Species Status Assessment Report for the Rayed Bean (*Villosa fabalis)*



Credit: G. Thomas Watters, Ohio State University

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This document was prepared by U.S. Fish and Wildlife Service (USFWS) biologist Angela Boyer (Ohio Field Office), with assistance from Jessica Pruden, Kaitlyn Kelly, 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), 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). And special thanks to all the peer reviewers: especially Megan Bradley (USFWS Genoa Fish Hatchery), Heidi Dunn (Ecoanalysts, Inc.), Robert Anderson (USFWS Pennsylvania Field Office), Kelly McNichols-O'Rourke (Fisheries and Oceans Canada), Kevin Eliason (WVDNR), and Daelyn Woolnough (Central Michigan University).

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U.S. Fish and Wildlife Service. 2022. Species status assessment report for the rayed bean (*Villosa fabalis*). May 2022 (Version 1.0). Ohio Ecological Services Field Office, Columbus, Ohio.

EXECUTIVE SUMMARY

The rayed bean is a small mussel usually less than 38 mm long (1.5 inches) and is elongate or ovate in males and elliptical in females. The life span of rayed bean is estimated to be approximately 15 years. It uses host fish to complete its reproductive cycle. The mussel is found in rivers, streams, creeks, or lakes, in areas of moderate flow, in sand and gravel substrate.

Currently the rayed bean occurs in 7 states, as well as the Canadian province of Ontario. We describe and analyze the distribution of the rayed bean 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 the rayed bean: Great Lakes, Ohio, and Tennessee. The species currently ranges across all three representation units.

We used the HUC8 at the subbasin scale to define a population of the rayed bean and conduct our current condition analysis. We analyzed current condition for extant populations (total of 19 populations). We assessed demographic population condition as high, moderate, low, or very low/functionally extirpated based on demographic criteria. For this assessment, a functionally extirpated population is defined as one that may have one or a few individuals still living but the population as too few individuals for the population to persist over time. We assigned an estimate of the probability of persistence over 30 years (approximately 2 life spans of rayed bean) for each population condition category based on the population's ability to withstand demographic stochastic events. For our current condition analysis, we also evaluated the five primary risk factors affecting the rayed bean (water quality/contaminants, hydrological regime, landscape, connectivity, and invasive species). We assigned these risk factors to three categories of high, moderate, and low risk and assigned a probability of persistence over 50 years for each of the risk categories.

The Great Lakes basin has nine extant populations; of these, eight are at high risk due to threats (one population in Canada was not able to be fully analyzed). The Ohio basin has nine extant populations, five of which are at high risk, three are at moderate risk, and one is at low risk. The Tennessee basin has one extant population which is at high 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 rayed bean 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. Under future Scenario 1, ten populations (53%) would be extirpated, two populations (11%) would be functionally extirpated, five populations (26%) would be in low demographic condition, and the

demographic condition of one population (5%) is unknown. Under future Scenario 2, nine populations (47%) would be extirpated, one population (5%) would be functionally extirpated, six populations (32%) would be in low demographic condition, two populations (11%) would be in moderate demographic condition, and the demographic condition of one population (5%) is unknown.

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CHAPTER 1. INTRODUCTION

1.1 Background

This report summarizes the results of a species status assessment (SSA) conducted for the rayed bean. 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 rayed bean, it has been prepared to inform decisions about recovery plan development and critical habitat designation. Decision makers will consider the information in this document (or referenced in this document), in combination with all relevant laws, regulations, and policies regarding those decisions. The public will be provided appropriate opportunities for input on the results of any decision.

1.2 SSA Framework and Analytical Approach

To conduct this assessment, we followed the U.S. Fish and Wildlife Service's (Service) 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 rayed bean to maintain populations in the wild over a biologically meaningful timeframe.

Using the SSA framework, we consider what the rayed bean 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 species' viability 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 species' current demographic and risk condition in terms of the 3Rs, 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 the species 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 rayed bean, including taxonomy, morphology, and 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.

2.1 Taxonomy and Genetics

The rayed bean is a member of the freshwater mussel family Unionidae and was originally described as *Unio fabalis* and accepted as *Paetulunio fabalis* by Lea in 1831 (pp. 86–87). The type locality (the location of the first identified specimen) is the Ohio River (Parmalee and Bogan 1998, p. 244), probably in the vicinity of Cincinnati, Ohio. We recognize *Unio capillus*, *U. lapillus*, and *U. donacopsis* as synonyms of *Villosa fabalis* (Butler 2002, p. 3).

2.2 Species Description

The rayed bean is a small mussel, usually less than 1.5 inches (3.8 centimeters) in length (Cummings and Mayer 1992, p. 142; Parmalee and Bogan 1998, p. 244; West et al. 2000, p. 248). The rayed bean is sexually dimorphic. The shell outline is elongate or ovate in males and elliptical in females, and moderately inflated in both sexes, but more so in females (Parmalee and Bogan 1998, p. 244). Key characters useful for distinguishing the rayed bean from other mussels are its small size, thick valves, unusually heavy teeth for a small mussel, and color pattern (Cummings and Mayer 1992, p. 142).

