bass, *Micropterus dolomieu*), and greater than 50mm (i.e., Silver Carp), respectively. Three concentrations of KOH were used: 2%, 5%, and 10% KOH. Transparency and intactness of the gills were evaluated at six different time points: 1, 5, 10, 20, 30, and 60 minutes. A black and white grid (cell size of $10 \times 10$ mm) was used to record transparency by determining when the transition line from black to white was visible through the gill filament. Transparency was noted for tips or edges of gill filament, mid-vein of filament, and whole filament. Intactness was determined by picking up the gill and recording if parts of it began to disintegrate.

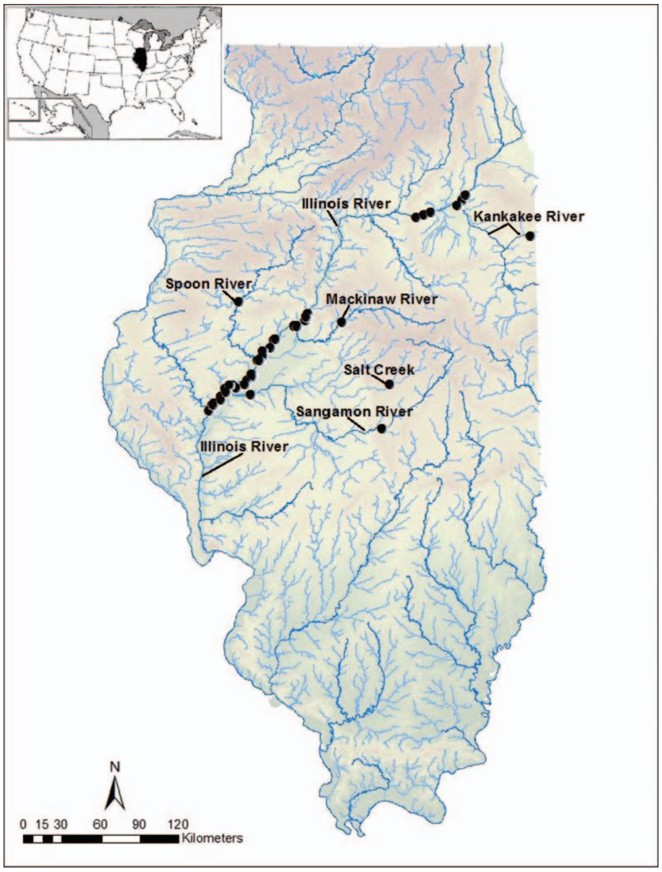


Figure 1: Fish collection sites on the Illinois River and its tributaries, Illinois, USA.

## RESULTS

Fishes were collected from 66 sites between 20 May 2014 and 22 July 2014 (Figure 1). We analyzed a total of 435 fishes for this study, including 145 non-natives (92 Silver Carp, 3 Bighead Carp, and 50 Common Carp) and 290 native fishes (12 species from 5 families) that are known to be host fish for the larval stage of at least one freshwater mussel species (Table 1). Of the total number of native fish collected, 10.7% were infected with glochidia, and five native fish species were infected. Infection rates among these species ranged from 12.5-100%. We recovered 242 glochidia from the 5 native fish species: Bluegill (*Lepomis macrochirus*), Largemouth Bass (*Micropterus salmoides*), Smallmouth Bass, Freshwater Drum (*Aplodinotus grunniens*), and Sauger (*Sander canadensis*) (Table 2). We identified the glochidial larval stage of 5 groups of freshwater mussels: Group A-Lilliput (*Toxolasma parvum*), Group B- Threeridge (*Amblema plicata*), Group C- Deertoe (*Truncilla truncata*) or Fawnsfoot (*Truncilla donaciformis*), Group D-Threehorn Wartyback (*Obliquaria reflexa*), and Group E- Fragile Papershell (*Leptodea fragilis*) (Table 3, Figure 2).

We recovered 9 types of non-glochidial parasites on 10 fish species. These parasites included: anchor worms (*Lernaea* sp.), black grub (*Neascus*), white grub (*Posthodiplostomum minimum*), yellow grub (*Clinostomum* sp.), monogenean trematodes (*Dactylogyrus* sp.), digenean trematode (*Bolbophorus* sp.), copepods, leeches, and nematodes. Telangiectasia were documented in eight fish species, and this phenomenon occurred more frequently in non-native fishes (Silver Carp and Common Carp) (Figure 3, Table 4). Non-native fishes were 4.3 times more likely to have telangiectasia than native fishes (95% confidence interval = 2.9 to 7.9 times more likely).

### Potassium hydroxide study for gill clarification optimization

The optimal concentration of KOH was determined to be a 5% KOH bath for 20 minutes. This concentration provided maximum transparency without causing excessive gill tissue deterioration. Smaller fishes and frozen Silver Carp gill specimens reached a sufficient level of transparency in less

Table 1. Native and non-native fishes collected in the Illinois River and its tributaries during May, June, and July of 2014.

| Common name | Scientific Name | Tributaries | Upper Illinois | Middle Illinois |
|---|---|---|---|---|
| Gizzard Shad | *Dorosoma cepedianum* | 0 | 1 | 0 |
| Red Shiner | *Cyprinella lutrensis* | 0 | 0 | 93 |
| Spotfin Shiner | *Cyprinella spiloptera* | 5 | 31 | 0 |
| Common Carp | *Cyprinus carpio* | 0 | 27 | 22 |
| Silver Carp | *Hypophthalmichthys molitrix* | 53 | 0 | 39 |
| Bighead Carp | *Hypophthalmichthys nobilis* | 1 | 0 | 2 |
| Spottail Shiner | *Notropis hudsonius* | 0 | 0 | 9 |
| Bluntnose Minnow | *Pimephales notatus* | 0 | 5 | 0 |
| Bullhead Minnow | *Pimephales vigilax* | 0 | 9 | 31 |
| Green Sunfish | *Lepomis cyanellus* | 0 | 1 | 0 |
| Bluegill | *Lepomis macrochirus* | 0 | 29 | 28 |
| Smallmouth Bass | *Micropterus dolomieu* | 0 | 4 | 0 |
| Largemouth Bass | *Micropterus salmoides* | 0 | 14 | 5 |
| Sauger | *Sander canadensis* | 0 | 1 | 0 |
| Freshwater Drum | *Aplodinotus grunniens* | 0 | 0 | 24 |

