ABSTRACT

ARCHAMBAULT, JENNIFER MICHELE. Contaminant-Related Ecosystem Functions and Services of Freshwater Mussels (Unionidae) and Public Views on Nature's Contributions to Water Quality. (Under the direction of Dr. W. Gregory Cope).

Ecosystem services are generally defined as benefits that humans derive from nature, and though human reliance on nature is timeless, the scientific study of these services has become part of mainstream ecology only in the last two decades. From the mid-19th to the mid-20th Centuries, freshwater mussel populations in North America (Unionida) were valued and exploited for their material uses in the pearling and button-making industries. However, the depletion of mussel populations through exploitation, habitat destruction, and water quality degradation reduced their availability, utility, and visibility to human communities, leaving native mussels largely forgotten by most people. However, freshwater mussel populations and human populations are inextricably linked through their mutual dependence on water – arguably the most precious of resources. Several modern studies have revealed that freshwater mussels perform a host of functions that are integral to maintaining surface water quality and keeping rivers and lakes properly functioning as ecosystems. These functions provide important ecosystem services related to maintaining water quality for human uses, but the capacity of mussels to contribute appropriately to ecosystem functioning and services is drastically hampered because a majority of mussel populations are declining and more than 70% of species are imperiled. Furthermore, though aquatic species are integral to ecosystem functioning and maintenance of water quality, most are not readily perceivable by the public, and people may not realize the relevance of these ecosystem components in regulating healthy waterways for human use and well-being. Native freshwater mussels in particular are not well understood by most people outside the aquatic sciences profession. One area of ecosystem services research that has

been underexplored is the role of native mussels in reducing aquatic pollution. Thus, I sought to advance the knowledge on ecosystem services of freshwater mussels from a contaminants perspective at both organismal and population scales, and I further explored human perceptions of floral and faunal influences on water quality.

First, I explored the feasibility of using existing data on mussel populations along with tissue concentrations of pollutants to estimate population-level pollutant sequestration as a potential ecosystem service. I investigated three scenarios that were selected based on my direct access to the rare resource of tissue contaminant data from mussels collected in the wild, and the availability of population estimates at these sites from the literature or from colleagues. These scenarios included Upper Mississippi River navigation pools, the Upper Neuse River watershed (North Carolina), and a polluted compared to a healthy mussel site in the Clinch River (Virginia and Tennessee). These scenarios represented a range of spatial scales, from wadeable streams to large river systems; contaminant datasets from metals to organic contaminants; mussel population sizes from tens of thousands to hundreds of millions; and population estimates based on data types that ranged from qualitative techniques (e.g., visual search of mussels) to robust, quantitative techniques (e.g., systematic sampling). Estimates of contaminant sequestration differed based on spatial scale, population size, and the kind of contaminant under consideration. We estimated that mussels in two navigation pools of the Upper Mississippi River sequestered approximately 15.6 tons of metals; mussels in the Upper Neuse River watershed sequestered between 2.4 and 5.8 billion ng of polycyclic aromatic hydrocarbons (PAHs); and Clinch River mussels at the polluted Pendleton Island site sequestered 24.2 billion ng of PAHs compared to 210 billion ng of PAHs sequestered by mussels at the healthier sites outside a mussel zone of decline – 10X greater capacity despite having much lower tissue concentrations. Estimating population-level sequestration by mussels using existing data varied in difficulty, from straightforward to highly conditional, based on the types of available population data. These efforts offer a proof-of-concept demonstration of the magnitude of pollution mussels are filtering out of the environment through their incidental exposure to contaminants. My findings suggest that contaminant sequestration may be interpreted as an ecosystem service, but mussels will only be able to remove contaminants so long as aquatic ecosystems are healthy enough to support their persistence.

After exploring population-level contaminant sequestration, I then addressed a central question in the discourse of contaminant related ecosystem services among mussel biologists: what happens to contaminants after mussels ingest them? Though there is scientific understanding that contaminants collect in soft tissue, as they do for humans and other exposed organisms, one area of research that has not been explored is the role of mussels in ecological partitioning of pollutants. I conducted 28-d laboratory experiments exposing mussels to environmentally relevant concentrations of Ni (0 to 100 µg/L) and Cd (0 to 2 µg/L) – two toxic heavy metals of both human and environmental health concern – to answer the following questions: what percentage of metals do mussels remove from water; how much is sequestered in soft tissue; how much is egested in biodeposits; how are filtration rates affected by metal exposure; and finally, how are these estimates affected by metal concentration or exposure duration? Mussels removed up to 36% of waterborne Ni and up to 77% of waterborne Cd and they sequestered metals in their soft tissue. Mussels also bound and bioconcentrated metals in egested biodeposits (e.g., feces). Ni concentrations in biodeposits were 2 to 7X higher than exposure concentrations, and Cd concentrations in biodeposits were 7 to 40X higher than Cd in the exposure water. These pollutant-processing functions fluctuated significantly within the environmentally relevant ranges of Ni and Cd concentrations over the course of 28-d exposures. Fluctuations in functional processing manifested differently for Ni and Cd. Mussels were more efficient at processing Ni at lower concentrations (i.e., when exposed to less pollution), while the duration of exposure was an important factor for Cd processing; these trends generally held for each metal even when mussels were exposed to both Ni and Cd. Moreover, this ability of mussels to influence the environmental fate and transport of metals was in turn affected by the metal concentrations to which they were exposed, suggesting that pollution may impede other beneficial ecosystem services that mussels provide. Ni exposure significantly reduced mussel filtration capacity at concentrations higher than 5 μg Ni/L. Filtration rates of mussels exposed to Cd were significantly reduced in the first two weeks of exposure compared to the last two weeks, but Cd concentration had no effect. Filtration rates of mussels under the stress of both metals were affected by metal concentration and exposure duration, suggesting an additive effect of the two pollutants. This work demonstrates the active role of mussels in environmental fate and transport of toxic heavy metals in aquatic ecosystems, and that pollution negatively affects freshwater mussel filtration and the ecosystem services they provide.

Finally, I engaged with local communities to investigate public perceptions of water quality's mediating factors. Though aquatic species are integral to ecosystem functioning and maintenance of water quality, most are not readily perceivable by the public, and people may not realize the relevance of these ecosystem components in regulating healthy waterways for human use and well-being. It is imperative to capture how these resources are valued by communities because improved understanding of community values is a critical component of promoting effective watershed management. Social science research methods are increasingly employed to investigate public understanding and beliefs about conservation and natural resource issues**.** A first step in understanding the community valuation of ecosystem services related to water quality is investigating public perceptions of water quality's mediating factors. Thus, I engaged 57 residents of central and eastern North Carolina in six focused small group discussions, using a series of photographs of plants and animals, including freshwater mussels, to examine communities' beliefs about whether and how those flora and fauna relate to maintenance of water quality. Several prevailing themes emerged from the focus group discussions, including positive effects that flora and fauna have on water quality, dualistic "good and bad" or negative impacts, flora and fauna as indicators of water quality, and balance in nature. Participants also expressed uncertainty at times, and we identified a number of misconceptions about flora and fauna. Participants also offered some comments on impacts to water quality by humans, and the photographs sparked some commentary about connections to values or well-being related to waterways and water quality. Participants regularly relied on their prior experiences to explain their understanding of factors affecting water quality. Findings from these focus group discussions provide baseline understanding of public beliefs and knowledge of ecosystem functioning related to water quality. Participants identified several effects that flora and fauna have on water quality, including ecosystem functions that provide essential ecosystem services (e.g., regulating services, such as water purification through filtering and cleaning, and provision of habitat for aquatic species). These findings suggest an encouraging congruence of public beliefs with expert science, offering some common ground, similar language, and opportunities for connecting with communities on important issues that highlight or threaten ecosystem functioning and resulting ecosystem services that link environmental and human well-being.

Contaminant-Related Ecosystem Functions and Services of Freshwater Mussels (Unionidae) and Public Views on Nature's Contributions to Water Quality

by

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DEDICATION

For my husband – William Brian Mullin

This journey and accomplishment would not have been possible without your love and support.

Thank you for enduring this wild ride and keeping me laughing and sane along the way.

BIOGRAPHY

I was born in Polk County, Florida, in the era of Circus World, and near enough to two juxtaposed aromatic industries whose blended scents were memorable even at an early age – the Donald Duck Orange Juice factory and a wastewater treatment plant. My earliest days were spent in tiny stack flip flops on Florida beaches. I remember at least one hard lesson about fire ants and the benefits of *Aloe vera* by age four…keep your feet on the big wheel pedals! I also hated grits. After a move to Michigan with my mom before age five, I spent my school years growing up in a Midwestern city, and my summers in the North Carolina mountains and then the Greater Atlanta area with my dad and step-mom, and eventually my younger sister and brother – always returning to Michigan sounding a bit more southern than when I left. I have been linguistically promiscuous since those formative years, earning both *y'all* and *yup* honestly.

Today, I am a scholar of ecology, environmental toxicology, science communication, and social sciences. My path to this point has been rich, winding, and sometimes difficult, and so I am much more than my scholarly accomplishments – I am a United States Marine Corps veteran, yogi, first generation college student and graduate student from a low income background, precision knitter, avid (though not always successful) angler, occasional baker, nature lover, wife to a phenomenal husband, a good but disgruntled chef (according to said husband), daughter and sister to a proud family, and mom to two full-throttle step-sons (ages 18 and 16), the sweetest jet black rescue Pyrador (age 4), and a forgiving sourdough starter (age 5 ½) named Betty after my late grandmother.

I don't recall any particularly defining moments as a child growing up in the city, which led me to a natural resources career. I grew up professing that I would be doctor from about age three. Despite the expectation of being called Dr. Archambault (MD) one day and lacking a defining moment along my path, the memories of my youth are dappled with moments passed in a swimming hole in the Smoky Mountains, gazing curiously at a garter snake in the city as it crossed my path, watching bats from my porch with wonder as they circled overhead at twilight, the smell of a local skunk that made its home in town, observing pheasants walk across a parking lot into the woods, and poking around the edges of a tiny, green, scum-covered pond. My respect and love for wildlife and natural landscapes was innate.

My life as a biologist officially began with a few well-timed decisions early on at Grand Rapids Community College. After my first semester and first zoology class, I was hooked! I wanted to learn all I could about wildlife. Thanks to the first among my many great advisors, Dr. Greg Forbes, I found the most appropriate path for me after my Associate's Degree – the Fisheries and Wildlife Program at NC State University. I earned my B.S. with a dual concentration in Fisheries and Wildlife in 2004 (advised by Dr. Pete Bromley and Dr. Phil Doerr) and then worked as a professional Wildlife and Wetlands Biologist at Dr. J. H. Carter III & Associates, Inc., Environmental Consultants, in the Sandhills Region of NC through 2010. Though it was a scary move to leave a full-time job and benefits, I returned to NC State that year to study freshwater mussels for the first time. I traded in my hiking boots for waders and a lab coat and studied the thermal ecology and toxicology of mussels, under the direction of Drs. Greg Cope and Tom Kwak, and I earned my M.S. in Zoology in 2012. I have remained a member of the Wolfpack family since then, working as a Research Associate in the Department of Applied Ecology, studying pollutants and their effects on freshwater mussels and freshwater snails. I began my current research in pursuit of my Ph.D. in 2016, studying ecosystem services of mussels and public understanding of water quality. It turns out I was right about being a doctor all those years ago; it just happens that I am earning the Ph.D. variety!

ACKNOWLEDGMENTS

My four-year journey of scholarship, research, teaching, and community engagement, toward this doctorate has been underpinned by a wide and wonderful network of supporters. Thanks to the five doctors who has served as my Graduate Advising Committee: Greg Cope, Catherine LePrevost, Jane Harrison, Tom Kwak, and Nick Haddad. Each one of them has contributed to my growth as a professional and scholar, and they have given their time and expertise any time I sought their help. Thanks especially to Greg for serving as my Chair and giving me the opportunity to earn my PhD continuing to study freshwater mussels, and for giving me the freedom to take the reins beyond our initial funding tasks to design and conduct the research that I envisioned in addressing pollutant-related ecosystem services and public perceptions about water quality. I also must express the deepest gratitude to Catherine. I'm not sure either of us realized the commitment that would manifest when I asked her to join my committee. She has been an exceptional mentor in my development as a social scientist and science communicator, and has truly devoted the time, effort, and mentorship of a Co-Chair in guiding the research that resulted in my third chapter (and more results to come).

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State University Student Subunit of the American Fisheries Society (aka Student Fisheries Society).

Thanks goes Teresa Newton, Patty Ries, Heidi Dunn, and Dan Scoggin – field assistance rock stars who helped me collect samples in the Upper Mississippi River from Wisconsin to Missouri; to my lab technicians – Meredith Shehdan and Clayton Lynch – for being test-solution making, mussel scrubbing, and mussel poop-scooping aficionados, and sticking out the long days and weeks to get the job done; to Sean Buczek, my colleague of several years for pitching in on lab experiments, brainstorming, and providing experimental advice; to Jim Rice, who let me borrow equipment and commandeer his laboratory for nearly three months to have space for my experiments; to Damian Shea, Xin-Rui (Summer) Xia, and Xiang Qing Kong (Anna), who supplied me with passive sampling devices and other equipment; to David Buchwalter for experimental advice and perspective on metals; to Dave Dickey, SAS and statistical consulting guru who helped me slog through the appropriate analysis approaches for my complex experimental data even as he retired; to Megan Bradley and Rachael Hoch for advice on mussel feeding; to Brena Jones, Trevor Hall, and Loretta Lutakas (NCWRC) for assistance and coordination in mussels for my experiments; to Cathy Dover and analytical staff at Shealy Environmental Services and Hope Taylor and staff at GEL Labs for organic contaminant analyses; to Frank Weber at RTI, International, for metals analysis and experimental sampling advice, and being generous with his time to a fellow Wolfpacker; and finally to Caryn Vaughn, whose advice, curiosities, and mentorship were inspiration for the study that eventually became a monumental experimental undertaking and Chapter 2 of my research herein.

I owe heartfelt thanks to the community organizers who were instrumental in helping me to gather focus group participants in Central and Eastern North Carolina, including Karen

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I am grateful to Matthew Morse Booker for helping me discover the past through environmental history, and allowing me to better understand ecology and environmental issues through this historical lens. His influence, guidance, thought-provoking conversations, and support directly led to my creation of the introductory chapter herein, and he has inspired me to keep history close at hand in my future research, teaching, and other professional endeavors. Let this be a proclamation and evidence for the necessity of the humanities disciplines in creating and maintaining good scientists and societies.

For guiding my development as an educator, I thank Brad Taylor and Benjamin Reading for providing teaching mentorship as I led their course laboratory sections. (Thanks also to Ben for his solidarity as a fellow Chicago sports fan!) I am especially grateful for the efforts and mentorship of Vanessa Doriott Anderson, who helped me to grow professionally as an educator through the Certificate in Teaching and Communication Program. Thanks also to The Graduate School for offering such wonderful programs and resources.

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INTRODUCTION

The Intersections of Exploitation, Visibility, and Conservation in an Environmental History of Freshwater Mussels

Freshwater mussel populations and human populations are inextricably linked through their mutual dependence on water – arguably the most precious of natural resources. As I venture into this comprehensive narrative on ecosystem functions of freshwater mussels, their connection to human populations through the beneficial services they provide, and the ways human communities currently understand aquatic organisms, this introductory provides background for the broad range of scholarly topics herein. Because the research presented in this dissertation is highly interdisciplinary – encompassing ecology, ecosystem services, toxicology, and social science topics – I believe readers will benefit from a primer outlining this network of disciplines as they relate to freshwater mussels. Moreover, freshwater mussels themselves – my naiad muses – are largely unknown to most people, even among many in the biological and ecological science disciplines. Following a brief primer on how each discipline is relevant to our current understanding of freshwater mussel fauna, I will demonstrate their timeless relevance in an environmental history of freshwater mussels that centers on their visibility, prior exploitation, and early efforts in aiding their conservation. This reflection of our past connections – and disconnections – with freshwater mussels should inform and illuminate a rationale for the research that follows.

The environmental history of freshwater mussel exploitation, visibility, and early conservation demonstrates that waterways and aquatic resources have always been coupled social-ecological systems. The enjoyment of ecosystem services as a mainstream field in ecological science over the past two decades shares some qualities with environmental history, such as human interaction with and reliance on nature and nature's agency to benefit society. The environmental history perspective of humans coupled with nature will be important to keep in mind in the following chapters because it aligns well with modern discussions of aquatic resource conservation and management as social-ecological systems

INTERDISCIPLINARY RESEARCH ON FRESHWATER MUSSELS

The freshwater mussels I study are those in the order Unionida – bivalve mollusks that occur in rivers and lakes around the globe, and comprise the native mussel fauna of North America. Similar to plant species that require animal vectors to disperse their seeds, unionid mussels require a host fish for successful development and dispersal of their offspring.[1] This life history strategy renders them unique among all other freshwater or marine bivalves, which have a free-living planktonic larval stage, and with which unionids are only distantly related (e.g., taxonomically separated at the order level, their relation to other bivalves is similar to that of humans' relation to whales). The southeastern United States (US) is home to the largest biodiversity hotspot of freshwater mussels in the world, and the native fauna in the US and Canada includes nearly 300 species (293 in Unionidae and 5 Margaritiferidae according to a recent taxonomic review).[2] North American freshwater mussel richness comprises nearly half of

[1] Lefevre G, WC Curtis. 1910. Reproduction and parasitism in the Unionidae. Journal of Experimental Zoology 9: 79-115; Bauer G, K Wächtler, eds. 2001. Ecology and evolution of the freshwater mussels Unionoida. Springer: Berlin.

[2] Williams JD, AE Bogan, RS Butler, KS Cummings, JT Garner, JL Harris, NA Johnson, GT Watters. 2017. A revised list of the freshwater mussels (Mollusca: Bivalvia: Unionida) of the United States and Canada. Freshwater Mollusk Biology and Conservation 20: 33-58.

all species in the globally distributed Unionidae, and approximately one-third of all extant species in the order Unionida.[3]

This biodiverse freshwater mussel fauna plays important ***ecological*** roles. Several modern studies have revealed that native mussels perform a host of functions that are integral to maintaining surface water quality and keeping rivers and lakes properly functioning as ecosystems. These functions include bioturbation (i.e., sediment mixing) and nutrient cycling, providing physical habitat and stabilizing sediments, filtering algae, bacteria, and other particles and manufacturing biodeposits that create local hotspots for supporting stream community biodiversity.[4]

Individual freshwater mussels can filter several liters of water per hour, so the ecosystem functions that populations perform may offer important ***ecosystem services*** related to maintaining and improving water quality.[5] Ecosystem services are generally defined as nature's goods and services that contribute to human well-being, and they have been codified according to four main categories, including: provisioning services (e.g., food, water, fiber, fuel), regulating services (e.g., climate/flood regulation, water purification), cultural services (e.g., spiritual, recreational, or aesthetic importance), and supporting services (e.g., soil formation, nutrient

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[3] Graf DL, KS Cummings. 2020. The freshwater mussels (Unionoida) of the world (and other less consequential bivalves). Available from: http://www.mussel-project.net/ [Accessed 24 January 2020].

[4] Vaughn CC, CC Hakencamp. 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biology 46: 1431-1446; Vaughn CC, KB Gido, DE Spooner. 2004. Ecosystem processes performed by unionid mussels in stream mesocosms: species roles and effects of abundance. Hydrobiologia 527: 35-47; Howard JK, KM Cuffey. 2006. The functional role of native freshwater mussels in the fluvial benthic environment. Freshwater Biology 51: 460-474; Spooner DE, CC Vaughn. 2006. Context-dependent effects of freshwater mussels on stream benthic communities. Freshwater Biology 51: 1016-1024; Vaughn CC, JS Nichols, DE Spooner. 2008. Community and foodweb ecology of freshwater mussels. Journal of the North American Benthological Society 27: 409-423; Allen DC, CC Vaughn, JF Kelly, JT Cooper, MH Engel. 2012. Bottom-up biodiversity effects increase resource subsidy flux between ecosystems. Ecology 93: 2165-2174.

[5] Kreeger DA, CM Gatenby, PW Bergstrom. 2018. Restoration potential of several native species of bivalve molluscs for water quality improvement in Mid-Atlantic watersheds. Journal of Shellfish Research 37: 1121-1157.

cycling.[6] The potential for ecological functions mussels perform to be of value as ecosystem services has only been explored in the last several years. Caryn Vaughn recently published an ecosystem service framework for mussel functions in which one of the most prevalent benefits for humans was water quality maintenance through their many regulating and supporting roles.[7] Mussels store and cycle nutrients, they sequester pathogens such as *Escherichia coli*, and they may filter out contaminants of emerging concern, including pharmaceuticals and personal care products.[8]

Despite the recognition that mussels provide ecosystem service benefits, the capacity of mussels to contribute appropriately to ecosystem functioning and services is drastically hampered because mussel populations have been substantially reduced from historic abundances.[9] North American native mussels have an imperilment rate greater than 70%, due to historical overharvesting, habitat destruction, and pollution, and they are particularly vulnerable to present-day chronic impacts, such as water quality degradation.[10] Mussels will likely suffer

[6] Millennium Ecosystem Assessment. 2005. Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC.
[7] Vaughn CC. 2018. Ecosystem services provided by freshwater mussels. Hydrobiologia 810: 15-27.
[8] Ismail NS, CE Muller, RR Morgan, RG Luthy, 2014. Uptake of contaminants of emerging concern by the bivalves Anodonta californiensis and Corbicula fluminea. Environmental Science & Technology 48: 9211–9219; Ismail NS, H Dodd, LM Sassoubre, AJ Horne, AB Boehm, RG Luthy, 2015. Improvement of urban lake water quality by removal of Escherichia coli through the action of the bivalve Anodonta californiensis. Environmental Science & Technology 49: 1664–1672; Ismail NS, JP Tommerdahl, AB Boehm, RG Luthy. 2016. Escherichia coli reduction by bivalves in an impaired river impacted by agricultural land use. Environmental Science & Technology 50: 11025-11033; Hoellein TJ, CB Zarnoch, DA Bruesewitz, J DeMartini. 2017. Contributions of freshwater mussels (Unionidae) to nutrient cycling in an urban river: filtration, recycling, storage, and removal. Biogeochemistry 135: 307–324.
[9] Vaughn CC, TJ Hoellein 2018. Bivalve impacts in freshwater and marine ecosystems. Annual Review of Ecology, Evolution, and Systematics 49: 183-208.
[10] Williams JD, ML Warren Jr, KS Cummings, JL Harris, RJ Neves. 1993. Conservation status of the freshwater mussels of the United States and Canada. Fisheries 18(9): 6-22; Strayer DL, JA Downing, WR Haag, TL King, JB Layzer, TJ Newton & SJ Nichols. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. BioScience 54: 429-439; Downing JA, P Van Meter, DA Woolnough. 2010. Suspects and evidence: a review of the causes of extirpation and decline in freshwater mussels. Animal Biodiversity and Conservation 33: 151-185; Haag WR, JD Williams. 2014. Biodiversity on the brink: an assessment of conservation strategies for North American freshwater mussels. Hydrobiologia 735: 45-60; FMCS [Freshwater Mollusk Conservation Society].

continued decline without measures to improve water quality because they are highly sensitive to many contaminants.[11] A concerted focus on mussel ***toxicology*** in recent decades has revealed that they are among the most sensitive organisms to several classes of contaminants that commonly occur in waterways and which span a range of toxic modes of action, including terrestrial herbicides and other pesticides, aquatic herbicides, copper and ammonia, and major ions.[12] Lopez-Lima and colleagues more recently reported that pollution is the most widely recorded global threat to freshwater bivalves for species that have been assessed by the International Union for Conservation of Nature.[13] Diminished mussel populations are clearly unable to provide the same magnitude of contribution to freshwater ecosystem functioning as

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2016. A national strategy for the conservation of freshwater mollusks. Freshwater Mollusk Biology and Conservation 19: 1-21.

[11] Cope WG, RB Bringolf, DB Buchwalter, TJ Newton, CG Ingersoll, N Wang, T Augspurger, FJ Dwyer, MC Barnhart, RJ Neves, E Hammer. 2008. Differential exposure, duration, and sensitivity of unionoidean bivalve life stages to environmental contaminants. Journal of the North American Benthological Society 27: 451-462.

[12] Conners DE, MC Black. 2004. Evaluation of lethality and genotoxicity in the freshwater mussel Utterbackia imbecillis (Bivalvia: Unionidae) exposed singly and in combination to chemicals used in lawn care. Archives of Environmental Contamination and Toxicology 46 :362-371; Bringolf RB, WG Cope, S Mosher, MC Barnhart, D Shea. 2007. Acute and chronic toxicity of glyphosate compounds to glochidia and juveniles of Lampsilis siliquoidea (Unionidae). Environmental Toxicology & Chemistry 26: 2094-2100; Newton TJ, MR Bartsch. 2007. Lethal and sublethal effects of ammonia to juvenile Lampsilis mussels (Unionidae) in sediment and water-only exposures. Environmental Toxicology & Chemistry 26: 2057–2065; Wang N, CG Ingersoll, IE Greer, DK Hardesty, CD Ivey, JL Kunz, WG Brumbaugh, FJ Dwyer, AD Roberts, T Augspurger, CM Kane, RJ Neves & MC Barnhart. 2007. Chronic toxicity of copper and ammonia to juvenile freshwater mussels (Unionidae). Environmental Toxicology and Chemistry 26: 2048-2056; Archambault JM, CM Bergeron, WG Cope, RJ Richardson, MA Heilman, JE Corey III, ME Netherland, RJ Heise. 2015. Sensitivity of freshwater molluscs to Hydrilla-targeting herbicides: providing context for invasive aquatic weed control in diverse ecosystems. Journal of Freshwater Ecology 30: 335-348; Zipper CE, PF Donovan, JW Jones, J Li, JE Price, RE Stewart. 2016. Spatial and temporal relationships among watershed mining, water quality, and freshwater mussel status in an eastern USA river. Science of the Total Environment 541: 603–615; Wang N, CD Ivey, CG Ingersoll, WG Brumbaugh, D Alvarez, EJ Hammer, CR Bauer, T Augspurger, S Raimondo, MC Barnhart. 2017. Acute sensitivity of a broad range of freshwater mussels to chemicals with different modes of toxic action. Environmental Toxicology & Chemistry 36: 786–796; Ciparis S, G Rhyne, T Stephenson. 2019. Exposure to elevated concentrations of major ions decreases condition index of freshwater mussels: comparison of metrics. Freshwater Mollusk Biology and Conservation 22: 98-108.

[13] Lopes-Lima M, LE Burlakova, AY Karatayev, K Mehler, M Seddon, R Sousa. 2018. Conservation of freshwater bivalves at the global scale: diversity, threats and research needs. Hydrobiologia 810: 1-14.

healthy populations, and mussels will likely suffer continued endangerment without measures to improve aquatic habitats and water quality.[14]

Water quality is as important for humans as it is for mussels. Water is an essential resource that humans derive from nature. Clean water is a necessity for survival, and surface water resources are arguably among the most important of social-ecological systems. Impaired water quality in the United States and around the globe has resulted in declining biodiversity and a reduction in the delivery of aquatic ecosystem services.[15] Though aquatic species are integral to ecosystem functioning and maintenance of water quality, most are not readily perceivable by the public, and people may not realize the relevance of these ecosystem components in regulating healthy waterways for human use and well-being. ***Social science*** research methods are increasingly employed to investigate public understanding and beliefs about conservation and natural resource issues.[16] In prior research asking communities to describe the quality of surface waters, respondents have relied extensively on the physical appearance of the water and other visible cues in immediate surroundings.[17] However, there is a gap in understanding people's knowledge and perceptions of functional ecology associated with water quality maintenance.

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[14] Jones J, T Lane, B Ostby, B Beaty, S Ahlstedt, R Butler, D Hubbs, C Walker. 2018. Collapse of the Pendleton Island mussel fauna in the Clinch River, Virginia: setting baseline conditions to guide recovery and restoration. Freshwater Mollusk Biology and Conservation 21: 36-56; Vaughn 2018.

[15] Baskett ML, BS Halpern. 2009. Marine ecosystem services. Part VI.7 in SA Levin, Ed. The Princeton Guide to Ecology. Princeton University Press. Princeton, NJ: 619-625; Palmer MA, DC Richardson. 2009. Provisioning services: a focus on fresh water. Part VI.8 in SA Levin, Ed. The Princeton Guide to Ecology. Princeton University Press. Princeton, NJ: 626-633.

[16] Moon K, D Blackman. 2014. A guide to understanding social science research for natural scientists. Conservation Biology 28: 1167-1177.

[17] David EL. 1971. Public perceptions of water quality. Water Resources Research 7:453-457; Dinius, SH. 1981. Public perceptions in water quality evaluation. Water Resources Bulletin 17: 116-121; Limburg KE, Luzadis VA, Ramsey M, Schulz KL, Mayer CM. 2010. The good, the bad, and the algae: perceiving ecosystem services and disservices generated by zebra and quagga mussels. Journal of Great Lakes Research 36:86-92; Artell, J, H Ahtianen & E Pouta. 2013. Subjective vs. objective measures in the valuation of water quality. Journal of Environmental Management 130: 288-296; West AO, Nolan JM, Scott JT. 2016. Optical water quality and human perceptions: a synthesis. WIREs Water 3:167-180.

Scientists must understand public knowledge and perceptions in order to bring to light the often invisible underlying ecological processes that regulate water quality.[18]

Human communities in North America (and beyond) have shared a long and rich history with unionid freshwater mussels, particularly in the broad Mississippi River Basin of the central United States. The field of ***environmental history*** incorporates human uses of and interactions with nature and explores societal views about nature, but it also emphasizes the role of nature's influence on human societies. The lexicon about human uses of and benefits from nature has entered a mainstream of study in the framework of ecosystem services, first in ecological economics and now in the ecology fields, and aquatic resources are increasingly (and appropriately) being framed as social-ecological systems governed by both ecological processes and human driven processes and values.[19] Therefore, environmental history and its analogous emphasis on human-environmental interactions in the past offers a useful framework to examine how we and mussels arrived at the current status of our shared trajectory, and offers insights into future directions.

AN ENVIRONMENTAL HISTORY OF FRESHWATER MUSSELS

North America's native freshwater mussel fauna is an ideal model for exploring exploitation, visibility, and conservation in environmental history. Of course, their history with humans extends prior to Colonial then US expansion, but they were seemingly of little

[18] West et al. 2016.

[19] Daily GC, ed. 1997. Nature's Services: Societal Dependence on Natural Ecosystems. Island Press: Washington, DC; Kinzig AP. 2009. Ecosystem Services. Part VI in SA Levin, Ed. The Princeton Guide to Ecology. Princeton University Press, Princeton, NJ; Costanza R, R de Groot, L Braat, I Kubiszewski, L Fioramonti, P Sutton, S Farber, M Grasso. 2017. Twenty years of ecosystem services: how far have we come and how far do we still need to go? Ecosystem Services 28 Part A: 1-16; Hand BK, CG Flint, CA Frissell, CC Muhlfeld, SP Delvin, BP Kennedy, RL Crabtree, WA McKee, G Luikart, JA Stanford. 2018. A social–ecological perspective for riverscape management in the Columbia River Basin. Frontiers in Ecology and the Environment 16(S1): S23-S33.

importance to new settlers in a developing country until commodified uses were discovered. Once a market for their use was established in the capitalist economic system, North America's freshwater mussel fauna was assumed to be of limitless bounty and overexploited for economic gain to their eventual detriment. Living on and buried in riverbeds, freshwater mussels literally are not visible unless one feels around the riverbed tactilely searching in shallow waters or dons a mask and snorkel or SCUBA gear to find them at depth. But the market demand for mussel-derived products increased their relevance, and thus their visibility via increased willingness to search. As exploitation led to their depletion, the visibility of freshwater mussels once again diminished. Finally, their conservation story takes compelling arcs. As we will discuss, the conservation of freshwater mussels began from a Progressive Era interpretation of maximum use that propelled artificial production efforts to satisfy economic interests. After more than a century of exploitation, habitat destruction and degradation, more than 70% of North America's ~300 species are considered imperiled, and conservation efforts have shifted to saving endangered species and restoring riverine habitats.[20]

In 1985, during the early years of environmental history as a field, Philip V. Scarpino published *Great River: An Environmental History of the Upper Mississippi, 1890 – 1950.* He discussed the relationship between the Upper Mississippi River and its people, and focused on how that relationship evolved through both human actions and nature's agency. The Upper Mississippi River is the portion upstream of the confluence with the Ohio River, near Cairo, Illinois (Figure 1). It is recognized separately from the Lower Mississippi River because of the distinct and historic channel differences upstream and downstream of this confluence. The Upper Mississippi River, especially the portion upstream of the Missouri River confluence at St. Louis,

[20] William et al. 1993; Strayer et al. 2004; FMCS 2016.

Missouri, was historically treacherous for commercial navigation and has been heavily modified since the early 20th Century with the construction of 29 locks and dams, numerous wing dam structures, and continuous dredging to aid navigation.[21] Scarpino briefly illustrated the pre-industrial conditions of the Upper Mississippi River, drawing on the accounts of early nineteenth-century explorers and surveyors. While some were motivated by adventures, other expeditions were explicitly designed to survey available resources and the potential for development in the Upper Mississippi River valley region.[22] Scarpino carefully described how prospectors, immigrants, and locals alike exploited the river's resources through the **freshwater mussel fishery** and later, hydroelectric ambitions – especially the Keokuk Hydroelectric Project. Scarpino also detailed the birth of the Izaak Walton League, a prominent conservation organization that drew its support largely from Midwestern outdoorsmen.[23]

Here I draw on Scarpino's work and the writings of several others to explore the themes of exploitation, visibility, and interpretations of conservation in environmental history, largely through the lens of freshwater mussels. Many stories of US environmental history find their origins in exploitation, or quickly arrive there, especially if told from a post-settlement perspective. Environmental historians have explored the changing visibility of nature both explicitly and implicitly – that is, whether and how people recognized and acknowledged the natural resources on which they relied. Historians have further demonstrated that *conservation*, a term dating back to the 14th century with a current definition of "a careful preservation and

[21] Anfinson JO. 2003. The River we have Wrought: A History of the Upper Mississippi. University of Minnesota Press: Minneapolis; Fremling CR. 2005. Immortal River: the Upper Mississippi in Ancient and Modern Times. The University of Wisconsin Press: Madison.
[22] Scarpino. *Great River: An Environmental History of the Upper Mississippi, 1890-1950* (University of Missouri Press, 1985): 3-5. Specifically, Scarpino describes accounts from Zebulon Pike and Stephen Long.
[23] Ibid. Chapter 4, *Conservation Crusade.*

protection of something; especially planned management of a natural resource to prevent exploitation, destruction, or neglect," has been applied to a spectrum of activities ranging from preservationist to irresponsibly exploitative.[24] I examine each of these themes – exploitation, visibility, and conservation – with examples of how they have been considered in environmental histories of freshwater mussels, and relate those discussions to the same themes as they are portrayed in environmental histories of other topics. Though freshwater mussels occur throughout the US and they were commodified through national markets, I have limited my focus to the Upper Mississippi River Valley, the region to which their known history with human populations in North America is largely confined, and where the bulk of harvesting and population impacts occurred (Figure 2).[25] The aims of environmental history are to situate human histories within their environments, give the environment voice as an actor in those histories, and to connect human actions with their environmental consequences for better or worse. My purpose then, is to give North America's native freshwater mussels their voice in history so that we may understand the stories that mussels and humans share, illustrate how these stories share themes with other environmental topics, and to demonstrate that that the present and future stories of mussels and human communities remain connected through the management and use of water resources.

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[24] Merriam-Webster. "Conservation." Dictionary by Merriam-Webster. Accessed November 20, 2017. https://www.merriam-webster.com/dictionary/conservation.
I use the term 'irresponsibly exploitative' not to pass judgment on cultures with differing views than my own or collectively different from modern views, but to capture the essence that much historic exploitation was shortsighted enough that rates of use could not sustain the economies they supplied.

[25] Pritchard: 16. US map of mussel survey and resources shows the main focus in Mississippi drainage.

Limitless Abundance and Exploitation

Before settlement of the Upper Mississippi Valley by colonial descendants and European immigrants, Native Americans harvested mussels for many uses. Mussels provided utilitarian benefits, including food, sturdy shell implements (e.g., tools, bowls, and spoons), and calcareous clay strengthener for pottery. Further, they imparted cultural status to their collectors, as implied by the use of shells as ornaments on ceremonial regalia, the collection of freshwater pearls, and the crafting of pearl jewelry known from archaeological sites, including burial grounds.[26] Despite their extensive use, it appears from the evidence we have that Native Americans did not overexploit mussels. As an example, in his chapter on human exploitation of North American freshwater mussels, malacologist Wendell Haag stated, “The pattern of accumulation of shells in some middens supports frequent but low-intensity utilization.”[27]

In *Great River*, Philip Scarpino painted an Upper Mississippi riverscape with “extremely clear” waters and “abundant fish and wildlife”.[28] Historians have illustrated the abundance of mussels with vivid pioneers’ descriptions of “finding the ‘bottom of the river covered in mussel shells.’”[29] With the arrival of railroads in the latter 19th century and subsequent human population growth, increased farming, industry, and shipping on the Upper Mississippi River, riverine habitat and water quality became degraded, leading to declines in fisheries by the 1890s. Still, settlers had no foresight of the rapid overexploitation to come. Despite changes already happening in the river, Wisconsin sheller J. P. Albee wrote to the state’s commissioner of

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[26] Carlander. Chapter 1 “Beginnings” 7-8.
Haag. Chapter 9 “Human Exploitation of Mussels” 288-295.
[27] Haag: 294.
[28] Scarpino: 4.
[29] Haag: 299. He uses quotations for part of the phrase with no attribution, so it is unclear if they may just be for emphasis.

fisheries in 1908 that he did not see "'the slightest danger' of commercially useful mussels becoming extinct."[30]

The first massive post-settlement impact to freshwater mussels was the first pearl rush in 1857. That year in a stream called Notch Brook near Patterson, New Jersey, a person collected a 23.25-carat pearl from inside the body of a mussel and sold it to Tiffany and Company, who then sold it to Empress Eugenie of France – the wife of Napoleon III – for $2500 (a more modern equivalent in 2009 USD of $67,000). Mussels in Notch Brook and the surrounding streams were summarily wiped out in the pearl rush that followed. [31] After this news and occasional future finds, Americans developed "pearl fever" on a decadal frequency, a trend that would march across at least nine other states and at least one Indian Territory throughout the Midwest and Southeast. Although Europeans had developed economical tools years beforehand that allowed them to gently pry open shells just enough to peer inside in search of pearls without killing mussels, Americans were content to extinguish millions of animals in their "treasure hunt" for elusive freshwater pearls. [32] Predictably, pearling did not last. Very few freshwater mussels contained marketable pearls (0.01% of them by one estimate), mussel populations were declining, and a new industry for freshwater mussel shell material – button production – was on the rise.[33] The pearl button industry quickly replaced pearling.

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[30] Scarpino: 89. This letter apparently was a rare instance of capturing the views of those who worked the rivers for mussels.

[31] Scarpino (p. 81) and Haag (p. 295) both tell this story. Both list the pearl as 93 grains, and I have converted that to carats for a more familiar context for the modern reader by using the formula of 4 grains/carat from PearlGuide.com (http://www.pearl-guide.com/forum/content.php?93-Pearl-Weight, accessed 12-6-2017). For perspective, such a pearl would be ~19 mm in diameter. Scarpino listed the sale price as $2500, and Haag provided the 2009 USD equivalent of $67,000. Haag also notes that a 400-grain pearl was found in a mussel around the same time and geographic area, but it was discovered only after being cooked in the mussel, having its luster and economic value ruined. Haag also provides the detail that Napoleon III was Empress Eugenie's husband.

[32] Scarpino, 82-83 lists pearl fever hunts in: OH, WI, AR, KS, MO, Choctaw Indian Territory, TN, GA, CT, NY, in that temporal order.

[33] Haag: 295-297.