2.3 Species Historical Distribution

The rayed bean historically occurred in at least 115 streams, lakes, and some human-made canals in 10 States: Illinois, Indiana, Kentucky, Michigan, New York, Ohio, Pennsylvania, Tennessee, Virginia, and West Virginia; and Ontario, Canada. The mussel occurred in parts of the upper (Lake Michigan drainage) and lower Great Lakes systems, and throughout most of the Ohio and Tennessee River systems. During historical times, the rayed bean was fairly widespread and locally common in many Ohio River system streams based on collections made over a several-decade period. The species was once fairly common in the Belle, South Branch Thames (Ontario, Canada), Detroit, Scioto, Wabash, and Duck Rivers; several tributaries in the Scioto system (Olentangy River, and Big Darby and Alum Creeks); and Tippecanoe Lake, based on literature and museum records (Call 1900, p. 459; Watters 1994, p. 105; West et al. 2000, p. 251; Badra 2002, pers. comm.). The rayed bean was last reported from some streams several decades ago (North Branch Clinton, Auglaize, Ohio, West Fork, Beaver, Shenango, Mahoning, Mohican, Scioto, Green, Barren, Salamonie, White, Big Blue, Tennessee, Holston, South Fork Holston, Nolichucky, Clinch, North Fork Clinch, and Powell Rivers; Wolf, Conewango, Oil, Crooked, Pymatuning, Mill, Alum, Whetstone, Deer, Lick, and Richland Creeks; and Buckeye, Tippecanoe, Winona, and Pike Lakes). The rayed bean population in Lake Erie was once

considerable (Ohio State University Museum of Biological Diversity (OSUM) collections) but has been eliminated, at least in part, by the zebra mussel (Butler 2002, p. 6).

2.4 Individual Needs

The general biology of the rayed bean is similar to other bivalved mollusks belonging to the family Unionidae. Adults are suspension-feeders, spending their entire lives partially or completely buried within the substrate (Call 1900, p. 459; Watters 1994, p. 105; West et al. 2000, p. 251; Badra 2002, pers. comm, p. 221). Unionids have an unusual mode of reproduction. Their life cycle includes a brief, obligatory parasitic stage on fish. Eggs develop into microscopic larvae called glochidia within special gill chambers of the female mussel. 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 into the host (fish) (Haag 2012, pp. 37–42). Following successful infestation of the host, glochidia are encapsulated by the fish and drop off as newly transformed juveniles. See Figure 2.1 for the general freshwater mussel life cycle. For further information on freshwater mussels, see Gordon and Layzer (1989, pp. 1–17).

Mussel biologists know relatively little about the specific life-history requirements of the rayed bean. Most mussels, including the rayed bean, have separate sexes. The age at sexual maturity is unknown for the rayed bean, though it likely occurs around five years of age. The rayed bean is thought to be a long-term brooder as the developing glochidia remain in the gill chamber from May through October until they mature and are ready for release (Parmalee and Bogan 1998, p. 108; Ecological Specialists, Inc. (ESI) 2000, p. 5; Woolnough 2002, p. 23).

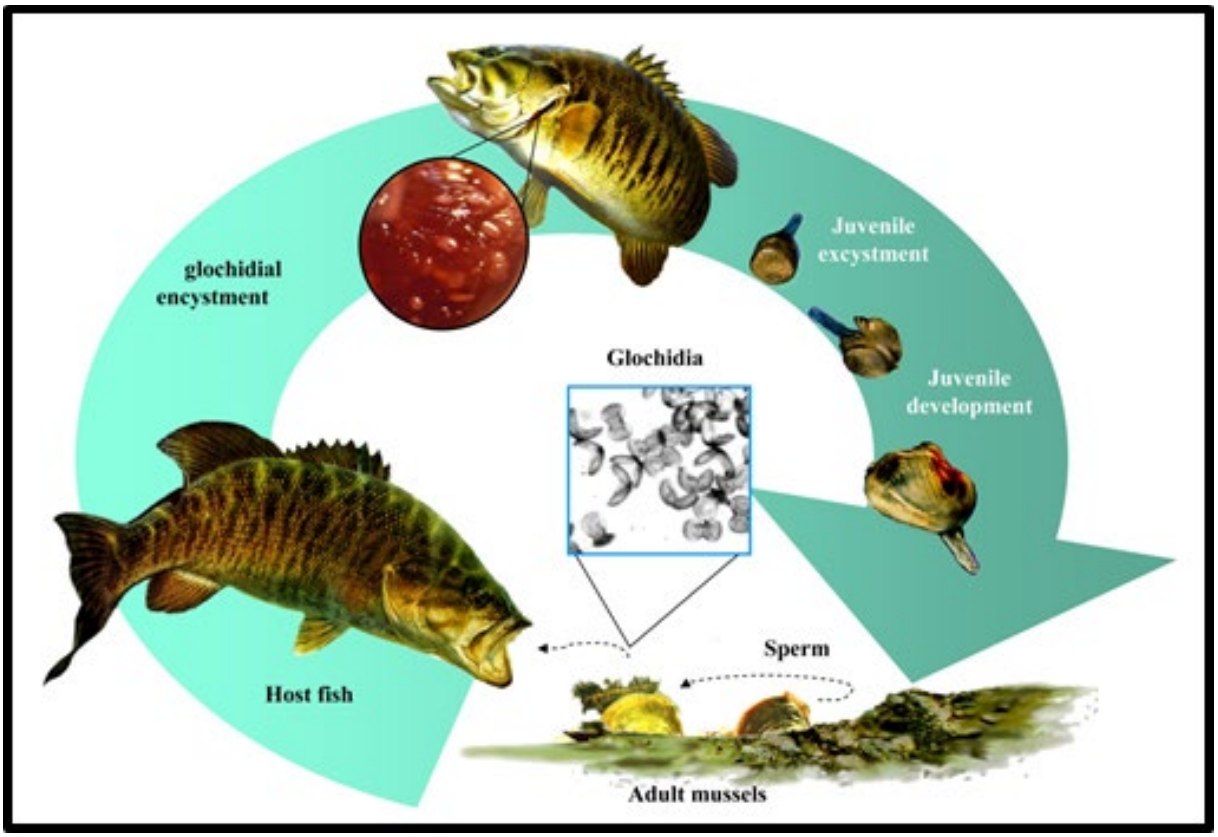


Figure 2.1. The life cycle of the rayed bean (Villosa fabalis). Credit Dr. M.C. Barnhart

Table 2.1. Individual needs for the rayed bean.