Table 2. Native fish species from the Upper and Middle Illinois River (ILR) with glochidia presence. N = number of native fish collected.

| Fish species | Upper ILR | Middle ILR | Total N | % infested | No. glochidia | Mean glochidia/fish |
|---|---|---|---|---|---|---|
| Bluegill | 16 | 3 | 57 | 33% | 178 | 9.4 |
| Smallmouth Bass | 1 | 0 | 4 | 25% | 1 | 1.0 |
| Largemouth Bass | 7 | 0 | 19 | 37% | 8 | 1.1 |
| Sauger | 1 | 0 | 1 | 100% | 38 | 38.0 |
| Freshwater Drum | 0 | 3 | 24 | 13% | 17 | 5.7 |

time, but the intactness of their gills was not negatively affected after 20 minutes of KOH exposure.

## DISCUSSION

We did not recover glochidia from any non-native fishes in this study. This result was not entirely unexpected for a number of reasons. First, if Asian carp were intercepting glochidia but were not suitable hosts, it is likely that the glochidia would slough from the carp within 1-4 days (Arey 1932a; Zale and Neves 1982a; Waller and Mitchell 1989). To document attachment, we would need to have collected fishes within a short period of time (i.e., <4 days) after they encountered the glochidia. Second, the Illinois River was in flood stage for most of the summer during 2014. Any glochidia that would have been present in the system, particularly in the water column, would have been more dilute than in a regular flow year. The flooding may have contributed to us not finding glochidia on any of our pelagic species (e.g., native cyprinids or Silver Carp). We also found no evidence of natural infestation on the benthic dwelling Common Carp. The gills of this species were rather difficult to process; the densely packed gill filaments of the Common Carp began to deteriorate in the KOH bath more quickly than any other species. This phenomenon could have decreased our ability to detect glochidia on Common Carp. Further, they are only considered marginal hosts for three native mussel species in Illinois that are considered host generalists: Rock Pocketbook (*Arcidens confragosus*), Flutedshell (*Lasmigona costata*), and Giant Floater (*Pyganodon grandis*) (Lefevre and Curtis 1910; Hove et al. 2011a; Hove et al. 2014). Thus glochidia would be subject to sloughing in a short time period in this case as well.

The majority of the native species that were infested with glochidia were collected in the Upper Illinois River, where recent surveys have seen a considerable rebound in mussel populations over the last decade (INHS Mollusk Collection; http://wwx.inhs.illinois.edu/collections/mollusk; accessed 1 July 2015). This recovery may be a response to improved water quality conditions brought forth by the Clean Water Act of 1972, as an extensive mussel survey in 1966 revealed extremely low mussel populations (Starrett 1971). Fifty-five percent of the Bluegill and 50% of Largemouth Bass collected from the Upper Illinois River were infected with glochidia. The best fit mussel species for identified glochidia are from the tribes Lampsilini and Amblemini. Lampsiline species typically have high fecundity, shorter life spans, and are host specialists that utilize only a single host fish species or a few species within a genus or family. Fragile Papershell, Deertoe, and Fawnsfoot are Freshwater Drum specialists and cannot metamorphose on any other fish species. Lilliput likely use *Lepomis* spp. and potentially darters as hosts (Mermilliod *in* Fuller, 1978; Hove, 1995; Watters et al. 2005). Threehorn Wartyback are known to metamorphose on cyprinids, but marginal metamorphosis success on fishes from several families has also been observed (Watters et al. 1998, B.R.

Table 3. Mean (±1 SE) and ranges (in parentheses) for measurements of glochidia recovered from naturally infested fishes of the Upper and Middle Illinois River (ILR). N = number of glochidia identified from each reach. Best fit species were assigned based upon established glochidial measurements from the literature and knowledge of the current mussel assemblage present in the ILR. Fish species = species from which the glochidia were obtained.

| Glochidia group | Height (μm) | Length (μm) | Hinge (μm) | N, Upper ILR | N, Middle ILR | Total N | Best fit species | Fish species |
|---|---|---|---|---|---|---|---|---|
| A | 176 ± 1 (163-188) | 158 ± 1 (146-171) | 87 ± 1 (78-97) | 52 | 0 | 52 | Lilliput | Bluegill, Sauger |
| B | 208 ± 1 (206-210) | 187 ± 1 (182-190) | 134 ± 1 (131-136) | 3 | 2 | 5 | Threeridge | Bluegill, Largemouth Bass, Freshwater Drum |
| C | 54 ± 1 (52-58) | 57 ± 3 (49-64) | 36 (36-36) | 0 | 7 | 7 | Deertoe, Fawnsfoot | Freshwater Drum |
| D | 263 ± 7 (251-281) | 251 ± 1 (243-259) | 147 ± 3 (139-154) | 0 | 15 | 15 | Threehorn Wartyback | Bluegill |
| E | 77 | 71 | 45 | 0 | 1 | 1 | Fragile Papershell | Freshwater Drum |

