Species Status Assessment Report for the Salamander Mussel (*Simpsonaias ambigua*)

Photo credit: Megan Bradley, USFWS

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Version 1.1 (May 2023) of the SSA report corrects minor typographical errors and inadvertent omissions from the Literature Cited in Version 1.0.

This document was prepared by U.S. Fish and Wildlife Service (USFWS) biologists Jessica Pruden and Kaitlyn Kelly (Michigan Field Office), with assistance from Carrie Tansy, Mandy Annis (Michigan Field Office), Barbara Hosler, Erik Olson, Laura Ragan, Sarah Bowman (Great Lakes Regional Office), Steve Choy, Tamara Smith, Nick Utrup (Minnesota-Wisconsin Field Office), Megan Bradley (Genoa National Fish Hatchery), Stephanie Martinez-Brewer, Amanda Goldsmith (Texas A&M University), Angela Boyer (Ohio Field Office), Sara Schmuecker (Illinois-Iowa Field Office), and Elliot Lustig (Branch of Data Integration).

The following individuals provided substantive information and insights for the Species Status Assessment: Scott Hicks (USFWS, Michigan Field Office), Andrew Henderson (USFWS, North Carolina Field Office), Josh Hundley (USFWS, Missouri Field Office), Brant Fisher (Indiana Department of Natural Resources), Monte McGregor (Kentucky Department of Fish and Wildlife Resources), Kevin Eliason and Alexander Silvis (West Virginia Division of Natural Resources), Cleyo Harris (Michigan Department of Natural Resources), Pete Badra (Michigan Natural Features Inventory), Janet Clayton (retired, West Virginia Division of Natural Resources), Andrew Phipps (USFWS, White Sulfur Springs National Fish Hatchery), Jordan Richards (USFWS, Southwest Virginia Field Office), Kendall Moles and Kelly Irwin (Arkansas Game and Fish Commission), Kelly Poole (Iowa Department of Natural Resources), Amy Benson (U.S. Geological Survey, Gainsville, Florida), Lisie Kitchel, Jesse Weinzinger (Wisconsin Department of Natural Resources), Todd Morris (Fisheries and Oceans Canada), Jason Wisniewski (Tennessee Wildlife Resources Agency), Anthony Ford (USFWS, Tennessee Field Office), Nevin Welte, Chris Urban (Pennsylvania Fish and Boat Commission), Steve McMurray (Missouri Department of Conservation), Andy Roberts (USFWS, Missouri Field Office), Robert Anderson (USFWS, Pennsylvania Field Office), Tom Augspurger (USFWS, Eastern North Carolina Field Office), Tobin Melvin (USFWS, Arkansas Field Office), Rebekah Ewing (USFWS, North Carolina Field Office), Dan Fitzgerald (USFWS, Headquarters), John Navarro (Ohio Department of Natural Resources), Amy Mahar, Jenny Landry (New York Department of Environmental Conservation), Cheryl Kaye, Aaron Jubar (USFWS, Sea Lamprey Control Program), Eric Stegmann (Missouri State University), Brian Metzke (Illinois Department of Natural Resources), Rich Baker (retired, Minnesota Department of Natural Resources), Mike Davis, Bernard Sietman, Bridget Henning-Randa, and Krista Larson (Minnesota Department of Natural Resources).

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EXECUTIVE SUMMARY

The Salamander Mussel (*Simpsonaias ambigua*) (Say 1825, p. 131, Frierson 1914, p. 7, p.40) is a small, thin-shelled, mussel about 42 mm long (2 inches) and about 20 mm (1 inch) high. The life span of Salamander Mussel is estimated to be approximately 10 years (Watson et al. 2001, entire). It is the only unionid (family of freshwater mussels) with a non-fish host, the mudpuppy (*Necturus maculosus*). The mussel is found in rivers, streams, creeks, or lakes, under flat rocks in areas of moderate flow, with varying substrate including bedrock, sand, gravel, or mud.

Currently Salamander Mussel occurs in 14 states, as well as the Canadian province of Ontario. We describe and analyze the distribution of Salamander Mussel in terms of watersheds occupied, delineated by the U.S. Geological Survey (USGS) based on surface hydrological features. These hydrological areas are identified as hydrological units at various geographic scales (referred to as HUC). We used the HUC2 scale to delineate our representation units for Salamander Mussel: Upper Mississippi, Ohio, Tennessee, Great Lakes, and Arkansas-White-Red. The species currently ranges across all five representation units.

We used the HUC8 at the subbasin scale to define a population of Salamander Mussel and conduct our current condition analysis. We categorized a population's status as extant, presumed extant, presumed extirpated, extirpated, or historical to assess the health, number, and distribution of populations through time. We analyzed current condition for extant and presumed extant populations (total of 66 populations). Given the paucity of data and lack of survey effort specifically for Salamander Mussel, we have minimal demographic data from a small number of populations across the range. We assessed demographic population condition as high, moderate, low, or functionally extirpated based on demographic criteria. We assigned an estimate of the probability of persistence over 20 years (approximately 2 generations of Salamander Mussel) for each population condition category based on the population's ability to withstand demographic stochastic events. For the majority of populations, we have data from incidental observations only, which does not allow us to evaluate demographic population condition (categorized as "unknown"). For our current condition analysis, we also evaluated the six primary risk factors affecting Salamander Mussel (water quality/contaminants, hydrological regime, landscape, connectivity, invasive species, and host species vulnerability). We assigned these risk factors to three categories of high, moderate, and low risk and assigned a probability of persistence over 20 years for each of the risk categories.

Of the 110 known populations of Salamander Mussel, 66 are currently occupied and 44 populations (40%) are either extirpated or historical. These populations are spread across the representation units unevenly, and a high percentage (98.5%) of populations are currently at high risk based on our risk factor analysis. Twenty-three current populations of Salamander Mussel are known from a single or couple records indicating an occupied river extent and, therefore, are more susceptible to extirpation from catastrophic events.

The Ohio River basin has 35 populations; of these, 27 are at high risk. The Upper Mississippi basin has 17 extant populations, all of which are at high risk. The Great Lakes basin has 8 populations with completed risk analyses, all of which are at high risk (three populations that cross the border with Canada were unable to be fully analyzed). The Arkansas-White-Red basin has one population that is presumed extant and at high risk. The Tennessee basin has two extant populations, both of which are at high risk. With few populations that are all at high risk, the Great Lakes, Tennessee, and Arkansas-White-Red representation units are all at risk of extirpation. Although the Upper Mississippi representation unit has 14 populations, all of them are at high risk, putting the unit at risk of extirpation. The Ohio basin is the only representation unit with populations experiencing moderate risk.

Lastly, we analyzed future condition by projecting each population's demographic condition in the future based on its current demographic condition as a baseline and the risk factor level projected for the future. Because there is substantial uncertainty regarding the magnitude, duration, and location of the risk factors, we forecasted future viability for the Salamander Mussel under two future scenarios that capture the range of plausible future conditions: (1) negative influences increase in magnitude/intensity 50 years into the future; and (2) current influences remain constant and/or improve 50 years into the future. We evaluated both scenarios where future threats determined the biological status of mussel populations and their habitats.

CHAPTER 1. INTRODUCTION

1.1 Background

We, the U.S. Fish and Wildlife Service (Service), were petitioned to list the Salamander Mussel (*Simpsonaias ambigua*) as an endangered or threatened species under the Endangered Species Act of 1973, as amended. This request was part of a petition to list 404 aquatic, riparian, and wetland species in the southeastern United States (Center for Biological Diversity et al. 2010, pp. 1067–1071).

This report summarizes the results of a species status assessment (SSA) conducted for Salamander Mussel (*Simpsonaias ambigua*). Importantly, the SSA report is not a decisional document; rather, it provides a summary of our analysis of the best available information as it relates to the species' biological condition. In the case of the Salamander Mussel, it has been prepared to inform decisions about the legal classification of this species. Decisionmakers will consider the information in this document (or referenced in this document), in combination with all relevant laws, regulations, and policies regarding classification decisions. The results of a proposed decision will be announced in the *Federal Register*, with appropriate opportunities for public input.

1.2 SSA Framework and Analytical Approach

To conduct this assessment, we followed the Service's SSA framework (USFWS 2016, entire), which is designed to be a gathering and scientific review of the best available information available about a species' biological needs and factors influencing the species, an evaluation of its biological status, and an assessment of the resources and conditions needed to maintain long-term viability. For this SSA, we define viability as the ability of the Salamander Mussel to maintain populations in the wild over a biologically meaningful timeframe.

Using the SSA framework, we consider what the Salamander Mussel needs to maintain viability by characterizing the status of the species in terms of the conservation biology principles of resiliency, redundancy, and representation, referred to hereafter as the 3Rs (Shaffer and Stein 2000, pp. 308–311).

Resiliency is "the ability of a species to withstand stochastic disturbance; resiliency is positively related to population size and growth rate and may be influenced by connectivity among populations" (Smith et al. 2018, p. 304). Highly resilient populations are better able to withstand disturbances, such as random fluctuations in birth rates (demographic stochasticity), variations in rainfall (environmental stochasticity), or the effects of anthropogenic activities.

Redundancy is an indication of "the ability of a species to withstand catastrophic events by spreading risk among multiple populations or across a large area" (Smith et al. 2018, p. 304), thereby reducing the likelihood that all populations are exposed simultaneously and possess similar vulnerabilities to catastrophes. Redundancy can be measured by the number, distribution, and connectivity of resilient populations across a species' range.

Representation is an indication of "the ability of a species to adapt to changing environmental conditions over time as characterized by the breadth of genetic and environmental diversity within and among populations" (Smith et al. 2018, p. 304). Representation reflects the evolutionary or adaptive capacity of the species and its ability to persist or adapt in the face of changes in the environment. In the absence of species-specific genetic and ecological diversity information, we can evaluate representation based on the extent and variability of habitat characteristics across the geographical range.

A species with a high degree of resiliency, representation, and redundancy is better able to adapt to novel changes and to tolerate environmental stochasticity and catastrophes. In general, species viability will increase with increases in resiliency, redundancy, and representation (Smith et al. 2018, p. 306).

Our analytical approach for assessing the viability of the salamander mussel involved three iterative stages. In Stage 1, we described the species' needs and ecological requirements for survival and reproduction at the individual, population, and species levels. In Stage 2, we determined the salamander mussel's current demographic and risk condition in terms of the 3 Rs, using the ecological requirements of the species identified in Stage 1 and the past and ongoing factors influencing viability that have led to the species' current demographic and risk condition. In Stage 3, we projected the future condition of salamander mussel using the baseline conditions established in Stage 2 and the predictions for future risk and beneficial factors.

CHAPTER 2. SPECIES LIFE HISTORY AND RESOURCE NEEDS

This chapter reviews biological and ecological information about the Salamander Mussel, including taxonomy, morphology, known life history traits that are important to viability now and into the future within the historical and current distribution. We have summarized that information in this chapter; for more background on the species biology and ecology see Appendix A.

2.1 Taxonomy and Genetics

The Salamander Mussel belongs to the family Unionidae, also known as the naiads or pearly mussels. The Salamander Mussel SSA report follows the most recently published and accepted taxonomic treatment of North American freshwater mussels as provided by Williams et al. (2017, entire). Salamander Mussel is the only living member of the genus *Simpsonaias*, and its phylogenetic position is obscure because it is not closely related to any other living species (Clarke 1985, pp. 60–68).

2.2 Species Description

Salamander Mussel is a small species, elliptical in shape, that is thin-shelled and that reaches approximately 48–51 mm long (1.5–2 inches) (Watson 2001, entire).

2.3 Species Historical Distribution

The Salamander Mussel was historically found across 14 states (Arkansas, Missouri, Tennessee, Kentucky, Iowa, Illinois, Indiana, Minnesota, Wisconsin, Michigan, Ohio, Pennsylvania, New York, and West Virginia) and one Canadian province (Ontario). It occurred in small streams to large rivers and in Lake Erie.

It has been extirpated within Iowa and can be found within the Mississippi river only along the eastern border of the state. Other portions of the range are historical as well including Lake Erie. Much of Illinois' historical range has been diminished to four populations.

2.4 Individual Needs

The Salamander Mussel is the only North American freshwater mussel species within the Unionidae family known to have a non-fish host, the mudpuppy (*Necturus maculosus*), for reproduction. Salamander Mussel has specific habitat requirements that overlap with the preferred habitats of the mudpuppy. See Figure 2.1 for the Salamander Mussel life cycle and Table 2.1 for a summary of its needs during each of its life stages. For additional information, see Appendix A.

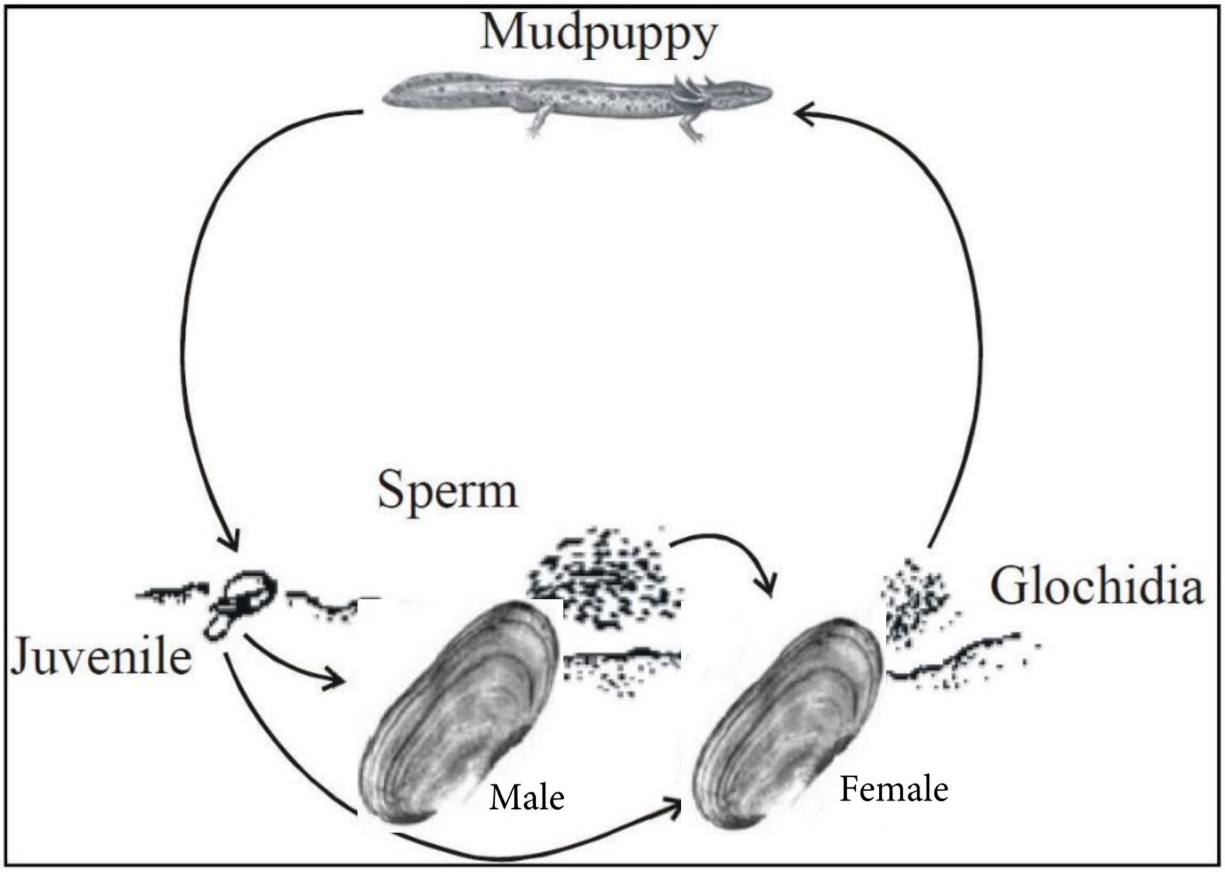

Figure 2.1. The life cycle of Salamander Mussel (Simpsonaias ambigua) (adapted from Watson 2001, p. 24).

Table 2.1. Individual Needs for Salamander Mussel (Simpsonaias ambigua).

Life Stage	Resources Needed to Complete Life Stage	Source
Fertilized eggs - late spring to summer	• Clear, flowing water • Sexually mature males in proximity to sexually mature females • Appropriate spawning temperatures	Berg et al. 2008, p. 397; Haag 2012, pp. 38–39
Glochidia - late summer released from female marsupial gills - develop on host fall to early spring	• Clear, flowing water • Presence of mudpuppy (host) for attachment • Flow to ensure glochidia encounter host	Strayer 2008, p. 65; Haag 2012, pp. 41–42; Clarke 1985, pp. 60–68
Juveniles - excystment (juveniles drop off from host)	• Clear, flowing water • Host dispersal • Appropriate interstitial chemistry: low salinity; high dissolved oxygen; absence of or non-toxic levels of contaminants, including ammonia, copper, chloride, and sulfate • Flat rocks and bedrock that provide crevices for shelter	Dimock and Wright 1993, pp. 188–190; Sparks and Strayer 1998, p. 132; Augspurger et al. 2003, p. 2,574; Augspurger et al. 2007, p. 2,025; Strayer and Malcom 2012, pp. 1,787–1,788
Adults - greater than 0.8 in (20 mm) shell length	• Clear, flowing water • Flat rocks and bedrock that provide crevices for shelter • Adequate food availability (phytoplankton and detritus) • High dissolved oxygen • Appropriate water temperature	Yeager et al. 1994, p. 221; Nichols and Garling 2000, p. 881; Chen et al. 2001, p. 214; Spooner and Vaughn 2008, p. 308

2.4.1 Reproduction

In general, reproduction in mussels starts with males releasing sperm into the water column and nearby females taking in sperm through their incurrent aperture (Figure 2.1). The sperm fertilize eggs in the suprabranchial chamber (dorsal part of the gills) as ova are passed from the gonad to the marsupia (Haag 2012, pp. 37–42). The developing larvae remain in the gill chamber until they mature into larvae called glochidia and are ready for release (Haag, pp. 37–42).

The Salamander Mussel lives for approximately 10 years. The age of sexual maturity is not known. Salamander Mussel spawns in the spring and is bradytictic, meaning it is a long-term brooder in which the female holds the glochidia in its marsupial gills over the winter until release the following spring or summer (Watson 2001, p. 5)

Salamander Mussel glochidia are considered “morphologically depressed,” meaning the valve height is less than or equal to the valve length. This is significant because this type of glochidia is less likely to contact a host, though once they do, they are more likely to stay clamped on to their host (Watson 2001, p. 5).

Time from encystment (where host tissue grows over glochidia that are attached to gills) to excystment (where metamorphosis occurs and glochidia transform to juveniles and break through tissue to drop off to substrate) based on propagation studies is approximately 19–28 days (Watson 2001, p. 5; M. Bradley, personal communication, 2021).

Salamander Mussel has an obligate parasitic relationship with its host the mudpuppy (Figure 2.1; Table 2.1). Mudpuppy (*Necturus maculosus*) is the only host of Salamander Mussel and is the only non-fish host used in North America. For Salamander Mussel to complete reproduction, mudpuppy must be present during glochidia release in the summer. Mudpuppies are more resident (do not travel long distances) during the portion of the year when Salamander Mussel release glochidia resulting in encystment and excystment on mudpuppy. It is thought that mudpuppies may consume Salamander Mussel adults and therefore become infested.

2.4.2 Nutrients

Adult freshwater mussels, including Salamander Mussel, feed by filtering suspended particles including phytoplankton, zooplankton, rotifers, protozoans, detritus, and dissolved organic matter from the water column or sediments (Table 2.1, Figure 2.2; Strayer et al. 2004, pp. 430–431). Juvenile mussels collect food items from sediments and water column (Vaughn et al. 2008, pp. 409–411). A very small amount of carbon is transferred from the fish to the cells that glochidia have clamped down on in the gills (M. Bradley, personal communication, 2021). Availability of nutrients is critical to the survival of mussels at the individual level. In general, the availability of nutrients is not considered a limiting factor except in cases where localized risk factors (for example, elevated water temperature, increased particle number, high flow causing aperture closing) are present that change the behavior of mussels’ filtering capacity or an invasive species is present in such abundance that competition for resources becomes an issue (for example, competition with Zebra mussels for food) (Strayer 1999, entire).

2.4.3 Clean, Flowing Water

Salamander Mussel inhabits rivers, streams, and in some cases lakes with natural flow regimes. Seasonal low flow is expected in some systems and can be tolerated by Salamander Mussel, though periodic drying or intermittent flow in lake and river habitats generally cannot support mussel assemblages. Appropriate flow and temperature are critical to delivering oxygen and nutrients for respiration and filtration, allowing glochidia to move to their host and encyst for reproduction, and removing silt and other fine sediments from within rock structures and crevices preventing mussel suffocation and degradation of mudpuppy shelter habitat (Table 2.1, Figure 2.2). Salamander Mussel inhabits rivers and streams with fairly swift velocities. Normal fluctuation in velocity is expected, but extreme changes can be detrimental. A significant and prolonged increase in velocity typically associated with flood conditions has the potential to dislodge mussels and move the bed load (particles that can be transported by flowing water along the stream bed) potentially destroying Salamander Mussel and mudpuppy habitat (Hastie et al. 2001, entire). High shear stress and areas of scour may cause instability of the rock structures themselves, creating unsuitable shelter habitat for Salamander Mussel and mudpuppy. Abnormally high velocities, for example from flood flows, have the potential to displace juveniles and adults, along with washing out free-floating glochidia resulting in mortality. Alternately, Salamander Mussel is a highly mobile and active mussel species with the capability to move to more suitable habitat (Stegman 2020, p. 12). Extreme low flow associated with drought or water withdrawal can impact reproduction, feeding, respiration, and in some cases result in dewatering and exposure and desiccation of the species.

2.4.4 Appropriate Water Quality and Temperature

Appropriate water quality is critical to the survival, reproduction, and persistence of all life stages of freshwater mussels. Point and non-point source contaminants result in water quality and habitat degradation. Contaminants alter the chemical, physical, and biological characteristics of a stream resulting in lethal and sub-lethal effects to mussels and their hosts. Although specific data for these parameters with respect to Salamander Mussel are not available, mussels in general are similar in terms of sensitivity to certain thresholds depending on the life stage exposed. Mussels in general need water temperature below about 86 degrees Fahrenheit (˚F) (30 degrees Celsius (˚C)), dissolved oxygen concentrations greater than 5 milligrams per liter (mg/L) (Pandolfo 2010, entire), and water quality concentrations below acute toxicity levels to mussels for contaminants including but not limited to total ammonia nitrogen, copper, chloride, and sulfate (see Appendix B for details).

2.4.5 Shelter

Salamander Mussel prefers shelter habitat with space under slab rock/ bedrock crevice type structures that are dark, where they are in contact with a solid surface, and there is stability from swift current (Table 2.1; Stegman 2020, p. 5). Often these rock structures have small amounts of sediment and silt present but are swept fairly clean of excessive silt and fine sediments (Watson 2001, p. 5). Salamander Mussel has been observed to be one of the most mobile mussels, capable of climbing up vertical surfaces, including plastic (Stegman 2020, p. 5). Being highly mobile may be an adaptation that helps them respond to substrate changes that might occur in swift current within rocky crevices or structures and allows them to seek more suitable habitat or disperse to new habitat from high density areas (Stegman 2020, p. 5).

2.4.6 Host

The mudpuppy is a fully aquatic salamander species that serves as a host to Salamander Mussel. The presence, abundance, distribution, and health of mudpuppies are critical in maintaining healthy populations of Salamander Mussel rangewide (Table 2.1, Figure 2.2). The mudpuppy is about 16 inches long and inhabits environments including small to large rivers, small ponds, lakes, and even large bodies of water like the Great Lakes (Murphy et al. 2016, p. 575). They are long lived, approximately 30 years (Murphy et al. 2016, p. 575). Age of sexual maturity is 5–10 years with breeding occurring in the fall (Virginia Herpetological Society 2021, webpage). Females store sperm over the winter in a spermatheca because ovulation and fertilization do not occur until the spring (Murphy et al. 2016, p. 575). After fertilization, females deposit eggs under large flat rocks. The eggs are attached to the underside of rocks, logs, and other cover objects by a stalk in a cluster of 50–100 (Fisher 2020, p. 6). Females guard the eggs until larvae hatch in early summer (Murphy et al. 2016, p. 575). They tend to be present within the same habitat preferred by Salamander Mussel during the summer and fall, transitioning to different foraging habitat in the winter and early spring.

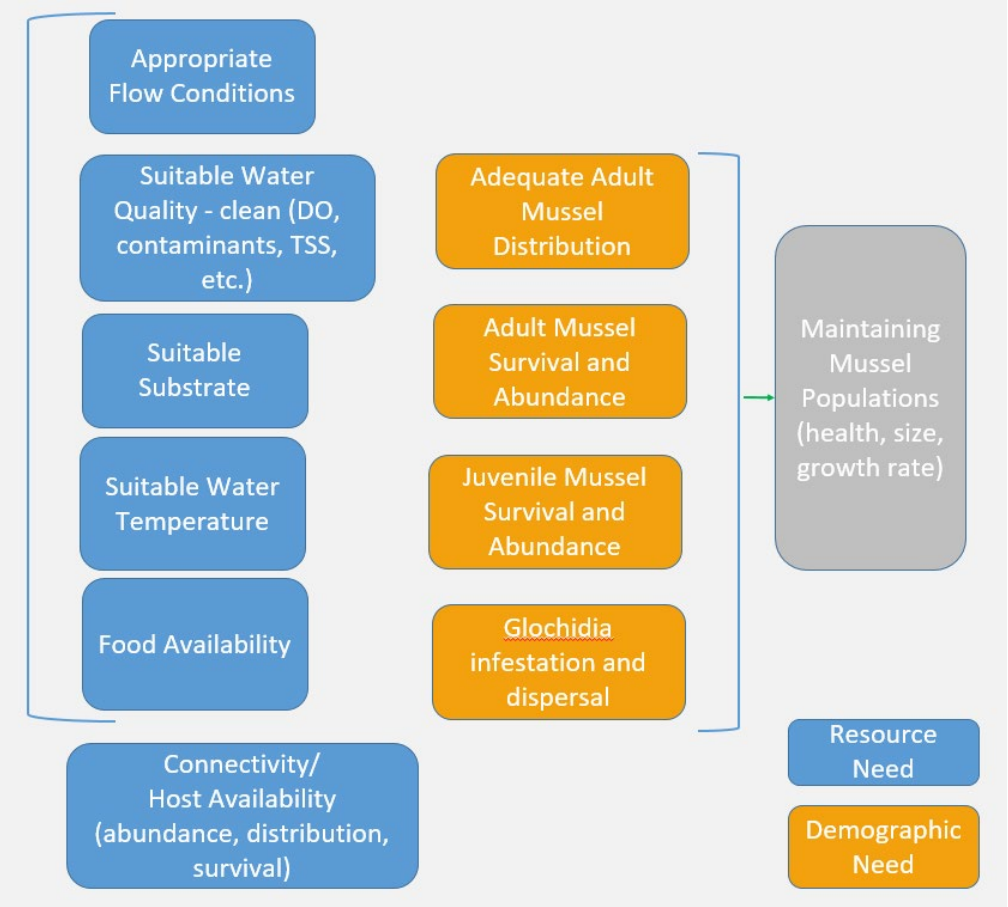

Figure 2.2. Conceptual model of the resource needs to support demographic needs to maintain Salamander Mussel populations.

2.5 Population and Species Needs

We defined populations by the watersheds through which occurrence streams flow, using the U.S. Geological Service (USGS) Hydrologic Unit Code (HUC) system. We used HUC8 watersheds as a representation for an area's potential capability for dispersal and interaction of individuals. Watershed boundaries and natural and artificial barriers constrain ecological processes, such as genetic exchange and ultimately adaptive capacity for aquatic species (Funk et al. 2018, entire). For the purposes of this assessment, Salamander Mussel populations were defined within the bounds of HUC8 watersheds.

However, Salamander Mussels are estimated to occupy limited reaches within streams, with occupied areas often confined by tributary confluences, impoundments, and/or areas of unsuitable habitat. Although a limited number of populations persist at the HUC8 scale, this approach provided additional resolution and allowed us to assess Salamander Mussel occurrences within a more ecologically appropriate context in regards to primary influences on viability (See Chapter 3). However, it is important to note that defining populations at the HUC8 watershed scale is not indicative of the level of genetic flow between populations as many populations are currently isolated within the watershed.

2.5.1 Population Connectivity

At a broader scale, suitable Salamander Mussel habitat constitutes stream reaches where mudpuppy is present and there is connectivity between localized populations to allow for mudpuppy and Salamander Mussel dispersal. Connectivity is characterized by suitable water quality, lack of barriers to dispersal (for example, perched culverts, hydropower dams, water control structures etc.), and presence of suitable shelter habitat and forage base for mudpuppies. Having multiple occupied sites within a high degree of habitat connectivity can provide a source of resiliency and redundancy that can benefit the viability of the Salamander Mussel. However, impoundments and other barriers to mudpuppy dispersal, such as river reaches with unsuitable water quality (for example, high concentrations of pollutants or temperature), effectively isolate populations from one another, making repopulation of extirpated locations from nearby populations unlikely without human intervention (in other words, active restocking).

2.5.2 Representation

Maintaining species representation in the form of genetic and ecological diversity is important in safeguarding the ability of Salamander Mussel populations to adapt to future environmental changes. Information regarding the genetic diversity of Salamander Mussel populations is not currently available. In the absence of species-specific genetic information, we can evaluate representation based on the extent and variability of environmental conditions within the species' geographic range. We considered geographic range as a surrogate for geographic variation and proxy for potential local adaptation and adaptive capacity because genetic information is not available. Therefore, Salamander Mussel representation was considered at the HUC2 watershed scale. We delineated five representation units for Salamander Mussel: Upper Mississippi, Ohio, Tennessee, Great Lakes, and Arkansas-White-Red basins.

2.5.3 Redundancy

The Salamander Mussel needs multiple resilient populations distributed throughout its range to reduce the risk of a single catastrophic natural or anthropogenic-induced event negatively affecting a large portion of the species' range at any given point in time. Species well distributed across their historical range are less susceptible to extinction and more likely to remain viable compared to species confined to a small portion of their historical range (Carroll et al. 2010, entire; Redford et al. 2011, entire).

CHAPTER 3. PRIMARY INFLUENCES ON VIABILITY

This chapter provides an overview of risk factors influencing past, current, and future Salamander Mussel population condition. The current and likely future extent and magnitude of the stressors and threats influence Salamander Mussel viability. For more detailed information on these stressors, see Appendix B.

Salamander Mussel populations are susceptible to several natural and anthropogenic stressors occurring within their watersheds. These stressors can influence one or more of the individual and population needs discussed in Chapter 2. Stressors can vary by degree of impact across the range of Salamander Mussel. The habitat risk factors represent these stressors. Habitat risk factors influence the demographics of a population, such as survival, reproduction, and recruitment. Populations with healthy demographics can offset some effects of these stressors. We identified contaminants, hydrological regime, landscape alteration, lack of connectivity, invasive species, and host vulnerability as the primary risk factors influencing the resources upon which the Salamander Mussel relies, either directly or indirectly (Figure 2.2). We also considered direct threats to the mussel, including the influence of mussel disease and the effect of catastrophic events on the Salamander Mussel.

3.1 Habitat Quality and Quantity Risk Factors

Freshwater mussels require habitat in sufficient quality and quantity to complete their life cycles and those of their host species. Populations experience natural changes in their habitat that influence demographic needs, such as survival. In addition, anthropogenic activities affect river system habitats and can have negative effects on both habitat quality and quantity. Further details about each of these risk factors are provided in Appendix B.

3.1.1 Contaminants

Sources - Sources of contaminants can include point (for example, wastewater treatment and industrial effluents, targeted lampricide treatment) and non-point (for example, runoff comprised of fertilizer, pesticide, road salts, grease, and oil) sources resulting from urbanization, agriculture, toxic spills, aquatic invasive species treatments, and resource extraction and mining (Gillis 2012, pp. 348–356; Gillis et al. 2014, pp. 134–143; Bringolf et al. 2007a, pp. 2086–2093; Wang et al. 2017, pp. 786–796; Augspurger et al. 2003, pp. 2569–2575). Contaminants in river systems are varied and widespread from past and current releases and will continue into the future with potential new chemicals of concern (for example, PFAS (polyfluoroalkyl substances)); Hazelton et al. 2012, pp. 1611–1620; Woolnough et al. 2020, 1625–1638). For example, in areas with heavy agriculture or urbanization, contaminants are generally more intense and prevalent.

Exposure avenues - The complex life history of freshwater mussels, including the existence of multiple early life stages (for example, glochidia, juveniles etc.), has been a challenge in determining the toxicity of contaminants, which depends on the life stage present and concentration of chemicals (Cope et al. 2008, pp. 451–462). Population and individual impacts and response can vary based on the magnitude, proximity to the contaminant source, sensitivity of host species, and mussel life stage exposed. Contaminants impact surface and pore water (water that occurs in the spaces between sediment/ soil particles) chemistry, sediment composition, and host species fitness and survival (Cope et al. 2008, pp. 451–462). All stages of freshwater mussels are directly exposed to contaminants when present in the system. Contaminants have the potential to affect several reproductive early life history processes including sperm viability, female fertility or brooding capabilities, or luring or glochidia release behavior (Cope et al. 2008, 451–462). Free glochidia are exposed through surface water for seconds, days, or weeks, depending on species and water temperature (Cope et al. 2008, p. 453). Partially encysted glochidia may be exposed to contaminants in surface water and when fully encysted may be exposed to toxicants in the host tissue (Cope et al. 2008, pp. 455–457). Exposure during encystment may influence the ability of glochidia to successfully transform into juveniles (Cope et al. 2008, pp. 457–458). Adults, however, can be exposed over years through surface water, pore water, sediment, and diet (Cope et al. 2008, pp. 452–453).

Effects - In acute toxicity tests, adults have not been found to be as sensitive as glochidia or juveniles. However, adults have a toxicity avoidance mechanism where they close up and cease respiration for a period of time. If toxic conditions persist, adults must cease avoidance behavior and can be similarly sensitive as early life stages when exposed to prolonged toxic conditions (Cope et al. 2008, pp. 452–453). Juveniles are exposed mainly through sediment, pore water, and diet as they are typically burrowed in the sediment up to 3 to 4 years although surface water may also contribute to exposure in juveniles (Cope et al. 2008, pp. 457–458). In summary, individual responses to contaminants may include reduced fitness, altered growth rate, mortality, impacts to reproduction, and disruption in glochidia release and transformation (Cope et al. 2008, pp. 451–462; Bringolf et al. 2010, pp. 1311–1318; Hazelton et. al. 2012, pp. 1611–1620; Hazelton et al. 2013, pp. 94–100). Contaminants may cause populations to have reduced reproduction and population growth rates, along with declines in abundance and distribution (see Appendix B for detail).

3.1.2 Sedimentation

Sediment is composed of both organic (biological material) and inorganic (sand, silt, clay) particulate matter formed through various processes including weathering, wind/wave/ice action, and tectonic uplift. Anthropogenic sources of sediment include agriculture (Peacock et al. 2005), logging (Beschta 1978, entire), mining (Seakem Group et al. 1992, p. 17), urbanization (Guy and Ferguson 1963, entire), and hydrological alteration (Hastie et al. 2001, entire). While all streams carry sediment, alterations in landscape may negatively impact aquatic ecosystems if sediment loads are excessive enough to alter channel formation and/or stream productivity, in turn degrading freshwater biota (USEPA 2007, p. 2–21; Gammon 1970, entire; Junoy and Vieitez 1990, entire).

Mussel declines have been partially attributed to sedimentation caused by anthropogenic activities (for example, decrease in vegetative and canopy cover and increase in urban and agricultural land; Peacock et al. 2005, entire; Guy and Ferguson 1963, entire). Increased sedimentation impacts both water quality and quantity, which can have direct and indirect impacts on the survival, reproduction, and growth of freshwater mussel populations (Brim Box and Mossa 1999; Goldsmith et al. 2021; Tuttle-Raycraft and Ackerman 2019, p. 2532; Tokumon et al. 2015, p. 201-203). As urbanization rapidly increases, it proves critical to understand sediment-mussel relationships. While, sediment thresholds exist within the literature, the impact of suspended sediment on freshwater mussels is insufficient to determine a sediment target for healthy streams. Many studies have examined sediment-mussel relationships; however, there has been little effort to standardize methodologies making results difficult to compare between studies. Additionally, many of the studies available do not place their results within environmental context, which is problematic as some TSS-thresholds may not translate to real-world scenarios. For more details, see Appendix B.

3.1.3 Water Temperature, Dissolved Oxygen, and Drought

Alteration to the natural thermal regime of mussels is one of the greatest threats freshwater ecosystems face today (Caissie 2006, p. 1389). Within coming years, this threat may be exacerbated due to climate change. In fact, impacts to organisms and ecosystems are already being observed and it is likely several regional changes in climate will occur in coming years including an increase in frequency and intensity of drought and precipitation in some regions (Intergovernmental Panel on Climate Change [IPCC] 2013, p. 177). Increased surface temperatures and decreases in precipitation will likely lead to elevated stream temperature and decreases in flow (Sinokrot and Gulliver 2000, p. 340). Water temperature and flow are key variables in maintaining riverine biota and if altered can strongly impact the distribution and ecology of freshwater mussels (Olden & Naiman 2010, p.87; Vannote & Sweeny 1980, p. 667; Khan et al. 2019, p. 2). Increased water temperature negatively affects mussel physiological processes (for example, protein damage, fluidity of the cellular membrane, and organ function), disrupting energy balance, growth, and reproduction (Ganser et al. 2015). Drought is a major environmental disturbance, especially in small streams. It is likely mussels are highly sensitive to the secondary effects of drought including low dissolved oxygen, low flow, high water temperature, and high biological oxygen demand (Haag and Warren 2008; p. 1165).

Low dissolved oxygen is a threat to freshwater mussels and is particularly an issue interstitial (spaces between individual particles) waters (Sparks and Strayer 1998). Low dissolved oxygen can be caused by excess sedimentation, nutrient loading, organic inputs, changes in flow, and higher temperatures (Sparks and Strayer 1998). Low dissolved oxygen can negatively affect mussels' metabolism.

Freshwater mussels need flowing water in order to survive. While mussels have evolved in habitats that experience seasonal fluctuations in discharge, global weather patterns can have an impact on the normal regimes. Even during naturally occurring low flow events, mussels can become stressed because either they exert significant energy to move to deeper waters or they may succumb to desiccations (Haag 2012, p. 109). Droughts during the late summer and early fall may be especially stress-inducing because streams are already at their naturally occurring lowest flow rate during this time. Hydrologic and thermal modifications through other factors such as irrigation may result in reduced water availability during these periods (Golladay et al. 2004, p. 494). A completely dry streambed eliminates habitat for mussels. Lowered flows can cause stagnant pools to form, which over a period of time can become unsuitable for mussels, as well as well as their host(s), as water temperatures increase and dissolved oxygen decreases (Vaughn et al. 2015, p. 1299; Gates et al. 2015, p. 622). Mussels may survive short periods of low flow, but if low flows persist, mussels face oxygen deprivation, increased water temperature, and, ultimately, stranding, reducing survivorship, reproduction, and recruitment in the population.

Anthropogenic activity coupled with climate change may result in shifts in mussel species' natural range and water temperature to which they are exposed (Caissie 2006, p. 1389). Within coming years, it is likely climate change will amplify these impacts as global surface temperatures are expected to rise >1.5˚C (35 ˚F) with some regions projected to experience even larger impact (Intergovernmental Panel on Climate Change [IPCC] 2013, p. 20). Within the Midwest, average annual temperatures have increased over the last several decades with the rate of increase accelerating in recent decades (USGCRP 2018, p. 888). It is suspected as surface temperatures increase, decreases in precipitation will be observed, likely resulting in elevated stream temperature, decreased dissolved oxygen, and decreased flows (Sinokrot and Gulliver 2000, p. 339; van Vliet et al. 2013, p. 740). Impacts such as these can negatively affect population performance (in other words, growth, reproduction, and survival) of freshwater mussel populations and lead to overall species decline.

3.1.4 Hydrological Regime

The hydrological regime, also known as the river flow or hydrological variation of a river, determines the dynamics of a river system by directing the processes that shape and organize its associated habitats and biotic communities, while in turn it is defined by distinct daily and seasonal patterns and climatic conditions (Zeiringer et al. 2018, pp. 67–69). These physical processes vary among rivers and are directly related to both water flow characteristics and the type and availability of transportable materials (Zeiringer et al. 2018, p. 69). Significant changes in the magnitude and frequency of these flows have critical impacts on biodiversity and ecosystem integrity (Zeiringer et al. 2018, pp. 69–70; Demaria et al. 2016, p. 309), as shifts in the volume and timing of flows can impact the native freshwater species that occupy aquatic habitats rely on predictable flow patterns for important transitions in their life cycles (Demaria et al. 2016, p. 309).

The presence of dams is considered by many to be the largest contributor to the decline in freshwater mussels in North America (Downing et al, 2010, pp. 155–160; Vaughn and Taylor, 1999, p. 915). Dams directly affect mussels through alterations in flow and habitat (Poff et al. 1997, pp. 772–774). This topic is explored more in the next Section (Section 3.2 Connectivity).

Historical land use change and associated water resource development has altered established patterns of hydrologic variation and associated dynamics of large river systems, resulting in long-term chronic stresses felt decades upon their initiation (Zeiringer et al. 2018, p. 70; Pyron et al. 2020, pp. 2, 6). Typical anthropogenic alterations to the naturally occurring hydrology of rivers and streams include construction of dams, water diversions, levees and other such structures for channelization.

Changes to a river's hydrology and ecological processes can increase or decrease water depths, decrease habitat heterogeneity, decrease substrate stability, block host passage, and isolate mussel populations from hosts resulting in a reduction or elimination of suitable mussel habitat and interfering with the mussel/host reproductive process.

3.2 Connectivity

Artificial barriers within streams and rivers (for example, dams, road crossings, water control structures, etc.) pose a great number of threats to freshwater mussels and are considered one of the primary reasons for their decline (Downing et al, 2010, pp. 155–160; Vaughn and Taylor, 1999, p. 915).

Artificial barriers affect freshwater mussels through direct effects (such as water temperature and flow changes and habitat alteration) and indirect effects (such as changes to food base and fish-host availability). Hydroelectric dams and similar water control barriers can create additional stressors by fluctuating flows to abnormal levels on a daily basis or at inappropriate times of year (Poff et al. 1997, pp. 772–774). Abnormally high stream flow can displace juvenile mussels and make it difficult for them to attach to the substrate (Holland-Bartels 1990, pp. 331–332; Layzer & Madison 1995, p. 335). Altered flow can destabilize the substrate, which is a critical requirement for mussel bed stability (Di Maio and Corkum 1995, p. 663). Barriers can also exacerbate the effects of drought, resulting in the stranding of mussels and drying of mussel beds (Fisher and LaVoy 1972, pp. 1473–1476).

Movement and presence of host species is critical to development and distribution of mussels (Watters 1992, pp. 485-486; Haag and Warren 1998, pp. 303–305). The presence of barriers has been linked to the extirpation of freshwater mussels (Vaughn and Taylor 1999, pp. 915–917; Watters 1996, p. 79) and reduction in density and species richness of fish assemblages and mussel beds (Gore and Bryant 1986, p. 333; Bain et al. 1988, pp. 389–390; Kinsolving and Bain 1993, p. 531; Scheidegger and Bain 1995, pp. 129–134). Haag and Williams (2012, pp 46-47) discuss the sensitivity of mussels to human alterations to the landscape, including dams. The systematic destruction of riverine habitat by dams and channelization is often described as the predominate cause of mussel extinctions in North America (Haag 2012, pp 328-330).

Unpaved road stream crossings impact ecosystems including, but not limited to, water quality degradation, changes in flow, and obstruction to host passage, all of which can limit access to certain stretches of river that are either not accessible or degraded to a point that lack of habitat essentially causes a barrier.

3.3 Invasive Species

Invasion of aquatic habitats within the United States by invasive species is one of the leading threats freshwater ecosystems face with about 42% of endangered and threatened species reported to be significantly affected (NCANSMPC 2015, p. 8–9; Dueñas et al. 2018, p. 3171). When introduced, nonnative species may outcompete (for example, crowd out or replace) native organisms, in turn negatively altering food web and ecosystem dynamics and ultimately severely damaging ecological health (Davis et al. 2000, p. 227). Invasion of non-native species may be due to lack of predation within the new environment and easier adaptation/tolerance to varying environments. Invasive species can impact native species in a multitude of ways including: (1) native species may become a source of food for invasive species; (2) invasive species may cause or carry diseases; (3) invasive species may prevent native species from reproducing and/or kill native species young; and (4) invasive species may outcompete native species for resources (for example, food, space; Sodhi et al. 2010, p. 318). The invasion of freshwater habitats within the United States has resulted in an imminent threat to mussel fauna within affected regions and thought to have contributed to the decline of mussel species (Ricciardi et al. 1998, p. 615).

Invasive species with detrimental effects to freshwater mussels include Zebra Mussel, Corbicula (freshwater clam), Black Carp, Rusty Crayfish, Spiny Waterflea, Brown Trout, Quagga Mussel, Common Carp, and Bighead Carp. While invasive species do pose a risk to Salamander Mussel, given their unique anatomy, habitat they occupy, and use of a non-fish host, we assessed the risk as either moderate or low. We do not feel there is a plausible situation in which invasive species would pose a risk for probability of persistence to be less than 60%. See Appendix B, C, and Table 4.4, for more information on each of these species and risk posed to Salamander Mussel.

3.4 Mussel Disease

Enigmatic declines and large-scale die-offs of mussel assemblages within otherwise healthy streams across large geographic regions have emerged as a very concerning risk factor (Haag and Williams 2014, pp. 45–60; Haag 2019, pp. 43–60; Waller and Cope 2019, pp. 26–42). Die-offs have been observed in Europe as well as both the western and eastern U.S. (Waller and Cope 2019, p. 27). In some cases (for example, Clinch River), dies-offs have occurred several years in a row. The mysterious documented decline in mussel populations in the U.S. between the 1970s and 1990s could be the result of a widespread virus, bacteria, fungi, parasite, or a suite of diseases affecting only freshwater mussels (Haag and Williams 2014, pp. 44–46; Haag 2019, pp.

44–45; Waller and Cope 2019, p. 26). More recently, unexplained mussel die-offs have been documented in the eastern U.S. in the Ohio and Tennessee River basins in the Clinch River and Big Darby Creek (Richard et al. 2020, p. 1–10; Waller and Cope 2019, p. 27). The die-off in Big Darby Creek affected all mussel species (Waller and Cope 2019, p. 27). In the Clinch River, the first die-off in 2016 affected only Pheasantshell (*Actinonaias pectorosa*) though die-offs in 2017, 2018, and 2019, impacted a wider variety of species and additional sites (Waller and Cope 2019, pp. 27–28).