A trifecta of factors coalesced to stimulate the birth of the freshwater pearl-button industry. The first was the 1890 McKinley Tariff, which substantially increased taxes on imported goods, thus creating a market for domestically produced buttons. The second was a growing mass-produced clothing industry in need of an increasing supply of buttons. The third and perhaps the most important factor was the timely arrival of Johann Boepple and his button-making ingenuity. Boepple brought experience from the button industry of his native Germany and a desire to take advantage of the plentiful stock material living in the Upper Mississippi's sediments. Without capital of his own to start a business, Boepple marketed his button lathe technology that eliminated the arduous task of hand-cutting button blanks.[34] Several writers and speakers have quoted Boepple as exclaiming, "mein buddons vill make you rich!"[35] Soon after securing investors, Johann Boepple opened the first freshwater mussel button factory in Muscatine, Iowa, in 1892. The number of button factories rose exponentially, starting slowly after Boepple's first opening, and exploding to nearly 200 factories over the next two decades. Muscatine was the central hub. Lasting through the 1930s, the button industry reached its zenith in 1916 when it employed 20,000 workers – a quarter of whom were women and girls – and produced "5.75 billion buttons valued at $230 million (2009 USD)."[36] "[B]y 1900, nearly 50%

[34] Scarpino: 84-85. Haag 297-300.
[35] Pritchard: 3. Scarpino: 85. Each time I encounter this quote, it is written phonetically to emphasize Boepple's German accent.
[36] Scarpino: 85-97. Haag: 298-300. Haag notes that button factories already existed along the Upper Mississippi prior to Boepple's, but ironically, they relied on a supply of marine mollusk shells from the east coast and lacked Boepple's efficient lathe technology. Quote is from Haag: 300. Scarpino lists the value, presumably in 1916 USD, at $12.5 million. Fascinating. Imagine the cost to ship shell inland. I wonder if these might be used oyster or clam shells, a byproduct of the massive live oyster trade that in turn followed the ice trade? Look back into that quote from Haag and then check out the ice trade timing. Did they ship shell or live oysters for consumption?

of America's buttons were manufactured from freshwater mussels"[37] and the total market share was estimated to be two-thirds at the height of the button industry (1905 – 1925) (Figure 3).[38]

Johann Boepple's button lathe was not the only technology that facilitated exploitation of freshwater mussels. Instruments known as the crowfoot or brailing hooks were invented in 1898 and made for efficient collection. These were pronged metal wires fastened to chains or ropes tied to a horizontal bar, and they were often constructed by hand (Figure 4). They could be deployed behind a johnboat and dragged along the bottom, where partially gaped mussels engaged in siphoning river water to feed on seston would clamp shut on the wires when disturbed.[39] Other technologies ranged from using long-handled tongs and rakes to harvest mussels while standing on river ice, to advances in button press machinery.[40]

It will come as no surprise that mussels were harvested by the tens of millions to support such an industry. Harriet Bell Carlander provided an impressive accounting of freshwater mussel harvests in her publication, *A History of Fish and Fishing in the Upper Mississippi River.* Written in 1954, her book is especially interesting because it offers the earliest history of the mussel industry; that is, she wrote it soon after the button industry collapsed and well before other historians or biologists took up the topic. Carlander reported that, early on, one ton of shells could be harvested from a mussel bed in a single day, and "[i]n 1899, it was the most important fishery in Wisconsin" with a harvest that year of more than 16 million pounds of

[37] Pritchard: 3.
[38] Haag, p. 309 lists two-thirds at the peak of the industry, citing Anthony and Downing (2001). I retrieved the range of years directly from Anthony & Downing. (2001). Exploitation trajectory of a declining fauna, a century of freshwater mussel fisheries in North America. *Canadian Journal of Fisheries and Aquatic Sciences*, 58:2071-2090.
[39] US Department of Commerce. 1921. Bulletin of the United States Bureau of Fisheries, Volume 36, 1917-1918: Washington, DC: 46-53. This document describes the mussel fishery, including how brailing hooks were used on brail bars, and instructions on how to make brailing hooks from wire.
[40] Ibid: 45-46; Carlander: 41; Haag: 303-305. Scarpino: 89-94.

shells. Though there is no reliable method for converting shell tonnage, such a figure represents an incomprehensible number of harvested mussels. “The operator of a ferry at New Boston [Illinois] in 1950 said that, when he was a boy, the clamming boats were lined up along the shore so solidly that one could walk from boat to boat for a distance of several blocks.”[41] Such intense harvesting of these invertebrates caused populations to decline within three years of the industry’s inception, such that by 1899 (Wisconsin’s boom year), people recognized that mussel populations were being impacted.[42]

Freshwater mussels exerted important agency on human populations as well. Farmers were losing help to musseling because entering that work force required no experience and very little investment in equipment. Furthermore, it often paid better and was less intensive than farm work, especially early on.[43] Though the button factories located themselves adjacent to thriving mussel beds in the Upper Mississippi River, their feverish local exhaustive consumption caused the industry to develop hinterlands of its own. In this way, mussels were harvested farther upstream, farther down tributaries, and eventually their shells were even shipped to button factories from other states in the Mississippi drainage. With humans’ excessive exploitation, musseling took on a nomadic quality and boomtowns blinked in and out of existence as workers eliminated populations of mussels from their natural place in the rivers. “[A] class of itinerant shellers rapidly developed, which followed the mussel frontier in shantyboats,” and workers typically established camps along the rivers. Such camps were often unwelcome to local communities because they smelled of the “sensory insults of an unregulated rendering plant” and

[41] Carlander: 40-41. Interestingly, no authors I’ve read attempt to enumerate mussels from shell tonnage.
[42] Pritchard: 3.
[43] Carlander: 40-41.

housed residents "who had a reputation for being shiftless, lawless, and even dangerous."[44] As the industry went on, other social issues arose. Hostility mounted between button factory workers and their employers in Muscatine, resulting in a strike that had to be quelled by the US Army. As mussel populations suffered wide extirpation, relations between industry and government agencies also became strained.[45] At the time of Carlander's writing in 1954, she reported that some people still participated in musseling in the Upper Mississippi River, but their primary objective was using the mussels' internal soft tissues as bait for their trotlines. Further, she noted: "The piles of shells which can be seen along the Mississippi River, and the abandoned buildings which once housed button factories show how the industry had a brief prosperity and then, in most places, disappeared as the shells disappeared from the river."[46]

The exploitation of freshwater mussels echoes the exploitative use of other natural resources in post-Colonial America. For example, abundant white-tailed deer (*Odocoilious virginianus*) populations that sustained indigenous peoples of New England became so depleted within mere decades of 17th Century Colonial settlement that "Massachusetts enforced the first closed season on their hunting in 1694," followed by a complete moratorium in the early 18th Century.[47] Forests were not only overused, but also squandered. While William Cronon pointed out that the Midwestern white pine forests were rapidly depleted in Chicago's 19th Century hinterlands, he also described how New Englanders egregiously wasted their forests two centuries before, where "many less-than-perfect trees were simply destroyed when larger ones

[44] Scarpino: 86-88.
[45] Pritchard: 4.
[46] Carlander: 51.
[47] Cronon, *Changes in the Land,* 101.

were felled…" and those "without market value could simply be burned where they fell."[48] Bison were turned into machine belts, and in the process were nearly driven to extinction.[49] Indeed, Harriet Bell Carlander compared the mussel fishery to these other exploitative industries: "The history of the mussel fishing and the pearl button industry on the [U]pper Mississippi River is brief and colorful. It shows the same 'feast or famine' philosophy which has characterized other industries" that "depended upon the use of natural resources—for example: lumbering, mining, and other fisheries—whaling, sturgeon, and salmon."[50] Environmental history also calls attention to technological advances that catalyzed exploitation of other resources. Donald Worster credited the development of motorized tractors and work-saving combines with expediting sodbusting and land consumption in the western short grass prairies. They were a factor that played prominently in leading to the environmentally, economically, and socially disastrous Dust Bowl.[51] Isenberg noted that commodification of bison was not possible until tannery technology advanced and "American manufacturers developed accurate large-bore rifles" that could penetrate their tough hides.[52] Likewise, Cronon credited the grain elevator for revolutionizing the wheat industry, calling the innovation "among the most important yet least acknowledged in the history of American agriculture.[53] Finally, Adam Rome highlighted the invention of his title character – the bulldozer – and the application of assembly-line production to homes to the rapid expansion of suburbia in the 1950s.[54] A significant element in each of these examples of environmental exploitation is that natural resources were not harvested to satisfy

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[48] Cronon, Changes in the Land, 111 in Chapter 6, Taking the Forest. Cronon also discusses the exploitation of the Midwestern white pine forests at length in Nature's Metropolis, especially in Chapter 4, The Wealth of Nature: Lumber.
[49] Isenberg, 2000, 130.
[50] Carlander, 1954, 40.
[51] Worster, *Dust Bowl: The Southern Plains in the 1930s.* Chapter 5 "Sodbusting": 80-97.
[52] Isenberg, 2000, 131.
[53] Cronon, *Nature's Metropolis.* (quote p. 111).
[54] Rome, *Bulldozer in the Countryside: Suburban Sprawl and the Rise of American Environmentalism, 2001.*

local consumption. Like mussels and their pearl and button products, they all were egregiously exploited to supply global markets.

Like economically desirable plants and animals, the waters and lands that supported them were similarly vulnerable to overuse and exploitation. Twentieth-century navigational alterations had even more extreme consequences on the Upper Mississippi River than did the industrial and population growth of the 19th Century. In *Great River's* first chapter, Scarpino introduces us to a swarm of great expectations by locals, shippers, and power companies alike to harness "waste[d]" energy and improve navigation on the Upper Mississippi River with the construction of the Keokuk Hydroelectric Project. Boosters promised an abundance of electricity that would be cheap for locals and spur industrial and economic growth in the region. Despite lack of interest from competing industries (e.g., white pine profiteers needed a flowing river) and with the help of federal agencies and foreign investors, they succeeded in constructing the Keokuk Dam in 1913. At more than a mile wide, this engineering monument would become the world's largest industrial dam in its time. [55] But the Keokuk dam and the many others that followed had unintended consequences as conditions for local people, mussels, and other fisheries tended to worsen rather than improve. Though the button industry was integral to the region's economy, the inundated slack water conditions proved harmful in the now-dammed Des Moines Rapids, which had been an important source of mussels.[56] Dams, increasing siltation from land clearing, and sewers that piped raw human waste into the river proved dreadful in combination. The

[55] Scarpino, Chapter 1 "The Keokuk, Iowa, Hydroelectric Project: Synchronizing the River with the Needs of an Industrial Society," quote: 40.
[56] Scarpino Chapter 3, "Shells, Sewage, and Silt."

river's water quality became unsuitable for many important fish species, negatively affected mussel reproduction, and helped to spread dangerous pathogens such as tuberculosis.[57]

Over and over, settlers and their descendants commodified and exploited nature to the extent that its products became divorced from the source. As William Cronon so eloquently described in *Nature's Metropolis, first nature* – that is the white pines, prairies, wetlands, or here, freshwater mussels – gave way to *second nature* – lumber, wheat fields, subdivisions, or buttons – through exploitation. Industrial commodification produced self-inflicting injuries that resulted in a loss of tangible goods and services provided by the then-depleted *first nature*. And along the way to market, *first nature* was forgotten and the visibility of mussels and other resources was masked.[58]

Changing Visibility of Nature's Bounty

The visibility of something – whether nature made or of human construct – seems governed principally by its relevance to and impact on human lives. Visibility changes not only with usefulness, but with time and with the sector of the human population considered. The visibility of freshwater mussels has oscillated in their shared history with humans. As I have already intimated in the discussion of uses by indigenous groups, freshwater mussels were highly visible to indigenous people in pre-settlement times, who regarded them for values ranging from utilitarian to cultural and spiritual. When settlers removed indigenous communities from their riparian lands in the Upper Mississippi Valley, the region's mussel fauna faded from human

[57] Scarpino, Chapter 2 "Unanticipated Consequences…" and Chapter 5 "Pollution of the River", especially pages 52 and 152.
[58] Cronon. *Nature's Metropolis.*

visibility.[59] [A tangential but important point is required to clarify my reference to settlers here and the faded visibility of mussels. The term should be construed as *White settlers* because Black or African-American people who were relocated under the duress of slavery or tenancy are notably absent from the available written histories of freshwater mussels.][60] Mussels were essentially unseen until their commodity potential for pearls and buttons was revealed. Even within the mussel fishery, shells were not valued during the pearling years and their lack of commodity usefulness rendered them waste to be tossed to the background of visibility.[61] Hugh Smith, President of the American Fisheries Society from 1907-1908 described the button industry as turning a "hitherto useless product into a valuable commodity." Though this sentiment illustrates that fisheries scientists held mussels as a commodity product, members of the American Fisheries Society also sought to protect the resource by advocating for improved water quality measures to ensure that mussels and other valuable fish species could thrive in the Upper Mississippi River.[62] My discussion of exploitation suggests specific ways in which freshwater mussels were visible: in the piles of shells along riverbanks, in the scores of boats clogging the waterway, in the construction of hundreds of factories, by the stench of rotting flesh along riverbanks, and in the migratory droves of shellers comprising intermittent camps. Mussels

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[59] Scarpino states that after the Indians were removed, mussel populations grew without pressure, though he is unclear for how long and does not present specific evidence to support this claim: 80 Nevertheless, settlers had no use for them at first. Haag offers a similar account: 296.

[60] Valencius CB. 2002. The health of the country: how American settlers understood themselves and their land. Basic Books: NY; Claassen C. 1994. Washboards, Pigtoes, and Muckets: Historic musseling in the Mississippi watershed. Historical Archaeology 28: i-v, 1-145. Histories of Midwestern settlement, such as that by Valencius of settlement in Missouri and Arkansas, reflect that African-Americans were often relegated to low-lying lands that were perceived as unhealthy. Such proximity to wetlands and aquatic habitats leads me to postulate that African-Americans have a shared history with mussels that has suffered implicit erasure or is yet to be told. Claassen's monograph has a few references to Black Americans, including pearlers and a pearling guide (38-41), and 15 Black union members in the shell button industry (102). This important gap in the environmental history of mussels should be investigated.

[61] Haag: 297.

[62] Scarpino: 105-111. Quote: 111.

are no longer conspicuous to modern communities in these tangible ways, as they were early 20th Century Americans.

While it is clear that mussels were a palpable commodity to those in the pearl and button industries, my research proved inadequate for answering questions about whether American culture at large recognized them outside the sphere of harvesting and manufacturing operations. Were button-down shirts advertised as having freshwater mussel buttons as an advantage over more expensive and less durable oyster buttons? Answers to such questions will require an intensive survey of archives and historic cultural articles, such as clothing catalogs. These culturally pertinent topics were not addressed in any of the literature on freshwater mussel history, and a search of internet images revealed little but art from a few button cards. Cultural aspects of histories are less often historically preserved due to their tentative relevance and lack of perceived importance for preservation when they are plentiful. Dissociation from *first nature* was and still is commonplace in societal regard for end products; this phenomenon was illustrated in *The Destruction of Bison* by Andrew Isenberg, in which he reported “an increasing demand for hides” that supplied “leather belting to animate…machinery” in the industrial expansion of the United States; he asserted this was a “primary cause of the bison’s near extinction.” [63] As was the fate of many other natural products, I suspect that the commodified products of mussels also became dissociated from their natural animal source in the eyes of consumers.

Water quality problems also were overlooked by the people living along the Upper Mississippi River until such problems manifested in tangible ways. Because sewage, silt, and

[63] Isenberg AC. 2000. The Destruction of the Bison: an Environmental History, 1750 – 1920. Cambridge University Press, New York, NY: 130.

eventual slack waters from dams required time to synergistically affect important fisheries, pollution went unchecked for decades. A particularly interesting element to consider is the river's agency in spatially altering the pollution problems of its cities. As upstream metropolitan areas such as Minneapolis and St. Paul piped away human waste to solve the sanitation and public health dilemma of trash and sewage in the streets, the river diluted and carried it downstream, thus decreasing its visibility. Operators of sawmills, meatpacking plants, and stockyards also dumped their waste in the river. But dams (and surely increasing human populations) threw a wrench in this strategy. Damming backed up and slowed the waters, creating stagnant and again visible waste that resulted in degraded water quality, fish kills, and human disease outbreaks. This conspicuous manifestation of pollution, such as a 45-mile stretch of dead river downstream of St. Paul, MN, eventually prompted riverside cities large and small to develop wastewater treatment facilities.[64]

This theme of changing visibility has been applied in other environmental histories. In fact, other mollusks share in this past. For example, shipworms – scantly-shelled marine bivalves in the family Teredinidae – burrowed into wooden pylons driven into San Francisco's tidelands and wreaked havoc on the shoreline docks.[65] Like the gradually worsening water quality in the Upper Mississippi River, invisible nature has a reputation for taking its citizens by surprise, and often acts as a disservice. The delayed detection of pollution's effects is paralleled in the history of diethylstilbestrol's generationally deferred cancerous consequences in the children of women who were convinced to take the drug during pregnancy.[66] In another account, highly erodible

[64] Scarpino, Chapter 5, especially 163-180. He mentions that one August, the dissolved oxygen concentration was too low to support any fish life for 45 miles downstream of St. Paul.
[65] Booker MM, *Down by the Bay: San Francisco's History between the Tides.* Chapter 2 "Ghost Tidelands": 53.
[66] Langston N, *Toxic Bodies: Hormone Disruptors and the Legacy of DES.*

soils lurked beneath the cotton and tobacco plantations of the Southeastern Piedmont's inhabitants.[67] A particularly unfortunate lesson was told in *Down by the Bay: San Francisco's History between the Tides*, where Matthew Morse Booker illuminated how the development of the city altered, consumed, and hid marshlands with "[a] veneer of cement", fooling people into dismissing their continued relevance. But in a city built so near a tectonic fault line, the tidelands demanded to be known again. Booker detailed the combined forces of humans and nature that produced consequences unthinkable to Bay residents. He shared the frenzied horror experienced by San Francisco's people in the famous 1906 San Francisco earthquake, where on one small piece of shoreline, "a three-story hotel had sunk two full stories into the filled marsh." The hotel, of course, was full of visitors, and firemen managed to rescue just two occupants. A massive portion of the city and her residents suffered similar fates from the quake and resulting fires.[68]

Sometimes appearances affect what we know of a place. This is true of myriad second-growth forests and other seemingly untouched landscapes. Sutter offered a convincing example from the agricultural South. "'Generations of southeasterners have grown up under the assumption that Piedmont streams naturally featured steep unstable banks and turbid waters, while the reality is that these conditions are a direct long-term (multi-millennial) consequence of poor farming practices.' A little more than a century of cotton culture, in other words, transformed the ecology, hydrology and geomorphology of many southern watershed in ways that may last for thousands of years."[69] Thus, it is not always nature that is hidden from view, but the work of humans in places we perceive as pristine.

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[67] Sutter P. *Let us Now Praise Famous Gullies*, especially Chapter 6 "Gullies and What they Mean."
[68] Booker. Introduction "Layers of History". Chapter 2 "Ghost Tidelands", quotes: 5 and 62.
[69] Sutter: 118-119. He references a report entitled, "A Southeastern Piedmont Watershed Sediment Budget" for the embedded quote.

The same sentiment of hidden human agency may be applied to water quality, especially as people rely heavily on visual cues (e.g., color or clarity) to convey its healthiness even in an era of increasing chemical use, from personal care products and pharmaceuticals to new industrial chemicals.[70] Surface waters may be crystal-clear, but heavily polluted; such is the case in the Clinch River in southwest Virginia and Tennessee – one of the most biodiverse rivers in all of North America. Mussels in portions of the Clinch River have suffered a 96% reduction in abundance compared to historic population estimates due to pollution in the watershed.[71] If looks can be deceiving, yet visual cues are perceived as a reliable source of information about nature, how do we reconcile the conservation and management needs of impaired but invisible nature, such as water quality and freshwater mussels?

The Conservation Paradox

Conservation of the less visible but impaired components of nature (e.g., clear but polluted waters; unseen fauna and the functions they provide) require public engagement by scientists to improve awareness of the issues as they relate to community interests (e.g., safe water or fishing).[72] But what does conservation mean? To the modern reader, that might seem a provocative question, and indeed Merriam-Webster would have you believe the answer is as straightforward as the definition I offered earlier. Likewise, the Society for Conservation Biology – a global community of conservation professionals – uses the word in their mission and values

[70] West et al. 2016.
[71] Cope WG, J Jones. 2016. Recent precipitous declines of freshwater mussels in the Clinch River: an in situ assessment of water quality stressors related to energy development and other land use. Final Full Completion Report, submitted to the US Fish and Wildlife Service Regions 4 and 5 offices, Cookeville, Tennessee and Abingdon, Virginia. 244 pp.
[72] Keeler BL, S Polasky, KA Brauman, KA Johnson, JC Finlay, A O'Neill. 2012. Linking water quality and well-being for improved assessment and valuation of ecosystem services. PNAS 109: 18619-18624.

statements as if conservation's meaning is implicit and static.[73] However, while preservation has been interpreted rather consistently over time as leaving natural resources untouched, conservation has been interpreted fluidly in our nation's history. In the early 20th Century, when planners sought to harness the power of the Upper Mississippi's Des Moines Rapids in the Keokuk Dam, they operated with the common Progressive Era interpretation of conservation, which was generally defined as 'wise use' to mean maximum exploitation and the avoidance of any waste.[74] Today we might reinterpret that definition as commodification, not conservation. It stands in stark contrast to modern applications of conservation to mean sustainable management for perpetuity, preservation of ecological integrity, and saving species or culturally important lands.[75]

Like the vision for maximum exploitation of the Upper Mississippi River, the early conservation focus on freshwater mussels was not for the sake of the creatures being exploited; it was an effort to save the button industry. Even scientists interpreted "full conservation" of fishes and mussels as full "utilization."[76] As such, the Bureau of Fisheries' efforts to intervene in nature by producing laboratory-reared mussels was to fulfill the promise of "saving an economically important, highly competitive industry from self-destruction." This intensive intervention by the Bureau of Fisheries began with investigations by Lefevre and Curtis in 1907 and progressed to commercial-scale propagation by 1912.[77] Such drastic measures were necessary because mussel populations were failing to reproduce on their own because of their complex life cycle in which

[73] Society for Conservation Biology. Available from: https://conbio.org/about-scb/who-we-are. [Accessed 8 March 2020].
[74] Merriam-Webster Online Dictionary. Rome: 8. Scarpino: 40-43, 60-75. Sutter: 51.
[75] Rome: 124. Scarpino: 115.
[76] Scarpino: 75.
[77] Scarpino: 100.

their larvae must parasitize species-specific fishes to complete metamorphosis to the juvenile life stage. Scientists recognized that damming of rivers exacerbated pollution's effects on mussels and also blocked the upstream migration of important host fish species.[78] After some legislative attempts to regulate the mussel fishery, the Bureau doubled-down on their utilization stance and recommended eliminating fishing restrictions "in accordance with the true concept of conservation as wise use of natural resources rather than simply "hoarding" or protecting."[79] But when full utilization of the river and freshwater mussel fauna resulted in degraded water quality and propagation efforts failed to reverse the widespread extirpation of mussels, conservation began to take on new meaning.

Still early in the 20th Century, the Izaak Walton League catalyzed a conservation movement to protect the Upper Mississippi watershed over concerns about water quality and fish and wildlife habitat. The shift in conservation focus was so important that Philip Scarpino devoted an entire chapter to the organization. "Waltonians thought in terms of scarcity rather than abundance and of recreation rather than production. They believed that contact with unspoiled nature had traditionally formed the finest qualities of the American character."[80] Their message resonated with the American public. Such interests and values are what present-day scientists would classify as cultural ecosystem services. Formed by 54 members in 1922, the Izaak Walton League ballooned to more than 175,000 members in 1928. As a point of emphasis, Scarpino compared their membership to that of more established conservation groups like the

[78] Carlander notes that the Keokuk Dam blocked the upstream movement of fish, including some very important mussel hosts. She mentions that one was the Skipjack Herring (*Alosa chrysochloris*), a primary host for economically important ebonyshell (*Fusconaia ebena*): 22.
[79] Carlander: 49.
[80] Scarpino: 115.

Sierra Club (founded in 1892) and the Audubon Society (founded in 1905). In just 6 years, the Izaak Walton League accumulated at least 25 times the membership of these other groups.[81] Focused on their message of saving wild spaces for America's *boys* to play, and strategically marketing directly to women and mothers to harness their freshly minted voting rights, one of the league's early conservation successes was convincing the United States Congress to create the Upper Mississippi River Wildlife and Fish Refuge.[82] While their efforts were not specifically directed toward conservation of freshwater mussels, the Izaak Walton League nonetheless led the way in promoting responsible use of the watershed. Fatefully, the artificial propagation technologies created to save the button industry are now a principal strategy employed by malacologists in endangered species recovery by augmenting remaining populations with laboratory-reared mussels.[83]

The shift in conservation's meaning from maximum use toward preservation was a national trend, and the experience of the Upper Mississippi River and its mussel populations mirrors that of other conservation narratives. The people of San Francisco Bay moved from converting marshlands into metropolis to creating wildlife habitat and attempting to restore some of the bay's original nature.[84] Rachel Carson sounded the alarm that efforts to protect desirable species with chemical pesticides at the expense of many others had to be remedied.[85] Suburban

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[81] Scarpino: 119.
[82] Scarpino: 116-135.
[83] Pritchard notes that biologists recognized the utility of artificial propagation for biodiversity conservation as early as 1982: 58. While they were specifically working on in-vitro propagation methods stimulating mussel metamorphosis in a nutrient medium, traditional host-fish propagation of is the most widely used method in freshwater mussel conservation today; FMCS 2016; Patterson MA, RA Mair, NL Eckert, CM Gatenby, T Brady, JW Jones, BR Simmons, JL Devers. 2018. Freshwater mussel propagation for restoration. Cambridge University Press: New York, NY.
[84] Booker: 151-155.
[85] Carson. *Silent Spring.*

development evolved from razing every possible acre to the environmental movement's call for saving open spaces.[86] Though the dictionary and professional conservation organizations seem to agree on conservation's modern definition, we must be cognizant of its transformation to understand environmental histories in their appropriate context.

Historical Synthesis

The intersections of visibility, exploitation, and conservation of freshwater mussels are representative of other chronicles in environmental history. Commodified use enhanced mussels' visibility, resulted in their exploitation, and defined the terms of their "conservation." The news of a few large pearls exponentiated mussels' visibility and demand. Technology enhanced collection of mussels and permitted the efficiency of transforming them to from water-purifying invertebrate bivalves to billions of opalescent buttons. Exploitation increased their relevance, but the aftermath and economic obsolescence also reduced their visibility and relevance to most publics. Exploitation spurred intervening conservation to maintain mussels as a commodity, and ultimately, to conserve species. As I highlight these intersections, we must recall that they are not passive – people are at the heart of these relationships with North America's mussels.

The environmental history of freshwater mussels fits well in the narrative of American western development and serves nicely as a geographic, temporal, and commodity extension of William Cronon's *Nature's Metropolis* in understanding the Mississippi Valley's connections to the East. It tells especially important industrial and conservation stories – in the shell button industry's importance in saving city economies in the Upper Mississippi Valley just as the white

[86] Rome. *Bulldozer in the Countryside.*

pine industry was waning; in the rise of damming for navigation and hydropower; and in a major shift in the meaning of conservation during these industrial developments.

Read together with other environmental histories, these works help to highlight the patterns of overexploitation of nature-turned-commodity. They illustrate that post-settlement American societies have operated in a mindset of 'seeing is believing' and are easily fooled when dangers are not apparent or when people become willingly complacent. Furthermore, they elucidate that conservation has been interpreted in wildly different ways – not as an anomaly in the exploitation by an unscrupulous few, but as a common cultural thread in the progression of the United States from its birth to the present. The same subjects could be framed with other disciplines, such as ecology, economics, or even traditional history. However, environmental history allows the latitude of a less anthropocentric focus than traditional history or economics, while allowing us to adopt a less anthropophobic perspective than ecology often takes. The arguably more holistic approach that environmental history follows then, may provide a critical link in a society where people often view themselves as separate from nature. That view has the potential to impede a full understanding of our own nature and actions, and environmental history may offer an antidote to that impediment.

MOVING FORWARD: IMPLICATIONS FOR MUSSEL CONSERVATION

The environmental history of freshwater mussel exploitation, visibility, and early conservation demonstrates that waterways and aquatic resources have always been coupled social-ecological systems. The enjoyment of ecosystem services as a mainstream field in ecological science over the past two decades shares some qualities with environmental history, such as human interaction with and reliance on nature and nature's agency to benefit society.

Looking back through our shared history with freshwater mussel through the lens of ecosystem services, we may identify provisioning services in the pearl and shell button industries, and cultural services for indigenous communities. The less visible and less appreciated benefits by the generations that preceded us (e.g., regulating and supporting services, such as water purification, nutrient cycling and storage) are now recognized thanks to our greater understanding of the functional roles of freshwater mussels.[87] The environmental history perspective of humans coupled with nature will be important to keep in mind in the following chapters because it aligns well with modern discussions of aquatic resource conservation and management as social-ecological systems.[88]

In the following chapters, my research examines the potential for native freshwater mussels to contribute to human well-being through filtration related ecosystem functions, along with an exploration of societal understanding about aquatic ecosystem services. Chapter 1 reports on contaminant sequestration of freshwater mussels, using an ecosystem services perspective and the scalability of such services to watersheds. Chapter 2 investigates the functional processing of toxic heavy metals by mussels, their role in the environmental fate of metals, and implications for freshwater ecology and ecosystem service delivery by mussels and waterways. Chapter 3 examines public understanding of floral and faunal influences on water quality, and suggests implications for engaging with communities about ecosystem services. Together, this research highlights the ongoing coupling of human communities with mussels and aquatic resources, how the visibility of mussels has once again oscillated down to a nadir in societal relevance, how mussels remain important to human well-being despite their invisibility

[87] Vaughn 2018.
[88] Hand et al. 2018.

through meaningful contributions to water quality, why conservation of freshwater mussels and water quality is mutually beneficial, and finally how public understanding of mussels is as important as in the past. As ecologists, we should strive more to operate within the social-ecological space by engaging with communities to cultivate awareness about, and build value for, the resources we care so deeply about. Only with the engagement of community partners and management action can we restore mussel populations from their imperiled status so they may thrive, contribute fully to aquatic ecosystem functioning and resilience, and support the aquatic resource benefits that people desire through their regulating and supporting services.

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FIGURES

Figure 1. The Upper Mississippi River [From Scarpino PV. 1985. Great River: An Environmental History of the Upper Mississippi, 1890-1950. University of Missouri Press: Columbia, MO].

Fig. 14. By 1914, surveys had revealed a great deal about mussel resources.
From R.E. Coker, "Fresh-Water Mussels and Mussel Industries" (1921).

Figure 2. United States map showing locations of mussel beds and tonnage of shells harvested within a year, from surveys spanning 1912-1914. [From RE Coker. 1919. Fresh-water mussels and mussel industries of the United States. Bulletin of Fisheries, Volume 36. Retrieved from J. Pritchard (2001). *An Historical Analysis of Mussel Propagation and Culture: Research Performed at the Fairport Biological Station.* A study supported by the US Army Corps of Engineers, in support of the Higgins Eye Pearlymussel Conservation Plan, Rock Island District. Ames, Iowa: page 16, Fig. 14.]

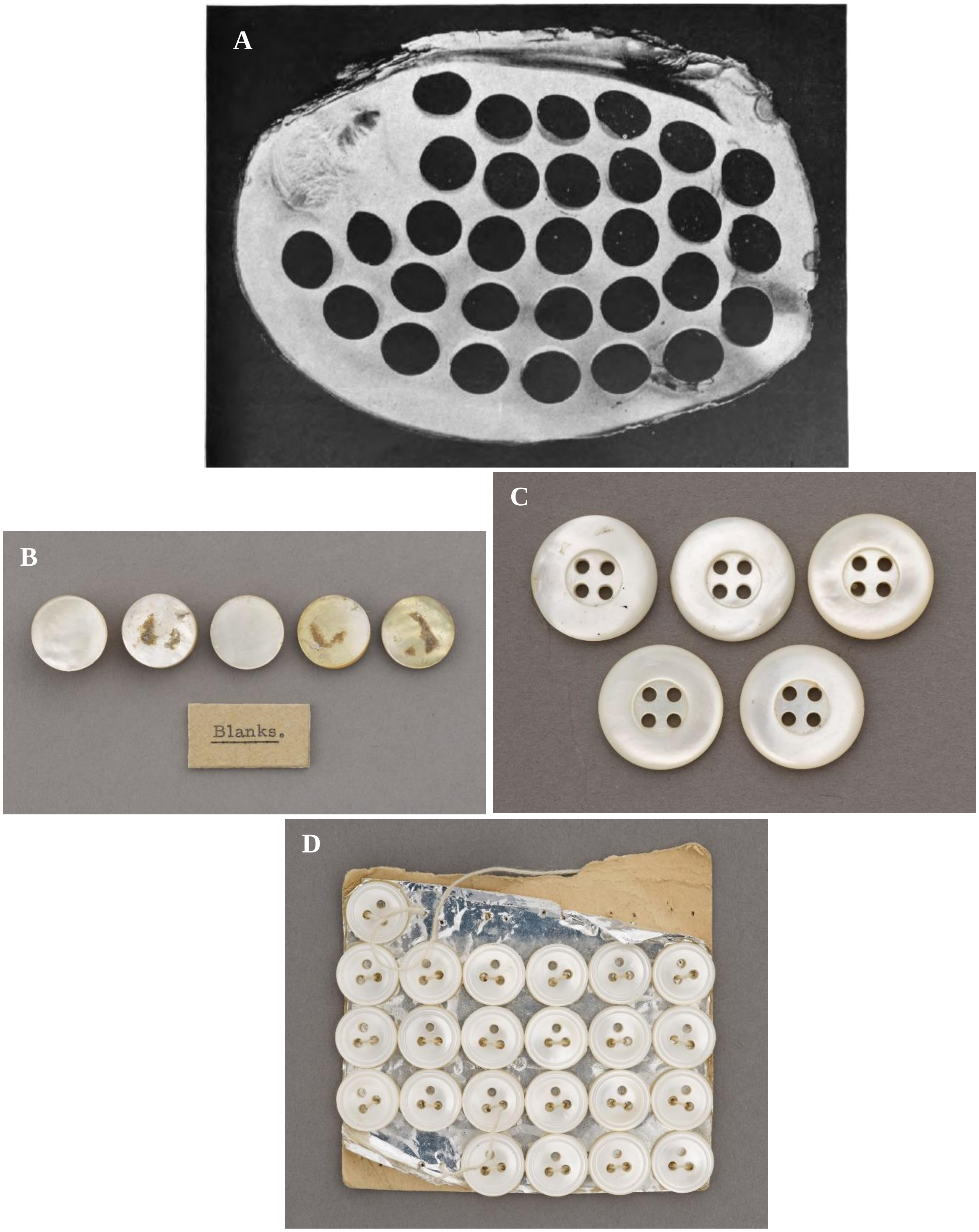

Figure 3. (A) A drilled Washboard (*Megalonaias nervosa*) mussel shell drilled for button blanks, (B) button blanks, (C) finished buttons, and (D) carded buttons ready for market. [(A) From RE Coker. 1919. Fresh-water mussels and mussel industries of the United States. Bulletin of Fisheries, Volume 36. (B-D) from the courtesy of the Birmingham Museum and Art Gallery via Flickr Creative Commons.]

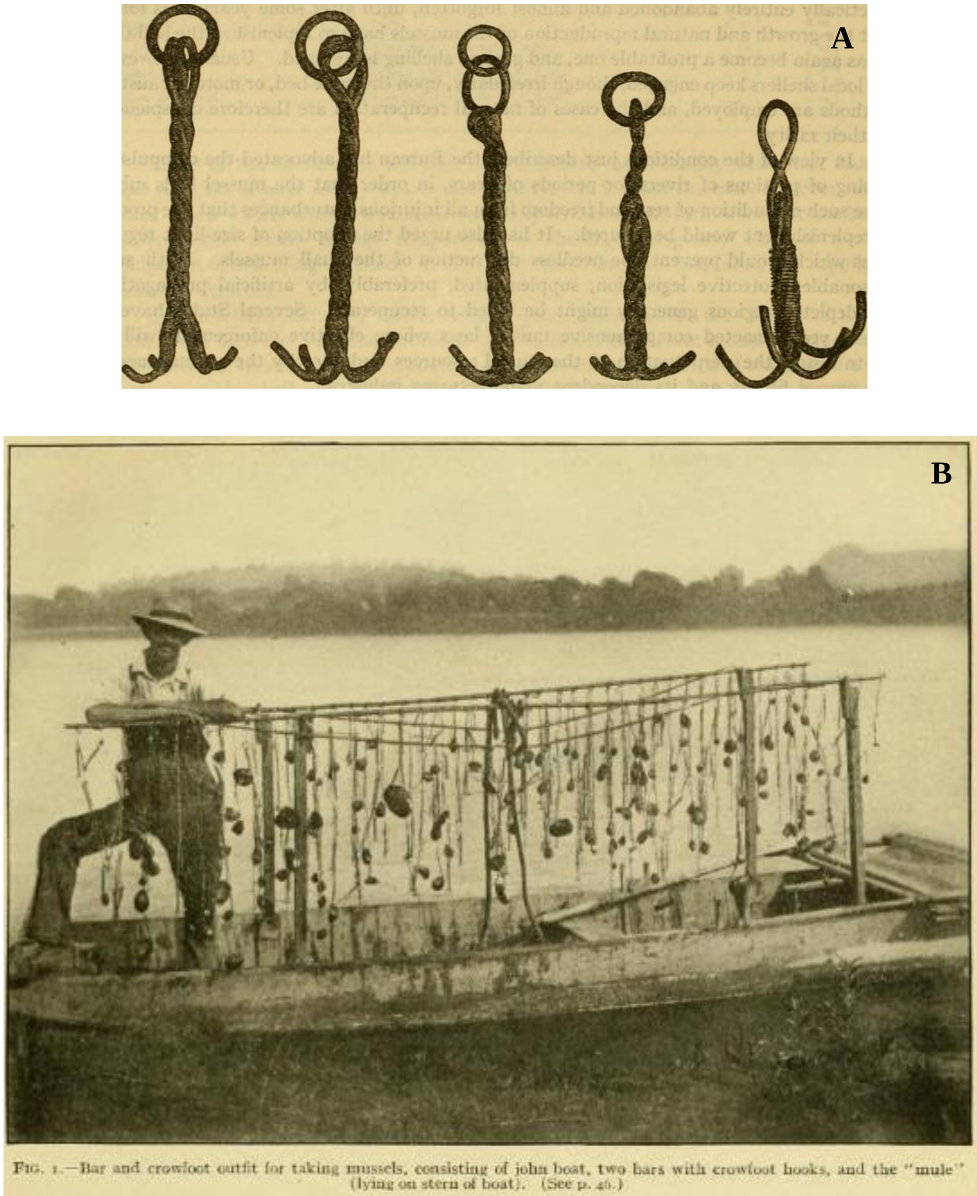

FIG. 1.—Bar and crowfoot outfit for taking mussels, consisting of john boat, two bars with crowfoot hooks, and the "mule" (lying on stern of boat). (See p. 46.)

Figure 4. (A) Brailing hooks or "crowfoot" used for harvesting freshwater mussels, and (B) mussel fishing setup on a johnboat with hooks on brailing bars and mussels attached. [From RE Coker. 1919. Fresh-water mussels and mussel industries of the United States. Bulletin of Fisheries, Volume 36.]

CHAPTER 1. Quantifying Sequestration of Aquatic Contaminants by Freshwater Mussels: an Ecosystem Services Perspective

ABSTRACT

Several modern studies have revealed that freshwater mussels (Unionida) perform a host of *functions* that are integral to maintaining surface water quality and to keeping rivers and lakes properly functioning as ecosystems. These functions offer important ecosystem *services* related to maintaining water quality for human uses. However, the capacity of mussels to contribute appropriately to ecosystem functioning and services is drastically hampered because a majority mussel populations are declining and more than 70% of species imperiled. This research explored the feasibility of using existing data on mussel populations along with tissue concentrations of contaminants to estimate contaminant sequestration by populations as a potential ecosystem service. I investigated three scenarios that were selected based on my direct access to the rare resource of tissue contaminant data from mussels collected in the wild, and the availability of population estimates at these sites from the literature or from colleagues. These scenarios included Upper Mississippi River navigation pools, the Upper Neuse River watershed (North Carolina), and a polluted compared to a healthy mussel site in the Clinch River (Virginia and Tennessee). These scenarios represented a range of spatial scales, from wadeable streams to large river systems; contaminant datasets from metals to organic contaminants; mussel population sizes from tens of thousands to hundreds of millions; and population estimates based on data types that ranged from qualitative techniques (e.g., visual search of mussels) to robust, quantitative techniques (e.g., systematic sampling). Contaminant sequestration differed based on spatial scale, population size, and contaminant type. Estimating population-level sequestration by

mussels using existing data varied in difficulty based on the types of available population data. We estimated that mussels in two navigation pools of the Upper Mississippi River sequestered approximately 15.6 tons of metals and mussels in the Upper Neuse River watershed sequestered between 2.4 and 5.8 billion ng of polycyclic aromatic hydrocarbons (PAHs). Clinch River mussels at the polluted Pendleton Island site sequestered 24.2 billion ng of PAHs compared to 210 billion ng of PAHs sequestered by mussels at the healthier sites outside a mussel zone of decline – 10X greater capacity despite having much lower tissue concentrations. Sequestration of pollutants by mussels is of interest to communities that rely on clean surface water resources for drinking water and recreation. However, a central tenet of native mussel conservation and recovery must be maintaining or improving water quality by promoting efforts of reciprocity – human services to maintaining ecosystems – to provide greater potential for mussel population recovery and sustainability of the ecosystem services they provide.

INTRODUCTION

The concept of humans benefiting from nature is potentially timeless – truly, nature was the only resource for survival and sustenance until human ingenuity fashioned new materials. Though societies have long recognized the value of natural resources (especially when the most relevant of them become scarce), the concept of enumerating the value of natural resources was only adopted into mainstream research and inquiry through ecological economics in the 1990s (Costanza et al. 1997; Daily 1997; Kinzig 2009). Generally defined as nature's goods and services that contribute to human well-being, ecosystem services have been codified according to four main categories, including: provisioning services that supply materials (e.g., food, water, fiber, fuel), regulating services that contribute beneficial processes (e.g., climate/flood regulation, water purification), cultural services that provide non-consumptive benefits (e.g., spiritual, recreational, or aesthetic importance), and supporting services that provide indirect benefits by maintaining the other three categories (e.g., soil formation, nutrient cycling; Millennium Ecosystem Assessment 2005). More recently, parallel efforts to refine ecosystem service categories and definitions have emerged (TEEB 2010, Costanza et al. 2017, Pascual et al. 2017, Haines-Young and Potschin 2018), but the broad concept of nature contributing to human well-being in a variety of ways remains the same. A primary focus of ecosystem research has been to place nature's benefits into a market-based economics framework in order to demonstrate the monetary value of maintaining, conserving, and restoring biological integrity for human well-being. Such efforts have worked well for some provisioning and regulating services (e.g., carbon sequestration, flood protection). However, researchers are recognizing that many of nature's benefits are difficult to capture in market-based analyses (e.g., indirect benefits that cannot be commodified), and that currency is not an appropriate measure of value for many of nature's benefits (e.g., cultural services; Costanza et al. 2017; Sanna & Eja 2017). Immense alteration, degradation, and depletion of natural resources by humans over the last few centuries has been a driving factor behind the intensive research focus since the 1990s to explicitly describe the value of dwindling resources, with a goal of influencing management that will contribute to sustaining societies.