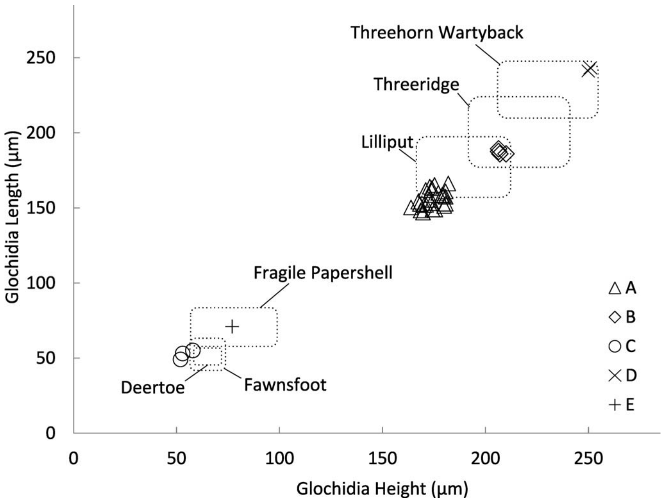


Figure 2. Bivariate plot of glochidia height and length, with assumed groups labeled as A, B, C, D, or E. Published size ranges for known glochidia are represented by labeled dotted lines.

Bosman, Texas Tech, personal communication). Amblemines are typically longer-lived, slower to reach sexual maturity, and can be host specialists or generalists (Watters et al. 2009). The Threeridge is a host generalist that can utilize many fish species, which is a factor that likely contributes to its dominance among mussel fauna within the Illinois River (Haag 2012).

We used a combination of morphological measurements and current species distribution to determine the most likely species assignment of attached glochidia. There were additional species that did overlap morphometrically with our glochidia but were eliminated based on known status of freshwater mussels in Illinois. Specifically, Spike (*Elliptio dilatata*) and Spectaclecase (*Margaritifera monodonta*) both have similar measurements as our designated Groups B and C, respectively, but have not been recovered alive from the Illinois since the early 20$^{th}$ century (Starrett 1971, Sietman et al. 2001). However, there have been recent discoveries of species considered extirpated, such as Scaleshell (*Leptodea leptodon*) (Illinois Natural History Survey Mollusk Collection; http://wwx.inhs.illinois.edu/collections/mollusk; accessed 1 July 2015), thus we cannot completely rule out the possibility that Spike or Spectaclecase may be attached to native fishes in the Illinois River.

Glochidia size can vary widely within a species throughout its range (Hove et al. 2011b, 2012, and M.C. Hove Macalester College, personal communication). An optimal situation would be to collect gravid females from the same waterbody and compare known glochidia morphology with the unknown glochidia from natural infestations. This was not an option in this study due to the flooding/sampling conditions at the time of fish collection; additionally, the availability of preserved specimens with mature glochidia from the Illinois River is also very limited, given the recent recolonization of this water body. Future research involving naturally infested glochidia should strive to include the collection of brooding females and glochidia from the areas of interest to allow for more accurate morphological comparisons.

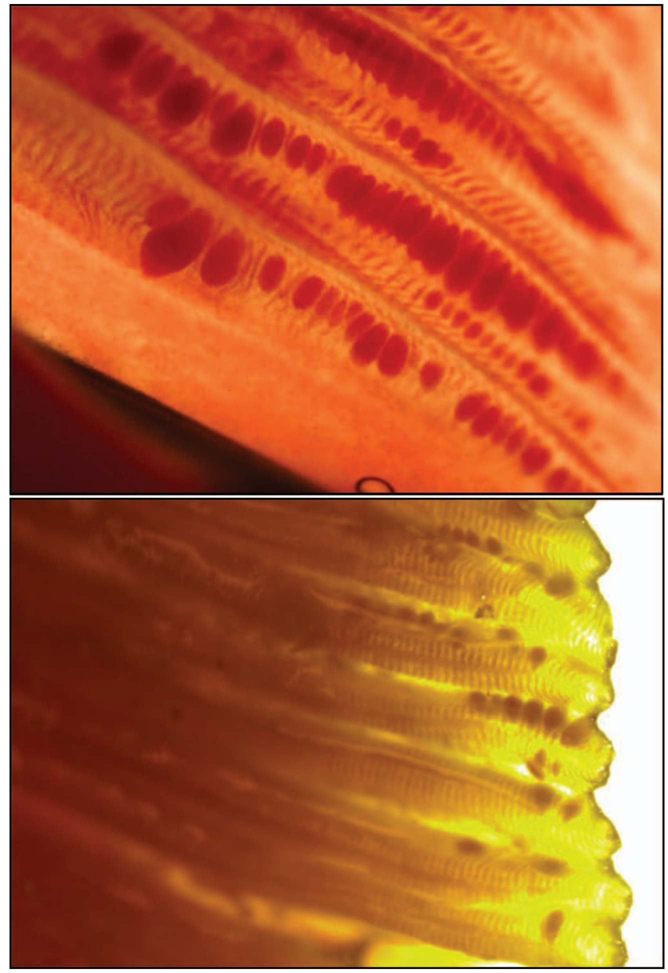

Figure 3. The occurrence of telangiectasia in gill filaments on Silver Carp. Top photo is of a freshly collected gill and the bottom photo is a gill that had been preserved in 95% EtOH.

In much of the literature with wild-caught fish, thousands of fish were examined to elucidate glochidial infestation levels (Zale and Neves 1982b; Neves and Widlak 1988; Weiss and

Table 4. Occurrence of telangiectasia on native and non-native fishes in the Illinois River (ILR) and its tributaries. Total N = total number of fish collected per species.

| Fish species | Tributaries | Upper ILR | Middle ILR | Total N |
|---|---|---|---|---|
| Red Shiner | – | – | 16 | 93 |
| Common Carp | – | – | 16 | 49 |
| Silver Carp | 7 | – | 39 | 92 |
| Bullhead Minnow | – | – | 1 | 40 |
| Bluegill | – | – | 3 | 57 |
| Smallmouth Bass | – | 2 | – | 4 |
| Largemouth Bass | – | – | 1 | 19 |
| Freshwater Drum | – | – | 6 | 24 |

Layzer 1995; Boyer et al. 2011). Thus, the fact that we found glochidia on a large percentage of the relatively low numbers of fishes examined during this study suggests that the Illinois River mussel community is recovering. However, it also means we would need to examine a substantial number of Asian carp to truly rule out the possibility that carp are not intercepting glochidia, since we only examined 145 carp during this study.