Little is known about mussel health, the role of microbiota and pathogens in mussel health, which makes it very difficult to understand how these factors may be impacting freshwater mussel populations. In 2018, the Freshwater Mollusk Conservation Society held a workshop: to increase awareness of, and encourage expanded research on, freshwater mollusk health and the potential role of disease by (1) identifying knowledge gaps in assessing mollusk health, (2) providing information on health assessment and diagnostic tools for mollusks, (3) aligning sampling and relocation protocols with those for health and disease assessment, and (4) promoting interdisciplinary cooperation and communication to advance knowledge of freshwater mollusk health (Bradley and Waller 2019, p. 25). The long-term outcomes of these goals will be critical in trying to address and potentially manage mussel health and disease issues given that mussel die-offs have the potential to result in population-level impacts.

3.5 Host Species Vulnerability

Mudpuppies are susceptible to many of the same threats that affect mussels including contaminants, habitat degradation and fragmentation, lack of water quality and quantity, known disease issues or die-offs, and potential overharvest and collection. These threats negatively impact the abundance, distribution, and survival of mudpuppy. The conservation status of mudpuppy varies across the 14 states where the range overlaps with Salamander Mussel. Therefore, it is difficult to determine what effect these activities are having at the mudpuppy's population level. Regardless, the magnitude of these factors has the potential to have a significant localized impact on the abundance and distribution of mudpuppies, thereby directly impacting the health and status of Salamander Mussel.

Threats to mudpuppies include disease and die-offs, collection, habitat degradation and fragmentation, contaminants, and climate change. See Appendix B for more information about each of these threats.

3.6 Catastrophic Risk Factors

3.6.1 Resource Extraction

Coal mining - Coal mining has the potential to result in accidental spills and contaminant runoff. Acid mine and saline drainage (AMD) is a major threat to aquatic ecosystems and is created from the oxidation of iron-sulfide minerals such as pyrite, forming sulfuric acid (Sams & Beer, p.

3). AMD may be associated with high concentrations of aluminum, manganese, zinc, and other constituents (Tennessee Department of Environment and Conservation (TDEC) 2014, entire).

The Surface Mining Control and Reclamation Act of 1977 (SMCRA) has played a significant role in reducing AMD during mining operations, though un-reclaimed areas mined prior to SMCRA continue to generate AMD. Abandoned mines are the source of pollution in more than 5,600 mi (9,102 km) of impaired streams in Pennsylvania; in West Virginia mine drainage affects 17 percent of stream miles; and in Kentucky surface mining has been identified as a source of impairment for approximately 775 mi of streams (Pennsylvania Department of Environmental Protection 2016, p. 51). Catastrophic events, such as black water release events and fly-ash spills, have occurred in some river systems (for example, upper Tennessee River) resulting in the extirpation of mussel populations within the watershed (Ahlstedt et al. 2016, p. 8).

Impacts from coal mining may result in direct mortality due to acute toxicity of introduced contaminants as well as impact growth and reproduction leading to population level changes in the form of local extirpations or significant population declines.

Oil and gas - Oil and gas exploration and extraction can result in accidental spills, discharges, and increased sedimentation. Discharge of untreated or poorly treated brine wastewater and inadvertent release during drilling of frack fluids high in chlorides and other chemicals can result in conditions that are acutely toxic to mussels (Patnode et al. 2015, p. 62). Excess sedimentation results when there is bank slippage and mudslides during pipeline construction, open trenching operations, construction of access roads, and well pads (Ellis 1936, p. 29; Anderson & Kreeger 2010, p. 2). Excessive suspended sediments and contaminants resulting from inadvertent releases or runoff can be acutely toxic, result in sublethal effects such as impairing feeding processes, and degrade and destroy suitable habitat for mussels.

3.7 Current Conservation Efforts

3.7.1 Mussel Conservation Propagation Programs

Conservation propagation is an important tool that is being used to augment and reintroduce Salamander Mussel populations in Pennsylvania, West Virginia, Wisconsin, and Kentucky. Two of USFWS's National Fish Hatcheries (Genoa and White Sulfur Springs) are actively propagating Salamander Mussel as well as other mussel species for conservation and recovery. In addition, several State wildlife agencies have developed mollusk conservation propagation programs, including The Kentucky Department of Fish and Wildlife Resources that established the Center for Mollusk Conservation in 2002 and have been propagating Salamander Mussel and other mollusks to aid conservation. These conservation propagation efforts have been critical in contributing significant conservation benefits to imperiled Salamander Mussel populations as well as enhancing our understanding of Salamander Mussel and mudpuppy reproduction and life history. These programs will continue to be an important conservation tool into the future for Salamander Mussel and mudpuppy conservation. For more information, see Appendix B.

3.7.2 Mudpuppy Conservation Efforts

Efforts to construct artificial mudpuppy habitats have been undertaken in several waterbodies, including Allegheny River in Pennsylvania (Welte 2020, entire); within the Detroit St. Clair Rivers, Lake St. Clair and Lake Erie in Michigan (Stapleton et al. 2018, entire); and at Guttenberg, Iowa (K. Hanson, personal communication, 2021). Mudpuppies have been observed using the constructed habitat within the first 6 months of installation (K. Hanson, personal communication, 2021). In Pennsylvania, structures were monitored one-year post-placement (structures placed in 2018, monitored in 2019); one live Salamander Mussel was observed under an artificial structure. No mudpuppies were observed, but silt may have obscured escaping mudpuppies during monitoring (Welte 2020, entire). In Michigan, mudpuppies were observed at two recent restoration sites where mudpuppies had not previously been detected, indicating that efforts to create mudpuppy artificial habitat have been successful (Stapleton et al. 2018, entire).

CHAPTER 4. CURRENT CONDITION

4.1 Species Current Distribution

We describe and analyze the distribution in terms of watersheds occupied. Watersheds are delineated by the U.S. Geological Survey (USGS) based on surface hydrological features. These hydrological areas are identified by hydrological units at various scales. The different scales are assigned Hydrologic Unit Codes (HUCs). The hydrological units start with a 2-digit code at the regional level, expanding from there to a finer scale. We used the HUC2 at the regional scale (representation unit) and the HUC8 at the subbasin (population) scale (https://nas.er.usgs.gov/hucs.aspx). Conducting our current condition analysis at the HUC8 scale allowed us to assess Salamander Mussel occurrences at an ecologically relevant scale for which we have data on the primary stressors affecting populations.

We developed categories that define a population's status as extant, presumed extant, presumed extirpated, extirpated, or historical to assess the health, number, and distribution of populations through time (Table 4.1). Because Salamander Mussel is a thin-shelled species, weathered dead shells are not expected to persist in a system for an extended time. Therefore, we classified weathered dead collections as an indicator of extant populations. We used the year 2000 as the cutoff for the extant category given incomplete survey data for Salamander Mussel across the range, lack of persistence of shell material, and the life span (approximately 10 years). Given the frequency of mussel surveys for other species within the range of Salamander Mussel, targeted or incidental observations of Salamander Mussel (live, fresh dead, or weathered individuals) would indicate the continued presence of the population. Given the paucity of data and lack of survey effort specifically for Salamander Mussel, we relied on the same methodology for presumed extant and determined that 1970–1999 was an appropriate time frame to presume potential presence. It is important to note a single observation of an individual in any condition can be considered an extant or presumed extant population depending on the observation year. We carried forward and analyzed current condition for both the extant and presumed extant (total of 66 populations).

Table 4.1. Definitions of status assigned to the Salamander Mussel populations.

Status	Definition
Extant (E)	Observation(s) from 2000 – 2020 live, fresh dead, or weathered dead
Presumed Extant (PE)	Observation(s) from 1970 – 1999 live, fresh dead, or weathered dead
Presumed Extirpated (PX)	Observation(s) from 1970 – 1999 live, fresh dead, or weathered dead that have been determined based on expert opinion to be likely extirpated due to significant survey effort within the river system since 1999 with no evidence of Salamander Mussel and/or general decline of entire mussel community within the HUC8 watershed indicating extirpation
Extirpated (X)	Documentation of extirpation from system based on acute event and/or expert opinion
Historical (H)	Observation(s) prior to 1970, categorized as historical within State Natural Heritage Data, and/or no additional data available after 1970 for the population.

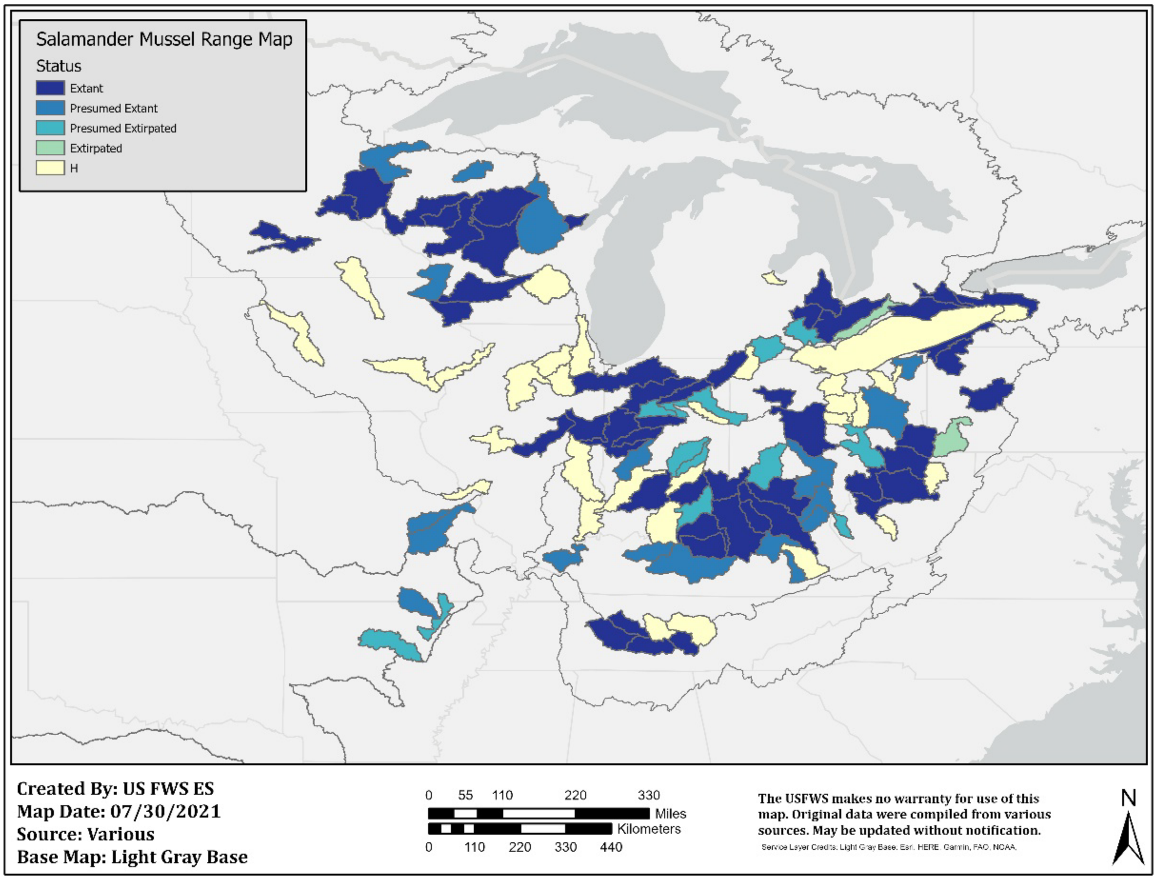

Figure 4.1. Salamander Mussel population status for the range.

Table 4.2. Summary of population status (HUC 8 watersheds) by representation unit (HUC 2 river basins) for Salamander Mussel.

Status	Extant (E)	Presumed Extant (PE)	**Current Population Total**	Presumed Extirpated (PX)	Extirpated (X)	Historical (H)	**Total**
Arkansas-White-Red	0	1	1	2	0	0	3
Great Lakes	9	2	11	2	1	6	20
Ohio	25	10	35	8	1	13	57
Tennessee	2	0	2	0	0	0	2
Upper Mississippi	12	5	17	0	0	11	28
Species Range Total	48	18	66	12	2	30	110

4.2 Population Resiliency

4.2.1 Population Factors Methodology

We have minimal demographic data from a small number of populations across the range, and most of these data are abundance information for localized populations. For these populations, we assessed demographic population condition (in HUC8 watersheds) as high, moderate, low, or functionally extirpated based on demographic criteria (Table 4.3). We used demographic data from targeted or semi-targeted surveys available for the last 20 years (2000–2020). These types of surveys are defined as someone looking specifically for Salamander Mussel (targeted) or spending a portion of the survey looking for Salamander Mussel while conducting other mussel surveys (semi-targeted). We considered populations with very low abundance (one to 10 live individuals found within the last 20 years) to be functionally extirpated (Table 4.3). We assigned an estimate of the probability of persistence over 20 years (approximately 2 generations of Salamander Mussel) for each population condition category based on best professional judgement of a population abilities to withstand demographic stochastic events. These opinions were provided by the Core SSA Teams (including species experts, a malacologist, SSA and recovery experts).

For most populations, we do not have demographic data that would allow us to evaluate population health in regard to the ability to withstand demographic stochastic events. For these populations, we have data from incidental observations only. These are surveys where Salamander Mussel was not the target species and suitable habitat was not surveyed. In these cases, evidence of Salamander Mussel in the form of shell or live individuals was incidental to targeted searches for other species. Due to this lack of data, we could not identify demographic conditions for these populations; therefore, we categorized them as unknown.

Table 4.3. Condition category descriptions for Salamander Mussel demographic factors using targeted and semi-targeted survey information.

Condition Category for Semi-targeted and Targeted Records	Demographic Factors	Probability of Persistence over 20 years
High	at least 1000 individuals (live, fresh dead, weathered, half valves) observed or collected over 20 years with no evidence of decline; evidence of reproduction (in other words, gravid) and/or recruitment (in other words, multiple age classes) in the last decade.	>80%
Moderate	500 to 1000 individuals (live, fresh dead, weathered, half vales) observed or collected (unless state experts estimate that relative level of effort equates to a predicted higher number of individuals) within the last 20 years with at least one live within the last 10 years	60–80%
Low	10 to 500 individuals (live, fresh dead, weathered, half valves) observed or collected in the past 20 years with at least 1 live in the past 20 years	30–60%
Functionally Extirpated	less than 10 live individuals within the last 20 years	<30%

4.2.2 Risk Factors Methodology

We evaluated the six primary risk factors affecting Salamander Mussel (water quality/contaminants, hydrological regime, landscape, connectivity, invasive species, and host species vulnerability) to assist in evaluating the current condition of each current population (Table 4.2). We assigned these risk factors to three categories of high, moderate, and low risk (Table 4.4). For further information on the methods used to evaluate metrics within each of these risk factors and the scoring system, see Appendix C. Similar to our demographic criteria, we also assigned a probability of persistence over 20 years (approximately 2 generations of Salamander Mussel) for each of the risk categories to create a common understanding of what we mean when we categorize a population as being at high, moderate, or low risk (Table 4.4). To assess overall current condition for the risk factors we developed a rule set as follows: if any one of the risk factors is high = overall population condition is high risk; if none of the risk factors are high an additive approach was used, scores 9–12 = moderate risk and scores 6–8 = low risk. These break points were based on three or more risk factors being categorized as moderate. In order to be considered an overall low risk, the majority of risk factors have to be categorized as low.

Table 4.4. Risk category descriptions for the six risk factors evaluated for the current condition of each Salamander Mussel population. See further descriptions of criteria for risk factors in Appendix C.

Risk Category	Contaminants	Landscape	Hydrological Regime	Connectivity	Invasive Species	Host Species Vulnerability	Estimation of probability of persistence over 20 years
High	Concentration exceeds acute toxicity levels >2% of samples (2000 - present)	Landscape condition severely altered by anthropogenic factors;	Extreme and exceptional droughts are severe annually for 3 or more consecutive years.	Habitat severely fragmented or unpaved road crossing density	N/A (invasive species do not present a high risk to Salamander mussel)	Sea lamprey control is administered frequently; collection of mudpuppy is not regulated	<60%
Moderate (2 points)	Concentration exceeds acute toxicity levels to mussels <2% of samples (2000 - present)	Moderate level of landscape alterations due to anthropogenic factors, isolated or widely distributed effects on aquatic biota survival	(Extreme and exceptional droughts occur for less than 3 consecutive years.	Some habitat fragmentation issues or unpaved road crossing density	Present in abundance	Sea lamprey control is administered infrequently; collection of mudpuppy is permitted, but have limits in place.	60–90%
Low (1 point)	Concentrations at levels below acute toxicity to mussels (2000 to present)	Landscape condition altered slightly due to anthropogenic factors, minimal to no known habitat fragmentation	Flows < 5th percentile for less than 4 consecutive weeks annually	Very little, if any, known habitat fragmentation issues or unpaved road crossing density	Present in moderation or absent	No known sea lamprey control; prohibition of mudpuppy collection.	>90%

4.2.3. Current Condition

Salamander Mussel occupies 66 HUC8 watersheds currently, though it was historically found in 110 (Figure 4.1, Table 4.2). The range spans across 14 states and one Canadian province. We evaluated risk factors for the 66 populations where data were available. We were not able to evaluate for risk factors for the three populations where watersheds cross into Canada. We also evaluated the demographic condition of the populations when we had survey data available. However, the majority of the populations have incidental surveys and therefore unknown demographic condition (Table 4.5) and have only risk factors evaluated.

Arkansas-White-Red Basin Representation Unit

Demographic condition - The Arkansas-White-Red basin spans portions of Arkansas and Missouri (Figure 4.2). There is one current population that occurs within this basin (the Spring population) (Table D.9). Two populations (the Lower Black and Little Red populations) are presumed extirpated based on state expertise; therefore, we did not evaluate the risk factors for those (K. Moles, personal communication, 2021). The Spring population has an unknown demographic population condition, but is presumed extant (K. Moles, personal communication, 2021). Three fresh dead individuals were found prior to 1987 (Figure 4.3), though recent survey effort has not resulted in any detections (Harris & Gordan 1987, entire).

Risk Factors - The Spring population is experiencing high risk due to lack of connectivity (Table D.9). There has not been evidence of Salamander Mussel in the last 20 years, and given the high risk from connectivity, we expect this population would at most be in low condition by 2040. Catastrophic risk for the representation unit includes high risk associated with oil and gas presence (Table D.10).

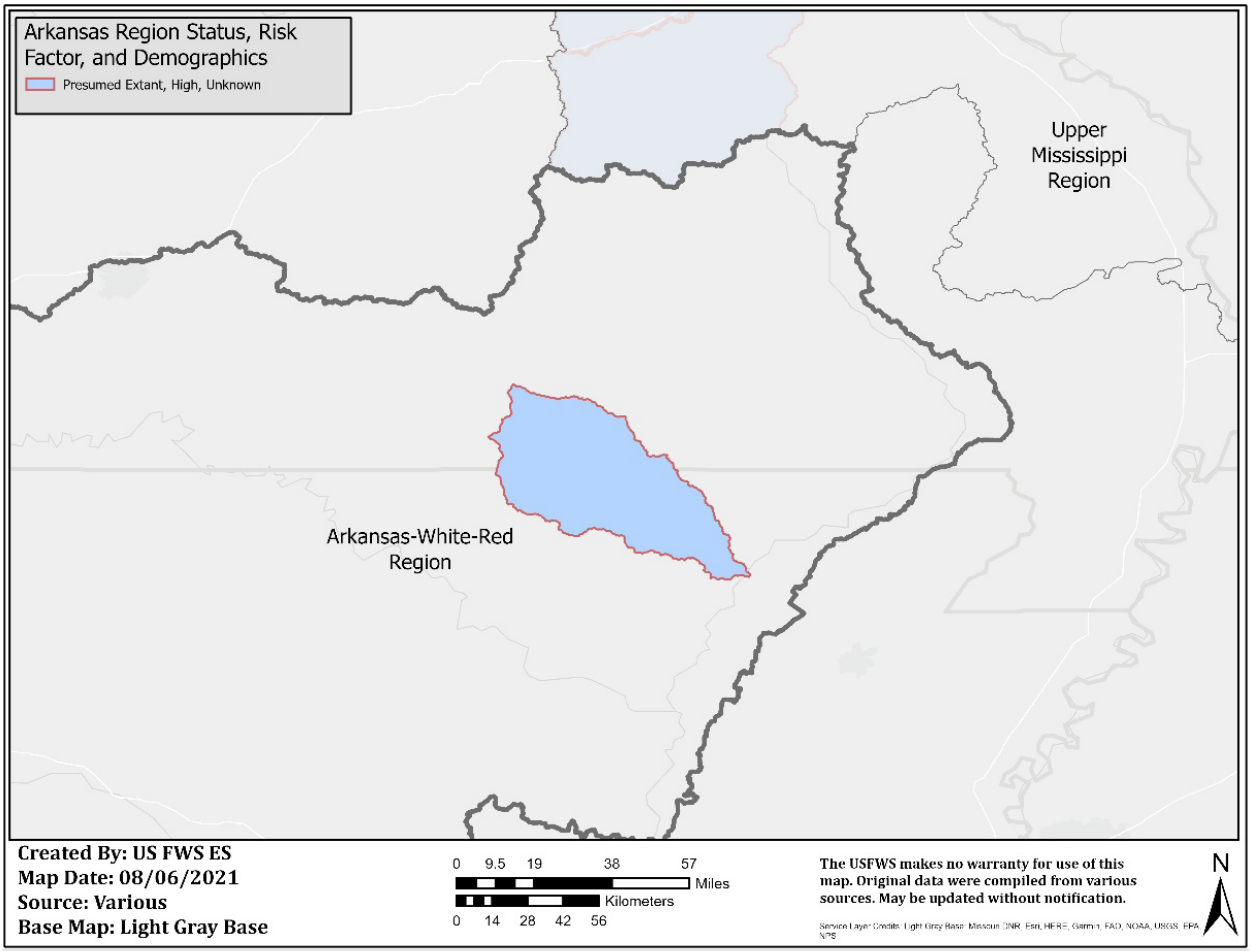

Figure 4.2. Extant and presumed extant populations within the Arkansas-White-Red basin for Salamander Mussel.

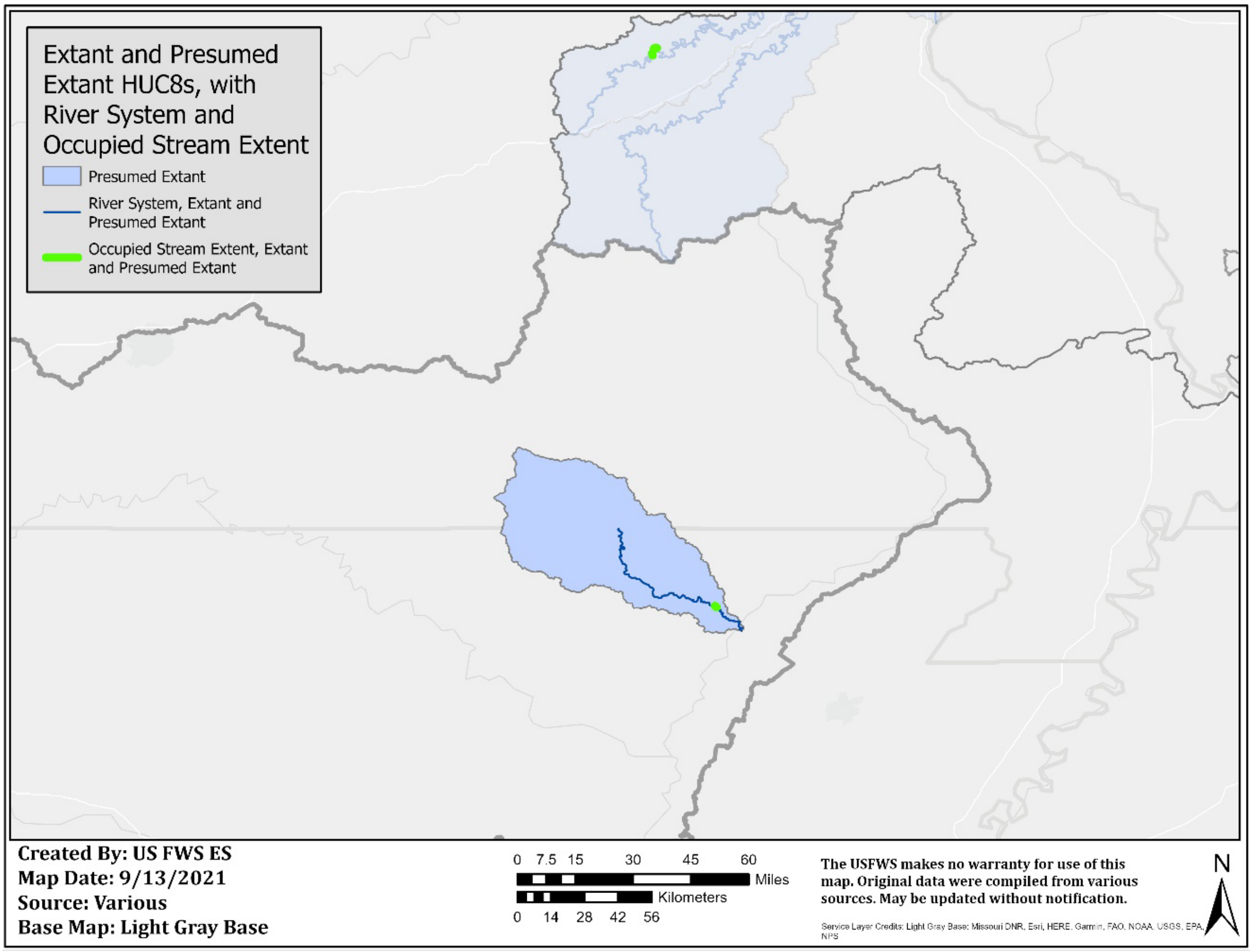

Figure 4.3. Occupied stream extent of extant and presumed extant populations within the Arkansas-White-Red basin for Salamander Mussel.

<u>Great Lakes Basin Representation Unit</u>

Demographic condition - The Salamander Mussel's range within the Great Lakes basin includes portions of Canada, New York, Pennsylvania, Ohio, Indiana, Michigan, and Wisconsin. Six populations are considered historical, two are presumed extirpated, and one is extirpated (Table 4.2). Eleven populations are still considered extant or presumed extant (Figure 4.2). Five extant populations have enough information to evaluate demographic condition; of these, four are functionally extirpated and one is in low condition (Figure 4.4, Table D.1). Three of the four categorized as functionally extirpated are represented by a single survey in a single year with a single individual (1 live, 2003; 1 weathered shell, 2019; 1 live, 2019). The survey in 2003 was conducted by 10 people looking for 2 hours and observed only 1 live individual. Of the remaining four populations with unknown demographic condition, two are represented by a single record (1 record, 2009; 1 fresh dead, 2018). Additionally, one of the four populations with unknown demographic condition is represented by 3 surveys all within April 2008 that were conducted in a single creek. A total of six populations (55%) are represented by a single observation or by a single year (Figure 4.5).

Risk factors - Risk factors could not be assessed for populations that overlapped into Canada based on the availability of datasets for risk metrics (see below). All populations that were assessed for risk (N=8) are in overall high-risk condition (Table D.1). Seven populations had complete datasets to assess contaminant risk. All seven populations were categorized at high risk for contaminants. The major drivers were nitrate, chloride, and copper exceedances. Additionally, 50% of the populations (N=4) are in high risk category for landscape threats, which were driven by agriculture, urbanization, and threats to the riparian vegetation and canopy cover. Two populations are within Wisconsin and categorized as high risk for host species vulnerability due to the lack of state regulations for mudpuppy collection. Two additional populations are at high risk for host species vulnerability due to lampricide treatments. For the populations within the U.S., catastrophic risk includes high risk associated to oil and gas presence (Table D.2).

Ontario, Canada (HUC8s Lower Thames [X], Lower Grand, Lake St. Clair (E), and Detroit)

Demographic condition - Seven populations cross the U.S.-Canada border, three of which are considered extant populations. Canada provided data to assess the current condition category for the demographic criteria for the Canadian populations. The Lake St. Clair population is in low population condition based on the demographic criteria. In 2019, one weathered dead individual was found in the Lower Grand population, and this population is functionally extirpated. For the Lower Thames and Detroit populations, we did not have any data within the last 20 years to assess the populations' demographic population condition (Table 4.3).

Risk factors - For the habitat risk factors, the spatial data sets used for assessment were not available for Canada. Therefore, to assess the overall current condition for the St. Clair, Lower Thames, Lower Grand, and Detroit populations within Canada, we are relying on the status assessment completed by the Committee on the Status of Species at Risk in Ontario (COSSARO) in June 2011 (COSSARO 2011, entire), which designated Salamander Mussel in Canada as endangered. This was based on presence of Salamander Mussel in a single river system in Canada, the Sydenham River (Lake St. Clair population). Live individuals have been found within the last 20 years within this river system. The Lake St. Clair population is in low demographic condition. The Lower Thames population is deemed to be extirpated by COSSARO. Based on the lack of records within the last 20 years, the risk factor analysis for the U.S. portion of the Detroit population and significant level of survey effort for the Detroit River on both the Canadian and U.S. portions of the river, we presume the Detroit population is extirpated.

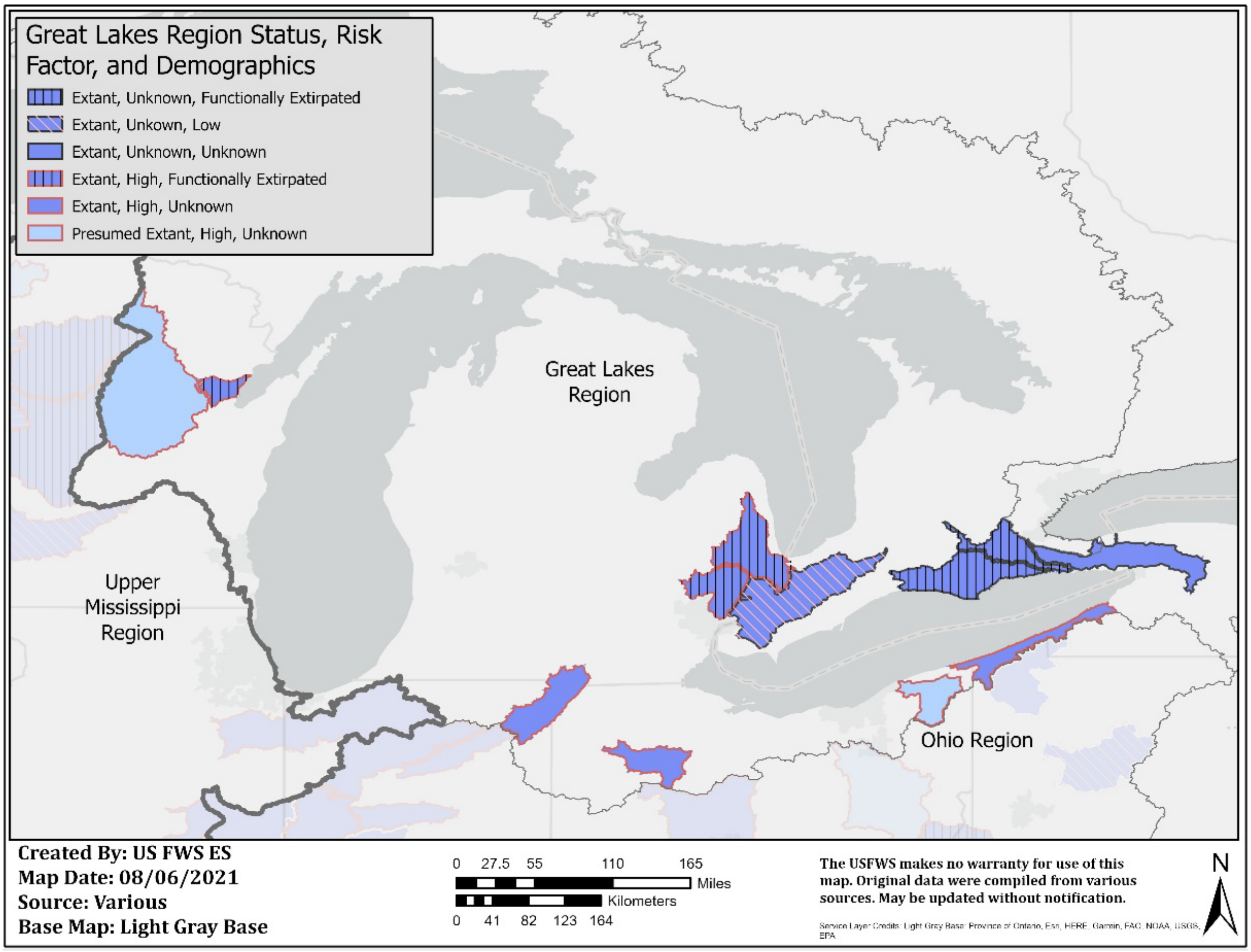

Figure 4.4. Extant and presumed extant populations within the Great Lakes basin for Salamander Mussel.

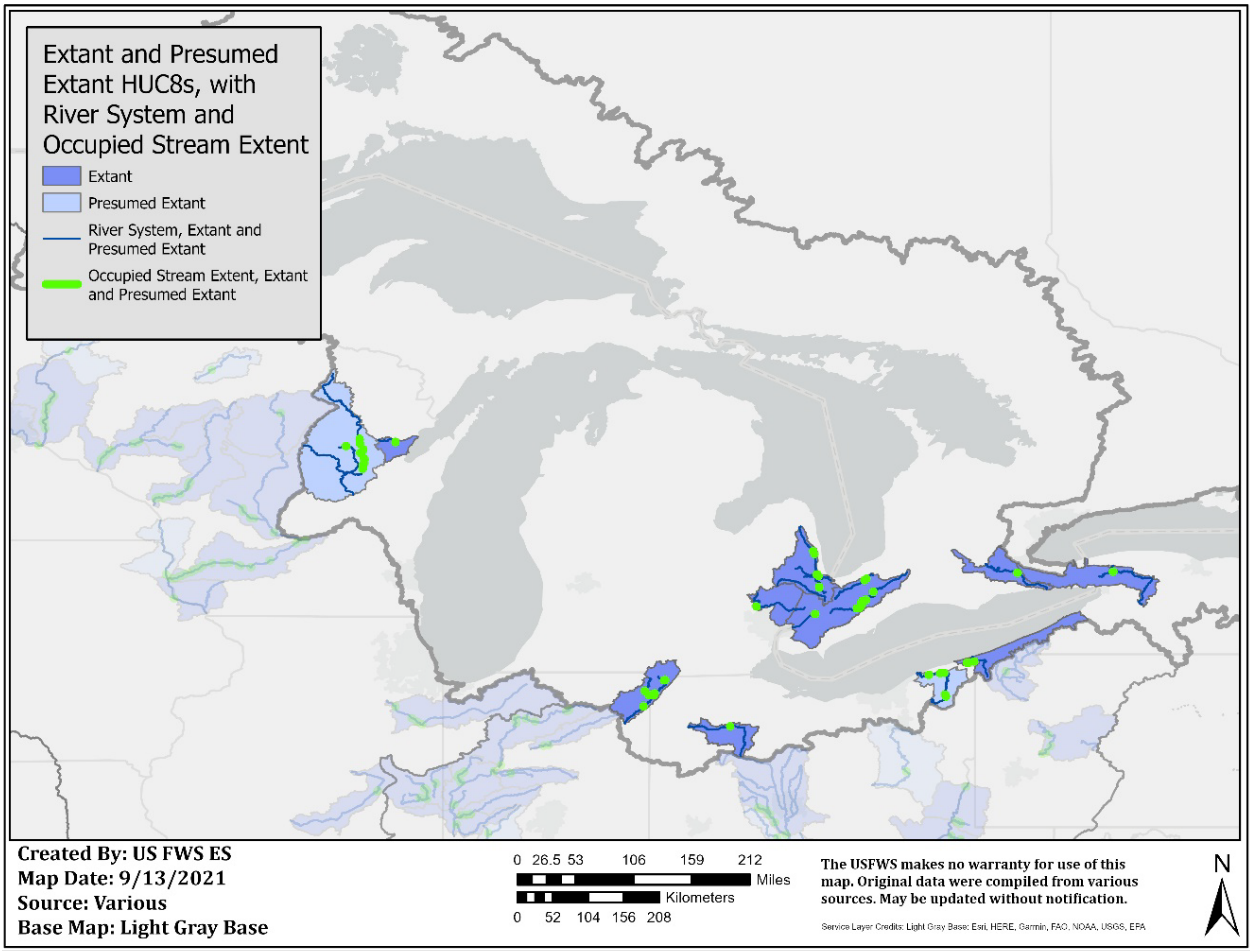

Figure 4.5. Occupied stream extent of extant and presumed extant populations within the Great Lakes basin for Salamander Mussel.

Ohio Basin Representation Unit

Demographic condition - The Ohio Basin includes portions of New York, Pennsylvania, West Virginia, Ohio, Kentucky, Tennessee, Indiana, and Illinois (Figure 4.6). The Ohio basin has the majority of Salamander Mussel populations. There are 35 populations within the basin, 25 extant and 10 presumed extant (Figure 4.6). Of the 35 populations, 29 are based on incidental surveys with a little over half (52%) determined to be occupied by only two observations for the entire population since 1970 (Figure 4.7). Six populations are considered historical, one is extirpated, and two are presumed extirpated. We can evaluate the demographic condition for six of the populations. Of these, two are functionally extirpated, three are in low condition, and one is in moderate condition (Figure 4.6, Table D.3). One of the two populations that is functionally extirpated has had 42 surveys completed in Fish Creek between 2010 and 2016, without finding any live individuals. The other has had significant survey effort for mudpuppies and two targeted surveys, though we do not have any data from any of this survey effort. The population in moderate condition is based on 10 semi-targeted and targeted surveys that have been conducted between 2009 and 2017 and found live individuals as well as mudpuppies. Of the populations in low condition, one is based on one targeted survey in 2013 that resulted in 125 live individuals, with 61 being found under one rock, and the other on one semi-targeted survey resulting in 36 live individuals. The other population in low condition is based on very few live individuals being found since 2000 with semi-targeted and targeted survey effort as well as significant survey effort for the broader mussel community. These populations have all had survey effort for other mussel species, mudpuppy, and targeted surveys for Salamander Mussel. However, in 3 of the 6 populations the demographic condition is based on either 1 or 2 surveys (creating single or small river extents) for the entire population (Figure 4.7).

Risk factors - Of the 35 populations, 27 are in overall high risk condition, and eight are at moderate risk condition (Figure 4.6; Table D.3). As such, approximately 77% of the populations within the Ohio Basin Representation Unit are at high risk. Of the populations at high risk overall, 96% are at high risk due to contaminants, with nitrate driving the high risk in almost all populations. A possible explanation for the nitrate source in this area is from fertilizers and animal manure, which are linked to agriculture types commonly found in the upper Midwest. Of these populations at high risk for contaminants, eight are also at high risk for other stressors including landscape, connectivity, and host species vulnerability. Catastrophic risk for the Ohio basin includes high risk associated with oil and gas presence (Table D.4).

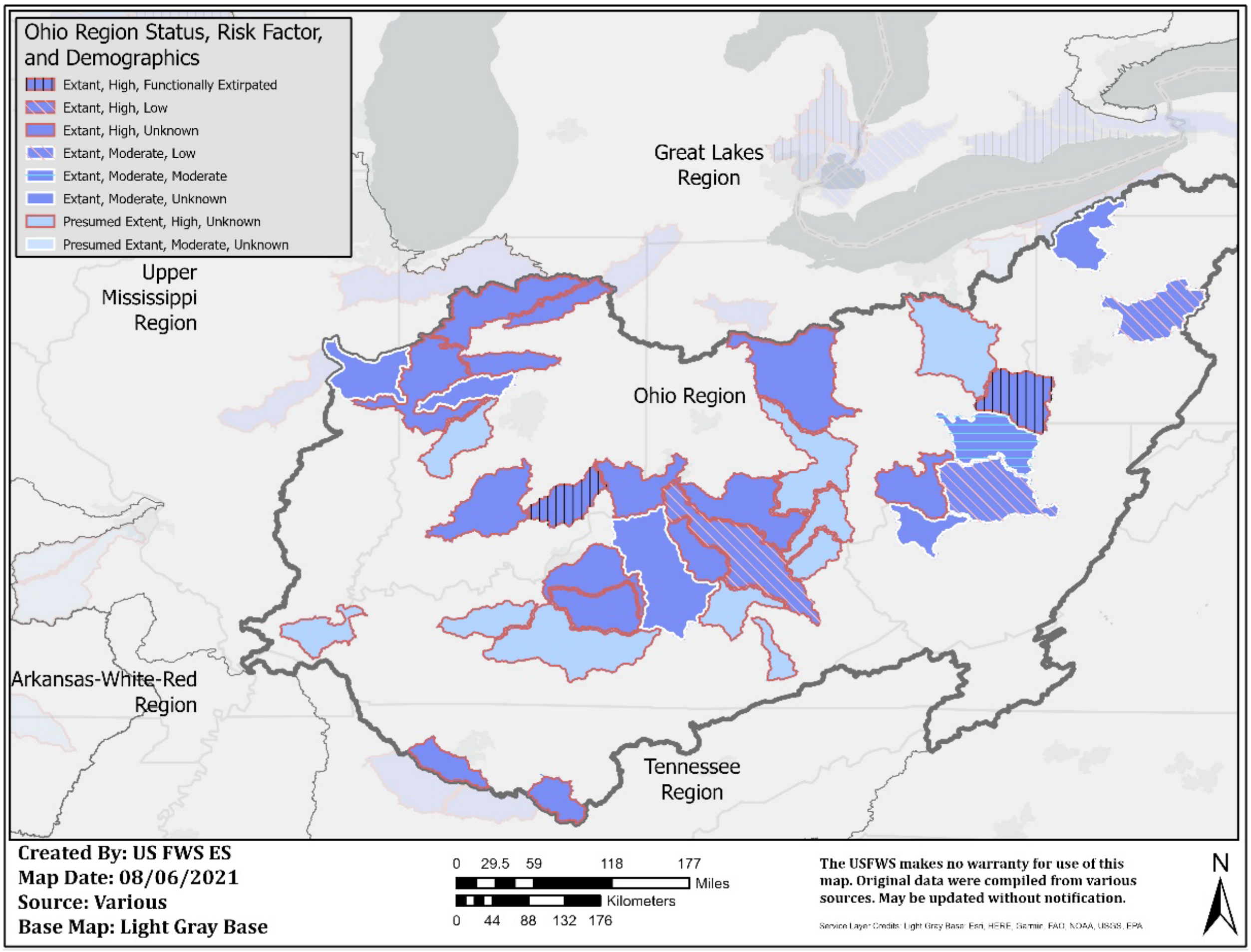

Figure 4.6. Extant and presumed extant populations within the Ohio basin for Salamander Mussel.

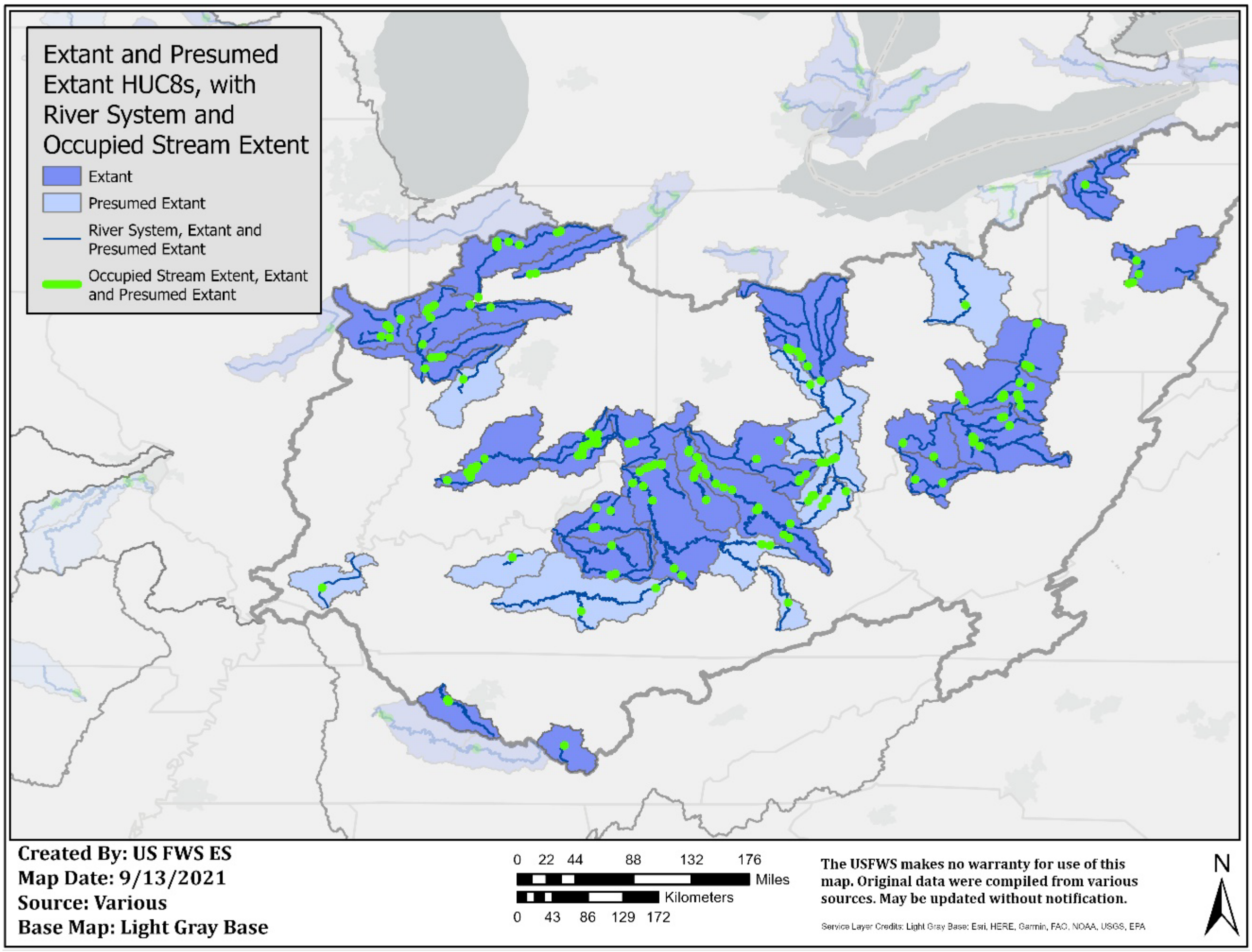

Figure 4.7. Occupied stream extent of extant and presumed extant populations within the Ohio basin for Salamander Mussel.

Tennessee Basin Representation Unit

Demographic condition - The Tennessee basin incorporates a small section of the Salamander Mussel's range within Tennessee (Figure 4.8). The two populations within this basin border the southern portion of the Ohio basin (Figure 4.8). The Upper Duck is represented by 2 introduction efforts in 2017. The Lower Duck is represented by two surveys (2003 and 2017). The 2017 survey found 1 fresh dead individual (Figure 4.9).

Risk factors - Both populations are in overall high level risk condition (Figure 4.8; Table D.5). Both populations are at high risk for connectivity due to unpaved road stream crossings, while the Upper Duck is also at high risk for contaminants from nitrate and copper exceedances, and both are at high risk due to host species vulnerability. Catastrophic risk for all populations in this basin includes high risk associated with oil and gas presence (Table D.6).