Life Stage	Resources Needed to Complete Life Stage	Source
Fertilized eggs - fall	● Clean, flowing water ● Sexually mature males upstream from sexually mature females ● Appropriate spawning temperatures.	Berg et al. 2008, p. 397 Haag 2012, pp. 38–39
Glochidia - late spring to early fall	● Clean, flowing water ● Presence of host fish for attachment.	Strayer 2008, p. 65 Haag 2012, pp. 41–42
Juveniles - excystment from host fish to approx. age 5 years	● Clean, flowing water ● Host fish dispersal ● Appropriate interstitial chemistry; low salinity, low ammonia, low copper and other contaminants, high dissolved oxygen. ● Appropriate substrate (clean gravel/sand/cobble) for settlement	Dimmock and Wright 1993, p. 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, p. 1,787–1,788
Adults - greater than ~ age 5 years	● Clean, flowing water ● Appropriate substrate (stable gravel and coarse sand free from excessive silt) ● 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 Food Availability

Adult freshwater mussels, including the rayed bean, feed by filtering suspended particles including bacteria, 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; Nichols and Garling 2000, p. 874). 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 2021, pers. comm.). 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).

2.4.2 Suitable 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 the rayed bean 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 (30 degrees Celsius), dissolved oxygen concentrations greater than 5 milligrams per liter (mg/L) (Pandolfo et al. 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).

2.4.3 Habitat Conditions, Suitable Substrate, and Flow Conditions

The rayed bean is generally known from smaller, headwater creeks, but occurrence records exist from larger rivers (Cummings and Mayer 1992, p. 142; Parmalee and Bogan 1998, p. 244). They are usually found in or near shoal or riffle (short, shallow length of stream where the stream flows more rapidly) areas, and in the shallow, wave-washed areas of glacial lakes, including Lake Erie (West et al. 2000, p. 253). In Lake Erie, the species was generally associated with islands in the western portion of the lake. Preferred substrates typically include gravel and sand. The rayed bean is oftentimes found among vegetation (water willow (*Justicia americana*) and water milfoil (*Myriophyllum sp.*)) in and adjacent to riffles and shoals (Watters 1988a, p. 15; West et al. 2000, p. 253). Specimens are typically buried among the roots of the vegetation (Parmalee and Bogan 1998, p. 245). Adults and juveniles appear to produce byssal threads (thin, protein-based fibers) (Woolnough 2002, pp. 99–100), apparently to attach themselves to substrate particles.

Mussels in streams occur chiefly in flow refuges, or relatively stable areas that display little movement of particles during flood events (Strayer 1999, pp. 471–472). Strayer thought that features commonly used in the past to explain the spatial patchiness of mussels (water depth, current speed, sediment grain size) were poor predictors of where mussels actually occur in streams. Rather, flow refuges conceivably allow relatively immobile mussels to remain in the same general location throughout their entire lives.

Generally speaking, rayed bean habitat is in rivers and streams with natural flow regimes. While mussels can survive seasonally low flows and (random) short-term, periodic drying events, intermittent stream habitats generally cannot support mussel populations. Because a lotic (that is, flowing water) environment is a critical need for the rayed bean and their host fish, perturbations that disrupt natural flow patterns (for example, dams) have a potential negative influence on rayed bean and host fish resilience metrics (Spooner et al. 2011, p. 1729). However, the rayed bean and their host fish may be able to tolerate altered flow regimes under certain circumstances.

For example, the rayed bean are found in several places in the navigable Allegheny River in Pennsylvania which contains a maintained navigation lock and dam pool system (Anderson 2022, pers. comm.).

Rayed bean habitat must have adequate flow to deliver oxygen, enable passive reproduction, and deliver food to filter-feeding mussels. Further, flowing water removes contaminants and fine sediments from interstitial spaces, preventing mussel suffocation. Stream velocity is not static over time, and variations may be attributed to seasonal changes (with higher flows in winter/spring and lower flows in summer/fall), extreme weather events (for example, droughts or floods), or anthropogenic influence (for example, flow regulation via impoundments). The rayed bean relies on visually luring fish as part of its life cycle; therefore, turbidity and high levels of suspended solids during critical reproductive period may impact glochidial attachment and ultimately decrease recruitment in any given population (McLeod et al. 2017, p. 348).

2.4.4 Host

Glochidia must come into contact with a specific host fish(es) for their survival to be ensured. Without the proper host fish, the glochidia will perish. Gravid rayed bean attract host fish with a modified mantle flap. Little has been published regarding host fishes of the rayed bean (Parmalee and Bogan 1998, p. 245; West et al. 2000, p. 254). The only published research identifies the Tippecanoe darter (*Etheostoma tippecanoe*) and spotted darter (*Etheostoma maculatum*) as a host fish for the rayed bean (White et al. 1996, p. 191; Gibson et al. 2011, p. 7). Other hosts are thought to include the greenside darter (E. blennioides), rainbow darter (*E. caeruleum*), mottled sculpin (*Cottus bairdi*), and largemouth bass (*Micropterus salmoides*) (Woolnough 2002, p. 51). Based on inference of closely related species (that is, purple bean, *V. perpurpurea*; Cumberland bean, *V. trabalis*), additional hosts may be suitable, including species in the *Etheostoma* subgenus *Nothonotus* (for example, bluebreast darter, *E. camurum*), sculpins (*Cottus spp.*), and fantail darter (*E. flabellare*) (J. Jones, pers. comm., 2002). The method of host fish attractant seems to be more appropriate for small predatory fishes like darters and sculpins.

In many species of mussels, a few weeks are spent parasitizing the fishes gill tissues. Laboratory tests showed that the encysted rayed bean glochidia from Canada metamorphosed after 7-14 days (Woolnough 2002, p. 25). One study by McNichols (2007, pp. 30-31) documented metamorphosis of rayed bean between 19 and 28 days. Newly metamorphosed juveniles drop off to begin a free-living existence on the stream bottom. Unless they drop off in suitable habitat, they will die. Thus, the complex life history of the rayed bean and other mussels has many weak links that may prevent successful reproduction and recruitment of juveniles into existing populations.