Clearly demonstrating how ecosystem functions translate to social values rather than just market economics is one strategy suggested by Olander et al. (2017) to make derived benefits more relevant to policy and decision-making. Clean water is a necessity for survival, and freshwater resources are arguably among the most important of social-ecological systems. Not only are freshwater ecosystems essential for the provision of drinking water, irrigation water, and food production through fisheries, they provide recreational and aesthetic benefits for well-being, and they supply important regulating and supporting services (e.g., nutrient processing, primary production) (Millennium Ecosystem Assessment 2005; Palmer & Richardson 2009). Declining freshwater biodiversity caused by impaired water quality in the US and around the globe has been linked to a reduction in the delivery of freshwater ecosystem services (Palmer & Richardson 2009). Keeler et al. (2012) highlighted the need for more research on quantifying and valuing ecosystem services related to water quality, many of which are mediated by freshwater biodiversity (Palmer & Richardson 2009). Such models provide an avenue for improving public understanding of the relationships between aquatic communities and human well-being, and may help to foster increased value for nature – especially for organisms and habitats where the connections are less obvious (Costanza et al. 2017). Freshwater mussels (Unionida) are one such cryptic component of aquatic systems that provide ecosystem service benefits (Strayer 2017; Vaughn 2018), and they are one of the most imperiled faunal groups and ecosystem service providers on the planet (Williams et al. 1993; Lydeard et al. 2004; Strayer et al. 2004; FMCS 2016).

The visibility and relevance of North America's native unionid freshwater mussels have oscillated in their shared history with people. They were relevant in America's pre-settlement period to some indigenous peoples, who recognized their multiple provisioning benefits, including food, sturdy shell implements (e.g., tools, bowls, and spoons), and calcareous clay strengthener for pottery. Mussels also were culturally meaningful in these communities, as implied by the use of shells that ornamented ceremonial regalia, the collection of freshwater pearls, and the pearl jewelry known from archaeological sites, including burial grounds (Carlander 1954; Haag 2012). Mussel fauna faded from relevance in North American societies when settlers removed these indigenous communities from their riparian lands, and mussels became essentially invisible until their commodity potential for pearls and buttons was revealed (Scarpino 1985; Haag 2012). Beginning in the 1850s, North American freshwater mussels were killed in massive numbers for freshwater pearls, despite the low occurrence of pearls in unionids (< 0.01% by one estimate; Haag 2012). Exploitation of mussels for the provision of buttons largely replaced pearling in the 1890s in an industry that lasted through the mid-1900s and employed 20,000 workers at its height (Scarpino 1985; Haag 2012). A recent estimate places the combined value of the pearl and button industries at around $10 billion (2017 USD; Strayer 2017).

Because freshwater mussels no longer support a thriving commodity market in North America, their visibility and relevance has once again waned even though mussels and humans remain connected through their mutual dependence on favorable water quality. Several modern studies have revealed that native mussels perform a host of functions that are integral maintaining surface water quality and to keeping rivers and lakes properly functioning as ecosystems. These functions include bioturbation (i.e., sediment mixing) and nutrient cycling (Vaughn & Hakencamp 2001; Vaughn et al. 2004: Vaughn et al. 2008), providing physical habitat and stabilizing sediments (Vaughn et al. 2008), filtering algae, bacteria, and other particles and manufacturing biodeposits that create local hotspots for supporting stream community biodiversity (Howard & Cuffey 2006; Spooner & Vaughn 2006; Vaughn et al. 2008; Allen et al. 2012).

Individual freshwater mussels can filter several liters of water per hour, so the ecosystem functions that populations perform may offer important ecosystem services related to maintaining and improving water quality (Kreeger et al. 2018). The potential for such activities to be of value as ecosystem services has only been explored in the last several years. Vaughn (2018) published an ecosystem service framework for mussel functions in which one of the most prevalent benefits for humans was water quality maintenance through their many regulating and supporting roles. Mussels store and cycle nutrients as mentioned above, including in urban areas where excess nutrients enter waterways through industrial effluents and non-point runoff (Hoellein et al. 2017); they sequester pathogens such as *Escherichia coli* (Ismail et al. 2015; 2016) and other fecal coliform bacteria (Turick et al. 1988); and they may filter out contaminants of emerging concern, including pharmaceuticals and personal care products (Ismail et al. 2014).

Despite the recognition that mussels are providing ecosystem service benefits (Vaughn & Hoellein 2018), the capacity of mussels to contribute appropriately to ecosystem functioning and services is drastically hampered because mussel populations have been substantially reduced from historic norms. North American native mussels have an imperilment rate greater than 70%, due to historic overharvesting, habitat destruction, and pollution, and they are particularly vulnerable to present-day chronic impacts, such as water quality degradation (Williams et al. 1993; Strayer et al. 2004; Downing et al. 2010; Haag & Williams 2014; FMCS 2016). Mussels will likely suffer continued decline without measures to improve water quality because they are highly sensitive to many contaminants (Cope et al. 2008). Further, mollusks are expected to experience increasingly fragmented populations (Inoue & Berg, 2017) and greater loss of suitable habitats resulting from climate change than any other freshwater group (Markovic et al., 2014). Researchers have already identified diminished ecological functioning and service provision by mussels associated with droughts (Vaughn et al. 2015; Dubose et al. 2019). Thus, highlighting the benefits that emanate from the persistence of unionid mussels is important for establishing a sense of value to promote their conservation (FMCS 2016; Strayer 2017) because they are simultaneously essential to the functional ecology of freshwater systems (Allen et al. 2012) and already critically imperiled (Williams et al. 1996; Lydeard et al. 2004; FMCS 2016).

Studies that focus on pathogen or contaminant removal (e.g., Ismail et al. 2014, 2015, 2016; Chaplin-Kramer et al. 2019) may be particularly appealing to people that use rivers and lakes for irrigation, subsistence fishing, or recreation (e.g., swimming, boating) and are in direct contact with water resources. Strayer (2017) suggested that sequestration of heavy metals and organic contaminants may be valuable ecosystem services if sequestration is substantial, and Vaughn (2018) noted a growing interest in this field of inquiry. Several recent publications have called for more ecosystem services to be quantified, valued, and scaled up to watersheds, and quantifying ecosystem services has been identified as one of the 10 priority research areas in *A National Strategy for the Conservation of Native Freshwater Mollusks* (FMCS 2016; Strayer 2017; Vaughn 2018). Pollution studies typically have not been designed with a goal of measuring contaminant sequestration in mind – so can we use existing data from studies centered on questions of environmental pollution and pair such data with population data to estimate this function? This research explores the feasibility of using existing data on mussel density or abundance along with tissue concentrations of contaminants to estimate contaminant sequestration by mussels at a population level as a potential ecosystem service. Though ingesting contaminants is an incidental act as a function of mussels' filter feeding ecology and can be detrimental to their health (e.g., copper (Wang et al. 2007); ammonia (US EPA 2013); pesticides (Bringolf et al. 2007)), this function may be perceived as providing an ecosystem service benefit to people and contribute to enhancing their visibility, value, and relevance for conservation action.

METHODS

We estimated the function of pollutant sequestration by freshwater mussels using existing data in three different scenarios: large navigational pools in the Upper Mississippi River, headwater streams in the Upper Neuse River watershed of central North Carolina, and the Clinch River in Virginia and Tennessee. We selected these scenarios based on our unique access to data on contaminant concentrations from mussel soft tissue and the availability of population estimates at these sites from the literature or from colleagues. These scenarios represented a range of spatial scales, from wadeable streams to large river systems; contaminant datasets from metals to organic contaminants; mussel population sizes from tens of thousands to hundreds of millions; and population estimates based on data types that ranged from qualitative techniques (e.g., visual search of mussels) to robust, quantitative techniques (e.g., systematic sampling) commonly used in mussel surveys (Strayer & Smith 2003).

Study Scenarios

Upper Mississippi River Navigational Pools

In the first scenario, we selected two navigational pools in the Upper Mississippi River for which mussel assemblages and population sizes were recently estimated (Newton et al. 2011). The Upper Mississippi River is the portion upstream of the confluence with the Ohio River, near Cairo, Illinois. It is recognized separately from the Lower Mississippi River because of the distinct and historic channel differences upstream and downstream of this confluence. The Upper Mississippi River, especially the portion upstream of the Missouri River confluence at St. Louis, Missouri, has been heavily modified since the early 20th Century with the construction of 29 locks and dams, numerous wing dam structures, and continuous dredging to aid navigation (Anfinson 2003; Fremling 2005). Navigational pools are sections of the river located between lock and dam structures, and are often delineated into upper (flowing), middle (slack water), and lower (impounded) pool reaches.

Native mussel species composition, richness, and diversity were recently determined in navigation pools 5 and 18 of the Upper Mississippi River (Newton et al. 2011). Pool 5 is bordered by southeastern Minnesota on its western banks and west-central Wisconsin to the east; nearby towns include Weaver, Minnesota, and Alma and Buffalo City, Wisconsin. Pool 5 runs 24 km and has a total area of 4,420 hectares between Lock and Dam 4 (upstream) and Lock and Dam 5 (downstream extent). Pool 18 is bordered by southeastern Iowa to the west, and northwestern Illinois to the east; nearby towns include New Boston, Keithsburg, and Oquawka, Illinois. Pool 18 runs 43 km, and has a total area of 4,713 hectares between Lock and Dam 17 (upstream) and Lock and Dam 18 (downstream; Newton et al. 2011).

For this scenario, robust, quantitative, systematic survey results and known species assemblage structure and population estimates were available from Newton et al. (2011). Briefly, Newton et al. (2011) employed a systematic random sampling technique, in which they surveyed 359 sites in Pool 5 and 379 sites in Pool 18, with two 0.25 m^2 quadrats per site. Survey sites were spaced ~300 m apart, and excavated to a depth of 15 cm. Mussels were then identified to species, measured, aged using external shell annuli, and sexed (in species with external dimorphic characteristics) (Newton et al. 2011).

Newton et al. (2011) reported a population estimate of 190 million native mussels in Pool 5 (range, 155 – 224 million; 95% confidence limits), with an average density of ~4.3 mussels/m^2; Threeridge (*Amblema plicata*) dominated the species assemblage, accounting for 56% of the pool-wide population. The researchers estimated that the native mussel population in Pool 18 numbered 212 million mussels (range, 169 – 256 million; 95% confidence limits), with an average density of ~4.5 mussels/m^2; the Threeridge, Threehorn Wartyback (*Obliquaria reflexa*), and Mapleleaf (*Quadrula quadrula*) mussels were the most abundant species, and each accounted for 18% of the pool-wide unionid population (Newton et al. 2011).

Soft tissue contaminant data were obtained from mussels collected in tandem with the Newton et al. (2011) study evaluating the population size and structure. Three abundant species were collected from the upper, middle, and lower reaches of each navigation pool, and frozen until tissue was analyzed for a suite of metals and organic contaminants for this project (details follow in the next section). While divers were available for the population surveys in both pools, malacologists collected mussels from Pool 5 in wadeable areas after the survey (i.e., qualified divers were not available for species collection), and they selected the abundant species that were accessible. Those included the Threeridge and Wabash Pigtoe (*Fusconaia flava*) – the most- and third-most abundant species in Pool 5, and the Plain Pocketbook (*Lampsilis cardium*) – another common species that was abundant in the wadeable areas. In Pool 18, the three equally abundant species mentioned previously were collected by malacologist divers at the time of the surveys (i.e., Threeridge, Threehorn Wartyback, and Mapleleaf) (Newton et al. 2011).

This Upper Mississippi River scenario represents contaminant sequestration in large populations at a large spatial scale, and the rigorous population survey methods provide the best possible accuracy in estimating the population size, and therefore, the best accuracy in estimating the mass of pollutants sequestered.

Upper Neuse River Watershed

In the second scenario, we used data from a relatively small watershed (1,686 km^2) in the Upper Neuse River Basin, which drains into the upper arms of Falls Lake Reservoir in the north-central Piedmont physiographic region of North Carolina. The main watercourses in the study area were the Eno, Little, and Flat Rivers, and their tributaries – all low-order wadeable streams.

Mussel population data were available from Levine et al. (2003, 2005), who assessed mussel distribution in relation to crossing structures. They used three 1-m wide one-pass visual surveys (one pass in the center of the channel and one near each bank) in 44 stream reaches, each spanning a distance of 300 m upstream and downstream of road crossings, for a total of 600 m surveyed per site (for a total of 26.4 river kilometers surveyed). The mean width of surveyed reaches was 9.9 m (range 3.4 – 26.5 m). Such qualitative methods are commonly used in field surveys as a rapid assessment of mussel presence in wadeable streams for population monitoring (e.g., Hastie et al. 2004) and for assessing potential impacts from proposed development projects (e.g., linear stream crossings, such as a bridge or utility pipeline) (e.g., Levine et al. 2003; USFWS 2008).

Levine et al. (2003, 2005) detected 26,131 mussels at the surface in the Upper Neuse watershed reaches they surveyed; 94% of those detected were Eastern Elliptio (*Elliptio complanata*; n = 25,413). To improve the estimation of mussels in the surveyed area, investigators applied corrections to the raw survey data to account for mussels in un-surveyed stream width (i.e., those areas between the center and bank transects) and to account for mussels that were likely not detected. Those in the un-surveyed area were accounted for by using mussel data from the center transect multiplied by total wetted width of each reach, minus the 3 m that were surveyed (Chris B. Eads, NC State University, unpublished data). Second, previous surveyor experience and research indicated that approximately 3 of every 4 mussels on the surface were likely to be detected in one pass; thus, a 0.75 correction factor was applied (i.e., number of mussel detected ÷ 0.75) to estimate total mussels on the surface, including those expected to be missed in a one-pass survey design (Eads and Levine 2013). Applying this correction yielded an estimate of 79,729 mussels on the streambed surface in the in the surveyed reaches.

Contaminant data were available from Archambault et al. (2018), who evaluated the influence of polycyclic aromatic hydrocarbons (PAHs) from road-crossings on mussels. Eastern Elliptio mussels were collected from the middle 100 meters at 20 of the 44 stream reaches (i.e., from 50 m upstream to 50 m downstream of the road crossing) during the original surveys, and tissue samples were analyzed for PAHs (Levine et al. 2005). To estimate PAH sequestration by mussels in the full area surveyed, we averaged the tissue PAH concentrations from mussels at all 20 sites (n = 40 concentrations, one each from the 50-m reaches upstream and downstream of each road crossing) and then scaled this average estimate of sequestered PAHs to the estimated population size. The average concentration of PAHs in mussel soft tissue from the Upper Neuse River watershed was 182 ng/g (dry weight; Levine et al. 2005; Archambault et al. 2018).

This Upper Neuse River Watershed scenario represents contaminant sequestration at a spatial scale comparable to that of most mussel data, which are often collected in small, low-order wadeable streams. While the survey technique in this scenario represents a lesser degree of precision in estimating population size – and therefore contaminant sequestration – than the first scenario, it is representative of the data quality typically available from natural resource agencies or consulting biologists, who often must conduct rapid population assessments to accommodate temporal and fiscal constraints (Hastie et al. 2004; Smith 2006; USFWS 2008), and whose data are similar in their qualitative nature. Accordingly, it is imperative that we understand the feasibility of using such existing data to derive estimates of mussel pollutant sequestration and other ecosystem services.

Clinch River

The third scenario we explored was in the Clinch River, where biologists have conducted long-term monitoring of mussel populations in a 164-km reach from southwestern Virginia to northeastern Tennessee since the 1970s (Jones et al. 2014, 2018; Ahlstedt et al. 2016). The Clinch River is within the Tennessee – Cumberland aquatic faunal province, known for the greatest unionid mussel and freshwater fish biodiversity in North America (Haag 2010). More than 135 freshwater species in the Tennessee – Cumberland province have been identified as priorities for conservation, and most are fishes and mollusks (Smith et al. 2002). The Clinch River alone has supported approximately half of the region's fish and mussel species (Smith et al. 2002; Zipper et al. 2014), and many of those fishes likely serve as hosts to the parasitic larval stage of native mussels (Barnhart et al. 2008). However, mussel populations have declined precipitously in sections of the Clinch River in the past 40 years. These declines were detected by quantitative population surveys at targeted sites of suitable habitat in the river, in which an 88-km zone of mussel decline has been identified, stretching from the confluence with Dumps Creek near the towns of Carbo, Virginia to around Clinchport, Virginia (Jones et al. 2014).

Approximately 18% of mussel fauna known from the Clinch River are believed to be extirpated (Jones et al. 2014). In total, at least 48 freshwater mussel and fish species in the river are imperiled or vulnerable, and many of those are federally listed as endangered or threatened (Master et al. 1998), including 20 endangered mussel species (Jones et al. 2014). Within the zone of mussel decline, Jones et al. (2018) reported a faunal collapse at Pendleton Island, where the abundance of mussels has been reduced by ~96%. Pendleton Island once supported the greatest mussel biodiversity and abundance in the Clinch River, with a historic baseline density of 25 mussels/m^2, but biologists found only ~1 mussel/m^2 during surveys conducted in 2014 (Ahlstedt et al. 2016; Jones et al. 2018). Results from multiple recent studies have implicated pollution from PAHs, major ions, and metals associated with fossil fuel mining in the watershed as likely causative factors in mussel declines (Johnson et al. 2014; Price et al. 2014; Archambault et al. 2017; Zipper et al. 2014), including Cope & Jones (2016), who found a strong correlation between decreased mussel density and increased tissue PAH concentrations.

In this scenario, we were particularly interested in comparing pollutant sequestration in mussel soft tissue from Pendleton Island – where the most catastrophic mussel losses have been documented – with an area of population stability in comparably-sized habitats outside and downstream of the recognized mussel zone of decline. We used the density and area data provided by Jones et al. (2018) to compare contaminant sequestration by mussels at Pendleton

Island (habitat area of 55,500 m^2) with sequestration by healthy mussel assemblages that occur downstream in Tennessee at the Wallen Bend, Kyle's Ford, and Frost Ford survey sites (combined area of 58,765 m^2). Jones et al. (2018) reported that these assemblages had a density of 29 mussels/m^2, proportionate to the historic baseline of 25 mussels/m^2 that Pendleton Island supported. Multiplying mussel density by each site area yielded an estimated 55,500 mussels at Pendleton Island and 1,704,185 mussels at the healthy sites.

We had contaminant data along with tissue masses from Pheasantshell mussels (*Actinonaias pectorosa*) that were collected from the same reach of the Clinch River in 2012-2013 during a collaborative project investigating potential causes of mussel decline (Cope & Jones 2016; Archambault et al. 2017). Pheasantshell was reported as abundant and as the dominant species in the Virginia section of the Clinch River by Jones et al. (2014). Tissue concentrations of several classes of contaminants were available from multiple study sites, including Pendleton Island and Wallen Bend. Here, we focus on a comparison of PAH sequestration because Cope and Jones (2016) reported that other contaminants (e.g., PCBs and pesticides) were of relatively low importance and presence in tissue and the river compartments (i.e., surface water and sediments). The average tissue concentration of PAHs from mussels collected at Pendleton Island was 852 ng/g, and the average from those collected at Wallen Bend was 239 ng/g (dry weight; Cope & Jones 2016).

The Clinch River scenario represents contaminant sequestration and comparison at targeted sites of interest, where quantitative mussel population data are available. Like the Upper Neuse River watershed scenario, mussel population data are at discrete locations and at a spatial scale comparable to that typically available from monitoring projects conducted by natural resource agencies, consultants, and researchers; it differs by having more reliable survey data on which to base population estimates, and therefore, allowing more precision in estimating ecosystem services. This scenario provides an opportunity to examine relative comparison of ecosystem services between sites or with historic data, and is a useful demonstration of adapting rich datasets, such as those available from long-term monitoring projects.

Contaminant Data and Ecosystem Service Calculations

For each of the three scenario locales, tissue contaminant data were from abundant mussel species in the reaches surveyed, and should provide a plausible representation of pollutant sequestration by the mussel assemblage. For the Upper Neuse River watershed and Clinch River scenarios, mussels were collected for previous projects in which the primary objective was to understand the risk of pollutants to mussels in the project areas. In the Upper Mississippi River scenario, mussels were collected to estimate pollutant sequestration. Twenty-seven composited tissue samples were available from each navigation pool for the Upper Mississippi River estimates (i.e., nine per species in each pool – three each from the upper, middle, and lower reaches). Two composited samples were available for each of the 20 sites for the Upper Neuse River watershed (Archambault et al. 2018). Three composited samples were available for each site for the Clinch River (Cope & Jones 2016). In each case, samples were a composite of three to five mussels at a given site; a total of 270 individuals representing the dominant species were collected from the Upper Mississippi River study areas; 100 Eastern Elliptio were collected from the Upper Neuse River study area (Archambault et al. 2018), 144 Pheasantshell were collected from the greater Clinch River study area (Cope and Jones 2016; here, we used average soft tissue weight of all those sampled, though only contaminant data from mussels at the two sites of interest are used). All samples were processed and analyzed according to standard methods and quality assurance. Though analysis of the Upper Mississippi River samples have not been previously reported, they were handled by the same toxicologists in the same manner as those from the other sites (Cope & Jones 2016; Archambault et al. 2018); quality assurance protocols were followed for all contaminant analyses, and quality control measures were within acceptable ranges.

For the Upper Mississippi River and Clinch River samples, available data included a suite of 22 metals and several classes of organic contaminants, including PAHs, PCBs, and legacy pesticides and current-use pesticides (78 individual organic compounds for the Upper Mississippi River and 138 for the Clinch River). For the Upper Neuse River watershed, only PAHs were available (Archambault et al. 2018). Metals were the only prevalent contaminants in the Upper Mississippi River samples; though we analyzed samples for organic contaminants, none were detected above reportable limits in any samples by the analytical laboratories. Metals and PAHs were the only prevalent contaminants in the Clinch River samples, and we focused on PAHs because of their strong inverse relationship with mussel density (Cope & Jones 2016). Metals may enter aquatic systems through natural processes such as weathering of rocks or volcanic activity, but anthropogenic activities, such as mining and industrial processes, agricultural practices, and urbanization (e.g., runoff and wastewater) have caused global contamination of soil and waterways. PAHs are a class of hydrophobic persistent organic pollutants that are ubiquitous in the aquatic environment, primarily because of anthropogenic activities including combustion of fossil fuels (Neff 1979).

We used average mussel soft tissue masses from each scenario to scale the mass-normalized tissue concentrations of prevalent contaminants (e.g., µg/g tissue) to an average whole mussel body burden (i.e., g/mussel). We then multiplied the average body burden by the population sizes to derive an estimate of contaminant sequestration at the population level.

RESULTS

Contaminant sequestration as a potential ecosystem service differs based on spatial scale, population size, and the kind of contaminant under consideration. Estimating population-level sequestration by mussels using existing data varied in difficulty, from straightforward to highly conditional. Rigorous, quantitative data were ready for use, while qualitative data required much more thought, careful consideration of factors, and correction factors based on knowledge in the literature and best professional judgment. The data used, conversions applied, and estimated contaminant sequestration for each scenario are summarized in Table 1.

Upper Mississippi River Navigation Pools

The availability of robust population estimates along with contaminant data and soft tissue masses from the dominant species in the Upper Mississippi River navigation pools made estimating sequestered contaminants relatively straightforward in this scenario, and no data conversions were required. We summed the 22 individual metals for each tissue sample, and computed the mean total metal concentration for each species in a given pool (i.e., averaged the upper, middle, and lower reach samples). In Pool 5, the mean total metal concentrations and associated soft tissue dry weights were 9,598 µg/g in Threeridge (range 8,317 – 10,336 µg/g; tissue weight = 2.50 g, ±0.22 g, SE); 12,102 µg/g in Plain pocketbook (range 10,674 – 13,548 µg/g; tissue weight = 9.90 g, ± 0.57 g); and 12,371 µg/g in Wabash Pigtoe (range 11,652 – 12,830 µg/g; tissue weight = 1.90 g ± 0.10 g). Given a pool-wide average total metal concentration of 11,357 µg/g and average tissue mass for the mussel assemblage of 4.77 g, the estimated 190 million mussels (± 37 million) in Upper Mississippi River Pool 5 had 11.3 tons of metals sequestered in soft tissues (range, 9.2 – 13.4 tons, accounting for population confidence limits; Table 1).

In Pool 18, the mean total metal concentrations and associated soft tissue dry weights were 6,824 µg/g in Threehorn Wartyback (range 5,859 – 6,626 µg/g; tissue weight = 0.81 g, ± 0.07 g); 9,780 µg/g in Mapleleaf (range 9,031 – 10,306 µg/g; tissue weight = 1.88 g, ± 0.18 g); and 10,385 µg/g in Threeridge (range 8,946 – 12,898 µg/g; tissue weight = 3.44 g, ± 0.35. g). Given a pool-wide average total metal concentration of 8,996 µg/g and average tissue mass for the mussel assemblage of 2.04 g, the estimated 212 million mussels (± 43 million) in Pool 18 sequestered approximately 4.3 tons of metals in soft tissues (range, 3.4 – 5.2 tons, accounting for population confidence limits; Table 1). These totals combine to approximately 15.6 tons of metals sequestered by mussels in the two Upper Mississippi River pools (range, 12.6 – 18.6 tons, if the low or high population limits are used).

Upper Neuse River Watershed

The nature of both the mussel survey and tissue data rendered the Upper Neuse River watershed scenario the least straightforward in making use of existing datasets. Several post-hoc corrections were required for estimation of contaminants sequestered in the 44 reaches of this study area. We mentioned earlier that biologists applied a correction to the visual one-pass survey data on providing it; this correction accounted for the incomplete coverage of stream reaches and typical detection ability of experienced field biologists, as noted above. Next, because mussels have a benthic ecology and surveyors employed a visual survey technique (i.e., no excavation of quadrats like was done for the Upper Mississippi River samples), we had to account for mussels that were burrowed beneath the sediment surface. Relevant, peer-reviewed studies addressing the comparison or conversion between visual survey data and those derived by excavation were limited. Heise et al. (2013) used correction factors to estimate burrowed mussels based on a comparison of visual and quadrat surveys conducted in a central North

Carolina stream. They estimated that an extra 50% to 140% more mussels were burrowed than were on the surface, and used correction factors that ranged from 1.5 – 2.4 (average 1.8, n = 15 for a given site and year) for all mussel species found. Their estimates may be appropriate because the study was conducted the same region as our study, and Heise et al. (2013) similarly found that Eastern Elliptio was the dominant species, accounting for 88 – 95% found at their sites. Balfour & Smock (1995) estimated 20% of *E. complanata* were burrowed in March/April in a Virginia headwater stream – another reasonable comparison for our southeastern sites. Eads and Levine (2007) conducted a mark-recapture study in a nearby central North Carolina stream (New Hope Creek, Cape Fear River Basin) that spanned the period of Upper Neuse River watershed study; the authors suggested a conservative correction of an extra 50% of Eastern Elliptio burrowed based on their findings (Chris B. Eads, NC State University, personal communication). Therefore, the range of estimates of burrowed mussels from the literature include a conservative estimate of an additional 20% (Balfour & Smock 1995) to a more liberal estimate of 2.4X as many mussels burrowed as on the surface (Heise et al. 2016).

Here, we report results based on wet weight from the availability of mussel measurements and tissue sample data from the original study. Tissue contaminant data were available in dry weight units, and the average dry weight proportion of tissue samples was known (9.58%), but the sample weights were unknown. The mean total wet weight was known for the mussels (43.85 g, including the shell), but the soft tissue weight was unknown. These circumstances required conversion of the contaminant data from dry to wet weight for scaling to body burden with the known mussel weights, and required a correction from whole body to tissue wet weight (i.e., where PAH concentrations were measured). First, the mean tissue PAH concentrations were converted to wet weight by applying the dry weight percentage (i.e., 182 ng/g dry weight ÷ 0.0958 = 1,900 ng/g wet weight). We then relied upon Eastern Elliptio measurements from a companion study (Chapter 2, herein) to figure out what proportion of the known whole mussel wet weight was likely soft tissue. We considered these mussels appropriate for comparison because their mean shell length (77.34 mm (± 7.83 mm, SD)) was similar to the Upper Neuse River mussels (67.93 mm (± 12.85 mm, SD)). We used measurements from 150 comparably sized mussels collected for experiments in Chapter 2 herein, for which we had data on whole body wet weight, tissue only, and shell weight. Eastern Elliptio soft tissue in those data averaged 36.4% of their total wet weight; thus, we applied a correction factor of 0.364 to the known whole body wet weight of the Upper Neuse River mussels. We were then able to scale PAH concentrations to estimate body burden (ng/mussel), and finally, scale up to the population estimate from the visual surveys (a minimum estimate of PAH sequestration) and to the totals that account for burrowed mussels. Scaling to just the surface estimate of 79,729 mussels, these mussels sequestered approximately 2.4 billion ng of PAHs. When we accounted for mussels that were likely burrowed in the surveyed reaches, using the low (x1.2) and high (x2.4) corrections available from the literature (Balfour & Smock 1995; Heise et al. 2013), we estimate that mussels sequestered between 2.9 and 5.8 billion ng of PAHs in the Upper Neuse River study reaches (Table 1).

Clinch River

The combination of site-specific quantitative survey data along with accurate estimates of site area and the availability of wet and dry tissue masses from the mussels collected, allowed for simple estimation of contaminants sequestered at the Clinch River sites. The only conversion required was translating the tissue PAH concentrations from dry to wet weight because dry tissue weights were not available for all of the tissue samples. We calculated the average dry weight

(8.31%) from tissue samples collected in the Clinch River study reach where both the wet and dry were weight available (n = 15, including samples from sites other than Pendleton Island and Wallen Bend). We applied the conversion to the average tissue PAH concentrations from our sites of interest; then, we were able to scale tissue PAH concentrations to body burden using the mean soft tissue weight of all Pheasantshell sampled (42.83 g). We estimated that the total mass of PAHs sequestered by mussels at Pendleton Island to be ~24.2 billion ng, and that mussels at the healthier sites outside the zone of decline sequestered ~210 billion ng of PAHs (Table 1). Despite having a much lower mean tissue PAH concentration (239 ng/g) – only 28% of that in mussels at Pendleton Island (852 ng/g) – the healthier assemblage had nearly 10 times more capacity to sequester PAHs from the aquatic environment.

DISCUSSION

Proof of Concept

We successfully quantified population-level estimates of pollutant sequestration by unionid mussels, using various types of existing field data. The degree of precision attainable in such calculations is admittedly limited, but our efforts offer a proof-of-concept demonstration of the magnitude of pollution mussels are filtering out of the environment and their incidental exposure to contaminants. Our scenario selection and ability to estimate pollutant sequestration was heavily influenced by the availability of both population data and tissue contaminant data. The latter is not normally monitored by natural resource agencies and may be prohibitively costly to obtain solely for the purposes of estimating contaminant sequestration; the contaminant data used here cost between $250 and $1,400 per sample analyzed in the original projects. Moreover, hundreds of mussels were collected and lethally taken for tissue analysis, even to achieve the general estimates calculated here. Collection of mussels may not be feasible in some habitats of interest, may not be optimal in areas where populations are in decline, and may require killing more mussels and spending more financial resources to achieve any greater precision in estimating contaminant burdens.

Utility and Challenges of Existing Data, and Some Lessons Learned

This exploration of quantifying pollutant removal using three existing datasets that represent a range of spatial scales and different survey techniques for estimating populations offers a comparison of the relative ease or difficulty in deriving ecosystem service estimates with re-purposed data. Just as systematic survey data are most reliable for estimating population size (Strayer & Smith 2003), the same is true for applying such totals to quantifying contaminant sequestration and other ecosystem functions and services provided by those populations. The population estimates in the Upper Mississippi River scenario that were derived from robust, quantitative survey data provided the soundest foundation on which to proceed with estimation of population-level ecosystem services. In that scenario, population confidence limits were also available, allowing us to quantify a range of pollutant sequestration, given the expected uncertainty in the population size. Similarly, population estimates derived from areas selected for their habitat suitability, like the quantitative surveys from the Clinch River sites, are reliable and highly applicable if applied appropriately to ecosystem service estimates in designated areas, and they may be especially useful for comparing ecosystem services among populations. However, targeted survey data may overestimate ecosystem functions and services if applied to river reaches beyond the surveyed areas, and investigators should take care in using existing data within its limitations.

Visual survey data like those represented in the Upper Neuse River watershed scenario presented the greatest challenges in attempting to account for the mussel population size and carrying an estimate forward for pollutant sequestration. Qualitative data are more imprecise and generally not recommended for deriving population estimates (Strayer & Smith 2003), but they may represent the only available information on mussels in many locales. Though visual surveys may exclude smaller individuals or may provide different assemblage results than quadrat excavation, they may be comparatively reliable for species estimates, such as richness, evenness, and diversity indices (Hornbach & Deneka 1996). Despite the fact that mussels spend much of their time burrowed and they regularly move within the sediment column, the body of knowledge on vertical migration, species differences, and temporal and environmental effects on this behavior is relatively small. The Upper Neuse River populations were dominated by Eastern Elliptio, and we were fortunate to find peer-reviewed (Balfour & Smock 1995; Heise et al. 2013) and grey literature (Eads & Levine 2007) that reported on the relative proportion of burrowed mussels for this common species in habitats similar to our scenario (wadeable, southeastern US streams).

Visual survey techniques are often selected to accommodate resource constraints (e.g., staff availability, project timing, or fiscal resources) and they may be sufficient for the intended project goals (Strayer & Smith 2003). However, opportunities for data collection on mussel populations are constrained, compared to data collection on fauna that are more frequently studied or are easier to access (e.g., birds, fish, other stream macroinvertebrates), because of their cryptic nature and the exceptionally small cohort of freshwater malacologists (e.g., ~500 members in the Freshwater Mollusk Conservation Society compared to ~8,000 in the American Fisheries Society). Additional effort to collect some quantitative data at a subset of sites for comparison with visual survey results when malacologists are already afield would increase data reliability and be worthwhile for the benefit of more extensive future use.

In a recent assessment of freshwater mussel conservation needs, Ferreira-Rodriguez et al. (2019) highlighted that information on population sizes, distributions, and trends were among the top 20 research priorities – an assessment that supports the need for more detailed survey efforts (and importantly, more resources for conducting surveys), which would contribute to ciphering out how mussel populations provide ecosystem services. At present, mussel survey data and population estimates are scarce and difficult to obtain (i.e., rarely available in peer-reviewed studies, and many datasets are likely tucked away in agency files), but they are the foundational information needed to estimate pollutant removal or other ecosystem functions and services provided by mussels. A centralized repository for these important population metrics would be beneficial for mussel conservation planning and for future endeavors in understanding the magnitude of their ecosystem functions and potential for providing beneficial services for human and environmental health. Ecosystem service delivery inherently depends on spatial extent, time scales (Scholes 2009), and biodiversity (Naeem 2009; Palmer & Richardson 2009); thus a better understanding of mussel population metrics will aid in quantifying benefits of mussels, and enhance future endeavors of modeling across such scales (e.g., Qiu et al. 2018).

Similar to our use of available population data, we encountered some benefits and challenges in using existing data on contaminant levels in mussel tissue. Two primary benefits were the significant cost savings of using available data, and eliminating the necessity of killing mussels for this purpose; alternately, these benefits may be considered as a value-added utility for tissue collection in future projects that investigate pollution. The main challenge we encountered with the tissue data was the availability of ancillary information for scaling concentrations to whole body burdens. Contaminant data are reported on a mass-normalized basis (e.g., ng/g), often in dry-weight terms, and are typically used in the same format for pollution studies (e.g., comparing concentrations among sites or treatments). Some metrics are normally measured when the mussels are collected (e.g., length), but are often only used for general summary reporting. The issues we encountered here, such as the lack of tissue weight or lack of sample wet and dry weights for conversion, highlight the need for recording all possible data when mussels are in hand. Such data may prove critical in another unplanned future context. For example, if tissue wet weights and sample weights were measured and recorded when the Upper Neuse River mussels were dissected for contaminant analysis, this post-hoc use of the data would have been more straightforward and reliable, and would not have required pulling metrics (i.e., soft tissue weights) from another project's dataset. Even in field surveys where mussels are not collected, measuring whole wet weight in addition to length (traditionally the easiest and preferred metric) when mussels are in hand would be beneficial, as biomass may be a more sensitive predictor of sublethal stress (Wang et al. 2011; Archambault et al. 2017) that could serve as an early warning sign about population health, their potential for decline, and their current and future ecosystem service capacity. Future assessments of contaminant sequestration may consider alternate methods that would eliminate the data availability issues we encountered. For example, Archambault et al. (2018) demonstrated a highly significant predictive relationship between PAH concentrations in passive sampling devices exposed to surface water and those in mussel tissue ($p < 0.0001$, $r^2 = .90$); pairing of such data with filtration rates of dominant species may yield adequate estimates of contaminant sequestration without killing mussels.

Finally, we elected to provide a range of pollutant sequestration only in cases where we had a range of population estimates. The range of mass-normalized contaminant concentrations, the range of soft tissue masses for mussels at a given site, population size, or all three could have been used to derive a range of estimates; however, varying all the components in concert may lead to unwieldy and difficult to interpret results. In these scenarios, we elected to base estimates on the average tissue concentrations and masses for a few reasons. Three species in the Upper Mississippi scenario were selected to represent between 16 and 23 species in the pool-wide assemblages; attempting greater 'precision' by calculating from a range of tissue concentrations may not have been any more representative than the average after scaling up to the populations. Furthermore, the species average tissue masses in that scenario ranged from < 1 g (Threehorn Wartyback) to nearly 10 g (Plain Pocketbook) dry weight, so an average tissue mass per pool should be the most reasonable approximation representing all species in the unionid populations. In the Upper Neuse watershed and Clinch River scenarios, the species selected were dominant in the assemblages and, therefore, representative. Finally, in the Upper Neuse watershed scenario, qualitative population data introduced enough imprecision that adding a range of estimates seemed imprudent. Quantifying the functions and services of any unionid population is likely to hinge on limited data, and such decisions will be required. We recommend careful consideration of the merits and limitations in each component, especially when using existing data, and transparently reporting assumptions that were made, how the data were applied, and the rationale for those decisions.

Interpretation of Contaminant Sequestration as an Ecosystem Service

There are a few important questions to consider in interpreting contaminant removal as an ecosystem service: what is the context compared to environmental availability of the contaminants? What happens after mussels sequester contaminants in their tissue? Is it bad for the mussels? (Strayer 2017; Vaughn 2018).

Discussing the sequestration of contaminants in context with their environmental concentrations or availability will aid in understanding the magnitude of their contribution to ecosystem health. For the scenarios we explored, useful data on environmental concentrations of contaminants were available. Organic contaminants were largely absent from water and sediment samples in the Upper Mississippi River pools; therefore there was no apparent service of organic contaminant sequestration even at a large spatial scale. However, a historic analysis might reveal substantial services when organic classes of contaminants were more prevalent (e.g., extensive 20th Century uses of polychlorinated biphenyls and organochlorine pesticides; Wiener et al. 1984; Cope et al. 1999). Tissue concentrations of PAHs in mussels were strongly correlated with surface water concentrations in the Neuse River and Clinch River scenarios (Archambault et al. 2018; Cope & Jones, 2016). Archambault et al. (2018) further demonstrated that PAH concentrations in PSDs had a significant predictive relationship with those mussels, suggesting the potential to monitor future mussel health and potentially the relative ability of contaminant sequestration without killing mussels. Further estimating the magnitude of contaminant sequestration and other ecosystem services in comparison to availability in surface water will require interdisciplinary team collaboration with a cross-section of experts, including hydrologists, and possibly economists, especially when considering larger spatial scales such as the Mississippi River.

Comparing mussel tissue contaminants with those in sediments may be instructive when such data are available. For the Upper Mississippi River, Pool 5 had an average sediment metal concentration of 15,576 µg/g, compared to the pool-wide average mussel metal concentration of 11,357 µg/g; on a mass-normalized basis, mussels accomplished about 73% of the metal binding as compared to sediment. In Pool 18, the average sediment concentration of metals was 22,461 µg/g, compared to the pool-wide average in mussels of 8,996 µg/g; on a mass-normalized basis, mussels there performed about 40% of the metal binding as sediment. Similarly, in the Neuse River watershed, mussels averaged 182 ng/g PAH in soft tissue compared to 428 ng/g in the sediment. On a mass-normalized basis, mussels were able to perform about 43% of the job of sediment in sequestering PAHs. Obviously, there is a much greater mass of sediment than mussels, even when only accounting for sediment near the water interface, but these comparisons show that mussels add capacity for environmental resilience to aquatic ecosystems, and that their sequestration potential is not inconsequential.

We know little about the environmental fate of contaminants once mussels ingest them. Most studies of toxicokinetics in bivalves have been focused at the organismal or tissue level, such as deriving uptake and depuration rate constants or quantifying contaminant partitioning among tissue types (Farris & Van Hassel 2007). Strayer (2017) noted that contaminants removed by mussels must remain out of the water to be a useful service. Vaughn and Hoellein (2018) highlighted several studies in which bivalves were deployed and removed from habitats as a means of bioremediation; however, that strategy is likely not feasible for the declining native mussel fauna in North America, where the conservation goal is to recover populations (FMCS 2016). Mussels may also egest contaminants in their biodeposits (Ismail et al. 2014) or inactivate pathogens (Ismail et al. 2015) as a means of permanent removal; this is a nascent area of research and needs to be further explored (see Chapter 2 on environmental fate of metals as mediated by mussels). A question that regularly arises in conversations of contaminant removal as an ecosystem service is: what happens when the mussels die. Though we are unaware of studies that measure the release of contaminants from dead tissue, the general presumption is that contaminants are released back into the water or are available for consumption by other organisms. Though this is likely the case, we suggest that standing healthy populations of these long-lived unionids can provide a reservoir of pollutant-removal capacity to aquatic ecosystems, so long as recruitment and mortality are balanced. Moreover, some contaminants, such as metals that may be processed similarly to calcium by mussels may get sequestered for long term storage (decades or centuries) in calcified shell material, and future studies should evaluate this potential pathway for contaminant-related ecosystem services, similar to studies on shell sequestration of carbon and nutrients (e.g., Hoellein et al. 2017; Atkinson et al. 2018). There is another important perspective related to the question about what happens when the mussels die. If mussel populations die *en masse* from disease or continue to decline due to modern threats including chronic pollution, we lose all the ecosystem functions and services related to environmental and human health. In our Clinch River scenario, we demonstrated that the impaired mussel fauna at Pendleton Island sequestered only ~10% of the PAHs compared to the healthy populations downstream (comparable in size to Pendleton's minimum historic baseline) despite harboring three times higher concentrations – an indication that other filtration services (e.g., nutrient cycling) were also lost. Indeed, Jones et al. (2018) tabulated a cumulative temporal loss of more than 67 million mussel-service years at this one site since survey records indicated declining populations in the 1970s, representing an unknown, but likely incomprehensible magnitude of lost ecosystem function and services. Contaminant sequestration may be interpreted as an ecosystem service, but mussels will only be able to remove contaminants so long as aquatic ecosystems are healthy enough to support their persistence. Finally, as we will see in Chapter 2, pollutant sequestration by mussels includes influencing the fate and transport of pollutants in waterways, and is a topic that may resonate with public audiences.