Silver, Bighead, and Common Carp did not carry many parasites, but Silver and Common Carp did have a substantially higher occurrence of telangiectasia hemorrhages compared to native fishes. This is a novel finding that is not well reported in the literature. It is unknown as to why non-native fishes may experience a higher degree of hemorrhages. There was also a higher occurrence of this condition in main stem fishes compared to tributaries, despite the fact that the fishes were collected with the same protocols and electrofishing settings. Variation in conductivity between the tributaries and the main stem may have contributed to the pattern; conductivity in the tributaries was generally lower than that in the main stem and pulsed DC-electrofishing can be affected by different conductivities (M.W. Fritts and R.M. Pendleton, Illinois Natural History Survey, C. Morgeson, Eastern Illinois University, personal communication). This may contribute to the increased presence of hemorrhages, but ultimately the cause of this phenomenon remains unresolved and will need additional research.

Asian carp are voracious filter-feeding consumers that can filter particles (e.g., plankton and algae) as small as 10-20 μm (Jennings 1988; Smith 1989; Vörös 1997). This feeding behavior introduces the potential for Asian carp to be consuming glochidia, an area of research that is currently unstudied. We focused our laboratory efforts on the filament structure of the gills, because this is the most likely location for glochidial gill-attachment in native fishes (Arey 1932b). However, the Asian carp could have been collecting glochidia with the fused, sponge-like gill rakers and subsequently ingesting the glochidia. Further studies should consider examining the gill rakers in addition to the filaments. It is unlikely that we would be able to detect glochidia in the stomach contents, but use of advance dietary studies, such as stable isotope analysis, may shed light on the potential for Asian carp to be ingesting glochidia.

Future work should include conducting laboratory host trials to evaluate the physiological ability of Asian carp to successfully transform any of our native mussel species, focusing on both host specialists and host generalists. This would be an efficient method to determine whether the Asian carp could successfully metamorphose glochidia to the juvenile life stage. If they are not suitable hosts, we could determine the period before untransformed glochidia are sloughed.

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ARTICLE

# TOXICITY OF SODIUM DODECYL SULFATE TO FEDERALLY THREATENED AND PETITIONED FRESHWATER MOLLUSK SPECIES

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*ABSTRACT*

**Anthropogenically caused physical and chemical habitat degradation, including water pollution, have caused dramatic declines in freshwater mollusk populations. Sodium dodecyl sulfate (SDS), a surfactant with no USEPA Water Quality Criteria (WQC), is commonly used in industrial applications, household cleaners, personal hygiene products, and herbicides. In aquatic habitats, previous SDS studies have associated deformities and death to mollusks found in these systems. The objective of this study was to determine $EC_{50}$ values for two freshwater juvenile unionids (*Villosa nebulosa* and *Hamiota perovalis*) and two freshwater caenogastropods (*Leptoxis ampla* and *Somatogyrus* sp.) endemic to the Mobile River Basin, USA, to SDS. Using the Trimmed Spearman-Karber method, $EC_{50}$ values were calculated. Results found that $EC_{50}$ values were: *V. nebulosa* = 14,469 μg/L (95% CI: 13,436 – 15,581 μg/L), *H. perovalis* = 6,102 μg/L (95% CI: 4,727 – 7,876 μg/L), *Somatogyrus* sp. = 1,986 μg/L (95% CI: 1,453 – 2,715 μg/L), and *L. ampla* = 26 μg/L (95% CI: 6 – 112 μg/L). Freshwater gastropods were more sensitive to SDS than freshwater unionids. *Leptoxis ampla* was the most sensitive species tested and had such a low $EC_{50}$ value that more protective regional criteria may be required. Therefore, future research should include additional testing on mollusk species, particularly regionally isolated species that may display increased sensitivity.**

***KEY WORDS*** - SDS, threatened, mollusk, Mobile River Basin, Water Quality Criteria

## INTRODUCTION

In North America, freshwater mollusks are the most imperiled aquatic fauna with 74% of 703 identified gastropods imperiled, followed by unionids with 72% of 298 identified species imperiled (Wilcove and Master 2008; Johnson et al. 2013). Many freshwater mollusk species are highly endemic, particularly in the Mobile River Basin, USA, which includes 139 endemic freshwater mollusk taxa (34 unionids and 105 gastropods) (Neves et al. 1997; Williams et al. 2008; Ó Foighil et al. 2011). Stenotypic species are often underrepresented in traditional toxicity testing that normally utilize broad ranging species, usually distributed across multiple drainage basins (Ó Foighil et al. 2011; Wang et al. 2010; Wang et al. 2011).

Declines in freshwater gastropod and unionid populations are attributed to increases in the human population, leading to alteration or destruction of habitat both physically and chemically (Villella et al. 2004; Johnson et al. 2013). Pollution is ranked as the second leading cause of stream impairment (USEPA 2009), following physical habitat alteration (Neves et al. 1997). Toxicity testing is important in protecting organisms by providing information on specific pollutant effects, such as reduced survival and growth or inhibited biological processes on a particular life stage (American Society for Testing and Materials (ASTM) 2013). Using data from these tests, criteria inclusive of imperiled organisms can be established to help protect remaining populations.