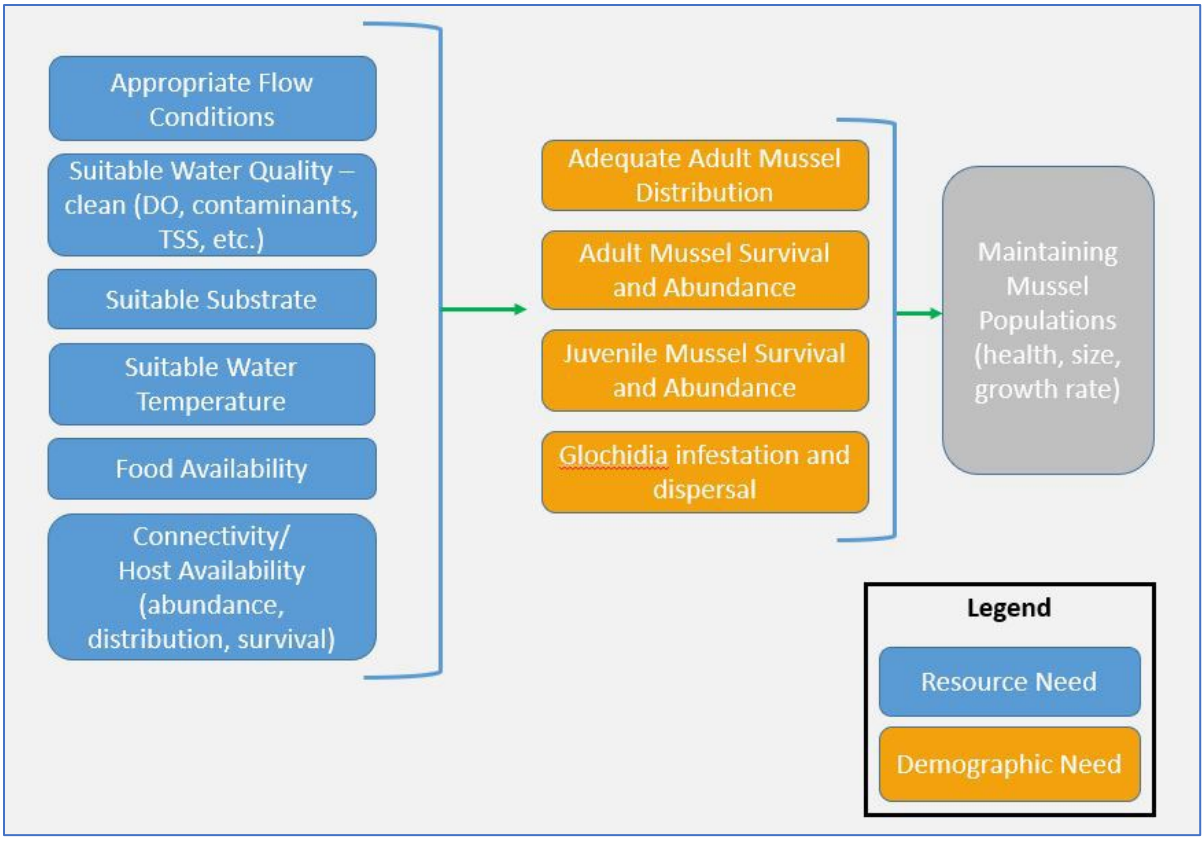


Figure 2.2. Conceptual model of the resource needs to support demographic needs to maintain rayed bean populations.

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. 2019, entire). For the purposes of this assessment, populations were defined within the bounds of HUC8 watersheds.

However, rayed bean 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 rayed bean occurrences within a more ecologically appropriate context in regard to primary influences on viability (See Chapter 3).

2.5.1 Population Connectivity

At a broader scale, suitable rayed bean habitat constitutes stream reaches where the host species are present and there is connectivity between localized populations to allow for both host and mussel dispersal. Connectivity is characterized by suitable water quality, lack of barriers to dispersal (for example, perched culverts, hydropower dams, water control structures), and presence of suitable habitat for host fish. 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 species. However, impoundments and other barriers to host species 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 the populations to adapt to future environmental changes. Information regarding the genetic diversity of rayed bean 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, representation was considered at the HUC2 watershed scale. We delineated three representation units for the rayed bean: Great Lakes, Ohio, and Tennessee basins.

2.5.3 Redundancy

The rayed bean needs multiple resilient populations distributed throughout its range to reduce the risk of a catastrophic, natural, or anthropogenic-induced event negatively affecting a large portion of the species' range at any given point in time. Catastrophic events may include, but are not limited to, prolonged drought, stream impounding, and spills. 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

Rayed bean 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 the species. 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, inadequate hydrological regime, landscape alteration, lack of connectivity, and invasive species as the primary risk factors influencing the resources upon which the rayed bean 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. An overview of risk factors influencing past, current, and future population condition is available in Appendix B.

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 occurrences at an ecologically relevant scale for which we have data on the primary stressors affecting populations.

We used the year 2000 as the cutoff to determine where the species is extant with an exception for two-pre-year 2000 occurrences (Stillwater River and Lake Maxinkuckee) where post-year 2000 survey data are lacking. It is important to note a single observation of an individual noted as live or fresh dead is considered an extant population. We carried forward and analyzed current condition for only the extant populations.

The rayed bean currently occupies 19 HUC8 watersheds, thus, for purposes of this analysis, we consider there to be 19 extant populations (Figure 4.1). Currently, the species is considered extant in 28 streams and 1 lake in seven states and 2 streams in one Canadian province: Indiana, Michigan, New York, Ohio, Pennsylvania, Tennessee, and West Virginia; and Ontario, Canada (total of 31 streams and lakes). The rayed bean historically occurred in at least 115 streams, lakes, and some human-made canals in 10 states: Illinois, Indiana, Kentucky, Michigan, New York, Ohio, Pennsylvania, Tennessee, Virginia, and West Virginia; and Ontario, Canada.