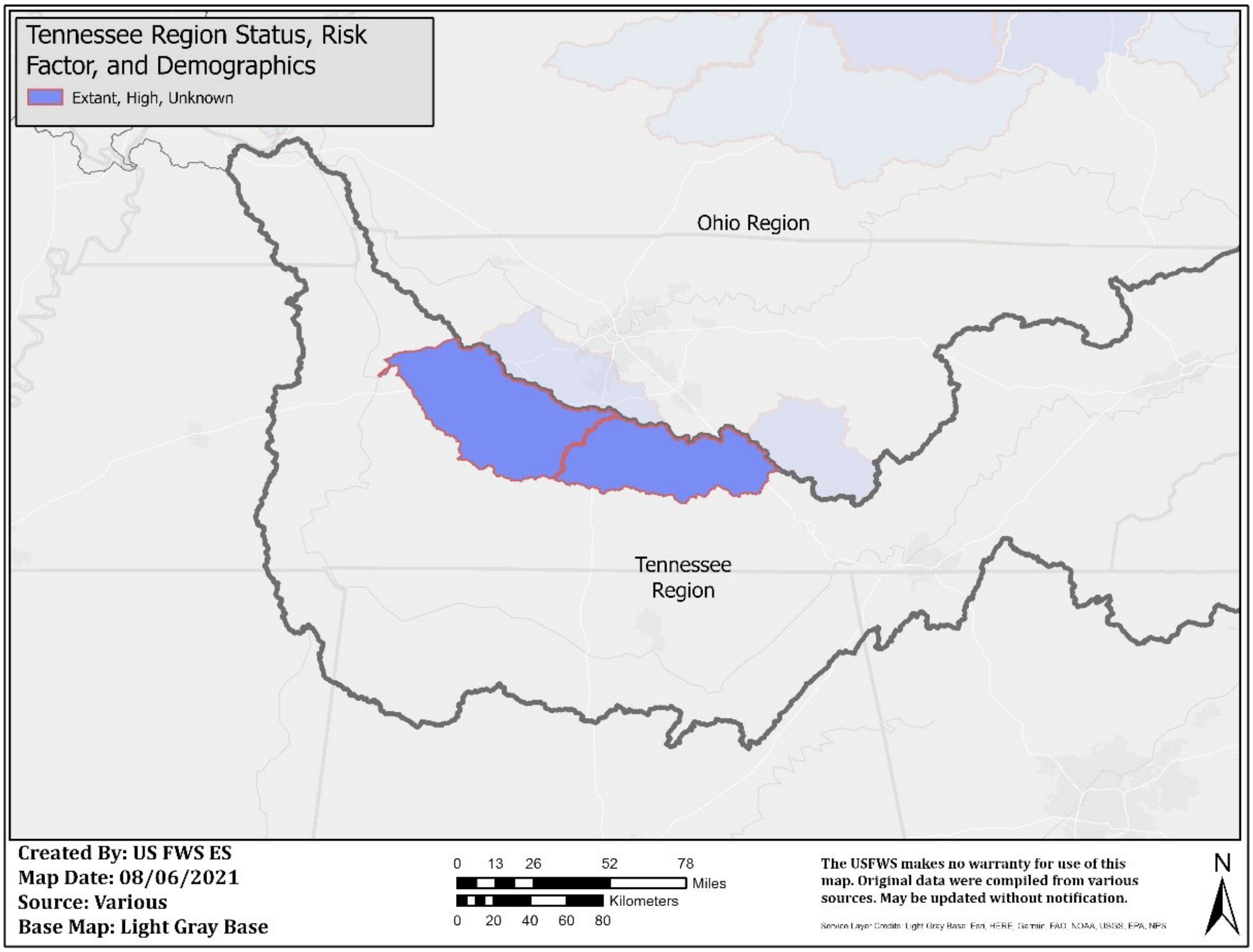

Figure 4.8. Extant and presumed extant populations within the Tennessee basin for Salamander Mussel.

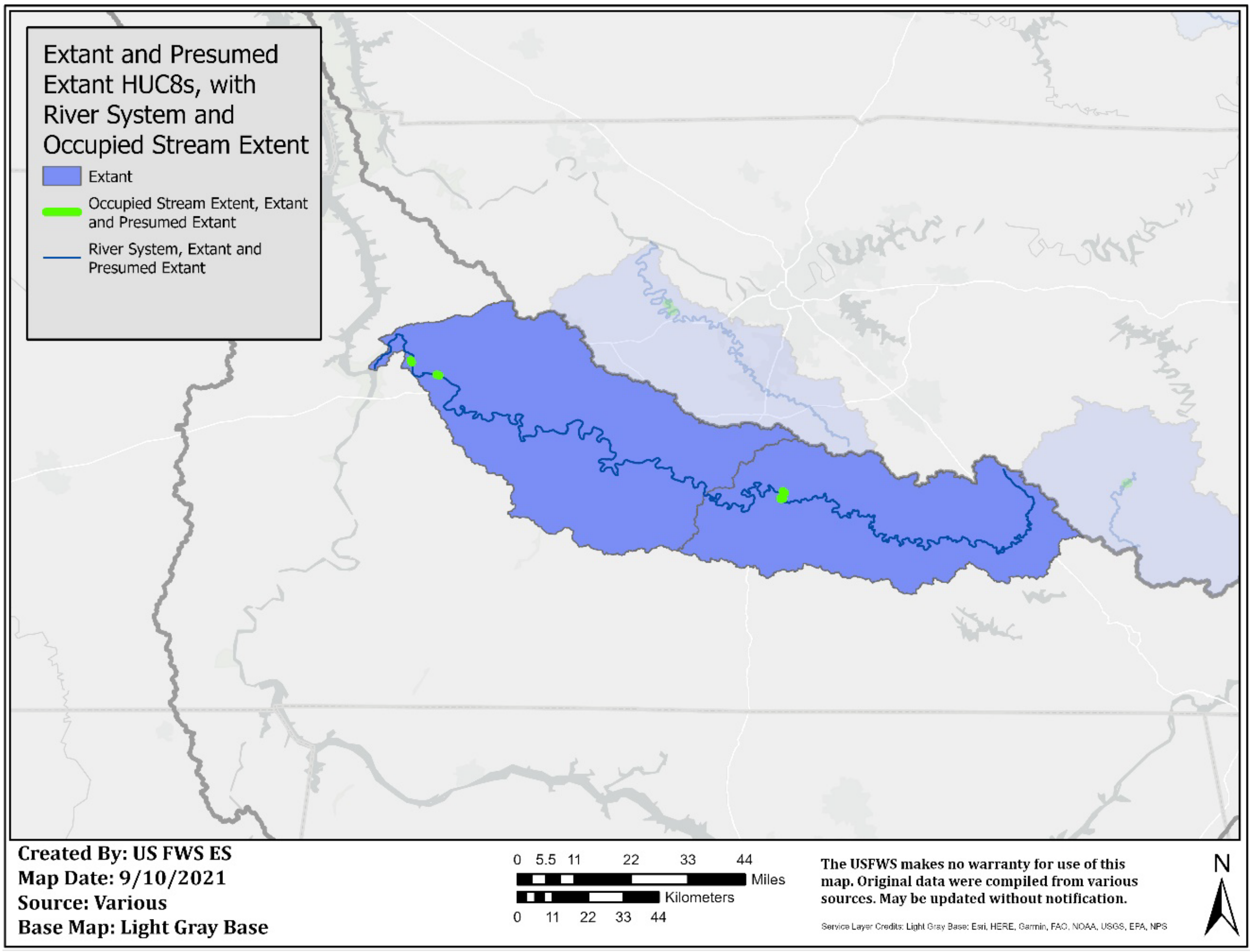

Figure 4.9. Occupied stream extent of extant and presumed extant populations within the Tennessee basin for Salamander Mussel.

Upper Mississippi Basin Representation Unit

Demographic condition - The Upper Mississippi Basin spans portions of Missouri, Illinois, Indiana, Iowa, Minnesota, and Wisconsin (Figure 4.10). The majority of Salamander Mussel populations within this basin are concentrated within Minnesota and Wisconsin (Figure 4.10). There are 17 populations within this basin; 12 are extant with the remaining 5 presumed extant. Seven of the 12 extant populations have survey information that allowed us to evaluate the demographic condition of the Salamander Mussel populations. Three populations are considered functionally extirpated. Of these three, two populations are based on a single survey with a single live individual. One population's survey was in 2010, while the other population's survey occurred in 2002. The third population considered functionally extirpated is based on two surveys from 2017 that found two fresh dead individuals. Similarly, out of the five extant populations with unknown demographic condition, three populations are represented by a single observation within the last two decades (1 weathered, 2004; 1 unknown condition, 2010; 1 unknown condition, 2014). Three presumed extant populations out of five are represented by a single observation (2 live, 1990; 2 dead, 1982; unknown, 1988). Within the Upper Mississippi basin, nine out of 17 populations (53%) are represented by a single observation or single year of data (Figure 4.11).

All 17 populations in this basin are in a high-risk condition for contaminants (Table D.7). The Upper Mississippi basin is currently experiencing high risk associated with nitrate thresholds for 16 populations (94%; excluding Grant-Little Maquoketa). Similarly to the Ohio basin, the possible explanation for the nitrate source in this area is from fertilizers and animal manure, which are linked to agriculture types commonly found in the upper Midwest.

Risk factors - Nine populations (within Wisconsin; 53% of total populations within Upper Mississippi basin) are categorized as high risk for host species vulnerability due to the lack of state regulations for mudpuppy collection. Additionally, five populations (30%) are at high risk for connectivity due to the number of dams within the watershed. Catastrophic risk for all populations in this basin includes high risk associated with oil and gas presence (Table D.8).

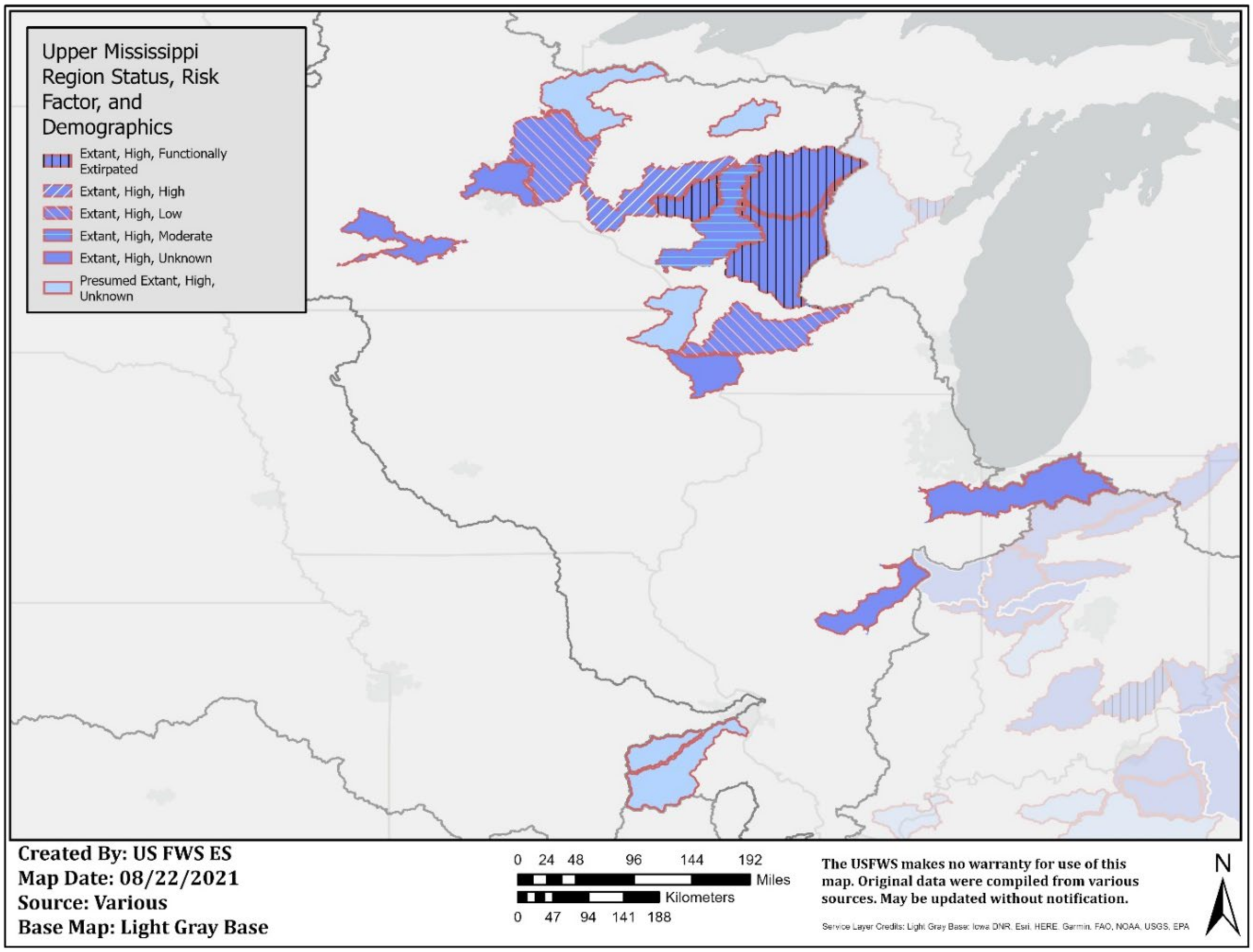

Figure 4.10. Extant and presumed extant populations within the Upper Mississippi basin for Salamander Mussel.

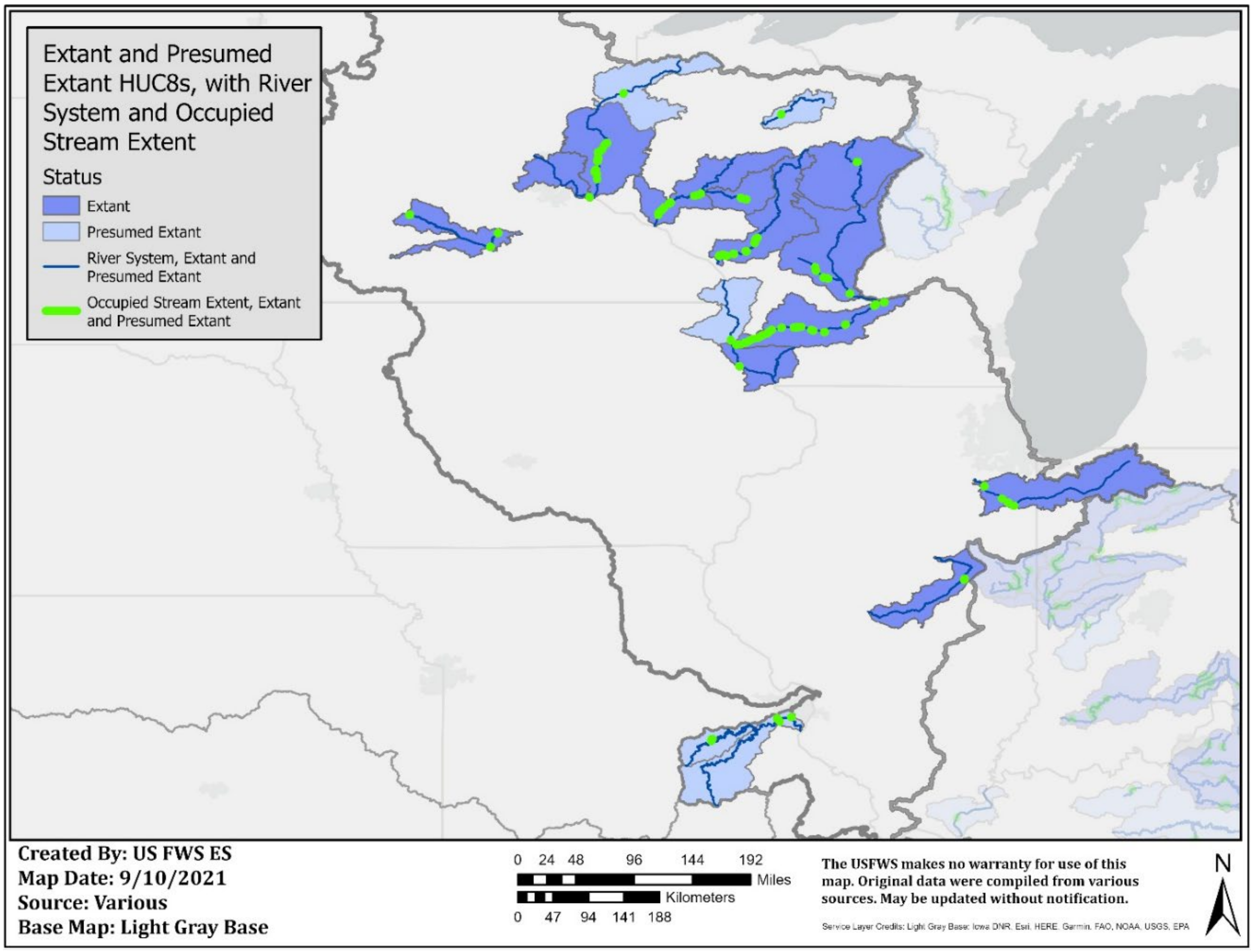

Figure 4.11. Occupied stream extent of extant and presumed extant populations within the Upper Mississippi basin for Salamander Mussel.

4.2.4 Population Resiliency Summary

Demographic condition - Of the 66 populations (extant and presumed extant), the majority (N=48; 73%) are in unknown demographic population condition. Demographic information is available for only 18 populations. Of these 18, half are considered functionally extirpated, a third are in low demographic population condition, and a sixth are in moderate or high (Table 4.5).

Risk factors - Over 80% of all 66 populations experience high risk, meaning there is a less than 60% chance of population persistence over 20 years (Figure 4.12, Table 4.6). This includes 42 populations with an unknown demographic condition (Table 4.7). Approximately 14% of all 66 populations experience moderate risk (5 unknown, 2 low, and 1 moderate demographic population condition; Table 4.7), meaning there is a 60–90% probability of population persistence over 20 years (Table 4.6). None of the populations across the range are experiencing low risk (Table 4.7). We did not have information to complete the risk factor analysis for three populations that cross the border with Canada (Table 4.6).

The number of populations has decreased 40% from historical numbers rangewide. The number of populations in the Ohio and Upper Mississippi basins declined by almost 40% while the number of Great Lakes basin populations has declined by 45% (Table 4.2). These three basins make up the core area for Salamander Mussel with approximately 40% of the populations having evidence of Salamander Mussel in the last two decades. Salamander Mussels have not been observed in the Arkansas-White-Red basin in the last two decades. One of the two populations in the Tennessee basin has had Salamander Mussels introduced the last two decades and is considered in the total number of extant populations.

Table 4.5. Summary of demographic condition for Salamander Mussel extant and presumed extant populations across the range.

Demographic Condition	**Number of Populations**
High	1
Moderate	2
Low	6
Functionally Extirpated	9
Unknown	48
Total	**66**

Table 4.6. Summary of risk factor condition for Salamander Mussel extant and presumed extant populations across the range.

Risk Factor Condition	Number of Populations
High Risk	55
Moderate Risk	8
Low Risk	0
Unknown	3
Total	**66**

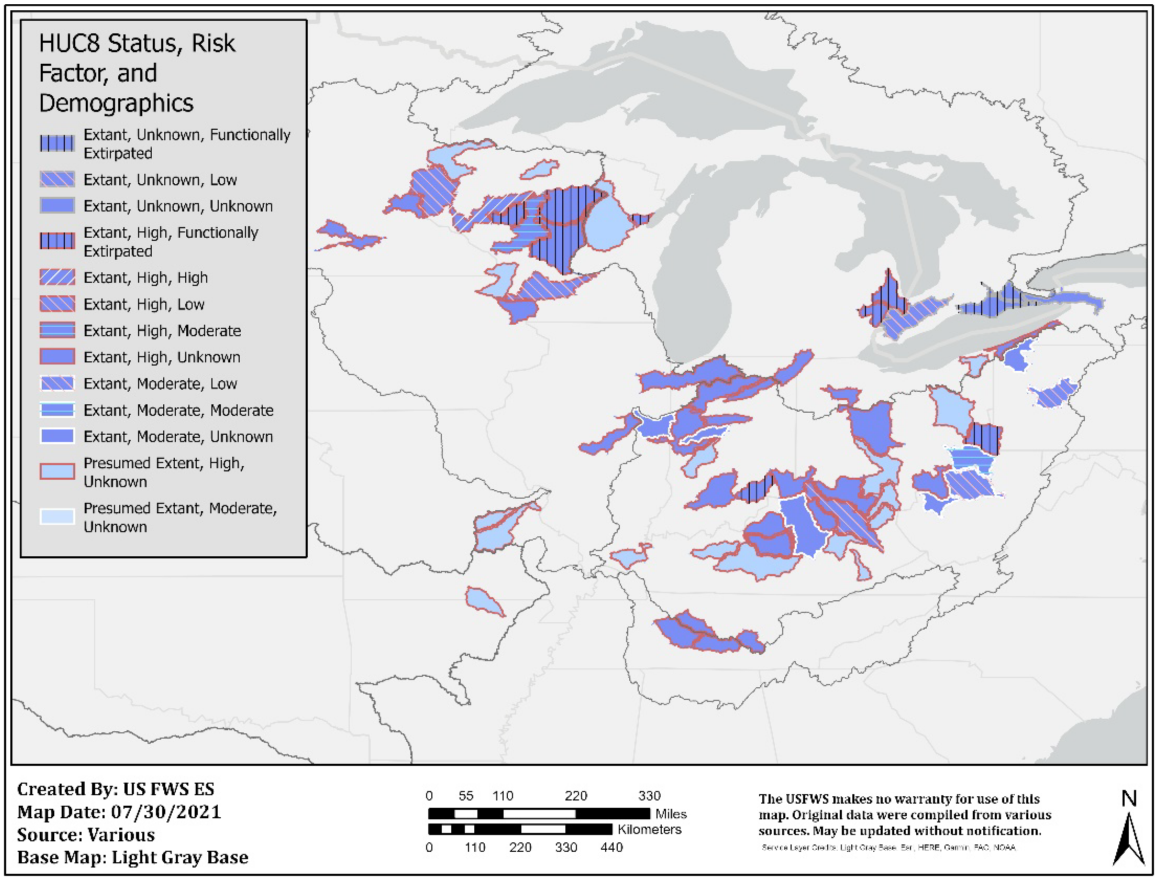

Figure 4.12. Salamander Mussel population status, risk factor condition, and demographic condition range wide for extant and presumed extant.

4.3 Species Representation

We used HUC2 river basins to delineate five representative units for Salamander Mussel: Upper Mississippi, Ohio, Tennessee, Great Lakes, and Arkansas-White-Red basins (refer to section 2.5.2). The species currently ranges across all five representation units (Figure 4.1; Table 4.7). The Ohio River basin has 35 populations; of these, 27 are at high risk (Table 4.7). The Upper Mississippi basin has 17 extant populations, all of which are at high risk (Table 4.7). The Great Lakes basin has 8 populations with risk analysis complete, all of which are at high risk (Table

4.7). We did not have information to complete the analysis for three populations that cross the border with Canada (Table 4.6). The Arkansas-White-Red basin has one population that is presumed extant and at high risk. The Spring population is the only remaining population left in the basin and the most recent record of Salamander Mussel is from over thirty years ago. The Tennessee basin has two extant populations, both of which are at high risk. With few populations that are all at high risk, the Great Lakes, Tennessee, and Arkansas-White-Red representation units are all at risk of extirpation. Although the Upper Mississippi representation unit has 14 populations, all of them are at high risk, putting the unit at risk of extirpation. The Ohio basin is the only representation unit with populations experiencing moderate risk (Table 4.7).

4.4 Species Redundancy

Of the 110 known populations of Salamander Mussel, 66 are currently occupied. These populations are spread across the representation units unevenly. The Ohio River basin contains 35 populations; the Upper Mississippi River basin contains 17 populations; the Great Lakes basin contains 11 populations; the Tennessee basin contains 2 populations; and the Arkansas-White-Red basin contains 1 population (Figure 4.1, Table 4.2). The total number of presumed extirpated, extirpated and historical populations by basin are: Ohio (22), Upper Mississippi (11), Great Lakes (9), Arkansas-White-Red (3), and Tennessee (0). Given the current status encompasses 66 populations throughout its range and all basins except one have more than one population, the species currently retains redundancy for withstanding and surviving potential catastrophic events. However, it is important to note that a high percentage (98.5%) of populations are currently at high risk. Further, 14% of populations are at high risk from both oil and gas activities as well as coal activities. Overall, the species has decreased redundancy across its range compared to its historical range due to the extirpation or historical status of 44 populations (40%). Twenty-three current populations (subbasins) of Salamander Mussel are known from a single or couple records occupied river extent and therefore are more susceptible to extirpation from catastrophic events.

*Table 4.7. Summary of Salamander Mussel population overall status, demographic condition, and risk category for the representation units. (*Three populations overlap with Canada and do not have an overall risk category assigned.) (U = Unknown, Fx = Functionally Extirpated, L = Low, M = Moderate, H = High)*

Status	**E**	**E**	**E**	**E**	**E**	**E**	**E**	**E**	**E**	**E**	**E**	**E Total**	**PE**	**PE Total**	**Grand Total**
Demographic Condition	**Fx**	**Fx**	**H**	**M**	**M**	**L**	**L**	**L**	**U**	**U**	**U**		**U**		
Risk Condition	**H**	**U**	**H**	**H**	**M**	**H**	**M**	**U**	**H**	**M**	**U**		**H**		
Arkansas-White-Red	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
Great Lakes	3	1*	0	0	0	0	0	1*	3	0	1*	9	2	2	11
Ohio	2	0	0	0	1	1	2	0	14	5	0	25	10	10	35
Tennessee	0	0	0	0	0	0	0	0	2	0	0	2	0	0	2
Upper Mississippi	3	0	1	1	0	2	0	0	5	0	0	12	5	5	17
Grand Total	8	1*	1	1	1	3	2	1*	24	5	1*	48	18	18	66

4.5 Description of Current Risks on Demographic Population Condition

The likelihood of all risks staying the same into the future is not a plausible future scenario. However, we can move beyond the snapshot of current condition by extrapolating a population's probability of persistence in 20 years from its current risks and the effects of those risks on a population (based on its current demographic population condition). In order to describe how the risk condition and demographic population condition may interact, we used the same ruleset that was developed for the plausible future scenarios, described below in 5.1.1 and Table 5.1. Looking at the current risks to the 66 extant and presumed extant populations, we can describe what that means for demographic population condition across the range and the impact to Salamander Mussel viability. Of the 18 populations for which we have current demographic population condition, 16 of those can be described 20 years into the future based on current risk condition. (We could not evaluate risk condition for the two populations with demographic data that are within Canada.) Of the 16 populations we could evaluate, 11 of those (~70%) would be extirpated due to current risks, 3 would be functionally extirpated (~18%), and 2 would be in low demographic condition (~12%, Table 4.8 Figure 4.13).

Forty-eight populations are in unknown current demographic condition (Table 4.8). However, 43 of these are experiencing high risk. At best, these populations would be in low condition in 20 years if they all were in high demographic population condition currently, which is unlikely. If we assume these unknown populations follow the pattern of the populations for which we do have data, 9 (18%) would be functionally extirpated and 34 (70%) would be extirpated.

Of the eight populations experiencing moderate risk (all within the Ohio basin), three populations would be in low condition (1) and functionally extirpated (2). The other five populations experiencing moderate risk have unknown demographic population conditions and would at best be in moderate condition in 20 years. Based on the pattern of known demographic populations, the Arkansas-White-Red, Tennessee, Great Lakes are at risk of being extirpated into the future given the current risk levels (Table 4.9, Figure 4.13). The Upper Mississippi is projected to have at least one population in low condition, along with the Ohio basin (Table 4.9, Figure 4.13).

*Table 4.8. Summary of projected demographic condition for Salamander Mussel extant and presumed extant populations given the current risk levels across the range. (*Two populations within Canada were not evaluated for risk levels and therefore could not have demographics projected.)*

Projected Demographic Condition if Current Risks Continue	Number of Populations Current Condition	Projected Number of Populations (2040)
High	1	0
Moderate	2	0
Low	6	2
Functionally Extirpated	9	3
Extirpated		11
Unknown	48	48
Total	**66**	**64***

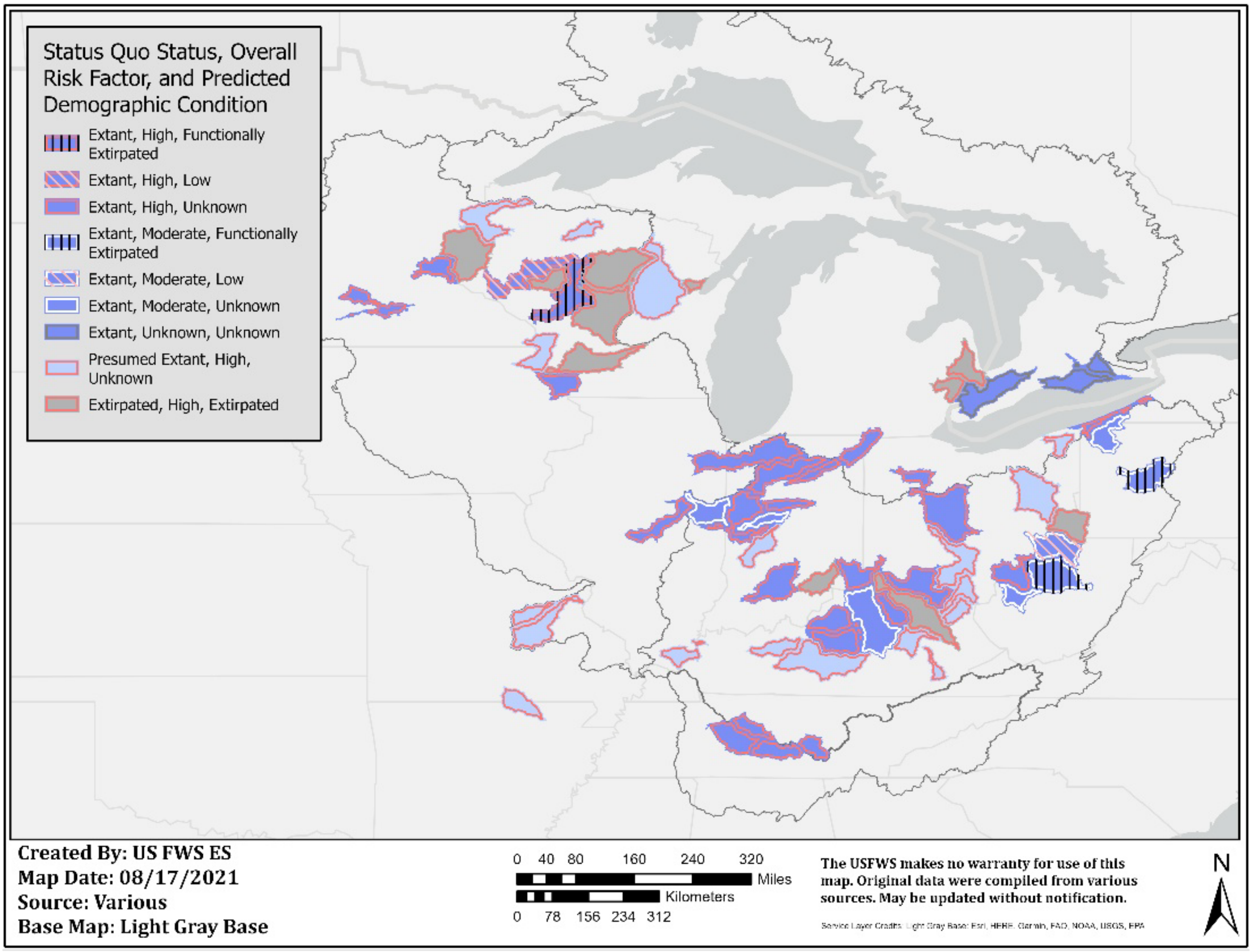

Figure 4.13. Salamander Mussel population projected status, risk factor condition, and demographic condition range wide for extant and presumed extant given the current risk conditions.

*Table 4.9. Summary of Salamander Mussel population projected overall demographic condition by representation unit. *two populations overlap with Canada and do not have an overall projected demographic category assigned. (U = Unknown, X = Extirpated, Fx = Functionally Extirpated, L = Low, M = Moderate, H = High)*

Representation Unit	**U**	**X**	**Fx**	**L**	**M**	**H**	**Total Population Count**
Arkansas-White-Red	1	0	0	0	0	0	**1**
Great Lakes*	6	3	0	0	0	0	**9**
Ohio	29	3	2	1	0	0	**35**
Tennessee	2	0	0	0	0	0	**2**
Upper Mississippi	10	5	1	1	0	0	**17**
Species Range Total	**48**	**12**	**2**	**2**	**0**	**0**	**64***

CHAPTER 5. FUTURE CONDITION

5.1 Future Projections of Influences on Viability

5.1.1 Population Factors Methodology

We created a ruleset using the SSA Core Team's best professional judgment to project each population's demographic condition in the future based on its current demographic condition as a baseline and the risk factor level projected for the future (Table 5.1). If high risk is projected into the future, the demographic condition will fall two levels (in other words, high current demographic condition will be projected to decline to a low demographic condition into the future). If moderate risk levels are projected into the future, the demographic condition will be projected to decline a single level. If low risk levels are projected into the future, the population will stay at the same demographic condition as identified in current condition. We recognize that a low risk level may provide the opportunity for successful reproduction and dispersal, which may improve the population's demographic condition. However, conservation efforts would likely have to be implemented (in addition to the low risk level) for populations in low or moderate demographic condition to generally increase in demographic condition.

Table 5.1 Projected future demographic condition rule set based on current demographic condition and projected future risk factor condition.

	Projected Future Risk Factor Condition		
Current Demographic Condition	**High**	**Moderate**	**Low**
High	Low	Moderate	High
Moderate	Functionally Extirpated	Low	Moderate
Low	Extirpated	Functionally Extirpated	Low
Functionally Extirpated	Extirpated	Extirpated	Functionally Extirpated

5.1.2 Habitat Risk Factors Methodology Overview

There is substantial uncertainty regarding the magnitude, duration, and location of effects related to hydrological regime, habitat degradation, contaminants, connectivity, invasive species, and mudpuppy vulnerability into the future. Because of this, we forecasted future viability for the Salamander Mussel under two future scenarios that represent the range of plausible environmental conditions and the projected consequences on the species' viability (Table 5.2). We projected out 50 years when information was available (2070; approximately 3-4 generations). We restricted our evaluation to 50 years primarily due to uncertainties regarding future land cover projections and limitations projecting non-modeled, extrapolated future conditions for water quality. We evaluated both scenarios where future threats determined the biological status of mussel populations and their habitats.

In this chapter, we considered climate change under various likely scenarios. Climate change directly or indirectly exacerbates the most relevant stressors (for example, water quality, hydrological regime, landscape alterations) to freshwater mussels wherever they occur. We expect climate change effects to occur throughout the Salamander Mussel range.

Table 5.2. Summary of risk factor metric projections for future scenarios. [1]primary contaminants [2]secondary contaminants. (FOREcasting Scenarios of Land Cover (FORE-SCE) IPCC Special Report on Emissions Scenarios (SRES); U.S. Global Roads Inventory Project (GRIP))

	Scenario 1	**Scenario 2**
Contaminants (2070)		
Ammonia[1]	Percent change for agriculture and development in FORE-SCE land cover change model SRES A2	Percent change for agriculture and development in FORE-SCE land cover change model SRES B1
Chloride[1]	Percent change for agriculture and development in FORE-SCE land cover change model SRES A2	Percent change for agriculture and development in FORE-SCE land cover change model SRES B1
Copper[1]	Percent change for development in FORE-SCE land cover change model SRES A2	Percent change for development in FORE-SCE land cover change model SRES B1
Lead[2]	Percent change for development in FORE-SCE land cover change model SRES A2	Percent change for development in FORE-SCE land cover change model SRES B1
Landscape (2070)		
% impervious surface	Percent change as modeled for development in FORE-SCE land cover change model SRES A2	Percent change as modeled for development in FORE-SCE land cover change model SRES B1
% vegetative cover within riparian buffer	Percent change as modeled for vegetative cover in FORE-SCE land cover change model SRES A2	Percent change as modeled for vegetative cover in FORE-SCE land cover change model SRES B1
% agriculture	Percent change as modeled for agriculture in FORE-SCE land cover change model SRES A2	Percent change as modeled for agriculture in FORE-SCE land cover change model SRES B1
% urbanization	Percent change as modeled for development in FORE-SCE land cover change model SRES A2	Percent change as modeled for development in FORE-SCE land cover change model SRES B1
% Canopy Cover within riparian buffer	Percent change as modeled for vegetative cover in FORE-SCE land cover change model SRES A2	Percent change as modeled for vegetative cover in FORE-SCE land cover change model SRES B1
Hydrological Regime (2040–2069)		
Drought	Warm Wet projections of the Cumulative Severe Drought Index (CDSI) developed by the U.S. Forest Service	Hot Dry projections of the Cumulative Severe Drought Index (CDSI) developed by the U.S. Forest Service
Connectivity (2040–2050)		
Number of Dams	No changes from current condition	Dam removal based on 2000–2020 trends
Unpaved road stream crossing density	increase density by 27.3% (GRIP Scenario SSP5)	increase density by 3.2% (GRIP Scenario SSP3)
Invasive Species		
Optimized Hotspot Analysis	Neighbor hotspot analysis	No changes from current condition
Host Species Vulnerability		
Lampricide Treatment	Increase in areas based on expert opinion	No changes from current condition
Collection, bag limits & fishing unintentional catch	No changes from current condition	Regulations increase protection for mudpuppy

5.2 Population Resiliency Future Assessment

Most populations' risk factor did not change from the current condition risk factor under Scenario 1 or Scenario 2 (Table 5.3, Table 5.4). The only changes occur in the Duck-Pensaukee population (Great Lakes basin) and the Eel population (Ohio basin) in which the overall risk is projected to decrease from high to moderate under Scenario 2 (Table 5.4, Figure 5.2).

Based on the projected overall risk factor, 14 populations, including the Duck-Pensaukee, are projected to be extirpated or functionally extirpated in both scenarios (Figure 5.1, Figure 5.2; See Appendix F for more detail). Only two populations are projected to be in low condition into the future, the Little Muskingum-Middle Island in the Ohio basin and the Lower Chippewa in the Upper Mississippi basin. Forty-seven of the populations have an unknown demographic condition in the future, though over 80% of these populations are at a high risk in both Scenario 1 and 2 (Table 5.3, Table 5.4), meaning there is a less than 60% chance of persistence over 20 years (Table 4.3). If we assumed that all of these populations were in a high demographic condition, they would at most be a low demographic population condition into the future. However, this is highly unlikely given that only one population within the range of Salamander Mussel is in high condition. Therefore, following the pattern of projected demographic condition of the populations for which we do have data (~70% extirpated, ~18% functionally extirpated, and ~12% low), it is more likely that these populations will be functionally extirpated or extirpated into the future in both Scenario 1 and Scenario 2. Approximately 12–15% of populations experience moderate risk across the two scenarios (Table 5.3, Table 5.4), meaning there is a 60–90% of persistence over 20 years (Table 4.3). None of the populations are projected to experience low risk into the future in Scenario 1 or 2. There are three populations that are not evaluated for risk factor condition because they cross the border with Canada and the analysis could not be completed for the entirety of the population area (Table 5.3, Table 5.4). Based on Scenario 1 and 2, Salamander Mussel populations are less resilient into the future based on the current condition and future projected risks.

5.3 Species Representation Future Assessment

Species representation is at risk of being lost in the future regardless of whether it is Scenario 1 or Scenario 2. The Spring population is the only remaining population left in the Arkansas-White-Red basin. The Spring population is still projected to be at high risk into the future and at most would be in low condition into the future, but more likely is at risk for being extirpated based on trends seen in populations with known demographic condition. As such, the Arkansas-White-Red basin representation unit may be extirpated, and the environmental variation would be lost. The Tennessee basin is also along the edge of the range represented with only two populations with unknown demographic condition that are at high risk into the future; therefore, it is uncertain to what extent this basin will continue to be represented without augmentation/ reintroduction (See Appendix D.3) that has occurred in the past. The Upper Mississippi basin will be reduced by approximately one-third based on the number of populations projected to be extirpated or functionally extirpated. Similarly, of the populations within the Great Lakes basin where risk could be assessed into the future, close to 40% of this basin is projected to be extirpated regardless of the scenario (Table 5.3, Table 5.4, Figure 5.1, Figure 5.2). The Ohio basin is the only representation unit experiencing moderate risk within a small number of populations. Risk remains high, though the majority of populations are unknown, it is clear that future projections result in reduced representation of Salamander Mussel across the range of the species.

5.4 Species Redundancy Future Assessment

The Salamander Mussel populations are spread across the representation units unevenly. The core area of the Salamander Mussel range remains the Ohio, Great Lakes, and Upper Mississippi basins. The Ohio basin and Upper Mississippi are projected to still contain the majority of the populations, with the Ohio basin having more than double the Upper Mississippi (Table 5.3, Table 5.4, Figure 5.1, Figure 5.2). However, the redundancy within the core basins for the populations where we could analyze demographic condition was cut by half or more. The Ohio basin lost 50% of the populations that had demographic condition in both Scenarios (Table 5.3, Table 5.4, Figure 5.1, Figure 5.2). All three of the populations with demographic condition in the Great Lakes are projected to be extirpated in both Scenarios (Table 5.3, Table 5.4, Figure 5.1, Figure 5.2). The Upper Mississippi basin was projected to have five populations (62%) extirpated in both Scenarios (Table 5.3, Table 5.4, Figure 5.1, Figure 5.2). In addition, more than 80% of the populations will be experiencing high risk in both future scenarios (Table 5.3, Table 5.4, Figure 5.1, Figure 5.2).

The Arkansas-White-Red and Tennessee basins are on the edge of the range (Figure 5.1, Figure 5.2). The population in the Arkansas-White-Red basin and the 2 populations in the Tennessee basin are projected to be at high risk in both scenarios Although we cannot project their future demographic condition, given they are experiencing high risk, they would be at risk of extirpation.

The expected declines in the number and distribution of resilient populations will likely make the Salamander Mussel more vulnerable to catastrophic events related to oil, gas, and coal, which are projected to remain in place regardless of frequency and intensity.

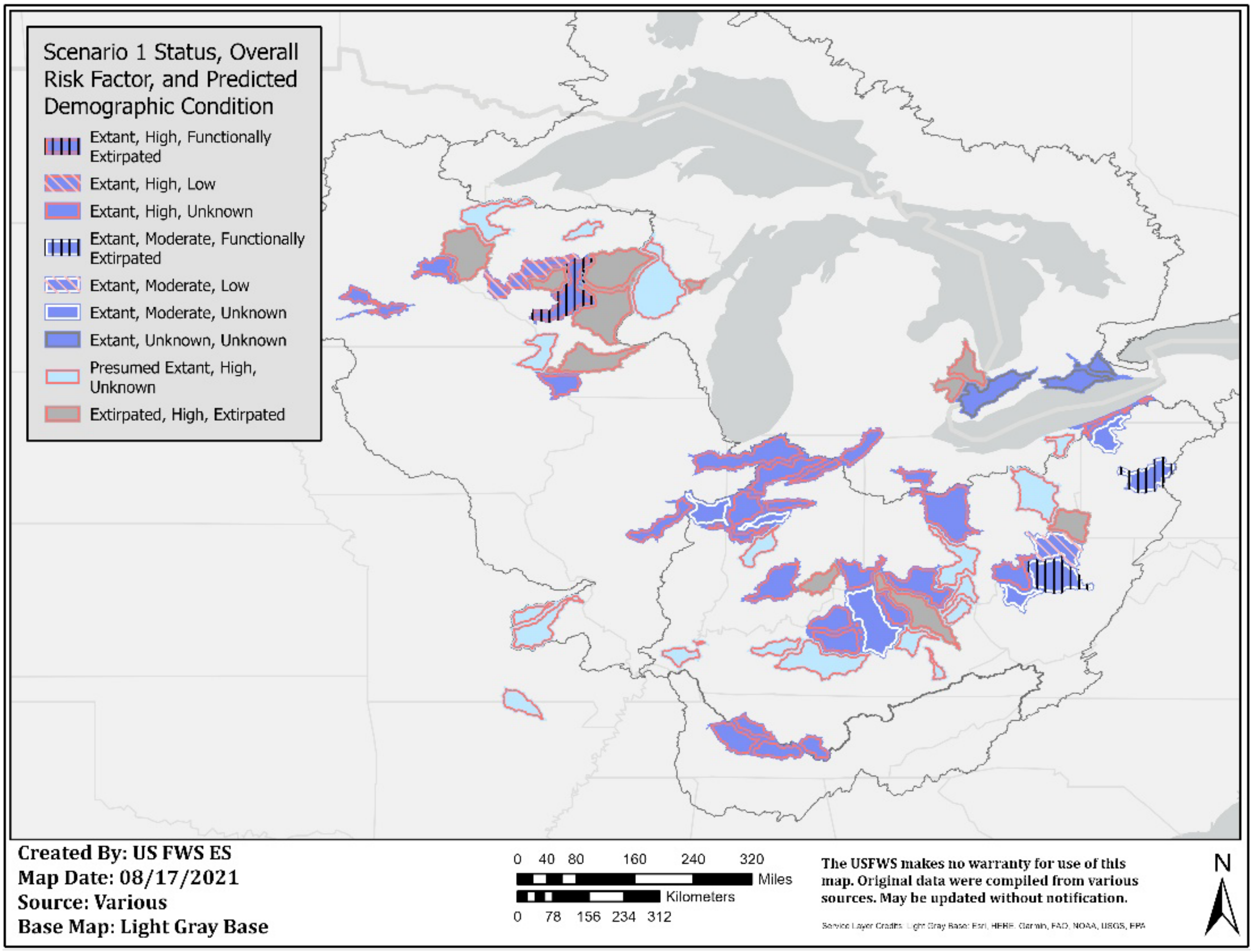

Figure 5.1. Salamander Mussel Future Scenario 1 population projected status, risk factor condition, and demographic condition range wide for extant and presumed extant.