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Sodium dodecyl sulfate (SDS), a surfactant, is the most widely used synthetic organic chemical found in detergents, shampoos, cosmetics, household cleaners, herbicides, and dispersants used in oil-spill cleanups (Cowan-Ellsberry et al. 2014). Sodium dodecyl sulfate is an alkylsulfate with sodium as the counter ion with a chain length of 12 carbons (Cowan-Ellsberry et al. 2014). Sodium lauryl sulfate (SLS) and SDS are often used synonymously in reporting of product ingredients (Singer and Tjeerdema 1993; NIH, 2014). Concentrations >67% SLS (active ingredients) can be found in household products, dispersants, and herbicides (Lewis 1991; Singer and Tjeerdema 1993; Kegley et al. 2014). Sodium dodecyl sulfate is also used in the cleanup of polycyclic aromatic hydrocarbons (e.g., oil and gas products). The major exposure route for SDS to aquatic environments is through contaminated waters, sediments, or soils, which threatens drinking water supplies or organisms living in these environments (Singer and Tjeerdema 1993). Contamination of groundwater by surfactants is caused primarily by leaching from industrial and municipal sewage systems, but can also be introduced to the environment by domestic and industrial effluents from discarded cleaning products (Singer and Tjeerdema 1993; Chaturvedi and Kumar 2010). In the United States, per capita detergent consumption is about 10 kg/year (Rebello et al. 2014), but consumption declined 3.9% per year during 2008 – 2013 (Cowan-Ellsberry et al. 2014). However, in 2008, 76% of alkylsulfate consumed in North America were found in household laundry detergents (59%) and personal care products (17%) (Cowan-Ellsberry et al. 2014).

Sodium dodecyl sulfate is not currently monitored in water systems or listed as a ground water contaminant (Kegley et al. 2014). Other surfactants with similar uses are monitored (reviewed by Rebello et al. 2014 and Singer and Tjeerdema 1993). In the United Kingdom, surfactant concentrations in surface waters have been recorded as high as 416 $\mu$g/L (Fox et al. 2000), while sewage effluents have had concentrations documented up to 1,090 $\mu$g/L (Holt et al. 1989). Treated sludge has been found to have concentrations of linear alkylbenzene sulfonate as high as 30,200,000 $\mu$g/kg (dry weight) (Berna et al. 1989). All monitored concentrations for sulfates exceed the predicted no-effect concentration value (250 $\mu$g/L) for surfactants by van de Plassche et al. (1999). In Massachusetts, the Town River had reported concentrations between 40 $\mu$g/L and 590 $\mu$g/L (Lewis and Wee 1983), while other major rivers in the United States had reported surfactant concentrations that ranged from 10 $\mu$g/L to 3,300 $\mu$g/L (A.D. Little Co. 1981) or 10 $\mu$g/L to 40 $\mu$g/L (Hennes and Rapaport 1989).

Sodium dodecyl sulfate was formally classified as ‘environmental friendly’ based on its readily biodegradable and low bioaccumulation properties, meaning it does not persist long in the environment (Belanger et al. 2004). However, some studies have suggested that SDS can be lethal in acute exposures (e.g., 19,040 $\mu$g/L for *Utterbackia imbecilis*, Keller 1993; summarized in Singer and Tjeerdema 1993; summarized in Kegley et al. 2014; Table 2). Because of its fast acting, nonselective, and consistent toxicity, SDS is commonly used as a reference toxicant in toxicity tests (USEPA 2002). Developmental abnormalities in *Illyanassa obsoleta* embryos, such as incomplete or inhibited formation of lobe-dependent structures (e.g., foot, operculum, and eyes) of gastropods have been attributed to SDS exposure (treatments ranging from 10,000 – 30,000 $\mu$g/L) (Render 1990). Tarazona and Nuñez (1987) reported that SDS exposure significantly decreased shell weights in lymnaeid gastropods and impeded normal shell deposition ($EC_{50} = 540$ $\mu$g/L for *Lymnaea vulgaris* and 610 $\mu$g/L for *Physa heterostropha*). When exposed to SDS ($EC_{50} = 31{,}400$ $\mu$g/L), *Corbicula fluminea* displayed avoidance behaviors and gill damage which decreased oxygen consumption and reduced siphoning activity (Graney and Giesy 1988).

Previous studies suggest early life stages of unionids are more sensitive than later life stages or other commonly used aquatic test organisms (Keller et al. 2007; Augspurger 2013). Until recently, freshwater unionid toxicity tests were not included in establishing Water Quality Criteria (WQC) due to limited information available (e.g., life cycle, host fish, sensitivity, populations), and the inability to culture them in sufficient numbers to support testing needs (Keller et al. 2007).

Table 1. Data from acute toxicity trials using SDS on four mollusk species, including toxicant concentrations, number of dead organisms, and number of organisms exposed.

| Species | Concentration (μg/L) | Dead | Number exposed |
|---|---|---|---|
| *Villosa nebulosa* | Control | 3 | 10 |
| | 5,000 | 1 | 30 |
| | 10,000 | 1 | 30 |
| | 15,000 | 16 | 30 |
| | 20,000 | 28 | 30 |
| | 30,000 | 30 | 30 |
| *Hamiota perovalis* | Control | 0 | 10 |
| | 5,000 | 11 | 30 |
| | 10,000 | 16 | 30 |
| | 15,000 | 17 | 30 |
| | 20,000 | 28 | 30 |
| | 30,000 | 30 | 30 |
| *Leptoxis ampla* | Control | 0 | 10 |
| | 1 | 3 | 30 |
| | 10 | 11 | 30 |
| | 100 | 21 | 30 |
| | 1,000 | 23 | 30 |
| | 10,000 | 18 | 30 |
| *Somatogyrus* sp. | Control | 0 | 10 |
| | 1,000 | 7 | 30 |
| | 3,000 | 20 | 30 |
| | 10,000 | 30 | 30 |
| | 30,000 | 30 | 30 |
| | 100,000 | 30 | 30 |