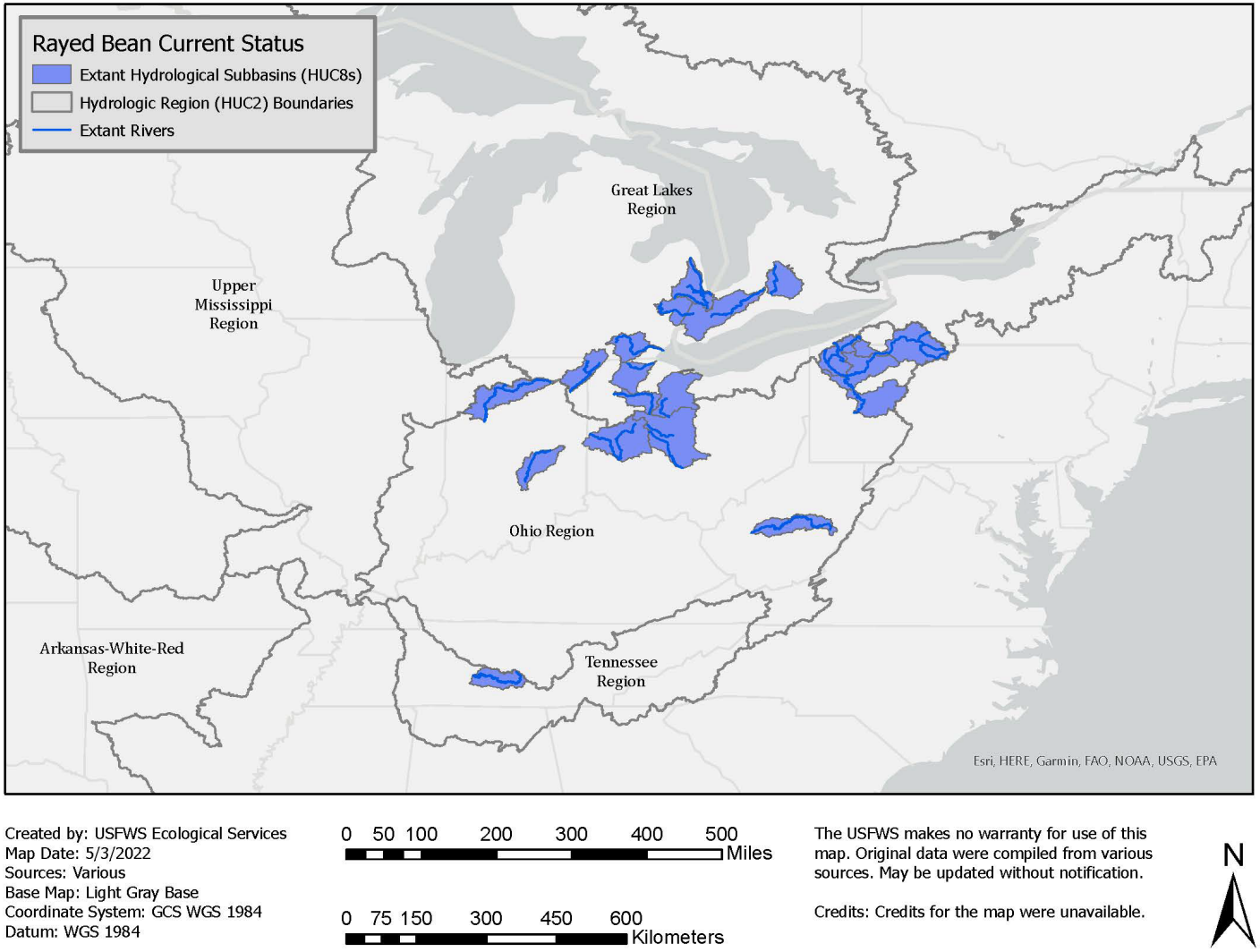


Figure 4.1. Rayed bean extant populations (HUC8 units).

4.2 Population Resiliency

4.2.1 Demographic Factors Methodology

We assessed demographic population condition (in HUC8 watersheds) as high, moderate, low, or very low/functionally extirpated based on demographic criteria (Table 4.1, Appendix C). We used demographic data from surveys available for the last 20 years (2000-2020). We considered populations with very low abundance (Survey efforts conducted over past 20 years have resulted in the collection of zero live or fresh dead individuals OR there have been no known surveys conducted within past 20 years and the last recorded observance was less than 5 live or fresh dead individuals) to be functionally extirpated (Table 4.1). We assigned an estimate of the probability of persistence over 30 years (approximately 2 life spans of rayed bean) for each population condition category based on best professional judgement of a population's abilities to withstand demographic stochastic events. These opinions were provided by the Core SSA Teams (including species experts, a malacologist, SSA and recovery experts).

Table 4.1. Condition category descriptions for rayed bean demographic factors in Appendix C.