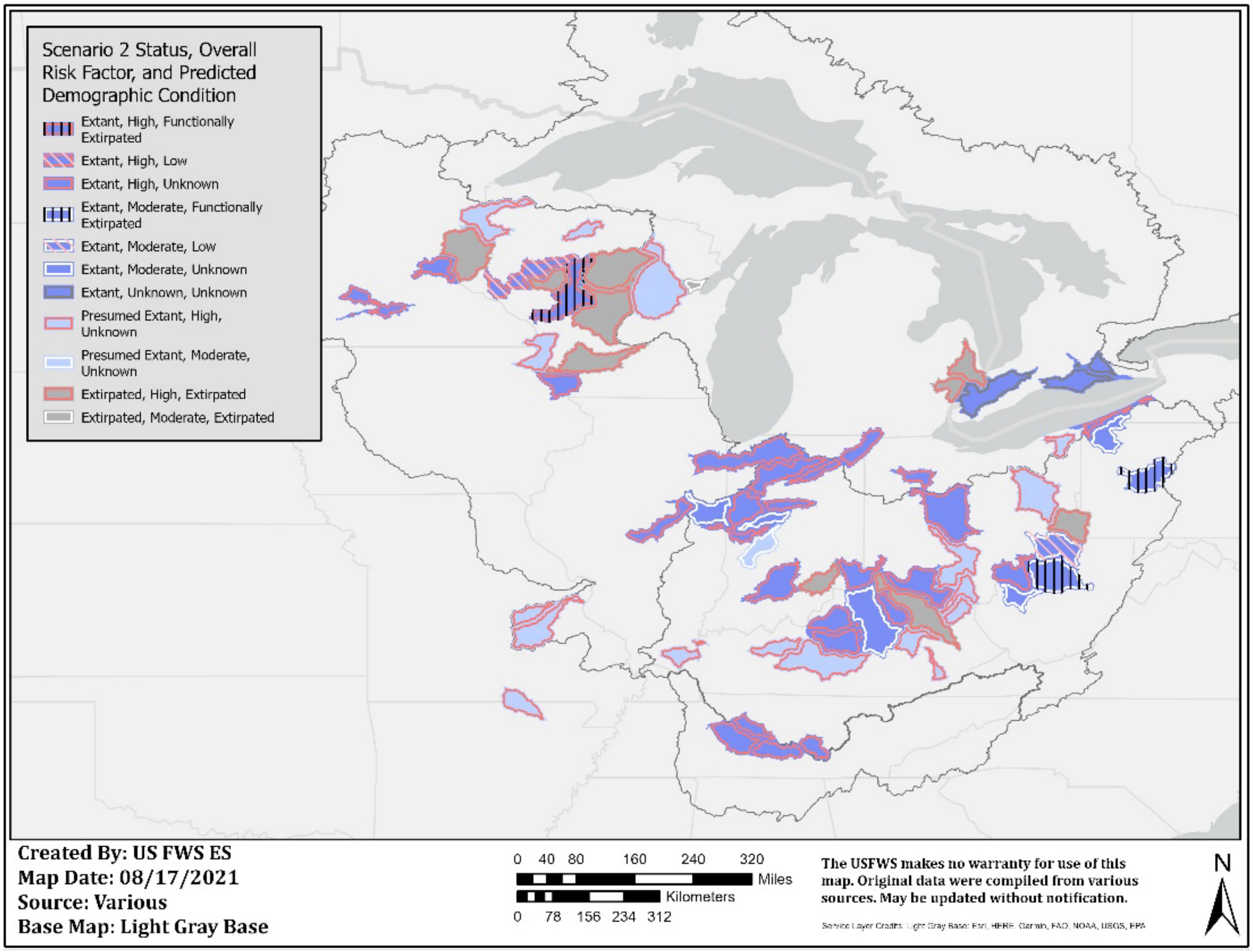

Figure 5.2. Salamander Mussel Future Scenario 2 population projected status, risk factor condition, and demographic condition range wide for extant and presumed extant.

*Table 5.3. Summary of Salamander Mussel population overall risk category by status and representation unit for Future Scenario 1. *three populations overlap with Canada and do not have an overall risk category assigned. (E=Extant, PE = Presumed Extant, X = Extirpated)*

Risk Category	**High**	**High**	**Moderate**	**Moderate**	**Low**	**Low**		**Total Population Count**
Representation Unit	**E**	**PE**	**E**	**PE**	**E**	**PE**	**X**	
Arkansas-White-Red	0	1	0	0	0	0		**1**
Great Lakes*	3	2	0	0	0	0	3	**5**
Ohio	14	10	8	0	0	0	3	**32**
Tennessee	2	0	0	0	0	0		**2**
Upper Mississippi	7	5	0	0	0	0	5	**12**
Species Range Total	**26**	**18**	**8**	**0**	**0**	**0**	**11**	**52***

*Table 5.4. Summary of Salamander Mussel population overall risk category by status and representation unit for Future Scenario 2. *three populations overlap with Canada and do not have an overall risk category assigned. (E=Extant, PE = Presumed Extant, X = Extirpated)*

Risk Category	**High**	**High**	**Moderate**	**Moderate**	**Low**	**Low**		**Total Population Count**
Representation Unit	**E**	**PE**	**E**	**PE**	**E**	**PE**	**X**	
Arkansas-White-Red	0	1	0	0	0	0		**1**
Great Lakes*	2	2	1	0	0	0	3	**5**
Ohio	14	9	9	0	0	0	3	**32**
Tennessee	2	0	0	0	0	0		**2**
Upper Mississippi	7	5	0	0	0	0	5	**12**
Species Range Total	**25**	**17**	**10**	**0**	**0**	**0**	**11**	**52***

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APPENDIX A. ECOLOGY BACKGROUND

A.1 Taxonomy and Genetics

The Salamander Mussel belongs to the family Unionidae, also known as the naiads or pearly mussels. The Salamander Mussel SSA report follows the most recently published and accepted taxonomic treatment of North American freshwater mussel as provided by Williams et al. (2017, entire). The Salamander Mussel was originally described by Say 1825 *Alasmodonta ambigua.* The type locality was described as "North-west Territory", the type specimens are not in the Academy of Natural Sciences of Philadelphia and have been lost (Clarke 1985, pp. 60–68; Watson 2001, entire). Salamander Mussel (*Simpsonaias ambigua)* is the only member of the genus *Simpsonaias* (Frierson 1914a, p. 7). According to Clarke (1985, pp. 60–68) *Simpsoniconcha* (Frierson, 1914b, p. 40) was proposed as a replacement name for *Simpsonaias* Frierson (1914a, p. 7) because it was thought to be preoccupied, though this was determined to be in error. Synonyms for *Simpsonaias ambigua* described in Clarke (1985, pp. 60–68) and Watson (2001, entire) include: *Simpsonaias ambigua* (Say 1825)*; Unio hildrethianus* (Lea 1834, p. 36); *Alasmodonta ambigua* (Say 1825, p. 131); *Margaritana ambigua* (Say 1825, p. 131; Kuster 1862, p. 300, p. 313); *Alasmodonta dubia* (Ferussac 1835, p. 26 as cited in Clarke 1985, p. 61); *Simpsoniconcha ambigua* (Say 1825, p. 131); *Hemilastena ambigua* (Say 1825, p. 131); *Margarita (unio) hildrethianus* (Lea 1834, p. 36); *Strophitus hildrethiana* (Lea 1834, p. 36); *Baphia hildrethiana* (Lea 1834, p. 36); and *Margaritana hildrethiana* (Lea 1834, p. 36). Clarke (1985, pp. 60–68) states that the phylogentic position is obscure because it is "not closely related to any other living species".

The currently accepted classification is (Turgeon et al. 1998, p. 35; Williams et al. 2017, p. 43):

- Phylum: Mollusca
- Class: Bivalvia
- Subclass: Palaeoheterodonta
- Order: Unionoida
- Family: Unionidae
- Subfamily: Anodontinae
- Genus: *Simpsonaias*
- Species: *Simpsonaias ambigua*

A.2 Species Description

Salamander Mussel is a small species, elliptical in shape, that is thin shelled and that reaches approximately 48–51 mm (1.5–2 inches) long (Watson 2001, entire). The shell is compressed in males and slightly inflated posteriorly in females. The anterior and posterior ends are both rounded, and the beak is located approximately one-quarter of the distance from anterior to posterior and is slightly elevated above the hinge line (Watson 2001, entire). The beak structure is composed of four to five double looped ridges, and the periostracum is smooth, yellowish tan to dark brown without any rays (Watson 2001, entire). The hinge teeth are small and incomplete, and the right valve has a single, small, low, rounded pseudocardinal tooth (tooth like structures along the hinge line of each valve) that rises from the shell wall as opposed to the hinge plate. The left valve of some specimens has an even smaller tooth that is posterior and below the umbo (the raised part at the dorsal margin of each valve) (Clarke 1985, pp. 60–68). Lateral teeth are absent (Clarke 1985, pp. 60–68). The nacre is the inner surface of the shell bluish white and sometimes tinged with salmon or purple (Clarke 1985, pp. 60–68).

APPENDIX B. PRIMARY INFLUENCES ON VIABILITY

B.1. Contaminants

Metals, Nutrients, and Major Ions

Freshwater mussels are among the most sensitive freshwater species to metals, ammonia, and ion constituents including copper, sulfate, alachlor, nickel, chloride, sulfate, zinc, and potassium (Wang et al. 2017, pp. 786–796). Representative species from different families or tribes had similar sensitivities to copper, sulfate, alachlor, nickel, chloride, sulfate, zinc, and potassium, regardless of mode of toxic exposure (Wang et al. 2017, pp. 786–796).

Heavy metals can cause mortality and affect biological processes, for instance, disrupting enzyme efficiency, altering filtration rates, reducing growth, and changing behavior of freshwater mussels (Jacobson et al. 1997, pp. 2384–2392; Keller & Zam 1991, pp. 539–546; Naimo 1995, pp. 341–362; Valenti et al. 2005, pp. 1242–1246; Wang et al. 2007a, pp. 2048–2056, pp. 2036–2047; Wang et al. 2010, pp. 2053–2063). Low but chronic heavy metal and other toxicant inputs may reduce mussel recruitment (Naimo 1995, pp. 352–354).

Both acute and chronic exposures to zinc and nickel demonstrated the sensitivity of mussels to these chemicals and chronic exposures increased mussel sensitivity to zinc (Kunz et al. 2016, p. 1). The USEPA has water quality criteria for six of the 10 chemicals tested in Wang et al. (2017, pp. 186–796). For ammonia, copper, and zinc, most of the species mean acute values were either similar to or less than the USEPA acute criteria (Wang et al. 2017, p. 786). Wang et al. (2017, p. 795) suggests that if the minimum data requirement for deriving water quality criteria required the inclusion of freshwater mussels, then water quality criteria would capture the high sensitivity of freshwater mussels to many chemicals and different exposure pathways. An example of this is the ammonia criterion that was updated to include mussels and since the acute criterion is 1.4-fold lower than the previous acute criterion (Wang et al. 2017, p. 792). Mussels exhibit differing sensitivities to chloride depending on genus, with one study using the *Epioblasma* genus, demonstrating it is the most sensitive (Gillis 2011, pp. 1702–1708). Current acute criteria may therefore not be protective of severely imperiled mussels. Furthermore, for chloride as well as other chemicals, concentrations in surface water in North America are increasing rather than decreasing (Gillis 2011, p. 1702) due to anthropogenic practices (for example, increase use of road salts; Gillis 2011, p.1702). Areas with elevated levels of chloride are acutely toxic to glochidia, if these areas are chronically exposed to chloride, population level effects will result.

Freshwater mussels are very sensitive to ammonia (Augspurger et al. 2003, pp. 2569–2575). Ammonia is widespread within the aquatic environment; typical sources include agricultural wastes (animal feedlots and nitrogenous fertilizers), municipal wastewater treatment plants, and industrial waste as well as precipitation and natural processes, such as decomposition of organic nitrogen (Augspurger et al. 2003, p. 2569; Goudreau et al. 1993, p. 212). Unionized ammonia is the most toxic to freshwater mussels (M. Bradley, personal communication, 2021). Sediment pore water concentrations of ammonia typically are higher than the surface water concentrations as well, which is of particular concern for freshwater mussels given the highest concentrations occur in mussel microhabitat (Augspurger et al. 2003, p. 2569). Ammonia can be acutely toxic to mussel in particular early life stages. Ammonia also causes sublethal effects, such as reduced respiration and feeding due to valve closure, impaired secretion of the byssal thread (used for substrate attachment), reduced ciliary action impairing feeding, depleted lipid, glycogen, and other carbohydrate stores, and altered metabolism (Augspurger et al. 2003, p. 2574; Goudreau et al. 1993, pp. 220–222; Mummert et al. 2003, p. 2545).

In addition to ammonia, phosphorus and nitrogen are the primary nutrient contaminants that occur in aquatic ecosystems when nutrient pollution is not properly managed. Nitrogen breaks down by various processes and produces nitrates, the nitrates react differently based on water hardness impacting the ionic charge and therefore impacts the bio availability affecting freshwater mussels. The amount of nitrate within river systems is one measure that can be used to assess water quality and toxicity to freshwater mussels.

Fertilizers and animal manure are both rich in nitrogen and phosphorus. If fertilizers are not applied properly or manure waste piles are not properly managed, water quality in nearby surface or ground water can be severely impacted leading to eutrophication and algal blooms. While food quantity may increase under moderate eutrophic conditions, the resulting algal community is often of lower quality, which may lead to decreased mussel growth and reproduction (Strayer 2014, p. 280). Increased algal productivity can produce toxic algal varieties and further degrade water quality by altering ammonia, oxygen, and pH levels, leading to further reductions in mussel reproduction through lost host and early life stage mortality and probable juvenile and adult mussel die offs (Strayer 2014, p. 280).

Organic Compounds and Contaminants of Emerging Concern

Contaminants of emerging concern (CEC) is a term that refers to a broad and diverse group of chemicals, often organic compounds, including pesticides, personal care products, pharmaceuticals, flame retardants, plasticizers, and industrial chemicals. These chemicals are found worldwide, but little information exists on the effects of this diverse array of chemicals and exposure pathways in sediment, pore water, and surface water (Woolnough et al. 2020, p. 1626). Pharmaceutical chemicals used in commonly consumed drugs increasingly occur in surface waters. Kolpin et al. (2002, pp. 1208–1210) detected the presence of numerous pharmaceuticals, hormones, and other organic waste products in nationwide sampling of 139 stream sites in 30 States downstream from urban development and livestock production areas. Eighty-three CECs were found in the sediment, water, and mussel samples tested from the Maumee River, indicating waterborne exposures to pharmaceuticals and sediment exposures to agricultural chemicals and personal care products were probable (Woolnough et al. 2020, p. 1631). Mussel tissues showed higher concentrations of pharmaceuticals indicating adult exposures had resulted in concentration of organic chemicals with unknown results.

Overall, mussels are considered to be less sensitive to organic compounds, but behavioral changes and reduced glochidia fitness have been noted in mussel species exposed to some agricultural chemicals, pharmaceuticals, and industrial compounds (Bringolf et al. 2007a, pp. 2086–2093; Hazelton et al. 2013, pp. 94–100; Hazelton et al. 2012, pp. 1611–1620). For example, the active ingredient in many prescription anti-depressants, which have selective serotonin reuptake inhibitors, are found in measurable concentrations in surface waters chemicals. At elevated levels these chemicals may disrupt the neuroendocrine pathways that control reproduction, impacting brooding glochidia within the marsupial gill as well as altering reproductive and avoidance behaviors (Bringolf et al. 2010, pp. 1311–1312; Hazelton et al. 2013, p. 95). Such alterations could lead to increased mortality and reduced reproduction.

Perfluoroalkyl acids (PFAAs) are another suite of organic chemicals that are prevalent and persistent in the landscape and are known to impact mussels. PFAAs repel water and oil and are found in a variety of products, including carpets, upholstery, paper, food containers, fabric, and fire suppressants (Hazelton et al. 2012, p. 1611). Perfluoroctanesulfonic acid (PFOS) and perfluoroctanoic acid (PFOA) bind to tissue, demonstrate biomagnification in aquatic food webs, and have been linked to decreased reproduction of aquatic species. Freshwater mussels are sensitive in both acute and chronic exposures to PFAAs (Hazelton et al. 2012, p. 1611–1620). Glochidia were the most sensitive organisms tested to date in acute toxicity exposures. Exposures to glochidia in marsupia demonstrated reduced viability and reduced ability to metamorphosis.

Research on agricultural chemicals that are currently in use further highlights the variability of mussel sensitivities to organic chemicals. Pesticide studies indicated that mussels were tolerant to active chemicals (for example, atrazine, chlorpyrifos, permethrin) in both acute and chronic mussel exposures. Conversely, chronic exposures of glyphosate formulations currently used containing surfactants to increase herbicide efficiencies resulted in mussels being highly sensitive to these ubiquitous herbicides highlighting the complexity of assessing the impacts of the thousands of organic chemicals found in mussel environments both singularly and in more ecologically relevant complex mixtures (Bringolf et al. 2007b, pp. 2094–2100). This suggests organic chemicals and CECs should not be overlooked as possible contributors to common and rare mussel declines.

Invasive Species Chemical Controls

Aquatic herbicides, algaecides, adjuvants, and lampricides are used to treat aquatic nuisance or invasive species within aquatic ecosystems. The majority of these chemicals do not have any data on toxicity to freshwater mussels. Copper is one chemical used to treat aquatic nuisance species, and depending on water chemistry, has the potential to be toxic to freshwater mussels and certain fish species (Bowman & Bush 2019, pp. 4–5; Wang et al. 2011, pp. 2115–2125). Other suites of chemicals such as endothall salts have had some studies conducted on freshwater mussels (Archambault et al. 2015, pp. 335–348; Keller 1993, pp. 696–702); however, more analysis is necessary to understand some of the effects.

In addition to nuisance aquatic plants, invasive sea lamprey (*Petromyzon marinus*) is present in many waterways in the Great Lakes Basin. Sea lamprey treatment and assessment activities using lampricides have the potential to negatively impact freshwater mussels and their host (for example, log perch, mudpuppy).

The USFWS Sea Lamprey Control Program uses the lampricides TFM (3-trifluoromethyl-4-nitrophenol) and Bayluscide® [active ingredient: 5-Chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide] to control sea lamprey in the Great Lakes Basin. TFM and the TFM/1% niclosamide mix is applied to streams to kill larval sea lampreys. The granular formulation of Bayluscide [Bayluscide 3.2% Granular sea lamprey Larvicide, granular Bayluscide (gB)] is applied in lake and river systems that are too large to be treated economically with the liquid lampricide formulations and to survey for larval sea lampreys in areas that are too deep to effectively electrofish with AbP-2 backpack electrofishing gear (C. Kaye, personal communication, 2020). Niclosamide, the active ingredient in Bayluscide, was first developed as a molluscide to kill snails. The granular form of Bayluscide targets benthic (bottom of river) larval sea lamprey habitat, which is the same habitat occupied by freshwater mussels and put themespecially at risk when present within the vicinity of Bayluscide applications.

Boogaard et al. (2015, pp. 1634–1641) tested the toxicity of TFM on multiple life stages of the Snuffbox Mussel *(Epioblasma triquetra*). The study evaluated the effects of TFM on Snuffbox glochidia, one week old juveniles, and logperch, as well as glochidia, one week old juveniles, and adults of the Ellipse Mussel (*Venustaconcha ellipsiformis*). The study also evaluated juvenile recruitment success from glochidia (larval) infested logperch exposed to multiple levels of TFM. This work demonstrated that there was minimal toxicity to the larval and juvenile stage of both the ellipse and Snuffbox Mussels, as well as the adult stage of the Ellipse Mussel at concentration ratios greater than what is required or typically used to kill larval sea lampreys in streams (USFWS 2013, pp. 1–44). A comparison of the results to Snuffbox glochidia indicates the life stage of the two species respond similarly to TFM. Survival was high among both species at concentrations greater than what would be encountered during treatments suggesting the risk from direct exposure to TFM is low (USFWS 2013, pp. 1–44). In the natural stream environment, glochidia are distributed directly on the gills of logperch by female Snuffbox. Glochidia that are inadvertently distributed into the water column (free-floating) have almost a 100 percent chance of dying (USFWS 2013, pp. 1–44). The viability test conducted during 2011 on free-floating Snuffbox glochidia demonstrated that viability decreased rapidly beginning at 12 hours after extraction (USFWS 2013, pp. 1–44). These results suggest, along with the recruitment tests where juvenile fall-off was not significantly different between the exposed and control fish, that there would be greater survival of glochidia encysted on gills (as opposed to free-floating) at concentrations tested (USFWS 2013, pp. 1–44). Results from toxicity tests on one week old juveniles suggest that there is no risk up to the highest concentration ratios for the snuffbox mussel and that Ellipse juveniles may be more at risk at higher concentrations, although these concentrations would not be applied in the field (USFWS 2013, pp. 1–44).

Boogaard et al. (2015, pp. 1643–1641) also looked at the toxicity of TFM to adult logperch. Logperch were exposed to concentration levels of TFM that are typically used in the field to kill sea lampreys in streams. Exposure duration was 12 hours followed by a 12-hour post-exposure period after which mortality of logperch was assessed. Minimum lethal concentrations [MLC; concentration of TFM required to kill 99.9% of sea lamprey larvae calculated from the pH and alkalinity of the test water (Bills et al. 2003, pp. 514–517) for sea lampreys in this study were 2.1 mg/L.

Mortality of logperch was 15% at 2.1 mg/L TFM, but 65% at 2.7 mg/L TFM (1.3 × MLC; concentration ratio = mean TFM concentration applied/predicted TFM MLC). However, several field studies and non-target mortality observations differ from these lab results including a study by Langdon and Fiske (1991, pp. 1–74) where logperch were captured at the same rate during 2 pre-treatment surveys and 1 post treatment survey in an area that had the highest mean concentration of TFM (4.7 mg/L TFM) and exposure time (11 h). Another long-term study (Schuldt et al. 1996, entire) reported 100% logperch survival in a cage study during five lampricide treatments. Until further tests are conducted and prove otherwise, logperch are considered sensitive to TFM, having the potential for high levels of mortality (85% mortality at treatments levels of 1.5 × MLC) based on laboratory results.

Mudpuppy have also been tested and been found to be very sensitive to TFM (see Appendix B.7). Toxicity tests on other host species within the Great Lakes Basin are important in understanding the potential impact of sea lamprey control on freshwater mussel communities given the critical role host species play in reproduction influencing the distribution and survival of these mussel assemblages.

A study conducted by Newton et al. (2017, pp. 370–378) investigated the risk of mortality and sub-lethal effects (probability and duration) as a function of exposure duration among adult and sub-adult mussel species exposed to environmentally relevant concentrations of niclosamide following a granular Bayluscide application. Eight species of mussels were chosen based on availability and potential overlap with larval sea lamprey habitat. At each exposure duration, mortality was estimated, and a suite of sub-lethal responses including siphoning activity, gaping valves, production of mucus, and rigid foot extension was recorded. Mortality, over all exposure durations, 21 days after exposure, averaged 42% in sub-adults (range, 23–54%) and 20% in adults (range, 3–44%). For those species tested as both sub-adults and adults (*O. olivaria and V. iris*), mortality was similar between life stages for *O. olivaria* (~23%) but more than twice as high for sub-adult (mean, 38%) compared to adults (mean, 14%) for *V. iris*. There were positive associations between duration of niclosamide exposure and mortality in all four species exposed as sub-adults, and in four of the six species exposed as adults. These results were the same positive associations seen between duration of exposure and sub-lethal responses in all four species of exposed sub-adults and four of the six species exposed as adults. Results indicate that the duration of exposure plays a significant role in the magnitude among mussels. The longer mussels are exposed to niclosamide, the greater the mortality and sub-lethal effects. Both adults and sub-adults were sensitive to exposure, but sub-adults were affected sooner (Newton et al. 2017, pp. 370–378).

While unionids absorb lampricides and experience narcotization (gaped shell and sometimes foot extended), toxicity studies have indicated that TFM exposure would not result in acute mortality at concentrations required to kill sea lamprey during stream treatments (Kaye 2021, pp. 1–50). Booggard and Waler (2004, p. 12). Bills et al. (1992) reported that 90% of Pink Heelsplitter survived when exposed to 3.5 mg·L-1 TFM (1.0 × MLC) for 12 hours. However, only 30% survived a 12-hour exposure of 5.25 mg·L-1 TFM (1.5 × MLC). The authors noted that static tests are a worst-case scenario and surmised that survival would be higher in a stream environment. Waller et al. (1998, pp.116–118) stated that both Threehorn Wartyback and Wabash Pigtoe would survive stream treatments at TFM concentration ratios of 1.3 and 1.4 × MLC. A study conducted to test several compounds for the potential control of zebra mussels (*Dreissena polymorpha*; Bills and Waller unpublished data) found no mortality of the Pimpleback (*Cyclonaias pustulosa*), Three Ridge (*Amblema plicata*), and Pink Papershell (*Potamilus ohiensis*) held in TFM concentrations up to 8.4 mg·L-1 (3.4 × MLC) for 12 hours, and 20% mortality of the Deertoe (*Truncilla truncata*) in 6.7 mg·L-1 (2.7 × MLC; MLC = 2.5 mg·L-1). These concentrations were much greater than what would be typically be applied to a stream (1.1–1.8 × MLC; Kaye 2021, pp. 5–6; L. Crieger, personal communication, 2020).

Boogaard et al. (2004, pp. 1–17) reported that TFM and TFM-1% niclosamide did not produce substantial mortality among three unionid mussel species [Giant Floater (*Pyganodon grandis*), Fragile Papershell (*Leptodea fragilis*), Pink Heelsplitter (*Potamilus alatus*)] tested at concentrations typically applied during stream applications to kill larval sea lamprey. Both lampricides were more toxic to larval sea lamprey than to any of the unionid species tested. The giant floater experienced the highest mortality, which the authors attributed to the added stress of handling and holding conditions in river water at temperatures as high as 27 °C. They stated that the species can often experience a natural die off during mid to late summer (Boogaard & Waller 2004, pp. 1–17).

Waller et al. (2003, pp. 546–550) found that acute mortality did not occur when Eastern Elliptio (*Elliptio complanata*) and Eastern Floater (*Pyganodon cataracta*) juveniles and adults were exposed to TFM up to 1.6 × MLC in a mobile bioassay trailer at the White River, tributary to the Bad River (Ashland County, Wisconsin). Acute mortality of Eastern Elliptio juveniles and adults did not occur when exposed to TFM-1% niclosamide up to 1.9 × MLC. Concentrations routinely applied in the Bad River system range from 1.0–1.7 × MLC (C. Gagnon, personal communication, 2020). Even at the highest concentrations, mortalities were not significantly different from the controls. However, survival was greater for Eastern Elliptio than for the Eastern Floater, and for adults relative to juveniles. Waller et al. (2003, p. 550) also found that trials conducted at lower water temperatures (13 °C versus 21 °C) resulted in higher mussel survival.

B.2 Sedimentation

River channel erosion, precipitation runoff, and wind transport account for 30% of the total sediment load in aquatic systems, while land-use activities such as agriculture (Peacock et al. 2005, p. 548), logging (Beschta 1978, entire), mining (Seakem Group et al. 1992, p. 17), urbanization (Guy & Ferguson 1963, entire), and hydrological alteration (Hastie et al. 2001, entire) account for the remaining 70% (Du Plessis 2019, pp. 86–87). Agricultural activities have been found to produce the most significant amount of sedimentation (for example, livestock grazing/trampling near water's edge; Nolte et al. 2013, p. 296). Excess sediment is listed as the most common pollutant in rivers, streams, lakes, and reservoirs and has been estimated to cause approximately US$16 billion in environmental damage every year (USEPA 2005, pp. 9–25; Du Plessis 2019, pp. 86–87).

In 1999, Brim Box and Mossa (1999, entire) reviewed sediment impacts to unionid mussels and reported sedimentation may lead to smothering, reduced fish abundance, and declines in feeding/respiration. Authors concluded suspended sediments negatively affect mussel reproduction, growth, and survival. However, Haag (2012, entire) in reviewing the effect of sedimentation on mussel populations found many studies conducted and reported within Brim Box and Mossa (1999, entire) review lacked controls and/or focused mainly on the effects of sudden sedimentation rather than gradual accumulations of sediment. To address uncertainty, a third review was conducted in which authors evaluated the effects of suspended sediment concentration (SSC), total suspended solids (TSS), and sediment deposition and scour on the population performance (in other words, growth, survival, and reproduction) of freshwater mussels (Goldsmith et al. 2021, entire). Authors found increases in SSC and/or TSS can impact mussels by decreasing food availability, physically interfering with filter feeding and respiration, as well as impact mussel-host fish relationships.

Sedimentation can result in negative impacts to mussel reproduction. Specifically, increased sedimentation within the water column can decrease mussel clearance rates (in other words, volume of water completely cleared of particles per unit time) and in turn interfere with the ability of female mussels to capture sperm within the water column, thus reducing fertilization success (Gascho Landis et al. 2013, entire). For example, Gascho Landis et al. (2013, p. 75) in evaluating the effects of suspended solids on Pondmussel (*Ligumia subrostrate*) found when TSS concentrations were greater than 8 mg/L, there was a sharp decline in clearance rates. It should also be noted, evidence shows species with low cilia density, often lentic taxa, and short-term brooders, which use all four gills to brood glochidia, may be more likely to endure respiratory stress, particularly during brooding periods (Gascho Landis et al. 2013, p. 71).

It has also been shown increased sedimentation may negatively impact mussel-host fish relations, further impacting mussel reproductive success. This relationship may be impacted via physical abrasion of the fish gills and/or decreased visibility within the water column. For example, the success of glochidial attachment of Fatmucket (*Lampsilis siliquoidea*) to Largemouth Bass (*Micropterus salmoides*), and metamorphic success was reduced from concentrations of montmorillonite clay ranging from 1,250 to 5,000 mg/L (Beussink et al. 2007, p 15–17). This may be due to physical abrasion to gill tissues from increased suspended sediment, increased fish mucus production in an attempt to protect the gill from physical abrasion, coughing (which may dislodge glochidia from the gills,), and/or declines in keratocytes (which help with encapsulation of glochidia) (Beussink et al. 2007, entire). In addition to physical abrasion, some mussels utilize lures or conglutinates to parasitize their respective host-fish (Barnhart et al. 2008, p. 374; Haag 2012, p. 171). Declines in visibility within the water column may lead to decreases in host fish encountering glochidia; however, no studies have been conducted to date (Goldsmith et al. 2021, p. 103). However, impacts to fish population performance (i.e., growth, reproduction, and survival) were observed between 20 to 5000 mg/L depending on testing a species ability to resurface after burial, clearance rate, filtration rate, etc. (Goldsmith et al. 2021, p. 10).

Increased sedimentation may result in decreases in feeding and respiration, which could result in negative alterations to mussel's energetic metabolism and ultimately growth (Dimock and Wright 1993, p. 183; La Peyre et al. 2019, p. 5). Specifically, as sedimentation increases, clearance rates decrease and pseudofeces increase to prevent gill filaments from clogging (Bayne & Newell 1983, entire; Madon et al. 1998, p. 401). If the stressor becomes long-term, mussels may find feeding gains to be outweighed by the energetic cost of sorting food vs. non-food material (Bayne & Widdows 1978, p. 137; Madon et al. 1998, p. 401). Clearance rates were negatively impacted when TSS concentrations were >8 mg/L, and respiratory stress was prevalent when TSS was about 600 mg/L (Goldsmith et al. 2021, pp. 102 and 104). Overtime, mussels may reduce clearance, nitrogen excretion, and respiration rate, as well as shift their metabolism to non-proteinaceous body stores (Aldridge et al. 1987, p. 25). This occurs when starvation sets in and may result in mussels prioritizing maintenance over reproduction and growth (Jokela & Mutikainen 1995, p. 129).

Finally, increased suspended sediment can alter river channel formation and habitat type through aggradation and degradation (Gordon et al. 2004, entire), which can lead to smothering and sometimes burial, ultimately impacting mussel survival. Impacts may affect different species and populations differently. For example, Ellis (1936, p. 39) examined the effects of silt deposition on four unionid mussel species within the Trinity River in Texas and found silt accumulations of 0.6–2.5 cm in depth resulted in approximately 90% mortality. Specifically, authors found *Lampsilis teres* to be the most sensitive, while the other three were the least sensitive (*Obliquria reflexa, Quadrula apiculata, Quadrula noblis*). Additionally, Imlay (1972, pp. 78–79) evaluating species response to smothering found sensitivities to differ between the three species being tested (*Pyganadon grandis* [least sensitive], *Ligumia recta* [second sensitive], and *Fusconaia flava* [most sensitive]). Localized bed degradation can impact mussels where suitable habitat is scoured, leading to individuals being washed away or habitat elimination (Goldsmith et al. 2021, p. 105). In the Little River in Oklahoma, mussel species richness and abundance were maximized in areas where chances for bed movement and particle entrainment (substrate particles being transported with the flow of water) were low (Allen and Vaughn 2010, entire). Richness and abundance were maximized when relative shear stress (RSS) was <1 (Allen & Vaughn 2010, p. 392).

In the Brazos and Trinity River basins in Texas mussel diversity was maximized at RSS values <1, and some species could persist at higher RSS values than others (Randklev et al. 2019, p. 392). Specifically, *Potamilus* and *Lampsilis* species were found to be more persistent than *Amblema*, *Cyclonaias*, and *Quadrula* species, which is likely due to differences in species traits (in other words, burrowing, morphology, and life history).

B.3 Water Temperature

Mussels are sedentary bottom dwelling ectotherms (dependent on external sources of body heat), and therefore exceedance of species thermal optima and decrease in flow will likely result in physiological impacts (Amyot & Downing 1997, p. 346) including altered heart rate, gape frequency, filtration rate, respiration rate (see dissolved oxygen), and reproductive success. Decreased flows may also result in increased toxicity levels within the water (for example ammonia; Khan et al. 2018, p. 2).

Additionally, mussels are obligate parasites, reliant on specific host-fish for dispersal who are also adversely impacted by altered flow and often equally sensitive to elevated water temperatures (Gates et al. 2015, p. 2). As a result of these host constraints, elevated water temperatures can quickly reach uninhabitable levels for mussel host species during periods of low flow and depending on the frequency and magnitude can have a profound negative impact on population persistence (Khan et al. 2019, p. 13–14)

Increased water temperature and altered flow patterns negatively affect water quality and quantity impacting mussel physiological processes (for example, protein damage, fluidity of the cellular membrane, and organ function), disrupting energy balance, growth, and reproduction (Ganser et al. 2015, p. 17). For example, factors that trigger glochidial release are unknown for many species; however, it is assumed the process is triggered by a combination of water temperature and photoperiod (Kautsky 1982, p. 149; Wieland et al. 2000, p. 452; Gascho Landis et al. 2012, p. 775). Thus, if the thermal regime of a river system is altered, timing of seasonal cues may shift and impact recruitment success (Hastie & Young 2003, p. 2107; Österling 2015, p. 1; Schneider et al. 2017, p. 267). Specifically, Schneider et al. (2017, pp. 267 and 283) evaluating temperature and host dependent reproduction within *Unio crassus* (Thick Shelled River Mussel) found the timing of glochidial release was delayed at both constantly low temperatures (in other words <10 °C) and higher-than-normal temperatures (in other words10–20 °C). Additionally, authors found moving mussels from the cold treatment (<10 °C) to natural temperatures (10–15 °C) resulted in the gravid females releasing their glochidia soon after

(Schneider et al. 2017, p. 283). Authors indicate this suggests there is a temperature threshold for glochidial release. Pandolfo et al. (2010, p. 964) observed significantly lower survival in several species of freshwater mussels at 37 ºC. Similar to mussels, temperature and photoperiod are thought to influence the location, abundance, and activity level of host fish as well as their immunity strength (Martel & Lauzon-Guay 2005, p. 420; Roberts & Barnhart 1999, entire; Gascho Landis et al. 2012, p. 776). Therefore, these variables may determine how well glochidia will transform to juveniles, as well as the chance of mussel and host-fish populations co-occurring. Research shows elevated thermal regime impacts both water quality and quantity, which can have direct impacts on the population performance of freshwater mussel populations.

B.4 Dissolved Oxygen

Low dissolved oxygen is a threat to freshwater mussels and is particularly an issue interstitial waters (waters between sand particles, sediment, gravel) (Sparks & Strayer 1998, p. 129). Low dissolved oxygen can be caused by excess sedimentation, nutrient loading, organic inputs, changes in flow, and higher temperatures (Sparks & Strayer 1998, p. 129). Alterations to flow directly affect the concentration of dissolved oxygen (DO) within a river system (Ganser et al. 2015, p. 17). Specifically, during high flow events, turbulent diffusion of atmospheric oxygen increases, while during low flow events, DO may drop to critically low levels (Chen et al. 2001, p. 209). Surface waters can be near saturation, while adjacent interstitial waters are far lower (Sparks & Strayer 1998, p. 129). Elevated water temperatures also affect dissolved oxygen concentrations in water bodies as well (Ganser et al. 2015, p. 17). Adults and juveniles that are buried in the sediment are particularly vulnerable to low dissolved oxygen for this reason (Sparks & Strayer 1998, p. 129).

The ability to maintain constant oxygen uptake during periods of low and high oxygen availability is essential to mussel population persistence. Mussels cannot maintain oxygen consumption rates when exposed to low levels of DO, so they may be forced to inefficiently bring oxygen into their bodies by activating anaerobic metabolism in their tissues (Gade & Grieshaber 1988, p. 255). While adults may be able to withstand some period of anoxia (absence of oxygen), there is the potential for these conditions to negatively impact their metabolism. Newly transformed juveniles that are entirely within interstitial waters may be exposed to prolonged periods of low dissolved oxygen that has the potential to significantly alter their behavior (for example, surfacing, gaping, extending their siphons and foot) leading to elevated levels of predation potential as well as direct mortality (Sparks & Strayer 1998, pp. 131–133).

Stegmann (2020, pp. 1–55) used hypoxia (oxygen deficiency) trials to evaluate the behavioral response of Salamander Mussel to cooler and warmer water hypoxic conditions. Mussels did not show a preference for cool water that is hypoxic or water with normal oxygen conditions, but under warm water conditions mussels did have a significantly higher tendency to occupy hypoxic waters compared to oxygenated waters. This could be because respiratory rate increases with increasing temperature, given that the mussels tended to stop moving in hypoxic waters, it could be more due to inability to move out of these areas, which could be compounded by temperature increases (Stegmann 2020, pp. 11–14). It is possible that these mussels depleted their oxygen stores reducing their ability to move or that they reduced movement to avoid additional depletion of their oxygen stores.

The ability to deal with alterations in DO levels may differ between species and populations. Oxygen regulation ability in unionids may be related to the degree of hypoxia a species normally experiences in its habitat type (Chen et al. 2001, pp. 209–214). Additionally, this ability may be enhanced at low temperatures (Chen et al. 2001, p. 209).

B.5 Hydrological Regime

The ecological responses to altered hydrology are overall described as "chronic and cumulative and profoundly negative (Poff et al. 1997, entire; Pyron et al. 2020, p. 3)," idiosyncratic, and can vary substantially with geography, geomorphology, type of land use, and engineering practices for each specific impacted river (Pyron et al. 2020, p. 3), and further worsened by the current and expected further changes of climate conditions (Addor et al. 2014, entire; Arnell 1999, entire; Brunner et al. 2019, entire; Horton et al. 2006, entire; Laghari et al. 2012, entire; Leng et al. 2016, entire; Milano et al. 2015, entire; Brunner et al. 2020, entire). Climatic changes to the hydrological regime are caused by changes in the seasonality and intensity of annual precipitation and changes in flood and drought characteristics (for example, the seasonality and magnitude of floods; the duration of droughts), as well as the seasonal shifts in melt contributions related to reduced snow and glacier storage (Middelkoop et al. 2001, entire; Farinotti et al. 2016, entire; Beniston et al. 2018, entire; Brönnimann et al. 2018, entire; Jenicek et al. 2018, entire; Brunner and Tallaksen 2019, entire; Brunner et al. 2020, entire). Being able to quantify these types of changes may assist in improving our understanding of further future changes in climatic extremes, which is crucial for adapting river conservation practices, especially those involving the management of existing river development. Specifically for freshwater mussel species, drought and flood conditions can shift energy allocation toward maintenance (for example, respiration) and therefore, may negatively impact the growth of individuals (Jokela and Mutikainen 1995, p. 129).

Drought

Varying temperature sensitivities can lead to feedback cycles that increase mortality during low flows and high temperatures. For example, Khan et al. (2020, entire) evaluating the upper thermal limits of three adult freshwater mussel species (Threeridge [*Amblema plicata]*, Guadalupe Orb [*Cyclonaias necki]*, and False Spike [*Fusconaia mitchelli]*) from the Guadalupe River in Texas, found thermal tolerance differences between species, with the most sensitive being *F. mitchelli*. The authors then related species thermal tolerance thresholds to daily discharge measurements to determine whether subsistence flows (i.e., represents infrequent, natural low flow events that occur for a seasonal period of time) were sufficient to offset thermal tolerance exceedances for the mussel species; however, summer subsistence flow standards inadequately addressed exceedance of upper thermal tolerances for their focal species (Khan et al. 2020, p. 14). Therefore, authors concluded current flow standards were insufficient to protect mussel populations during low flows and severe droughts.

During periods of low flow and temperature exceedance, water quality may degrade as contaminants become more concentrated. This may be problematic for freshwater mussels because they are particularly sensitive to ammonia (Augspurger et al. 2003, p. 2569; Spooner and Vaughn 2008, entire). As surface water temperatures increase, toxicity of ammonia increases, which may result in sublethal or lethal impacts to mussels (USEPA 2013, p. 6). For example, Augspurger et al. (2003, p. 2571) examining current water quality guidance for protection of freshwater mussels from ammonia exposure found concentrations as low as 0.7 ppm total ammonia nitrogen were lethal to juveniles and concentrations as low as 2.4 ppm total ammonia nitrogen were lethal to glochidia. Authors concluded current U.S. EPA criteria for continuous concentration of total ammonia (1.24 mg/L) may not be protective of mussels.

Thermal tolerance and avoidance strategies are thought to differ among species as well as population. For example, Gough et al. (2012, entire) assessed the linkage between physiological tolerance, behavioral response, and survival of three species of freshwater mussels subjected to drought: Pondhorn (*Uniomerus tetralasmus)*, Rough Fatmucket (*Lampsilis straminea)*, and Giant Floater (*Pyganodon grandis*). Authors observed and identified strategies each mussel species used to deal with drought and consequently thermal intolerance (Gough et al. 2012, p. 2357). The three strategies observed included: tracking (i.e, track receding water; intolerant), track and then burrow (semi-tolerant), and burrowing (tolerant). Both *U. tetralasmus* and *L. straminea* burrowed in response (shallowly – approximately 3–4cm), while *P. grandis* rarely burrowed. Survival results suggest drought and elevated water temperatures pose the greatest threat to intolerant trackers, while tolerant burrowers are the most resistant to drought conditions. This suggests mussel species capable of burrowing in response to stress may have a greater ability to persist.

Prolonged Stream Drying

Prolonged stream drying occurs during periods of extreme drought as a result of climate change and may occur across river systems at varying levels depending on the rate in which climatic impacts are accelerated (Gates et al. 2015, p. 622; Aldous et al. 2011, p. 233), but can also occur as a result of land use activities such as water withdrawal for oil and gas extraction, irrigation for agriculture, and other municipal/industrial purposes (Poff et al. 1997, pp. 772–774). Although seasonal drying occurs as a natural component to the hydrological regime, these periods of drought may prolong, increase in frequency and severity, and become unpredictably timed as climatic conditions are expected to change as a result of rising surface temperatures and other factors (Gates et al. 2015, p. 622; Mukherjee et al. 2018, p. 1).

Low water levels may be endured for short periods of time (Pyron et al. 2020, p. 5), though such lower flows can cause stagnant pools to form, which overtime, can become unsuitable for freshwater mussels and their host fish, especially during the summer months, as water temperatures increase and dissolved oxygen decreases (Gates et al. 2015, p. 622). A completely dry streambed not only can eliminate habitat for freshwater mussels, but it also has the ability to fragment population connectivity. Salamander Mussel occurs in varying depths across the range, in Missouri is typically found in shallow habitat along the fringe of riffles (A. Roberts personal communication, 2021), whereas in Pennsylvania it can be found in very deep pools (B. Anderson, personal communication, 2021).

Inundation

Stream inundation typically occurs as a result of water impoundment and retention from dams, further exacerbated by extreme flooding via climate change (Zeiringer et al. 2018, p. 72; Hastie et al. 2003, pp. 42–43). Dams are the most obvious direct modifiers of hydrological regime (Zeiringer et al. 2018, p. 72). Dams capture both high and low flows, as well as accumulate sediment, and are responsible for coarsening (thicker and heavier substrate particles) streambeds (Zeiringer et al. 2018, p. 72). Reservoirs and other types of artificially ponded areas provide poor conditions for freshwater mussels (for example, increased siltation and sediment deposit; temperature changes), and can result in direct smothering when large amounts of sediment are deposited along the bed. Deep water in particularly large reservoirs is additionally known to be cold and can often be devoid of necessary nutrients. If cold enough (<11 °C (52 °F)) growth of any freshwater mussel occupants could be stunted; these individuals likely never reproduce or may reproduce less frequently (Vaughn & Taylor 1999, pp. 915–916).

Increased Flashiness

Increased stream flashiness is another result of extreme flooding via climate change and can impact associated river habitats by destabilizing and disrupting natural substrate transportation by means of increased water velocity, further worsened by the overwhelming presence of impervious surfaces as a consequence of development; stream destabilization has the ability to undercut stream banks, blow out crucial riffle habitats, and wash scour substrate (Hinck et al. 2011, p. 6; Gangloff & Feminella 2007, p. 69; Zeiringer et al. 2018, p. 70). We expect for freshwater stream and river habitats within or near urban areas to be most affected by flashiness as a result of frequent surface runoff, though we understand that extreme flooding events have the ability to impact any reach throughout a specific river system. Impacts to native biota tend to be localized; though as development increases across the natural landscape into the future, we should expect for the effects of increased flashiness to spread and to become more severe. Miller and Lyon (2021, p. 7) also found a correlation between cropland drainage tiles and increase flashiness in streams during rain events, making drainage tile runoff another potential contributor to stream destabilzation, especially in agricultural areas.

B.6 Invasive Species

*Zebra Mussels (*Dreissena polymorpha*)*

The Zebra Mussel (*Dreissena polymorpha*) is a freshwater bivalve native to the Black, Caspian, and Azov Seas and was likely introduced to North America via commercial cargo ships traveling from the north shore of the Black Sea to the Great Lakes (McMahon 1996, p. 358). Due to the species ability to passively drift at the larval stage and attach to boats, the Zebra Mussel rapidly dispersed throughout the Great Lakes and major river systems and now inhabiting all the Great Lakes, all large navigable rivers within the eastern United States, and many lakes within the Great Lakes region. Zebra Mussels have been found to have a profound effect on the ecosystems they invade through biofouling (accumulation of organisms on surfaces) and reducing the amount of phytoplankton within the water column significantly (Holland 1993, p. 622; Fahnenstiel et al. 1995, p. 471; Caraco et al. 1997, p. 597). With a 90% filter efficiency rate and the ability to filter particles less than 1um in diameter (with preference for larger particles), Zebra Mussels have been found to be more efficient at filtration than unionids (Sprung & Rose 1988, p. 526; Noordhuis et al. 1992, p. 108).

The invasion of freshwater habitats within the United States poses an imminent threat to mussel fauna (Ricciardi et al. 1988, p. 615). Zebra Mussel invasion can result in the loss of entire native mussel beds through direct attachment to mussel shells (Strayer et al. 1999, pp. 75–80). By attaching themselves in large numbers to native mussel beds, the invasive species negatively impacts locomotion, valve-movement, and native species energy stores, depleting food concentrations to levels too low to support reproduction or survival of native species (Strayer et al. 1999, pp. 75–80). Because mussel species filter phytoplankton at higher concentrations than native freshwater mussels, habitat for native freshwater mussels also may degrade over time with an increased deposit of Zebra Mussel psueudofeces (undigested waste material passed out of the incurrent siphon) that foul benthic habitat. Additionally, Zebra Mussels may impact native mussel fauna by filtering their sperm and/or glochidia from the water column, thus negatively altering reproductive potential (77 FR 14913). Currently, Zebra Mussels are established within the upper Mississippi, lower St. Croix, Ohio, and Tennessee Rivers overlapping much of the current range of native freshwater mussel species and likely have already reduced mussel species populations in heavily infested waters.