Table 2. Median effective concentrations ($EC_{50}$) for 96 h acute toxicity tests of sulfate surfactants on mollusk species.

| Mollusk species | Common name | Age | Hardness (mg $CaCO_3$/L) | 96 h $EC_{50}$ ($\mu$g/L) | References |
|---|---|---|---|---|---|
| **Bivalves** | | | | | |
| *Corbicula fluminea* | Asiatic Clam | NR | NR | 31,400 | Graney and Gisey 1988 |
| *Utterbackia imbecillis* | Paper Pondshell | Juveniles | NR | 19,040[a] | Keller 1993 |
| *Hamiota perovalis* | Orangenacre Mucket | Juveniles | 43 | 6,102 | Current Study |
| *Villosa nebulosa* | Alabama Rainbow | Juveniles | 43 | 14,469 | Current Study |
| **Gastropods** | | | | | |
| *Physa heterostropha** | Pewter Physa | NR | NR | 34,161 | Patrick et al. 1968 |
| *Lymnaea peregra** | Wandering Pond Snail | NR | NR | 15 | Misra et al. 1984 |
| *Lymnaea vulgaris** | Great Pond Snail | Juveniles | 35.6 | 540 | Tarazona and Nuñez 1987 |
| *Lymnaea vulgaris** | Great Pond Snail | Juveniles | 35.6 | 610 | Tarazona and Nuñez 1987 |
| *Leptoxis ampla* | Round Rocksnail | Juveniles | 43 | 26 | Current Study |
| *Somatogyrus* sp. | Cahaba Pebblesnail | Adults | 43 | 1,986 | Current Study |

NR = not reported
[a] = 48 h $LC_{50}$ value
* = pulmonate gastropod

Similarly, caenogastropods (respire using a gill or ctenidia) are considered among the most sensitive aquatic organisms to contaminants (Besser et al. 2009), but are rarely used for toxicity testing due to slow growth and low reproductive rates, which make them difficult to culture or test in the laboratory (Besser et al. 2009). New propagation and rearing techniques have been recently developed that allow sufficient numbers of organisms to support formal toxicity testing (Barnhart 2006). The goal of the current study was to evaluate acute SDS exposure to four Mobile River Basin endemic mollusks, two freshwater juvenile unionids and gastropods. These data may eventually contribute to the development of a specific WQC for SDS for freshwater mollusks.

## METHODS

Two lotic freshwater unionids (*Hamiota perovalis* and *Villosa nebulosa*) and two lotic freshwater caenogastropods (*Leptoxis ampla* and *Somatogyrus* sp.) endemic to the Mobile River Basin were used. *Hamiota perovalis* (Orangenacre Mucket) and *Leptoxis ampla* (Round Rocksnail) were federally listed as threatened under the Endangered Species Act (ESA) (USFWS 1993, 1998). *Villosa nebulosa* (Alabama Rainbow) has been formally petitioned for federal protection under the ESA (Center for Biological Diversity 2010). The specific taxonomic position of *Somatogyrus* sp., Cahaba Pebblesnail, is unclear (E.E. Strong and P.D. Johnson, Alabama Aquatic Biodiversity Center (AABC), personal observation).

Unionids were propagated by the AABC, Marion, Alabama, using host-fish infections and standard culturing methods (Barnhart 2006). *Villosa nebulosa* adults (n=6) were collected from South Fork Terrapin Creek in Celburne County, Alabama while *H. perovalis* adults (n=4) were collected from Rush Creek in Winston County, Alabama. Both species used Largemouth Bass (*Micropterus salmoides*) for transformation. Unionids were fed a diet of Nanochloropsis, Shellfish diet (Reed Mariculture) at a concentration of ~50K cells/mL.

For gastropods, *Leptoxis ampla* was propagated by AABC, while *Somatogyrus* sp. was collected from the Cahaba River (Latitude: 32° 57.577′ N, Longitude: 87° 08.441′ W). The AABC commonly uses this location for reintroductions, restocking, and translocations of threatened and endangered mollusk species. Juvenile unionids were 30 – 60 days post-transformation, and gastropods were 5 – 8 months post hatch. *Somatogyrus* sp. is considered to be an annual species with juveniles hatching in spring. Adults die soon after the reproductive season is concluded (Johnson et al. 2013). Test organisms were kept in an aquarium with dilution water prepared following ASTM (2007) guidelines and used in testing within 14 days of arrival, so feeding was not necessary (ASTM 2013). Since it can absorb contaminants (Newton and Bartsch 2007), sediment was not used during toxicity testing, which reduced the likelihood of organisms being exposed to contaminants that may be present in sediment or uncontrolled chemical reactions occurring within the sediments.

### Experimental Conditions

Static renewal toxicity tests for both classes of organisms were completed following ASTM Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels (E2455-06) (ASTM 2013). Dilution water recipe additions included sodium bicarbonate ($NaHCO_3$), calcium sulfate ($CaSO_4 \cdot H_2O$), magnesium sulfate ($MgSO_4$), and potassium chloride (KCl) following ASTM Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians (E729-96) (ASTM 2007). The mean physiochemical variables of the dilution water were as follows: pH - 7.3 (7.1 – 7.4), hardness -

41 (40 – 42) mg $CaCO_3$/L, alkalinity - 33.5 (33 – 34) mg $CaCO_3$/L, and conductivity - 168 (130 – 189) $\mu\Omega$/cm. Dissolved oxygen saturation measured $\geq$90%, temperature was kept at 25 $\pm$ 1 °C, and ambient light from overhead fluorescent laboratory lights was used with a photoperiod of 16L: 8D. Test organisms were acclimated in the dilution water for at least 24 hours before trials by adjusting the temperature no more than 3 °C/h until reaching 25 °C (Wang et al. 2007; ASTM 2013). Ten individuals were used in triplicate per concentration (n = 30). Chambers (600 mL Pyrex® beakers) were filled with 300 mL of either dilution water (controls) or toxicant solution and were changed at 48 h (Table 1). Stock SDS toxicant solutions (Laboratory grade, Lot # AD-14008-56, Carolina Biological Supply) analyses were not conducted to quantify concentrations in toxicant exposure; therefore, $EC_{50}$ values are based on nominal concentrations.