Ecosystem Services in an Era of Faunal Decline

A recent review of emerging and persistent threats to freshwater biodiversity suggests that a changing climate, emerging contaminants, and cumulative stressors are among the most important challenges in conserving freshwater biodiversity (Reid et al. 2018). Coupled with the assessment that mussels will experience increased fragmentation and loss of suitable habitats (Markovic et al. 2014; Inoue & Berg 2017) are findings that hydrologic fragmentation may impair mussel physiology (Galbraith & Vaughn 2011), and increasing temperature and drought may further threaten their survival (e.g., Golladay et al. 2004; Haag & Warren 2008; Pandolfo et al. 2010; Archambault et al. 2013, 2014a, 2014b). Researchers have already reported that climatic factors, including drought, could affect ecosystem service delivery. For example, Spooner and Vaughn reported that mussels exhibit altered resource assimilation and excretion rates under different thermal regimes, and Vaughn et al. (2015) and Dubose et al. (2019) both reported on drought-induced losses of ecosystem function and services related to biofiltration, nutrient cycling, and nutrient storage. Advances in freshwater mussel toxicology over the last two decades have revealed that unionids are among the most sensitive organisms to several classes of contaminants entering surface waters (e.g., copper (Wang et al. 2007), ammonia (Newton et al. 2003; Newton & Bartsch 2007; US EPA 2013), and pesticides (Conners & Black 2004; Bringolf et al. 2007; Archambault et al. 2015)), and mussels are exposed differentially through various routes during development from their parasitic larval (upon a host fish) life stage to their benthic juvenile and adult life stages (Cope et al. 2008). As mussels are already imperiled, we need a greater focus on elucidating how their ecosystem functions translate to services and how those functions and services are hampered by persistent and emerging threats, so that we can build a robust narrative about their value, and make the case for conservation action.

Conservation and Policy Decisions in an Era of Uncertainty

As a community of trained scientists, we are most comfortable with data and interpretations made with a high degree of certainty (e.g., 95%); even then, our tendency is to humbly qualify our findings. Precision in understanding population or ecosystem-level processes is rarely a luxury we are afforded; yet at human community and society scales and in socio-ecological systems such as water resources, decision-making timelines are urgent, stakeholders have conflicting interests, and scientific data are incomplete – a combination that Funtowicz and Ravetz (1993) characterized nearly three decades ago as post-normal science. Ainscough et al. (2018) recently conducted a review of ecosystem services research and suggested that post-normal science is a useful framework for the field. Such a framework may be practical in the pursuit of identification, quantification, and valuation of freshwater mussel ecosystem services. As we have demonstrated, scaling available data to relevant spatial scales may be difficult and imprecise; yet pursuing this endeavor despite its difficulties may be the most effective way to provide representation and visibility for unionid resources. The Freshwater Mollusk Conservation Society (2016) and Strayer (2017) suggested that mussel biologists have an integral role to play in establishing a public narrative of value by quantifying and valuation of services when the methodology is ready, but also by simply increasing awareness of their existence and offering a narrative of the benefits they provide. Costanza et al. (2017) substantiated that raising awareness and interest are among the spectrum of uses for ecosystem service valuation, and that low precision estimates at regional to global scales is appropriate for those goals. Scenario-building exercises are regularly used in ecosystem service evaluation to explore the impacts of various management actions (e.g., water purification or fisheries models available in open-source software such as InVEST [Integrated Valuation of Ecosystem Services and Tradeoffs], https://naturalcapitalproject.stanford.edu/). Constructing scenarios to demonstrate freshwater mussel services (e.g., services lost with continued faunal decline or gained with restoration to historic population sizes), and collaborating across disciplines to incorporate mussel filtration into existing models may be another way to advocate for their value. Though our exploration into quantifying contaminant removal at mussel population levels is definitely incomplete, we believe that it demonstrates one of the many ecosystem service benefits mussels provide and is an appealing component for the public narrative that freshwater mussels maintain and improve water quality.

Ecosystem services are a valuable tool for cultivating social value and providing context for management decisions, especially when social benefits are emphasized (e.g., water clarity; fishing opportunities; Olander et al. 2015, 2018). Though establishing value for non-consumptive benefits to humans is more difficult than for tangible goods, services such as water quality maintenance (e.g., through reduction of pathogens or contaminants) tend to have greater shared societal impacts (Small et al. 2017). Without accounting for services that freshwater mussels provide, we leave open the risk that they will be discounted or left out of important management and policy decisions that affect their conservation. One regionally illustrative example that would have benefited from recognition, quantification, or valuation of ecosystem services, is a highway construction project that will transect endangered mussel habitat in central North Carolina. The expansion of Interstate 540 around the greater Raleigh area captured regular media attention and has been controversial for several years because of the potential environmental impacts and conflicting values about alternative impacts to established human communities. Of nearly 20 routes considered, the final selection was the most environmentally damaging to federally endangered Dwarf Wedgemussel (*Alasmidonta heterodon*) and to stream and wetland habitats. Though an endangered species mitigation plan was negotiated, a proper accounting of aquatic ecosystem damages and services lost may have resulted in a different decision. Another possibility is that a clearer understanding of the ecological consequences and lost services could have resulted in a more comprehensive mitigation agreement that would not have resulted in litigation by conservation groups. Incorporating ecosystem services into decision-making has been practiced for more than a decade by federal agencies in the US and abroad (Olander & Maltby 2014), but their application has not become standard despite concerted efforts to establish guidance (Olander et al. 2015).

Conclusions

Much more effort and research on illuminating ecosystem services by native mussels is needed, with a continued emphasis on explicit links between their ecosystem functions and socially desirable metrics (Keeler et al. 2012; Olander et al. 2018). We further need to make interdisciplinary strides toward coupling the messages of the benefits of mussel conservation with those focused on the social benefits of water quality and water resources (Keeler et al. 2012; Anderson et al. 2019). Public understanding of ecosystem contributions to maintaining water quality are likely specific to people's lived experiences and regional influences (see Chapter 3); thus, leveraging existing public knowledge about other bivalves (e.g., oysters) may be useful in educating people about the basic ecology and functions native freshwater fauna, their contributions to ecosystem health, and how those contributions may translate to beneficial outcomes for water quality and human well-being. Therefore, messages about the benefits of mussel conservation may be best achieved by highlighting their contributions to water quality, including pollutant removal.

While sequestration of pollutants by mussels may be of interest to human populations that rely on clean surface water resources for drinking water and recreation, allowing pollution to persist is not in the best interest of human or environmental well-being. Because a primary ecosystem function of mussels is to filter water as a necessity for fulfilling their basic life history needs of acquiring food and exchanging gametes, and they are largely sedentary and restricted to patchy areas of suitable habitat, a central tenet of freshwater mussel conservation and population recovery must be to maintain and improve water quality by reducing societal pollution (e.g., corporate responsibility; improvements in wastewater technology). Promoting efforts of reciprocity – services of humans in maintaining ecosystems (Comberti et al. 2015) – for pollution reduction would produce improved conditions for all aquatic species, greater potential for population recovery (Reid et al. 2018), and allow for sustainability of ecosystems through improved environmental health, including that of aquatic fauna.

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Table 1. Summary of tissue concentration, tissue mass, population estimate data, and unit conversions used in calculating population-level contaminant sequestration estimates for total metals in the Upper Mississippi River scenario and total polycyclic aromatic hydrocarbons (PAHs) in the Upper Neuse River Watershed and Clinch River scenarios. [a] Mean (± 95% confidence limits (CL)) from Newton et al. (2011). [b] Surface mussel population from Levine et al. (2005), adjusted for detection. [c] Site area and mean density from Jones et al. (2018). Metals converted to tons for more accessible communication (e.g., 1.03×10^{13} µg is difficult to convey, compared to 11.3 tons of metals).

Site	Species	Tissue Concentration	Tissue Mass	Number of Mussels	Contaminant Sequestration
Upper Mississippi River		*Total Metals (µg/g dry weight)*	*Dry Weight (g)*	*Population Estimate [a] (Quantitative)*	***Total Metals (µg → tons)***
Pool 5	Threeridge	9,598	2.50		
	Wabash Pigtoe	12,371	1.90		
	Plain Pocketbook	12,202	9.90		
Pool-wide		*11,357*	*4.77*	190 million (± 37 million, 95% CL)	**11.3 tons (range 9.2 – 13.4)**
Pool 18	Threeridge	10,385	3.44		
	Threehorn Wartyback	6,824	0.81		
	Mapleleaf	9,780	1.88		
Pool-wide		*8,996*	*2.04*	212 million (± 43 million; 95% CL)	**4.3 tons (range 3.4 – 5.2)**
Upper Neuse River Watershed		*Total PAHs (ng/g, Dry → Wet)*	*Wet Weight (g) (Body → Tissue)*	*Surface Population [b] (x burrowed)*	***Total PAHs (ng)***
Combined sites	Eastern Elliptio	182 (÷ 0.0958)	43.85 (x 0.364)	79,729 (x1.2 – 2.4)	**2.4 billion (2.9 – 5.8, with burrowed)**
Clinch River		*Total PAHs (ng/g, Dry → Wet)*	*Wet Weight (g)*	*Population Estimate [c] (Density x Site Area)*	***Total PAHs (ng)***
Pendleton Island	Pheasantshell	852 (÷ 0.0831)	42.83 g	55,500	**24.2 billion**
Tennessee Sites	Pheasantshell	239 (÷ 0.0831)		1,704,185	**210 billion**

CHAPTER 2. Functional Processing of Toxic Heavy Metals by Mussels (Unionidae) and Implications for Freshwater Ecology and Ecosystem Service Delivery

ABSTRACT

Studies on the functional ecology of freshwater mussels (Unionida) have established that mussels are essential to the ecological integrity of freshwater ecosystems. One area of research that has not been explored, and that we study here, is the role of mussels in ecological partitioning of pollutants. Mussels actively influence the distribution of nutrients in rivers, even to the extent of affecting aquatic-terrestrial linkages. This leaves open the possibility that mussels play an active role in the fate and transport of waterborne pollutants through the same functional mechanisms of filtration, retention, and biodeposition. We conducted 28-d laboratory experiments exposing mussels to environmentally relevant concentrations of Ni (0 to 100 µg/L) and Cd (0 to 2 µg/L) – two toxic heavy metals of both human and environmental health concern – to answer the following questions: what percentage of metals do mussels remove from water; how much is sequestered in soft tissue; how much is egested in biodeposits; how are filtration rates affected by metal exposure; and finally, how are these estimates affected by metal concentration or exposure duration? Mussels removed up to 36% of waterborne Ni and up to 77% of waterborne Cd and they sequestered metals in their soft tissue. Mussels also bound and bioconcentrated metals in egested materials (e.g., feces). Ni concentrations in biodeposits were 2 to 7X higher than exposure concentrations, and Cd concentrations in biodeposits were 7 to 40X higher. These pollutant-processing functions fluctuated significantly within the environmentally relevant ranges of Ni and Cd concentrations over the course of 28-d exposures. Fluctuations in functional processing manifested differently for Ni and Cd. Mussels were more efficient at

processing Ni at lower concentrations (i.e., when exposed to less pollution), while the duration of exposure was an important factor for Cd processing; these trends generally held for each metal even when mussels were exposed to both Ni and Cd. Moreover, this ability of mussels to influence the environmental fate and transport of metals was in turn affected by the metal concentrations to which they were exposed. Metal exposure reduced their filtration capacity, and filtration rates were affected differently under the stress of both metals compared to just Ni or Cd, suggesting that pollution may impede other beneficial ecosystem services that mussels provide. This work demonstrates the active role of mussels in environmental fate and transport of toxic heavy metals in aquatic ecosystems, and that pollution negatively affects freshwater mussel filtration and the ecosystem services they provide.

INTRODUCTION

Studies on the functional ecology of freshwater mussels (Unionida) have firmly established that these benthic bivalves are essential to the ecological integrity of freshwater ecosystems. Through their basic biological functions of filter feeding and movement, unionid mussels regulate a wide range of aquatic and benthic environmental parameters. Mussels can filter several liters of water per hour, with estimates dependent on species and body size (Naimo 1995; Kreeger et al. 2018), and at large population or watershed scales, mussels can filter millions of cubic meters of water (i.e., billions of liters) per day (Newton et al. 2011; Kreeger et al. 2018). Though mussels are generally considered sessile or sedentary, they migrate both horizontally and vertically in bed sediments (Balfour & Smock 1995; Amyot & Downing 1997; Watters et al. 2001). Research has confirmed that these movements lead to sediment mixing (i.e., bioturbation) and nutrient cycling (Vaughn & Hakencamp 2001; Vaughn et al. 2004; Vaughn et al. 2008). Through nutrient retention and deposition, unionid mussels alter and slow the downstream transport of nutrients, thus exerting control on foundational tenets in stream ecology (e.g., nutrient spiraling; Elwood et al. 1983). As they filter surface waters, mussels remove algae, bacteria, and other particles, and manufacture biodeposits creating local, nutrient rich hotspots that support stream community biodiversity (Howard & Cuffey 2006; Spooner & Vaughn 2006; Vaughn et al. 2008; Allen et al. 2012). Moreover, these freshwater fauna regulate primary production in waterways, and such influences are dependent on traits that differ among species (e.g., thermal optima) in this highly biodiverse faunal group (Spooner & Vaughn 2012). Their living bodies and spent shells further provide physical habitat and stabilize surrounding sediments, especially in large aggregations called mussel beds (Gutierrez et al. 2003; Vaughn et al. 2008). Because native freshwater mussels often comprise a large proportion of benthic biomass in their habitats, the functions they carry out are integral to maintaining surface water quality and to keeping rivers and lakes properly functioning as ecosystems. However, unionid fauna are in steep decline throughout much of their native range in North America and around the globe.

The southeastern United States (US) is home to the largest biodiversity hotspot of freshwater mussels in the world, and the native fauna in the US and Canada includes nearly 300 species (293 in Unionidae and 5 Margaritiferidae according to a recent taxonomic review; Williams et al. 2017). North American freshwater mussel richness comprises nearly half of all species in the globally distributed Unionidae, and approximately one-third of all extant species in the order Unionida (Graf & Cummings 2020). Unionid mussels are unique among all other bivalves in that they have a parasitic larval life stage (as opposed to a planktonic veliger common in other bivalves) and they require a host fish for successful development and dispersal of their offspring (Lefevre & Curtis 1910; Bauer & Wächtler 2001). Though status assessments are lacking for many species, and global research on the fauna is more limited outside of North America, patterns of global decline are widely recognized (Lydeard et al. 2004; Lopez-Lima et al 2018; Ferreira-Rodriquez et al. 2019). North American native mussels have an imperilment rate of more than 70%, due to historic overharvesting, habitat destruction, and pollution, and they are particularly vulnerable to present-day chronic impacts, such as water quality degradation (Williams et al. 1993; Strayer et al. 2004; Downing et al. 2010; Haag & Williams 2014; FMCS 2016). In outlining the timing of North American mussel extinctions and extirpation of populations from native habitats, Haag and Williams (2014) asserted that habitat fragmentation from damming and channelization was the most pervasive reason for their precipitous decline in the 20th Century. Along with loss of habitat, a primary factor in the imperilment of freshwater mussels is habitat degradation, including impaired water quality from toxic chemicals. Downing et al. (2010) found in a review of unionid studies that the most frequently cited cause of local extirpations was habitat alteration and destruction, in which water quality issues such as pollution was mentioned most often, along with generalized degradation, dams/impoundment, and hydrologic change also among the most common specific reasons. Lopez-Lima et al. (2018) more recently reported that pollution is the most widely recorded global threat to freshwater bivalves for species that have been assessed by the International Union for Conservation of Nature, and Ferreira-Rodriquez et al. (2019) listed understanding toxicological responses of mussels to contaminants among the top 20 research priorities for the conservation of unionid mussels. Diminished mussel populations are clearly unable to provide the same magnitude of contribution to freshwater ecosystem functioning as healthy populations (Jones et al. 2018; Vaughn 2018), and mussels will likely suffer continued endangerment without measures to improve aquatic habitats and water quality.

Unionid mussels are highly sensitive to many contaminants because of their complex life history and multiple exposure routes (Cope et al. 2008). A concerted focus on mussel toxicology in recent decades has revealed that they are among the most sensitive organisms to several classes of contaminants that commonly occur in waterways and which span a range of toxic modes of action (Wang et al. 2017), including terrestrial herbicides and other pesticides (Connors and Black 2004; Bringolf et al. 2007); aquatic herbicides (Archambault et al. 2015); copper and ammonia (Newton & Bartsch 2007; Wang et al. 2007); and major ions (Zipper et al. 2016; Ciparis et al. 2019). The bulk of toxicological research has necessarily focused on an effects-level perspective (i.e., identifying concentrations that elicit a harmful effect). The benefits of this relatively new focus on mussel toxicology has begun to pay dividends; since methodological toxicity testing guidelines were established for the fauna (ASTM 2006), the US EPA began including mussel studies in reviews of water quality criteria for aquatic life protection, and inclusion of mussels has prompted revisions to improve protection of the fauna (e.g., updated ammonia criteria; US EPA 2013).

One area of contaminants research that has not been studied and that is the focus of this research is the role of mussels in ecological partitioning of pollutants. The fate and transport of contaminants is often described and discussed in a passive manner, in which chemical fate is determined by physical-chemical properties (e.g., water solubility), environmental factors (e.g., temperature or pH), or environmental processes (e.g., photolysis or hydrolysis), and some potential for biotransformation (e.g., microbial activity; Shea 2010). Mussels and other organisms are often discussed as passive participants subjected to contaminants through aqueous or dietary exposure, and studies have focused on elucidating toxicokinetic endpoints, such as uptake and elimination rates, bioaccumulation, and metabolic processes to understand contaminant effects on organismal health (e.g., Thorsen et al. 2007). Mussels are intimately connected with their aquatic and benthic habitats, and we know they actively influence the distribution of nutrients in rivers as discussed above, even to the extent of affecting aquatic-terrestrial linkages (e.g., Allen et al. 2012; Atkinson et al. 2014). This leaves open the possibility that mussels play an active role in the fate and transport of waterborne pollutants through the same functional mechanisms of filtration, retention, and biodeposition. Keeler et al. (2012) highlighted the need for more research on quantifying and valuing ecosystem services related to water quality.

In considering the best approach for investigating the role of mussels in ecological partitioning of contaminants, we looked to the bivalve ecotoxicological literature as a means for selecting an experimental design (Farris & Van Hassel 2007). Bivalves have little ability to metabolize organic contaminants, as evidenced by elimination rates that are orders of magnitude lower than uptake rates (Thorsen et al. 2007). Thus, mussels may truncate the transport of organic contaminants and serve as a long-term storage compartment, but they may not contribute appreciably to active fate and transport of organic contaminants on informative temporal scales, except potentially in cases of mass mortality events. However, bivalves have some ability to metabolize metals. They can regulate some essential metals (e.g., Ca and Zn) and they are able to metabolize and eliminate some non-essential metals (e.g., Ag, Cd, and Pb) via induction of metallothioneins (a family of metal-binding proteins; Newton & Cope 2007). In a recent field study, Cope & Jones (2016) reported that mussels collected from a river harbored lower tissue metal concentrations in a year with less waterborne metal pollution than those collected the previous year. These findings coupled with the current state of knowledge on bivalve toxicokinetics suggest that mussels may exert appreciable influences on the environmental fate and transport of metals over meaningful temporal scales. Therefore, we investigated the influence of mussel functional ecology on the environmental fate and transport of contaminants through the lens of metals. Specifically, we selected Ni and Cd because they are toxic heavy metals of both environmental and human health concern (i.e., relevant to ecosystem service benefits for humans) and they are on the US EPA Priority Pollutants List (40 CFR § 423, Appendix A).

The purpose of this research was to examine the role of unionid mussels in the environmental fate and transport of contaminants through their daily ecological functions. This research addresses three of the 10 priority areas of *A National Strategy for the Conservation of Native Freshwater Mollusks*, including understanding mollusk ecology – specifically in describing ecological functions, understanding the impacts of stressors, and identifying their ecosystem services (FMCS 2016). The aims of this research were to measure physiological partitioning of metals by mussels (e.g., sequestration and biodeposition) and examine variation in such processes as a function of environmental exposure concentration and duration. Investigating their functional role in the ecological processing of contaminants will help us understand how mussels contribute to the environmental distribution of contaminants, elucidate the ecological cascade of consequences from pollution and benefits of water quality maintenance and improvement, and may help us understand how estimates of pollutant sequestration by mussels as a potential ecosystem service (as explored in Chapter 1) may fluctuate with under different environmental scenarios. This research further should help to demonstrate how mussel ecosystem functions translate to social values related to water quality (e.g., Castro et al. 2016) and improve our ability to emphasize their relevance in conservation policy and decision-making (Olander et al. 2017).

METHODS

We conducted laboratory experiments exposing mussels to environmentally relevant concentrations of Ni and Cd to answer the following questions: what percentage of metals do mussels remove from water?; what percentage of metals is sequestered in soft tissue?; how much is egested in biodeposits?; how are filtration rates affected by metal exposure; and finally, how are these estimates affected by metal concentration or exposure duration?

Test Organisms

We selected the Eastern Elliptio (*Elliptio complanata*) mussel for use in experiments because they are abundant in local streams (i.e., ease of collection and transport to the laboratory at NC State University) and are one of the most common species that occurs in Atlantic Slope drainages of the eastern United States. This latter quality renders them a suitable representative of unionids for studying ecosystem processing because they often comprise a substantial proportion of mussel assemblages in this region. Moreover, because Eastern Elliptio is a common and abundant species, they have been studied in greater detail than some of their less common counterparts. Thus generating data on their contribution to ecosystem processing of metals may be paired with other relevant knowledge (e.g., known health metrics or behaviors) and prove more useful because there is a body of knowledge for context and synthesis.

We collected 50 Eastern Elliptio per experiment for each of three experiments in September and October 2018, from relatively uncontaminated streams in the Cape Fear River Basin in the central Piedmont physiographic region of North Carolina. For the first experiment with Ni, we collected mussels from the Rocky River, downstream of Reeves Lake and Hoosier Dam (Chatham County, NC; 35.63357° N, 79.20901° W) on 4 September 2018. Because mussels were less plentiful than anticipated at the Rocky River site, we collected 100 Eastern Elliptio for the Cd and NiCd experiments (conducted simultaneously) on 16 October 2018 from Mill Creek near Mebane, Alamance County, NC (36.11812°N, 79.27192° W), in the Upper Cape Fear drainage.

During each collection trip, we targeted mussels that were approximately 75 cm in length to ensure they were large enough for tissue analysis after the experiments. At the time of the experiments, mussels (n = 150) had an average shell length of 77.34 mm (± 7.83 mm, SD; range 61.12 – 102.43 mm) and a mean whole body (live) wet weight of 63.95 g (± 22.56 g; range 30.11 – 157.17 g). After collection from each site, mussels were immediately transported to the laboratory at North Carolina State University (Raleigh, NC) inside coolers and wrapped in damp dive bags and towels with cool-packs, in accordance with recommended procedures for safely translocating mussels (Cope et al. 2003). On arrival to the laboratory, mussel shells were scrubbed with a soft-bristled brush to remove sediment or organic matter that could bind metals, and placed into a cooler with aerated river water. Mussels were then slowly acclimated from river water to laboratory reconstituted moderately hard water (US EPA 2002) over 24 h by placing them in a 50:50 solution of river/laboratory water for ~5 hours, then further diluting the river water to a 25:75 ratio with laboratory water, and held for another ~5 hours, before being placed in 100% laboratory water. Moderately hard water was selected for its similarity to the hardness benchmark referenced in the US EPA Ambient Water Quality Criteria for Ni and Cd, which are hardness dependent and expressed in values normalized to 100 mg $CaCO_3$/L (US EPA 1995, 2016).

Experimental Design and Conditions

We conducted a Ni test, a Cd test, and a two-metal test containing both Ni and Cd. Each experiment was a 28-d aerated static-renewal exposure consisting of five metal treatment concentrations, with nine replicate aquaria per treatment (Figure 1, Table 1). Each aquarium contained one mussel and 3 L of test solution, comprised of reconstituted moderately hard water (US EPA 2002) and an appropriate spike of metal stock solution to achieve the target treatment concentrations (Figure 1, Table 1). A 100% solution renewal was conducted daily in an effort to keep metal concentrations near target treatment levels as mussels filtered the water; this 3-L daily renewal design was informed by the procedures of Mosher et al. (2012), who detected a >95% reduction in metals after mussels filtered 2-L metal solutions for 48 – 72 hours in their test with lead. Aquaria were wiped clean of accumulated biofilm at the end of each week to reduce the possibility of metals adsorbing to organic matter. Prior to starting each test, mussels were measured (length, height, width) to the nearest 0.01 mm, patted dry, and weighed to the nearest 0.1 g, and distributed into aerated aquaria containing 3 L of fresh laboratory water for acclimation to the test holding conditions at least 12 h prior to metal exposures. During the test, mussels were fed daily, immediately following renewal of the test solutions. Mussels were fed a mixture of 2 mL Instant Algae® Shellfish Diet and 1 mL *Nannochloropsis* (Nanno 3600) concentrate diluted in 1 L of reconstituted water (Reed Mariculture, Campbell, California, USA). Approximately 44 mL of food mixture was added to each replicate, yielding over 1 million cells/mL of test solution (administered concentrations of 58,667 cells/mL Shellfish Diet and 997,333 cells/mL Nanno 3600). Assuming constant filtration by the mussels (which is unlikely), this feeding rate provided 44,000 cells/mL/h over 24 h, exceeding the minimum constant feeding rate of 30,000 cells/mL recommended by freshwater mussel propagation and culture specialists (Rachael Hoch, personal communication, NC Wildlife Resources Commission), and protecting against stress from underfeeding during the tests. Water quality conditions (i.e., alkalinity, hardness, conductivity, pH, and dissolved oxygen) were monitored twice weekly and all metrics were within acceptable ranges throughout the experiments.

In total, 45 of the 50 mussels collected for each test were used in the exposures; the remaining five mussels from each test batch were frozen at -20°C on test day 0 for soft tissue analysis to establish baseline contaminant concentrations. Tests were conducted bench-top in ambient indoor light- and room-temperature conditions to accommodate the large size and number of aquaria (daylight ranged from 12.75 h to 10.25 h between 5 September – 15 November 2018; ambient room temperature was 20 – 22°C) .

Nickel stock solutions (10 mg Ni/L) were prepared with reconstituted moderately hard water and nickel chloride hexahydrate ($NiCl_2 \cdot 6H_2O$). We selected concentrations that were environmentally relevant and well below median lethal concentrations that have been reported in experiments testing nickel toxicity to juvenile freshwater mussels (173 µg/L to >1.5 mg/L; Wang et al. 2017; Popp et al. 2018) to ensure mussels would survive the metal challenge. Ni test concentrations were 0, 5, 25, 50, and 100 µg Ni/L. These concentrations are relevant to environmental and human health guidelines for waterborne Ni. The low concentration of 5 µg/L is in the range of measured concentrations in pore water and surface water in a recent study from a southeastern US stream (Cope & Jones 2016); 50 µg/L is near the 30-day Criterion Continuous Concentration of 52 µg/L in the ambient water quality criteria set for aquatic life by the US EPA (1995). A Criterion Continuous Concentration is defined as "an estimate of the highest concentration of a material in the water column to which an aquatic community can be exposed indefinitely without resulting in an unacceptable effect" (40 CFR § 132.2). Our highest selected concentration of 100 µg/L is the EPA lifetime health advisory for drinking water (US EPA 2018).

Cadmium stock solutions (1 mg/L) were prepared with reconstituted moderately hard water and cadmium chloride ($CdCl_2$, anhydrous). Similar to our Ni concentration selections, we selected concentrations that were environmentally relevant and well below median lethal concentrations that have been reported in experiments testing cadmium toxicity to juvenile freshwater mussels (Wang et al. 2010). Cd test concentrations were 0, 0.25, 0.5, 1, 2 µg Cd/L. Again, we selected concentrations that were relevant to environmental and human health metrics. The lower two Cd concentrations were below the US EPA 4-day 'chronic' Water Quality Criterion of 0.72 µg/L for aquatic life (there is no 30-day chronic Criterion Continuous Concentration for Cd), and the higher two concentrations were above the 4-day metric. The highest concentration of 2 µg Cd/L is equivalent to the established acute Water Quality Criterion set in 2001 (US EPA 2001), and near the recently adjusted acute criterion of 1.8 µg/L (US EPA 2016). Cope et al. (2008) considered this range of Cd concentrations relevant to those measured in surface waters, and recent research reported in a southeastern watershed, where Cd was regularly found in very low concentrations (e.g., < 0.1 µg/L), further confirms that assessment (Cope & Jones 2016). In relation to human health metrics, our test concentrations were below the US EPA's Maximum Contaminant Level and Maximum Contaminant Level Goal of 5 µg/L for Cd in drinking water (EPA 2018).

In the Ni + Cd test, we combined the concentrations of the individual tests in ascending order, such that the lowest treatment was the NiCd control (i.e., 0 µg/L for both metals) and the highest treatment contained 100 µg Ni/L and 2 µg Cd/L.

Data Collection and Processing

<u>*Treatment Concentration Verification and Metal Removal*</u>

Test solutions were sampled weekly at the beginning of each week (Days 1, 8, 15, and 22) to verify treatment concentrations and monitor removal of waterborne metals by mussels. Water samples collected for treatment verification were taken shortly after test solutions were renewed (i.e., within 30 minutes of renewal) and before mussels were fed. This allowed the metal stock spike to time to mix via aeration, yet little time for mussels to filter the test water, so that samples would be representative of the prepared concentration. Water samples for monitoring removal of metals by mussels were taken just before solutions were renewed (i.e., ~24 h after the previous removal) to measure the amount of metal that mussels removed over a 24-h period each week. For both sample types, we collected 20 mL of water from each of three randomly selected replicates per treatment into a large syringe for one composited 60 mL sample per treatment. Half of the composited sample was dispensed through a syringe filter (0.45 µm pore size, PP Membrane EZFlow® HP Syringe Filter, Foxx Life Sciences, Salem, NH) to remove algae and other organic material, and the other half was dispensed unfiltered into a sample collection tube. We performed the filtration to account for any metal binding by organic material in the water. This yielded one filtered and unfiltered treatment verification sample (just after renewal), and one filtered and unfiltered metal removal sample (24 h after a renewal) per week. Water samples were preserved by pipetting 200 µL of nitric acid into each sample tube to ensure a pH of < 2.0 and refrigerated at 4°C until metal analysis. This sampling scheme yielded 20 total water samples per week per test ($n = 80$ samples/experiment; 240 samples overall) to account for all metrics of interest, but no replication for a given time/treatment combination.

Metal Deposition

Biodeposits were sampled daily from aquaria before they were moved for solution renewal to measure the amount of metals that mussels processed and egested. Biodeposits consisted of feces, pseudofeces (undigested material), mucus, and any organic material egested by the mussels; they were typically comingled and settled at the bottom of the aquaria. Biodeposits were collected separately for each replicate aquarium, and daily deposits were composited into one weekly sample per replicate as a measure of the total metals removed per week by a given mussel. We used disposable, long transfer pipettes to remove biodeposits from the bottom of aquaria, taking care not to disturb and distribute the deposits into the water column. Collecting some test water along with the biodeposits was unavoidable. Excess water was decanted from collection tubes as needed during each sampling week, typically before a collection so that the previous day's samples were settled to the bottom of the tube. At the end of each week (i.e., Day 7, 14, 21, and 28) biodeposit samples and any overlying water were preserved with nitric acid and refrigerated, as previously described for water samples. Due to the mixed nature of the biodeposit samples, they were analyzed for metals in the same way as water samples, and the metal contribution from biodeposits was calculated by subtracting metal concentrations from the unfiltered water samples taken before solution renewal to match the condition of overlying water in the biodeposit samples (i.e., biodeposited metal = concentration from biodeposit sample – concentration from unfiltered water sample). This sampling scheme yielded nine replicate samples per treatment in Week 1, six per treatment in Week 2, and three per treatment in each of Weeks 3 and 4, with replicate samples repeated weekly for all mussels that were not removed in a prior week (n = 105 samples per experiment and 315 total biodeposit samples).

Metal Accumulation

Mussels in three replicates per treatment were removed at the end of experimental Weeks 1, 2, and 4 (i.e., Day 7, 14, and 28) to evaluate metal accumulation in mussel tissue over time (n = 15 mussels/time point). Each mussel was measured and weighed in the same manner as before the test, and then placed in a food-grade zip-sealable plastic freezer bag, labeled with the metal test, date, test day, treatment and replicate number. Mussels were then immediately placed into a freezer at -20°C and stored for processing after the experiments. Soft tissue processing consisted of thawing mussels, shucking the soft tissue from the shells, weighing shell and soft tissue separately, homogenizing all tissues in a blender, and re-freezing for metal analysis. This yielded three replicate samples per treatment for each week mussels were collected, and five representative baseline samples (n = 50 tissue samples/experiment; 150 tissue samples overall).

Filtration Rates

Measurement of mussel filtration rates was performed to confirm periodically that animals were adequately fed throughout the experiment, but also provided a mechanism to understand sublethal mussel responses to environmentally relevant metal exposure. Test solution sampling for filtration analysis was conducted approximately weekly, and samples were analyzed for the algal concentration using an automated particle counter (Multisizer 4e Coulter Counter, Beckman Coulter, Brea, CA). We collected 1 mL of test solution from each of three replicates in each treatment and combined them for one composite sample per treatment. Samples were taken immediately after mussels were fed (for a baseline algal concentration) and then at 2 h and 24 h after feeding. A 1-mL aliquot from each sample was placed in the particle counter, which scanned the sample and produced an average cell count (cells/mL) from three reads of the sample. We were most interested in particles ranging 1.79 – 6.09 µm in size, appropriately representing the size for algal foods that we administered.

Filtration data were limited in the Ni test (the first test conducted) due to processing and other constraints. However, after the first filtration samples were analyzed in week 2 of the Ni test, we determined that 24-h samples were necessary to detect a substantial reduction in the volume of algal food particles administered, and to confirm adequate feeding over the entire day between feedings. From the cell count reading given by the particle counter (cells/mL), we derived the particle filtration rate (cells/mL/min) based on the sample time point. Then, using the known volume of water in each test chamber, we calculated the volume of solution cleared (i.e., the filtration rate, expressed as a volume in mL/h). This volume is the most relevant to ecological measures. We evaluated the effect of metal concentration and exposure duration on short- and longer-term trends in filtration, using the 2-h and 24-h filtration rates.

Metal Analysis and Quality Control Measures

Analyses of metal concentrations were performed using standard methods and approved protocols, including EPA Methods 3050B and 7473 (Thermo X-Series II ICP-MS, Thermo iCAP6500 ICP-OES; RTI International, Research Triangle Park, North Carolina, USA). The rigorous quality assurance protocol followed for metals analyses included reagent blanks, reagent blank spikes, duplicates, matrix spikes, and surrogate internal standards. Average recovery in surrogate standards was 100% (range 96 – 102%), relative percent difference (RPD) of duplicates averaged 3% (range 0 – 12%) for water and biodeposit samples (processed like water samples) and 9% (range 0 – 35%) for tissue samples, recovery of matrix spikes averaged 96% (range 48 – 122%) for water and biodeposit samples and 84% (range 37 – 124%) for tissue samples, reagent blanks were uncontaminated, and average recovery from reagent blank spikes was 90% (range 83 – 94%).

Statistical Analyses

For metals removed from water, metals accumulated in tissue, and mussel filtration rates, we used analysis of variance (ANOVA) to test for differences among metal concentrations or over time, along with a Tukey's post-hoc test in cases where significant differences among treatments were detected (PROC GLM, SAS v.9.4; SAS Institute, Cary, NC, USA). With the mussel tissue bioaccumulation data, we further compared results to baseline mussels using a Dunnett's test to detect any differences from mussels that were not experimentally exposed. For biodeposit samples, we used a repeated-measures ANOVA (PROC MIXED, SAS v.9.4), again using a Tukey's post-hoc test to investigate the effects in cases of significance. Because the water data and filtration data each stemmed from composite sampling, and thus had no replication for a given treatment/time point combination, we examined main effects only for those two endpoints. Finally, we investigated whether mussel filtration rates were related to metal accumulation and biodeposition using correlation analyses (PROC CORR, SAS v.9.4).

RESULTS

Exposure Accuracy

Mean exposure accuracy in the Ni test was 104% (range 97 – 118%) whereas mean exposure accuracy in the Cd test was 21.4% (range 9 – 51%) of target Cd concentrations. In the Ni + Cd test, the mean exposure accuracy of Ni was 76.9% (range 67 – 88%) and for Cd, the mean accuracy was 20.1% (range 9 – 54%). The Ni results are reported in terms of target concentrations. We are uncertain as to the cause of the under-recovery of Cd in our experiments, but the most likely explanation for the deviance from target concentrations is loss in the stock solution and in test chambers via adsorption to glassware and biofilms. Despite using pre-treated glassware throughout the experiments, mixing the Cd stock solution on a stir plate, and using fresh Cd stock solution testing higher than target (14.5 mg/L for a target stock concentration of 10 mg/L), the stock solution was verified to 3.1 mg/L after one week. Another possible explanation may be rapid uptake by mussels; mussels were regularly observed with apertures open within a few minutes after solution renewal, so they quickly may have assimilated Cd from the 3 L of solution in aquaria before water samples for verification were collected (within ~30 minutes of renewal). The lower Cd concentrations are still comparable to environmental concentrations in surface waters, and we still achieved observable results in our test endpoints. Because Cd deviated so far below target concentrations, we report the results based on the average measured concentrations (0, 0.05, 0.1, 0.2, and 0.4 µg Cd/L).

Nickel Removal, Accumulation, and Deposition

<u>Reduction of Waterborne Nickel by Unionid Mussels</u>

Among the Ni exposures of 5 to 100 µg Ni/L, mussels removed 21 to 36% of Ni in solution over a 24-h period after the first day of exposure. Efficiency of Ni removal from solution generally trended downward over time, with mussels removing 0 to 17% of Ni by the beginning of Week 4 (Day 22), depending on exposure concentration. The reduction in efficiency appeared negatively associated with increasing Ni concentration. Whereas mussels exposed to 5 µg Ni/L went from removing 20.7 to 17.4% of Ni over time (a decrease of about 3%), mussels exposed 25, 50, and 100 µg Ni/L respectively decreased in effectiveness of Ni removal from solution by 17%, 25%, and 30% over time (Figure 2A). Results in the NiCd test were similar to those in the Ni-only test, where mussels removed 16 to 36% of Ni in the first day of exposure, and removal efficiency trended downward over time, except in the lowest concentration. Mussels exposed to the NiCd mixture containing 5 µg Ni/L maintained the same Ni removal efficiency by Week 4 (35% of Ni removed compared to 36% at the beginning of Week 1), whereas mussels exposed to 25, 50, and 100 µg Ni/L respectively decreased in effectiveness of Ni removal from solution by about 16%, 24%, and 30% over time (Figure 2B).

Week was the only significant factor in an ANOVA of Ni removal by mussels in the Ni-only test, and exposure duration explained 47% of the variation in percent of Ni removed ($F_{3,12}$ = 3.49, $p = 0.0502$). As mentioned in the summary above, we observed a decrease in the efficiency of Ni removal over time. In Tukey's post-hoc analysis of treatment means, we found that the percent of Ni removed at the beginning of Week 1 was significantly higher than at the Week 4 time point ($p < 0.05$). The high variance among treatments at Week 2 may have precluded any detection of a significant difference between that and other weeks. We were unable to detect an effect of Ni concentration on removal in the Ni test (Figure 2A). However, Ni data from the combined metal test were opposite, wherein we detected an influence of Ni concentration on the percent of Ni removed, but did not detect an effect of exposure time ($F_{3,12} = 7.61$, $p = 0.0041$, $r^2 = .66$; Figure 2B). Tukey's post hoc analysis of differences among the metal treatment means in the NiCd test revealed that mussels exposed to the lowest Ni treatment were significantly more efficient at removing Ni from solution than those in all other metal treatments ($p < 0.05$). We found no other differences among treatments. The results of both tests taken together suggest that we might expect Ni sequestration to be influenced by concentration and time in the environment.

Accumulation of Waterborne Nickel in Mussel Soft Tissue

Mussels generally accumulated more Ni in their tissue over time and with higher waterborne Ni concentrations in both the Ni and NiCd tests (Figure 3). Baseline mussel tissue concentrations of Ni were 417 ng/g (± 110 ng/g, SE) for the Ni test and 364 ng/g (± 182 ng/g, SE) for the NiCd test. Tissue concentrations of Ni ranged from 205 ng/g to 2,755 ng/g in mussels from the Ni test and from 156 ng/g to 1,988 ng/g in mussels from the NiCd test. We found no difference between concentrations of Ni in the tissue from baseline mussels compared to those exposed to the control treatments (0 µg Ni/L) in either test.