*Corbicula Clam (*Corbicula fluminea*)*

The Corbicula (*Corbicula fluminea*) is a freshwater bivalve native to tropical southern Asia west to the eastern Mediterranean, Africa, and southeast Asian islands south into central and eastern Australia (Morton 1986, p. 114). The species was first reported within the United States in 1938 along the banks of the Columbia River, Washington (Counts 1986, pp. 18–19). While the mechanism for dispersal within the United States is unknown, the species is currently found in 46 states as well as Lake Erie, Lake Michigan, and Lake Superior (USEPA 2008, p. 35). The most prominent effects the introduction of Corbicula has had on native mussel fauna and habitats include biofouling, altering benthic substrates, and outcompeting (especially juvenile mussels) for food, nutrients, and space (Leff et al. 1990, p. 415; Neves & Widlak 1987, p. 6). Additionally, it has been suggested Corbicula may filter native freshwater mussel sperm, glochidia, and/or newly metamorphosed juveniles reducing native freshwater mussel reproductive potential (Strayer 1999, p. 82; Yeager et al. 2000, p. 255). Corbicula actively disturb sediment altering benthic substrates and ultimately reduce habitat for juvenile native mussels (Strayer 1999, p. 82).

Research suggests invasion of Corbicula tends to occur in areas where native freshwater mussel density is low or declining (Strayer 1999, pp. 82–83; Vaughn & Spooner 2006, pp. 332–336). It appears Corbicula cannot successfully invade dense, healthy mussel beds in small-scale habitats (Vaughn & Spooner 2006, pp. 334–335). However, while Corbicula may not be a factor in the decline of native freshwater mussels in dense beds, the invasive species has the potential to result in the decline of populations that are stressed or in decline through competition for resources and space (Vaughn & Spooner 2006, pp. 335–336). Therefore, Corbicula are considered a low threat to native freshwater mussel species.

*Black Carp (*Mylopharyngodon piceus*)*

The Black Carp (*Mylopharyngodon piceus*) is a fish species native to major Pacific drainages of eastern Asia and to the Honghe or Red Rivers of northern Vietnam (Nico et al. 2005, p. 337). The species was first introduced within the United States in the early 1970s when it was imported with grass carp stocks and then was subsequently introduced in the early 1980s when it was used as fish food and a biological control to fight the spread of Yellow grub (*Clinostomum margaritum*) in aquaculture ponds (Nico et al. 2005, p. 337). Currently, the Black Carp has been reported in Arkansas, Illinois, Mississippi, and Missouri (Nico et al. 2005, p. 337). The species negatively alters native aquatic ecosystems by preying on and subsequently reducing juvenile and adult unionid and snail populations, many of which are considered endangered or threatened (Nico et al. 2005, p. 337). This predation has the potential to restructure benthic communities. Additionally, due to the Black Carp's large size (can reach more than 4ft long) and life span (~15 years), the species has the potential to persist for many years and cause significant declines in native bivalve populations in North American streams and lakes (Nico et al. 2005. p. 337). In fact, research has shown the foraging rate of a four-year-old Black Carp can average three to four pounds a day and can ultimately consume ~10 tons of native unionids over its lifetime (Mississippi Interstate Cooperative Resource Association (MICRA) 2005, p. 1). While the Black Carp has not invaded all waters with native freshwater mussels and currently is not considered an immediate threat, it has been suggested the species has the potential to become a threat of high magnitude if introduced into more systems with native freshwater mussels.

*Rusty Crayfish (*Faxonius rusticus*)*

The Rusty crayfish (*Faxonius rusticus*) is a freshwater crustacean native to the Ohio River Basin across tributaries in Ohio, Indiana, Kentucky, and northern Tennessee as well as Lake Erie

(Creaser 1931, entire; Hobbs 1974, entire; Momot et al. 1978, pp. 10–35; Hobbs et al. 1989, p. 300; Taylor 2000, p. 140). The species was likely introduced both unintentionally through dumping of angler bait buckets and use of the species in schools and biological supply houses and intentionally by commercial crayfish harvesters and as a means to remove nuisance weeds (Kilian et al. 2012, p. 1469; Gunderson 2008, entire; Wilson et al. 2004, p. 2256; Magnuson et al. 1975, p. 67). The introduction of Rusty crayfish can cause significant population declines in native unionid mussel populations through direct predation resulting in a cascade of impacts to food web dynamics (Klocker & Strayer 2004, pp. 174–175). Currently, the species is found in 20 states and can live at high densities (Klocker & Strayer 2004, p. 168). Thus, the increase and spread of this predator population can result in negative impacts to threatened unionid populations inhabiting the same area (Klocker & Strayer 2004, pp. 174–175).

*Spiny Waterflea (*Bythotrephes longimanus*)*

The Spiny Waterflea (*Bythotrephes longimanus*) is a large cladoceran native to the Baltic Nations, Norway, northern Germany, the European Alps, the British Isles, the Causcasus region, and Russia (USFWS 2013, p. 1). The species was likely introduced from ship ballast water and diapausing eggs from sediment in ballast tanks (Berg et al. 2002, p. 275; Evans 1988, p. 235). The species is responsible for significant declines and shifts in plankton communities and directly competing with small fish and bivalves that rely on these food stocks (USEPA 2008, p. 37). Currently, the species is found in all the Great Lakes and many inland lakes within the region. Specifically, densities have been reported to be low in Lake Ontario, southern Lake Michigan, and offshore areas of Lake Superior, moderate to high in Lake Huron, and very high in the central basin of Lake Erie (Barbiero et al. 2001, p. 147; Vanderploeg et al. 2002, p. 1222; Brown & Branstrator, 2004, pp. 1–8). Because the species has high generation turnover, population densities can rapidly increase, negatively affecting mussels within the region (Brown 2008, pp. 1–8). Therefore, when occupying the same waterway, the Spiny Waterflea is considered a threat to native freshwater mussel populations.

*Brown Trout (*Salmo trutta*)*

The Brown Trout (*Salmo trutta*) is a fish species native to Europe, northern Africa, and western Asia (Page & Burr 1991, p. 42). The species was first reported in the United States in 1833 and since then, has been stocked in virtually all states (Courtenary et al. 1984, pp. 41–77; MacCrimmon et al. 1970, pp. 811–818). Since its introduction, the species has contributed to the decline of native fish species, especially other salmonids, through direct predation, displacement, and food competition (Taylor et al. 1984, pp. 322–373). Competition with native fish species has the potential to impact host-fish stocks and ultimately impact freshwater mussel's reproductive potential. Gall and Mathis (2010, entire) determined that the response of larval hellbender to chemical cues from introduced predatory trout was generally weak and could result in increased predation on the species, mudpuppies could have a similar response those this study did not investigate mudpuppy response. Due to mussel's unique reproductive strategy, without the presence of host fish, mussel species cannot reproduce. Currently, natural reproduction of Brown Trout is low in many states; however, many states maintain fish populations by restocking. Therefore, Brown Trout pose an indirect threat to unionid populations inhabiting the same communities due to their predation of host-fish populations.

*Quagga Mussel (*Dreissena rostriformis bugensis*)*

The Quagga Mussel (*Dreissena rostriformis bugensis*) is a small freshwater bivalve native to the Dneiper River drainage of Ukraine and Ponto-Caspian Sea (Mills et al. 1996, p. 271). The species was likely introduced through ballast water within the Great Lakes, and due to its high potential for rapid adaptation and ability to passively drift, the species was able to rapidly expand and colonize the United States (Mills et al. 1996, p. 275). Similar to Zebra Mussels, the Quagga Mussel can be harmful to aquatic ecosystems through biofouling and utilization of the same food resource as freshwater unionids (Karatayev et al. 2015, p. 104). While less information is available regarding the impact of Quagga Mussels on native freshwater mussels (Lucy et al. 2014, p. 241), information suggests the Quagga Mussel may have smaller impacts on native freshwater mussels than the Zebra Mussel (Karatayev et al. 2015, p. 14; Sherman 2013, p. 208). Zebra Mussels are much more commonly found on native freshwater mussel shells than Quagga Mussels even in areas where Quagga Mussels are more abundant than Zebra Mussels (Karatayev et al. 2015, p. 104). Yet, if affixed to the shell of a native freshwater mussel, Quagga Mussels can impact native freshwater mussel locomotion, ability to gape, and food storage. Additionally, Quagga Mussels have the potential to remove large quantities of phytoplankton and suspended particulate matter from the water, thus decreasing the food source and altering the food web (Claxton & Mackie et al. 1998 p. 1210). Because Quagga Mussels filter high concentrations of phytoplankton, the quality of habitat will likely degrade due to an increase in psueudofeces. Finally, Quagga Mussels may impact native mussel fauna by filtering their sperm and/or glochidia from the water column, thus negatively altering reproductive potential. Despite the threats the Quagga Mussel may pose, it was found the number of dreissenids attached to native freshwater mussels was lower in lakes dominated by Quagga Mussels suggesting the ongoing replacement of Zebra Mussels by Quagga Mussels within the Great Lakes may reduce impacts to native freshwater mussels (Karatayev et al. 2015, p. 104). Currently, the Quagga Mussel is found within the lower Great Lakes and harbor and nearshore areas of Lake Superior. Research suggests if occupying the same reach as native freshwater mussels, the Quagga Mussel has the ability to negatively impact native freshwater mussels by outcompeting the native mussels for resources (in other words, food and space); however, research also suggests the replacement of Zebra Mussels by Quagga Mussels may reduce impact to native freshwater mussels and aid in species recovery (Karatayev et al. 2015, p. 104).

*Common Carp (*Cyprinus carpio*)*

The Common Carp (*Cyprinus carpio*) is a fish species native to Eurasia (Page & Burr 1991, entire; Balon 1995, p. 5). The species was likely first brought to the United States from France in

1831; but in 1877, the U.S. Fish Commission began to import Common Carp from Germany to begin stocking and distributing the species as fish food (DeKay 1842, Part IV; Cole 1905, entire). The Common Carp poses a risk to aquatic ecosystems because of its widespread abundance and tendency to destroy vegetation, resulting in increased turbidity and deterioration of habitat (Laird and Page 1996, pp. 13–14). Additionally, Common Carp prey on the eggs of other fish species, negatively impacting species recruitment (Miller and Beckman 1996, pp. 338–340). Mudpuppy eggs may be more protected in shelter habitats from Common Carp predation; however, some predation may still occur. Recruitment of other fish populations have also been impacted by Common Carp's degradation of their breeding grounds (McCarraher and Gregory 1970, pp. 700–707). This suggests Common Carp may have indirect threats to native freshwater mussel populations due to degradation of host spawning grounds and predation of host eggs, ultimately impacting freshwater mussel reproductive potential. Currently, Common Carp have been recorded in all states but Alaska and is widely distributed in the Great Lakes Basin (Bailey and Smith 1981, pp. 1539–1561). Common Carp are considered a moderate threat to Salamander Mussel populations when the invasive species occupies the same reach as their host (mudpuppy).

*Bighead Carp (*Hypophthalmichthys nobilis*)*

Bighead Carp is an invasive fish species native to southern and central China (Li and Fang 1990, pp. 244–250; Robins et al. 1991, p. 243). The species was introduced to the United States in 1973 in Arkansas. Sightings of Bighead Carp that had escaped from aquaculture facilities into open waters began in the early 1980s (Jennings 1988, entire). Because the species is planktivorous and can reach large sizes, Bighead Carp have the potential to deplete plankton stocks in turn impacting native fish, mudpuppy, and mussel populations that rely on the same food source (Laird & Page 1996, pp. 13–14). Ultimately this can lead to declines in body condition and impact the reproduction, growth, and survival of native freshwater mussels and host populations. Currently, the species has been recorded in 18 states with evidence of natural reproducing populations in the middle and lower Mississippi and Missouri Rivers (Pflieger 1997, p. 372; Burr et al. 1996, entire). Bighead Carp are considered a threat to native freshwater mussel and host populations occupying the same reach.

B.7 Host species – Mudpuppy Species Vulnerability

Mudpuppy Disease and Die-offs

Little is known about existing or novel amphibian pathogens that have the potential to impact mudpuppies. *Batrachochytrium dendrobatidis (Bd)* is a fungal pathogen which can cause chytridiomycosis, a highly infectious amphibian disease associated with mass die-offs, population declines and extirpations, and potentially species extinctions on multiple continents (Berger et al. 1998, pp. 9031–9036; Bosch et al. 2001, pp. 331–337; Lips et al. 2006, pp. 3165–3166). *Bd* attacks the keratinized tissue of amphibian skin and can lead to thickened epidermis, lesions, body swelling, lethargy, loss of righting reflex, and death in all life stages (Berger et al. 1998, pp. 9031–9036; Bosch et al. 2001, p. 331; Carey et al. 2003, p. 130). A high prevalence of

Bd has been found in fully aquatic salamanders, including the genera Necturus (mudpuppy genus) (Chatfield et al. 2012, p. entire). Necturus had the second highest prevalence of *Bd* out of the four genera sampled across three states (Chatfield et al. 2012, p. 2). Little to no information exists on the effects of *Bd* on wild mudpuppies, but it is possible that mudpuppies are vulnerable to infection, which could be amplified by other stressors (Chatfield et al. 2012, pp. 1–5).

Another fungal pathogen, *B. salamandrivorans* (*Bsal*), invaded Europe from Asia around 2010 and is responsible for causing mass die-offs of fire salamanders (*Salamandra salamandra*) in northern Europe (Martel et al. 2014, p. 631; Fisher 2017, p. 300–301). Given extensive unregulated trade and the recent discovery of *Bsal* in amphibians, there is concern about the introduction of a novel pathogen causing extirpations of naive salamander populations in North America (Yap et al. 2017, entire). While we still do not have a clear understanding of all of the salamander species that will be susceptible to *Bsal*, there is concern that mudpuppies could be impacted.

Ranaviruses are another emerging group of pathogens affecting amphibian populations worldwide. *Ranavirus* is one of five genera in the family Iridoviridae, a family of viruses known to infect a diversity of invertebrate and ectothermic (cold-blooded) vertebrate hosts. Ranaviruses were originally detected in frogs (Granoff et al. 1965, pp. 237–255; Rafferty 1965, pp. 11–17) but are now known to infect and cause disease in fish, reptiles, and other amphibians (Marschang & Miller 2012, p. 1). Ranaviruses are often virulent and can cause systemic infections in amphibians (Daszak et al. 1999, p. 742). Mortality caused by ranaviruses has been reported from five continents and in most of the major families of frogs and salamanders (Gray et al. 2009, pp. 243–244).

Amphibian larvae seem to be the developmental stage most susceptible to ranaviruses (Daszak et al. 1999, p. 742), with physical characteristics of infections in larval stages including skin hemorrhages, ulcers, and bloating (Marschang & Miller 2012, p. 1). Overt signs of infection may not be exhibited in juvenile and adult stages (Daszak et al. 1999, p. 742), but when present typically include skin abnormalities (e.g., sloughing, hemorrhaging) and sometimes necrosis (tissue death) of digits and limbs (Cunningham et al. 1996, pp. 1539, 1541; Jancovich et al. 1997, p. 163). The exact mechanism by which *Ranavirus* infections cause amphibian mortalities remains unclear, but hemorrhaging in skeletal tissue (Daszak et al. 1999, p. 743) and extensive necrosis in the liver, spleen, kidneys, and digestive tract have been observed in infected individuals (Gray et al. 2009, p. 253). It is also postulated that viral infections may suppress the immune system, resulting in secondary invasion by opportunistic pathogens (Miller et al. 2008, p. 448).

Similar to freshwater mussels, mudpuppies have suffered mass die-offs in recent years that have largely gone unexplained (for example, Detroit River and Lake St. Clair, MI) (Stapleton et al. 2018, p. 8). In the Detroit Lakes region of Minnesota between 2012 and 2018, tens to hundreds of mudpuppies were reported washing up dead during July and August. Pathology examinations found *Edwardsiella bacteria* consistently cultured from dead mudpuppies (USGS unpublished data 2020). Similarly, documented die-offs of mudpuppies in 2006 in Canada have been associated with *Edwardsiella tarda* (USGS unpublished data 2020). Further work on the die-offs revealed that they were attributed to *Edwardsiella pscicida* not *E. tarda*. Ongoing work to study these die-offs in the Upper Midwest and Canada will potentially inform the cause and severity that such events pose to mudpuppy populations (USGS unpublished data 2020).

Overharvest and Collection

Direct mortality or removal of mudpuppy from the wild results from a variety of sources including unintentional catch during recreational fisheries, collection for the biological supply or pet trade industry, collection for use as bait, or illegal collection/ harvest (Craig et al. 2015, p. 926). Across the 14 states occupied by Salamander Mussel, 5 states completely prohibit collection, 7 states have daily bag limits or restrictions on collection, and 2 states have no limits or collection restrictions. Mudpuppies are often unintentionally caught especially during the ice fishing season. Myths about mudpuppies being poisonous or competing with local fishermen for fish can result in mudpuppies being tossed on the ice to freeze and die. Mudpuppies may also suffer mortality through unintentional hooking during recreational fishing and frog gigging. Collection for biological supply for use by academic institutions has resulted in the removal of hundreds of mudpuppies and likely millions historically in parts of the Great Lakes (Stapelton et al. 2018, p. 6). It is likely that this type of collection occurred range-wide. Collection for the pet trade and biological supply company is ongoing in many of the states with no prohibitions or limited restrictions, and illegal collection is also occurring across the species range. Removal of adults, which are long lived and reach sexual maturity later compared to other species, will impact the stability and persistence of populations subject to such collection pressure.

Mudpuppy Habitat Degradation and Fragmentation

Risk factors described above that pose a threat to freshwater mussels, including excess sedimentation, increased stream temperature, changes in flow regimes (for example, drought/ floods), and loss of connectivity, also have the potential to fragment, degrade, and destroy mudpuppy adult, larval, and egg habitat.

Impoundments are barriers to migration, change streamflow, increase sedimentation, create sediment deficits in other cases, potentially contribute to low dissolved oxygen within aquatic ecosystems, and thermal changes. These impacts can create a barrier to mudpuppy movements, preventing potential dispersal into both unoccupied and occupied habitats and resulting in limited gene flow and population isolation. Dams are present throughout the range of mudpuppy and have likely caused population declines in certain areas and perhaps even local extirpations (Murphy et al. 2018, pp. 407–419).

Changes in the hydrologic regime of river systems described above in section 3.1.4 have the potential to impact mudpuppies as well as freshwater mussels. These factors may negatively impact the amount and quality of mudpuppy shelter habitat, substrate stability, nesting and larval habitat, as well as ability to disperse to other suitable locations depending on stream flow and inundation. All these factors have the potential to affect reproduction, abundance, and stability of mudpuppy populations.

Contaminants Influences on Amphibians

Due to their semi-permeable skin, amphibians are particularly susceptible to certain contaminants (Fisher 2020, p.14). Given that mudpuppies are fully aquatic salamanders, they are considered bioindicators of aquatic ecosystems and are highly sensitive to contaminants and changes in water quality (Craig et al. 2015, pp. 926–927).

Treatment of invasive species with certain chemicals have detrimental impacts on mudpuppy survival. Mudpuppies are very sensitive to TFM, a lampricide used to treat streams in the Great Lake Basins for infestation by invasive sea lamprey. The Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin (USFWS 2012, pp. 1–14) cultured mudpuppies for cage studies at four locations in the Sturgeon River: (1) Control site that was upstream of the primary lampricide application point (AP); (2) Site A (high concentration) that was 200–300 m downstream of an AP ; (3) Site B (intermediate concentration) that was equidistant between APs; and (4) Site C (low concentration) that was 200–300 m above an AP (USFWS 2012, p. 2). Cages in the treated area were removed about 12 hours after TFM had passed each site (3–5 days total in the river) and control cages were removed after about 3 days in the river (USFWS 2012, pp. 3–8). At Site C, total mortality averaged 17.0% at an average concentration ratio of 1.39 × MLC. Mudpuppies died in two of the seven cages; one of three mudpuppies died in one cage (33.3%) and two of three mudpuppies died in a second cage (66.7%). No mortality occurred at Site A (1.34 × MLC), Site B (1.48 × MLC), or the control site (USFWS 2012, pp. 3–8). Two other studies have investigated the toxicity of lampricides to mudpuppies. Boogaard et al. (2003, pp. 529–541) concluded that lampricide treatment levels at 1.5 × MLC for sea lampreys may cause some mortality among adult mudpuppies, and young of the year and age-1 mudpuppies were more sensitive to lampricides than adults. The collections and treatment data from the Grand River and Conneaut Creek in Ohio indicate that most mortality of mudpuppies occurred downstream of application points and at night when there was a shift in pH. High mudpuppy mortality also occurred in the mudpuppy sanctuary (an area designated by Tim Matson, Cleveland Museum of Natural History) on the Grand River where mudpuppy densities are relatively high compared to other sections of the river.

Increased nutrification of aquatic ecosystems can result from point and non-point sources (for example, unproperly treated effluent from wastewater treatment facilities, runoff of excess fertilizer from agriculture or residential development, and manure). Many of these sources (for example, sewage and livestock waste) also contain contaminants such as ammonia, bacteria, and organic matter all of which reduce dissolved oxygen levels if present in excess. Low dissolved oxygen can alter habitat and directly impact mudpuppies. Amphibians are particularly sensitive to nitrate exposure, which can interfere with growth and development, reduce swimming speed, change feeding behavior, and result in direct mortality (Chamber 2009, p. 14).

Conductivity is a measure of electrical conductance in water and results from the presence of ions in the water column (Chamber 2009 p. 3). Conductivity is influenced by the geology of the stream and surrounding areas, but also by contaminants in particular de-icing chemicals (Chamber 2009, pp. 3–4; Allan and Castillo 2007, pp. 61-62). Estimates of salt compounds applied to roads annually in North America are approximately 14 million tons (Chamber 2009, pp. 3–4). Changes in conductivity have been shown to negatively impact growth, development, behavior, and survival of amphibians. "Conductivity below 278 milli Siemens per cm (ms/cm) (178,000 Total Dissolved Solids (TDS) in parts per million (ppm) for the 640 scale) was the strongest predictor of hellbender persistence at 24 historical sites, and that conductivity was negatively correlated to tree canopy cover (Pitt et al. 2017, p. 972).

Endocrine disrupting chemicals (EDCs) are a suite of chemicals (for example, pharmaceuticals, pesticides, nonionic surfactants, environmental pollutants) that interfere with normal endocrine or reproductive function (Hayes et al. 2002, pp. 5477-5478). They disrupt normal endocrine function by either mimicking normal hormones or disrupting the production or function of normal hormones (Kiesecker 2002, pp. 9902-9903; Forson and Storfer 2006, pp. 2328-2329; Hayes et al. 2006, pp. 29-30; Brodkin et al. 2007, pp. 81-82). EDCs accumulate in fatty tissue; if an organism is exposed over their lifetime, these chemicals will bioaccumulate (concentrated inside the body of living things). Given that mudpuppy is a fairly long lived species and fully aquatic salamander similar to the Eastern Hellbender, these types of chemicals pose an increased risk to these aquatic salamanders (Diamanti-Kandarakis et al. 2009, pp. 3-4). They pose a significant threat to amphibians from sublethal effects including male feminization, increased susceptibility to disease, and even direct mortality (Kiesecker 2002, pp. 9902-9903; Forson and Storfer 2006, pp. 2328-2329; Hayes et al. 2006, pp. 29-30; Brodkin et al. 2007, pp. 81-82). Point and non-point sources contribute EDCs to aquatic systems. The Eastern hellbender has been described and being particularly susceptible to EDCs given that it is a completely aquatic salamander (White et al. 1994, p. 176), mudpuppies would be similarly susceptible.

Road construction and resource extraction (for example, coal mining activities) may result in excess levels of heavy metals (for example, manganese, zinc, and aluminum) and are another threat to the health of amphibians in aquatic ecosystems affected by these chemicals (TDEC 2014, p. 72).

Climate Change Impacts on Mudpuppy

We were unable to conduct any modeling to determine the potential impact of climate change on mudpuppy due to a lack of data. However, we did draw from some of the analysis and information in the Eastern Hellbender SSA because we would assume many if not all of the impacts would be similar. The range of mudpuppy is much larger than Eastern hellbender, though there is overlap. Climate change will likely result in increased average temperatures throughout the range of mudpuppy, with potentially more periods of drought as well as increased and more intense precipitation (Karl et al. 2009, pp. 44, 107, 111-112, 117-118). These changes will result in increased stream temperature resulting in lower dissolved oxygen as well as dramatic shifts in stream flow, increased flashiness, and inundation. Similar changes could be seen in lake environments inhabitated by mudpuppy, as well as changes in the amount of ice cover in the northern extent of the range. These types of changes to riverine systems have the potential to negatively impact Eastern hellbender growth, immune function, survival, and reproductive success (Raffel et al. 2006, pp. 823-826. While Eastern hellbender may be more sensitive than mudpuppy, we would still expect mudpuppies to be negatively impacted by these changes to riverine systems due to climate change. Sutton et al. (2015) looked at predicted changes in climatic niche and climate refugia for salamanders of conservation priority including Hellbender (pp. 1–26). All of the salamander species were predicted to lose some of their climatic niche ranging from 3–100%, with Hellbender projected to lose 61%. Mudpuppy was not one of the priority species modeled, but certainly this study indicates that the salamander species modeled are vulnerable to climatic changes.

B.8 Mussel Conservation Propagation Programs

Kentucky

The Kentucky Department of Fish and Wildlife Resources established the Center for Mollusk Conservation to restore and recover rare and imperiled freshwater mollusks. Conservation propagation for the Salamander Mussel has resulted in the production of approximately 800 individuals for augmentation and reintroduction. The Duck and Licking Rivers have been the prime focus of conservation propagation efforts. Research on in vitro culture is being explored and is a promising tool that could be used for large-scale augmentation or reintroduction programs.

Pennsylvania

In 2015 and 2016, the Pennsylvania Department of Environmental Protection (PADEP) directed commercial sand and gravel mitigation monies to White Sulfur Springs National Fish Hatchery (WSSNFH) to rear Salamander Mussel to recover the population in Dunkard Creek (PA) after a mine discharge caused a lethal golden algae bloom that killed all of the mussels. A total of 20 mudpuppies were trapped from the Allegheny and Ohio rivers during 2018 and 2019. Following this collection, 10 Salamander Mussels were collected for broodstock for the WSSNFH propagation efforts (Welte 2020, p. 3). This effort was unsuccessful due to loss of broodstock in holding.

On October 7, 2020, WSSNFH collected 6 gravid females from the Allegheny River in Pool 6 near Cogley Island. Gravid females were held until March of 2021 when host could be acquired. Three females were used to infest three host species. The three infestations yielded ~1400 juvenile mussels. These animals are being produced for restoration efforts in Pool 5 of the

Allegany. WSSNFH will continue to produce Salamander Mussel for PADEP for the next 3 years. The Pennsylvania Fish and Boat Commission FBC has plans to continue propagation efforts with the development of a state hatchery geared towards propagation of mussels for the Dunkard Creek restoration project, with plans to propagate Salamander Mussel.

Recent propagation studies on Salamander Mussel have tested (in captivity) two additional species of salamanders for host suitability in captivity, axolotls (*Ambystoma mexicanum*) and tiger salamanders (*Ambystoma tigrinum*) (Moore et al. 2021, pp. 26–27). Glochidia were able to transform on both salamander species and juveniles survived and grew for 1 week (Moore et al. 2021, pp. 26–27). This has implications for future conservation propagation programs.

Wisconsin and Minnesota

The Genoa National Fish Hatchery (GNFH), Genoa, Wisconsin, has been propagating Salamander Mussel since 2013. The program started with collection of gravid female Salamander Mussels in 2013 and in 2014. Approximately 100 mudpuppies were infested with Salamander Mussel glochidia. The mudpuppies were then moved to Pool 4 of the Mississippi River and placed in 10 culture cages to allow the juvenile mussels to drop off and develop throughout the summer. Juveniles were then stocked in the Chippewa River. Since the start of this propagation effort Salamander Mussels have been regularly propagated to augment the Chippewa River population. Genoa has also been a leader in groundbreaking research on Salamander Mussel reproduction and their relationship to their host, mudpuppy. Much of what we know about Salamander Mussel reproduction has been learned through the conservation propagation program at GNFH.

APPENDIX C. METHODS FOR CURRENT CONDITION RISK FACTOR ANALYSIS

We developed a rule set to guide how to assess overall current condition for the risk factors. If any one of the risk factors is high, then the overall population condition is categorized as high risk, based on the importance of each risk factor in influencing the survival and persistence of freshwater mussels. If none of the risk factors are high, then we used an additive approach to assessing the overall population condition. Using the scores in Table 4.4, for additive scores 9–12, the overall population condition is categorized as moderate risk and for additive scores 6–8, the overall population condition is categorized as low risk. These break points were based off of three or more risk factors being categorized as moderate. In order to be considered an overall low risk, the majority of risk factors have to be categorized as low.

C.1 Contaminants

We evaluated a suite of chemicals based on the availability of acute toxicity data that indicated that freshwater mussels are sensitive to these chemicals. In the absence of toxicity data specific to Salamander mussel, we used toxicity studies from other freshwater mussel species as a surrogate, with the assumption that Salamander mussel would be either equally or more sensitive than the species tested given that acute toxicity tests included non-listed species and listed species deemed to be more sensitive. Primary contaminants were identified as the chemicals posing the greatest risk to freshwater mussels. The primary contaminants we evaluated were ammonia, chloride, nitrate, and copper (Table C.1). We developed a rule set to guide how we evaluated contaminant risk metrics. If any of the primary contaminant risk metrics were determined to be high for the population, then the overall contaminant risk for that population is considered at high risk. We applied the same rule for moderate risk: if any of the primary contaminant risk metrics are moderate, then the overall contaminant risk is considered moderate. If all of the primary contaminant risk metrics are low, then we used the secondary risk metrics to evaluate the risk for contaminants using an additive scoring approach. Secondary contaminants are chemicals that also have an effect on freshwater mussels, but do not present a high risk if simply one is present at acutely toxic thresholds. The secondary contaminants we evaluated were lead, potassium, sulfate, zinc, aluminum, and cadmium (Table C.1). Based on our rule set, any secondary risk metrics that were considered high received 3 points, moderate risk was assigned 2 points, and low risk was assigned 1 point. The six secondary risk metric scores were added together to get a total score for the population. A total score of 14–18 across all secondary contaminant risk metrics (lead, potassium, sulfate, zinc, aluminum, and cadmium) results in an overall contaminant risk of high; a score of 9–13 is an overall contaminant risk of moderate; and 6–8 is an overall contaminant risk of low. The cutoffs for the risk metrics for secondary contaminants whereas based on the majority of contaminants fall within that risk category.

Ambient water quality data from 2000–2020 available from the National Water Quality Monitoring Council's Water Quality Portal was used to evaluate the risk for each chemical. We established thresholds for high, moderate, and low risk for each chemical based on a review of the literature, input from contaminant experts within the Service, and toxicity studies on aquatic organisms (Table C.1). We qualitatively analyzed chemicals that pose a risk to freshwater mussels, but for which we do not have acute toxicity data that establish thresholds for quantitative evaluation (See Appendix B).

To compare concentrations of primary contaminants to water quality criteria, we filtered the Water Quality Portal to include only surface waters of reservoirs, streams, rivers, impoundments, and ditches to capture possible mussel habitat. We also geographically filtered the data to include only samples collected within 12-digit HUC watersheds immediately draining into extant rivers. We did not have enough water quality data to make meaningful assessments of individual reaches of streams and rivers currently occupied by mussels. In addition to concentrations of contaminants, we also queried the Water Quality Portal for hardness, pH, and temperature to adjust for watershed and site-specific water chemistry. For metals such as copper, toxicity is influenced by hardness, which impacts the bioavailability of metals in water. Ammonia toxicity is impacted by pH and temperature. There was not enough hardness data to calculate water quality criteria for metals specific to each data point, so we averaged the hardness for individual watersheds in the study area to calculate hardness-dependent water quality criteria at the 8-digit HUC scale. For approximately half (76 of 133 total) of the watersheds, there were pH and temperature data collected concurrently with ammonia samples to calculate site-specific water quality criteria for ammonia. For watersheds lacking concurrent ammonia, pH, and temperature data, we used the same approach as we used for metals and averaged the pH and temperature across each watershed. We provide brief rationales for the water quality criteria we used compare to ambient water quality data in Table C.2.

It should be noted that there is a degree of uncertainty associated with assessing contaminants risks to Salamander Mussels, and while efforts were made to provide assessments protective of endangered mussels considerable knowledge gaps remain on which to base evaluations. For example, the data represent a snapshot of water quality. As a result, we were not able to compare concentrations of contaminants to chronic water quality and we have an incomplete understanding of risk. Assessments were limited largely to acute lethal dose 50 concentrations (LD50 or the dose concentration in which 50% of mussels died during laboratory tests) due to limited datasets for chronic mussel sensitivity as well as limited ambient water quality measurements on which to compare effects. The Environmental Protection Agency guidelines indicate that freshwater aquatic life should be protected if the 24-hour average (acute) and four-day average concentrations (chronic) do not respectively exceed the acute and chronic criteria (Stephen et al. 1985). This would require an average of 4 consecutive ambient water quality samples, yet current data are limited to single sampling events corresponding to acute testing. LD50s were used in this assessment to allow comparisons to other risk assessments. However, the authors acknowledge there is concern that LD50s may not be protective of species of special concern, and further understanding of sublethal effects (that is, reproductive, behavioral) would be valuable to better understand the full impact of contaminants.

We also have relatively few data points for occupied reaches of extant rivers. Instead, we conducted our assessment at the watershed scale and conditions at that scale may not be representative of conditions where mussels are. Another impact of limited data is that we were not able to calculate specific water quality criteria for metals based on hardness and had to rely on averages of hardness across whole watersheds. This may result in water quality that are overly conservative, or too high. Additionally, water quality criteria for freshwater mussels were developed in controlled laboratory studies using common species. Threatened and endangered species may be more or less sensitive than laboratory test organisms. Sensitivity of the Salamander Mussel to the assessed chemicals was not available for comparisons to current water quality conditions as toxicology data for rare species is limited. Use of mussel data from common species is generally accepted. Wang and others (2017) have shown the Fatmucket (*Lampsilis siliquoidea*) to be a suitable surrogate for several species with Fatmucket sensitivities within 2–3-fold of that of other assessed species and chemicals. However, there are known notable exceptions that suggest this may not be appropriate for all species. Research by Gillis (2011) indicates the federally endangered Northern Riffleshell (*Epioblasma torulosa rangiana*) is 8x more sensitive to chloride than Fatmucket. Finally, our contaminants assessments are limited to surface water borne contaminants and do not account for additional pathways of exposure through food, and most notably sediments and pore water, where a considerable portion of both juvenile and adulthood may experience exposure to contaminants. Such data for environmental levels and associated mussel sensitivity are currently limited. Despite these limitations, we believe our analysis provides valuable insight into potential limiting factors and threats to freshwater mussels with respect to contaminants.

As stated in Section 3.1.3, suitable water temperature and dissolved oxygen levels are essential to Salamander Mussel population persistence. Anthropogenic activity coupled with climate change may result in shifts in mussel species natural range and water temperature to which they are exposed (Caissie 2006, entire). The shifts in temperature and dissolved oxygen beyond suitable ranges can negatively impact growth, reproduction, and survival. Thermal sensitivity can vary within a species depending on the life stage. Salamander Mussel appears to not be sensitive to thermal changes within propagation facilities (M. Bradley, personal communication, August 2021). Sand and muck occupied by Salamander Mussel has been observed to be cooler than the surrounding water in the Chippewa River, Wisconsin, indicating a possible relationship with habitat and groundwater ingress (M. Bradley, personal communication, August 2021). While we do not know the thermal lethal temperature for Salamander Mussel, there haves been extensive research on other species of mussel across life stages. This research indicates there is likely a thermal lethal limit for Salamander Mussel. Ganser et al. (2013, entire) found elevated water temperatures and elevated water temperature over time negatively impacted the survival, heart rate, and growth of juvenile freshwater mussels when exposed to the elevated water temperature and elevated water temperate over time. Survival of Fatmucket (*Lampsilis siliquoidea)* was affected at temperatures as low as 19.6°C. Ganser et al. (2015, entire) conducted a similar study using adult mussels representing four different species and found the higher the temperature the greater the oxygen consumption. Oxygen consumption is impacted by temperature thereby impacting metabolic activity that affects survival and growth. It has been suggested that mussel assemblages may already be living near their upper thermal limits (Ganser et al. 2013, p. 1168). Additionally, the ability to deal with alteration in DO levels may differ between species and even populations. Chen et al. (2001, entire) examined how oxygen consumption is impacted by low dissolved oxygen and temperature in nine different species that inhabit different habitats. Chen et al. (2001, entire) concluded that oxygen consumption is related to the normal amount of hypoxia (low oxygen) a species experiences in the natural environment and is improved when temperatures are lower (16.5° C). As such, we concluded with no research completed for the thermal sensitivity or DO limits of Salamander Mussel and with no closely related relatives, it would be difficult to quantify temperature and dissolved oxygen in a meaningful way to incorporate in the resiliency analysis of populations.

For nitrate, we used an extremely conservative acute value intended to be protective of the most sensitive aquatic life (Camargo et al. 2005, entire). However, Camargo et al. 2005 (entire) did not use freshwater mussel species in their study and new literature indicates that acute values for nitrate for mussels is orders of magnitude higher (524–905 mg/L NO3-N; Moore & Bringolf 2020, entire). As a result, we have overestimated the risk posed by nitrate concentrations to Salamander Mussels in this SSA. Unfortunately, we were not able to incorporate this new data in time, and we recommend using new data to inform acute values for nitrate in future SSAs of freshwater mussel species.

Table C.1. Indicator descriptions for the four primary contaminant risk metrics[1] and the six secondary risk metrics[2] used to evaluate the overall contaminant risk to Salamander Mussel populations. ([3] See EPA Aquatic Life Ambient Water Quality Criteria for Ammonia - Freshwater 2013, Tables 5b & 6 for pH and temperature normalized criteria.)

Current Condition Indicator - Contaminants	**Ammonia[1]**	**Chloride[1]**	**Nitrate[1]**	**Copper[1]**
Description of Indicator	Temperature and pH normalized[3] ammonia concentration in surface water within HUC12s draining into extant rivers from 2000-2021.	Chloride concentration in surface water within HUC12s draining into extant rivers from 2000-2021.	Nitrate concentration in surface water within HUC12s draining into extant rivers from 2000-2021.	Copper concentration in surface water within HUC12s draining into extant rivers from 2000-2021.
High Risk	Water quality concentration exceeds acute toxicity levels >2% of samples (2000 - present), generally exceeds acute toxicity levels multiple times/year.	Water quality concentration exceeds acute toxicity levels >2% of samples (2008-2018), generally exceeds acute toxicity levels multiple times/year. Based on Michigan R57 review, range for mussels is 244-2,246mg/L with hardness of 95-115 mg/L $CaCO_3$	Water quality concentration exceeds acute toxicity levels >2% of samples (2000 - present), generally exceeds acute toxicity levels multiple times/year. Maximum nitrate (mg/L) concentrations protective of mussels = 2.0. Nitrite (mg/L) 96 h LC50 for most sensitive mussel = 55.8.	Water quality concentration exceeds acute toxicity levels >2% of samples (2000 - present), generally exceeds acute toxicity levels multiple times/year. Lowest EPA genus mean acute value for mussel species = 11.33 ug/L at standard biotic ligand model chemistry.
Moderate Risk (2 points)	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2000 - present), generally exceeds acute toxicity levels <1X/year.[3]	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2008-2021), generally exceeds acute toxicity levels <1X/year. Based on Michigan R57 review, range for mussels is 244-2,246mg/L with hardness of 95-115mg/L $CaCO_3$.	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2000 - present), generally exceeds acute toxicity levels <1X/year. Maximum nitrate (mg/L) concentrations protective of mussels =2.0. Nitrite (mg/L) 96 h LC50 for most sensitive mussel =55.8.	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2000 - present), generally exceeds acute toxicity levels <1X/year. Lowest EPA genus mean acute value for mussel species =11.33 ug/L at standard biotic ligand model chemistry at standard biotic ligand model chemistry.
Low Risk (1 point)	Water quality concentrations at levels below acute toxicity to mussels (1990 - 2018).[3]	Water quality concentrations at levels below acute toxicity to mussels (2008-2021). Based on Michigan R57 review, range for mussels is 244-2,246mg/L with hardness of 95-115mg/L$CaCO_3$	Water quality concentrations at levels below acute toxicity to mussels (1990 - 2018). Maximum nitrate (mg/L) concentrations protective of mussels = 2.0. Nitrite (mg/L) 96 h LC50 for Most Sensitive Mussel = 55.8.	Water quality concentrations at levels below acute toxicity to mussels (1990 - 2018). Lowest EPA genus mean acute value for mussel species = 11.33 ug/L at standard biotic ligand model chemistry.

Table C.1. (continued) Indicator descriptions for the four primary contaminant risk metrics[1] and the six secondary risk metrics[2] used to evaluate the overall contaminant risk to Salamander Mussel populations.

Current Condition Indicator - Contaminants (cont.)	**Lead[2]**	**Potassium[2]**	**Sulfate[2]**
Description of Indicator	Hardness normalized lead concentrations in surface water within HUC12s draining into extant rivers from 2000-2021.	Potassium concentration in surface water within HUC12s draining into extant rivers from 2000-2021.	Sulfate concentration in surface water within HUC12s draining into extant rivers from 2000-2021.
High Risk (3 points)	Water quality concentration exceeds acute toxicity levels >2% of samples (2000 - present), generally exceeds acute toxicity levels multiple times/year. Acute toxicity hardness normalized (50 mg/L as $CaCO_3$) Lead (mg/L) EC50 range = 0.205 - >0.362. Used Michigan EGLE formula for hardness adjusted acute value to determine HUC8-specific water quality criteria.	Water quality concentration exceeds acute toxicity levels >2% of samples (2008-2018), generally exceeds acute toxicity levels multiple times/year. EC 50 of 31-48 mg/L	Water quality concentration exceeds acute toxicity levels >2% of samples (2008-2018), generally exceeds acute toxicity levels multiple times/year. Based on Michigan R57 review, range for mussels is 1378-2709mg/L with hardness of 103-106 mg/L $CaCO_3$
Moderate Risk (2 points)	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2000 - present), generally exceeds acute toxicity levels <1X/year. Acute toxicity hardness normalized (50 mg/L as $CaCO_3$) Lead (mg/L) EC50 range = 0.205 - >0.362. Used Michigan EGLE formula for hardness adjusted acute value to determine HUC8-specific water quality criteria.	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2008-2021), generally exceeds acute toxicity levels <1X/year. EC50 31-48 mg/L	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2008-2021), generally exceeds acute toxicity levels <1X/year. Based on Michigan R57 review, range for mussels is 1378-2709mg/L with hardness of 103-106 mg/L $CaCO_3$
Low Risk (1 point)	Water quality concentrations at levels below acute toxicity to mussels (1990 - 2018). Acute toxicity hardness normalized (50 mg/L as $CaCO_3$) Lead (mg/L) EC50 range = 0.205 - >0.362. Used Michigan EGLE formula for hardness adjusted acute value to determine HUC8-specific water quality criteria.	Water quality concentrations at levels below acute toxicity to mussels (2008-2021), EC50 31-48 mg/L.	Water quality concentrations at levels below acute toxicity to mussels (2008-2021). Based on Michigan R57 review, range for mussels is 1378-2709mg/L with hardness of 103-106 mg/L $CaCO_3$.

Table C.1. (continued) Indicator descriptions for the four primary contaminant risk metrics[1] and the six secondary risk metrics[2] used to evaluate the overall contaminant risk to Salamander Mussel populations.

Current Condition Indicator - Contaminants (cont.)	**Zinc[2]**	**Aluminum[2]**	**Cadmium[2]**
Description of Indicator	Hardness normalized zinc concentration in surface water within HUC12s draining into extant rivers from 2000-2021.	Aluminum concentration in surface water within HUC12s draining into extant rivers from 2000-2021.	Hardness normalized cadmium concentration in surface water within HUC12s draining into extant rivers from 2000-2021.
High Risk (3 points)	Water quality concentration exceeds acute toxicity levels >2% of samples (2000 - present), generally exceeds acute toxicity levels multiple times/year. Acute toxicity hardness normalized (50 mg/L as $CaCO_3$) zinc (mg/L) EC50 range = 0.120 - 0.295. Used Michigan EGLE formula for hardness adjusted acute value to determine HUC8-specific water quality criteria.	Water quality concentration exceeds acute toxicity levels >2% of samples (2008-2018), generally exceeds acute toxicity levels multiple times/year. EC 50 of 29.5mg/L with hardness of 100 mg/L $CaCO_3$	Water quality concentration exceeds acute toxicity levels >2% of samples (2000 - present), generally exceeds acute toxicity levels multiple times/year. Lowest EPA acute value for mussel species = 35.73 ug/L at 100mg/L $CaCO_3$.
Moderate Risk (2 points)	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2000 - present), generally exceeds acute toxicity levels <1X/year. Acute toxicity hardness normalized (50 mg/L as $CaCO_3$) zinc (mg/L) EC50 range = 0.120 - 0.295. Used Michigan EGLE formula for hardness adjusted acute value to determine HUC8-specific water quality criteria.	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2008-2021), generally exceeds acute toxicity levels <1X/year. EC50 29.5 mg/L with hardness of 100 mg/L $CaCO_3$	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2000 - present), generally exceeds acute toxicity levels <1X/year. Lowest EPA acute value for mussel species = 35.73 ug/L at 100mg/L $CaCO_3$.
Low Risk (1 point)	Water quality concentrations at levels below acute toxicity to mussels (1990 - 2018). Acute toxicity hardness normalized (50 mg/L as $CaCO_3$) zinc (mg/L) EC50 range = 0.120 - 0.295. Used Michigan EGLE formula for hardness adjusted acute value to determine HUC8-specific water quality criteria.	Water quality concentration at levels below acute toxicity to mussels (2008-2021), EC50 29.5 mg/L with hardness of 100 mg/L $CaCO_3$	Water quality concentrations at levels below acute toxicity to mussels (1990 - 2018). Lowest EPA acute value for mussel species = 35.73 ug/L at 100mg/L $CaCO_3$.

Table C.2. Rationale for water quality criteria used to compare with ambient water quality data. The Analyte column lists the contaminant we analyzed, the Acute Value column provides the acute value (i.e. water quality criteria) we compared with ambient water quality, the Basis column lists the mussel species used to derive the water quality criteria (if applicable), the Source column identifies the reference, and the Rationale provides our reasoning for choosing the specific acute value for each contaminant.