Condition Category	Demographic Factors			Estimation of Probability of Persistence over 30 years
	Population Size	Population Extent	Reproduction/ Recruitment	
High	Cumulative numbers at high end of range (> 75) individuals observed in the past 20 years	Distributed throughout 30 or more river miles	Juveniles observed in past 5 years (2015-2020) AND in the preceding 5 years (2010-2015) (At least 2 recruitment events)	>90%
Moderate	Moderate numbers (>25) of individuals observed in the past 20 years	Distributed throughout 10 to 30 river miles	Juveniles or > 5 gravid females observed in the past 10 years (1 recruitment event)	60-90%
Low	Low numbers (1 - 25) of individuals observed in the past 20 years	Occurs in < 10 river mi	No juveniles or <5 gravid females observed in the past 10 years	10-60%
Very Low/Functionally Extirpated	Survey efforts conducted over past 20 years have resulted in the collection of zero live or fresh dead individuals OR there have been no known surveys conducted within past 20 years and last survey(s) found <5 live or fresh dead individuals	Unknown	No juvenile individuals or gravid females identified within past 20 years.	<10%

4.2.2 Risk Factors Methodology

We grouped the risk factors into five primary categories (water quality/contaminants, landscape, hydrological regime, connectivity, and invasive species) to assess the current condition of each population. Water quality/contaminants include four primary contaminants (ammonia, chloride, nitrate, and copper) and six secondary contaminants (lead, potassium, sulfate, zinc, aluminum, and cadmium). To evaluate the effects of various land use activities, we assessed a suite of landscape metrics derived from the 2016 National Landcover Dataset (Jin et al. 2019, entire). Specific metrics include percent imperviousness mean within the population; percent vegetative cover remaining within a 108-meter riparian buffer; and percent urban, percent agriculture, and canopy cover within a 108-meter riparian buffer. We used U.S. Drought Monitoring Data to assess drought risk for the hydrological regime; and we used the number of dams and density of unpaved roads to evaluate connectivity. The invasive species assessment included twelve species known to impact native freshwater mussels: zebra mussel, *Corbicula fluminea* (Asian clam), five species of invasive carps (silver, bighead, black, grass, common), rusty crayfish, spiny waterflea, brown trout, quagga mussel, and hydrilla.

Risks associated with each of the five factors were assigned as either high, moderate, or low risk (Table 4.2). Similar to our demographic criteria, we assigned a probability of persistence over 50 years for each risk category to create a common understanding of what we mean when we categorize a population as being at high, moderate, or low risk (Table 4.2). 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 5–7 = low risk, 8–10 = moderate risk, and 11–15 = high risk (Table D.9). 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.

For further information on the methods used to evaluate metrics within each of these risk factors and the scoring system, see Appendix D.

Table 4.2. Summary of risk category descriptions for the five risk factors evaluated for the current condition of each population. A detailed description of the risk factor categories is in Appendix D.

Risk Category	Water Quality/Contaminants[1]	Landscape[2]	Hydrological Regime[3]	Connectivity[4]	Invasive Species[5]	Estimation of probability of persistence over 50 years
High	Concentration of primary and/or secondary contaminants exceeds acute toxicity levels in >2% of samples	Landscape condition severely altered by anthropogenic factors	Hydrological regime highly altered by anthropogenic factors	Habitat severely fragmented by dams and road crossing density	Present in abundance	<60%
Moderate	Concentration of primary and/or secondary contaminants exceeds acute toxicity levels in <2% of samples	Landscape condition moderately altered by anthropogenic factors	Hydrological regime slightly altered by anthropogenic factors	Some habitat fragmentation due to dams and road crossing density	Present in moderation	60-90%
Low	Concentration of primary and/or secondary contaminants at levels below acute toxicity to mussels	Landscape condition slightly altered or unaltered due to anthropogenic factors	Hydrological regime characteristic of natural conditions, unaltered by anthropogenic factors	Few, if any, known habitat fragmentation issues	Absent	>90%

[1] See Tables D.1 and D.2 for details
[2] See Table D.3 for details
[3] See Table D.4 for details
[4] See Table D.5 for details
[5] See Table D.8 for details

4.2.3. Current Condition

The rayed bean currently occupies 19 HUC8 watersheds. The range spans across seven states and one Canadian province. We evaluated current risk factors for the 19 populations where data were available. We were not able to evaluate for current risk factors for one population, the Upper Thames River, in Canada due to a lack of available data metrics for our analysis. We also evaluated the current demographic condition of all the populations.

Great Lakes Basin Representation Unit

Demographic condition – The rayed bean's range within the Great Lakes basin includes a portion of Canada, Indiana, Michigan, and Ohio. Nine populations are considered extant in the Great Lakes basin (Figure 4.2). The demographic condition of four populations is categorized as high and the other five populations are categorized as low (Figure 4.3, Table E.2)

Risk Factors – Current risk factors could not be assessed for one population that occurs in Canada based on the availability of datasets for risk metrics (see below). All the populations that were assessed for risk (N=8) are in overall high-risk condition (Table E.1). Over 62% of the eight populations (N=5) are in high-risk category for landscape threats (Table E.1). Also, none of the eight populations analyzed had a low landscape risk. Additionally, 75% (N=6) of the populations were categorized at high risk for contaminants (Table E.1). For the populations within the U.S., catastrophic risk includes high risk associated with oil and gas presence (Table E.2). For detailed information on risks for each population, see Tables G.11, G.12, G.13, G.14, G.15, and G.16.