In the Ni test, we detected significant effect of both waterborne Ni concentration ($p < 0.0001$) and time (i.e., week; $p = 0.0052$) on the accumulation of Ni in mussel soft tissue ($F_{6,37} = 9.49$, $p < 0.0001$, $r^2 = 0.61$); there was no interaction between these factors (Figure 3A). In examining for differences over time, we found that mussels accumulated significantly more Ni in tissue by the end of Week 4 compared to Week 1 ($p = 0.0036$). We did not detect differences in Ni accumulation between Weeks 1 and 2 or Weeks 2 and 4. For a given time point, mussels exposed to 100 µg Ni/L accumulated significantly more Ni than those exposed to 0, 5, and 25 µg

Ni/L ($p < 0.0001$, $p = 0.0005$, and $p = 0.0290$, respectively). Similar to the highest treatment, mussels exposed to 50 µg Ni/L accumulated significantly more Ni than those in the lowest two treatments (i.e., control (0 µg Ni/L; $p = 0.0001$) and 5 µg Ni/L ($p = 0.0026$)). Mussels exposed to 50 µg Ni/L also tended to accumulate more Ni than those to 25 µg Ni/L, but there was no significant difference in Ni accumulation between mussels in those treatments ($p = 0.0978$; Figure 3A).

In the NiCd test, we found trends in accumulation of Ni by mussels similar to those in the Ni only test. Again, both waterborne Ni concentration ($p< 0.0001$) and exposure duration ($p=0.0095$) were significant predictors of Ni accumulation in tissue (model $F_{6,38} = 15.35$, $p < 0.0001$), explaining 71% of the variation in data ($r^2 = 0.708$; Figure 3B). In this case, data required ln transformation to satisfy the model assumption of independent and identically distributed variance. Mussels again accumulated significantly more Ni by Week 4 compared to Week 1 ($p = 0.0081$), and there was a marginal increase in Ni accumulation after two weeks ($p = 0.0880$). For a given time point, mussels exposed to 100 µg Ni/L accumulated significantly more Ni than those exposed to 0 ($p < 0.0001$) and 5 µg Ni/L ($p = 0.0034$). Moreover, mussels had significantly higher tissue concentrations in all Ni treatments, compared to controls ($p < 0.0001$ for all, except for 0 vs. 5 µg Ni/L, where $p = 0.0009$; Figure 3B).We detected no other differences in accumulation of Ni in tissue among waterborne Ni concentrations for a given time point; high variation in the amount of Ni accumulated by mussels exposed in the 100 µg Ni/L treatment may have masked any other effects of Ni concentration (Figure 3B).

Biodeposition of Nickel by Unionid Mussels

One of the most salient outcomes we observed from collecting biodeposits was that mussels substantially concentrated Ni in the materials they egested (Figure 4). We observed the most pronounced bioconcentration of Ni in the lowest exposure concentration and least pronounced bioconcentration in the highest exposure concentration. Mussels exposed to 100 µg Ni/L exhibited little to no concentration of Ni in their biodeposits in the Ni test (average = 1.0X, range 0.7 – 1.4X more Ni in biodeposits than test water; Figure 4A), and those exposed to the same concentration in the NiCd test bioconcentrated Ni, but to a lesser degree than in other treatments (average = 2.1X, range 1.8 – 2.3X more Ni in biodeposits than test water; Figure 4B). In contrast, mussels exposed to the lowest concentration of 5 µg Ni/L averaged 4.4X more Ni in their biodeposits than in test water (range 3.8 – 5.0X; Figure 4A), and those in the NiCd test averaged 6.3X more Ni in their biodeposits than in test water (range 5.5 – 6.8X; Figure 4B).

Though bioconcentration was inversely related to exposure, mussels biodeposited more Ni overall as exposure concentration increased in both the Ni and NiCd tests (Figure 5). Exposure duration was also an important predictor; however, we did not observe monotonic trends over time in either test (Figure 5). Ni concentrations in mussel biodeposits ranged from 1.25 µg/L to 136 µg/L in the Ni test and from 1.17 µg/L to 190 µg/L in the NiCd test. (Recall that biodeposit samples were processed as water samples; hence, we report biodeposited metal concentrations in units of µg/L). Transformation (ln) was required for statistical analysis of the Ni biodeposition data in both the Ni and NiCd tests to satisfy model assumptions of variance normality.

In the Ni test, a repeated measured analysis revealed that both Ni exposure concentration and exposure time were significant predictors of Ni detected in mussel biodeposits ($p < 0.0001$ for both; Figure 5A). For a given week, mussels biodeposited significantly more Ni in the higher treatments of 25, 50, and 100 µg Ni/L ($p \leq 0.006$) than in the lowest treatment of 5 µg Ni/L, and mussels in all treatments biodeposited significantly more Ni than those in the controls (p <

0.0001 for all). Biodeposition of Ni trended upward with increasing waterborne Ni concentration, no statistical differences were detected among the biodeposits from mussels exposed to the three higher treatments (25, 50, 100 µg Ni/L). Comparisons among weeks revealed that mussels biodeposited more Ni in Week 2 than in other weeks (p range < 0.0001 – 0.02). There were no other differences detected among treatment weeks ($p = 0.18$ – 0.90; Figure 5A). Lastly, we found a significant first-order autoregressive (estimate 0.63, $p = 0.04$). Thus, residuals were predictable from their predecessors by an addition of 63% to the estimate. We note this finding here for completeness; though it is not interpretable, it emphasizes the importance of accounting for the repeated measures in the test.

In the NiCd test, autoregression was not detected, therefore, repeated measures were not necessary, and we proceeded with a mixed model analysis. The mixed model analysis indicated that both exposure concentration ($p < 0.0001$) and duration ($p=0.0005$) were important predictors of Ni in mussel biodeposits. Except for one pairwise comparison of Ni concentration at 25 vs. 50 µg Ni/L ($p = 0.5462$), all others were significantly different for a given time point (all $p < 0.0001$, except for 50 vs. 100 µg Ni/L, where $p = 0.0011$). In pairwise comparisons over time for a given concentration, Week 1 differed from Weeks 2 ($p = 0.0025$) and 3 ($p = 0.0098$), though the magnitude of differences appear inconsequential (Figure 5B). There were no other differences among time points.

Cadmium Removal, Accumulation, and Deposition

<u>Reduction of Waterborne Cadmium by Unionid Mussels</u>

Trends in the percent of Cd removal over a 24-h period were strikingly similar among exposure concentrations and varied widely, but similarly, over time in both the Cd and NiCd tests. Reduction of waterborne Cd took a non-monotonic U-shaped formation that trended lower in Weeks 2 and 3 in both experiments, with mussels exhibiting the greatest efficiency in Cd removal at the beginning and end of the tests, regardless of the Cd exposure concentration (Figure 6). In the Cd test, mussels removed 19 to 43% of Cd from overlying water after the first day of exposure, followed by a low of 0 to 13% at the beginning of Week 2, and rebounded to removing 67 to 77% of Cd from water by Week 4 (Figure 6A). In the NiCd test, mussels similarly removed 25 to 41% of Cd in water after the first day in the NiCd test, followed by a low of 0 to 12% in Week 2, and then rebounded to removing 40 to 53% of Cd by Week 4 (Figure 6B).

Exposure duration explained 93% of variation in the amount of waterborne Cd mussels removed in the Cd test ($F_{3,12} = 49.54$, $p < 0.0001$; Figure 6A). Mussels removed more Cd in a 24-h period the beginning of Week 1, then removed very little in Week 2, after which their removal of waterborne Cd increased steadily in all concentrations. Tukey's post-hoc analysis revealed that mussels removed significantly less Cd in Week 2 than any other time point, and they removed significantly more Cd over a 24-hr period in Week 4 than at any other time point ($p < 0.05$). Removal of waterborne Cd by mussels was similar at the Week 1 and 3 time points.

We observed similar trends in the percent of Cd mussels removed in the NiCd test, where exposure duration was the only significant predictor of waterborne Cd removal ($F_{3,12} = 68.08$, $p < 0.0001$)), and it explained 94% of variation in the data. The proportion of Cd removed was again suppressed in the middle of the test. Again, Tukey's post-hoc analysis revealed that mussels removed a significantly greater proportion of Cd over a 24-hour period in Week 4 than at all other time points ($p < 0.05$). Cd removal also was significantly higher at the Week 1 time point than in Weeks 2 and 3 ($p < 0.05$). Mussels removed the least amount of waterborne Cd in Weeks 2 and 3 in the NiCd test, and data from these time points were not statistically different.

Accumulation of Waterborne Cadmium in Mussel Soft Tissue

Mussels generally accumulated more Cd in their tissue with the highest waterborne Cd concentration in both the Cd and NiCd tests (Figure 7). Baseline tissue concentrations of Cd were 142 ng/g (± 22 ng/g, SE) for the Cd test and 170 ng/g (± 21 ng/g, SE) for the NiCd test. Tissue concentrations of Cd ranged from 111 ng/g to 342 ng/g in mussels from the Cd test and from 59 ng/g to 237 ng/g in mussels from the NiCd test. We found no significant difference ($p \leq 0.05$) between concentrations of Cd in the tissue from baseline mussels compared to those exposed to the control treatments (0 µg Cd/L) in either test.

In the Cd test, tissue accumulation of Cd was influenced by the exposure concentration ($p = 0.0003$) and duration ($p = 0.0002$); further, there was an interactive effect of Cd exposure and week ($p = 0.0332$; overall model $F_{14,30} = 5.16$, $p < 0.0001$, $r^2 = 0.71$; Figure 7A). On inspecting the Tukey post-hoc comparisons of each time by treatment pair, the 28-day tissue Cd concentration in the highest treatment was the driving data point in the model, and may have contributed substantially to the outcome. Within a given week, we detected significant differences among Cd treatments in Week 2 ($p = 0.0348$) and Week 4 ($p < 0.0001$); there were no differences among Cd treatments at the Week 1 time point (Figure 7A). Mussels exposed to the highest Cd concentration further accumulated significantly more Cd over time ($p < 0.0001$). We also detected a significant difference in tissue Cd among mussels in the control treatment over time ($p = 0.02$). In comparison to baseline tissue samples, tissue concentrations of Cd were significantly higher in the highest treatment in Week 2 ($p = 0.02$) and Week 4 ($p < 0.0001$; Figure 7A). No other treatment differed significantly from baseline at any time, though the control mussels that were sampled in Week 4 had marginally higher Cd in their tissue ($p = 0.08$). Control solution concentrations of Cd were essentially zero and consistent throughout the exposure (0.0002 – 0.0003 µg Cd/L), so the most plausible explanation for a higher tissue concentration in control mussels is contamination from their river habitat prior to collection.

In the NiCd test, the only factor explaining accumulation of Cd in soft tissue was the Cd exposure concentration ($F_{4,40} = 3.87$, $p = 0.0095$; Figure 7B). This model explains only 28% of variation in Cd accumulated in tissue. Mussels in the highest Cd concentration accumulated significantly more Cd than mussels in any other treatment ($p < 0.05$). However, none of the mussels in Cd treatments had significantly higher tissue Cd concentrations than those measured in baseline mussels. The low Cd exposure concentrations we achieved may be limiting our ability to detect more obvious trends. However, the results we were able to achieve suggest that as waterborne Cd increases, accumulation in tissue increases, and that there may be a time-dependent relationship.

Biodeposition of Cadmium by Unionid Mussels

Like the results we found for Ni biodeposition, one of the most salient outcomes we observed from collecting biodeposits was that mussels substantially concentrated Cd in the materials they egested (Figure 8). The Cd trends were even more pronounced than that of Ni, as mussels bioconcentrated Cd 6X to 42X higher than the concentrations to which they were exposed. Here, we observed that bioconcentration of Cd was similar among Cd concentrations, but varied in pattern over time (similar to Cd removal from water). Here, it oscillated higher and lower in a zigzag fashion over the test duration in both the Cd and NiCd tests, and its bioconcentration factors were lower in Weeks 1 and 3 relative to Weeks 2 and 4. In the Cd test, mussels averaged 23X more Cd in their biodeposits in Week 1 than test water (range 14 – 42X). In Week 2, they biodeposited an average of 34X more Cd than in their exposure water (bioconcentration range, 28 – 36X more). In Week 3, mussels biodeposited an average of 14X more Cd (range 8 – 22X), and in Week 4, bioconcentration increased to an average of 34X more Cd in biodeposits than test water (range 29 – 39X more Cd; Figure 8A). This trend of high, but oscillating bioconcentration of Cd in biodeposits compared to exposure water was also observed in the NiCd test, with averages as follows: 14X more Cd in Week 1 (range 13 – 16X); 30X more in Week 2 (range 19 – 42X); 7X more in Week 3 (range 6 – 9X); and 27X more in Week 4 (range 26 – 30X; Figure 8B).

The average concentrations of Cd in materials biodeposited by mussels mirrored the non-monotonic U-shaped trend over time that we observed in the proportion of Cd that mussels removed from water. However, for biodeposition, Cd concentration was also an important factor (Figure 9). Cd concentrations in mussel biodeposits ranged from 0.14 µg/L to 22.4 µg/L in the Cd test and from 0.14 µg/L to 17.3 µg/L in the NiCd test. These results exemplify the bioconcentration of Cd discussed above; recall that verified exposure concentrations ranged between 0 and 0.4 µg Cd/L. As with the Ni biodeposit data, natural log transformation was required for statistical analysis of the Cd biodeposition data in both the Cd and NiCd tests to satisfy model assumptions of variance normality.

A mixed model analysis of Cd in biodeposits egested by mussels in the Cd test exposure revealed significant effects of Cd concentration ($p < 0.0001$) and time ($p < 0.0001$), along with an interaction between the predictors ($p = 0.0035$; Figure 9A). Further examination of the simple effects revealed differences among concentrations within each weekly time point (all $p < 0.0001$) and differences over time within every given concentration ($p < 0.0001 – 0.0024$).

Mussels deposited more Cd in the first and last weeks than in the middle of the test, matching their pattern of waterborne Cd removal.

Results of the Cd deposited by mussels in the NiCd test were similar to those in the Cd test. Again, Cd concentration ($p < 0.0001$) and week ($p < 0.0001$) were important predictors of Cd in biodeposited materials, along with an interaction between the predictors ($p = 0.0005$; Figure 9B). Similarly, there were differences among concentrations within each weekly time point (all $p < 0.0001$) and differences over time within every given concentration ($p < 0.0001$ – 0.0040), wherein mussel deposition of Cd exhibited a same U-shaped pattern of concentrations. Mussels again deposited more Cd in the first and last weeks than in the middle of the test.

Influence of Metal Exposure on Mussel Filtration

Despite limited information on mussel filtration in the Ni test, we were able to detect statistical trends in filtration rates (Figure 10). Ni concentration was a significant predictor of filtration volume 2 h after renewal and feeding ($F_{4,10} = 7.08$, $p = 0.0057$, $r^2 = 0.74$). Despite the range of variation within the 0 and 5 µg Ni/L treatments, The Tukey's post-hoc analysis revealed that mussels exposed to 25, 50, and 100 µg Ni/L had significantly lower filtration rates than those in the 5 µg Ni/L treatment ($p < 0.05$). Trends in mussel filtration were similar between the controls and low Ni treatment of 5 µg/L. Limited data likely precluded detection of any significant deviation in filtration rates between the controls and higher Ni treatments (Figure 10A). Ni concentration also was a significant predictor of filtration volume 24 h after feeding ($F_{4,5} = 51.03$, $p = 0.0003$, $r^2=0.98$). Again, trends in mussel filtration were similar between the controls and lowest Ni treatment, and the Tukey's post-hoc analysis revealed that mussels exposed to 25, 50, and 100 µg Ni/L had significantly lower filtration volumes than those exposed to 0 or 5 µg Ni/L ($p < 0.05$). We did not detect an effect of exposure duration on 2-h or 24-h filtration rates in the Ni test; however, our ability to detect any such effects was probably limited in this truncated dataset.

Correlation analyses with other test responses showed that the 2-h filtration rates were positively correlated with Ni removed from water ($\rho_s = 0.65$, $p = 0.04$), negatively correlated with Ni biodeposition ($\rho_s = -0.59$, $p = 0.02$), and had a marginal negative correlation with tissue accumulation of Ni ($\rho_s = -.59$, $p = 0.07$). The 24-h filtration rates were also negatively correlated with Ni biodeposition $\rho_s = -.70$, $p = 0.03$.

In the Cd test, Cd exposure concentrations had no effect on either the 2-h ($F_{4,15} = 0.20$, $p = 0.93$) or 24-h ($F_{4,15} = 0.14$, $p = 0.97$) filtration rates. However, exposure duration was an important predictor of filtration both at the 2-h (Model $F_{3,16} = 13.48$, $p = 0.0001$, $r^2 = 0.72$) and 24-h ($F_{(3,16)} = 3.21$, $p = 0.0514$; $r^2 = 0.38$) time points (Figure 11). Other than relatively steady temporal filtration by mussels in the controls, temporal trends of filtration in Cd treatments superficially resembled the zigzag pattern observed in Cd bioconcentration. At both 2 h and 24 h after Cd renewal and feeding, the trends in filtration were similar, in which mussels filtered less in Weeks 1 and 2 compared to Weeks 3 and 4. Tukey comparisons revealed these trends were statistically significant at the 2-h time point (i.e., filtration in Week 1 and 2 lower than in Week 3 and 4; $p < 0.05$; Figure 11A).That trend was not statistically significant at the 24-h time point (Figure 11B). Within-week variation in filtration among Cd treatments was greater at the 24-h time point, and likely hampered our ability to detect a significant difference among time points.

Correlation analyses with other test responses showed that the 2-h and 24-h filtration rates had a positive linear relationship with Cd removed from water, though the correlation at 24 h was statistically marginal (ρ_p = 0.73, p < 0.01 at 2 h, and ρ_p = 0.50, p = 0.06 at 24 h). Filtration rates were not correlated with Cd biodeposition or accumulation in tissue.

In the two-metal test, both NiCd treatment (p = 0.01) and Week (p = 0.04) had a significant effect on the 2-h filtration rates ($F_{7,12}$ = 4.61, p = 0.0103; r^2 = 0.73; Figure 12A). Further examination of the main effects showed that mussel filtration volume decreased with increasing metal concentration, similar to the trends observed in the Ni test (Figure 10). Mussels exposed to the highest metal treatment (NiCd-E, with 100 µg Ni/L and 0.4 µg Cd/L) exhibited significantly lower 2-h filtration (least squares mean (LSM) filtration volume = 127.5 mL/h) than mussels exposed to the control (NiCd-A, 0 µg/L Ni and Cd; LSM filtration = 447.5 mL/h) and middle treatment (NiCd-C, with 25 µg Ni/L and 0.1 µg Cd/L; LSM filtration = 440.5 mL/h) (both p = 0.04). Those in NiCd-E also had marginally lower filtration than mussels in the lowest metal treatment (NiCd-B, with 5 µg Ni/L and 0.05 µg Cd/L; LSM filtration = 407.8 mL/h; p = 0.08; Figure 12A). Comparisons among weeks showed that mussels had significantly lower 2-h filtration volume in Week 2 (LSM filtration = 156.4 mL/h) compared to Week 4 (425.8 mL/h; p = 0.04); Week 2 filtration by mussels also trended lower than that in Week 3 (380.4 mL/h; p = 0.09; Figure 12A), similar to the exposure duration effects observed in the Cd test (Figure 11). Though we did not have sufficient degrees of freedom to test for an interaction between metal treatment and week, an interaction plot showed no crossed lines for week among metal treatments (PROC GLM, SAS v.9.4), indicating there might not have been an interaction and effects of Ni and Cd on filtration rates may be additive.

Like at the 2-h filtration time point, NiCd treatment concentration had a significant effect on the filtration rates of mussels 24 h after NiCd renewal and feeding ($F_{4,15}$ = 10.87, p = 0.0002, r^2 = 0.74); however, test exposure duration was not an important predictor of 24-h filtration volume ($p = 0.45$; Figure 12B). Mean filtration volume decreased with increasing metal concentrations, ranging from 144.5 mL/h in the controls to a low of 60.50 mL/h in the highest NiCd treatment. Tukey comparisons revealed that mussels in the two treatments with the highest metal concentrations (NiCd-D and NiCd-E) had significantly lower filtration rates than mussels in the control (NiCd-A) and lowest metal treatment (NiCd-B). The middle treatment (NiCd-C) was not significantly different from any other treatment (Figure 12B).

The 24-h filtration rates had stronger relationships with the metal removal, biodeposition, and tissue accumulation endpoints, especially for Ni. Correlation analyses with these test responses showed that the 24-h filtration rates had a positive linear relationship with Ni removed from water ($\rho_p = 0.61$, $p = 0.04$) and a negative linear response with Ni accumulated in mussel tissue ($\rho_p = -0.58$, $p = 0.02$). Filtration rates both the 2 h and 24 h after metal renewal and feeding were negatively correlated with Ni and Cd biodeposition, though the 24-h rates were more strongly correlated. The relationships between filtration and biodeposited Cd were more curvilinear ($\rho_s = -0.51$, $p = 0.02$ for 2-h filtration; $\rho_s = -0.77$; $p < 0.01$ for 24-h filtration) than the predominantly linear trends between filtration and biodeposited Ni ($\rho_p = -0.58$, $p = 0.01$ for 2-h filtration; $\rho_p = -0.80$, $p < 0.01$).

DISCUSSION

Mussels substantially reduced the concentration of metals in water by filtering, they sequestered metals in their soft tissue, and they concentrated metals through biodeposition of egested organic materials (e.g., feces, pseudofeces, and mucus products). In natural environments, these laboratory outcomes translate into mussels actively influencing the environmental fate and transport of metals – and altering their bioavailability – by reducing metal concentrations in surface waters, sequestering metals in aggregate mussel populations, and biodepositing concentrated metals into stream and lake bed sediments.

Our findings show that these pollutant-processing functions fluctuated significantly within environmentally relevant ranges of Ni and Cd concentrations over the course of 28-d exposures. The fluctuations in functional processing manifested differently for Ni and Cd. Mussels were more efficient at processing Ni at lower concentrations (i.e., when exposed to less pollution), while the duration of exposure was an important factor for Cd processing; these trends generally held for each metal even when mussels were exposed to both Ni and Cd.

Moreover, this ability of mussels to influence the environmental fate and transport of metals was in turn affected by the metal concentrations to which they were exposed. Metal exposure reduced their filtration capacity, and filtration rates were affected differently under the stress of both metals compared to just Ni or Cd. This work helps us begin to understand the active role of mussels in environmental fate and transport of toxic heavy metals in aquatic ecosystems and gives additional perspective on implications for water quality management for the conservation of freshwater mussels and the ecosystem services they provide.

Functional Roles of Mussels in Metal Processing

The most salient trends that we uncovered in mussels' processing capacity of Ni were that they most consistently and efficiently removed waterborne Ni in the lowest concentration of 5µg/L over the test duration. Further, their ability to reduce waterborne Ni became less efficient over time, especially at higher exposure concentrations. The accumulation of Ni in soft tissue increased with increasing exposure concentration and over time, and biodeposition similarly increased positively with exposure concentration. The fact that mussels were able to bioconcentrate Ni more strongly at lower exposure concentrations may have helped them maintain lower tissue concentrations at these lower exposures as well. Finally, mussel filtration rates were near control levels only at the lowest Ni concentration. Ni hampered filtration rates at concentrations of 25 to 100 µg/L, indicating a potential threshold of tolerance to Ni exposure even below the US EPA 30 day CCC of 52 µg/L (US EPA 1995). Little is known about the toxicokinetics of Ni in bivalves (Thorsen et al. 2007), and our findings may inform future research in that area of study. Taken together, the results of our Ni exposures suggest that mussels can remove waterborne Ni most efficiently in less polluted waterways, and are likely to assimilate and biodeposit more Ni with greater exposure.

The impact of Ni on filtration rates suggests that Ni pollution reduces the capacity of mussels to perform this essential function for nutritive intake, which could lead to lasting impacts on sublethal measures of stress, such as growth. A study with juvenile mussels that were caged and deployed in the Clinch River supports this notion; Cope and Jones (2016) found that higher concentrations of nine metals, including Ni, were strongly correlated with lower growth rates in mussels. It further suggests that such an impact would affect filtration-related ecosystem functions and services provided by mussel populations, such mediating water clarity (Chowdhury et al. 2016), denitrification (Hoellein et al. 2017), and removal of waterborne pathogens and other contaminants (e.g., *E. coli* and pharmaceuticals; Ismail et al. 2014, 2015, 2016).

Mussels responded to Cd exposure much differently than Ni. The duration of exposure to Cd was the most important factor affecting their efficiency in removal of waterborne Cd, in contrast to Ni's driving effect of concentration. We suspected that the lower percentages of waterborne Cd removal during the middle of the tests were indicative of an avoidance response by mussels, in which they “clammed up” and filtered less Cd out of the water. A short-term (4-d) study of Cd exposure to adult mussels at environmentally relevant concentrations (0 – 5 μg/L supports this interpretation. Cope et al. (2008) measured mussel respiration in response to Cd exposure and found that mussels exhibited avoidance to Cd by closing, and that the avoidance response was greatest (i.e., respiration was lowest) within the first day of the exposure (Cope et al. 2008). Mussels in their 4-d study accumulated less Cd in tissue at the highest concentration because of avoidance behavior. Our findings that mussels had depressed filtration rates after the first two weeks of exposure to Cd (i.e., from samples taken on Days 7 and 14) confirm that mussels were filtering less at the time points matching our findings of diminished performance in waterborne Cd removal (i.e., beginning of Weeks 2 and 3, with samples taken on Days 8 and 15). Moreover, the our findings of higher filtering rates at the ends of both Weeks 3 and 4 suggest that mussels exhausted their capacity for avoidance after two weeks of exposure and needed to return to higher filtration to satisfy their basic requirements. Mussels biodeposited more Cd as exposure concentrations increased, though the amount deposited was dependent on the duration of exposure, and Cd biodeposition was depressed in the middle of the test when mussels were likely avoiding exposure. The return of greater Cd biodeposition by the end of the month in every exposure concentration mirrored the pattern of waterborne Cd removal. The peculiar zigzag pattern of Cd bioconcentration above test concentrations may suggest a lag response in the ability of mussels to bind and egest Cd. For example, bioconcentration increased substantially after two weeks of exposure despite evidence from other endpoints suggesting that mussels were avoiding Cd exposure after 1 week. Though our findings on Cd accumulation in mussel tissue were less congruent between tests, possibly due to prior field contamination and lower than anticipated exposure concentrations, mussels exposed in the highest concentration of 0.4 µg/L accumulated the most Cd in both tests.

In summary, both Cd exposure concentration and duration of exposure affected the capacity of mussels to influence the fate and transport of Cd in the environment. Mussels appear to avoid Cd at the onset of exposure (e.g., a pulse or spill), but they must resume filtering for sustaining their basic life requirements. Importantly, these avoidance responses were detected at concentrations (0.05 – 0.4 µg Cd/L) far below the recently updated US EPA 4-day “chronic” water quality criterion of 0.72 µg Cd/L (US EPA 2016). At higher filtration rates, mussels removed nearly 80% of waterborne Cd, regardless of exposure concentration and their patterns of Cd biodeposition followed that of Cd removal. Taken together, our findings suggest that any level of environmental Cd pollution is potentially harmful to mussels and their ability to sustain their normal activity, and thus contribute to ecosystem functioning and ecosystem service delivery. They suggest that native mussels are at risk of accumulating substantial burdens of Cd from sustained exposure in waterways, and that their tendency to avoid exposure for relatively long durations (e.g., two weeks) may be accompanied by costly consequences for fitness and periods of impaired functionality and mobility, leading to the loss of other ecosystem services. Starvation prompted by prolonged closure responses could have cascading consequences, such as depletion of glycogen energy stores (Patterson et al. 1999; Huong et al. 2011), which could be especially detrimental if timed before a period of food scarcity (e.g., winter or drought) or when such resources are needed for reproduction. Furthermore, extended shell closure could disrupt mussel population dynamics if the avoidance responses were co-timed with phenologically sensitive activities, such as spawning periods, availability of host fishes, or increased predator activity.

Findings from the two-metal test indicated that this contaminant mixture affected mussel filtration differently than exposures to Ni or Cd individually. Whereas exposure concentration was the only factor explaining the mussel filtration response 2 h after feeding in the Ni test, and duration of exposure (test week) was the only factor explaining the response in the Cd test, both factors were important predictors in the NiCd test, suggesting an additive effect of the two metals on short-term mussel filtration. Though it is unclear why test week was not an important predictor of filtration 24 h after feeding, it could be that the avoidance response of mussels was most pronounced shortly after renewing the metal solutions, similar to the more conspicuous avoidance reported by Cope et al. (2008) in their acute Cd test.

Another possibility is that mussels attempted to balance avoidance and filtering over the 24-h period. In a review of aquatic organisms' responses to disturbance, Bae & Park (2014) listed a number of bivalve responses to toxicants, including prolonged closure, reduction of shell gape distance during filtering, and increased activity (more frequent opening and closing). The addition of food soon after metal renewal was not a powerful enough stimulus for mussels to overcome their avoidance response within the first two hours. An artifact of our sequentially ordered Ni and Cd concentrations was that they were autocorrelated; therefore, modeling exposure to both metals as predictors of the removal, accumulation, and biodeposition responses would have been redundant, and attempts to investigate the influence of one metal as a covariate of processing the other were not were not fruitful. However, the similarity in our findings between the single metal and NiCd tests suggests that the effects of Ni and Cd were not interactive, additive, or synergistic for removal of waterborne metals by mussels, accumulation in their tissue, or bioconcentration and deposition activity – at least over the 28-d duration of the experiments. Nonetheless, the results of an apparent combination effect on the filtration activity of mussels suggests that this essential life function and mode of ecosystem service delivery may be more adversely affected by the typical condition of contaminant mixtures present in surface waters than by exposure to individual contaminants.

Ecological Considerations of Pollutant-Related Mussel Functions

This research demonstrates the active role of freshwater mussels in environmental fate and transport of toxic heavy metals in aquatic ecosystems. If filtration rates are sufficient relative to stream discharge or lake/pond volume, mussels may be reducing waterborne contaminant exposure for other freshwater organisms (e.g., fishes, amphibians, rock-dwelling aquatic insects, and freshwater snails). However, bioconcentration and deposition of metals may produce sediment pollution hotspots that are analogous to, and co-located with, those nutrient-rich hotspots in mussel beds that create favorable habitat for benthic invertebrates (Howard & Cuffey 2006; Spooner & Vaughn 2006; Vaughn et al. 2008), including juvenile mussels (Irmscher & Vaughn 2018; Ries et al. 2019). Might this incidental function of greater pollutant deposition by mussels create a population sink for progeny and habitat associates that are drawn by resource availability? Or might it result in subsequent biomagnification in consumers of invertebrates from mussel bed habitats, including those in terrestrial habitats (e.g., consumers of emergent insects)? One study using an "artificial mussel" passive sampling device detected hotspots of heavy metals in locations where they were deployed, suggesting these are possibilities (Kibria et al. 2012). We also observed that metals were also bioconcentrated in mussel tissue compared to test waters. Through we did not calculate bioconcentration factors for these field collected mussels, an inspection shows that Ni concentrations in tissue were ~1 to 3 orders of magnitude higher than test water and Cd tissue concentrations were about 3 orders of magnitude higher by the end of the exposures. A possible consequence of pollutant sequestration and bioaccumulation in mussel tissue, then, is biomagnification in molluscivorous consumers in both aquatic and terrestrial environments (e.g., sturgeons (*Acipenser* spp.), Freshwater Drum (*Aplodinotus grunniens*), Muskrats (*Ondatra zibethica*), and River Otters (*Lutra canadensis*)). Finally, the diminished filtration patterns we observed could result in reduced nutrient retention, cycling, and deposition in more polluted environments. Therefore, mussel beds may offer less potential benefit and more potential harm for associated species in more polluted waters. Such open questions would benefit from future research to improve our understanding of pollutant cascades in freshwater mussel ecosystem functioning.

Implications for the Conservation of Freshwater Mussels and their Ecosystem Services

By investigating and demonstrating the pollutant-related ecological functions of mussels, we also discovered that Ni and Cd elicited significant reductions in mussel filtration below concentrations that are considered protective of aquatic life (US EPA 1995; US EPA 2016). That we were able to detect significant changes to this most essential function over 28 days suggests implications for mussel health, population resilience, and any benefits they provide over long-term or lifetime exposures, even at low concentrations. It is unclear if exposure alone or a toxic effect from accumulated metals resulted in lower filtration rates, but the limited research on metal toxicokinetics shows that depuration rates are relatively slow (Thorsen et al. 2007). A study by Cooper et al. (2013) compared the survival of two groups of Giant Floater (*Pyganodon grandis*) mussels transplanted to a Canadian lake contaminated with Cd, Cu, and Zn; one group was sourced from an uncontaminated lake and the other was sourced from a lesser contaminated lake. The group that was sourced from an uncontaminated lake fared better over time, with 80% survival after 860 days, compared to 0% survival in the group that originated from the lesser contaminated lake. Cooper et al. (2013) suggested that prior exposure to pollution contributed to lower resilience of mussels to cope with an added stressor of translocation along with continued pollution stress. Alternatively, Pheasantshell mussels (*Actinonaias pectorosa*) collected from the Clinch River harbored lower tissue metal concentrations in a year with less waterborne metal pollution than mussels collected the previous year (Cope & Jones 2016). Such findings suggest an encouraging dynamic in which social-ecological management strategies that reduce pollution could allow mussel populations to recover from previous exposure. In addition to the lower tissue burdens reported by Cope & Jones (2016), reducing pollution can reduce the probability of detrimental sediment pollution hotspots, and lead to improved water filtration capacity, ecosystem functioning, and ecosystem service delivery by mussel populations.

We recently explored the feasibility of using existing data on mussel population sizes and tissue concentrations of pollutants to estimate population-level pollutant sequestration as a potential ecosystem service (Chapter 1). Here, we expanded on this concept by investigating their functional processing as a means of understanding how such population-level estimates might fluctuate under different environmental scenarios (e.g., more or less pollution). We showed that the ecosystem functions of metal removal and processing varied within an environmentally relevant range of metal concentrations, and that this can directly affect the provision of mussel ecosystem services by influencing their filtration rates. Therefore, estimates of pollutant removal by mussels may be temporally dynamic under variable environmental conditions (e.g., pulses of pollution (Cope & Jones 2016)), even within a spatially discrete mussel population. Mussel filtration performance in wild populations exposed to complex contaminant mixtures could be a key indicator assessing population health and ecosystem functional capacity. Hartmann et al. (2016) suggested that filtration activity should be considered as an important behavioral biomarker in ecotoxicological studies, and Bae and Park's (2014) review of behavioral monitoring included bivalve studies going back more than 30 years. One possible application of our findings, then, could be using in-stream monitoring or streamside studies to evaluate the filtration capacity of wild mussels as they relate to local water quality conditions. Such research might serve as a warning sign or a potential recovery metric for populations. Furthermore, Buchwalter et al. (2017) suggested that field data should be incorporated in modernizing the development of regulatory water quality criteria, and focusing on mussel filtration as an endpoint of aggregate stressors may be a useful diagnostic.

Monitoring changes in filtration activity over time also may allow evaluation of sufficient water quality improvement for reintroduction efforts. Kreeger et al. (2018) pointed out that knowledge on unionid filtration rates is limited and that existing studies lack breadth for a spectrum of factors that may affect clearance rates. Filtration research should expand to include more species, performance under a range of temperatures, and studies using natural seston (Kreeger et al. 2018). The field studies we suggest above could address some of those research goals, while producing useful population data relative to water quality conditions. Studies of other ecosystem functions (e.g., nutrient cycling) have demonstrated relationships with mussel life history traits (e.g., thermal preference; Spooner & Vaughn 2008, 2012). Future research focusing on the influence of species traits or phylogeny on filtration behavior and contaminant processing (e.g., patterns in metal binding affinity) may help to generalize and broaden the application of our findings to improve estimates of ecosystem functions and services across species assemblages. Such research would be applicable to a wide range of filtration-related ecosystem functions beyond our contaminant focus here.

Gillis (2012) reported that Flutedshell (*Lasmigona costata*) mussels exposed to contaminant mixtures downstream of urban runoff and multiple municipal wastewater discharge sites had significantly higher metal concentrations in their gill tissue and suffered lower body condition and shorter life spans than those in locations upstream of an urban area (Grand River, Ontario, Canada). In follow up studies, Gillis et al. (2014) showed that mussel populations affected by these urban-derived contaminant mixtures suffered greater oxidative stress and higher biomarkers of exposure (elevated metallothionein). Their findings of organismal stress translated to population-level impacts; urban-impacted populations had 60% lower abundance than reference populations, including one that measured a 98% reduction of abundance immediately below a wastewater treatment plant discharge (Gillis et al. 2017). Moreover, mixtures often contain multiple classes of contaminants. A review of the co-toxic effects of metal-PAH mixtures by Gautier et al. (2012) concluded that synergistic impacts were more likely than additive effects, and that the current paradigm of environmental risk assessment is ill equipped to consider such interactions. Contaminant mixtures (as we explored with Ni and Cd) is just one consideration among a myriad of other environmental stressors relevant to mussel functioning and conservation. Mollusks are expected to experience increasingly fragmented populations (Inoue & Berg 2017) and greater loss of suitable habitats resulting from climate change than any other freshwater group (Markovic et al. 2014). Moreover, studies on the thermal sensitivity of mussels suggest that water temperatures regularly recorded in areas of high mussel biodiversity already may be exerting lethal or sublethal stress (Pandolfo et al. 2010; Archambault et al. 2013, 2014; Ganser et al. 2015). Researchers studying mussel nutrient dynamics have already identified diminished ecological functioning and service provision associated with droughts and water flow management (Vaughn et al. 2015; Dubose et al. 2019). Those research teams emphasized that mussel population decline or mass die-offs associated with these factors result in a long-term loss of important ecosystem functions (e.g., biofiltration, nutrient retention;

Vaughn et al. 2015; Dubose et al. 2019). The population-level impacts that Gillis et al. (2017) identified from water quality stressors also certainly translate to lost ecosystem functioning and attending benefits provided by mussels. Because unionid mussels are long-lived invertebrates with most having multi-decadal life spans and slow maturity to reproductive age (Haag 2012), understanding how contaminants may contribute to the alteration of their ecosystem functioning when coupled with other stressors would provide insightful data for prioritizing conservation measures.

Conclusions

W demonstrated the active role of mussels in the fate and transport of contaminants through filtration, sequestration, and deposition. We showed that low-level contaminants have an appreciable impact on mussels' essential function of filtration. The mounting evidence that freshwater mussels are exceptionally sensitive to some contaminants has made pollution reduction a significant priority for the conservation of this already highly imperiled faunal group (FMCS 2016). We further discovered the potential for mussel populations to act as catalysts for ecosystem-level consequences of human pollution through bioaccumulation and bioconcentration of contaminants. Thus, the possibility exists for human-derived pollution in waterways to biomagnify (e.g., from mussel bioaccumulation or deposition of concentrated metals in sediments to invertebrates to fishes) and cycle back to human communities through fish consumption. Haag and Williams (2014) proffered an argument to restore and conserve mussel fauna for the benefit of freshwater ecosystems, given their importance in maintaining ecosystem functionality and integrity. Here we go a step further to suggest that maintaining and improving surface water quality by reducing pollution allows mussels to more successfully carry out their ecological functions, which in turn may provide some benefits to human communities that rely on these waterways for drinkable, fishable, swimmable sources of sustenance and recreation.

Emphasizing the role of pollution in the demise of mussels and other freshwater fauna is crucial in this tumultuous era of water policy in the United States, where unionid mussel biodiversity is highest, and yet the fauna continues to decline. The objective of the Clean Water Act as a guiding document for managing the social-ecological system of aquatic resources "is to restore and maintain the chemical, physical, and biological integrity of the Nation's waters" (33 USC §§ 1251 *et seq.*). On 23 January 2020, under the direction of Executive Order 13788 issued by the Trump Administration (Trump 2017), the US EPA reversed a myriad of protections based on sound-science and decades of precedent in enforcing the Clean Water Act (US EPA 2020b). Many organizations, including the Consortium of Aquatic Science Societies – which represents more than 20,000 aquatic science professionals from nine professional societies, including the Freshwater Mollusk Conservation Society and American Fisheries Society – have criticized this legislation, calling "inconsistent with the best available science" and a threat to water quality and aquatic ecosystem services (CASS 2020). Our findings that mussels' basic function of filtration was affected by metal concentrations below US EPA water quality criteria (US EPA 1995; US EPA 2016) suggest the need for greater protections for water quality – not less. Regarding the water quality criteria, our findings highlight that the practice of single-contaminant management and reliance on coarse metrics (e.g., survival) for setting guidelines may not be adequate for the persistence of populations or the conservation of functionally intact aquatic ecosystems. Modernizing methods for deriving water quality criteria (Buchwalter et al. 2017) and restoring the full measure of the Clean Water Act would be valuable steps toward protecting ecosystem functions performed by aquatic fauna, and therefore, recovering aquatic fauna, and restoring ecological integrity and the resulting ecosystem service benefits to human communities (Downing et al. 2010; Reid et al. 2018). Better water quality protections will results in improved success in the conservation of aquatic fauna – including freshwater mussels – strengthen ecosystem resilience to natural disturbance and anthropogenic stressors, and restore or increase their capacity to provide ecosystem services.

Ecosystem services research on native freshwater mussels is still in early stages, and more focus on this topic may provide a stronger argument for their protection, restoration, conservation (FMCS 2016; Strayer 2017; Vaughn 2018). Scientists must continue to emphasize research on the ecological functions performed by freshwater mussels to improve our understanding of their essential contributions to ecosystem functioning, while explicitly communicating how those functions are connected to socially desirable benefits, such as healthy water resources (Keeler et al. 2012; Olander et al. 2018). The malacological community further needs to make interdisciplinary strides to couple messaging about the benefits of mussel conservation with those focused on the social benefits of water resources, because this declining faunal group is currently irrelevant to most people (Keeler et al. 2012; Anderson et al. 2019). A base of knowledge about freshwater mussel ecology and their positive impacts on environmental and human well-being can help cultivate a diverse set of values (e.g., existence value, indirect use value for their regulating services) to improve public understanding of their necessity to aquatic ecosystems, their current dire status, and their need for conservation focus and funds (Daily 1997; Perrings 2007; Strayer 2017). Efforts of public engagement by scientists and aquatic professional societies will further empower communities with the awareness and proficiency in aquatic resource issues they need to address inequities in natural resource decision making – especially for the most vulnerable and marginalized populations (Berbez-Blazquez et al. 2016; Vallet et al. 2019). Inclusive societal participation to develop policies emphasizing favorable water quality and restoration of aquatic habitats for both human and environmental health will aid in the conservation of unionid mussel fauna and may improve equity in the access to and benefits of aquatic ecosystem services by human communities.