Analyte	**Acute Value**	**Basis**	**Source**	**Rationale**
Ammonia	Temperature and pH dependent	11 genera representing 16 species of freshwater mussels (including 4 federally listed species)	USEPA 2013	Mussels are the most sensitive taxa to ammonia. Sixteen species of mussels were used to derive the EPA criteria; since listed species are present in the dataset and mussels were among the most sensitive species used to derive the EPA acute value, we used the acute value (based on temperature and pH) for comparison to ambient water concentrations.
Aluminum	29,492 ug/L	Lampsilis siliquodea	Wang et al. 2016; Wang et al. 2018 as cited in EPA 2018	Mussels are not among the most sensitive species (top 4). The EPA formula for hardness-dependent aluminum criteria were based on Daphnia, which we felt was overly conservative for mussels. As a result, we used lowest acute value for mussels listed in EPA 2018.
Cadmium	35.73 ug/L	Lampsilis siliquoidea	Wang et al. 2010 as cited in EPA 2016	Mussels are not among the most sensitive species (top 4). The EPA formula for hardness-dependent cadmium criteria were based on fish (trout), which we felt was overly conservative for mussels. As a result, we used lowest acute value for mussels listed in EPA 2016.
Chloride	244 mg/L	9 genera representing 12 species of freshwater mussels (including 1 federally listed species)	Gillis 2011	Mussels are sensitive to chloride and as a result, we used the lowest acute value for freshwater mussels from Gillis 2011.

Table C.2. (continued) Rationale for water quality criteria used to compare with ambient water quality data. The Analyte column lists the contaminant we analyzed, the Acute Value column provides the acute value (i.e. water quality criteria) we compared with ambient water quality, the Basis column lists the mussel species used to derive the water quality criteria (if applicable), the Source column identifies the reference, and the Rationale provides our reasoning for choosing the specific acute value for each contaminant.

Analyte	Acute Value	Basis	Source	Rationale
Copper	11.33 ug/L	2 genera representing 2 species of freshwater mussels	EPA 2007	Mussels are not among the most sensitive species (top 4). Ambient water quality data were missing paired parameters in order to use the Biotic Ligand Model to calculate site-specific thresholds for aquatic organisms. Therefore, ambient copper concentrations were compared to the lowest genus mean acute value for mussel species in USEPA 2007 at standard biotic ligand model chemistry.
Lead	(EXP(0.9859*(LnH) +0.4892))*CFc^{D}	Lampsilis siliquoidea	Michigan EGLE 2020	We used Michigan EGLE formula using average hardness across each HUC8.
Nitrate	2 mg/L	No mussel species represented in this study	Camargo et al. 2005	EPA and Michigan EGLE acute values were not available so we used values protective of the most sensitive aquatic species in Camargo et al. 2005 (see Section C.1 for more details).
Potassium	31 mg/L	Amblema plicata	Wang et al. 2017	EPA and Michigan EGLE acute values were not available. We therefore used 31 mg/L, which was the lowest acute value of 5 species tested in Wang et al. 2017.
Sulfate	1,378 mg/L	4 genera representing 5 species of freshwater mussel	Wang et al. 2017	EPA acute value were not available so we used acute values derived for freshwater mussels in Wang et al. 2017.
Zinc	(EXP(0.8473*(LnH) +0.884))*0.978^{D}		Michigan EGLE 2020	EPA acute values for freshwater mussels were not available, so we used Michigan EGLE formula which accounts for hardness.

C.2 Landscape

To evaluate the effects of various land use activities and the resulting risk to each population, we assessed a suite of landscape metrics derived from the 2016 National Landcover Dataset (Jin et al. 2019, entire). Specific metrics were selected to determine overall landscape risk: percent imperviousness mean within the population, percent vegetative cover remaining within a 108-meter riparian buffer, percent urban, percent agriculture, and canopy cover within a 108-meter riparian buffer (Table C.3). Vegetative and canopy cover (%) are considered as they have the potential to reduce erosion through the following ways: (1) provides cover from direct erosive precipitation; (2) improves the porosity and capacity of the soil so greater infiltration may occur; and (3) slows runoff allowing sediment to drop out (USEPA 1990, p. IV-1; Abari et al. 2017, p. 375). Thus, preserving vegetative and canopy cover as well as revegetating areas can serve as an indicator of how well a site is protected from erosion or can act as a means of erosion control. Beyond sediment removal and erosion control, riparian forest cover protects water quality and buffers extreme water temperature through moderation of shade (Broadmeadow & Nisbet 2004, p. 286). Additionally, percent urban and agricultural land use can serve as indicators of the quantity of sediment that rivers and streams may experience. When developing urban settings, much of the disturbed soil becomes sediment in streams. This alteration of land from permeable to impervious land can result in increased flooding and washing of sediment / contaminants into waterways (Guy 1970, p. E7). Additionally, the development of agricultural land may increase the sediment load in areas due to livestock grazing near the water's edge (increases impaction and erosion of soil) and may increase stream temperature and further increase sediment load due to the clearing of tree / riparian vegetation to make room for more crops (decreases vegetative cover and allows for more runoff; Broadmeadow & Nisbet 2004, p. 286; Nolte et al. 2013, p. 296). To determine the current condition of our mussel population, we examined these four categories to analyze the impact sedimentation may have on the population performance (in other words growth, reproduction, and survival) of our species.

The NLCD depicts land cover across the United States through an overlay of 30-meter by 30-meter grids (in other words raster cells). Each grid represents a classification of land cover. We based our riparian buffers on the EnviroAtlas developed by EPA as it utilizes 108 meters for riparian buffers (www.epa.gov/enviroatlas). We used an additive scoring approach to determine the overall risk to a population posed by the landscape risk factor for these metrics. A population that is at overall low risk due to landscape changes has a score between 5–7; a population that is at overall moderate risk due to landscape changes has a score of 8–11; and a population that is at an overall high risk due to landscape changes has a score of 12–15. These metric scores were then used to categorize the overall risk to the population posed by landscape factors (Table 4.4).

Urban imperviousness is available at the same the same 30m by 30m resolution as NLCD 2016 data with each raster cell representing the percent imperviousness at that location, ranging from 0% impervious to 100% impervious (meaning that no water would be absorbed on that surface; Yang et al. 2003, entire). We used the Zonal Statistics as Table tool to calculate the average imperviousness value of all raster cells at the 8-digit HUC scale to calculate the average imperviousness of the landscape for each population.

We calculated the percent of vegetative cover within the 108m riparian zone of extant river for each population by first combining the National Hydrographic Dataset (NHD) Area shapefile with a 10m buffered version of the NHD Flow Line shapefile to get a more accurate delineation of the footprint of extant rivers (USGS 2019, entire). The NHD delineates waterbodies of the United States including rivers and streams. We buffered the resulting shapefile to create a 108m buffer around each extant river and used this shapefile as a mask to extract NLCD 2016 raster cells within the riparian zone (Jin et al. 2019, entire). Land cover types we considered to be "vegetative" include: 41 – Deciduous Forest; 42 – Evergreen Forest; 43 – Mixed Forest; 52 – Shrub/Scrub; 71 – Grassland/90 - Woody Wetlands; 95 – Emergent Herbaceous Wetlands. We calculated the total number of cells representing land (as opposed to water) as well as the number of cells representing vegetative cover to calculate the percent of all land cells that represent vegetative cover within the riparian zone of extant rivers for each population.

We calculated the amount of agricultural and developed land cover for populations by using NLCD 2016 and the Zonal Histogram tool to count the total number of raster cells within a watershed representing each land cover type (Jin et al. 2019, entire). We then tallied the total amount of raster cells representing land, agricultural land cover (81 – Pasture/Hay; 82 – Cultivated Crops), and developed land cover (21 – Developed, Open Space; 22 – Developed, Low Intensity; 23 – Developed, Medium Intensity; 24 – Developed High Intensity) to calculate the percent cover of each.

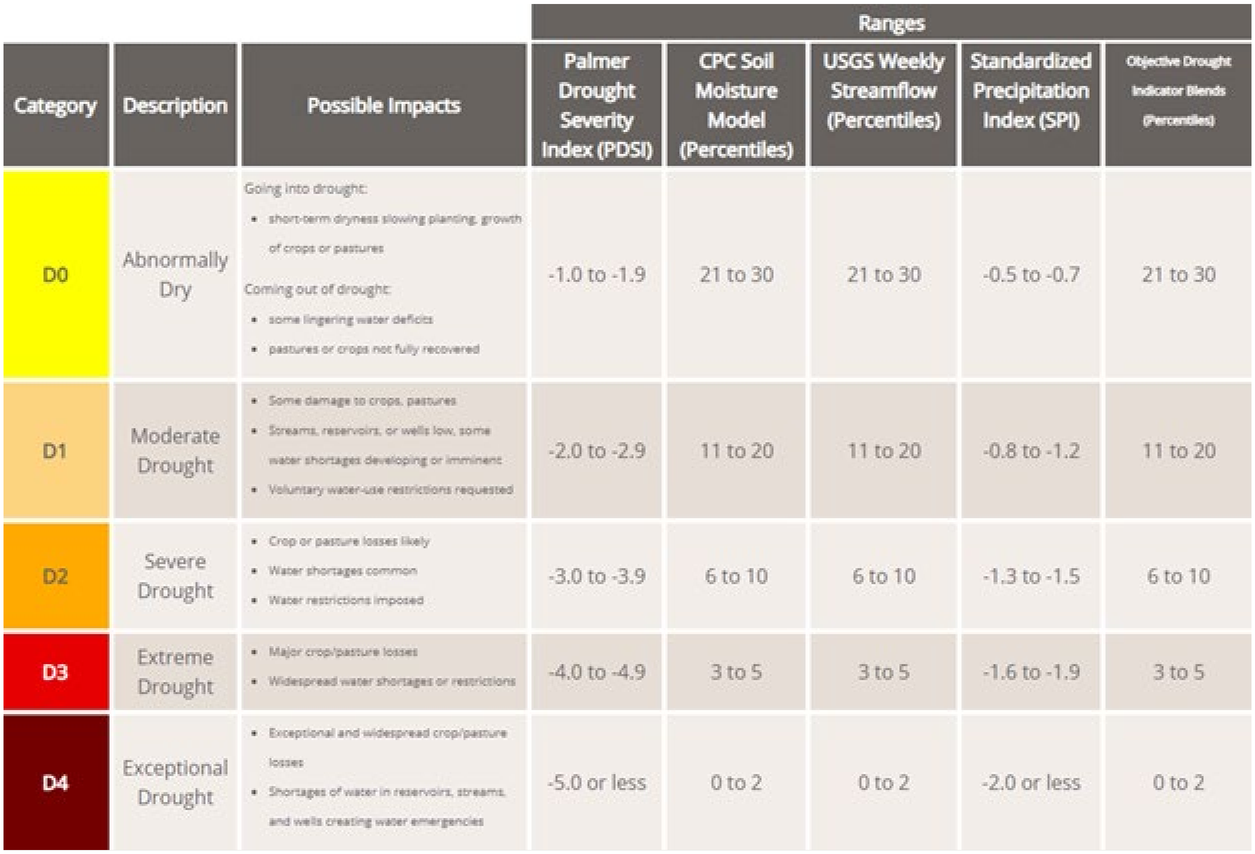
To measure the amount of canopy cover over extant rivers, we downloaded the NLCD 2016 USFS Tree Canopy Cover raster dataset (Coulston 2012, entire). The value of each raster cell in the Tree Canopy Cover dataset represents the percent canopy cover at that location. We then used the same merged NHD Flow Line shapefile and NHD Area shapefile we created to calculate vegetative cover in the 108m riparian zone as the mask for the Extract by Mask tool and extracted all of the Tree Canopy Cover raster cells coincident with extant rivers. Lastly, we used the Zonal Statistics as Table tool to calculate the average value of all Tree Canopy Cover raster cells to find the average tree canopy cover over the footprint of extant rivers for each population.

Table C.3. Indicator descriptions for the five landscape risk metrics used to evaluate the overall landscape risk to Salamander Mussel populations.

Current Condition Indicator - Landscape	**% Imperviousness, Mean in WS (2016)**	**% Vegetative Cover remaining in 108m riparian buffer**	**% Urban in WS (2016)**	**% Ag in WS (2016)**	**% Canopy Cover remaining in 108m riparian buffer**
Description of Indicator	Percent of the HUC8 with developed impervious cover. Calculated as the mean value of percent in the HUC8.	Calculated as the forest area in the riparian zone divided by the total area of the riparian zone.	Percent of the HUC8 classified as urban cover. Calculated as urban area divided by HUC8 area.	Percent of the HUC8 classified as agriculture. Calculated as agriculture area in the HUC8 divided by HUC8 area.	The mean value of NLCD canopy cover in the 108m riparian buffer of occupied rivers.
High Risk (3 points)	>15	<50	>10	>40	<50
Moderate Risk (2 points)	10–15	50–75	5–10	25–40	70–50
Low Risk (1 point)	<10	>75	<5	<25	>70

C.3 Hydrological Regime

To assess the condition of the hydrologic regime, we used the U.S. Drought Monitoring Data (USDM) to evaluate drought risk. The USDM classifies drought into five categories: D0, D1, D2, D3, and D4 (Figure C.1.). Per our assessment of risk to Salamander Mussel (see 3.1.4), categories with USGS weekly streamflow below 5% were included in our analysis (in other words Extreme Drought [D3] and Exceptional Drought [D4]). To evaluate the frequency of drought that each population experienced, we examined weekly percent drought data from 4 January 2000 to 4 January 2021 (Accessed on May 28, 2021). The specific metrics for high, moderate, and low risk are outlined in Table C.4.



Category	Description	Possible Impacts	Ranges Palmer Drought Severity Index (PDSI)	CPC Soil Moisture Model (Percentiles)	USGS Weekly Streamflow (Percentiles)	Standardized Precipitation Index (SPI)	Objective Drought Indicator Blends (Percentiles)
D0	Abnormally Dry	Going into drought: • short-term dryness slowing planting, growth of crops or pastures Coming out of drought: • some lingering water deficits • pastures or crops not fully recovered	-1.0 to -1.9	21 to 30	21 to 30	-0.5 to -0.7	21 to 30
D1	Moderate Drought	• Some damage to crops, pastures • Streams, reservoirs, or wells low, some water shortages developing or imminent • Voluntary water-use restrictions requested	-2.0 to -2.9	11 to 20	11 to 20	-0.8 to -1.2	11 to 20
D2	Severe Drought	• Crop or pasture losses likely • Water shortages common • Water restrictions imposed	-3.0 to -3.9	6 to 10	6 to 10	-1.3 to -1.5	6 to 10
D3	Extreme Drought	• Major crop/pasture losses • Widespread water shortages or restrictions	-4.0 to -4.9	3 to 5	3 to 5	-1.6 to -1.9	3 to 5
D4	Exceptional Drought	• Exceptional and widespread crop/pasture losses • Shortages of water in reservoirs, streams, and wells creating water emergencies	-5.0 or less	0 to 2	0 to 2	-2.0 or less	0 to 2

Figure C.1. U.S. Drought Monitor severity classification system.

Table C.4. Indicator descriptions for the drought risk metric used to evaluate the overall hydrological regime risk to Salamander Mussel populations.

Current Condition Indicator – Hydrological Regime	**Drought**
Description of Indicator	Consecutive weeks of extreme low flow and multi-year droughts classified as severe/exceptional.
High Risk	Flows <5th percentile for greater than 6 consecutive weeks annually; extreme and exceptional droughts occur for 3 or more consecutive years.
Moderate Risk	(1) Flows <5th percentile for greater than 4 consecutive weeks but less than 6 consecutive weeks annually; extreme and exceptional droughts occur less than 3 consecutive years. OR (2) Flows <5th percentile for greater than 6 consecutive weeks annually; extreme and exceptional droughts occur for less than 3 consecutive years.
Low Risk	Flows < 5th percentile for less than 4 consecutive weeks annually

C.4 Connectivity

We evaluated the number of dams and the density of unpaved road stream crossings to evaluate connectivity within each population (Table C.5). The number of dams within a population was evaluated using the 2012 National Anthropogenic Barrier Dataset (Ostroff et al. 2013, entire). To calculate the number of barriers for each population, we analyzed the 2012 National Anthropogenic Barrier Dataset (Ostroff et al. 2013, entire). We then used the Summarize Within Tool in ArcGIS Pro to count the number of dams within each population.

Unpaved road stream crossings impact ecosystems including, through degradation of water quality, changes in flow, and obstruction to host passage, physically limiting access to certain stretches of river or are degraded to a point that lack of habitat essentially causes a barrier. Density of unpaved stream crossings per kilometer of stream was evaluated using spatial datasets from state transportation agencies. To calculate the density of unpaved stream crossings, we used spatial datasets from state transportation agencies. Most states had comprehensive road data while others only contained state-maintained roads. We filtered each state's data using a definition query to include only unpaved roads and merged the data to create a single unpaved road layer. Next, we filtered the NHD Flow Line shapefile using a definition query to retain only features with Geographic Names Information System (GNIS) names and classified as streams and rivers, artificial paths, and canals and ditches (FTypes 460, 558, and 336, respectively). We used the calculate geometry function to calculate the total kilometers NHD features in each watershed. Then, we used the intersect tool to identify all crossings of unpaved roads and NHD features, used the Summarize Within Tool to count the number of crossings in each watershed, and divided the number of crossings by the kilometers of named stream in the watershed.

To determine the overall risk posed by loss of connectivity for each population, we decided that if one of the two metrics was high risk and the other moderate risk, then the overall risk condition for the population would be high. If one was moderate risk and the other low risk, then the overall risk condition for the population would be moderate. If one metric was low and one metric high, then the overall risk condition would be moderate.

Table C.5. Indicator descriptions for the risk metrics used to evaluate the overall connectivity risk to Salamander Mussel populations.

Current Condition Indicator - Connectivity	**Count Dams**	**Unpaved road stream crossing density**
Description of Indicator	The number of dams in the population unit.	The number of unpaved road crossings in the HUC8 divided by area of management unit stream length in the population unit.
High Risk	>30	> 0.40
Moderate Risk	10–30	0.21–0.40
Low Risk	<10	0–0.20

C.5 Invasive Species

We assessed the impact of invasive species with the use of Optimized Hotspot Analysis in ArcGIS Pro 2.8.0. We downloaded invasive species occurrence data (both incidental/opportunistic observations and conducted presence/absence surveys) for the Zebra Mussel, *Corbicula*, Black Carp, Rusty Crayfish, Spiny Waterflea, Brown Trout, Quagga Mussel, Common Carp, and Hydrilla for our occupied HUC12 watersheds from the USGS nonindigenous aquatic species database.

Instead of running individual hotspot analyses for each species, we chose to group and categorize species by their common impact to Salamander Mussel where they occur (Table C.6). We prepared the downloaded data by first merging invasive species into categories (Table C.6), then aggregating the positive (the species is present) occurrence records by occupied HUC12 watersheds for each of our occupied populations. Afterwards, we tested each aggregated invasive species category for significant clustering using Spatial Autocorrelation (Table C.6). Each invasive species category had a low, but positive Index, a high positive ZScore and a near or at 0 PValue, indicating that any hot and/or cold spots created by the analysis tests are highly statistically significant. The results of these optimized hotspot analyses will indicate higher than normal numbers of significant clustering via hot spots (confidence level/Gi Bin of 1 to 3) and lower than normal numbers of significant clustering via cold spots (-1 to -3). Confidence levels of 0 are insignificant (Table C.7). Risk levels were based on the presences of hotspots in the analysis (Table C.8, See Section 3.3 for additional information).

Table C.6. Invasive species grouped and categorized by impacts on mussel species.

Category	Impact	Species
Direct competition	Competition pressure for resources; often can outcompete and displace	Zebra Mussel; Quagga Mussel; *Corbicula*; Spiny Waterflea
Reduction of reproductive potential	Displaces host species via competition and predation (including eggs)	Common Carp; Brown Trout; Rusty Crayfish
Disturbance to ecosystems and/or reduction of habitat quality	Feeding habits are known to alter habitat by increasing siltation, uprooting/displacing native vegetation/algae-grazing snails, altering benthic substrates, etc.	Zebra Mussel; Quagga Mussel; *Corbicula*; Common Carp; Brown Trout; Black Carp; Rusty Crayfish; Hydrilla
Direct harm/predation	Includes smothering and predation	Zebra Mussel; Black Carp; Common Carp

Table C.7. Results of the Global Moran's I (Spatial Autocorrelation) for each invasive species category.

Category	Index	ZScore	PValue
Direct competition	0.152131	11.26856	0
Reduction of reproductive potential	0.068038	4.768954	0.000002
Disturbance to ecosystems and/or reduction of habitat quality	0.078681	8.150607	0
Direct harm/predation	0.053724	3.448528	0.000564

Table C.8. Indicator descriptions for the risk metric used to evaluate the overall invasive species risk to Salamander Mussel populations.

Current Condition Indicator - Invasive Species	**Invasive Species**
Description of Indicator	Optimized Hotspot Analysis using invasive species occurrence data for occupied HUC and categorized by common impacts to mussel species.
High Risk	N/A (invasive species do not present a high risk to Salamander mussel)
Moderate Risk	Present in abundance
Low Risk	Present in moderation or absent

C.6 Host Species Vulnerability

We did not have enough information to do either a qualitative or quantitative assessment of the health and abundance of mudpuppy populations within the range of Salamander Mussel. However, we conducted a qualitative assessment of disease and die-off events, effects of contaminants, and habitat loss and degradation.

We have sufficient information to evaluate the effects of lampricide treatments and State regulation regarding collection quantitatively. For the Great Lakes Representation Unit, we evaluated the risk posed by lampricide control because mudpuppy is particularly sensitive to lampricides. We used treatment history data provided by the Services' Sea Lamprey Control Program to determine if treatment occurred within that population and if so, how frequently (Table C.9). Given we do not have information regarding collection numbers and frequency of unintentional catch for each state, we reviewed State laws and regulations for mudpuppy collection or possession (Table C.9). For these metrics we used the highest risk for either metric to determine the overall current risk to the population. For example, if the lampricide metric was high, but the collection and possession limit was low, the overall risk condition for that population would be high.

Table C.9. Indicator descriptions for the risk metrics used to evaluate the risk to host species and the overall risk to Salamander Mussel populations.

Current Condition Indicator - Host Species Vulnerability	**Lampricide treatment**	**Collection, bag limits & Fishing unintentional catch**
Description of Indicator	The frequency that lampricide treatment is conducted within the population.	Evaluated using the presence or absence of bag limits and regulations of each state.
High Risk	Stream treatments routinely between 1 and 5 years	No possession limits for either recreational and/or commercial purposes.
Moderate Risk	Stream treatments no more than every 5–10 years	Commercial and/or recreational collection permitted, but possession limits in place.
Low Risk	No known lampricide activities	Prohibition on all recreational and commercial collection.

C.7 Catastrophic Events

Coal mining - To evaluate the risk posed by coal mining we analyzed whether coal mining activities were present within the HUC8. If there were no known coal mining activities within the HUC8, coal mining was considered a low catastrophic risk to that population and if there were known coal mining activities within the HUC8, the population was considered at high risk of a catastrophic event.

Oil and gas - To evaluate the risk posed by oil and gas exploration and extraction we analyzed the number of oil and gas wells present within a HUC8. If there were no known oil and gas exploration/ extraction activities within the HUC8 oil and gas activities were considered a low catastrophic risk to that population and if there were known oil and gas activities within the HUC8, the population was considered at high risk of a catastrophic event.

APPENDIX D. CURRENT CONDITION

D.1 Great Lakes Basin Representation Unit

The Duck-Pensaukee population in Wisconsin is extant but considered functionally extirpated. This population is experiencing high risk due to the state's regulations not prohibiting or limiting collection or capture of mudpuppy. Contaminant data were available only for ammonia and nitrate. Data were not available for the other eight metrics. Therefore, the low condition for water quality is based on the available contaminants data. If the risk threshold continues, this population is expected to be extirpated by 2040.

The St. Clair population in Michigan/Canada is considered functionally extirpated. The one survey in 2003 resulted in one live individual found with 10 people surveying for two hours. The thresholds for chloride were exceeded for this population putting it at high risk. Additionally, this population experiences high risk due to high agricultural landcover within the population and low tree canopy cover within the riparian buffer, along with moderate risks associated with urbanization, impervious surfaces, and vegetative cover in the riparian buffer. If the high risk continues, this population is expected to be extirpated by 2040.

The Clinton population in Michigan is considered functionally extirpated. A survey in 2019 produced one live individual of Salamander Mussel. Contaminant levels for chloride, nitrate, and copper exceed the thresholds for high risk. Landscape is a contributing risk as well. The Clinton population exceeds high risk thresholds for urbanization, impervious surface, vegetative cover, and tree canopy in the riparian buffer. If the contaminant and landscape high risks continue, this population is expected to be extirpated by 2040.

The St. Joseph population spans portions of Indiana, Michigan, and Ohio. There are 7 incidental reports of Salamander Mussel for this population leading to an unknown demographic status. This population experiences high risk from contaminant and landscape risks. The threshold for chloride exceeds high risk within this population for Salamander Mussel. Additionally, the high risk thresholds were surpassed for urbanization, agriculture, and canopy cover within the riparian corridor. Given this population experiences high risk, the best demographic population condition it is expected to be in 2040 is low.

One incidental record from 2009 occurs in the Blanchard population within Ohio giving the population an unknown demographic status. Nitrate and copper risks are high for this population. Additionally, the Blanchard population experiences high risk from high density of agriculture on the landscape and lack of canopy and vegetation in the riparian corridor. Given this population experiences high risk, the best demographic population condition it is expected to be in 2040 is low.

The Chautauqua-Conneaut population within portions of New York, Ohio, and Pennsylvania experiences high risk from copper and routine lampricide treatments. Additionally, state regulations limit mudpuppy collection. The Chautauqua-Conneaut population has three incidental surveys completed within 2008, all within Conneaut Creek in Ohio. Given this population experiences high risk, the best demographic population condition it is expected to be in 2040 is low.

The Niagara population is in New York and Canada. This population is represented by one fresh shell found incidentally in 2018 at one location in Tonawanda Creek, New York. The assessment completed for the Canada populations did not include the Niagara population as the incidental fresh shell was found in New York. Because of differences in data availability from the U.S. and Canada, it was difficult to evaluate the risk metrics in a comparable way for the current condition analysis.

The Wolf population in Wisconsin is presumed extant. There were numerous incidental surveys prior to 2000. One incidental survey occurred in the Embarrass River and 16 incidental records were recorded along approximately 25 miles of the Wolf River prior to 2000. The demographic population condition is unknown. The high risks within this population include threats to mudpuppy from lack of state collection or capture regulations and nitrate levels over thresholds. There has not been evidence of Salamander Mussel in the last 20 years, and given the high risk from threats to mudpuppy and nitrates, we expect the demographic population condition would at most be in low by 2040.

The Grand population in Ohio is presumed extant. One incidental record is from 1966; the remaining 5 incidental surveys occurred between 1995–1998. The Grand population experiences high risk from nitrate levels and routine lampricide treatment. There has not been evidence of Salamander Mussel in the last 20 years, and given the high risk from threats to mudpuppy, we expect the demographic population condition would at most be in low by 2040.

*Table D.1. Current condition risk factor analysis for Salamander Mussel populations within the Great Lakes basin representation unit. (*data was limited for metric analysis; CN = Canada, E = Extant, PE = Presumed Extant)*

State	HUC 8 Name	HUC 8 Code	Status	Contaminant	Landscape	Hydrological Regime	Connectivity	Invasive Species	Host Species Vulnerability	Overall Current Risk
WI	Duck-Pensaukee	04030103	E	Low*	Moderate	Low	Low	Moderate	High	High
WI	Wolf	04030202	PE	High	Moderate	Moderate	Moderate	Moderate	High	High
CN, MI	St. Clair	04090001	E	High	High	Low	Low	Moderate	Moderate	High
CN, MI	Lake St. Clair	04090002	E							Unknown
MI	Clinton	04090003	E	High	High	Low	Moderate	Moderate	Moderate	High
IN, MI, OH	St. Joseph	04100003	E	High	High	Moderate	Moderate	Moderate	Moderate	High
OH	Blanchard	04100008	E	High	High	Low	Low	Moderate	Moderate	High
OH	Grand	04110004	PE	High	Moderate	Low	Moderate	Low	High	High
NY, OH, PA	Chautauqua-Conneaut	04120101	E	High	Moderate	Low	Moderate	Moderate	High	High
CN, NY	Niagara	04120104/ 04270101	E							Unknown
CN	Lower Grand	04250005	E							Unknown

Table D.2. Current condition summary of demographic, risk factor, catastrophic event analysis for Salamander Mussel populations within the Great Lakes basin representation unit. (CN = Canada; E=Extant; PE = Presumed Extant)

State	**HUC8 Name**	**HUC8 Code**	**Status**	**# of Targeted/ semi-targeted surveys**	**# of incidental surveys**	**Demographic Condition**	**Overall Current Risk**	**Risk of Catastrophic event - Coal**	**Risk of Catastrophic event - Oil and Gas**
WI	Duck-Pensaukee	04030103	E	2	2	Functionally Extirpated	High	Low	High
WI	Wolf	04030202	PE	0	16	Unknown	High	Low	High
CN, MI	St. Clair	04090001	E	1	5	Functionally Extirpated	High	Low	High
CN, MI	Lake St. Clair	04090002	E	15	0	Low	Unknown	Low	High
MI	Clinton	04090003	E	1	0	Functionally Extirpated	High	Low	High
IN, MI, OH	St. Joseph	04100003	E	0	7	Unknown	High	Low	High
OH	Blanchard	04100008	E	0	1	Unknown	High	Low	High
OH	Grand	04110004	PE	0	6	Unknown	High	Low	High
NY, OH, PA	Chautauqua-Conneaut	04120101	E	0	3	Unknown	High	Low	High
CN, NY	Niagara	04120104 / 04270101	E	0	1	Unknown	Unknown		
CN	Lower Grand	04250005	E	1	0	Functionally Extirpated	Unknown		

D.2 Ohio Basin Representation Unit

The French population within portions of New York and Pennsylvania is considered extant. One incidental record is from 2015, with 4 live individuals found at one location. The demographic population condition is unknown. All the risk factors were moderate except for hydrological regime (drought) and invasive species posing a low risk. Given this population experiences moderate risk, the best condition this population is expected to be in 2040 is moderate.

The Middle Allegheny-Redbank population in Pennsylvania is considered extant, but in low demographic population condition based on one semi-targeted survey completed in 2013 that found 36 live individuals and 6 gravid females found during 5.5 person hours of search time. All the risk factors were moderate with the exception of hydrological regime (drought) and host species vulnerability, posing a low risk. If the moderate risk continues, this population is expected to be functionally extirpated by 2040.

The Upper Ohio-Wheeling population in portions of Ohio, Pennsylvania, and West Virginia, is considered functionally extirpated. There has been a significant amount of survey effort with 42 total surveys completed in Fish Creek between 2010 and 2016. Contaminants pose a high risk, with copper and sulfate exceeding the thresholds for high risk. If the high risk continues, this population is expected to be extirpated by 2040.

The Little Muskingum-Middle Island population in portions of Ohio and West Virginia is considered extant and in moderate demographic population condition, based on a total of 10 semi-targeted and targeted surveys conducted from 2009 through 2017 that found live individuals. Mudpuppies of various age classes were also present in 2017. The population has high species diversity across the present mussel assemblage with a total of 27 species being found during surveys and 33 different species found in the mainstem river system. All of the risk factors are moderate with the exceptions of hydrological regime (drought) and host species vulnerability, both of which were low. Copper and nitrate are both considered primary contaminant risk metrics and are at moderate thresholds. Two secondary contaminant risk metrics (potassium and lead) exceed the high threshold levels. If the moderate risk continues, the demographic population condition is expected to be low by 2040.

The Upper Ohio-Shade population in portions of Ohio and West Virginia is considered extant. There is one incidental record of a weathered dead shell from 2008. Other surveys found low species diversity of 7 different species documented. The demographic population condition is unknown, and this population is at high risk due to contaminants (chloride, nitrate, and potassium). Given this population experiences high risk, the best demographic population condition that is expected is low by 2040.

The Little Kanawha population in West Virginia is extant. The population is in low demographic population condition based on semi-targeted and targeted survey data collected since 2000, with very few live individuals found, though there has been a significant amount of survey effort for the broader mussel community within this watershed. The risk posed by all of the risk factors to this population is moderate with the exception of low risk for hydrological regime (drought) and host species vulnerability. If the moderate risk continues, the demographic population condition is expected to be functionally extirpated by 2040.

The Tuscarawas population in Ohio is presumed extant. The demographic population condition is unknown based on only one incidental survey. This population is at high risk from contaminants, specifically chloride, copper, and cadmium, and landscape factors, including impervious surfaces, urbanization, and lack of vegetation and canopy cover in the riparian corridor. There has not been evidence of Salamander Mussel in the last 20 years. Given the high risk from multiple contaminants and landscape factors, we expect the demographic population condition would at most be in low by 2040.

The Lower Kanawha population in West Virginia is extant. The demographic population condition is unknown. There is an incidental record of one weathered dead shell from 1982. A survey in July 2020 that was approximately 150m in length found a single weathered valve, which was estimated to be dead 1–2 years. Otherwise, there is only one other incidental record of a live individual from 1913. This population is at moderate risk for all the risk factors with the exception of hydrological regime (drought) and host species vulnerability, both of which are low risk.

The Upper Scioto population in Ohio is extant. Historically, the Salamander Mussel occurred in 5 waterways, including Big Darby Creek, Big Walnut Creek, Alum Creek, and Olentangy River, but in the last two decades, it has only been found in Little Darby Creek as a single weathered shell during an incidental survey in 2000. During this survey, they noted mortality from the 1999 drought was high in the series of riffles surveyed for approximately a quarter mile. Within the Upper Scioto population, four incidental surveys in the 1960s and 1970s, as well as a survey in 1996 documented the presence of Salamander Mussel; however, no data on the number or condition of individuals are available for these records. The demographic population condition is unknown. This population is at high risk from contaminants, specifically nitrate, copper, zinc, and cadmium, and landscape factors, including urbanization, agriculture, and lack of canopy and vegetative cover in the riparian zone.

The Lower Scioto population in Kentucky and Ohio is presumed extant. There are no data available on the number or condition (for example, live, fresh dead, weathered dead) of individuals observed. Therefore, the demographic population condition is unknown. This population is at a high risk from copper, nitrate, cadmium, aluminum, and zinc. There has not been evidence of Salamander Mussel in the last 20 years, and given the high risk from multiple contaminants, we expect the demographic population condition would at most be low by 2040.

The Little Scioto-Tygarts populations in portions of Kentucky, Ohio, and West Virginia is presumed extant. The demographic population condition is unknown based on a small number of incidental records from the 1970s and 1980 primarily in Kentucky. The population is at a high risk from nitrate and copper. There has not been evidence of Salamander Mussel in the last 20 years, and given the high risk from multiple contaminants, we expect this population would at most be in low demographic population condition by 2040.

The Little Sandy population in Kentucky is presumed extant. The demographic population condition is unknown based on a small number of incidental records from the 1970s and 1980s in which several of the individuals documented were weathered dead. Contaminants pose a high risk to this population, with nitrate exceeding the threshold for high risk. There has not been evidence of Salamander Mussel in the last 20 years, and given the high risk contaminants, we expect the demographic population condition would at most be low by 2040.

The Ohio Brush-White Oak population in portions of Kentucky and Ohio is extant. The demographic population condition is unknown with the most recent incidental record from 2007 in which two live individuals were found. Contaminants pose a high risk to this population, with nitrate exceeding the threshold for high risk. Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Middle Ohio-Laughery population in portions of Indiana, Kentucky, and Ohio is extant. There are two incidental records from Indiana, one of which is from 2010, and one incidental record from the Ohio River in Kentucky that is pre-1980. Based on this, the demographic population condition is unknown. This population is at high risk from nitrate. Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Licking population in Kentucky is extant. In 2013 a total of 125 live individuals have been found, with 61 individuals found under one boulder during a targeted survey in 2013. The demographic population condition is low; however, Kentucky considers this population to be a stronghold for the State. Individuals from this population were used as broodstock for a conservation propagation program in Kentucky. Mudpuppies in this population are considered to be stable. This population is at a high risk from nitrate. If the high risk continues, the demographic population condition is expected to be functionally extirpated by 2040.

The South Fork Licking population in Kentucky is considered extant. The population condition is unknown. This population is at high risk from nitrate and landscape factors, including agricultural lack of canopy and vegetative cover in the riparian zone, and impervious surfaces. Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Middle Fork Kentucky population in Kentucky is presumed extant. There is one incidental record of a fresh dead individual in 1996. The population condition is unknown. This population is at high risk from nitrate. There has not been evidence of Salamander Mussel in the last 20 years, and given the high risk from contaminants, we expect the demographic population condition would at most be low by 2040.

The Upper Kentucky population in Kentucky is presumed extant. There are 4 incidental records, with the most recent being one fresh dead individual in 1996. The demographic population condition is unknown based on 4 incidental records. Contaminants pose a high risk to this population with nitrate exceeding the threshold for high risk. There has not been evidence of Salamander Mussel in the last 20 years, and given the high risk from contaminants, we expect the demographic population condition would at most be low by 2040.

The Lower Kentucky population in Kentucky is extant. There are 14 incidental records for this population. The most recent record is from 2018 in which one fresh dead individual was found in Drennon Creek. One weathered dead individual was found in Eagle Creek in 2007. All of the other incidental records are from the 1980s and 1990s. Based on this information, the demographic population condition is unknown. This population is at moderate risk for all of the risk factors. Given this population experiences moderate risk, the best demographic population condition expected is moderate by 2040.

The Upper Green population in Kentucky is presumed extant. The demographic population condition is unknown based on 5 incidental survey records, 3 of these incidental surveys have a note that when those sites were visited later to conduct surveys for other mussels no evidence of Salamander Mussel was detected. This population is considered at high risk posed from nitrate. There has not been evidence of Salamander Mussel in the last 20 years, and given the high risk from contaminants, we expect this population would at most be in low demographic population condition by 2040.

The Rough population in Kentucky is presumed extant. The only record for this populations is of a weather dead valve incidentally found in 1993. Therefore, the demographic population condition is unknown. Contaminants pose a high risk to this population with nitrate exceeding the threshold for high risk. There has not been evidence of Salamander Mussel in the last 20 years, and given the high risk from contaminants, we expect the demographic population condition would at most be low by 2040.

The Eel population (HUC8 05120104) in Indiana is extant. There are 3 incidental records, the most recent observation is of a weathered dead individual in 2013. The other two records are of a weathered dead individual from 1999 and one fresh dead individual from 1997. Based on this, the demographic population condition is unknown. This population is at high risk from nitrate and copper and landscape factors, including agriculture and lack of canopy and vegetative cover in the riparian zone. Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Tippecanoe population in Indiana is extant. The population condition is unknown. Ten incidental records have been documented within this population. Most recently, weathered dead individuals were found in 2016 and 2010. The other incidental records are from the 1990s and are represented mainly by weathered dead shells. This population is at high risk from nitrate and potassium. Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Wildcat populations in Indiana is extant. The population condition is unknown based on one incidental record of a weathered dead individual found in 2009. Contaminants, specifically nitrate, and landscape factors, including agriculture, lack of canopy in the riparian zone, and urbanization, pose a high risk to this population. Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Middle Wabash-Little Vermillion population in portions of Illinois and Indiana is extant. The demographic population condition is unknown. Incidental records are all of weathered dead individuals in the Indiana portion of the population with the most recent record from 2013. Contaminants, specifically nitrate, potassium, and aluminum, pose a high risk to this population. Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Vermillion population in portions of Illinois and Indiana is extant. The demographic population condition is unknown. This population is at moderate risk from all of the risk factors. Though, within the individual risk factor metrics one metric exceeded the threshold for high risk which was potassium. Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Sugar population in Indiana is extant. The demographic population condition is unknown based on three incidental records, the most recent of which is a weathered dead individual from 2009. This population is at moderate risk for all risk factors. Given this population experiences moderate risk, the best demographic population condition expected is moderate by 2040.

The Eel population (HUC8 05120203) in Indiana is presumed extant. The demographic population condition is unknown and based on a single incidental record of a fresh dead individual from 1999. This population is at high risk for landscape factors, including agriculture and lack of canopy and vegetative cover within the riparian area. There has not been evidence of Salamander Mussel in the last 20 years, and given the high risk from contaminants, we expect the demographic population condition would at most be in low by 2040.

The Muscatatuck population in Indiana is functionally extirpated. While this is one of the known reproducing populations (B. Fisher, personal communication, 2020), no data are available on the condition or number of individuals from two targeted surveys since 2000. Mudpuppies are thought to be in decline based on a significant level of survey effort, which would likely have also revealed Salamander Mussel if present. This population is at a high risk posed by contaminants with nitrate exceeding the threshold for high risk. If the high risk continues, the demographic population condition is expected to be extirpated by 2040.

The Lower East Fork White population in Indiana is extant. The demographic population condition is unknown, but there are 20 incidental records for this population, with some representing fresh dead shells that may indicate a reproducing population (B. Fisher personal communication 2020). The risk posed by contaminants for this population is high with nitrate exceeding the threshold for high risk. Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Collins population in Tennessee is extant. The demographic population condition is unknown based on two incidental records in 2004 and 2008, though no number or condition of individuals is available. The risk posed by contaminants for this population is high based on nitrate exceeding the threshold for high risk. This population is also at high risk for host species vulnerability based on a lack of possession or collection limits for mudpuppy. Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Harpeth population in Tennessee is extant. The demographic population condition is unknown based on two incidental records. The population is at high risk based on several factors including contaminants, (nitrate and cadmium), connectivity, (unpaved road crossings and dams), and host species vulnerability (no possession or collection limits for mudpuppy). Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Salt population in Kentucky is extant. The demographic population condition is unknown, with the most recent incidental record from 2005. This population is at high risk due to contaminants, with nitrate exceeding the threshold for high risk. This population is also at high risk based on landscape factors including agriculture and urbanization, as well as lack of canopy cover within the riparian area. Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Rolling Fork population in Kentucky is extant. There are three incidental records for this population. A record from 2004 was considered to be a 1- to 2-year-old individual, perhaps indicating reproduction. The other most recent record is of a fresh dead individual from 2008. Based in this information, the demographic population condition is unknown. This population is at high risk from nitrate. Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Lower Ohio-Bay population in portions of Kentucky and Illinois is presumed extant. The demographic population condition is unknown, with only one incidental record from before 1985 without condition information available. The risk posed by contaminants is high with ammonia, nitrate, and potassium exceeding the threshold for high risk. There has not been evidence of Salamander Mussel in the last 20 years, and given the high risk from contaminants, we expect the demographic population condition would at most be low by 2040.