Endpoint determinations were completed after the 96 h tests, unionids were placed under a microscope to view heartbeat or foot movement. If no movement was observed after five-minutes, the unionid was classified as dead. Gastropods were classified as dead if no movement was detected within a five-minute observation period (Archambault et al. 2015) or after the "tickle" test, performed by touching the organisms with a soft pick to provoke a stimulus response. An eyelash stick was used to prevent any excess pressure being placed on the foot and observing a false reaction. Non-decaying individuals were placed in fresh dilution water for thirty minutes and rechecked for survival. Control survivorship had to exceed 90% at the end of each trial for results to be acceptable (ASTM 2013) (Table 1).

### Data Analysis

The 96 hour $EC_{50}$ values for SDS were determined using ToxStat® 3.5 from West, Inc. (downloaded from https://www.msu.edu/course/zol/868/) using the Trimmed Spearman-Karber method (TSK). The $EC_{50}$ values of test organisms were then compared to $EC_{50}$ values of other species in the published literature.

## RESULTS

In accordance with ASTM (2013) protocols, control survivability was $\geq$90% for each unionid trial and 100% for each gastropod trial. Gastropods were active and scaling the beaker walls within control test chambers. The $EC_{50}$ value for *Hamiota perovalis* was 6,102 $\mu$g/L (95% CI: 4,727 – 7,876 $\mu$g/L), and *Villosa nebulosa* was 14,469 $\mu$g/L (95% CI: 13,436 – 15,581 $\mu$g/L), more than double the $EC_{50}$ value of *H. perovalis* (Table 2). In all treatments containing SDS, juvenile unionids of both species purged a mucus-like substance that coated the entire organism. While little foot movement was observed, a heartbeat was always detected in live unionids. However, in high concentrations of SDS, no soft tissue was observed in the shells at the end of the 96 h acute toxicity tests.

Gastropods tested in the current study were more sensitive to SDS than unionid species evaluated. *Leptoxis ampla* had an $EC_{50}$ value of 26 $\mu$g/L (95% CI: 6 – 112 $\mu$g/L), which was the lowest $EC_{50}$ value calculated in this study. *Somatogyrus* sp. had an $EC_{50}$ value of 1,986 $\mu$g/L (95% CI: 1,453 – 2,715 $\mu$g/L) (Table 2). Similar to unionids, soft tissues dissolved or completely separated from the shell in the highest concentrations, and most dead gastropods had begun to decompose so death was easily determined. Living gastropods from lower concentrations appeared to begin normal activity once transferred to water lacking SDS. Movement was observed without "tickling" in most low concentrations.

## DISCUSSION

Sodium dodecyl sulfate is commonly found in high concentrations in detergents and household cleaners and contaminates drinking water and aquatic ecosystems (Cowan-Ellsberry et al. 2014); however, it has no WQC. Keller (1993) reported a 48 h $LC_{50}$ value of 19,040 $\mu$g/L for juvenile *Utterbackia imbecillis*, a species classified as having a stable conservation status (Williams et al. 2008). Both juvenile unionid species exposed to SDS in the current study had 96 h $EC_{50}$ values (*V. nebulosa*: 14,469 $\mu$g/L (federally petitioned); *H. perovalis*: 6,102 $\mu$g/L (federally threatened)) below the value reported by Keller (1993). In a related study, Graney and Giesy (1988) reported a 96 h $LC_{50}$ value of 31,400 $\mu$g/L for SDS using *Corbicula fluminea* (Asiatic clam), which is 2x and 5x higher than the $EC_{50}$ values determined for *V. nebulosa* and *Hamiota perovalis*, respectively, in the current study.

The gastropod species used in the current study, *L. ampla* and *Somatogyrus* sp., were generally more sensitive to SDS as compared to other published studies, but Tarazona and Nuñez (1987) reported a 96 h $LC_{50}$ value of 540 $\mu$g/L for SLS using *Lymnaea vulgaris*, a pulmonate gastropod. This reported value was higher than the 96 h $EC_{50}$ value for *Leptoxis ampla* reported in the current study (26 $\mu$g/L), but *Somatogyrus* sp. had a higher 96 h $EC_{50}$ value at 1,986 $\mu$g/L, suggesting it may be more tolerant than *Lymnaea vulgaris*. Patrick et al. (1968) reported a $LC_{50}$ value of 34,161 $\mu$g/L for alkylbenzene sulfonate using the pulmonate gastropod *Physa heterostropha*, which was a greater concentration than any $EC_{50}$ value reported in this study. Misra et al. (1984) reported an $EC_{50}$ value of 15 $\mu$g/L (endpoint: calcium uptake) for alkylbenzene sulfonate using *Lymnaea peregra*, which was similar to the $EC_{50}$ value for *Leptoxis ampla*. No other published $EC_{50}$ or $LC_{50}$ values were close to the $EC_{50}$ value of *Leptoxis ampla*, suggesting that this highly stenotypic species (Cahaba River Basin endemic) could be one of the most sensitive aquatic species to SDS tested to date. These reported values suggest that caenogastropods display increased sensitivity over pulmonate gastropods to SDS contamination.