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Table 1. Treatment concentrations for the Ni, Cd, and NiCd Experiments. NiCd treatment concentrations were simply an admixture of the corresponding Ni and Cd concentrations in ascending order. Cd verified concentrations are shown in parentheses, and Cd results are reported with verified rather than nominal concentrations due to low exposure accuracy in both the Cd and NiCd tests.

Ni Test Concentrations (μg Ni/L)	**Cd Test Concentrations (μg Cd/L)**	**NiCd Test Concentration ID**
0 (control)	0 (control)	A (control)
5	0.25 (= 0.05 verified)	B
25	0.5 (= 0.1 verified)	C
50	1 (= 0.2 verified)	D
100	2 (= 0.4 verified)	E

FIGURES

Figure 1. Photograph of experimental set up and aerated static-renewal design, with nine replicate aquaria in each of five metal treatments, and one mussel per aquarium (two experiments shown, one on either side of the lab bench).

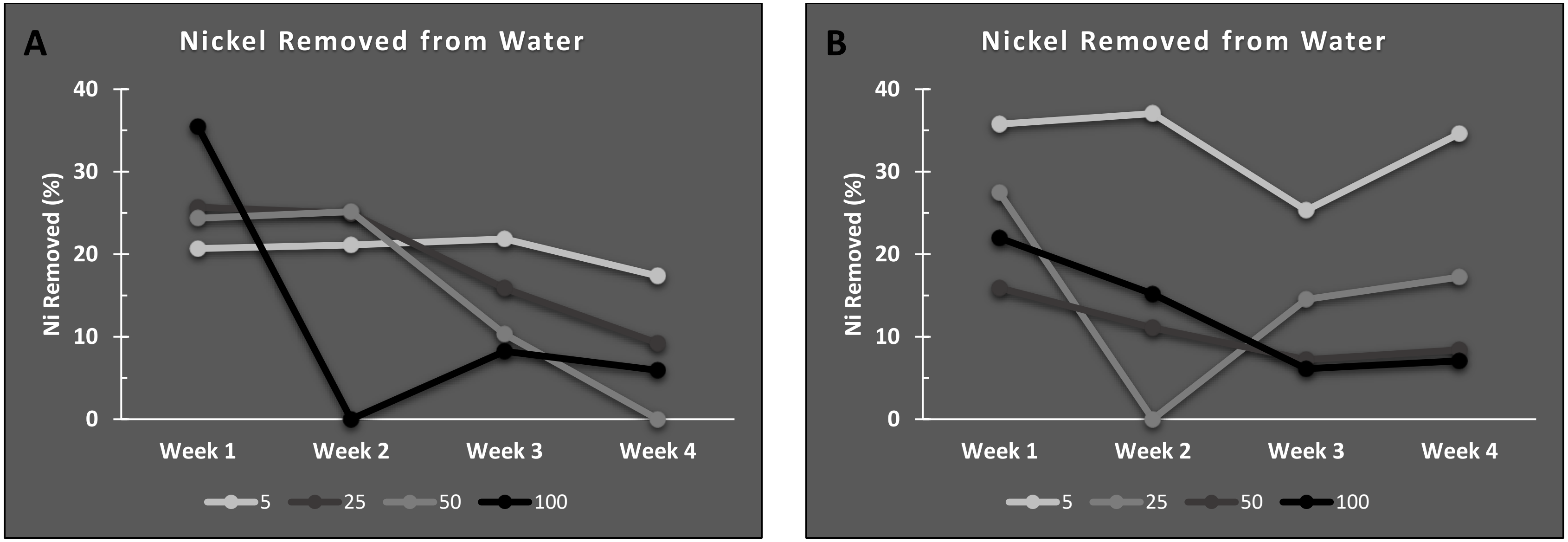
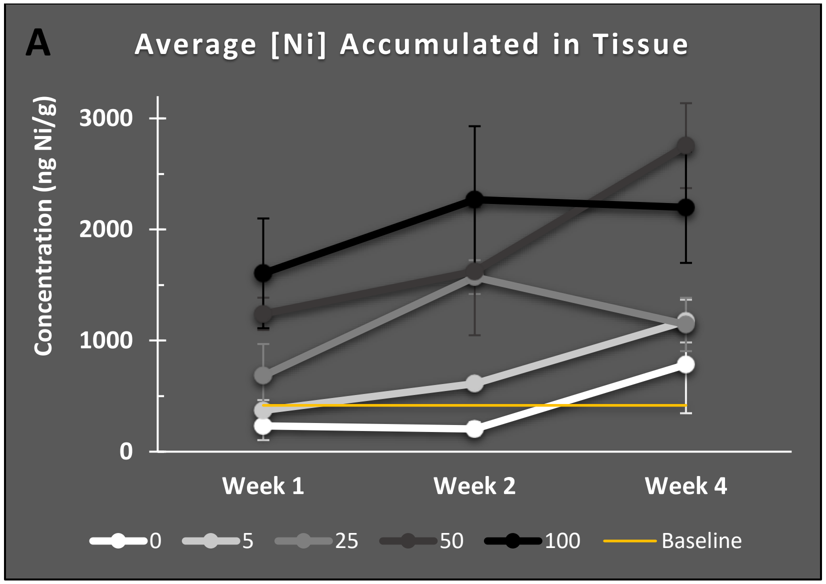


Figure 2. Removal of waterborne Ni from the Ni (A) and NiCd (B) tests, showing the percent of Ni removed by mussels over a 24-h period each week. Data lines are colored from lightest to darkest corresponding with low to high Ni concentrations.



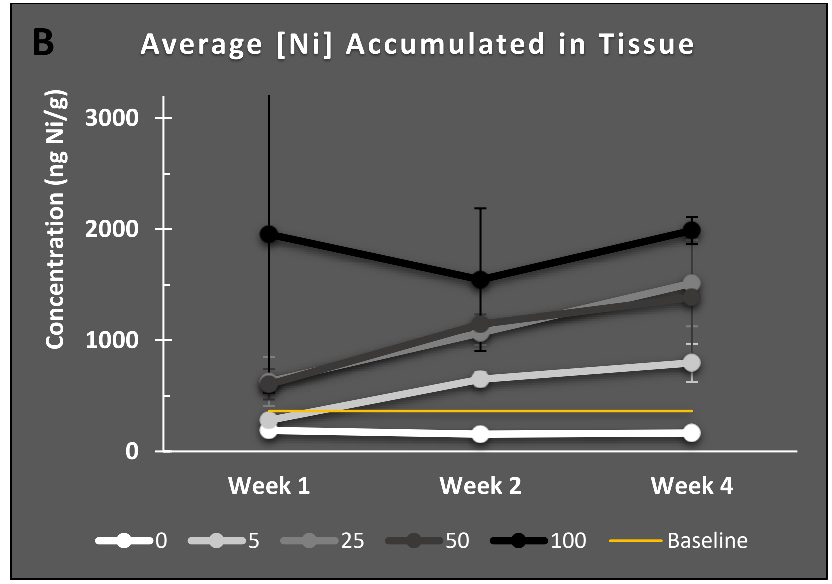


Figure 3. Mean (± SE) accumulation of Ni in mussel tissue from the Ni (A) and NiCd (B) tests. Darker data lines correspond to higher Ni exposure concentrations, and the thin yellow-orange line represents the mean concentration of Ni in baseline mussel tissue.

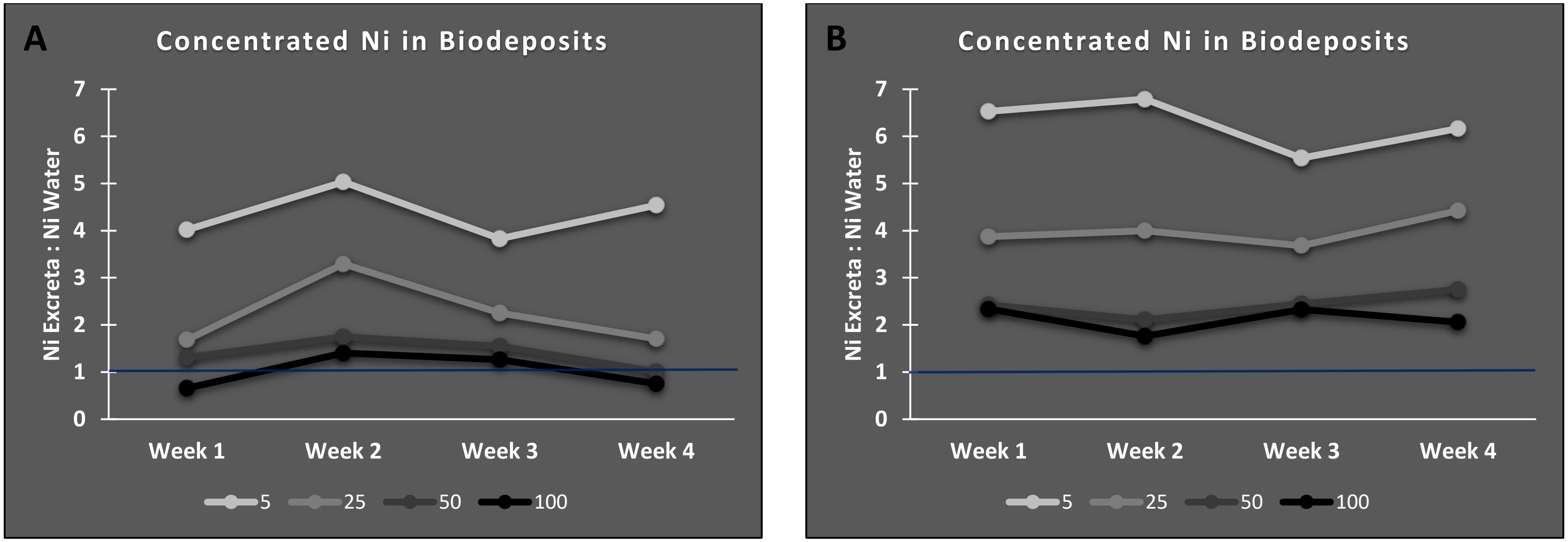


Figure 4. Bioconcentration of Ni in egested biodeposits from the Ni (A) and NiCd (B) tests. Darker data lines correspond to higher Ni exposure concentrations, and the thin blue line represents a 1:1 ratio (i.e., no bioconcentration). Data above the 1:1 line shows biodeposits contained higher concentrations of Ni than exposure water (e.g., data point at 4 = 4X higher Ni in biodeposits than in water).

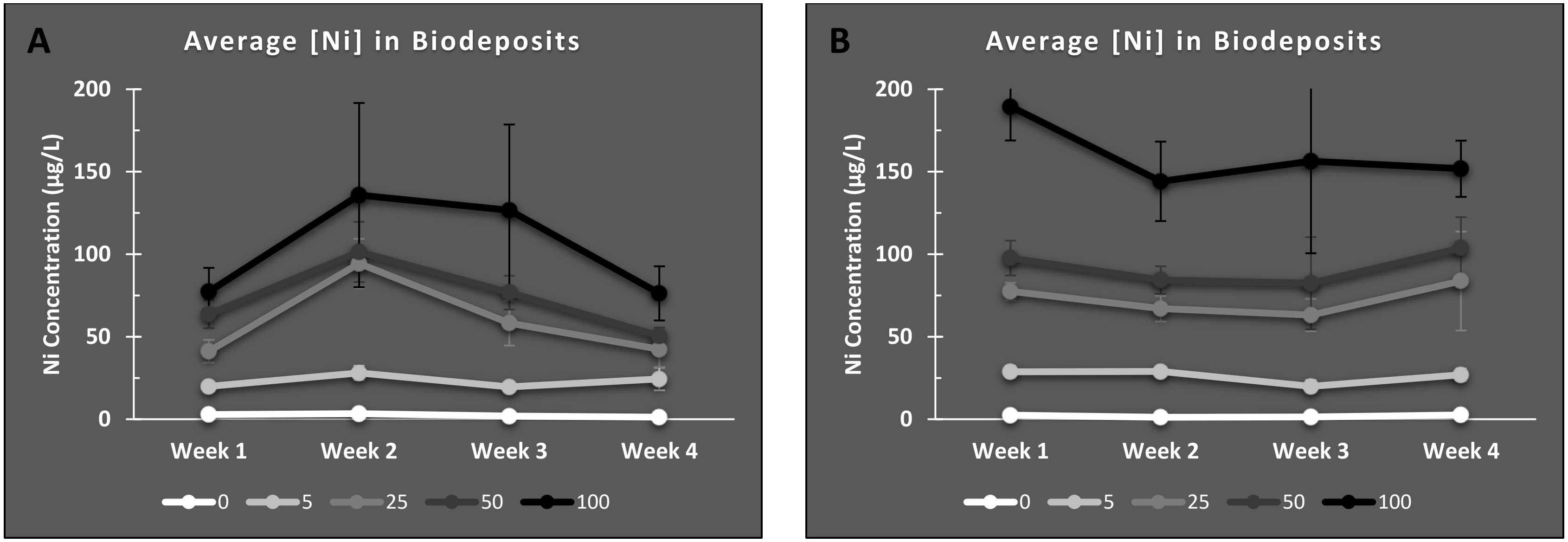


Figure 5. Mean (± SE) concentration of Ni in biodeposits from the Ni (A) and NiCd (B) tests. Darker data lines correspond to higher Ni exposure concentrations.

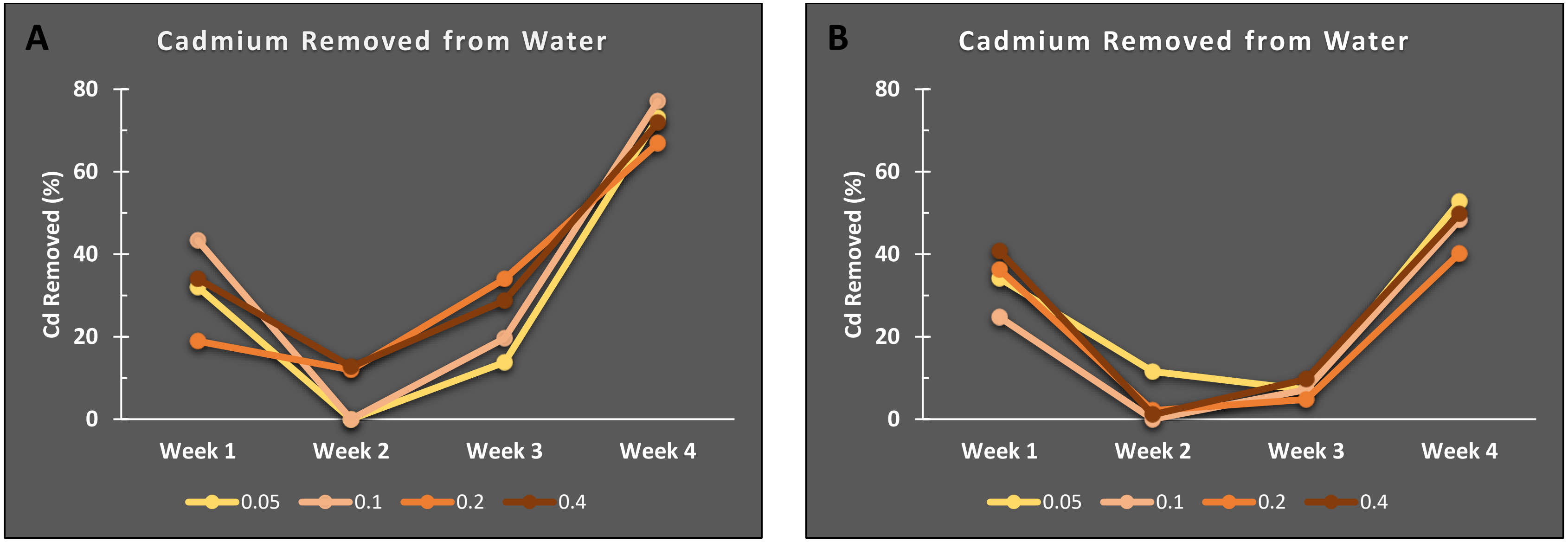
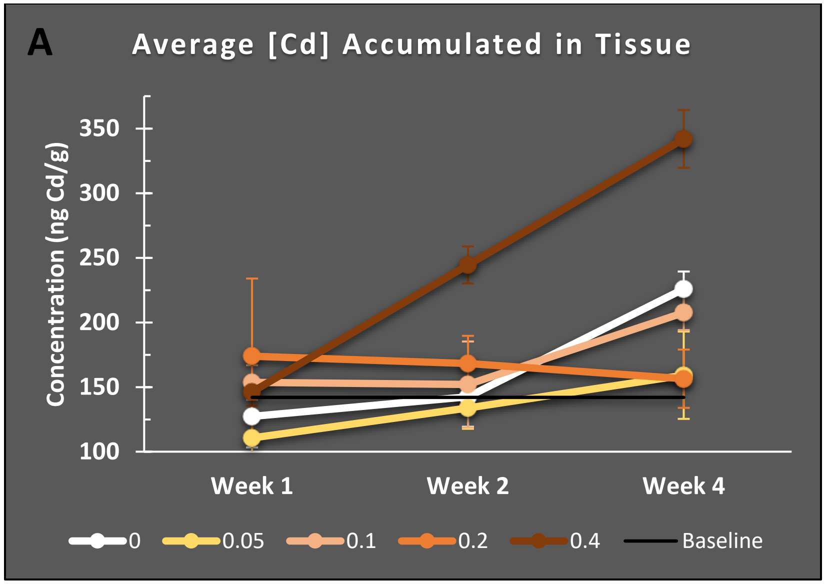


Figure 6. Removal of waterborne Cd from the Cd (A) and NiCd (B) tests, showing the percent of Cd removed by mussels over a 24-h period each week. Data lines are colored from lightest to darkest corresponding with low to high Cd concentrations.



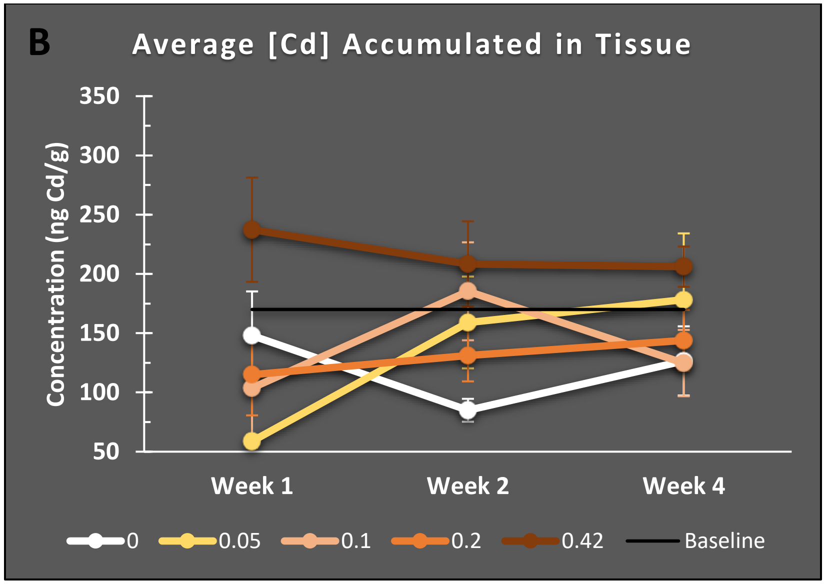


Figure 7. Mean (± SE) accumulation of Cd in mussel tissue from the Cd (A) and NiCd (B) tests. Darker data lines correspond to higher Cd exposure concentrations, and the thin black line represents the mean concentration of Cd in baseline mussel tissue.

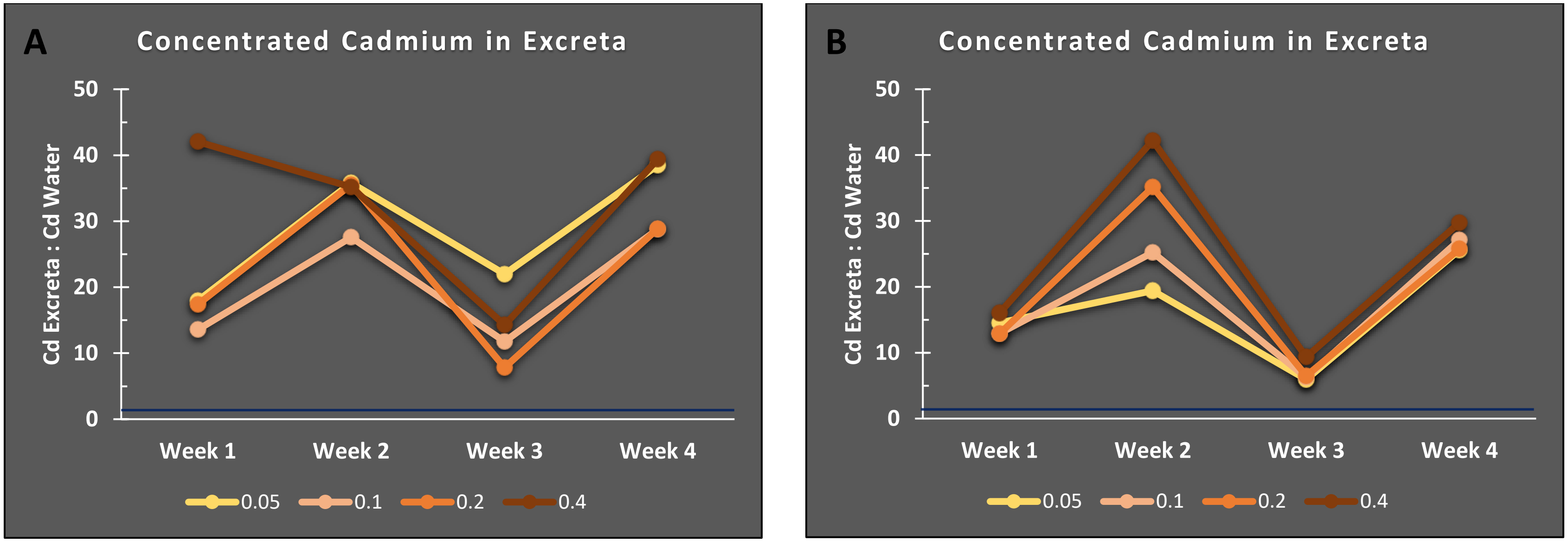


Figure 8. Bioconcentration of Cd in egested biodeposits from the Cd (A) and NiCd (B) tests. Darker data lines correspond to higher Cd exposure concentrations, and the thin blue line represents a 1:1 ratio (i.e., no bioconcentration). Data above the 1:1 line shows biodeposits contained higher concentrations of Cd than exposure water (e.g., a data point at 10 = 10X higher Cd in biodeposits than in water).

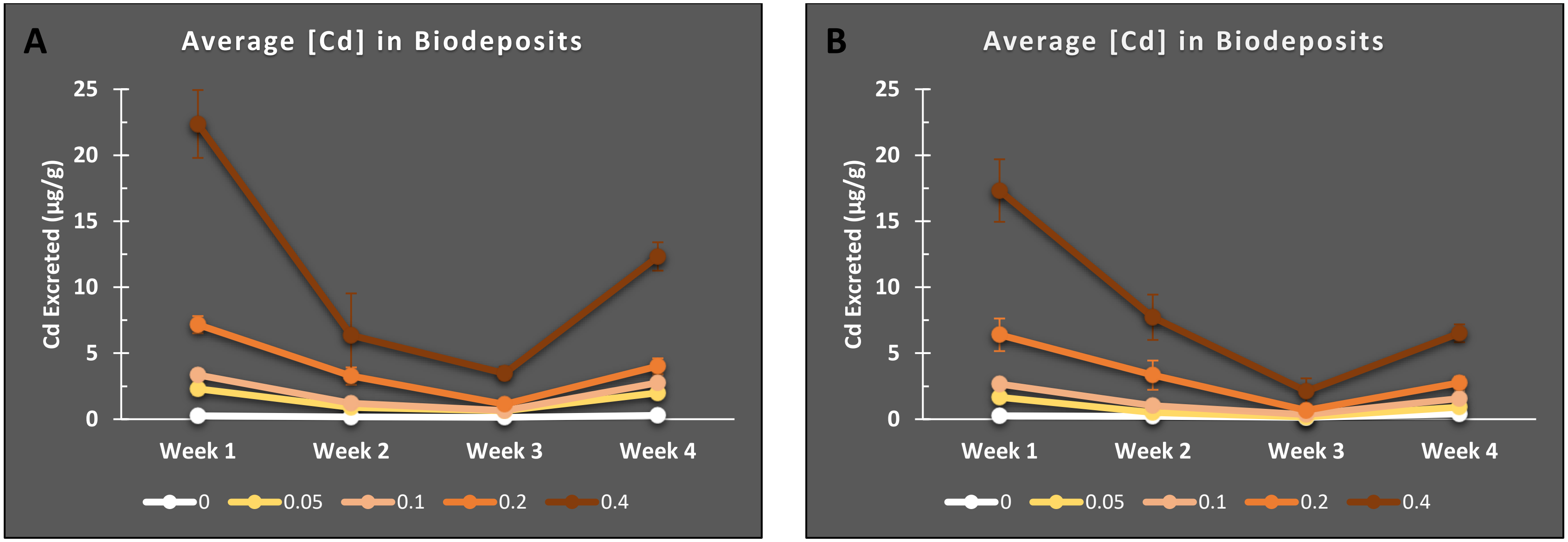


Figure 9. Mean (± SE) concentration of Cd in biodeposits from the Cd (A) and NiCd (B) tests. Darker data lines correspond to higher Cd exposure concentrations.

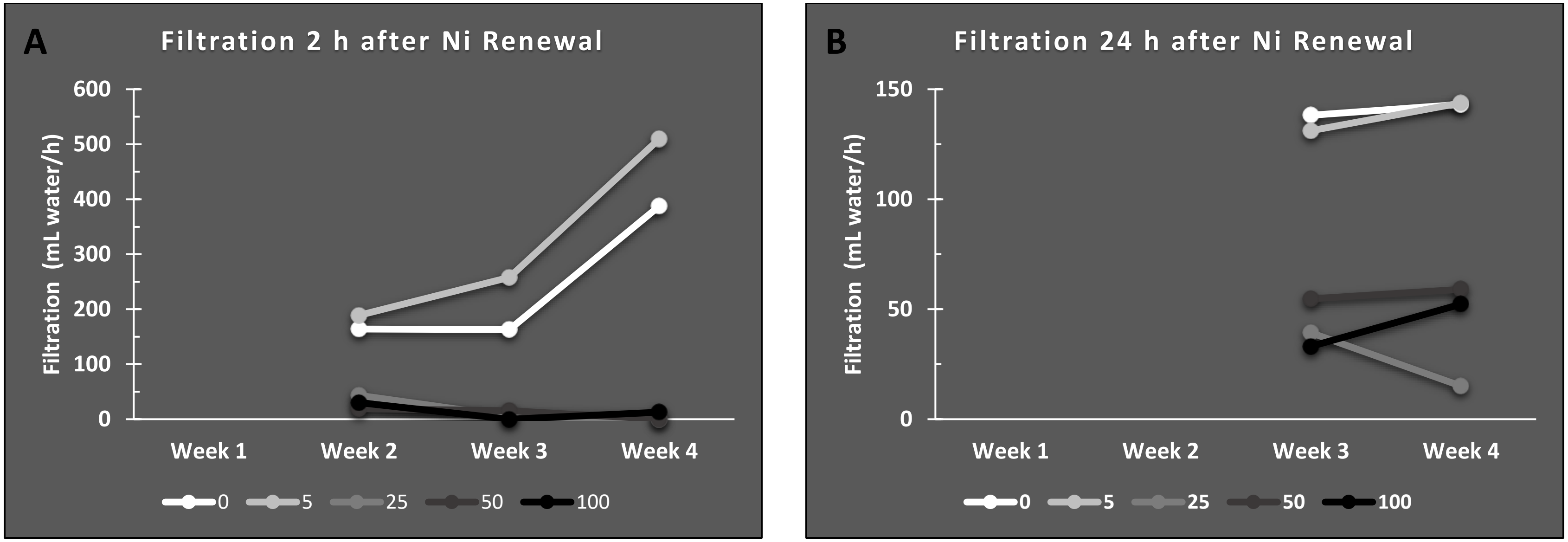


Figure 10. Filtration rates of mussels 2 h (A) and 24 h (B) after feeding in the Ni test. Data lines are colored from lightest to darkest corresponding with low to high Ni concentrations. (Note that the vertical axes are on different scales, and data were not available in Week 1 for the 2-h time point or for Weeks 1 or 2 at the 24-h time point.)

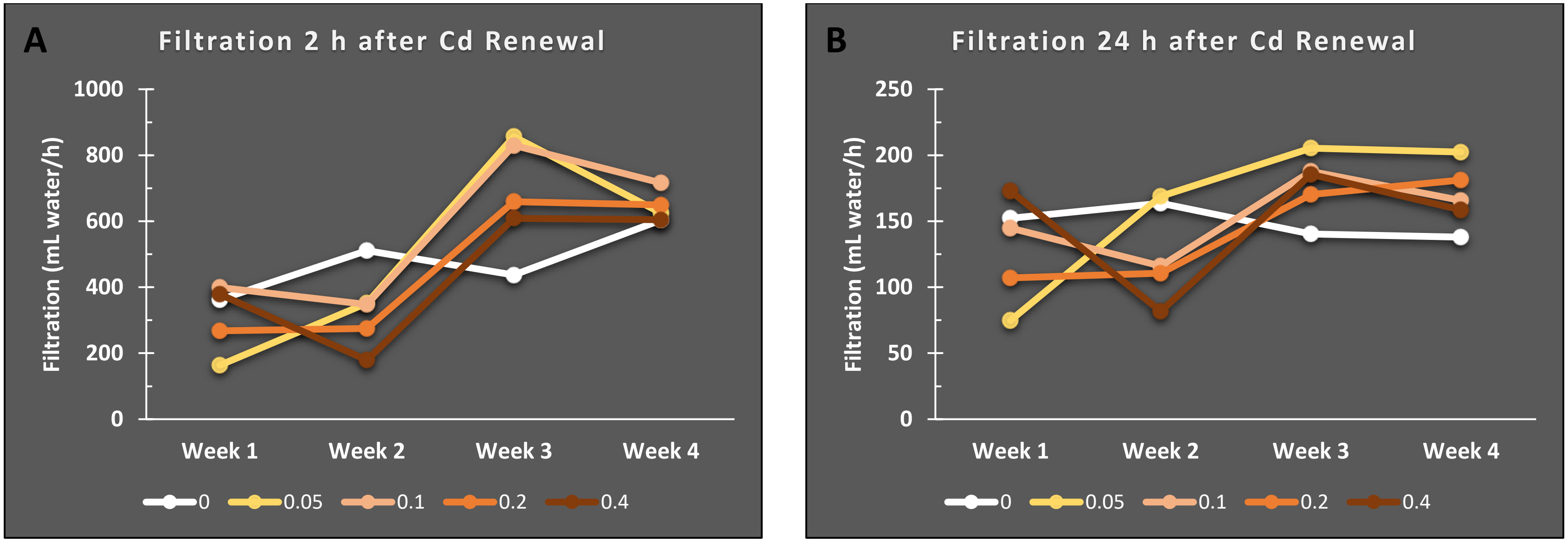


Figure 11. Filtration rates of mussels 2 h (A) and 24 h (B) after feeding in the Cd test. Data lines are colored from lightest to darkest corresponding with low to high Cd concentrations. (Note that the vertical axes are on different scales.)

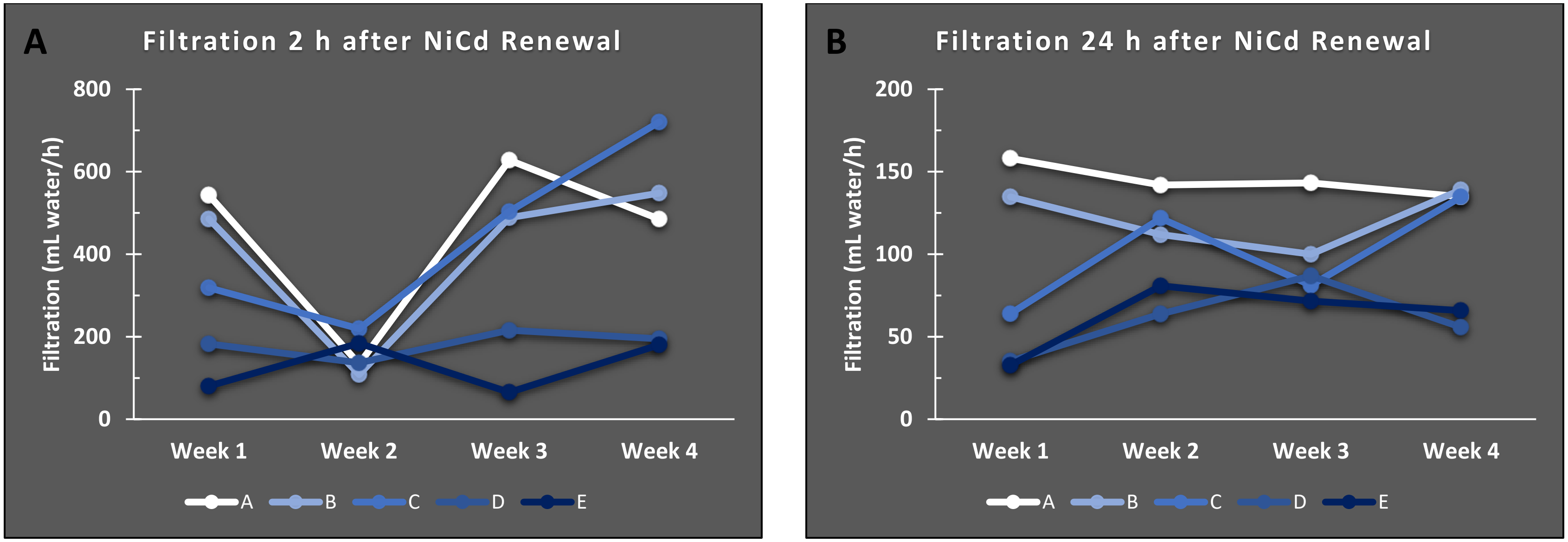


Figure 12. Filtration rates of mussels 2 h (A) and 24 h (B) after feeding in the NiCd test. Data lines are colored from lightest to darkest corresponding with low to high NiCd concentration combinations. (Note that the vertical axes are on different scales.)

CHAPTER 3. Public Understanding of Floral and Faunal Influences on Water Quality: Implications for Communication about Ecosystem Services

ABSTRACT

Though aquatic species are integral to ecosystem functioning and maintenance of water quality, most are not readily perceived by the public, and people may not realize the relevance of these ecosystem components in regulating healthy waterways for human use and well-being. It is imperative to capture how these resources are valued by human communities because improved understanding of community values is a critical component of promoting effective watershed management. Social science research methods are increasingly employed to investigate public understanding and beliefs about conservation and natural resource issues. A first step in understanding the community valuation of ecosystem services related to water quality is investigating public perceptions of water quality's mediating factors. We engaged 57 residents of central and eastern North Carolina in six focused small group discussions, using a series of photographs of plants and animals to examine communities' beliefs about whether and how those flora and fauna relate to maintenance of water quality. Several prevailing themes emerged from the focus group discussions, including positive effects that flora and fauna have on water quality, dualistic "good and bad" or negative impacts, flora and fauna as indicators of water quality, and balance in nature. Participants also expressed uncertainty at times, and we identified a number of misconceptions about flora and fauna. Participants also offered some comments on impacts to water quality by humans and about connections to values or well-being. Participants regularly relied on their prior experiences to explain their understanding of factors affecting water quality. Findings from these focus group discussions provide baseline understanding of

public beliefs and knowledge of ecosystem functioning related to water quality. Our findings show that people identified several effects that flora and fauna have on water quality, including ecosystem functions that provide essential ecosystem services (e.g., provision of habitat and regulating services, such as water purification through filtering and cleaning). These findings suggest an encouraging congruence of public beliefs with expert science, offering some common ground, similar language, and opportunities for connecting with communities on important issues that highlight or threaten ecosystem functioning and resulting ecosystem services that link environmental and human well-being.

INTRODUCTION

Water is an essential resource that humans derive from nature. Clean water is a necessity for survival, and surface water resources are arguably among the most important of social-ecological systems. Not only are freshwater ecosystems essential for the provision of drinking water, irrigation water, and food production through fisheries, they also provide recreational and aesthetic benefits for well-being and supply important regulating and supporting ecosystem services, such as nutrient processing and primary production) (Millennium Ecosystem Assessment 2005; Baskett & Halpern 2009; Palmer & Richardson 2009). Generally defined as nature's goods and services that contribute to human well-being, ecosystem services have been codified according to four main categories, including: provisioning services that supply materials (e.g., food, water, fiber, fuel), regulating services that contribute beneficial processes (e.g., climate/flood regulation, water purification), cultural services that provide non-consumptive benefits (e.g., spiritual, recreational, or aesthetic importance), and supporting services that provide indirect benefits by maintaining the other three categories (e.g., soil formation, nutrient cycling) (Millennium Ecosystem Assessment 2005). Impaired water quality in the United States and around the globe has resulted in declining biodiversity and a reduction in the delivery of aquatic ecosystem services (Baskett & Halpern 2009; Palmer & Richardson 2009). An example of aquatic ecosystem services is filter-feeding bivalves that improve water clarity by consuming algal species and that alter nutrient concentrations and their spatial dynamics through excretion and uptake (Vaughn 2018; Vaughn & Hoellein 2018). Through consumption and excretion, many aquatic species (e.g., fishes, bivalves, gastropods) can alter the spatial distribution of nutrients, thereby influencing spatial patterns of nutrient uptake, availability to other organisms, and ultimately water quality (e.g., suppressing algal blooms) (Vaughn et al. 2004, 2008;

McIntyre et al. 2008; Brown & Lydeard 2010). The presence of invasive species may upset the balance of water quality metrics. For example, invasive zebra and quagga mussels (*Dreissena* spp.) promote harmful algal blooms through preferential feeding (Limburg et al. 2010), and dense stands of the invasive aquatic weed, hydrilla (*Hydrilla verticillata*), alter several water quality metrics including reduction of dissolved oxygen and increased sedimentation (Langeland 1996). Though aquatic species are integral to ecosystem functioning and maintenance of water quality, most are not readily perceivable by the public, and people may not realize the relevance of these ecosystem components in regulating healthy waterways for human use and well-being.

Social science research methods are increasingly employed to investigate public understanding and beliefs about conservation and natural resource issues (Moon & Blackman 2014). In prior research asking communities to describe the quality of surface waters, people have relied extensively on the physical appearance of the water and other visible cues in immediate surroundings (David 1971; Dinius 1981; Limburg et al. 2010; Artell et al. 2013; West et al. 2016). For example, in surveys of Wisconsin residents (David 1971), Lake Ontario residents (Limburg et al. 2010), and "laymen" who took part in a visual perception test (Dinius 1981), participants considered murkiness or clarity as important factors. The color of water was another major indicator of quality, as perceived by the public. Discoloration and the presence of algae blooms affecting water color influenced perceptions of water quality (David 1971; Dinius 1981; Limburg 2010). In a recent review of research on water quality perceptions, West et al. (2016) found that people associated waters in the blue and blue-green hues as healthy, and those with hues in the yellow and brown ranges unhealthy or less healthy. People have used various other visible components of aquatic habitats to judge water quality, including litter and surface suds (David 1971, Dinius 1981, West et al. 2016). While some of these studies focused solely on visual indicators (e.g., Dinius 1981; Cottet et al. 2013), the trend of people to understand and assign water quality using visual cues was persistent throughout the literature. Beyond perception studies of water quality status, others have attempted to find linkages between publicly perceived and scientifically measured water quality (e.g., dissolved oxygen concentration, turbidity; Artell et al. 2013). However, there is a gap in understanding people's knowledge and perceptions of functional ecology associated with water quality maintenance.

Clearly demonstrating how ecosystem functions translate to social values is one strategy to make derived ecosystem benefits more relevant to policy and decision-making (Olander et al. 2017). Aquatic resources are social-ecological systems governed by both ecological processes and human driven processes and values (e.g., Hand et al. 2018; Colding & Barthel 2019), highlighting the need for natural resource professionals to have some proficiency in understanding social science research (Moon & Blackman 2014). Values are determined, in part, by people's existing mental models, which are informed by their lived experiences, beliefs, and knowledge of these systems. Therefore, research on how people understand aquatic resources, functions, and services and the role of social filters (e.g., influences of prior knowledge, Ambrose et al. 2010) in understanding, will help natural resource professionals engage with communities in appropriate and relevant ways (Baron 2010; Alda 2017; AAAS 2020).

Keeler et al. (2012) highlighted the need for more scientific research on quantifying and valuation of ecosystem services related to water quality because water quality supports many aspects of well-being, from provision of food and water to recreation. Not only is it important to account for the multiple regulating, provisioning, and cultural ecosystem services that aquatic resources provide, but also it is imperative to capture how these resources are valued by communities. Improved understanding of nature's services and community values are both critical components for promoting effective watershed management. A first step in understanding the community valuation of ecosystem services related to water quality is investigating public perceptions of water quality's mediating factors. In this study, we used a series of photographs of plants and animals to examine communities' beliefs about whether and how those flora and fauna relate to maintenance of water quality. This research elucidates how people believe flora and fauna relate to water quality maintenance, provides insight on commonalities with expert ways of knowing, and highlights opportunities for engagement with public audiences on ecosystem service benefits and the underlying ecosystem functions that support desirable outcomes.