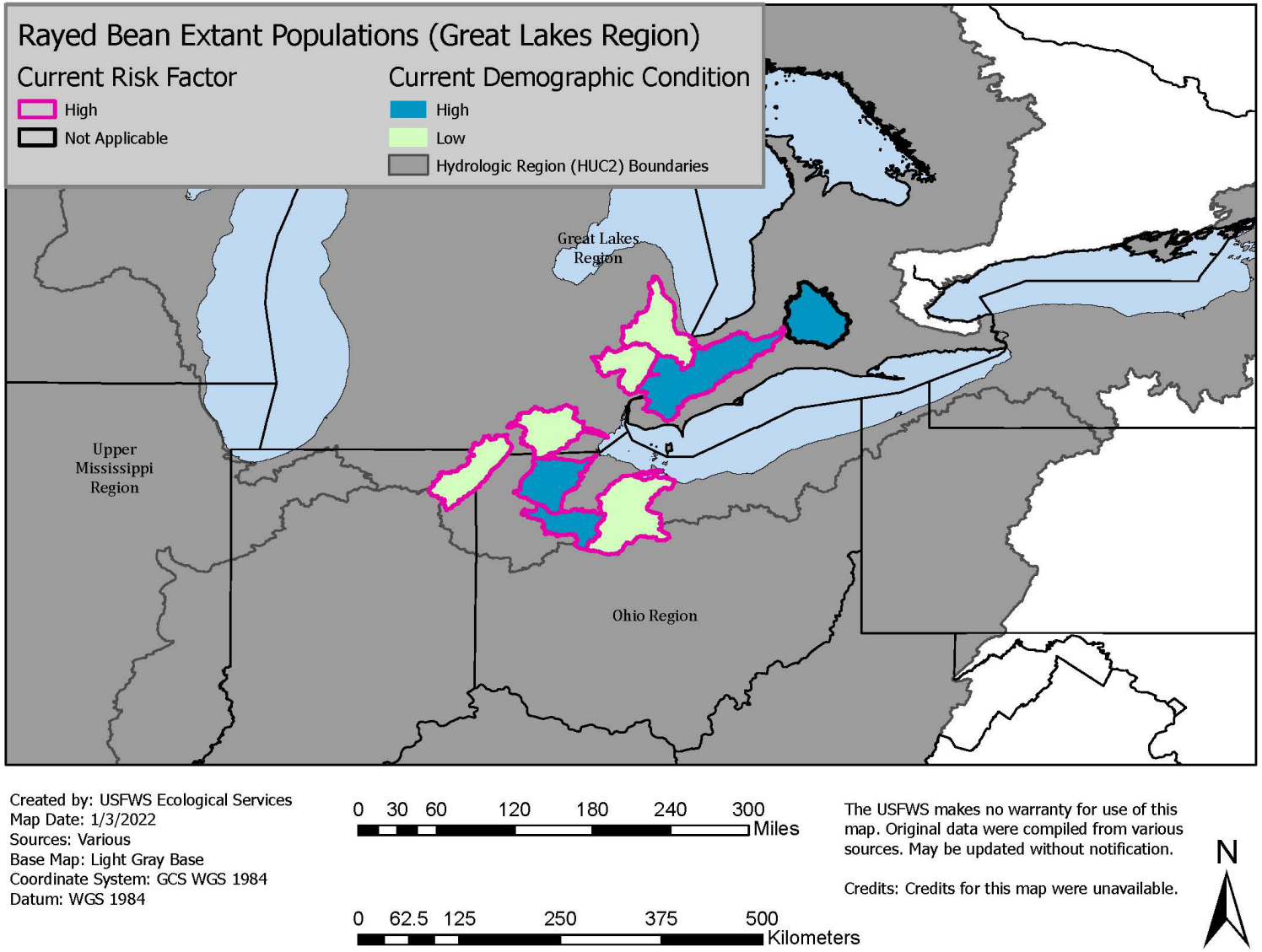


Figure 4.2. Extant populations within the Great Lakes basin for the rayed bean.

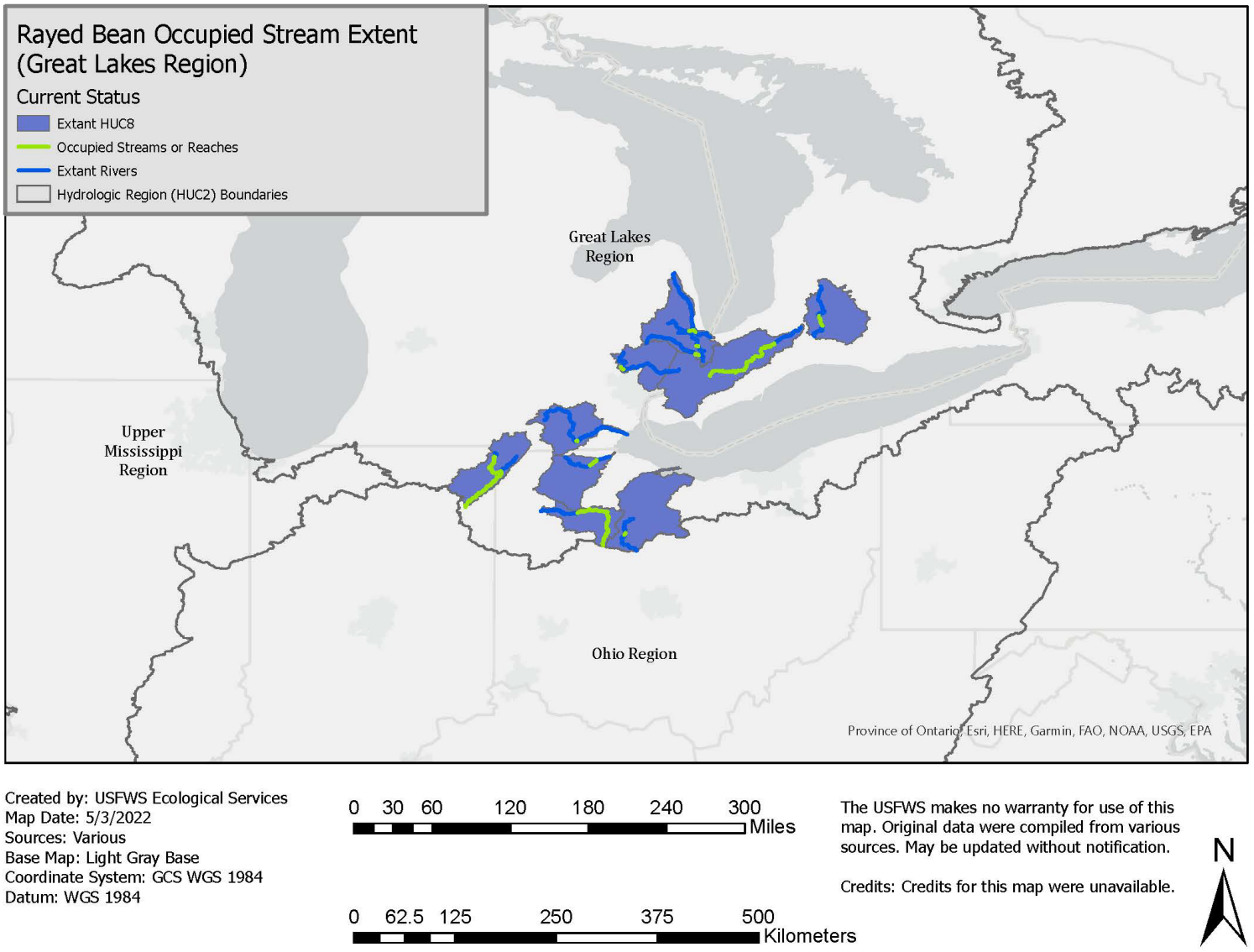


Figure 4.3. Occupied stream extent within the Great Lakes basin for the rayed bean.