Table D.3. Current condition risk factor analysis for Salamander Mussel populations within the Ohio basin representation unit. (E=Extant; PE = Presumed Extant)

State	**HUC8 Name**	**HUC8 Code**	**Status**	**Contaminant**	**Landscape**	**Hydrological Regime**	**Connectivity**	**Invasive Species**	**Host Species Vulnerability**	**Overall Current Risk**
NY, PA	French	05010004	E	Moderate	Moderate	Low	Moderate	Moderate	Low	Moderate
PA	Middle Allegheny-Redbank	05010006	E	Moderate	Moderate	Low	Moderate	Moderate	Low	Moderate
OH, PA, WV	Upper Ohio-Wheeling	05030106	E	High	Moderate	Low	Moderate	Moderate	Low	High
OH, WV	Little Muskingum-Middle Island	05030201	E	Moderate	Moderate	Low	Moderate	Moderate	Low	Moderate
OH, WV	Upper Ohio-Shade	05030202	E	High	Moderate	Low	Moderate	Moderate	Moderate	High
WV	Little Kanawha	05030203	E	Moderate	Moderate	Low	Moderate	Moderate	Low	Moderate
OH	Tuscarawas	05040001	PE	High	High	Low	Moderate	Moderate	Moderate	High
WV	Lower Kanawha	05050008	E	Moderate	Moderate	Low	Moderate	Moderate	Low	Moderate
OH	Upper Scioto	05060001	E	High	High	Low	Moderate	Moderate	Moderate	High
KY, OH	Lower Scioto	05060002	PE	High	Moderate	Moderate	Moderate	Moderate	Moderate	High
KY, OH, WV	Little Scioto-Tygarts	05090103	PE	High	Moderate	Moderate	Moderate	Moderate	Moderate	High
KY	Little Sandy	05090104	PE	High	Moderate	Moderate	Moderate	Moderate	Moderate	High
KY, OH	Ohio Brush-Whiteoak	05090201	E	High	Moderate	Moderate	Moderate	Moderate	Moderate	High
IN, KY, OH	Middle Ohio-Laughery	05090203	E	High	Moderate	Moderate	Moderate	Moderate	Moderate	High
KY	Licking	05100101	E	High	Moderate	Moderate	Moderate	Moderate	Moderate	High
KY	South Fork Licking	05100102	E	High	High	Moderate	Low	Moderate	Moderate	High
KY	Middle Fork Kentucky	05100202	PE	High	Low	Moderate	Moderate	Low	Moderate	High
KY	Upper Kentucky	05100204	PE	High	Moderate	Moderate	Moderate	Moderate	Moderate	High
KY	Lower Kentucky	05100205	E	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate

Table D.3. (continued) Current condition risk factor analysis for Salamander Mussel populations within the Ohio basin representation unit. (E=Extant; PE = Presumed Extant)

State	HUC8 Name	HUC8 Code	Status	Contaminant	Landscape	Hydrological Regime	Connectivity	Invasive Species	Host Species Vulnerability	Overall Current Risk
KY	Upper Green	05110001	PE	High	Moderate	Moderate	Moderate	Moderate	Moderate	High
KY	Rough	05110004	PE	High	Low	Moderate	Moderate	Moderate	Moderate	High
IN	Eel	05120104	E	High	High	Moderate	Low	Moderate	Moderate	High
IN	Tippecanoe	05120106	E	High	Moderate	Moderate	Moderate	Moderate	Moderate	High
IN	Wildcat	05120107	E	High	High	Moderate	Low	Low	Moderate	High
IL, IN	Middle Wabash-Little Vermillion	05120108	E	High	Moderate	Moderate	Moderate	Moderate	Moderate	High
IL, IN	Vermillion	05120109	E	Moderate	Moderate	Moderate	Low	Moderate	Moderate	Moderate
IN	Sugar	05120110	E	Moderate	Moderate	Moderate	Low	Moderate	Moderate	Moderate
IN	Eel	05120203	PE	Moderate	High	Moderate	Moderate	Low	Moderate	High
IN	Muscatatuck	05120207	E	High	Moderate	Moderate	Moderate	Moderate	Moderate	High
IN	Lower East Fork White	05120208	E	High	Moderate	Moderate	Moderate	Moderate	Moderate	High
TN	Collins	05130107	E	High	Moderate	Moderate	Moderate	Low	High	High
TN	Harpeth	05130204	E	High	Moderate	Moderate	High	Low	High	High
KY	Salt	05140102	E	High	High	Moderate	Moderate	Moderate	Moderate	High
KY	Rolling Fork	05140103	E	High	Moderate	Moderate	Moderate	Moderate	Moderate	High
IL, KY	Lower Ohio-Bay	05140203	PE	High	Moderate	Moderate	Moderate	Moderate	Moderate	High

Table D.4. Current condition summary of demographic, risk factor, catastrophic event analysis for Salamander Mussel populations within the Ohio basin representation unit. (E=Extant; PE = Presumed Extant)

State	HUC8 Name	HUC8 Code	Status	# of Targeted/ semi-targeted surveys	# of incidental surveys	Demographic Condition	Overall Current Risk	Risk of Catastrophic event - Coal	Risk of Catastrophic event - Oil and Gas
NY, PA	French	05010004	E	0	1	Unknown	Moderate	Low	High
PA	Middle Allegheny-Redbank	05010006	E	1	4	Low	Moderate	High	High
OH, PA, WV	Upper Ohio-Wheeling	05030106	E	5	0	Functionally Extirpated	High	High	High
OH, WV	Little Muskingum-Middle Island	05030201	E	10	1	Moderate	Moderate	High	High
OH, WV	Upper Ohio-Shade	05030202	E	0	3	Unknown	High	Low	High
WV	Little Kanawha	05030203	E	8	3	Low	Moderate	Low	High
OH	Tuscarawas	05040001	PE	0	1	Unknown	High	High	High
WV	Lower Kanawha	05050008	E	0	2	Unknown	Moderate	Low	High
OH	Upper Scioto	05060001	E	0	5	Unknown	High	Low	High
KY, OH	Lower Scioto	05060002	PE	0	2	Unknown	High	Low	High
KY, OH, WV	Little Scioto-Tygarts	05090103	PE	0	5	Unknown	High	Low	High
KY	Little Sandy	05090104	PE	0	4	Unknown	High	Low	High
KY, OH	Ohio Brush-Whiteoak	05090201	E	0	5	Unknown	High	Low	High
IN, KY, OH	Middle Ohio-Laughery	05090203	E	0	3	Unknown	High	Low	High
KY	Licking	05100101	E	1	17	Low	High	High	High
KY	South Fork Licking	05100102	E	0	9	Unknown	High	Low	High
KY	Middle Fork Kentucky	05100202	PE	0	2	Unknown	High	High	Low
KY	Upper Kentucky	05100204	PE	0	4	Unknown	High	High	High

Table D.4. (continued) Current condition summary of demographic, risk factor, catastrophic event analysis for Salamander Mussel populations within the Ohio basin representation unit. (E=Extant; PE = Presumed Extant)

State	**HUC8 Name**	**HUC8 Code**	**Status**	**# of Targeted/ semi-targeted surveys**	**# of incidental surveys**	**Demographic Condition**	**Overall Current Risk**	**Risk of Catastrophic event - Coal**	**Risk of Catastrophic event - Oil and Gas**
KY	Lower Kentucky	05100205	E	0	14	Unknown	Moderate	Low	High
KY	Upper Green	05110001	PE	0	5	Unknown	High	Low	High
KY	Rough	05110004	PE	0	1	Unknown	High	Low	High
IN	Eel/05120104	05120104	E	0	3	Unknown	High	Low	High
IN	Tippecanoe	05120106	E	0	10	Unknown	High	Low	High
IN	Wildcat	05120107	E	0	3	Unknown	High	Low	High
IL, IN	Middle Wabash-Little Vermillion	05120108	E	0	7	Unknown	High	Low	High
IL, IN	Vermillion	05120109	E	0	1	Unknown	Moderate	Low	High
IN	Sugar	05120110	E	0	3	Unknown	Moderate	Low	High
IN	Eel/05120203	05120203	PE	0	3	Unknown	High	High	High
IN	Muscatatuck	05120207	E	2	13	Functionally Extirpated	High	Low	High
IN	Lower East Fork White	05120208	E	0	20	Unknown	High	High	High
TN	Collins	05130107	E	0	2	Unknown	High	Low	High
TN	Harpeth	05130204	E	0	2	Unknown	High	Low	High
KY	Salt	05140102	E	0	8	Unknown	High	Low	High
KY	Rolling Fork	05140103	E	0	3	Unknown	High	Low	High
IL, KY	Lower Ohio-Bay	05140203	PE	0	3	Unknown	High	Low	High

D.3 Tennessee Basin Representation Unit

The Upper Duck is extant. The Kentucky Department of Fish and Wildlife provided Salamander Mussels, produced by the Center for Mollusk Conservation from Licking River, Ohio brood stock, for release at two locations in the Upper Duck River in 2017. The demographic population condition is unknown because surveys were not completed during the reintroductions. The Upper Duck population experiences high risk from nitrate and copper, unpaved road stream crossings, and increased sedimentation. The future of this population is dependent on the reintroduction efforts. Given the population experiences high risk, we would expect them to be at most in low demographic population condition if supplementation is not continued.

The Lower Duck population is extant. The Lower Duck is at high risk from unpaved road stream crossings, dams, and a lack of regulations limiting recreational or commercial collection of mudpuppy. Given the population experiences high risk, we would expect them to be at most in low demographic population condition by 2040.

Table D.5. Current condition risk factor analysis for Salamander Mussel populations within the Tennessee basin representation unit. (E=Extant; PE = Presumed Extant)

State	HUC8 Name	HUC8 Code	Status	Contaminant	Landscape	Hydrological Regime	Connectivity	Invasive Species	Host Species Vulnerability	Overall Current Risk
TN	Upper Duck	06040002	E	High	Moderate	Moderate	High	Moderate	High	High
TN	Lower Duck	06040003	E	Moderate	Moderate	Moderate	High	Moderate	High	High

Table D.6. Current condition summary of demographic, risk factor, catastrophic event analysis for Salamander Mussel populations within the Tennessee basin representation unit. (E=Extant; PE = Presumed Extant)

State	HUC8 Name	HUC8 Code	Status	# of Targeted/ semi-targeted surveys	# of incidental surveys	Demographic Condition	Overall Current Risk	Risk of Catastrophic event - Coal	Risk of Catastrophic event - Oil and Gas
TN	Upper Duck	06040002	E	0	2	Unknown	High	Low	High
TN	Lower Duck	06040003	E	0	2	Unknown	High	Low	High

D.4 Upper Mississippi Basin Representation Unit

The Twin Cites population in Minnesota is considered extant due to an incidental record of a weathered shell found in 2004. The demographic population condition is unknown. The high risks for this population include chloride and landscape, which includes threats associated with imperviousness, urbanization, canopy cover and vegetation cover in the riparian corridor. Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Middle Minnesota population in Minnesota is considered extant. There were 2 incidental surveys in 2006 and 2012. Both surveys found subfossil shells, 2 valves and 1 valve respectively. The demographic population condition is unknown. The only high risk the Middle Minnesota population is experiencing is due to high nitrate concentrations. Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Lower St. Croix population in Minnesota and Wisconsin has numerous surveys along the St. Croix River in 2019 and 2020. Juveniles were present in the 2019 surveys indicating reproductive success; however, no age class or measurements were provided. The demographic population condition is low. In addition to nitrate, this population exceeds high risk thresholds for copper. If the high risk continues, this population is expected to be extirpated by 2040.

The Black population in Wisconsin has two targeted surveys completed in 2018 and 2020. . The surveys accounted for over 500 individuals, with two juveniles found in 2018 and 18 in 2020. Additionally, one mudpuppy was observed during the 2020 survey. The population condition is moderate. The population is at high risk from dams and lack of regulations regarding collection of mudpuppy. If the high risk continues, the demographic population condition expected is functionally extirpated by 2040.

The Lower Chippewa population in Wisconsin is extant. A single juvenile was found at each of two sites in 2018. Approximately 700 juveniles were released at two locations in 2020. During these introductions, there were 12 mudpuppies observed between the two sites. The demographic population condition is high. Beyond the high risk from nitrate, the Lower Chippewa population also experiences high risk from the lack of collection regulations for mudpuppy. If the high risk continues, the demographic population condition expected is low by 2040.

The Eau Claire population in Wisconsin is considered extant. A single live individual was found during a 2002 targeted survey. The demographic population condition is functionally extirpated. The population is at high risk from nitrate and chloride and lack of collection regulations for mudpuppy. If the high risk continues, this population is expected to be extirpated by 2040.

The Grant-Little Maquoketa population is considered extant. The demographic population condition is unknown. The population is at high risk from contaminants, including ammonia, chloride, and copper. This is the only populations in the Upper Mississippi basin not to exceed thresholds for nitrate. Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Lake Dubay population in Wisconsin is considered extant. During a 2010 monitoring survey, one live individual was found. Therefore, we evaluated the demographic population condition as functionally extirpated. The population is at high risk from nitrate, dams, and lack of regulations for collection of mudpuppy. If the high risk continues, this population is expected to be extirpated by 2040.

The Castle Rock population in Wisconsin is extant. Two surveys were conducted in 2017 and found 2 fresh dead Salamander Mussel individuals. Therefore, we evaluated the demographic population condition as functionally extirpated. The population is at high risk from nitrate, chloride and copper and lack of regulations for mudpuppy collection. If the high risk continues, this population is expected to be extirpated by 2040.

The Lower Wisconsin population in Wisconsin is extant. There have been approximately 50 surveys completed in the population; however, only four have been targeted surveys (one in 2016, one in 2017, and two in 2020). From these four surveys, approximately 50 individuals have been observed along with three juveniles at one of the sites in 2020. Based on this information, the demographic population condition is low. The population is at high risk from nitrate and lack of regulations prohibiting or limiting mudpuppy collection. If the high risk continues, this population is expected to be extirpated by 2040.

The Kankakee population spans portions of Illinois, Indiana, and Michigan. One incidental observation along the Kankakee River within Illinois since 2000 make up this extant population. There are three other incidental observations prior to 2000 in the Kankakee River. The population is at high risk from nitrate and copper. Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Upper Sangamon population in Illinois is considered extant from a single incidental observation in 2000. The population is at high risk from nitrate and chloride. Given this population experiences high risk, the best demographic population condition expected is low by 2040.

The Upper St. Croix population in Minnesota and Wisconsin is presumed extant with an incidental observation from 1988. The population condition is unknown. The population is at high risk from nitrate, dams, and lack of regulations protecting mudpuppies from collection. There has not been evidence of Salamander Mussel in the last 20 years and given the high risk from contaminants, connectivity, and host species vulnerability, we expect the demographic population condition would at most be low by 2040.

The South Fork Flambeau population in Wisconsin is presumed extant with an incidental observation of 2 live individuals from 1990. The demographic population condition is unknown. The population is at high risk due to nitrate and lack of regulations protecting mudpuppies from collection. There has not been evidence of Salamander Mussel in the last 20 years, and given the high risk from contaminants, we expect the demographic population condition would at most be low by 2040.

The Coon-Yellow population spans portions of Iowa, Minnesota, and Wisconsin. There is one incidental survey in the Mississippi River from 1982 that found 2 dead individuals in separate dives. The population condition is unknown. The population is at high risk due to nitrate and lack of regulations protecting mudpuppies from collection. There has not been evidence of Salamander Mussel in the last 20 years, and given the high risk from contaminants, we expect the demographic population condition would at most be low by 2040.

The Meramec population in Missouri is presumed extant. The Meramec River system has not been comprehensively surveyed for Salamander Mussel or within suitable habitat since 1997 (A. Roberts and B. Simmons, personal communication, 2021). A study in 1984 found them at 4 sites in the Meramec (a total of 11 animals) (ESEI 1987). In 1997, Salamander Mussel was detected at two additional sites in the Meramec where 14 and 5 live specimens were collected (Roberts et al 2000). The demographic population condition is unknown. The population is at high risk from chloride exceedances and number of dams within the watershed. There has not been evidence of Salamander Mussel in the last 20 years, and given the high risk from contaminants and connectivity, we expect the demographic population condition would at most be low by 2040.

The Bourbeuse population in Missouri is presumed extant, the demographic population condition is unknown. This population was extensively surveyed in 1979 and 1997. In 1979, the Salamander Mussel was found at one site where 5 live individuals were found (Buchanan 1979, 1980). No evidence of the species was found in 1997 (Roberts et al 2000). The 1979 site was surveyed in 2018 for a bridge replacement project and a relocation was done in 2019, the species was not found (A. Roberts, personal communication, 2021). There are other reaches of the Bourbeuse River that provide suitable Salamander Mussel habitat that have not been searched. The population is at high risk from nitrate, dams, and unpaved road stream crossings. There has not been evidence of Salamander Mussel in the last 20 years, and given the high risk from contaminants and connectivity, we expect the demographic population condition would at most be low by 2040.

Table D.7. Current condition risk factor analysis for Salamander Mussel populations within the Upper Mississippi basin representation unit. (E=Extant; PE = Presumed Extant)

State	HUC8 Name	HUC8 Code	Status	Contaminant	Landscape	Hydrological Regime	Connectivity	Invasive Species	Host Species Vulnerability	Overall Current Risk
MN	Twin Cities	07010206	E	High	High	Low	Moderate	Moderate	Low	High
MN	Middle Minnesota	07020007	E	High	Moderate	Moderate	Moderate	Moderate	Low	High
MN, WI	Upper St. Croix	07030001	PE	High	Low	Moderate	High	Moderate	High	High
MN, WI	Lower St. Croix	07030005	E	High	Moderate	Moderate	Moderate	Moderate	Low	High
WI	Black	07040007	E	High	Moderate	Moderate	High	Moderate	High	High
WI	South Fork Flambeau	07050003	PE	High	Low	Low	Moderate	Moderate	High	High
WI	Lower Chippewa	07050005	E	High	Moderate	Moderate	Moderate	Moderate	High	High
WI	Eau Claire	07050006	E	High	Moderate	Moderate	Moderate	Moderate	High	High
IA, MN, WI	Coon-Yellow	07060001	PE	High	Moderate	Moderate	Moderate	Moderate	High	High
IA, WI	Grant-Little Maquoketa	07060003	E	High	Moderate	Moderate	Low	Moderate	Moderate	High
WI	Lake Dubay	07070002	E	High	Moderate	Moderate	High	Moderate	High	High
WI	Castle Rock	07070003	E	High	Moderate	Low	Moderate	Moderate	High	High
WI	Lower Wisconsin	07070005	E	High	Moderate	Moderate	Moderate	Moderate	High	High
IL, IN, MI	Kankakee	07120001	E	High	Moderate	Moderate	Moderate	Moderate	Moderate	High
IL	Upper Sangamon	07130006	E	High	Moderate	Moderate	Low	Moderate	Moderate	High
MO	Meramec	07140102	PE	High	Moderate	Moderate	High	Moderate	Moderate	High
MO	Bourbeuse	07140103	PE	High	Moderate	Moderate	High	Moderate	Moderate	High

Table D.8. Current condition summary of demographic, risk factor, catastrophic event analysis for Salamander Mussel populations within the Upper Mississippi basin representation unit. (E=Extant; PE = Presumed Extant)

State	HUC8 Name	HUC8 Code	Status	# of Targeted/ semi-targeted surveys	# of incidental surveys	Demographic Condition	Overall Current Risk	Risk of Catastrophic event - Coal	Risk of Catastrophic event - Oil and Gas
MN	Twin Cities	07010206	E	0	1	Unknown	High	Low	High
MN	Middle Minnesota	07020007	E	0	2	Unknown	High	Low	High
MN, WI	Upper St. Croix	07030001	PE	0	1	Unknown	High	Low	High
MN, WI	Lower St. Croix	07030005	E	11	49	Low	High	Low	High
WI	Black	07040007	E	15	13	Moderate	High	Low	High
WI	South Fork Flambeau	07050003	PE	0	2	Unknown	High	Low	High
WI	Lower Chippewa	07050005	E	9	13	High	High	Low	High
WI	Eau Claire	07050006	E	1	3	Functionally Extirpated	High	Low	High
IA, MN, WI	Coon-Yellow	07060001	PE	0	1	Unknown	High	Low	High
IA, WI	Grant-Little Maquoketa	07060003	E	0	4	Unknown	High	Low	High
WI	Lake Dubay	07070002	E	1	1	Functionally Extirpated	High	Low	High
WI	Castle Rock	07070003	E	2	7	Functionally Extirpated	High	Low	High
WI	Lower Wisconsin	07070005	E	4	47	Low	High	Low	High
IL, IN, MI	Kankakee	07120001	E	0	4	Unknown	High	Low	High
IL	Upper Sangamon	07130006	E	0	5	Unknown	High	Low	High
MO	Meramec	07140102	PE	0	1	Unknown	High	Low	High
MO	Bourbeuse	07140103	PE	0	7	Unknown	High	Low	High

D.5 Arkansas-White-Red Basin Representation Unit

The Lower Black and Little Red populations are presumed extirpated, both have unknown demographic population condition (K. Moles, personal communication, July 23, 2021). The Arkansas Game and Fish Commission (AGFC), along with the USFWS, have spent time looking in these rivers for a different species, but within the same habitat Salamander Mussel would occupy.

The Spring population is presumed extant because the mussel fauna is still intact within this system and surveys have not be exhaustive (K. Moles personal communication July 23, 2021). Three fresh dead individuals were found prior to 1987 (Harris & Gordan 1987, entire). The AGFC completed some targeted searching for Salamander Mussel in the Spring population recently, but did not find any individuals (K. Moles, personal communication, July 23, 2021). The demographic population condition is unknown. The Spring population is experiencing high risk from a high number of dams and moderate level of unpaved road crossings threatening movement of the Salamander Mussel and mudpuppy, along with increased sedimentation into the system (Table D.9). There has not been evidence of Salamander Mussel in the last 20 years, given the high risk from connectivity, we expect the demographic population condition would at most be low by 2040.

Table D.9. Current condition risk factor analysis for Salamander Mussel populations within the Arkansas-White-Red basin representation unit. (E=Extant; PE = Presumed Extant)

State	HUC8 Name	HUC8 Code	Status	Contaminant	Landscape	Hydrological Regime	Connectivity	Invasive Species	Host Species Vulnerability	Overall Current Risk
AR, MO	Spring	11010010	PE	Moderate	Moderate	Moderate	High	Moderate	Moderate	High

Table D.10. Current condition summary of demographic, risk factor, catastrophic event analysis for Salamander Mussel populations within the Great Lakes basin representation unit. (E=Extant; PE = Presumed Extant)

State	HUC8 Name	HUC8 Code	Status	# of Targeted/ semi-targeted surveys	# of incidental surveys	Demographic Condition	Overall Current Risk	Risk of Catastrophic event - Coal	Risk of Catastrophic event - Oil and Gas
AR, MO	Spring	11010010	PE	0	1	Unknown	High	Low	High

APPENDIX E. METHODS FOR FUTURE CONDITION RISK FACTORS

E.1 Contaminants

Because there is not currently a way to directly predict the presence or concentrations of contaminants in surface waters, we used land cover as a proxy for future condition (Table 5.2). The presence and concentration of certain contaminants, including ammonia, are correlated with specific land cover types, and land cover is an important variable in predicting the occurrence of contaminants in surface waters (Baker 2003, entire; Kiesling et al. 2019, entire; Rothenberger et al. 2009, entire; Zhongwei et al. 2009, entire). Although the strength of the relationship between land cover and occurrence of contaminants may vary by geography due to large ranges in concentrations and laboratory reporting methods, we believe our approach to qualitatively predict where concentrations of contaminants may increase or decrease due to projected changes in land cover to be reasonable based on these studies and what we know about sources of contaminants. We used the FORE-SCE land cover change model to project how land cover may change in the future relative to current condition under a worst-case (IPCC Special Report on Emissions Scenarios (SRES) A2) and best-case (SRES B1) scenario and assumed the occurrence and concentration of contaminants would increase or decrease relative to current condition along with increases or decreases in the percent cover of certain land cover types. For example, ammonia has both agricultural and industrial applications and is a component of municipal effluent discharges (USEPA 2013, p. 5–7) and urban and agricultural land cover has been positively correlated with concentrations of ammonia (Baker 2003, pp. 2–3; and Rothenberger et al. 2009, p. 520). Therefore, we would expect the presence and concentrations of ammonia in surface water to increase with projected increases in the percent cover of developed and agricultural land cover types, although we cannot predict by exactly how much. Similarly urban and agricultural land cover also has a statistically significant relationship to concentrations of chloride (Zhongwei et al. 2009, p. 76) as sources of chloride include deicing salt, urban and agricultural runoff, and discharges from wastewater treatment facilities (USEPA 1988, p. 1). Therefore, we would also expect chloride to increase where developed and agricultural land cover types are projected to increase. Anthropogenic inputs of copper, lead, and other metals into surface waters come primarily from mining and manufacture of alloys, metal products, electrical equipment (Baker 2003, pp. 2–3; Zhongwei et al. 2009, p. 76). We associated metals with developed land cover types (Tchounwou et al. 2012, p. 3–18).

Within coming years, climate change will likely amplify these impacts as global surface temperatures are expected to rise greater than 1.5 ˚C, relative to 1850 to 1900 for all RCP scenarios except RCP2.6, with some regions projected to experience even larger impact (Intergovernmental Panel on Climate Change [IPCC], 2013, p. 20). As surface temperatures increase, decreases in precipitation may occur, likely resulting in elevated stream temperature, decreased dissolved oxygen, and decreased flows (Sinokrot & Gulliver 2000, pp. 349–359; van Vliet et al. 2013, pp. 450–464). Morrill et al. (2005, pp. 139–146) studied the empirical relationship between steam and air temperature and how these relationships impact water temperature and potential changes in dissolved oxygen. For every 1˚C increase air temperature, water temperature increased 0.6–0.8 ˚C for the majority of streams, but few of these streams had a linear relationship of 1:1 for air/water temperature trend. B on this modeling, an increase in air temperature of 3–5 ˚C would cause surface water temperature to increase 2–3 ˚C (Morrill et al. 2005, pp. 139–146). Dissolved oxygen levels are lower at higher water temperatures, so as stream temperatures increase, dissolved oxygen will decrease. We used the USGS NCCV (https://www2.usgs.gov/landresources/lcs/nccv/maca2/maca2_counties.html) to assess potential increases in air temperature for the emission scenarios RCP4.5 and RCP8.5. Under RCP8.5, the mean change in air temperature across the range of Salamander Mussel is projected to be approximately between 3.12–4.02 ˚C. Under RCP4.5, the mean temperature change is projected to be approximately 2.08–2.77 ˚C. Based on these climate projections, stream temperature will likely increase in many geographic areas, and dissolved oxygen will decrease, severely impacting aquatic ecosystems and freshwater mussels.

E.2 Landscape

To project landscape conditions under Future Scenario 1, we used the Forecasting Scenarios (FORE-SCE) model (Sohl et al. 2007, entire) SRES A2 to predict future land cover for agriculture, development, and vegetative cover in the riparian buffer. For landscape conditions under Future Scenario 2, we used SRES Scenario B1. We calculated the percent of land cover for each HUC in 2050 and 2070. Because the projected data from SRES is based on modeling, and our current condition was calculated using NLCD 2016 dataset, we calculated the percent change using modeled land cover in 2005, 2050, and 2070 and applied the percent change to the NLCD 2016 data. Using the modeled 2005 historic data, we calculated the percent change for each land cover in 2050 and 2070. We then applied the percent change to the current condition to get a projected percentage of land cover types for each HUC in 2050 and 2070. We used agricultural land cover to project out the percent of agriculture within HUCs. We assumed that as development increases, percent urban and percent imperviousness would increase at the same rate. We used the change in vegetation cover within the riparian buffer for vegetative cover and assumed the rate of change in vegetation would apply to the change in canopy cover within the riparian buffer.

E.3 Hydrological Regime

Although we used U.S. Drought Monitor data to quantitatively evaluate current condition, we could not use this data to project future conditions because not all of the indices used to derive drought category can be modeled or predicted. Rather, we used projections of the Cumulative Severe Drought Index (CDSI) developed by the U.S. Forest Service to determine qualitatively how drought severity may change in the future (Peters & Iverson 2015, p. 57). The CDSI uses Palmer Drought Severity Index (PDSI) values calculated for individual spatial grids (in other words, raster cells) across the continental United States, assigns weights to each PDSI drought category, and sums the weighted occurrences across time. The more severe drought categories receive higher weighting. For example, if a raster cell has a calculated PDSI value between -2.0 to -2.99 (indicating moderate drought) for a single occurrence in a given period of time, a weight of 1 is applied to that raster cell for that occurrence for total CDSI value of 1 (that is, 1 occurrence multiplied by a weight of 1). If for the next occurrence the PDSI value decreases to between -3.0 to -3.99 (indicating severe drought), a weight of 2 is applied for that occurrence and the total CDSI value for that period encompassing both occurrences is 3. This process allows weighted occurrences to be summed up over different time periods, facilitating comparison of many locations over multiple time periods (Peters & Iverson 2019, p. 21). We used CDSI projections for the continental United States from Peters and Iverson (2019, p. 21) in the form of in 4km x 4km resolution rasters. To account for variability among climate models, Peters and Iverson (2019, p. 21) developed four overarching future scenarios: Warm Wet, Hot Wet, Hot Slightly Dry, and Hot Dry. We used the book end scenarios of Warm Wet and Hot Dry to compare CDSI values for the current time period (1980–2009) to the time period 2040–2069. This time period was chosen to be consistent with our other future condition analyses and because we felt that time frame was more biologically relevant. We calculated the average CDSI value of all raster cells within a HUC8 corresponding to a population in the current time period across all scenarios to create an average baseline and compared those values with the projected averaged CDSI values for that watershed between 2040 and 2069 to determine how drought severity may change under both the Warm Wet and Hot Dry scenarios. Increasing CDSI values indicate increasing drought severity.

The results indicate that in the foreseeable future (2040–2069), averaged CDSI values increased in almost all watersheds (99%) under the Warm Wet scenario and in most watersheds (58%) under the Hot Dry scenario. The average percent change in CDSI was +111% under the Warm Wet scenario and +35% under the Hot Dry scenario. Although this seems counterintuitive given the name of the scenarios, the scenarios are named for the final time period of the projections (2070–2099), where averaged CDSI values increased in all watersheds under both scenarios and average percent change in CDSI was +246% under the Warm Wet scenario and +435% under the Hot Dry scenario. We did not project out 2070–2099 because we did not think it was as biologically relevant. These watershed-scale results comport with the regional-scale results presented in Peters and Iverson (2019, entire) which suggest that drought may decrease in the immediate future (2010–2039) due to increased precipitation but may also become more frequent and intense during the second half of the century. Due to the conditions of the projected scenarios in the foreseeable future, we included the Warm Wet scenario in Scenario 1 and Hot Dry scenario in Scenario 2 even though the second half of the century would place them opposite. While projections of temperature, precipitation, and other drought-related factors vary across models, and thus methods for projecting drought inherently carry some amount of uncertainty, these models (Peters & Iverson 2019, entire; Mishra & Cherkauer 2010, entire; Cook et al. 2014, entire; Wehner et al. 2011, entire; Zhao & Dai 2017, entire; Cook et al. 2020, entire) all indicate a tendency towards increasing drought at the turn of the century.

E.4 Connectivity

In some areas of Salamander Mussel, barriers have been removed from river systems. We assumed construction of new barriers is unlikely. Therefore, under Scenario 1, we projected no change from the current number of dams. For Scenario 2, we assumed that the rate at which barriers were removed in the last 2 decades would continue into the future for two decades. Therefore, we projected dam removals out to 2040 based on the number of dams removed between 2000–2020.

To assess future changes in unpaved road density, we used the U.S. Global Roads Inventory Project's (GRIP). For Future Scenario 1 we used SSP5 (27.3%; projections for increases in road (all types) length (km) in the U.S. by 2050 (Meijer et al. 2018, Table S6). For Future Scenario 2, we used GRIP Scenario SSP3 (3.2%; which projects a 3.2% increase in road density (Meijer et al. 2018, Table S6). These projections assume that unpaved road density will increase at the same rate as all road types in the U.S. The GRIP Scenarios extend to 2050. We applied the SSP percent increase to the current density to get a projected unpaved road density in 2050 for each HUC8 (considered the population).

E.5 Invasive Species

We assessed the risk of negative impacts because of invaders worsening in the future by identifying the number of hot spots in neighboring HUC8 watersheds (HUC8 watershed was the level considered for populations and associated analyses) directly adjacent to HUC8 watersheds we determined in the current condition risk assessment to be at a low risk of invasive species impacts (Table 5.2). We included neighboring watershed assuming there was risk of dispersal into current populations. We do not consider a future in which invasive species impacts are improved (dropped from high risk to moderate risk, or from moderate risk to low risk), but instead one in which these impacts likely worsen (bumped from low risk to moderate risk, or from moderate risk to high risk); however, we do also consider a future in which the risk in any population may remain unchanged (remains unchanged from current condition) and does not increase because of mitigation efforts, minimal invader access (for example, being upstream from an impacted neighboring HUC8 watershed), or any other reason.

We consider any low-risk population with one or more neighbors with a hotspot in any invasive species category (for example, direct competition, reduction of reproductive potential, disturbance to ecosystems, direct harm/predation) to increase in risk (from low risk to moderate risk) sometime in the future populations determined to already be at moderate risk will remain unchanged for this future condition assessment (Table E.1).

Table E.1. Indicator descriptions used to evaluate the risk of invasive species impacts worsening or remaining unchanged in current populations in the future.

Future condition indicator – Invasive Species	**Metrics/description**
Description of Indicator	Optimized Hotspot Analysis using invasive species occurrence data for occupied HUC8 and categorized by common impacts to mussel species
High Risk (3 points)	N/A
Moderate Risk (2 points)	Hot spots were identified in one or more neighboring HUC regardless of number or confidence levels. No cold spots were identified to occur in any neighboring HUC8
Low Risk (1 point)	No hotspots were identified to occur in any neighboring HUC8 AND/OR cold spots were identified in one or more neighboring HUC8 regardless of number or confidence levels

Scenario 1 will be the hotspot and neighbor analysis from current condition (Table 5.2). If there is a hotspot neighbor to a low risk population, we are making the assumption the frequency and abundance necessary to cause that hotspot would mean the dispersal into the low risk population is sufficient to move it into a moderate risk.

Future Scenario 2 is the same invasive rate as it is now (Table 5.2).

The risk did not change for either future scenario 1 or 2. All populations stayed at the same risk factor of either moderate or low. We believe this is due to the fact that the moderate risk is our highest category and so any increase risk is capture and overall, we do not think that invasive species invasions pose a high risk to Salamander Mussel. For the populations that remained low, we hypothesize that there are other factors potentially influencing increased risk that we are unable to determine (for example, barrier to invasion, flow dynamics, habitat suitability, etc.). It is also possible that a lack of detections in the neighboring HUC8 watersheds due to a lack of survey effort/ detections is not reflective of the true current risk posed by neighboring HUC8 watersheds. Once again this is not something that we are able to determine. Therefore, there is no change in the risk posed by invasive species across all populations into 2070.

E.6 Host Species Vulnerability

We had limited data with which to assess future viability of mudpuppies as such we were not able to evaluate many stressors to mudpuppies (e.g., climate change, disease). To determine the future vulnerability to mudpuppies, we estimated plausible continued use of lampricide and changes to bag and collection regulations. Future Scenario 1 projects no changes in state regulations for collection or bag limits, with the assumption that current restrictions would not lessen given that there seems to be more of widespread concern ab out potential decline of mudpuppy rangewide and more focus on conservation efforts evidenced by additional species protection in certain states (for example, mudpuppies are now considered special concern in Michigan) (Table 5.2). Given that the trend for mudpuppy conservation has been increasing, future Scenario 2 projects more protection for mudpuppies by implementing additional state regulations and bag limits (Table 5.2). Lampricide application into the future is difficult to project. For future Scenario 1 we also assume that sea lamprey control activities will increase based on increased sea lamprey infestation (Table 5.2). In Future Scenario 2, we assume there are no changes in the application frequency or quantity (Table 5.2). We used the expertise of the Services' Sea Lamprey Control Program to inform our Future Scenarios (A. Jubar, personal communication, 2021). This expertise is based on knowledge of sea lamprey assessment and treatment activities in the past and understanding of potential treatment sites into the future.

E.7 Catastrophic Events

We relied on U.S. Energy Information Administration (EIA) analyses to qualitatively project how the risk of catastrophic events may change in the future. The EIA's Annual Energy Outlook 2021 provides analyses of the energy market for policy makers and public understanding (EIA 2021, p. 2). Energy consumption in the U.S. is expected to increase over the next 30 years. The primary sources of that energy, however, are dependent on oil and gas supplies and prices. In a low oil and gas supply case, electricity generation from natural gas decreases by a third by 2030 and stays level. In a high oil and gas supply case, electricity generation from natural gas more than doubles by 2050. Similarly, production of crude oil and gas plant liquids increases until 2040 and levels off under a high oil and gas supply scenario and decrease slightly in a low oil and gas supply scenario. In both scenarios, electricity generation from coal spikes in the near-term but continues a downward trend (EIA 2021, pp. 8–19). We make the assumption that energy infrastructure (for example, pipelines, wells, and mines) increases and decreases along with consumption and production and that those changes are geographically explicit. For example, under the high oil and gas supply case, the EIA projects that production of crude oil will increase, and we assume that the number of pipelines may also increase. Furthermore, we assume that increase would occur only in areas with existing pipeline infrastructure. In other words, if an 8-digit HUC currently has no pipelines running through it, we would not assume the risk of an oil spill increases in this watershed because there was no related infrastructure to begin with. For pipelines and oil and natural gas wells, the worst-case scenario for catastrophic events would be the high oil and gas supply scenario where production and consumption increase, and the risk of a catastrophic event also increases. The best-case scenario would be the low oil and gas supply scenario where production and consumption decrease and the risk of a catastrophic event decreases. Since electricity generation from coal is expected to decrease in either scenario, we consider a worst-case scenario to be no change in risk and the best-case scenario to be a reduction in risk.

APPENDIX F. FUTURE CONDITION

Scenario 1

Great Lakes Basin Representation Unit

Based on the overall risk levels in the Great Lakes basin, we project three populations will be extirpated – Duck-Pensaukee, St. Clair, and Clinton (Figure 5.1, Table F.1) – all of which are currently in functionally extirpated condition. Two populations that had demographic data evaluated overlap with Canada were not fully assessed to project risk levels. The remaining six populations in the Great Lakes basin have unknown demographic population status currently (Figure 5.1, Table F.1). However, given they experience high risk, we project they will not be in any better condition than low in Scenario 1. We highlight the projected changes for populations within the Great Lakes basin for Scenario 1 in the following paragraphs and Table F.2.

Ammonia and chloride levels are projected to increase for all populations in the Great Lakes basin in Scenario 1. Copper and lead levels are projected to increase in four populations: Duck-Pensaukee, St. Joseph, Grand, and Chautauqua-Conneaut. All populations, except the Duck-Pensaukee, which had incomplete data for current condition, remain at a high level of risk for contaminants as nitrate levels remain high for Wolf, Clinton, Blanchard, and Grand populations. Chloride is the driving risk and increasing in St. Clair, Clinton, and St. Joseph populations. Copper is the driving risk and increasing in Blanchard and Chautauqua-Conneaut populations. The Clinton population currently experiences high risk from copper levels but is projected to decrease. Lake St. Clair population remains at a moderate level of risk due to levels of nitrate.

The Chautauqua-Conneaut is the only population with projected changes to the landscape risk factor, with the risk level increasing from moderate to high. Percent agriculture and percent tree canopy in the riparian buffer are expected to increase from moderate to high risk, along with percent vegetative cover in the riparian buffer increasing from low to moderate risk.

Six of the populations experience low risk for drought, but the CDSI is projected to increase ranging from 77.24–194.86%, averaging 132% increase. The other two populations, Wolf and St. Joseph, experience moderate risk, and the CDSI is projected to increase 85% and 113% respectively.

The Clinton and St. Clair populations are projected to have increased efforts in lampricide treatment. Given this projection, these two populations moved from low to moderate risk within that risk factor. The overall host vulnerability remained at moderate risk due to averaging with the bag and collection limit risk metric.

All populations in the Great Lakes basin are currently at high risk for catastrophic events from oil and gas. Scenario 1 projects an increase in production and consumption of oil and gas in areas that already have infrastructure. Therefore, the risk of a catastrophic event increases. We project no change in risk for catastrophic events related to coal, and all populations within the Great Lake basin remain at a low risk.

*Table F.1. Summary of projected demographic condition for Salamander Mussel extant and presumed extant populations given Scenario 1 and Scenario 2 risk levels within the Great Lakes basin. (*two populations within Canada were not evaluated for risk levels and therefore could not have demographics projected.)*

Projected Demographic Condition – Great Lakes basin	**Current Condition HUC8 Count**	**Projected HUC8 Count (2040–2070)**
High		
Moderate		
Low	1	
Functionally Extirpated	4	
Extirpated		3
Unknown	6	6
Total	11	9*

Table F.2. Scenario 1 future projections for risk factors for extant and presumed extant populations of Salamander Mussel in the Great Lakes basin. Bold text indicates changes from the current condition risk factor condition. (CN = Canada; E= extant, PE = presumed extant)

State	**HUC8 Name**	**Status**	**Current Demographic Condition**	**Contaminant**	**Landscape (2070)**	**Hydrological Regime (% change in CDSI)**	**Connectivity (2040 & 2050)**	**Invasive Species**	**Host Species Vulnerability**	**Overall Current Risk**
WI	Duck-Pensaukee	E	Functionally Extirpated	Low* ↑	Moderate	Low (107.5)	Low	Moderate	High	High
WI	Wolf	PE	Unknown	High ↑	Moderate	Moderate (85.76)	Moderate	Moderate	High	High
CN, MI	St. Clair	E	Functionally Extirpated	High ↑	High	Low (127.57)	Low	Moderate	Moderate	High
CN, MI	Lake St. Clair	E	Low							Unknown
MI	Clinton	E	Functionally Extirpated	High ↑	High	Low (123.09)	Moderate	Moderate	Moderate	High
IN, MI, OH	St. Joseph	E	Unknown	High ↑	High	Moderate (112.94)	Moderate	Moderate	Moderate	High
OH	Blanchard	E	Unknown	High ↑	High	Low (77.24)	Low	Moderate	Moderate	High
OH	Grand	PE	Unknown	High ↑	Moderate	Low (194.86)	Moderate	Low	High	High
NY, OH, PA	Chautauqua-Conneaut	E	Unknown	High ↑	**High**	Low (162.01)	Moderate	Moderate	High	High
CN, NY	Niagara	E	Unknown							Unknown
CN	Lower Grand	E	Functionally Extirpated							Unknown

Ohio Basin Representation Unit

We project the Middle Allegheny-Redbank and Little Kanawha populations will be functionally extirpated, given their current low demographic condition and moderate risk projected into the future under Scenario 1 (Figure 5.1, Table F.3). We expect the Upper Ohio-Wheeling, Licking, and Muscatatuck populations will be extirpated, given that they are either currently functionally extirpated or in low demographic condition and facing high risk into the future under Scenario 1 (Figure 5.1, Table 5.5). The Little Muskingum-Middle Island population is currently in moderate demographic condition and is projected to experience moderate risk under Scenario 1; therefore, we project it will be in low demographic condition (Figure 5.1, Table F.3).

Twenty-nine populations are currently in unknown demographic condition. Therefore, we could not project their condition into the future. However, twenty-four of these populations are projected to face high risk in the future under Scenario 1. Therefore, we project they would be at most in low condition. Lastly, the Sugar, Vermillion, Lower Kentucky, Lower Kanawha, and French populations have unknown current demographic condition; however, because they are projected to face moderate risk under Scenario 1, we project they would at most be in moderate demographic condition in the future. We highlight the projected changes for populations within the Ohio basin for Scenario 1 in the following paragraphs and Table F.4.

The Ohio basin is projected in Scenario 1 to have an increase of ammonia and chloride levels in almost all populations. The Vermillion and Sugar populations are projected to decrease in ammonia and chloride. Thirteen populations are projected to decrease in copper and lead levels, with 22 showing an increase. All populations continue to be at high risk as levels for nitrate remain high in the majority of the population. The following populations are at moderate risk for contaminants in Scenario 1: French, Middle Allegheny-Redbank, Little Muskingham-Middle Island, Little Kanawha, Lower Kanawha, and Eel. Levels of ammonia, chloride, copper, and lead are projected to increase in the future in these populations, but we lack the data to assess whether the increases in these four contaminants meets or exceeds the threshold for high risk. The Lower Kentucky population is currently at moderate risk, with increases projected for ammonia and chloride. The Vermillion and Sugar populations are also at moderate risk, but ammonia, chloride, copper, and lead are projected to decrease. Again, we were unable to determine whether this decrease in the four contaminants in the future would change the risk category.

Projected landscape changes in the Lower Scioto, Little Scioto, Middle Ohio-Laughery, Tippecanoe, Lower East Fork of the White, Collins, and the Harpeth populations increase the risk level of these populations from moderate to high. Projected changes in agriculture and tree canopy cover in the riparian buffer from moderate to high risk increase the Rough population’s risk from low to moderate. The projected increase in percent vegetative cover in the riparian buffer increased the additive score for the overall landscape risk factor to change the Middle Ohio-Laughery and Tippecanoe populations’ risk from moderate to high risk. The percent agriculture in the Lower Scioto, Lower East Fork of the White River, and the Harpeth populations are projected to increase, changing these populations' risk from moderate to high. The East Fork of the White River, Collins, and Harpeth populations also moved to high risk based on a projected decrease in percent vegetative cover in the riparian buffer. The Little Scioto population is projected to move from low to moderate risk for percent agriculture and from moderate to high risk for vegetative cover in the riparian buffer.

The projected 27.3% increase in unpaved road density would change the Upper Ohio-Shade population's connectivity risk from moderate risk to high.

Nine of the populations currently experience low risk from drought, but the CDSI is projected to increase by 109.46–193.68%. The other 26 populations currently experience moderate risk from drought, with projected increases in CDSI ranging from 8.85–223.08%.

Oil and gas supply are projected to increase in areas that already have infrastructure. With the exception of the Middle Fork of the Kentucky, all populations in the Ohio basin are currently at high risk for catastrophic events related to oil and gas. Scenario 1 projects an increase in oil and gas production and consumption, which increases the risk of a catastrophic event. We project no changes in coal production; therefore, there is not expected to be a change to any population's current risk of a catastrophic event related to coal.

Table F.3. Summary of projected demographic condition for Salamander Mussel extant and presumed extant populations given Scenario 1 and Scenario 2 risk levels within the Ohio basin.

Projected Demographic Condition – Ohio basin	Current Condition HUC8 Count	Projected HUC8 Count (2040–2070)
High		
Moderate	1	
Low	3	1
Functionally Extirpated	2	2
Extirpated		3
Unknown	29	29
Total	35	35

Table F.4. Scenario 1 future projections for risk factors for extant and presumed extant populations of Salamander Mussel in the Ohio basin. Bold text indicates changes from the current condition risk factor condition. (E= extant, PE = presumed extant)

State	HUC8 Name	Status	Current Demographic Condition	Contaminant	Landscape (2070)	Hydrological Regime (% change in CDSI)	Connectivity (2040 & 2050)	Invasive Species	Host Species Vulnerability	Overall Current Risk
NY, PA	French	E	Unknown	Moderate ↑	Moderate	Low (146.83)	Moderate	Moderate	Low	Moderate
PA	Middle Allegheny-Redbank	E	Low	Moderate ↑	Moderate	Low (193.68)	Moderate	Moderate	Low	Moderate
OH, PA, WV	Upper Ohio-Wheeling	E	Functionally Extirpated	High ↑	Moderate	Low (147.62)	Moderate	Moderate	Low	High
OH, WV	Little Muskingum-Middle Island	E	Moderate	Moderate ↑	Moderate	Low (156.57)	Moderate	Moderate	Low	Moderate
OH, WV	Upper Ohio-Shade	E	Unknown	High ↑	Moderate	Low (116.81)	**High**	Moderate	Moderate	High
WV	Little Kanawha	E	Low	Moderate ↑	Moderate	Low (109.46)	Moderate	Moderate	Low	Moderate
OH	Tuscarawas	PE	Unknown	High ↑	High	Low (159.93)	Moderate	Moderate	Moderate	High
WV	Lower Kanawha	E	Unknown	Moderate ↑	Moderate	Low (150.22)	Moderate	Moderate	Low	Moderate
OH	Upper Scioto	E	Unknown	High ↑	High	Low (113.85)	Moderate	Moderate	Moderate	High
KY, OH	Lower Scioto	PE	Unknown	High ↑	**High**	Moderate (73.99)	Moderate	Moderate	Moderate	High
KY, OH, WV	Little Scioto-Tygarts	PE	Unknown	High ↑	**High**	Moderate (86.24)	Moderate	Moderate	Moderate	High
KY	Little Sandy	PE	Unknown	High ↑	Moderate	Moderate (96.41)	Moderate	Moderate	Moderate	High
KY, OH	Ohio Brush-Whiteoak	E	Unknown	High ↑	Moderate	Moderate (63.53)	Moderate	Moderate	Moderate	High
IN, KY, OH	Middle Ohio-Laughery	E	Unknown	High ↑	**High**	Moderate (66.6)	Moderate	Moderate	Moderate	High

Table F.4. (continued) Scenario 1 future projections for risk factors for extant and presumed extant populations of Salamander Mussel in the Ohio basin. Bold text indicates changes from the current condition risk factor condition. (E= extant, PE = presumed extant)

State	HUC8 Name	Status	Current Demographic Condition	Contaminant	Landscape (2070)	Hydrological Regime (% change in CDSI)	Connectivity (2040 & 2050)	Invasive Species	Host Species Vulnerability	Overall Current Risk
KY	Licking	E	Low	High ↑	Moderate	Moderate (111.91)	Moderate	Moderate	Moderate	High
KY	South Fork Licking	E	Unknown	High ↑	High	Moderate (122.3)	Low	Moderate	Moderate	High
KY	Middle Fork Kentucky	PE	Unknown	High ↑	Low	Moderate (215.9)	Moderate	Low	Moderate	High
KY	Upper Kentucky	PE	Unknown	High	Moderate	Moderate (192.32)	Moderate	Moderate	Moderate	High
KY	Lower Kentucky	E	Unknown	Moderate ↑	Moderate	Moderate (145.27)	Moderate	Moderate	Moderate	Moderate
KY	Upper Green	PE	Unknown	High ↑	Moderate	Moderate (223.08)	Moderate	Moderate	Moderate	High
KY	Rough	PE	Unknown	High ↑	**Moderate**	Moderate (129.08)	Moderate	Moderate	Moderate	High
IN	Eel/05120104	E	Unknown	High ↑	High	Moderate (65.78)	Low	Moderate	Moderate	High
IN	Tippecanoe	E	Unknown	High ↑	**High**	Moderate (54.7)	Moderate	Moderate	Moderate	High
IN	Wildcat	E	Unknown	High ↑	High	Moderate (51.5)	Low	Low	Moderate	High
IL, IN	Middle Wabash-Little Vermillion	E	Unknown	High ↑	Moderate	Moderate (27.8)	Moderate	Moderate	Moderate	High
IL, IN	Vermillion	E	Unknown	Moderate ↓	Moderate	Moderate (8.85)	Low	Moderate	Moderate	Moderate
IN	Sugar	E	Unknown	Moderate ↓	Moderate	Moderate (34.51)	Low	Moderate	Moderate	Moderate
IN	Eel/05120203	PE	Unknown	Moderate ↑	High	Moderate (54.98)	Moderate	Low	Moderate	High

Table F.4. (continued) Scenario 1 future projections for risk factors for extant and presumed extant populations of Salamander Mussel in the Ohio basin. Bold text indicates changes from the current condition risk factor condition. (E= extant, PE = presumed extant)

State	**HUC8 Name**	**Status**	**Current Demographic Condition**	**Contaminants**	**Landscape (2070)**	**Hydrological Regime (% change in CDSI)**	**Connectivity (2040 & 2050)**	**Invasive Species**	**Host Species Vulnerability**	**Overall Current Risk**
IN	Muscatatuck	E	Functionally Extirpated	High ↑	Moderate	Moderate (80.03)	Moderate	Moderate	Moderate	High
IN	Lower East Fork White	E	Unknown	High ↑	**High**	Moderate (57.97)	Moderate	Moderate	Moderate	High
TN	Collins	E	Unknown	High ↑	**High**	Moderate (74.86)	Moderate	Low	High	High
TN	Harpeth	E	Unknown	High ↑	**High**	Moderate (176.57)	High	Low	High	High
KY	Salt	E	Unknown	High ↑	High	Moderate (117.53)	Moderate	Moderate	Moderate	High
KY	Rolling Fork	E	Unknown	High ↑	Moderate	Moderate (169.82)	Moderate	Moderate	Moderate	High
IL, KY	Lower Ohio-Bay	PE	Unknown	High ↑	Moderate	Moderate (97.23)	Moderate	Moderate	Moderate	High

Tennessee Basin Representation Unit

The two populations in the Tennessee basin have unknown demographic conditions (Figure 5.1, Table F.5). However, based on the overall risk levels in the Tennessee basin, we can assume that the Upper and Lower Duck populations will at best in a low demographic condition and facing high risk into the future. We highlight the projected changes for populations within the Tennessee basin for Scenario 1 in the following paragraphs and Table F.6.