METHODS

This study was designed to engage residents of central and eastern North Carolina in an effort to learn about their views on nature's contributions to water quality (i.e., ecosystem services). Central and eastern North Carolina offer a demographically diverse range of communities for engagement (e.g., urban to rural, coastal to inland). Furthermore, communities are hydrologically connected along Atlantic Slope river basins that begin as headwaters in north-central North Carolina and flow to the coast, and these river basins were in areas prioritized for research on education and awareness of water quality and conservation issues by the Water Resources Fund (North Carolina Community Foundation 2018). Finally, the geographic area was reasonably accessible to the research team, given fiscal and temporal considerations, and investigators had community connections that helped in recruiting participants.

We used focused small group discussions as the primary means of data collection. Focus groups are one of several qualitative research methods in the social sciences; they are especially useful for research involving beliefs and studying the language that communities use in in talking about the topic of interest (Berg & Lune 2012). Further, focus groups are effective tools for gathering rich information from many participants with a relatively low time commitment for participants and researchers (Berg & Lune 2012). Focus groups have been used in other studies to understand cultural attitudes about water quality and its regulation, to learn about behaviors related to surface waters, and to learn about contaminant concerns (Langford et al. 2000; Henrich et al. 2015). Further, West et al. (2016) suggested that integrating social science with hydrology can foster better communication between scientists and public audiences (e.g., communities or policy makers) and inclusion of public views for more holistic water quality management and water resource policy decisions. The integration of social and environmental aims have recently been achieved in several studies: for example, in understanding stakeholder views of water quality problems in a Florida watershed (Borisova et al. 2012); beach visitor perceptions of water quality communications (Pratap et al. 2013); farmer perceptions of participating in environmentally friendly agriculture programs (Taylor & Van Grieken 2015); and perceptions of surface water quality and related behaviors in British Columbia (Henrich et al. 2015). Finally, because existing information is scarce regarding public perceptions of ecosystem services related to water quality in North Carolina and generally, other social science methods (e.g., surveys, quantitative metrics) were less feasible or not appropriate for the current state of knowledge on the topic. Given that focus groups have been applied successfully in studies of water quality perceptions as described above, we deemed them the most suitable method for gathering data for this study. The qualitative nature of focus groups allowed us to capture participants' understanding of concepts of water quality and ecosystem services in their own words, and the flexible structure allowed additional relevant topics outside the scope of the topic guide to arise.

We recruited residents of central and eastern North Carolina to participate in focus groups by connecting with community-based organizations or outreach and service providers, including county Cooperative Extension offices, a local museum, a conservation organization, community college instructors, and a community leader. Our goal was to hold two focus groups in the urban/suburban headwaters, two in the rural mid-watershed area, and two in coastal communities to gain a variety of perspectives. We hypothesized that attitudes might be more similar within geographic regions than between portions of the hydrologic continuum. Criteria for participation included the following: (a) resident of North Carolina, (b) speak English, and (c) age 18 or older. Study participants consented to participate, using a protocol approved by the Institutional Review Board of NC State University (IRB#15515) and we provided financial compensation for their time and participation in the study.

The focus group discussions lasted between 60 and 88 minutes, and were audio recorded, transcribed by a third party (Verbal Ink, New York, New York), and coded. A set of questions was used to guide the conversation on participants' perceptions of judging water quality, factors that affect water quality, the relationship of pictured flora and fauna to water quality, and how water resources affect their well-being (see Topic Guide, Appendix 1). Here, we report on an exercise in which we asked participants to discuss any water quality connections with photographs of the following items: a streamside forest, fishes, beavers, algae, underwater vegetation, freshwater snails, and freshwater mussels (Figure 1). Photographs were selected to represent a range of familiar to less familiar objects and range of scale from individual flora and fauna to a natural community (i.e., forest). We displayed each in turn on a large easel, printed in color on 11-x-17-inch paper, and for each photograph, we asked the groups, "if in your mind [photographed object] has something to do with water quality, what is the connection?" We anticipated that the photographs and verbal prompt would elicit responses from participants showing their understanding of ecological principles related to these organisms and organismal influences water quality. The focus group transcriptions yielded approximately 285 single-spaced pages of transcription data, including approximately 107 pages from the photograph exercise. We analyzed the contents of the transcriptions by identifying prevalent themes within and across discussions. Debriefing sessions between the facilitator and research team took place within 24 h of discussions to compare impressions of recorded observations. The focus group transcriptions were coded using *a priori* codes derived from research questions and data-driven codes that were grounded in themes or concepts that emerged during initial reviews of the focus group discussions (Corbin & Strauss 2008; Saldaña et al. 2009; DeCuir-Gunby et al. 2011; Berg & Lune 2012). We (two members of the research team) coded one full transcript together to establish code categories that classified prevailing themes in the participant discussion, and sub-codes that captured the language participants used to describe their impressions. We then separately coded three additional photograph exercise transcripts, meeting after each one to review emergent codes or themes and achieve consensus in coding; we then determined that we had reached code saturation and coder alignment (see Codebook, Appendix 2). I coded the remainder of the transcripts, reviewed all transcripts and coding, drafted detailed memos of emerging themes within and among focus groups, discussed progress and impressions several times with another team member, and interpreted the findings. I used a qualitative data analysis software to aid in organizing, coding, and interpreting the discussions, with an iterative approach to discover trends grounded in the data (ATLAS.ti version 8.4, ATLAS.ti Scientific Software Development GmbH, Berlin, Germany; Friese 2019). The full qualitative analysis encompassed all activities from initial inter-researcher debriefing sessions through the final interpretation of the participant discussions (Corbin & Strauss 2008; Berg & Lune 2012). Two research team members conducted a peer review to ensure that interpretations were reasonable and defensible. One of the team members attended all six focus groups along with me (the Principal Investigator and discussion facilitator) and another attended four; both reviewed preliminary findings and conclusions and found the interpretations to be supported by the data. Below, we report on the prevailing themes from focus group discussions about the relation between water quality and the flora and fauna we showed. Themes align with the code categories that emerged in the qualitative analysis (Codebook, Appendix 2).

RESULTS

Participation and Demographic Representation

We engaged 57 people in central and eastern North Carolina in six focus groups, and participants included people from a range of different backgrounds. In the coastal region, we talked to coastal fishermen (a mix of commercial and recreational anglers, charter captains and tour operators, aquaculturists, and tackle shop owners; n = 11) and students at a community college (n = 6). In the central portion of our study area, we engaged a group of farmers (a mix of row crop and livestock farmers and smaller operations; n = 11) and members of a tribal community (n = 16). In the upper watershed portion of our study area, we talked with members of a conservation group (n = 3) and residents who were patrons of programs (e.g., parent support, garden club) at an urban Cooperative Extension Center (n = 10). The average age of individuals from all focus groups was 51 years (range 18 – 78), although about one-third of participants fell into each of three broad US Census age categories of 18 – 44, 45 – 64, and 65 or older. A majority of participants (66%) had completed no more than an associate's degree, and despite holding three sessions in town or city centers, only 22% of participants identified the area where they live as suburban or urban. Participation was largely representative of the racial and ethnic demographics in the study area. However, only 2% of participants identified as Hispanic; participation among Hispanic individuals in this study was below the US Census Bureau's 2018 estimate of ~9.6% Hispanic or Latinx in North Carolina (US Census Bureau 2020). Additional details and demographics are reported in Table 1.

Prevailing Themes Discussed by Participants

Several prevailing themes emerged from the focus group discussions when we asked participants what connection, if any, existed between water quality and the photographed plants and animals. Participants often talked about **positive effects that flora and fauna have on water quality**. They also discussed dualistic **"good and bad" impacts** by some flora or fauna as they offered thoughts on **negative impacts to water quality**. Across all the focus group discussions, participants emphasized that the objects pictured served as **indicators of water quality**. Specifically, participants in most groups identified three to five of the pictured items as indicators of water quality, and the conservation group members identified all photographs as representing indicators. Another commonly expressed theme was **balance in nature**, though balance was a more prevalent concept in the discussions with the tribal community and conservation group members than with others. Participants in each group also expressed **uncertainty** at times related to *how* flora or fauna affected water quality, lack of familiarity with species (especially mollusks), or researcher language, and we identified a number of **misconceptions** about flora and fauna in the discussions. Additionally, though the photograph exercise was intended to elicit comments about effects of the items pictured – and most of the responses were closely related to flora and fauna – participants did offer some comments on

beneficial and detrimental impacts to water quality by humans. Human impacts were most commonly conveyed by coastal fishermen and farmers. Finally, there was some discussion about connections to participants' **values or human well-being**. These major themes were often discussed congruently in the course of the focus group discussions (Figure 2), and people regularly relied on their **prior experiences** to explain their understanding of factors affecting water quality.

Perceptions of Nature's Positive Contributions to Water Quality

Participants in every group offered substantial commentary on water quality maintenance or improvement by flora and fauna. Four groups of participants (coastal fishermen, tribal community members, urban Cooperative Extension patrons, and community college students) emphasized positive contributions to water quality more than any other identified theme. Farmers talked about positive effects with similar frequency as negative effects and indicator themes, and conservation group members mentioned positive effects with similar frequency as negative effects and less so than indicator themes. Focus group participants discussed positive influences of flora and fauna mainly in terms of specific effects they had on water quality, but there were a few comments in which participants expressed that flora or fauna were generally positive, without specifying an effect (e.g., "It's a positive," about the streamside forest; "Fish are good, you know? It's good to have fish. More are better," about fishes; and "They're important. They are," about mussels). However, these general comments typically led to more detailed conversation about positive effects or other themes (e.g., indicators of water quality or balance in nature).

The predominant specific positive effects identified by participants included *filtering, cleaning*, *habitat*, and other benefits for species (e.g., food source). Another common water quality contribution that participants discussed was sediment maintenance, variously referred to as “erosion control,” “stability,” or “stop[ping] sediment”. Other important but less frequently specified benefits recognized by attendees included influences on water temperature regulation (i.e., “cooling,” streamside forest), “oxygen” (i.e., underwater vegetation), and mediating water flow (i.e., runoff, beavers). Participants in three groups introduced the term *buffer* when discussing the streamside forest (coastal fishermen, urban Cooperative Extension patrons, and farmers); this term was typically used in conjunction with many of the other specific benefits mentioned here.

Descriptions of filtering and cleaning co-occurred in a few cases where participants used both terms to describe a benefit. For instance, attendees of the farmer group explained: “That freshwater mussel is first cousin to an oyster. – Do the same jobs. – They *filter*. – Oyster *cleans* the bottom of the ocean… – That’s right. – and a mussel *cleans* the water…creek” (female and 4 males, italics added for emphasis). Participants talked about cleaning most often to describe benefits provided by freshwater mussels and snails. Snails were discussed especially in terms of cleaning (e.g., “They’re like little vacuum cleaners... – Yeah, there’s something similar to how the algae eaters do it” (community college students, female and male), referencing a similarity to *Plecostomus* spp. catfishes in home aquariums). People in two groups used a pop culture reference to the SpongeBob SquarePants cartoon as a lens through which they understood aquatic snails and their functions. In one case, a participant explained how Gary, the snail character, was instrumental in cleaning up in an episode entitled “Fungus Among Us”:

I mean, it might be like a dumb connection, but whenever you watch SpongeBob, I always wondered like how there was a snail underwater, but then like the episode where they had all that green stuff on them – Yes! – and then he just comes and like, eats it off of them. – Yes! I know exactly what you're talking about! (community college students, 2 females).

A participant in another group mentioned the photograph made her think of Gary, the snail character in the show. Notions of cleaning were also mentioned occasionally to describe benefits provided by fishes (e.g., bottom feeders) and streamside forests (e.g., "soaking up" pesticides/fertilizers).

People regularly used either cleaning or filtering to describe the water quality benefits of mussels, and filtering was associated with mussels more than any other organism we showed (e.g., "they filter;" "they're filter feeders;" "they're water purifiers, filtration;" "they're like industrial strength filtration systems"). However, filtering was mentioned by at least one participant for every photo. Participants regularly associated filtration benefits with streamside forests (e.g., "Filtering out sediments and other nutrients or things that might go into the water. Buffer" (farmer, female)). They also commonly noted filtration benefits of fishes (e.g., "the shad are filter feeders;" "some are algae eaters") and underwater vegetation (e.g., "Yeah, it's the same thing as a marsh; it's a filtration" (coastal fisherman, male)). Participants made a few comments about beavers contributing to water filtration by making dams or indirectly by providing more habitat for underwater vegetation to filter, and a participant in one group commented that "good algae" help filter the water.

When talking about benefits to other species, focus group participants referred to habitat in ways that connoted shelter or space. Participants in all groups identified underwater vegetation as beneficial for providing habitat for other species. Some participants also identified habitat benefits provided by streamside forests and beavers, though to a lesser extent. People talked about underwater vegetation providing “a hibernation point or cover for fish,” and habitat for frogs, snails, dragonflies, and other insects. The coastal fishermen shared a wealth of habitat benefits provided by underwater vegetation:

> *There’s clams in that. – Yeah, scallops, too. – Believe it or not, grouper will grow in them. – Yeah, I mean you see a lot of offshore fish will actually come in and spawn in the shallows and stay there for the first little bit of lives and then go back out so it’s…. – The grass is a good thing. – The grass is a very good thing (5 males).*

Unlike other groups, the community college students keyed into the vegetation shown in one of the fish photographs and offered that fish “depend on the vegetation for safety” and that fish require “that lower ecosystem” for survival. This group then interpreted other benefits when discussing the vegetation photograph (e.g., serves as a water quality indicator). Participants in two groups (urban Cooperative Extension patrons and conservation group members) pointed out that the streamside forest provided habitat for wildlife. Beavers were credited with creating habitat for themselves (community college students) and other flora and fauna (conservation group members).

In addition to habitat, participants spoke about different kinds of benefits for other species or cases of species/ecological interactions. A few people talked about fish eating algae during discussions of either the fish or algae photograph. These comments ranged from benefits that fish provide (e.g., “eating the bad algae”) to the benefit of algae providing a food source (e.g., “my fish eat the algae. I have koi”). Other comments included underwater vegetation as a food source for fish and snails or mussels eating algae, as participants described mussel cleaning and filtering benefits. People from two groups articulated that underwater vegetation provides oxygen (e.g., “I believe that the underwater vegetation puts off oxygen…so that the fish can maintain” (tribal community member, male)). In one case, a participant pointed out an aquatic-terrestrial linkage, as she perceived that “the stream helps hydrate the trees that are out there” (urban Cooperative Extension patron). In a different example of aquatic-terrestrial linkages, as a conservation group discussed the forest as habitat, she talked about cascading benefits for the stream: “It [the forest] gives it more of a habitat for animals to live near the river, and so then if they’re near the river, then they can interact with it [the stream] in a natural way that could benefit it.”

Nature’s Negative Impacts on Water Quality and Nature as “Good and Bad”

Participants in every group thought that some of the flora and fauna we showed could negatively affect water quality or possessed both *good and bad* qualities, often using that phrase. This theme occasionally intersected with the other main themes of balance in nature or indicators of water quality (e.g., in reference to algae: “I’m thinking of it as a bad thing if it gets to be a lot” (tribal community member, female)). Focus group participants concentrated mostly on algae and beaver dams in describing negative and dualistic qualities. The first statements that participants offered following the prompt for the algae picture captured the range of perceptions expressed in conversations about algae: “Algae blooms and fish kills” (farmer); “Some algae is fine. Too much algae means it’s not balanced. Too much nitrogen” (conservation group member); “Negative, it’s a negative” (tribal community member); “Well, algae is a good thing and a bad thing” (urban Cooperative Extension patron); “Isn’t algae more of a pollution...” (community college student). Participants expressed concern about toxic red tides, invasive species, and impacts on oxygen in waterways. The following dialogue among the coastal fishermen, when they were presented with the algae photo, exemplifies the interacting themes of nature's negative effects, nature as good and bad, and balance:

> *I mean it's bad news. – Mmm-hmm. – Yep. – But we've gotta have it. You know that. – We need some algae. [Laughs] – But not that much. – No. – Certain kinds is good. – Those big blooms hurt you. – Yeah (9 males, with the remaining participants agreeing audibly or nonverbally).*

Discussions of the beaver photograph often reflected the notions of good and bad, with participants offering that beaver dams offered benefits such as slowing erosion and creating habitat, but that they also disrupt water flow, cause stagnation, and flood land (e.g., "they help filter – but they also block waterways" (community college students, 2 females). Compared to the typical responses, one participant offered an alternate non-anthropocentric view in considering beavers, that "if we weren't here, they'd be here, and maybe these things should be clogged up" (conservation group member, male). Finally, the discourse about beavers included perceptions by participants in three other groups that farmers had conflict with or negative connotations about beavers (e.g., "they cause a lot of trouble for farmers and landowners;" "farmers like to get rid of them;" "the farmers don't have much use for them"). Although the farmers we talked to did mention some negative impacts of beaver dams and a couple of members expressed not seeing any benefit, others in the group commented about beavers stopping sediment and slowing erosion "drastically". They articulated a wide range of views on beavers, from useful irrigation ponds to a "need to eradicate them," and expressed a "love/hate relationship," in part because they "slow erosion down…but they also back up water into the fields" or "destroy timber." However, comments about beavers from our farmer group did not appear to be appreciably different from the other groups, and we found it interesting that this was the only occasion when members of multiple groups put forth perceptions about how another stakeholder group may feel about flora or fauna.

Negative comments about other flora and fauna were minimal. Participants in a few groups pointed out potential issues with underwater vegetation, including overgrowth and invasive species. Only the conservation group members discussed freshwater snails and mussels in a negative context. One member of this group was familiar with native mussels and their imperiled status, but the group largely associated mussels and snails with invasive species, particularly from their prior experiences living in other geographic regions where invasive mollusks are problematic (e.g., Florida and invasive snails; New England and invasive mussels). Conservation group members also associated snails with population explosions in fish tanks and thought large populations of small snails in waterways was probably bad.

Flora and Fauna as Water Quality Indicators

Participants in every focus group used the term *indicator* in describing the relationship between the featured plants and animals and water quality. Across all of the focus groups, the term arose in the discussion of each photograph, though to a lesser extent in the discourse surrounding the streamside forest and beaver photographs than for others. Specifically, the comments about beavers centered on their presence as an indicator of good water quality; beavers indicated “clean areas,” a “somewhat clean body of water,” or “healthy environment.” Indicator comments about the streamside forest were more about runoff than in-stream water quality (e.g., references to the pictured adjacent farm field and comments about whether “soil quality” or “lush vegetation” condition was related to runoff). Fishes were described as an indicator of water quality more so than all the other items, and participants explained how fishes could signal good or poor water quality. One person described the intimate connection between fishes and water simply, yet eloquently:

> *Fish are the barometer of water quality. They tell you how good the water is – the quality of your water, if you will. – How so? – Healthy fish equals healthy water (tribal community member, male, and focus group facilitator).*

Another person expressed how fish may avoid poor water quality with a relatable analogy.

> *Well ya gotta look at it – as far as water quality, I think it's sort of like us going to a restaurant. If you go to a restaurant and it's dirty, or stuff on the floor, or it's got a C rating or something, most likely you're not going back. Well, that's the same deal with these fish. They're not going up these rivers and creeks with nasty water (coastal fisherman, male).*

Together, these quotations capture much of the sentiment expressed by other participants who referenced the presence of living, healthy looking fish as indicators of good water quality. Alternately, people talked about fish kills, the disappearance of fishes, or unhealthy looking fishes (e.g., with "sores or bumps") as indicators of poor water quality.

All six groups referenced algae as an indicator of water quality, and half of the groups (conservation group members, tribal community members, and urban Cooperative Extension patrons) commented on algae in terms that evoked themes of balance in nature (e.g., "…if that was a lake, that little bit of algae is not too bad, but for a pond it's way too much" (urban Cooperative Extension patron, female). Similar to her statement, others referenced the visibility of algae as an indicator of water quality issues, such as excess nitrogen, low oxygen conditions, or just generally "a negative indicator."

Vegetation was referenced as an indicator with similar frequency to algae, as was water clarity, even though water clarity in the photographs was not specifically highlighted by researchers. Participants thought healthy underwater vegetation was a sign of a healthy ecosystem (e.g., "it looks like that body of water is in a more natural state than if it is just a muddy bottom" (conservation group member, female). People also said it indicated habitat for other species, such as fishes, clams, dragonflies, or nursery grounds. Some people focused on changes they have observed in condition or quantity of underwater vegetation as an indicator of water quality issues:

> *Yeah, we don't have the grass to the banks like we used to back there in the early '90s – that long eelgrass; I don't know what kind it was…but in the 90s it was there – there's little spots, but it's not like it…it's not big – no, not like it used to be (coastal fishermen, 3 males).*

Others discussed overgrowth of underwater vegetation as an indicator of a system "out of balance," unhealthy looking plants as an indicator of pollution, or questioned whether species belonged there, particularly in the context of freshwater habitats (e.g., lakes and ponds). Some participants noted the water clarity shown in the underwater vegetation photograph or referred to their own prior observations related to water clarity. A typical position was that water clarity was important for healthy vegetative growth, and participants tended to interpret clear water, along with other cues (e.g., presence of flora or fauna), as healthy in their observations of the photographs and discussion of prior experiences. For example, in reference to the fish photographs, one person commented:

> *Well, it's a picture of two healthy waterways. I mean that's obvious. – Okay. And what makes it obvious to you? – 'Cause there's fish. – Living fish and everything looks healthy.*

– Clarity of the water. – And the clarity of the water, I mean… (farmers, 3 males, and focus group facilitator).

An Emphasis on Balance

The theme of balance in nature was emphasized in multiple contexts over the course of the focus group discussions, and we grounded our definition of this theme in the way participants used the term *balance* or talked about the quantity or prevalence of ecosystem components. As mentioned above in the negative impacts and indicator sections, one central idea was that participants perceived that too much (e.g., algae, underwater vegetation, or beaver dams) or the absence of a component (e.g., fish or underwater vegetation) signaled an ecosystem out of balance. We identified expressions of balance in nature in the discussions of each photograph to some extent, and it was most frequently associated with algae and fishes. The typical narratives about balance and algae centered on having too much. In talking about balance and fishes, group attendees regularly mentioned *invasive species* or used similar language (e.g., introductions). Invasive species concerns were also revealed for algae, snails, and mussels. One participant said of fishes that diversity matters, hinting at the need for functional balance in aquatic ecosystems:

> *I think there needs to be a variety of species because if it's just one, then it's probably, I don't know, an invasive species, or it's imbalanced. Whether there's too many predators, or too many bottom feeders or something. You just want a good balance of species that serve different purposes in that waterway (conservation group member, female).*

Participants in each group talked about balance in nature to some extent, but this theme was conveyed most commonly and consistently by the conservation group members and tribal community members. One conservation group member suggested that "catastrophic events," such as “tsunamis” or “hurricanes that can wipe out wildlife populations” are a part of balance. Balance was discussed in a management context by the farmers; they considered themselves an important factor in maintaining balance of wildlife, particularly underwater vegetation in ponds and for beavers (e.g., “you kind of got to manage them and knock them down to a healthy level”). At times, it was evident that some participants lacked surety about what constituted natural balance in a heathy system or held misconceptions about species or their interactions, explained below.

Expressions of Uncertainty

Participants in each group expressed some degree of uncertainty while discussing what, if any, connections photographed flora and fauna had to water quality. We identified these expressions of uncertainty evenly among most photographs, but uncertainty about freshwater mussels was common and it was rare for fishes. We also identified different kinds of uncertainty. There was some confusion about the language used by the researchers (e.g., “If it’s fresh water it wouldn’t be muddy, would it? It looks muddy.” (tribal community member, female); “I have a question. What is a streamside forest?” (urban Cooperative Extension patron, female)). Sometimes people were uncertain about how a component of nature might affect water quality. For example, people discussed pollen, leaves, or whole trees falling into rivers, noting the connection to aquatic systems, but they were unsure about potential impacts to water quality:

> *What I think of is, when I see all these trees and think about the spring here is these things, the pollen that comes from them…and whether or not…because it just will lay on the stream of water. So, what does that do to the water? I don’t know. – But, we’ve always had trees (tribal community members, 2 females).*

Sometimes participants identified a benefit to water quality, but they were uncertain about the process by which that benefit occurred (e.g., stating that mussels clean the water, and then expressing uncertainty when asked about how they clean water). People were occasionally unsure what purpose flora/fauna served, or they assumed it had a purpose in the ecosystem but expressed uncertainty about what it was. For example, in reference to mussels, a participant commented:

> *I would be curious…what is their purpose? In the freshwater, like in the creeks or tributaries. What is the purpose they serve, yeah. Because it's got to be something, I would imagine. Most of the places I've seen, a lot of 'em it's…the water is pretty clear (tribal community member, male).*

In this example, the participant later asked in jest, "Can I cheat? Because I just have to find out," and then shared the results of his smartphone internet search with the group. This was a memorable and singular instance during the study in which a participant took it upon himself in the moment to gain knowledge about an uncertainty.

Another related uncertainty we identified in the discussions was lack of familiarity with fauna featured in a photo. This was especially true for freshwater snails and freshwater mussels. The snail and mussel photograph discussions were the only occasions when people expressed that they were unfamiliar with the flora/fauna, had never seen one, or had never heard of them. One notable conversation about these lesser-known fauna implied a lack of educational opportunities or inadequate or poorly focused communication about freshwater mollusks (e.g., "don't seem to get a lot of good press"), where issues about invasive species are perceived as emphasized over information about native species:

But I can't remember seeing anything that told me, "Well, these are the good guys." And, you know, "If you see this type, you know that it's a good, healthy stream system." I don't know. For my mind, it all seemed like they're invasive. Because I don't hear what are the guys that are supposed to be there. – Yeah. We don't really hear about the shellfish or invertebrates that are in waterways. It's all about like usually the fish, or the plant life. But not the smaller creatures that have a role in the system. So, I think I would like to learn more about that, eventually. Or people should emphasize that a lot more. – You never hear about these things…unless they're a problem. – Hmm, yeah. – Unless they're a problem. And oftentimes, it's when the problem's gotten to a point where it's a dire problem. So, yeah, if you could…if people knew a little more about what maybe supposed to be there…like, your kids might see it because you're interested in it. But there would be other kids that would never see it because it's just not in their little world. So they don't know good or bad, so they got to run on the limited information that's given them (conservation group, 2 females and male).

Misconceptions about Nature

Participants conveyed a limited number of misconceptions relative to the overall content of the discussions. The misconceptions that we identified typically revealed gaps in ecological literacy that may be insightful for natural resource professionals to consider in their future public engagement and education efforts. One misconception that arose in both the urban Cooperative Extension and community college groups was that beavers are predators rather than herbivores; a few participants commented about beavers preying on fish or other species, and these comments either were substantiated in crosstalk or were uncontested. In the course of discussing the relation of fishes to water quality, one participant offered that they help to clean because "most fish are bottom feeders" and another participant echoed that sentiment (urban Cooperative Extension patrons). There was also a misconception about the connection between algae and oxygen by a participant in this group. In some dialogue about algae serving as an indicator of low oxygen, he commented: "what they do to get rid of algae is they put oxygen in – oxygenate – …. They force the air into it and it kills the algae." He seemed to have some knowledge of algae management (e.g., in ponds), but he misconstrued the mechanisms. Interestingly, throughout the conversation about algae and oxygen, no one in the group mentioned that algae makes oxygen. In the community college group, we heard participants discuss ongoing adaptation of flora and fauna to pollution (i.e., vegetation) or from the ocean to freshwater (i.e., fishes and algae). The comments implied rapid adaptation of individuals rather than species adaptation over generations, and there were no references to particular flora/fauna to indicate otherwise (e.g., migratory diadromous fish species).

Misconceptions were sometimes paired with expressions of uncertainty. For example in the discussion of beavers, one participant was unsure exactly what they ate, though her verbiage implied certainty about them being predators. In another instance, a misconception expressed by a conservation group member that large freshwater snails "seem like they should be there" but that seeing an abundance of "really small" snails is bad was coupled with an expressed uncertainty about their size (i.e., "And I don't know, maybe they stay that small"). Members of the conservation group also had a general perception that freshwater snails are bad, although this group had the fewest participants and we may not have exhausted possible perspectives on snails. There was also one hypothetical comment in this group about algae in lakes affecting people's wells, indicating some misconception about surface water and groundwater dynamics.

The only commentary farmers that we interpreted as a potential misconception was in reference to the photograph of the schooling fish (Figure 1, image 2, left panel), when two members said "the stocking rate…is too high – Yeah, a little heavy." This comment seemed to reflect the group's general emphasis on direct management. We did not identify any misconceptions expressed by coastal fishermen or tribal community members during the discussion of the photographs.

Commentary about Human Influences on Water Quality

Although we asked specific questions about human impacts on water quality in a portion of the focus group discussions prior to the photograph review (not reported here), and our questions about the photographs were directed toward considering impacts of the natural components featured (Topic Guide, Appendix 1), members of all groups mentioned human impacts to some degree during the discussion of the photographs. Human impacts were most commonly expressed by coastal fishermen and farmers when reviewing the photographs. However, all groups discussed human impacts on water quality at length when we inquired about the topic in other portions of the discussion. Though a few comments were on topics tangential to the photograph being discussed, most were about human influences related to the objects pictured. All comments were relevant to water quality, and negative human impacts were more frequently referenced than positive.

In general, comments about negative impacts by humans were clustered mostly in conversations about the algae photo, and they referenced pesticides or fertilizers or land management (e.g., high-density housing and other impermeable surfaces). Based on their own observations, members of the fisher group were particularly concerned about runoff from landscaping or agricultural pesticides (i.e., “Roundup,” “Dicamba,” and “mosquito spray”) and their impacts on algae, crabs, or other aquatic organisms (e.g., “That mosquito spray they use…is pretty toxic to peeler crabs, ain’t it?... And it killed bulls…three or four years ago.”). They also perceived that urbanization and areas of greater impervious density were associated with recent harmful algal blooms and associated fish and wildlife kills in the news (e.g., red tide in Florida was discussed during the fish photographs). A few other groups brought up negative impacts during conversations about the streamside forest (pesticide/fertilizer runoff) and mussel photographs (e.g., “cow poop is getting in the streams”).

Farmers discussed positive and negative human impacts equally, with an emphasis on management in both cases. They were particularly concerned about negative impacts of excess nitrogen applied by homeowners and golf courses in reference to the algae photo. The conservation group members specifically mentioned excess nitrogen as well, though they did not reference any particular source. Positive management themes highlighted by the farmer group included reducing algae; managing populations of beaver and underwater vegetation to reduce flooding or irrigation problems; and controlling, repairing, or preventing erosion to protect water quality (e.g., with tillage practices, mechanical repair, or riparian buffers). Other groups that mentioned positive human impacts during the photographs exercise (coastal fishermen, conservation group members, and tribal community members) expressed similar themes of land, water, or wildlife management, and the conservation group also talked about education (e.g., as demonstrated earlier in the quotation on native species information).

Connections to Values and Human Well-being

After the photograph portion of the focus group discussions, we specifically asked questions about the ways in which participants valued water resources and how water quality was related to their well-being. However, participants in every group talked about values or well-being during the photograph review before our prompts. Although comments about value or well-being comprised a small proportion of the photograph discussion, the fact that these issues arose illustrates that the photographs stimulated discussion about such connections to nature. The themes of value and well-being were expressed in conversations of every photo, but mussels were an exception. The only potential value connected with mussels was food provisioning. People related freshwater mussels to oysters or other marine bivalves and often discussed eating them, avoidance of eating them (e.g., "I know I don't eat a mussel"), or uncertainty about them (e.g., "Is this the ones we eat in stores? 'Cause I eat mussels. Is that the ones we eat?" (community college student, female)). In one case, it seemed clear that the participant was sure of eating freshwater mussels in the past (farmer group), and the coastal fishermen were confident they were not eating freshwater mussels. Where participants described eating or avoiding eating mussels, it was usually clear by the context of conversations that people were confusing freshwater mussels with marine species. Other than eating, the only value-related statement about mussels was conflict associated with environmental impacts of a highway construction project in the area and the mussels' presence in the watershed delaying or halting the project. "They'll stop a road" was the first reaction to the mussel photograph in the farmer group. We found it interesting that they were the only group to mention the highway project and conflict with environmental impacts (i.e., endangered mussel habitat and other stream/wetland impacts) because the issue had received extensive media coverage and two other groups were located geographically closer to the construction area.

Participants' commentary related to value and well-being typically pertained to aesthetic, recreational, or occupational qualities of water or the flora and fauna. A few participants appreciated beavers or the aesthetic beauty of their ponds. Other comments about irrigating from beaver ponds evoked an occupational value. One person said that algae was ugly and that snails have "beautiful shells." A few groups discussed recreational fishing while reviewing the photographs (e.g., "I love to fish;" "Fish can bring people to a river or lake to fish"). A participant in one group suggested that streamside forests in developed neighborhoods might help in "getting kids outside" and establish connections with nature "that they would then carry into their adult voting or civic engagement" (conservation group member, male). In talking about the benefit of underwater vegetation as habitat, coastal fishermen expressed notions that it also supports occupational well-being while providing recreational opportunities (potentially with aesthetic value) for tourists:

> *And also too, I put it on my side because of the ecotours…that healthy vegetation right here also is an ecosystem for a lot of the smaller little tiny fish that really grow in those grass flats. – The [tourists] love to see it. – They love seeing that…they love that (male and 2 females).*

In contrast to the potential value of nature's benefits to well-being, the photograph discussions sparked concerns about well-being by participants in four groups. An urban Cooperative Extension patron shared a story about the hazards of a beaver dam breaking, with the flooding trapping friends of hers in a cave downstream for nearly a day. While talking about snails as an indicator, farmers thought snails would not be found in the Neuse River because the water quality is “bad,” and one person expressed avoidance of the river, saying “I wouldn’t go in it.” The conversation continued into discussion about fish consumption advisories related to mercury and polychlorinated biphenyl contamination: “Well, you know it’s bad when they come and tell you don’t consume more than one catfish per month from the Neuse River ’cause of the mercury levels. I mean that’s bad” (male). This tangential conservation went on at some length, as some participants were unaware of the advisory and others were well informed. The snail photograph led to a conversation about food chains among the community college students, in which a couple of participants questioned whether “toxins in the water…affects us, too” when they consume fish or other seafood. A tribal community member also brought up concerns about fish consumption as she recalled that the James River was contaminated with Kepone (an organochlorine insecticide and now globally banned persistent organic pollutant) in the 1970s when she lived in Virginia. On reviewing the algae photo, another tribal community member said: “when I see something like that, I don't know what it consists of, so I don’t go in the water, and I don’t wanna fish out of it” (male). A college student was also concerned about algae, referencing recent news coverage of dogs that died after swimming in a pond contaminated with toxic algae.

DISCUSSION

Findings from these six focus group discussions provide baseline understanding of public beliefs and knowledge of ecosystem functioning related to water quality in central and eastern North Carolina. Our findings show that people identified several effects that flora and fauna have on water quality, including ecosystem functions that provide essential ecosystem services (e.g., provision of habitat and regulating services, such as water purification through filtering and cleaning). Such underlying functions are integral to the provision of benefits that people desire from surface waters and associated landscapes (e.g., drinking water resources, safe contact for recreation, and habitat for wildlife; Castro et al. 2016; Weber & Ringold 2019). Overall, our findings suggest an encouraging congruence of public beliefs with expert science, offering some common ground, similar language, and opportunities for connecting with communities on important issues that highlight or threaten ecosystem functioning and resulting ecosystem services that link environmental and human well-being.

Relation of Participant Beliefs to Ecosystem Functions and Services

Focus group participants shared a wealth of information detailing their beliefs about floral and faunal connections to maintenance, improvement, and degradation of water quality. Their most prevalent views reflected that participants in all groups had some understanding of water quality maintenance and improvement provided by flora and fauna, especially through functions of filtering and cleaning. Beliefs about these positive effects on water quality align well with scientific understanding about functional ecology in aquatic systems, especially for filtering freshwater mussels and other bivalves (Haag 2012; Vaughn & Hoellein 2018), grazing snails (Brown & Lydeard 2010), beneficial native underwater vegetation (Weller 1994; Palmer & Richardson 2009), and adjacent riparian vegetative communities (Lowrance 1998; Table 2). Native freshwater mussels perform a host of functions that support water quality, including filtration of algae, bacteria, and other particles (Haag 2012). Snails can comprise a majority of invertebrate biomass in some freshwater systems, such that their grazing activity is an important mediator of nutrient cycling and algal productivity (Brown & Lydeard 2010; Johnson et al. 2013). Conservation of vegetated habitats along waterways has been a focal management strategy for nutrient reduction (i.e., filtering capacity) in impaired waterways in many states, including several river basins in central and eastern North Carolina (NC DEQ 2020).

Participants' identification of habitat as an important function of underwater vegetation also aligns well with the scientific consensus that submerged aquatic vegetation is an essential habitat component of estuaries, freshwaters, and emergent wetlands. These habitats are plentiful in coastal North Carolina (within our project area), where the Albemarle and Pamlico Sounds join to make the second largest estuarine complex in the conterminous United States (US EPA 2001), providing half of the fish nursery habitat along the US eastern coast (Mallin et al. 2000). While participants associated riparian forests with habitat to a lesser extent than underwater vegetation – probably because the nature of our questions focused on water quality – these observations were consistent with expert knowledge that riparian areas serve as habitat for terrestrial, semiaquatic, and amphibious species. We also found it interesting that the only two groups to identify the streamside forest as habitat were those in the most urbanized locations (urban Cooperative Extension patrons and conservation group members), where habitat for wildlife is more scarce and fragmented. Such field borders are known for providing habitat in addition to the filtering functions mentioned above, and they have been incorporated into federal habitat management strategies (e.g., Conversation Reserve Program, Wildlife Habitat Incentives Program, administered by USDA through the US Farm Bill legislation; USDA 2020). Riparian areas also function as important corridors that connect areas of habitat for a wide variety of species in an increasingly fragmented landscape, resulting from more intensive human land uses (e.g., urbanization) (Beier & Noss 2008; Calçada et al. 2013; Haddad et al. 2015).

The ecological functions identified by participants as providing benefits for, or maintenance of, water quality underpin several regulating and habitat-related ecosystem services recognized by the scientific community, including water purification, erosion control, sediment retention, erosion prevention and regulation, biodiversity, refugia (i.e., nursery or migration habitats), and life cycle maintenance (Costanza et al. 2017). Moreover, the comments that participants offered related to their values and well-being translate to several well-documented cultural ecosystem services, including aesthetic and educational values, recreation and ecotourism, or physical and experiential interactions with nature (Millennium Ecosystem Assessment 2005; Costanza et al. 2017; Sanna & Eja 2017). Both freshwater and marine surface water ecosystems support these benefits to human communities, including food production of high quality protein (i.e., fisheries), water supply, carbon and nitrogen sequestration and cycling, recreation, flood control and habitat, among others (Brauman et al. 2007; Baskett & Halpern 2009; Palmer & Richardson 2009). Engagement with communities about *conserving* ecosystem services, then, may benefit from straightforward communication highlighting the connections between desired benefits and the ecosystem components that function to support them (Keeler et al. 2012).

The potential for nature's components to cause water quality degradation as expressed by our participants – particularly by algae and invasive species – are similarly concordant with concerns within the scientific community about ecological disservices. Research on harmful algal blooms has been an active area of research for decades and continues to be relevant because of the increasing prevalence and duration of blooms affecting drinking water sources, diminishing safe contact with recreational waters, upsetting the balance of ecosystem functioning, and threatening biodiversity (Huisman et al. 2018; Reid et al. 2018). Harmful algal blooms have been linked to excess nutrient input from human land uses (urban/suburban lands, agricultural areas) or a shift in nutrient availability (e.g., release from phosphorous limitation). The general distaste for algae expressed by participants may be useful in contextualizing messaging about responsible use of consumer fertilizer products, especially in suburban locales.

Similarly, invasive species introductions in aquatic habitats have been a pervasive issue, from angler introductions of sport- or bait-fish releases to more calamitous introductions, such as dreissenid mussels into the Laurentian Great Lakes system and Asian Carp (*Hypophthalmichthys* spp.) into the Mississippi River Basin (USGS 2020). These issues highlight the social-ecological nature of aquatic ecosystem functioning and resilience, and opportunities for improved human management practices to contribute to water quality improvement and provision of desirable ecosystem service outcomes.

Water Quality Indicators

Participants' emphasis on flora and fauna as indicators of water quality has several important implications. First, their focus on indicators aligns with previous research on metrics that people have used in judging water quality. For example, others have found that people perceive the presence of algae as an indicator of polluted waters (David 1971; Gartin et al. 2010), and waterways with underwater vegetation have been perceived as healthier (House & Sangster 1991; Cottet et al. 2013). Second, participants' attention to flora and fauna relates closely to some scientific methods used for monitoring waterways and identifying water quality problems (e.g., fish or macroinvertebrate indices of biotic integrity (IBI, Karr 1981; Barbour et al. 1999); the use of mussels for environmental biomonitoring (Van Hassel & Farris 2007)). Water quality assessment tools, such as various IBIs, capture metrics that integrate species presence/absence, abundance, richness, and biodiversity. Such natural constituents are equivalent to features that focus group members said were indicators of water quality status or natural balance. Third, there is an entire field of research and a scientific journal (i.e., Ecological Indicators) dedicated to defining, measuring, quantifying, and monitoring ecological factors that inform development of reliable indicators for ecological management or the presence, status, and magnitude of ecosystem services. However, there seems to be limited research highlighting public recognition or use of flora and fauna as indicators of water quality. West et al. (2016) emphasized the need for interdisciplinary research linking ecology and public perceptions and suggested that public and expert mental models of water quality may be more similar than realized. We assert that our findings lend support to that notion.