Fish have more frequently been subjected to SDS acute toxicity testing than freshwater mollusks and tend to have slightly higher $LC_{50}$ values than mollusks (Table 3). However,

Table 3. Median effective concentrations ($EC_{50}$) for 96 h acute toxicity tests of sulfate surfactants on macroinvertebrate and fish species.

| Species name | Common Name | Hardness (mg $CaCO_3$/L) | 96 h $EC_{50}$ ($\mu$g/L) | References |
|---|---|---|---|---|
| **Invertebrates** | | | | |
| *Daphnia magna* | Cladoceran | NR | 10,300 | Keller 1993 |
| *Daphnia magna* | Cladoceran | NR | 5,400 – 15,000[a] | Lewis and Weber 1985 |
| *Daphnia pulex* | Cladoceran | NR | 1,400 – 15,200[a] | Lewis and Weber 1985 |
| **Fish** | | | | |
| *Danio rerio* | Zebrafish | NR | 7970 | Fogels and Sprague 1977 |
| *Danio rerio* | Zebrafish | NR | 8810[a] | Fogels and Sprague 1977 |
| *Danio rerio* | Zebrafish | NR | 9,900 – 20,100 | Newsome 1982 |
| *Cichlasoma nigrofaciatum* | Convict Cichlid | NR | 16,100 – 30,000 | Newsome 1982 |
| *Ctenopharyngodon idella* | Grass Carp | NR | 7,700 | Susmi et al. 2010 |
| *Cynopoecilus melanotaenia* | Killfish | NR | 14,900 | Arenzon et al. 2003 |
| *Cyprinus carpio* | Common Carp | NR | 1,310 | Verma et al. 1981 |
| *Jordanella floridae* | Flagfish | NR | 8,100 | Fogels and Sprague 1977 |
| *Macrones vittatus* | Asian Striped Catfish | NR | 1,390 | Verma et al. 1978 |
| *Menidia beryllina* | Inland silverside | NR | 9,500 | Hemmer et al. 2010 |
| *Oncorhynchus mykiss* | Rainbow Trout | NR | 4,620 | Fogels and Sprague 1977 |
| *Piaractus brachypomus* | Red Pacu | 24 | 11,290 | Reátegui-Zirena et al. 2013 |
| *Pimephales promelas* | Fathead Minnow | NR | 6,600 | Conway et al. 1983 |
| *Pimephales promelas* | Fathead Minnow | NR | 10,000 – 22,500 | Newsome 1982 |
| *Pimephales promelas* | Fathead Minnow | NR | 8,600 | USEPA 2002 |
| *Poecilia reticulartus* | Guppy | NR | 13,500 – 18,300 | Newsome 1982 |

NR = not reported
[a] = 48 h $LC_{50}$ value

invertebrates tend to be more sensitive than fish to SDS, and few toxicity studies have focused on freshwater unionids using SDS. The cladoceran *Daphnia magna* had 48 h $LC_{50}$ value of 10,300 $\mu$g/L (Keller 1993), while *Daphnia pulex* had 48 h $LC_{50}$ values ranging between 1,400 $\mu$g/L and 15,200 $\mu$g/L (Lewis and Weber 1985; Singer and Tjeerdema 1993) (Table 3). These lower values compared to the $EC_{50}$ values of *Somatogyrus* sp. reported in the current study suggest that *D. pulex* and *Somatogyrus* sp. may have similar SDS sensitivity. Few toxicity studies have been conducted on caenogastropods (Besser et al. 2009; Johnson et al. 2013), and previous investigations with surfactants have used pulmonate gastropods (e.g., Patrick et al. 1968; Misra et al. 1984; Tarazona and Nuñez 1987).

Other surfactants have been studied more extensively on freshwater mollusks. Ostroumov and Widdows (2006) examined surfactants hindering filter feeding for unionids by reporting a drop in clearance rates after a 10 minute exposure. Bringolf et al. (2007b) examined the components of Roundup® using *Lampsilis siliquoidea* and found that MON 0818, the polyethoxylated tallow amine (POEA) surfactant blend that helps the active ingredients penetrate the waxy coverings of plant leaves, was the most toxic component of that herbicide. In a similar study, Bringolf et al. (2007a) further examined MON 0818 and reported that unionids during their earlier life stages (i.e., glochidia and juvenile stages) are among the most sensitive organisms tested.

The $EC_{50}$ values of the current study were lower than most reported in the literature (Tables 2 – 3) for SDS, suggesting that some freshwater mollusks may be among the most sensitive aquatic species tested to date. The majority of mollusk species previously tested have broad geographical ranges and occur across multiple basins and are, therefore, probably adapted to a broad range of physical and chemical water quality. In contrast, many federally listed freshwater mollusks have distributions limited to a distinct, regional drainage, such as the Mobile River Basin species utilized in the current study. These regionalized species would be adapted to specific regional physical and chemical parameters. Freshwater unionids and caenogastropods in the current study were found to be sensitive to SDS, particularly the Cahaba River Basin endemic, *Leptoxis ampla* had the lowest $LC_{50}$ value recorded to date. Threatened species, such as *L. ampla* or *H. perovalis*, often demonstrate increased sensitivity to environmental changes than other broad ranging species. The stenotypic biology of small range endemic mollusks with unique generic placement (e.g., *Hamiota*) may represent increased sensitivity to a variety of toxicants (Gibson 2015) than wider ranging species adapted to a broad range of normal water quality variables (e.g., *Lampsilis siliquoidea*).

Toxicity tests are important tools that provide information for risk assessment of chemicals and are used when determining USEPA WQC. This study is one of the few testing toxicity of SDS using Mobile River Basin freshwater mollusks, specifically using federally threatened or petitioned species. Many mollusks have a narrow endemic range which may increase sensitivity to water quality as compared to the broader ranging species. Further testing is urged for regional mollusk species, and especially determination of WQC for SDS, which currently does not exist. Criteria may need to be established using a suite of organisms from various river basins to include stenotypic species (e.g., Cahaba River Basin endemics) that may display increased sensitivity to SDS.

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