The Upper Duck population in the Tennessee basin is projected in Scenario 1 as high risk for contaminants from the projected increase in ammonia and chloride and a decrease in lead and copper in the future. Due to the projected increase in ammonia and chloride and a decrease in lead and copper, the Lower Duck population is projected to be at a moderate risk in the future for contaminants. Nitrate, copper, and lead are currently moderate and seem to be drivers of the overall risk for contaminants for this population. We do not have the data to project whether the increase or decrease for the four chemicals would meet or exceed threshold levels, thereby affecting the overall risk category. Instead, we were able to project only whether the contaminant risk would be increasing or decreasing for a specific chemical. However, given that the Upper Duck population is already at high risk and ammonia and chloride are increasing, the risk would remain high for this population even if we did know the potential increase with respect to thresholds.

Projected landscape changes for the Upper and Lower Duck populations increase the risk level of these populations from moderate to high. The Lower Duck population is projected to have the percent agriculture changed from a low to a high risk into the future. Both populations are projected to have a decrease in vegetative cover in the riparian area.

Both populations experience moderate risk for hydrological regime with a CDSI increase of 82.71% for the Upper Duck population and 140.65% for the Lower Duck population.

There was no change in the projected risk resulting from invasive species, connectivity, or host species vulnerability.

Oil and gas supply are projected to increase in areas that already have infrastructure. The Upper and Lower Duck populations are at high risk for catastrophic events for oil and gas. Scenario 1 projects an increase in production and consumption, therefore increasing the risk of an event. We project no change in risk for catastrophic events related to coal because both populations remain at a low risk.

Table F.5. Summary of projected demographic condition for Salamander Mussel extant and presumed extant populations given Scenario 1 and Scenario 2 risk levels within the Tennessee basin.

Projected Demographic Condition – Tennessee basin	**Current Condition HUC8 Count**	**Projected HUC8 Count (2040–2070)**
High		
Moderate		
Low		
Functionally Extirpated		
Extirpated		
Unknown	2	2
Total	2	2

Table F.6. Scenario 1 future projections for risk factors for extant and presumed extant populations of Salamander Mussel in the Tennessee basin. Bold text indicates changes from the current condition risk factor condition. (E= extant, PE = presumed extant)

State	HUC8 Name	Status	Current Demographic Condition	Contaminant	Landscape (2070)	Hydrological Regime (% change in CDSI)	Connectivity (2040 & 2050)	Invasive Species	Host Species Vulnerability	Overall Current Risk
TN	Upper Duck	E	Unknown	High ↑	**High**	Moderate (82.71)	High	Moderate	High	High
TN	Lower Duck	E	Unknown	Moderate ↑	**High**	Moderate (140.65)	High	Moderate	High	High

Upper Mississippi Basin Representation Unit

Based on the overall risk levels in the Upper Mississippi basin, we project the Black population will be functionally extirpated given the moderate demographic condition and high risk to this population into the future (Figure 5.1, Table F.7). We expect the Lower St. Croix, Eau Claire, Lake Dubay, Castle Rock, and Lower Wisconsin populations will be extirpated given that they are either currently functionally extirpated or low demographic condition and facing high risk into the future (Figure 5.1, Table F.7). The Lower Chippewa population is projected to be in low condition into the future given that the demographic condition is high, but the population is facing a high risk into the future (Figure 5.1, Table F.7). There are ten populations that are categorized as unknown for demographic population condition, and therefore we were unable to project their demographic population condition into the future. However, all ten populations experience high risk. Therefore, we can assume even if they are in high demographic population condition now (which is unlikely), they would be at most in low condition into the future. We highlight the projected changes for populations within the Upper Mississippi basin for Scenario 1 in the following paragraphs and Table F.8.

All populations in the Upper Mississippi basin are projected in Scenario 1 to continue to remain at high risk for contaminants. In almost all populations within this basin, there is an increase in ammonia and chloride levels. The Twin Cities, Kankakee, and Upper Sangamon populations are projected to decrease in ammonia and chloride. There is a mix of populations that are increasing and decreasing for copper and lead. All populations continue to be at high risk for contaminants as nitrate levels remain high in the majority of the populations and appear to be the driver for the high contaminant risk in this basin. We do not have the data to determine whether increases in the four contaminants meets the threshold for high risk into the future. Instead, we were able to project only whether the contaminant risk would be increasing or decreasing for a specific chemical.

The Twin Cities is the only population at high risk due to landscape factors. Fourteen populations are at moderate risk, and two are at low risk due to landscape factors in the Upper Mississippi basin. The overall risk did not change for any of these populations from the overall risk currently posed by these landscape factors and the overall risk in Scenario 1 that is projected into the future. In a small number of these populations, the risk posed by an individual metric did change; but in these cases, it did not change the overall risk posed by landscape factors to the population.

The Coon-Yellow population increased from a moderate risk to high in the overall connectivity risk factor. This is due to an increase in unpaved road density moving from a low risk to a moderate risk from a 27.3% increase.

The Twin Cities, South Fork, and Castle Rock populations all experience a low risk with a wide range in the CDSI. Twin Cities population is projected to have an increase in 139.94% and the Castle Rock population an increase of 119.23%. However, the South Fork population is projected to have a decreased drought severity of –4.73%. The other fourteen population experience moderate risk with a CDSI increase ranging from 13.42% - 220.11% averaging an increase of 82.71%.

Oil and gas supply are projected to increase in areas that already have infrastructure. All populations in the Upper Mississippi basin are at high risk for catastrophic events for oil and gas. Scenario 1 projects an increase in production and consumption and therefore increases the risk of an event. We project no change in risk for catastrophic events related to coal given all the populations within the Upper Mississippi basin are at a low risk.

Table F.7. Summary of projected demographic condition for Salamander Mussel extant and presumed extant populations given Scenario 1 and Scenario 2 risk levels within the Upper Mississippi basin.

Projected Demographic Condition – Upper Mississippi basin	Current Condition HUC8 Count	Projected HUC8 Count (2040–2070)
High	1	
Moderate	1	
Low	2	1
Functionally Extirpated	3	1
Extirpated		5
Unknown	10	10
Total	17	17

Table F.8. Scenario 1 future projections for risk factors for extant and presumed extant populations of Salamander Mussel in the Upper Mississippi basin. Bold text indicates changes from the current condition risk factor condition. (E= extant, PE = presumed extant)

State	**HUC8 Name**	**Status**	**Current Demographic Condition**	**Contaminant**	**Landscape (2070)**	**Hydrological Regime (% change in CDSI)**	**Connectivity (2040 & 2050)**	**Invasive Species**	**Host Species Vulnerability**	**Overall Current Risk**
MN	Twin Cities	E	Unknown	High ↓	High	Low (139.94)	Moderate	Moderate	Low	High
MN	Middle Minnesota	E	Unknown	High ↑	Moderate	Moderate (138.77)	Moderate	Moderate	Low	High
MN, WI	Upper St. Croix	PE	Unknown	High ↑	Low	Moderate (99.89)	High	Moderate	High	High
MN, WI	Lower St. Croix	E	Low	High ↑	Moderate	Moderate (95.31)	Moderate	Moderate	Low	High
WI	Black	E	Moderate	High ↑	Moderate	Moderate (63.8)	High	Moderate	High	High
WI	South Fork Flambeau	PE	Unknown	High ↑	Low	Low (-4.73)	Moderate	Moderate	High	High
WI	Lower Chippewa	E	High	High ↑	Moderate	Moderate (41.97)	Moderate	Moderate	High	High
WI	Eau Claire	E	Functionally Extirpated	High ↑	Moderate	Moderate (35.66)	Moderate	Moderate	High	High
IA, MN, WI	Coon-Yellow	PE	Unknown	High ↑	Moderate	Moderate (196.89)	**High**	Moderate	High	High
IA, WI	Grant-Little Maquoketa	E	Unknown	High ↑	Moderate	Moderate (220.11)	Low	Moderate	Moderate	High
WI	Lake Dubay	E	Functionally Extirpated	High ↑	Moderate	Moderate (54.94)	High	Moderate	High	High

Table F.8. (continued) Scenario 1 future projections for risk factors for extant and presumed extant populations of Salamander Mussel in the Upper Mississippi basin. Bold text indicates changes from the current condition risk factor condition. (E= extant, PE = presumed extant)

State	HUC8 Name	Status	Current Demographic Condition	Contaminant	Landscape (2070)	Hydrological Regime (% change in CDSI)	Connectivity (2040 & 2050)	Invasive Species	Host Species Vulnerability	Overall Current Risk
WI	Castle Rock	E	Functionally Extirpated	High ↑	Moderate	Low (119.23)	Moderate	Moderate	High	High
WI	Lower Wisconsin	E	Low	High ↑	Moderate	Moderate (196.27)	Moderate	Moderate	High	High
IL, IN, MI	Kankakee	E	Unknown	High ↓	Moderate	Moderate (31.98)	Moderate	Moderate	Moderate	High
IL	Upper Sangamon	E	Unknown	High ↑	Moderate	Moderate (13.42)	Low	Moderate	Moderate	High
MO	Meramec	PE	Unknown	High ↑	Moderate	Moderate (73.29)	High	Moderate	Moderate	High
MO	Bourbeuse	PE	Unknown	High ↑	Moderate	Moderate (71.5)	High	Moderate	Moderate	High

Arkansas-White-Red Basin Representation Unit

The Spring population is unknown for demographic condition; therefore, we were unable to project demographic condition into the future (Figure 5.1, Table F.9). However, based on the overall risk levels in the Arkansas-White-Red basin, we project that the Spring population will be at most be in a low demographic condition and facing high risk into the future. We highlight the projected changes for the population within the Arkansas-White-Red basin for Scenario 1 in the following paragraphs and Table F.10.

The Spring population is projected in Scenario 1 as moderate risk for contaminants into the future, though there is expected to be an increase in ammonia, chloride, lead, and copper. Nitrate seems to be the driver of the moderate risk for this population. We do not have the data to determine whether increases or decreases in the four chemicals would meet or exceed threshold levels, thereby affecting the overall risk category. Instead, we were able to project only whether the contaminant risk would be increasing or decreasing for a specific chemical.

The Spring population is projected to have the CDSI increase by over 100% into the future, increasing the risk of drought to Salamander Mussel. However, we cannot determine if this increase would move the risk factor into the high threshold.

Oil and gas supply are projected to increase in areas that already have infrastructure. The Spring population is at high risk for catastrophic events for oil and gas. Scenario 1 projects an increase in production and consumption and therefore increases the risk of an event. The Spring population is at high catastrophic risk due to coal activities in the future, but this is not a change from current condition.

Table F.9. Summary of projected demographic condition for Salamander Mussel extant and presumed extant populations given Scenario 1 and Scenario 2 risk levels within the Arkansas-White-Red basin.

Projected Demographic Condition – Arkansas-White-Red basin	**Current Condition HUC8 Count**	**Projected HUC8 Count (2040–2070)**
High		
Moderate		
Low		
Functionally Extirpated		
Extirpated		
Unknown	1	1
Total	1	1

Table F.10. Scenario 1 future projections for risk factors for extant and presumed extant populations of Salamander Mussel in the Arkansas-White-Red basin. Bold text indicates changes from the current condition risk factor condition. (E= extant, PE = presumed extant)

State	**HUC8 Name**	**HUC8 Code**	**Status**	**Current Demographic Condition**	**Contaminant**	**Landscape (2070)**	**Hydrological Regime (% change in CDSI)**	**Connectivity (2040 & 2050)**	**Invasive Species**	**Host Species Vulnerability**	**Overall Current Risk**
AR, MO	Spring	11010010	PE	Unknown	Moderate ↑	Moderate	Moderate (101.8)	High	Moderate	Moderate	High

Scenario 2

Great Lakes Basin Representation Unit

Based on the overall risk levels in the Great Lake basin, we project the same demographic population conditions as Scenario 1 (Figure 5.2, Table F.1). The Duck-Pensaukee population is projected to be in moderate risk in Scenario 2; however, this still leads to the projection of the population to be extirpated. The remaining six populations in the Great Lakes basin have unknown demographic population condition currently (Figure 5.2, Table F.1). However, given they experience high risk, we can assume they will not be in any better condition than low in Scenario 2. We highlight the projected changes for populations within the Great Lakes basin for Scenario 2 in the following paragraphs and Table F.11.

Ammonia, chloride, copper, and lead levels are projected to increase in all occupied populations, with the exception of one, in the Great Lakes basin in Scenario 2. The Blanchard population increases in chloride and ammonia, but not copper and lead in Scenario 2. All populations, except the Duck-Pensaukee, which had incomplete data for current condition, remain at a high level of risk for contaminants as nitrate levels remain high for Wolf, Clinton, Blanchard, and Grand populations. Chloride is the driving risk and increasing in St. Clair, Clinton, and St. Joseph populations in addition to copper, which is also high in the Clinton and increasing. Lake St. Clair population is at a moderate level of risk due to levels of nitrate but increasing for all four chemicals.

There is no change in the risk category as a result of landscape factors based on current condition risk factor analysis and projections for Scenario 2 for any of the Great Lakes basin populations. In a small number of these populations, the risk posed by an individual metric did change, but in these cases, it did not change the overall risk posed by landscape factors to the population.

The Chautauqua-Conneaut population decreased from a moderate risk to low risk in the overall connectivity risk factor. This is due to the projected removal of four dams within the population from moderate to low risk to a high risk from a 27.3% increase.

Six of the populations experience low risk for drought, with the CDSI actually projected to have a decreased drought severity for Scenario 2. These six populations have a projected percent decrease ranging from -10.15% to -80.92%. The Wolf population has a moderate risk and a CDSI percent decrease of 72.98%. The St. Joseph population is projected to be a moderate risk and has a CDSI percent decrease of 40.72%.

Lampricide treatment is expected to remain unchanged from the current treatment intensity and frequency; therefore, no change is expected from the current risk posed by lamprey control. However, The Duck Pensaukee population moved from a high to moderate risk given that mudpuppy conservation is expected to improve within Scenario 2. This change resulted in a change to the overall current risk for this population to moderate. The Wolf population also moved from high to moderate risk based on increased conservation, but it did not change the overall risk for the population. The St. Clair, Clinton, St. Joseph, and Blanchard populations all were at a moderate risk and moved to a low risk, though not changing the overall condition for the populations. The Grand population moved from a high risk to a low risk, and the Chautauqua-Conneaut population remained at a high risk.

Oil and gas supply are projected to decrease in Scenario 2 leading to a decrease in the frequency and intensity of potential catastrophic events. However, the infrastructure is still present. Despite the projection that oil and gas production will decrease, all populations in the Great Lakes basin are at high risk for catastrophic events from oil and gas, based on the thresholds we set for presence/absence of oil and gas infrastructure. We project a decrease in coal activity in Scenario 2 but no change in risk for catastrophic events related to coal, given that all populations within the Great Lakes basin are currently at low risk.

Table F.11. Scenario 2 future projections for risk factors for extant and presumed extant populations of Salamander Mussel in the Great Lakes basin. Bold text indicates changes from the current condition risk factor condition.(CN = Canada; E= extant, PE = presumed extant)

State	**HUC8 Name**	**Status**	**Current Demographic Condition**	**Contaminant**	**Landscape (2070)**	**Hydrological Regime (% change in CDSI)**	**Connectivity (2040 & 2050)**	**Invasive Species**	**Host Species Vulnerability**	**Overall Current Risk**
WI	Duck-Pensaukee	E	Functionally Extirpated	Low* ↑	Moderate	Low (-80.92)	Low	Moderate	**Moderate**	**Moderate**
WI	Wolf	PE	Unknown	High ↑	Moderate	Moderate (-72.98)	Moderate	Moderate	**Moderate**	High
CN, MI	St. Clair	E	Functionally Extirpated	High ↑	High	Low (-13.83)	Low	Moderate	**Low**	High
CN, MI	Lake St. Clair	E	Low							Unknown
MI	Clinton	E	Functionally Extirpated	High ↑	High	Low (-24.48)	Moderate	Moderate	**Low**	High
IN, MI, OH	St. Joseph	E	Unknown	High ↑	High	Moderate (-40.72)	Moderate	Moderate	**Low**	High
OH	Blanchard	E	Unknown	High ↑	High	Low (-39)	Low	Moderate	**Low**	High
OH	Grand	PE	Unknown	High ↑	Moderate	Low (-10.15)	Moderate	Low	**Low**	High
NY, OH, PA	Chautauqua-Conneaut	E	Unknown	High ↑	Moderate	Low (17.27)	**Low**	Moderate	High	High
CN, NY	Niagara	E	Unknown							Unknown
CN	Lower Grand	E	Functionally Extirpated							Unknown

Ohio Basin Representation Unit

Based on the overall risk levels in the Ohio basin, we project the same projected demographic conditions as Scenario 1 (Figure 5.2, Table F.3). Twenty-nine populations are in unknown demographic condition (Figure 5.2, Table F.3). Therefore, we could not project their condition into the future. However, in Scenario 2, twenty-three of these populations are facing high overall risk into the future and therefore we could assume they would be at most in low condition given our rule set. Lastly, the Sugar, Vermillion, Lower Kentucky, Lower Kanawha, French, and Eel populations are unknown for current demographic population condition, however, because they experience moderate risk, we can assume they would be in moderate demographic condition into the future at most. We highlight the projected changes for populations within the Ohio basin for Scenario 2 in the following paragraphs and F.12.

In the Ohio basin in Scenario 2, ammonia and chloride levels are projected to increase in almost all occupied populations, except for a projected decrease in the Middle Fork Kentucky, Lower Kentucky, Sugar, and Collins populations. Copper and lead levels are projected to decrease in 6 populations and increase in 29. Most of the populations continue to be at high risk for contaminants, except for the following populations, which are projected to be at moderate risk for contaminants: the French, Middle Allegheny, Little Muskingham-Middle Island, Little Kanawha, Lower Kanawha, Lower Kentucky, Vermillion, Sugar, and Eel populations. The Lower Kentucky and Sugar are the only populations currently at moderate risk and do not show an increase for all four contaminants, however, we are unable to determine if based on this decrease in the four contaminants into the future if this would change the risk category for these two populations.

The Lower Scioto and Harpeth populations’ risk increased from moderate to high for landscape based on an increase in percent agriculture and percent of vegetative cover in the riparian buffer. The Rough population changed from a low to a moderate risk based on landscape factors. The Eel is the only population that decreased in risk from high to moderate based on a decrease in risk due to the percent vegetative cover in the riparian buffer. The Rough population experiences changes in landscape levels that increase the risk from low to moderate based on the percent change in agriculture and urbanization. The projected risk in Scenario 2 for all other populations in the Ohio basin stayed the same as the risk for current condition for landscape factors.

Nine of the populations experience low risk for drought. Of these nine populations, the Tuscarawas population had a decrease of 3.15% in the drought severity index, and the Upper Scioto population decreased in severity by 26.46%. The range of the increase in drought severity index for the low-risk populations was between 2.47% and 125.67%. The other twenty-six populations experience moderate risk with a CDSI ranging from a decrease of 3.17% to an increase up to 150.89%.

In Scenario 2, mudpuppy conservation efforts are projected to improve. This led to host species vulnerability decreasing to a low risk for 33 of the 35 populations. The Collins and Harpeth populations moved from a high risk to a moderate risk.

Oil and gas supply as well as coal mining activities are projected to decrease in Scenario 2 leading to decrease in the frequency and intensity of potential catastrophic events. However, the infrastructure is still present. All populations in the Ohio basin are at high risk for catastrophic events for oil and gas, with no change projected for Scenario 2. Almost all populations are at low risk of catastrophic event due to coal activity. Once again while coal activities will decrease, the infrastructure is still present, which is why for some of the populations the risk remains high despite a decrease in production/activity.

Table F.12. Scenario 2 future projections for risk factors for extant and presumed extant populations of Salamander Mussel in the Ohio basin. Bold text indicates changes from the current condition risk factor condition. (E= extant, PE = presumed extant)

State	HUC8 Name	Status	Current Demographic Condition	Contaminant	Landscape (2070)	Hydrological Regime (% change in CDSI)	Connectivity (2040 & 2050)	Invasive Species	Host Species Vulnerability	Overall Current Risk
NY, PA	French	E	Unknown	Moderate ↑	Moderate	Low (2.47)	Moderate	Moderate	Low	Moderate
PA	Middle Allegheny-Redbank	E	Low	Moderate ↑	Moderate	Low (104.9)	Moderate	Moderate	Low	Moderate
OH, PA, WV	Upper Ohio-Wheeling	E	Functionally Extirpated	High ↑	Moderate	Low (74.07)	Moderate	Moderate	Low	High
OH, WV	Little Muskingum-Middle Island	E	Moderate	Moderate ↑	Moderate	Low (100.09)	Moderate	Moderate	Low	Moderate
OH, WV	Upper Ohio-Shade	E	Unknown	High ↑	Moderate	Low (83.73)	Moderate	Moderate	**Low**	High
WV	Little Kanawha	E	Low	Moderate ↑	Moderate	Low (123.46)	Moderate	Moderate	Low	Moderate
OH	Tuscarawas	PE	Unknown	High ↑	High	Low (-3.15)	Moderate	Moderate	**Low**	High
WV	Lower Kanawha	E	Unknown	Moderate ↑	Moderate	Low (125.67)	Moderate	Moderate	Low	Moderate
OH	Upper Scioto	E	Unknown	High ↑	High	Low (-26.46)	Moderate	Moderate	**Low**	High
KY, OH	Lower Scioto	PE	Unknown	High ↑	**High**	Moderate (28.13)	Moderate	Moderate	**Low**	High
KY, OH, WV	Little Scioto-Tygarts	PE	Unknown	High ↑	Moderate	Moderate (70.82)	Moderate	Moderate	**Low**	High
KY	Little Sandy	PE	Unknown	High ↑	Moderate	Moderate (88.97)	Moderate	Moderate	**Low**	High
KY, OH	Ohio Brush-Whiteoak	E	Unknown	High ↑	Moderate	Moderate (48.44)	Moderate	Moderate	**Low**	High

Table F.12. (continued) Scenario 2 future projections for risk factors for extant and presumed extant populations of Salamander Mussel in the Ohio basin. Bold text indicates changes from the current condition risk factor condition. (E= extant, PE = presumed extant)

State	HUC8 Name	Status	Current Demographic Condition	Contaminant	Landscape (2070)	Hydrological Regime (% change in CDSI)	Connectivity (2040 & 2050)	Invasive Species	Host Species Vulnerability	Overall Current Risk
IN, KY, OH	Middle Ohio-Laughery	E	Unknown	High ↑	Moderate	Moderate (12.75)	Moderate	Moderate	**Low**	High
KY	Licking	E	Low	High ↑	Moderate	Moderate (70.68)	Moderate	Moderate	**Low**	High
KY	South Fork Licking	E	Unknown	High ↑	High	Moderate (69.81)	Low	Moderate	**Low**	High
KY	Middle Fork Kentucky	PE	Unknown	High ↓	Low	Moderate (150.89)	Moderate	Low	**Low**	High
KY	Upper Kentucky	PE	Unknown	High ↑	Moderate	Moderate (116.18)	Moderate	Moderate	**Low**	High
KY	Lower Kentucky	E	Unknown	Moderate ↓	Moderate	Moderate (90.91)	Moderate	Moderate	**Low**	Moderate
KY	Upper Green	PE	Unknown	High ↑	Moderate	Moderate (97.82)	Moderate	Moderate	**Low**	High
KY	Rough	PE	Unknown	High ↑	Moderate	Moderate (91.44)	Moderate	Moderate	**Low**	High
IN	Eel/05120104	E	Unknown	High ↑	High	Moderate (-29.4)	Low	Moderate	**Low**	High
IN	Tippecanoe	E	Unknown	High ↑	Moderate	Moderate (-9.76)	Moderate	Moderate	**Low**	High
IN	Wildcat	E	Unknown	High ↑	High	Moderate (-29.56)	Low	Low	**Low**	High
IL, IN	Middle Wabash-Little Vermillion	E	Unknown	High ↑	Moderate	Moderate (-9.39)	Moderate	Moderate	**Low**	High
IL, IN	Vermillion	E	Unknown	Moderate ↑	Moderate	Moderate (-3.18)	Low	Moderate	**Low**	Moderate

Table F.12. (continued) Scenario 2 future projections for risk factors for extant and presumed extant populations of Salamander Mussel in the Ohio basin. Bold text indicates changes from the current condition risk factor condition. (E= extant, PE = presumed extant)

State	HUC8 Name	Status	Current Demographic Condition	Contaminant	Landscape (2070)	Hydrological Regime (% change in CDSI)	Connectivity (2040 & 2050)	Invasive Species	Host Species Vulnerability	Overall Current Risk
IN	Sugar	E	Unknown	Moderate ↓	Moderate	Moderate (-35.11)	Low	Moderate	**Low**	Moderate
IN	Eel/05120203	PE	Unknown	Moderate ↑	**Moderate**	Moderate (-27.62)	Moderate	Low	**Low**	**Moderate**
IN	Muscatatuck	E	Functionally Extirpated	High ↑	Moderate	Moderate (-3.17)	Moderate	Moderate	**Low**	High
IN	Lower East Fork White	E	Unknown	High ↑	Moderate	Moderate (9.58)	Moderate	Moderate	**Low**	High
TN	Collins	E	Unknown	High ↓	Moderate	Moderate (100.96)	Moderate	Low	**Moderate**	High
TN	Harpeth	E	Unknown	High ↑	**High**	Moderate (130.26)	High	Low	**Moderate**	High
KY	Salt	E	Unknown	High ↑	High	Moderate (82.26)	Moderate	Moderate	**Low**	High
KY	Rolling Fork	E	Unknown	High ↑	Moderate	Moderate (111.23)	Moderate	Moderate	**Low**	High
IL, KY	Lower Ohio-Bay	PE	Unknown	High ↑	Moderate	Moderate (88.52)	Moderate	Moderate	**Low**	High

Tennessee Basin Representation Unit

The two populations in the Tennessee basin have unknown demographic conditions (Figure 5.2, Table F.5). However, based on the overall risk levels in the Tennessee basin, we can assume that the Upper and Lower Duck populations will at best in a low demographic condition and facing high risk into the future. We highlight the projected changes for populations within the Tennessee basin for Scenario 2 in the following paragraphs and Table F.13.

The Upper Duck population in the Tennessee basin is projected in Scenario 2 as high risk for contaminants into the future. There is expected to be an increase in ammonia, chloride, lead, and copper. The lower Duck population is projected to be at a moderate risk into the future for contaminants. Nitrate, copper, and lead are currently moderate and seem to be drivers of the overall risk for contaminants for this population. Given that the Upper Duck population is already at high risk and all four chemicals are increasing, the risk would remain high for this population even if we did know the potential increase with respect to thresholds.

The Upper and Lower Duck populations remain at a moderate risk into the future for landscape risk factors. The only change that resulted when we evaluated the individual landscape metrics for the Lower Duck population is that the percent agriculture changed from a low to a high risk into the future; however, this did not change the overall risk into the future.

The Upper Duck population is at a moderate risk based on evaluation of the hydrological regime with a projected increase in risk as a result of drought severity of 91.11%. The percent increase in drought risk severity for the Lower Duck population is 128.22%.

The host specific vulnerability risk changed from high to moderate based on increased mudpuppy conservation efforts for Scenario 2 for both the Upper and Lower Duck populations.

Oil and gas supply as well as coal mining activities are projected to decrease in Scenario 2, leading to decrease in the frequency and intensity of potential catastrophic events. The Upper and Lower Duck populations are at high risk for catastrophic events for oil and gas. We project no change in risk for catastrophic events related to coal because both populations remain at a low risk.

Table F.13. Scenario 2 future projections for risk factors for extant and presumed extant populations of Salamander Mussel in the Tennessee basin. Bold text indicates changes from the current condition risk factor condition. (E= extant, PE = presumed extant)

State	**HUC8 Name**	**Status**	**Current Demographic Condition**	**Contaminant**	**Landscape (2070)**	**Hydrological Regime (% change in CDSI)**	**Connectivity (2040 & 2050)**	**Invasive Species**	**Host Species Vulnerability**	**Overall Current Risk**
TN	Upper Duck	E	Unknown	High ↑	Moderate	Moderate (91.11)	High	Moderate	**Moderate**	High
TN	Lower Duck	E	Unknown	Moderate ↑	Moderate	Moderate (128.22)	High	Moderate	**Moderate**	High

Upper Mississippi Basin Representation Unit

Based on the overall risk levels in the Upper Mississippi basin, we project the same projected demographic conditions as Scenario 1 (Figure 5.2, Table F.7). There are ten populations that are categorized as unknown for demographic condition; therefore, we were unable to project their demographic condition into the future. However, all ten populations experience high risk. Therefore, we can assume even if they are in high demographic condition now (which is unlikely), they would be at most in low condition into the future. We highlight the projected changes for populations within the Upper Mississippi basin for Scenario 2 in the following paragraphs and Table F.14.

All populations in the Upper Mississippi basin are projected in Scenario 2 to continue to remain at high risk for contaminants. Ammonia, chloride, copper, and lead levels increase in almost all populations within this basin, with the exception of the Meramec population that decreases for lead and copper. All populations continue to be at high risk for contaminants as nitrate levels remain high in all but one of the populations and appear to be one of the main drivers for the high contaminant risk in this basin.

The Twin Cities is the only population at high risk due to landscape factors. Fourteen populations are at moderate risk, and two are at low risk due to landscape factors in the Upper Mississippi basin for Scenario 2. The overall risk did not change for any of these populations from the overall risk currently posed by these landscape factors. In a small number of these populations, the risk posed by an individual metric did change; but in these cases, it did not change the overall risk posed by landscape factors to the population.

The Twin Cities, South Fork, and Castle Rock populations all experience a low risk with drought severity decreasing in the Twin Cities (7.24%), South Fork (13.14%), and the Castle Rock (56.69%). The other fourteen populations experience moderate risk with a decreasing drought severity index of 12.12–59.08%, with only two populations showing an increase in drought severity of 55.19% for the Meramec population and the Bourbeuse population of 49.95%.

Host species vulnerability risk changed for Scenario 2 where mudpuppy conservation efforts are projected to improve. The Twin Cities and Middle Minnesota populations' risk stayed the same as current condition for Scenario 2. However, the nine populations in Wisconsin all moved from a high risk for current condition to a moderate risk for Scenario 2. The Grant Little Maquoketa, Kankakee, Upper Sangamon, Meramec, and Bourbeuse populations all moved from a moderate risk to a low risk for Scenario 2.

Oil and gas supply as well as coal mining activities are projected to decrease in Scenario 2, leading to a decrease in the frequency and intensity of potential catastrophic events. All populations in the Upper Mississippi basin are at high risk for catastrophic events for oil and gas. We project no change in risk for catastrophic events related to coal because all populations remain at a low risk.

Table F.14. Scenario 2 future projections for risk factors for extant and presumed extant populations of Salamander Mussel in the Upper Mississippi basin. Bold text indicates changes from the current condition risk factor condition. (E= extant, PE = presumed extant)

State	HUC8 Name	Status	Current Demographic Condition	Contaminant	Landscape (2070)	Hydrological Regime (% change in CDSI)	Connectivity (2040 & 2050)	Invasive Species	Host Species Vulnerability	Overall Current Risk
MN	Twin Cities	E	Unknown	High ↑	High	Low (-7.24)	Moderate	Moderate	**Low**	High
MN	Middle Minnesota	E	Unknown	High ↑	Moderate	Moderate (9.61)	Moderate	Moderate	**Low**	High
MN, WI	Upper St. Croix	PE	Unknown	High ↑	Low	Moderate (-26.14)	High	Moderate	**Moderate**	High
MN, WI	Lower St. Croix	E	Low	High ↑	Moderate	Moderate (-27.06)	Moderate	Moderate	Low	High
WI	Black	E	Moderate	High ↑	Moderate	Moderate (-55.58)	High	Moderate	**Moderate**	High
WI	South Fork Flambeau	PE	Unknown	High ↑	Low	Low (-13.14)	Moderate	Moderate	**Moderate**	High
WI	Lower Chippewa	E	High	High ↑	Moderate	Moderate (-49.85)	Moderate	Moderate	**Moderate**	High
WI	Eau Claire	E	Functionally Extirpated	High ↑	Moderate	Moderate (-52.87)	Moderate	Moderate	**Moderate**	High
IA, MN, WI	Coon-Yellow	PE	Unknown	High ↑	Moderate	Moderate (-12.45)	Moderate	Moderate	**Moderate**	High
IA, WI	Grant-Little Maquoketa	E	Unknown	High ↑	Moderate	Moderate (-14.41)	Low	Moderate	**Low**	High
WI	Lake Dubay	E	Functionally Extirpated	High ↑	Moderate	Moderate (-59.08)	High	Moderate	**Moderate**	High

Table F.14. (continued) Scenario 2 future projections for risk factors for extant and presumed extant populations of Salamander Mussel in the Upper Mississippi basin. Bold text indicates changes from the current condition risk factor condition.(E= extant, PE = presumed extant)

State	HUC8 Name	Status	Current Demographic Condition	Contaminant	Landscape (2070)	Hydrological Regime (% change in CDSI)	Connectivity (2040 & 2050)	Invasive Species	Host Species Vulnerability	Overall Current Risk
WI	Castle Rock	E	Functionally Extirpated	High ↑	Moderate	Low (-56.69)	Moderate	Moderate	**Moderate**	High
WI	Lower Wisconsin	E	Low	High ↑	Moderate	Moderate (-43.42)	Moderate	Moderate	**Moderate**	High
IL, IN, MI	Kankakee	E	Unknown	High ↑	Moderate	Moderate (-32.15)	Moderate	Moderate	**Low**	High
IL	Upper Sangamon	E	Unknown	High ↑	Moderate	Moderate (-12.12)	Low	Moderate	**Low**	High
MO	Meramec	PE	Unknown	High ↑	Moderate	Moderate (55.19)	High	Moderate	**Low**	High
MO	Bourbeuse	PE	Unknown	High ↑	Moderate	Moderate (49.95)	High	Moderate	**Low**	High

Arkansas-White-Red Basin Representation Unit

The Spring population is unknown for demographic condition; therefore, we cannot project demographic condition into the future (Figure 5.2, Table F.9). However, based on the overall risk levels in the Arkansas White-Red basin, we can assume that the Spring population will be at most in a low demographic condition and facing high risk into the future. We highlight the projected changes for the population within the Arkansas-White-Red basin for Scenario 2 in the following paragraphs and Table F.15.

The Spring population in the Arkansas White-Red basin is projected in Scenario 2 as moderate risk for contaminants into the future, though there is expected to be an increase in ammonia, chloride, lead, and copper. Nitrate and copper seem to be the drivers of the moderate risk for this population.

The Spring population remains at a moderate risk into the future. Although the percent agriculture did increase from moderate to high, the overall risk for landscape factors did not change in Scenario 2.

The drought severity index for the Spring population is at a moderate risk with a projected increase of 133.05% for Scenario 2.

The host species vulnerability based on a projected increase in mudpuppy conservation is projected to change from a moderate risk to a low risk into the future.

Oil and gas supply as well as coal mining activities are projected to decrease in Scenario 2 leading to decrease in the frequency and intensity of potential catastrophic events. The Spring population is at high risk for catastrophic events for oil and gas. We project no change in risk for catastrophic events related to coal because the Spring population remains at low risk.

Table F.15. Scenario 2 future projections for risk factors for extant and presumed extant populations of Salamander Mussel in th Arkansas-White-Red basin. Bold text indicates changes from the current condition risk factor condition.(E= extant, PE = presumed extant)

State	**HUC8 Name**	**Status**	**Current Demographic Condition**	**Contaminants**	**Landscape (2070)**	**Hydrological Regime (% change in CDSI)**	**Connectivity (2040 & 2050)**	**Invasive Species**	**Host Species Vulnerability**	**Overall Current Risk**
AR, MO	Spring	PE	Unknown	Moderate ↑	Moderate	Moderate (133.05)	High	Moderate	**Low**	High

Table F.16 Summary of current demographic population condition, with the future risk factors for Scenarios 1 and 2 and the predicted demographic population conditions.

State	HUC2 Representation Unit	HUC8 Name	HUC8 Code	Status	Current Demographic Condition	Scenario 1 Overall Risk Factor	Scenario 1 Predicted Demographic Condition	Scenario 2 Overall Risk Factor	Scenario 2 Predicted Demographic Condition
AR, MO	Arkansas-White-Red	Spring	11010010	PE	Unknown	High	Unknown	High	Unknown
WI	Great Lakes	Duck-Pensaukee	04030103	E	Functionally Extirpated	High	Extirpated	*Moderate*	Extirpated
WI	Great Lakes	Wolf	04030202	PE	Unknown	High	Unknown	High	Unknown
CN, MI	Great Lakes	St. Clair	04090001	E	Functionally Extirpated	High	Extirpated	High	Extirpated
CN, MI	Great Lakes	Lake St. Clair	04090002	E	Low	Unknown	Unknown	Unknown	Unknown
MI	Great Lakes	Clinton	04090003	E	Functionally Extirpated	High	Extirpated	High	Extirpated
IN, MI, OH	Great Lakes	St. Joseph	04100003	E	Unknown	High	Unknown	High	Unknown
OH	Great Lakes	Blanchard	04100008	E	Unknown	High	Unknown	High	Unknown
OH	Great Lakes	Grand	04110004	PE	Unknown	High	Unknown	High	Unknown
NY, OH, PA	Great Lakes	Chautauqua-Conneaut	04120101	E	Unknown	High	Unknown	High	Unknown
CN	Great Lakes	Lower Grand	04250005	E	Functionally Extirpated	Unknown	Unknown	Unknown	Unknown
CN, NY	Great Lakes	Niagara	04120104/ 04270101	E	Unknown	Unknown	Unknown	Unknown	Unknown
NY, PA	Ohio	French	05010004	E	Unknown	Moderate	Unknown	Moderate	Unknown
PA	Ohio	Middle Allegheny-Redbank	05010006	E	Low	Moderate	Functionally Extirpated	Moderate	Functionally Extirpated

Table F.16 (continued) Summary of current demographic population condition, with the future risk factors for Scenarios 1 and 2 and the predicted demographic population conditions.

State	HUC2 Representation Unit	HUC8 Name	HUC8 Code	Status	Current Demographic Condition	Scenario 1 Overall Risk Factor	Scenario 1 Predicted Demographic Condition	Scenario 2 Overall Risk Factor	Scenario 2 Predicted Demographic Condition
OH, PA, WV	Ohio	Upper Ohio-Wheeling	05030106	E	Functionally Extirpated	High	Extirpated	High	Extirpated
OH, WV	Ohio	Little Muskingum-Middle Island	05030201	E	Moderate	Moderate	Low	Moderate	Low
OH, WV	Ohio	Upper Ohio-Shade	05030202	E	Unknown	High	Unknown	High	Unknown
WV	Ohio	Little Kanawha	05030203	E	Low	Moderate	Functionally Extirpated	Moderate	Functionally Extirpated
OH	Ohio	Tuscarawas	05040001	PE	Unknown	High	Unknown	High	Unknown
WV	Ohio	Lower Kanawha	05050008	E	Unknown	Moderate	Unknown	Moderate	Unknown
OH	Ohio	Upper Scioto	05060001	E	Unknown	High	Unknown	High	Unknown
KY, OH	Ohio	Lower Scioto	05060002	PE	Unknown	High	Unknown	High	Unknown
KY, OH, WV	Ohio	Little Scioto-Tygarts	05090103	PE	Unknown	High	Unknown	High	Unknown
KY	Ohio	Little Sandy	05090104	PE	Unknown	High	Unknown	High	Unknown
KY, OH	Ohio	Ohio Brush-Whiteoak	05090201	E	Unknown	High	Unknown	High	Unknown
IN, KY, OH	Ohio	Middle Ohio-Laughery	05090203	E	Unknown	High	Unknown	High	Unknown
KY	Ohio	Licking	05100101	E	Low	High	Extirpated	High	Extirpated
KY	Ohio	South Fork Licking	05100102	E	Unknown	High	Unknown	High	Unknown
KY	Ohio	Middle Fork Kentucky	05100202	PE	Unknown	High	Unknown	High	Unknown

Table F.16 (continued) Summary of current demographic population condition, with the future risk factors for Scenarios 1 and 2 and the predicted demographic population conditions.

State	HUC2 Representation Unit	HUC8 Name	HUC8 Code	Status	Current Demographic Condition	Scenario 1 Overall Risk Factor	Scenario 1 Predicted Demographic Condition	Scenario 2 Overall Risk Factor	Scenario 2 Predicted Demographic Condition
KY	Ohio	Upper Kentucky	05100204	PE	Unknown	High	Unknown	High	Unknown
KY	Ohio	Lower Kentucky	05100205	E	Unknown	Moderate	Unknown	Moderate	Unknown
KY	Ohio	Upper Green	05110001	PE	Unknown	High	Unknown	High	Unknown
KY	Ohio	Rough	05110004	PE	Unknown	High	Unknown	High	Unknown
IN	Ohio	Eel/05120104	05120104	E	Unknown	High	Unknown	High	Unknown
IN	Ohio	Tippecanoe	05120106	E	Unknown	High	Unknown	High	Unknown
IN	Ohio	Wildcat	05120107	E	Unknown	High	Unknown	High	Unknown
IL, IN	Ohio	Middle Wabash-Little Vermillion	05120108	E	Unknown	High	Unknown	High	Unknown
IL, IN	Ohio	Vermillion	05120109	E	Unknown	Moderate	Unknown	Moderate	Unknown
IN	Ohio	Sugar	05120110	E	Unknown	Moderate	Unknown	Moderate	Unknown
IN	Ohio	Eel/05120203	05120203	PE	Unknown	High	Unknown	*Moderate*	Unknown
IN	Ohio	Muscatatuck	05120207	E	Functionally Extirpated	High	Extirpated	High	Extirpated
IN	Ohio	Lower East Fork White	05120208	E	Unknown	High	Unknown	High	Unknown
TN	Ohio	Collins	05130107	E	Unknown	High	Unknown	High	Unknown
TN	Ohio	Harpeth	05130204	E	Unknown	High	Unknown	High	Unknown
KY	Ohio	Salt	05140102	E	Unknown	High	Unknown	High	Unknown
KY	Ohio	Rolling Fork	05140103	E	Unknown	High	Unknown	High	Unknown
IL, KY	Ohio	Lower Ohio-Bay	05140203	PE	Unknown	High	Unknown	High	Unknown
TN	Tennessee	Upper Duck	06040002	E	Unknown	High	Unknown	High	Unknown
TN	Tennessee	Lower Duck	06040003	E	Unknown	High	Unknown	High	Unknown

Table F.16 (continued) Summary of current demographic population condition, with the future risk factors for Scenarios 1 and 2 and the predicted demographic population conditions.

State	HUC2 Representation Unit	HUC8 Name	HUC8 Code	Status	Current Demographic Condition	Scenario 1 Overall Risk Factor	Scenario 1 Predicted Demographic Condition	Scenario 2 Overall Risk Factor	Scenario 2 Predicted Demographic Condition
MN	Upper Mississippi	Twin Cities	07010206	E	Unknown	High	Unknown	High	Unknown
MN	Upper Mississippi	Middle Minnesota	07020007	E	Unknown	High	Unknown	High	Unknown
MN, WI	Upper Mississippi	Upper St. Croix	07030001	PE	Unknown	High	Unknown	High	Unknown
MN, WI	Upper Mississippi	Lower St. Croix	07030005	E	Low	High	Extirpated	High	Extirpated
WI	Upper Mississippi	Black	07040007	E	Moderate	High	Functionally Extirpated	High	Functionally Extirpated
WI	Upper Mississippi	South Fork Flambeau	07050003	PE	Unknown	High	Unknown	High	Unknown
WI	Upper Mississippi	Lower Chippewa	07050005	E	High	High	Low	High	Low
WI	Upper Mississippi	Eau Claire	07050006	E	Functionally Extirpated	High	Extirpated	High	extirpated
IA, MN, WI	Upper Mississippi	Coon-Yellow	07060001	PE	Unknown	High	Unknown	High	Unknown
IA, WI	Upper Mississippi	Grant-Little Maquoketa	07060003	E	Unknown	High	Unknown	High	Unknown
WI	Upper Mississippi	Lake Dubay	07070002	E	Functionally Extirpated	High	Extirpated	High	Extirpated
WI	Upper Mississippi	Castle Rock	07070003	E	Functionally Extirpated	High	Extirpated	High	Extirpated
WI	Upper Mississippi	Lower Wisconsin	07070005	E	Low	High	Extirpated	High	Extirpated

Table F.16 (continued) Summary of current demographic population condition, with the future risk factors for Scenarios 1 and 2 and the predicted demographic population conditions.

State	HUC2 Representation Unit	HUC8 Name	HUC8 Code	Status	Current Demographic Condition	Scenario 1 Overall Risk Factor	Scenario 1 Predicted Demographic Condition	Scenario 2 Overall Risk Factor	Scenario 2 Predicted Demographic Condition
IL, IN, MI	Upper Mississippi	Kankakee	07120001	E	Unknown	High	Unknown	High	Unknown
IL	Upper Mississippi	Upper Sangamon	07130006	E	Unknown	High	Unknown	High	Unknown
MO	Upper Mississippi	Meramec	07140102	PE	Unknown	High	Unknown	High	Unknown
MO	Upper Mississippi	Bourbeuse	07140103	PE	Unknown	High	Unknown	High	Unknown