One promising aspect of potential concurrence between public and expert mental models of water quality and its indicators is that professionals can leverage the public recognition of generalized indicators to improve communication and educational materials about ecosystem services. Professionals may also emphasize the relation of public observations to expert metrics, thus enhancing ecological literacy and improving public confidence in their observations. There also may be opportunities here for engaging and partnering with communities about their water quality concerns, such as using participatory action research, in which communities drive research questions and participate in investigations (Berg & Lune 2012). Such research endeavors may help improve their ecological perceptions as they learn from experts, while scientists benefit from becoming more informed about social issues important to water quality stakeholders. Finally, ecosystem components that serve as water quality indicators may be an important ecosystem service in their own right. The body of research on “ecosystem service indicators” revolves around experts using ecological indicators to describe, quantify, value, or otherwise assess ecosystem services (e.g., Czúcz et al. 2018). Among the many ecosystem services compiled by Costanza et al. (2017), which includes multiple categorization paradigms, none lists a service of indicators for the public. The closest approximation is the ecological benefit of science as a cultural value (Costanza et al. 1997), but that implies use for professionals with society as a beneficiary. We suggest that indicators of water quality (e.g., indicators of safe contact, signals of alarm, imbalance, or reduction in ecosystem resilience) and other natural communities should be considered within the realm of cultural ecosystem services, potentially as part of knowledge systems or educational values (Millennium Ecosystem Assessment 2005).

Prior Experience – An Important Filter

Lived experience and locally relevant events (e.g., hurricanes, algae blooms) are influential in people's understanding of the world around them (Ambrose et al. 2010; Jones et al. 2011), and prior experiences were an important filter through which participants in our groups perceived functions of the flora and fauna they reviewed. Members in every group relied upon their own previous observations as they derived answers to our prompt about organismal connections to water quality. Although this may seem like common sense, research on teaching has emphasized the importance of activating prior knowledge for learning (Ambrose et al. 2010), and natural resource professionals would benefit from learning about their audiences and using this technique when engaging with the public on technical topics. Participants in every group expressed some basic level of technical knowledge that influenced their responses (e.g., underwater vegetation needs sunlight, fishes that do different jobs); technical knowledge was especially evident in talking with coastal fishermen and farmers, who relied on occupational experiences as they assessed the influence of flora and fauna on water quality. Coastal fishermen had detailed knowledge of aquatic ecosystems, and farmers' perceptions were influenced by their close ties with and management focus on working lands. Urban Cooperative Extension patrons mentioned prior experiences markedly less than other groups during the photograph exercise. These participants simply may have chosen not to share prior experiences, or they may have fewer or different prior experiences than other participants and more limited access to waterways. Understanding an audience's background is important for establishing rapport and effective communication, especially between experts and non-experts, regardless of the subject matter (Morgan et al. 2002; Ozesmi & Ozesmi 2004; Ambrose et al. 2010; Baron 2010; Alda 2019). Issues experienced through news or other media outlets also influenced perceptions (e.g., red tide, toxic algae, and invasive species), as did pop culture references in a couple groups. Natural resource professionals should be cognizant that news, media, or pop culture connections may be the only relevant connections some people possess, especially for lesser-known fauna (e.g., SpongeBob and aquatic snails). Moreover, we must be aware of differences in lived experiences and locally impactful events to leverage these potential connections and enhance understanding. Such considerations may be equally important in communicating about ecosystem services with policy and decision makers and legislators, especially at the local, municipal, state levels.

Knowledge Gaps and Opportunities for Engagement

The misconceptions and uncertainties that we identified in this study signal opportunities for addressing knowledge gaps when communicating with communities about ecological functions and services. People are sometimes reluctant to be wrong or share their uncertainty, and our observations of these knowledge gaps are limited to what participants expressed. Most misconceptions were mentioned only one time. The candor and willingness of our participants in this regard in the comfort of a familiar group setting allowed us to learn more about misconceptions and uncertainties that may be pervasive in public knowledge about natural resources, their functions, and their benefits. For example, on recognizing that two groups had misconceptions about beavers being predators or piscivores, we conducted an internet search of *beaver diet*. The first of Google's *people also ask* suggestions on that day was *do beavers eat fish*, suggesting this may be a relatively common misconception or uncertainty. We believe that it is important to be aware of such misconceptions, even if some were not directly related to our research questions about water quality. While we cannot conclude that these are widely held beliefs, taken together, they may indicate that the public has misconceptions about the basic biology and ecology of the flora, fauna, or habitats represented in our photographs, and may have misconceptions about other parts of ecosystems as well. Though less prevalent than other information our participants shared, misconceptions and uncertainties potentially highlight expert blind spots; scientists and resources managers should include information they may take for granted as common knowledge (e.g., beavers are herbivores) when engaging with communities about particular species, habitats, or ecosystems.

Just as knowledge gaps, inaccurate information, and misconceptions can hinder learning in formal settings (Ambrose et al. 2010), they also may hinder public understanding of ecosystem functioning and, ultimately, services related to water quality. The awareness of the conservation group members that information about freshwater mollusks was lacking in their own experience and generally in the media (e.g., emphasis on invasive species) may provide insight for our comparatively greater detection of uncertainty about snails and mussels among the groups. It is striking that such comments came from a conservation group – people who may seek knowledge and feel more informed about nature. For them, mollusks were a known unknown; for communities that lack access or are less connected with nature, such topics may be unknown unknowns. Moreover, we found it thought provoking that the most common benefits participants mentioned – filtering and cleaning – were most frequently associated with mussels and snails – the fauna people expressed uncertainty about. These terms, *filtering and cleaning*, were typically used in a generalized way. This may signal an example of both common ground for engaging the public on ecological functions (i.e., meet them where they are), and an opportunity for sharing more specific information (e.g., removal of pathogens, nutrient retention) to empower a greater understanding of how such functions relate to ecosystem benefits they desire (e.g., swimmable waterways, water clarity; Weber et al. 2019).

Limitations

Although the photographs included in focus group discussions were researcher-introduced and may limit the full applicability of participant responses (e.g., photographs could have included an organismal microscope photograph of algae, rather than a visible (albeit small) bloom), overall, we think that photographs helped participants think about the potential water quality associations, especially for lesser-known flora/fauna they may never have seen before (e.g., snails, mussels, underwater vegetation). While caution should be used in generalizing the results of our study, they may provide some insight on attitudes beyond our study area (e.g., southeastern US Piedmont and Coastal Plain regions or beyond). Follow up research in a wider geographic area and use of additional validation techniques (e.g., interviews or content analyses) with appropriate representation of all populations (e.g., younger, Hispanic/Latinx, or different primary language populations) and informed by the views expressed by our participants may help elucidate the breadth of their utility.

Conclusions

We conducted a study investigating public understanding of floral and faunal connections to water quality maintenance by listening to participants' views in six focus group discussions across central and eastern North Carolina. Participants identified several functions performed by flora and fauna that mediate water quality and contribute to ecosystem maintenance and resilience, and a particular focus on algae as a detriment to water quality. They also identified these ecosystem components as important indicators of water quality. Many of their determinations were informed by their prior knowledge, and we discovered some misconceptions and knowledge gaps along the way. Our findings and participants' expressions may help to inform the design of similar studies across regions or development of broader scale studies on water quality topics (e.g., survey instruments). Importantly, many of the public impressions about ecosystem functioning aligned with professional (expert) knowledge. Coming from a background of training in ecology, this research has been an enriching exercise that has made me a better scientist by working closely with communities on a mutually important topic of water quality. Seeking deeper understanding of social perspectives may help experts improve their relatability in engagement with communities to cultivate awareness of and social value for species that support aquatic systems and well-being, especially for lesser-known fauna, such as freshwater mollusks. Furthermore, engaging and partnering with communities in research that addresses their concerns about ecosystem-mediated well-being may enhance their understanding, thus improving knowledge equity and empowering them to be more influential in decision-making power relations (Berbés-Blázquez et al. 2016). Given that much ecological research currently includes little of social dynamics beyond societal effects on study systems (e.g., urbanization), ecological research and management communities may benefit by improving on their awareness of social science research relevant to their goals and working to incorporate more holistic socio-ecological approaches to support desirable outcomes for the conservation and sustainability of the inherently inseparable constructs of ecosystems and society.

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TABLES

Table 1. Participant Demographics. Some category percentages may total slightly higher than 100% due to rounding. Two participants did not turn in a demographic survey.

Demographic Categories	Count	Percent
Gender		
Female	32	56%
Male	25	44%
Age		
18 – 24	6	11%
25 – 44	12	22%
45 – 64	20	36%
65 and over	17	31%
Race		
American Indian	16	29%
Asian	1	2%
Black or African American	12	22%
Two or more races	1	2%
White	25	46%
Ethnicity		
Hispanic	1	2%
Not Hispanic	40	70%
Did not answer	16	28%
Community Type		
Rural	43	78%
Suburban	10	18%
Urban	2	4%
Educational Attainment		
Some high school-no degree	3	5%
High School or Equivalent	7	13%
Some College-no degree	13	24%
Trade School	1	2%
Associates	12	22%
Bachelors	12	22%
Masters	3	5%
Doctorate	3	5%
Other	1	2%
Annual Household Income		
Less than $20,000	11	21%
$20,000 – 39,999	7	14%
$40,000 – 59,999	13	25%
$60,000 – 79,999	9	17%
$80,000 or more	12	23%

Table 2. Participant impressions of major floral and faunal connections to water quality, the photographs they associated connections with most frequently, and examples that show alignment of participant beliefs with expert knowledge about aquatic flora and fauna, with references to literature sources.

Water Quality Connection	Floral/Faunal Association	Examples of Expert Knowledge	References
Filtering	Freshwater Mussels	Filtration of algae, bacteria, particulates	Haag 2012; Vaughn & Hoellein 2018
	Freshwater Snails	Grazing mediates nutrient cycling and algal productivity	Brown & Lydeard 2010
	Underwater Vegetation	Nutrient and carbon sequestration	Weller 1994; Palmer & Richardson 2009
	Streamside Forest	Nutrient and pollutant filtration	Lowrance 1998
Cleaning	Freshwater Snails	Grazing mediates nutrient cycling and algal productivity	Brown & Lydeard 2010; Johnson et al. 2013
Habitat	Underwater Vegetation	Essential fish habitat, nursery grounds	Mallin et al. 2000; US EPA 2001; Palmer & Richardson 2009
	Streamside Forest	Habitat on field borders, corridors for wildlife movement	USDA 2020; Beier & Ross 2008; Haddad et al. 2015

FIGURES

Figure 1. Focus group photographs. 1. Streamside Forest; 2. Fishes; 3. Beavers; 4. Algae; 5. Underwater Vegetation; 6. Freshwater Snails; 7. Freshwater Mussels.


1


2A


2B


3

4

5

6

Note. Photograph credits and sources: streamside forest by Duk, Wikimedia Commons via US Department of Agriculture Southern Research Station; schooling fish by Will Parson, Chesapeake Bay Program via Flickr Creative Commons; Largemouth Bass by Fishinpedia; Beaver by J J, Flickr Creative Commons; Algae by Minnesota Pollution Control Agency (MPCA Photos) via Flickr Creative Commons; Underwater Vegetation by Will Parson, Chesapeake Bay Program via Flickr Creative Commons; Freshwater Snails by Tim Lane, Flickr Creative Commons; Mussel in sand by Gary Peeples, US Fish and Wildlife Service via Flickr Creative Commons; Mussels with calipers photo by Tim Menard, US Fish and Wildlife Service via Flickr Creative Commons.

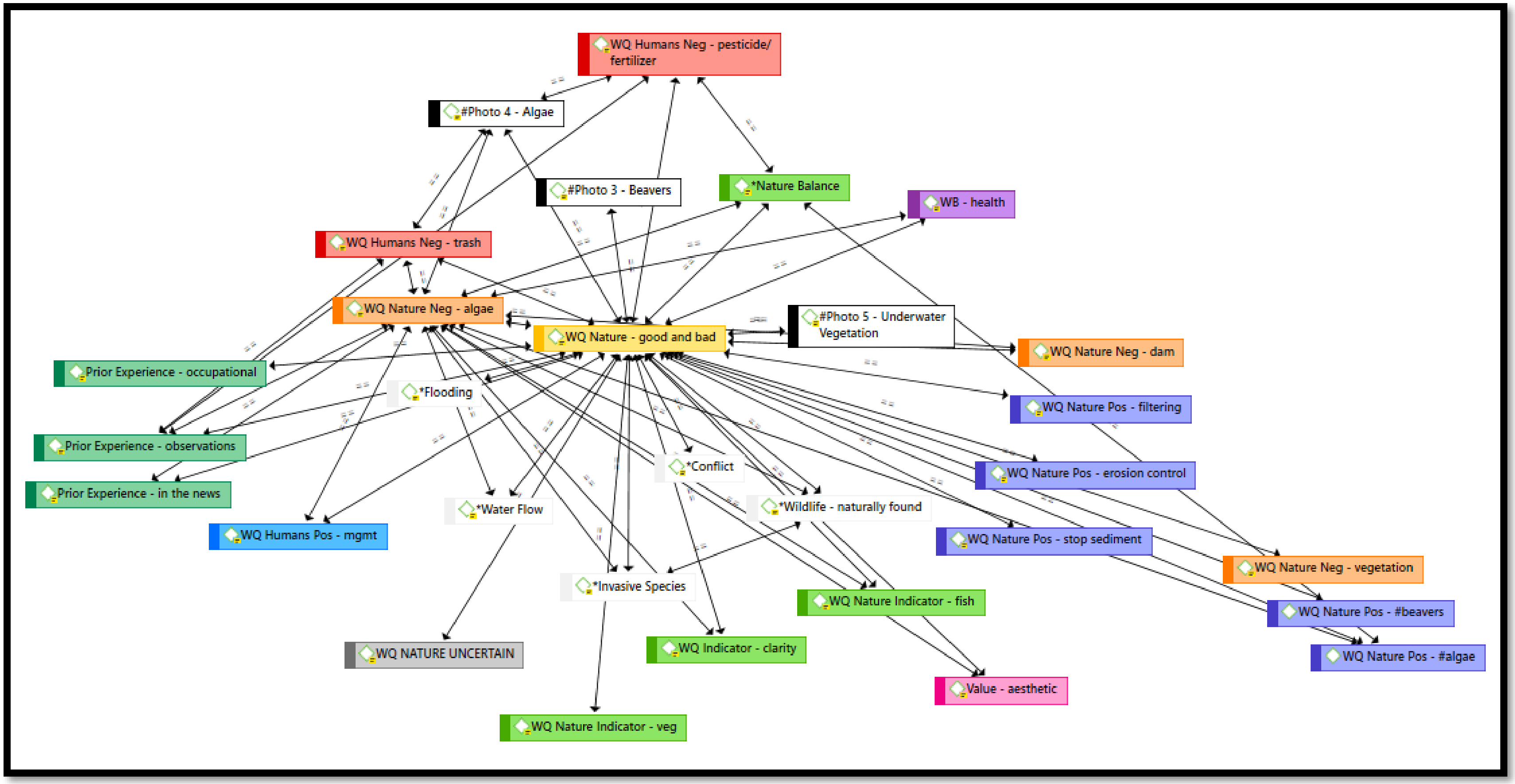

Figure 2. Example code network of co-occurring major themes throughout the project, showing the relationship of *nature good and bad* with photos, code categories and their sub-codes (categories are colored the same), and administrative codes (uncolored with asterisk) associated with coded quotations. Abbreviations key: WB = well-being, WQ = water quality; Pos = positive; Neg = negative.

APPENDICES

Appendix 1. Focus Group Topic Guide

A) Informed Consent

Before beginning the focus group, we will register participants and solicit verbal consent. Staff will read the consent form aloud, answer any questions and get verbal consent from participants. In this case, the principal investigator will sign to affirm that participants provided verbal consent to participate. Any participant who does not consent to participate will be allowed to leave.

B) Introduction to discussion

We will introduce ourselves and let participants know our study is focused on waterways, aquatic life, and how people and nature interact. We will then inform them that the overarching goal of this project is to understand people's beliefs about factors that impact water quality for better and worse, and what they value about freshwater resources. We will ensure participants that we are there to learn from them and that there are no wrong answers. Finally, we will explain that all information shared in the session will be treated confidentially, and no participants will be named in any future use of this information.

<u>Introductions</u>: What was your most recent experience near a freshwater stream, river, lake, or pond? What were you doing there?

C) Main Questions

The first question will help us understand how you judge a waterway's quality.

1. Thinking back to your last experience with a stream, river, lake, or pond, how would you describe the water's quality?
    - Was it generally good or bad?
    - What kinds of things made you think of the water quality as good or bad?
    - How did your thoughts about the water quality affect your activities?

The next two questions will help us understand what you think about things that cause water quality to change.

2. What are the main things you believe negatively affect the health of rivers, streams, lakes, or ponds?
    - *Probe for a variety of answers; try to exhaust the potential sources participants can think of.*
    - Are there things that humans do that negatively affect the health of rivers, streams, lakes, or ponds?

- Are there things that other living things do? What about naturally-occurring events?

3. What are the main things you think of that positively affect the water in waterways?
    - *Again probe for a variety of answers.*
    - Are there things that humans do that positively affect the health of rivers, streams, lakes, or ponds?
    - Are there things that other living things do? What about naturally-occurring events?

<u>Photo Exercise:</u> Introduce a photo exercise, where participants will view a variety of printed pictures and be asked whether they think the object in the photo affects water quality, and if so, whether it has a positive or negative impact and why. If, in their mind, the object has something to do with water quality, we'd like them to tell us the connection. Individually, participants will be given a few moments to look at the photos (they may choose to jot notes or put photos into stacks of objects with no impact, with a positive impact, or a negative impact). The facilitator will then show each photo from the sorting exercise one-by-one and ask participants to share in which stack they sorted the photo and why. The final photo will be of freshwater mussels, to allow ample discussion time.

- For this photo, we will also ask: "What do you know about freshwater mussels?"

The last set of questions will help us to understand how you feel about the relationship between water resources and your well-being.

4. How does water quality in your area affect your well-being? Your family's?
    - As a drinking water source? Occupational?
    - For recreation?
    - For any cultural, spiritual, or aesthetic benefits?
    - Are there any drawbacks or negative impacts of water quality in your area?

5. Thinking back over the last year, what water-related experiences have you valued the most?
    - Why? What made those experience valuable to you?
    - What could improve the value of future water-related experiences?

6. Over the last year, have any of your water related experiences been concerning or detrimental?
    - Why? What made those experiences concerning or detrimental?
    - Did you avoid water-related activities out of concern?

D) Wrap-Up

Following these questions, the facilitator will summarize the participants' comments and ask for any clarifications. After any input is recorded (on a flip chart or notepad), the facilitator will administer a paper-based **sociodemographic survey** (i.e., gender, birth year, level of formal education, household income, race, ethnicity, urban/suburban/rural resident). As with informed consent, a staff person will read categories aloud. We will collect these anonymous surveys in an envelope. Finally, the facilitator will thank participants and provide them with compensation.

Appendix 2. Codebook containing code categories and data driven sub-codes applied in the qualitative analysis of focus group transcripts. Code totals within each category that aligned with prevailing themes are listed at the top in the order they are discussed in the results. Code Categories follow in all capital letters, with sub-codes listed below each code category. Sub-code names were selected to reflect participants' own words. Grounded is the number of times a code was assigned to a transcript quotation.

Code	Code Definition, Comments, and Examples	Grounded
	Prevailing Themes and Code Totals within Themes	
Water Quality (WQ) Nature Positive, Effects	All codes within the WQ NATURE POSITIVE category that specified effects (e.g., filtering, cleaning).	114
WQ Nature Positive, General	All codes within the WQ NATURE POSITIVE category that coded general comments, and were sub-coded with just the photo they occurred with (i.e., the quotation did not say *how* something was good, just that it was).	21
WQ Nature Negative	All codes within the WQ NATURE NEGATIVE category.	69
WQ Nature Indicator	All codes within the WQ NATURE INDICATOR category (i.e., flora/fauna).	90
WQ Indicator	All codes within the WQ INDICATOR category (e.g., clarity)	20
WQ Humans Negative	All codes within the WQ HUMANS NEGATIVE category.	34
WQ Humans Positive	All codes within the WQ HUMANS POSITIVE category.	20
Value or Well-Being (WB)	All codes within the VALUE or WELL-BEING categories.	28
Prior Experience	All codes within the PRIOR EXPERIENCE category.	70
Total	**Sum of times that prevailing theme codes were assigned to quotations.**	**466**
	Code Categories and Sub-codes (Listed Alphabetically)	
• MUSSEL FAMILIARITY	Comments about what people know about freshwater mussels, or what they do not know - mostly captures comments responding to "what do you know about freshwater mussels" and ensuing conversation (e.g., "aren't they protected?"; "bivalves, they call them."	30
• Mussel Familiarity - eating	Comments or conversation about eating mussels. Often about whether or not people have eaten them. Some comments capture people talking about eating mussels, that may actually be about marine mussels, but they are not recognizing the difference (e.g., "I ain't never cooked that;" "it's similar to an oyster, have you tried those?").	27

Code	Code Definition, Comments, and Examples	Grounded
• Mussel Familiarity - oyster/clam	Comments where people compare freshwater mussels to other bivalve species they may be familiar with, such as oysters, clams, other mussels, or try to make sense of them with reference to other bivalves (e.g., "that freshwater mussel is first cousin to an oyster"; "they do the same jobs". *Once all groups are coded, inspect for frequency of comments about "marine mussels" to discern if there is some additional value in that association as opposed to bivalves of other names.	21
• PRIOR EXPERIENCE	Code Category for comments describing prior experiences or how participants related a concept to something familiar to them.	0
• Prior Experience - in the news	Comments about things people recall encountering in the news or indirectly, but did not experience themselves (e.g., red tide in FL, water crisis in Flint, MI, deadly algae killing dogs in ponds).	6
• Prior Experience - natural event - hurricane	Comments about experiencing a hurricane.	3
• Prior Experience - natural event - rain	Comments about negative experiences with rain	2
• Prior experience - none	Comments about having no prior experience with something (e.g., "I don't recall ever seeing one").	4
• Prior Experience - observations	Comments about specific things people remember observing; may be specific to a location or a time -- has some level of specificity beyond (e.g., "as a boy I walked across Mill Creek during June & July when there was no water"; "but I mean, I've seen them on the ground every now and then. I feel like I see them more now than before with all this rain").	48
• Prior Experience - occupational	Comments about experiences related to work (e.g., "we irrigate out of two beaver ponds"; "I used to trap beavers..."; "it used to be loads of grass when we were scalloping").	7
• Prior experience - pollution	Comments about experiences related to pollution (e.g., used to live in Richmond and Kepone was in the water).	1
• Prior Experience - recreation	Comments about recreational experiences; may be co-coded with "Well-being" or "Value" codes if those apply, but may also stand alone if the comments just notes something they were doing (e.g., we stayed at the creek a lot hunting and fishing";	4

Code	Code Definition, Comments, and Examples	Grounded
• Prior experience - water quantity	Comments about experiences related to the amount of water, such as flooding, drought, or high water (e.g., "I was near the family pond and noticed how high it's been in the last 18 months"; "it's never been wet this long"). Typically co-coded with codes that describe the reasons for water quantity (e.g., Prior experience - natural event - hurricane) or the context (e.g., "concern").	1
• VALUE	Code Category. This category and the codes within it are not yet well grounded. May work well for comments that align with types of ecosystem service values (i.e., cultural, provisioning, regulating, or supporting services) Occupational may be provisioning, but might be something a little different than consumptive. Alternatively, it may be that these were things people talked about after being asked about what they value.	5
• Value - aesthetic	Comments about appreciating the beauty, view, or sound of something; language that suggests a value for aesthetic reasons; may also be in response to prompts about what people value (e.g., "the ding batters love to see it").	7
• Value - provisioning	Comments about a tangible good or service (direct use) that nature provides (e.g., 'we irrigate out of two beaver ponds' expresses the provision of water for irrigation...that one is co-coded w/ Value/WB - occupational to show the connection to occupation).	5
• VALUE/ WELL-BEING (WB)	Code category for comments that relate to things that have value for improving, contributing to, and maintaining well-being. For comments where it is difficult to discern a difference between value and well-being, or that something is valued because of its contribution to well-being. May become clearer with more coding.	1
• Value/WB - occupational	Comments about how water quality/nature affects occupational well-being/livelihoods or how people value water resources because of their contribution to occupation (e.g., “we can't farm without good water"; and if his water ain't right, he ain’t gonna buy his product"; comment about healthy water being important for ecotours so the animals can live and be seen there).	4
• Value/WB - recreation	Comments about how water quality affects recreational well-being; opportunities for recreation, or how recreational opportunities are valued (e.g., "well if you ain't got good water, you ain't gonna have no fish"; "and it's to get away. You know, just take your mind of what's worrying you"). May be in response to prompts about value and well-being or unprompted comments that express recreational value, enjoyment, or connection to well-being.	7

Code	Code Definition, Comments, and Examples	Grounded
• WELL-BEING (WB)	Code Category for comments about how water is related to well-being. Careful in applying these codes or making new ones - consider whether WB or Value/WB is the best fit. Could be in response to prompts about how water relates to well-being or unprompted comments about nature or water that evokes connotations about impacts to well-being, either positive, negative, or neutral. This category and its codes should be limited to those comments that seem strictly about well-being.	1
• WB - avoidance	Comments about avoiding water or nature activities out of concern for well-being (e.g., "I wouldn't go in it" in reference to the Neuse River; "algae to me...when I see something like that...I don't go in the water, and I don't want to fish out of it").	4
• WB - drinking water	Comments about how drinking water relates to well-being. The current quotation that is coded discusses not wanting to have bad drinking water, being on city water or a well and trusting who manages the water. May also apply to comments about positive or negative issues related to drinking water and well-being (e.g., "well, it's safe to drink, but don't drink it. That's what they tell us. The gray water... the boil advisory.").	1
• WB - health	Comments about how water/nature may affect well-being related to health (e.g., "I can't help but think that algae does have some kind of healing qualities...for the skin"). Not yet well grounded...might consider changing this to a Value/WB category if the quotations warrant it.	3
• WQ HUMANS	Code Category for the ways in which humans may affect water quality. May collect quotations here that do not have a good home yet, and then examine collected quotes for commonalities to determine if new sub-codes are warranted.	0
• WQ Humans - farming	Observations/comments related to farming that do not have a positive or negative connotation (e.g., you've got a farm field right next to it..."). The only time we have used this code so far was someone describing the streamside forest photo.	5
• WQ Humans - management	Observations/comments related land/water management that do not have a positive or negative connotation. The only use so far was a story about developing land and using other land as an offset.	5
• WQ Humans - pesticide/fertilizer	Comments about pesticide/fertilizer use that are either neutral, or do not have a pos/neg connotation. (e.g., (you've got a farm field right next to it so we know that there's being industrial fertilizers and things.")	3

Code	Code Definition, Comments, and Examples	Grounded
● WQ Humans - uncertain	Comments in conversation about human impacts where someone expresses uncertainty. (e.g., in relation to pesticide runoff "I don't have a good handle on that.").	1
● WQ Humans - urbanization	Comments about urbanization. May be co-coded with impervious surfaces or others, but urbanization seems to evoke a potentially broader suite of impacts. (e.g., I mean you got urbanization heavy duty there till you get up around Corolla.").	4
● WQ HUMANS NEG	Code Category for the ways in which humans may negatively affect water quality. May collect quotations here that do not have a good home yet, and then examine collected quotes for commonalities to determine if new sub-codes are warranted.	2
● WQ Humans Neg - contaminants	Comments about contaminants (either in general or specific) polluting waterways (e.g., "contaminants that's coming out of the air....and gets in waterways"). Differs from "Prior Experience - neg - pollution" code that describes someone's past experiences with pollution/contaminants.	2
● WQ Humans Neg - farming	Negative comments about farming affecting water quality (e.g., "farmland, pesticides, fertilizers running through the stream...killing the fish and water life.") So far, co-coded w/ WQ Humans - neg - pesticide/fertilizer; may be useful to have both now because pesticides/fertilizers could be discussed separate from farming.	6
● WQ Humans Neg - golf courses	Negative comments related to golf courses during water quality discussions (e.g., comments about nitrogen leading to "and the golf courses"; "They irrigate on rainy days. I ain't lying.").	2
● WQ Humans Neg - houses	Negative comments related to houses or housing developments during water quality discussions ("The subdivisions are gonna direct the water into one spot"; and in reference to nitrogen "It's coming from those houses around here. It's those lawns.")	2
● WQ Humans Neg - impermeable surfaces	Comments about negative effects on water in relation to impermeable surfaces, such as concrete, etc. (e.g., "Hey I wanted to add impermeable surfaces to the negative."; But it's getting more urbanized; hardened all the time.").	3
● WQ Humans Neg - mgmt	Comments about negative effects on water in relation to land use, land management, or wildlife management - human management of natural resources (e.g., in reference to pesticide application to the land "there's a lot of cases directly in the water"; "I'd say the stocking rate on that one...is too high."; "....when you see algae in the water you got a nitrogen problem.").	6

	Code	Code Definition, Comments, and Examples	Grounded
•	WQ Humans Neg - pesticide/fertilizer	Comments about negative effects on water in relation to pesticide/fertilizer use (e.g., "effects from fertilizers from agriculture near streams...	18
•	WQ Humans Neg - pollution	Comments about negative effects on water in relation to pollution, where the participant speaks in more general terms of "pollution" or names something different than the specific subcode that have commonly come up (e.g., "the people are polluting. Like say when you go out and you just throw out your drink or whatever into the ground and eventually the runoff will get into the water..").	1
•	WQ Humans Neg - trash	Comments about negative effects on water in relation to trash (e.g., "the trash is unreal, I mean..."; "and it's not just the trash people are throwing out really. These trucks run up and down the road.").	2
•	WQ HUMANS POS	Code Category for they ways in which humans may positively impact water quality. May collect quotations here that don't have a good home yet, and then examine collected quotes for commonalities to determine if new sub-codes are warranted.	1
•	WQ Humans Pos - education	Comments about positive effects educational opportunities or efforts may have on water quality.	3
•	WQ Humans Pos - mgmt	Comments about positive effects on water (from the participants' perspectives) in relation to land use, land management, or wildlife management - human management of natural resources (e.g., "with agriculture you can kind of help determine cleaning out the waterways..."; "but it has a pump in it and everything, so the water is continuously moving").	14
•	WQ Humans Pos - technology	Comments about positive effects on water quality with use of technologies (e.g., "technology is kind of stepping up...")	1
•	WQ Humans Pos - tillage	Comments about positive effects on water quality with use of tilling practices, such as low till or no till farming (e.g., "strip till planting, you conserve a lot of water.")	1
•	WQ Indicator - clarity	Comments where people indicate they use the clarity of the water as an indicator, when prompted about how they judge water quality, or they are talking about an experience or photo where they have keyed in on the clarity of the water (e.g., "the pond was extremely clear and clean at that time"; "....and I just noticed the stream was...the river was so pretty. The water was clear.").	18
•	WQ Indicator - color	Comments where people indicate they use the clarity of the water as an indicator, when prompted about how they judge water quality, or they are talking about an experience or photo where they have keyed in on the clarity of the water (e.g., "yellow river").	2

	Code	Code Definition, Comments, and Examples	Grounded
●	WQ Indicator - muddy water	So far, this code has captured a couple comments about muddy or silty water in response to a prompt asking what signs made them think the water quality was generally good or bad. May eventually collapse into the 'clarity' indicator category, but may be useful to keep for now to note the different language participants are using (talking about mud/silt vs, mentioning clarity/clear water).	1
○	WQ NATURAL EVENTS	Code Category for the ways in which natural events may affect water quality. May collect quotations here that do not have a good home yet, and then examine collected quotes for commonalities to determine if new sub-codes are warranted.	1
●	WQ Natural Events - neg - rain/storms	Comments about how rain, storms, or hurricanes may negatively impact water quality; may be in response to prompts about natural events affecting WQ. Somewhat redundant to rain, and co-coded in at least one place, but sticking close to participants words. (e.g., "the storms have took away the creek"; ever since Fran, Floyd and all those, it has devastated the area we're in.")	3
	WQ NATURE	Code Category for the ways in which nature (flora, fauna, ecosystems, etc.) may affect water quality. May collect quotations here that do not have a good home yet, and then examine collected quotes for commonalities to determine if new sub-codes are warranted.	0
●	WQ Nature - good and bad	Comments that mention both positive and negative aspects of flora, fauna, ecosystems (e.g., "if you fish a lot you enjoy the vegetation somewhat...but if you're trying to irrigate...it's a problem"; "they cause a lot of trouble for the farmers and landowners, ...but I've seen the prettiest ponds ever been...and it's because of the beavers".	20
●	WQ NATURE INDICATOR	Code Category for the ways in which nature (flora, fauna, ecosystems, etc.) may be an indicator of water quality. May collect quotations here that do not have a good home yet, and then examine collected quotes for commonalities to determine if new sub-codes are warranted. Reviewed 2/6/2020: OK with the quotations left here. One is more indicator-balance, one is wildlife generally, and the third is crayfish. All were in the conservation group.	3

Code	Code Definition, Comments, and Examples	Grounded
• WQ Nature Indicator - algae	Comments about algae being a sign water quality either good or bad, or when comments suggest people are using algae as an indicator to make a judgment about water quality. Often in response to the algae photo prompt. (e.g., "yeah, it's a negative indicator"; "now like you said, algae is on that water. You see it on the fish pond; you see it in certain parts of the creek -- it just, if the water is still and not moving.").	16
• WQ Nature Indicator - beavers	Comments about beavers being a sign water quality either good or bad, or when comments suggest people are using beavers as an indicator to make a judgment about water quality. (e.g., "but there's still obviously stuff there for him to eat or he won't be there, so it's got to be somewhat healthy to start with."	5
• WQ Nature Indicator - fish	Comments about fish being a sign water quality either good or bad, or when comments suggest people are using fish as an indicator to make a judgment about water quality (e.g., "first, it's a sign you've got good water quality, the fish are alive."; "I mean a lot of people judge water quality by fish kills.")	31
• WQ Nature Indicator - forest	Comments about terrestrial vegetation being a sign water quality either good or bad, or when comments suggest people are using terrestrial plants as an indicator to make a judgment about water quality. Often in response to the streamside forest photo (e.g., “looking at it might tell you what's in the soil or might -- what's affecting it.").	3
• WQ Nature Indicator - mussels	Comments about mussels or other bivalves being a sign water quality either good or bad, or when comments suggest people are using mussels as an indicator to make a judgment about water quality. Often in response to mussel photo (e.g., in response to Q about how did participant interpret lots of mussels in relation to WQ "it was very positive, because I knew then that they were much cleaner than they are today..."; "you know, like oysters and stuff, they don't grow on a dead bottom. No clams there.")	9
• WQ Nature Indicator - snails	Comments about snails being a sign water quality either good or bad, or when comments suggest people are using snails as an indicator to make a judgment about water quality. Often in response to the snail photo (e.g., "They're very positive if you see them anywhere."; "if you didn't find them anywhere, it would probably tell you one way or the other whether the water is bad").	6

Code	Code Definition, Comments, and Examples	Grounded
• WQ Nature Indicator - veg	Comments about underwater vegetation being a sign water quality either good or bad, or when comments suggest people are using underwater vegetation as an indicator to make a judgment about water quality. Often in response to the underwater vegetation photo (e.g., "and it does show good water quality to a point"; "It's a world class ecosystem when you see submerged aquatic vegetation like we have on the banks.")	21
• WQ NATURE NEG	Code Category for the ways in which nature (flora, fauna, ecosystems, etc.) may negatively affect water quality or waterways. Language may not specify how the impact is negative or be unclear for interpretation, leaving us to keep the quotations in this generalized category (e.g., "It's between the beavers and the storms we've had, ever since Fran, Floyd and all those it's just devastated the area."). May also be delineating the negative portion of a WQ Nature - good and bad code. May collect quotations here that do not have a good home yet, and then examine collected quotes for commonalities to determine if new sub-codes are warranted.	3
• WQ Nature Neg - algae	Negative comments related to algae during water quality discussions (e.g., "those big blooms hurt you"; "I mean it's bad news").	27
• WQ Nature Neg - dam	Negative comments related to beaver dams during water quality discussions (e.g., "they dam up a lot...wildlife officers blowing up the dams...because they were blocking the water runoff).	26
• WQ Nature Neg - mussels	Negative comments related to mussels during water quality discussions (e.g., "they definitely have a negative connotation in the water"). There are only a couple quotations for this code and they are both in the conservation group - there was discussion mostly of invasive species. Only one quick comment about filtering and then right to invasive.	2
• WQ Nature Neg - other species	Negative comments related to flora/fauna impacts on other species during water quality discussions (e.g., "if there's oysters on a pile or something, after a while there won't be any oysters left. The mussels just take over."; "and they was starting to get other creatures migrating and living in them areas.")	7
• WQ Nature Neg - snails	Negative comments related to snails during water quality discussions (e.g., "they definitely have a negative connotation in the water").	4
• WQ Nature Neg - vegetation	Comments about negative aspects of vegetation (e.g., "it'll stop up everything if you're trying to irrigate...")	4

Code	Code Definition, Comments, and Examples	Grounded
• WQ NATURE NEUTRAL	Code Category for the ways in which nature (flora, fauna, ecosystems, etc.) may affect water quality or waterways, without giving a clue as to which direction (e.g., in response to whether snails have a connection to water quality, "oh yeah").	4
• WQ Nature Neutral - tree pollen/leaves/debris	Comments about tree pollen, leaves or debris impacting water quality that do not insinuate positive or negative impacts (e.g., Something else in looking at it, it's the leaves and the debris from the trees falling into the water.") The one example we have seems to be an acknowledgment that trees could affect water quality, trying to make sense of the streamside forest photo. There is not a ton of uncertainty or judgment either way, just an acknowledgment.	1
• WQ NATURE NO BENEFIT	Comments that a natural entity has no benefit or the participant either cannot find or does not think there's any benefit to water quality. Lacks any insinuation of negative impact, though. (e.g., "We've had healthy streams before beavers on our property, so I don't see that they've done much improvement to our farm.").	3
• WQ NATURE POS	Code Category for the ways in which nature (flora, fauna, ecosystems, etc.) may positively affect water quality or waterways. Language may not specify how the impact is positive or be unclear for interpretation, leaving us to keep the quotations in this generalized category (e.g., "the grass is a good thing"; "so they have a purpose"). May also be delineating the positive portion of a WQ Nature - good and bad code. May collect quotations here that do not have a good home yet, and then examine collected quotes for commonalities to determine if new sub-codes are warranted.	0
• WQ Nature Pos - #algae	General positive comments about algae	4
• WQ Nature Pos - #beavers	General positive comments about beavers	5
• WQ Nature Pos - #fish	General positive comments about fishes	2
• WQ Nature Pos - #forest	General positive comments about streamside forests	5
• WQ Nature Pos - #mussels	General positive comments about mussels	2
• WQ Nature Pos - #veg	General positive comments about vegetation	3
• WQ Nature Pos - buffer	Comments about how natural things may positively affect water quality or waterways by acting as a buffer (e.g., I was getting ready to say the same thing. It's a buffer zone.")	11
• WQ Nature Pos - cleaning	Comments about how natural things may positively affect water quality or waterways by cleaning (e.g., "they suck in dirty water and spit out clean water"; "I would assume they eat up all the gross stuff").	24

Code	Code Definition, Comments, and Examples	Grounded
• WQ Nature Pos - cooling	Comments about how natural things may positively affect water quality or waterways by regulating temperature (the only coded comment so far was specifically about cooling - name may need to change to temperature if others arise. There will be other quotations, but not enough to split into different temperature categories). Example: "yeah it keeps it cool which would probably mean the water creatures would be more plentiful..."	2
• WQ Nature Pos - erosion control	Comments about how natural things may positively affect water quality or waterways by providing erosion control (e.g., "it does slow a lot of erosion"; "it protects the banks of that stream from erosion").	7
• WQ Nature Pos - filtering	Comments about how natural things may positively affect water quality or waterways by filtering (e.g., "it's the same thing as a marsh; it's a filtration"; "filtering out sediments and other nutrients...").	32
• WQ Nature Pos - habitat	Comments about how natural things may offer benefits by providing habitat - more specifically when they are referring to offering shelter/space (e.g., "there's clams in that"; "it's a hibernation point for the fish..."). Use the "-other species" sub-code if the comments are about other benefits (food, etc.).	27
• WQ Nature Pos - other species	Comments about how natural things may offer benefits to other species (e.g., "it feeds the fish"; "underwater vegetation puts off oxygen so the fish can maintain"). May also capture instances where species were perceived to benefit the water (e.g., in forest photo, both conservation and urban Extension groups discussed it providing habitat for species that in turn positively benefited the stream). May also capture species interactions (in fish photo - fish eat the algae, or veg - provides oxygen - sometimes directionality is different than we might infer (e.g., in the fish photo, the fish isn't benefiting the algae by eating it, but the species interaction is captured, and we've classified it under the WQ Nature Pos code because it was perceived as a positive benefit of regulating algae).	17
• WQ Nature Pos - oxygen	Comments about how natural things may benefit water quality by supplying oxygen (in reference to vegetation ("I think it's just like the trees out here, where the trees put off oxygen..."	2
• WQ Nature Pos - stability	Comments about how natural things may benefit water quality by providing stability (e.g., "it helps hold everything together"; "when you have all of that undergrowth, it causes a bit of stability under the water").	3

Code	Code Definition, Comments, and Examples	Grounded
• WQ Nature Pos - stop sediment	Comments about how natural things may benefit water quality by stopping sediment (e.g., "they probably stop a lot of sediment"; "I think it probably has some impact on sediment movement. It stabilizes the sediment"). Hmm, that last one could also be stability...we might consider combining those codes to something like 'sediment stability'.	4
• WQ Nature Pos - water flow	Comments about how natural things may benefit water quality by influencing water flow (e.g., "it more or less churns the water, keeps that water moving...and it ain't stalemated"; "they can [have something to do w/water quality] by building dams and slowing runoff").	2
• WQ NATURE UNCERTAIN	Code Category for participants being unsure about the ways in which nature (flora, fauna, ecosystems, etc.) may affect water quality or waterways. Language may be generalized or unclear for interpretation, leaving us to keep the quotations in this generalized category (e.g., "I don't know anything about freshwater snails"; "It's muddy, that's all you know. It's brown."). May collect quotations here that do not have a good home yet, and then examine collected quotes for commonalities to determine if new sub-codes are warranted.	36