Ohio Basin Representation Unit

Demographic condition – The rayed bean's range within the Ohio basin includes a portion of Indiana, Pennsylvania, New York, Ohio, and West Virginia. Nine populations are considered extant in the Ohio basin (Figure 4.4). The demographic condition of three populations is categorized as high, two populations are categorized as moderate, and four populations are categorized as low (Figure 4.5, Table E.4).

Risk factors – Of the nine populations, five are in overall high-risk condition, three are in moderate condition, and one is in low-risk condition (Figure 4.4; Table 4.4, Table E.3). As such, five of the populations within the Ohio basin representation unit are at high risk. Of the populations at high risk overall, four are at high risk due to contaminant risks (Table E.3). Furthermore, of all nine population, only one population has a low connectivity risk (Table E.3). All but one of the nine populations are also at moderate (N=5) to high risk (N=4) from contaminants. Catastrophic risk for eight of the nine populations in this basin includes high risk associated with oil and gas presence (Table E.4). For detailed information on risks for each population, see Tables G.11, G.12, G.13, G.14, G.15, and G.16.

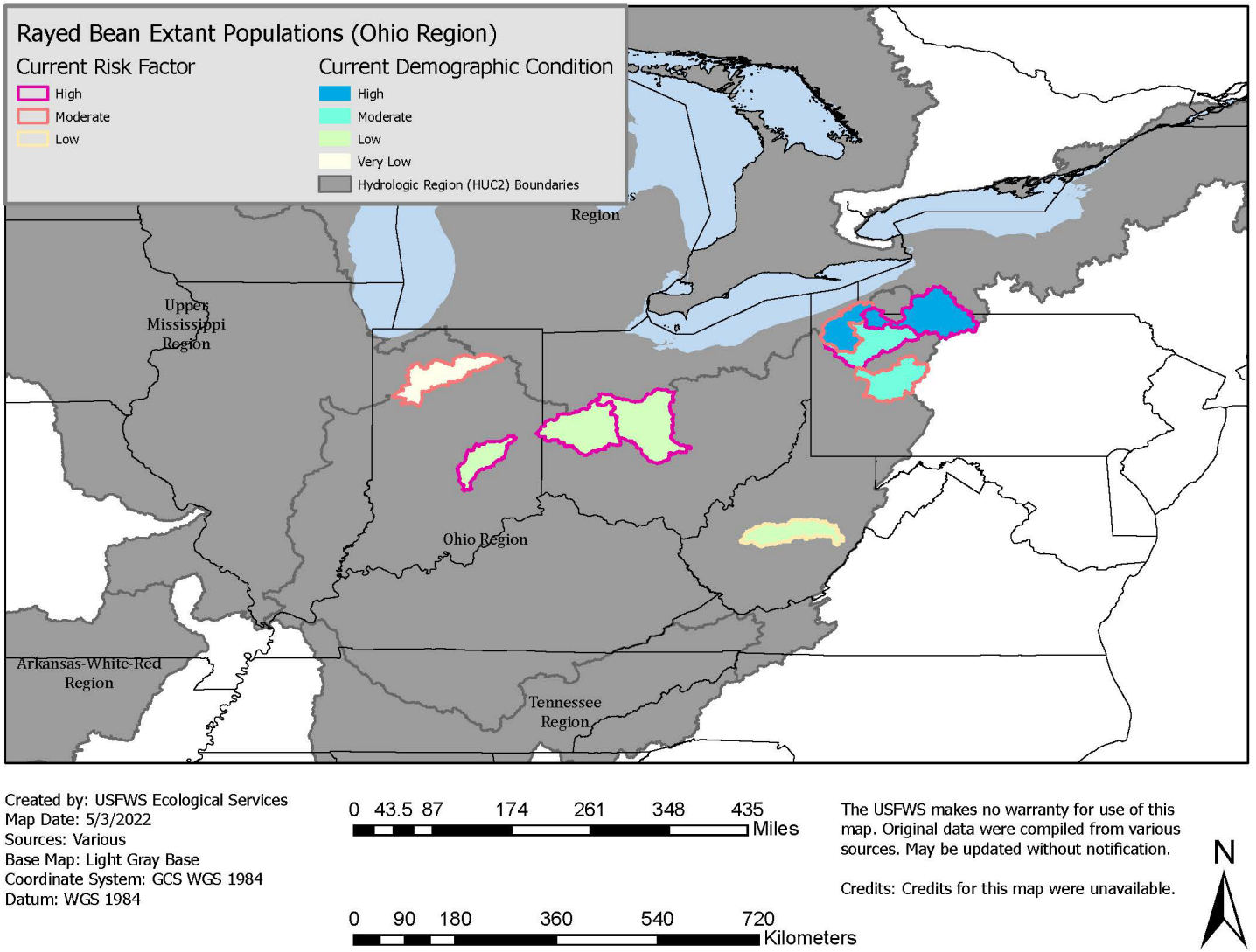


Figure 4.4. Extant populations within the Ohio basin for the rayed bean.

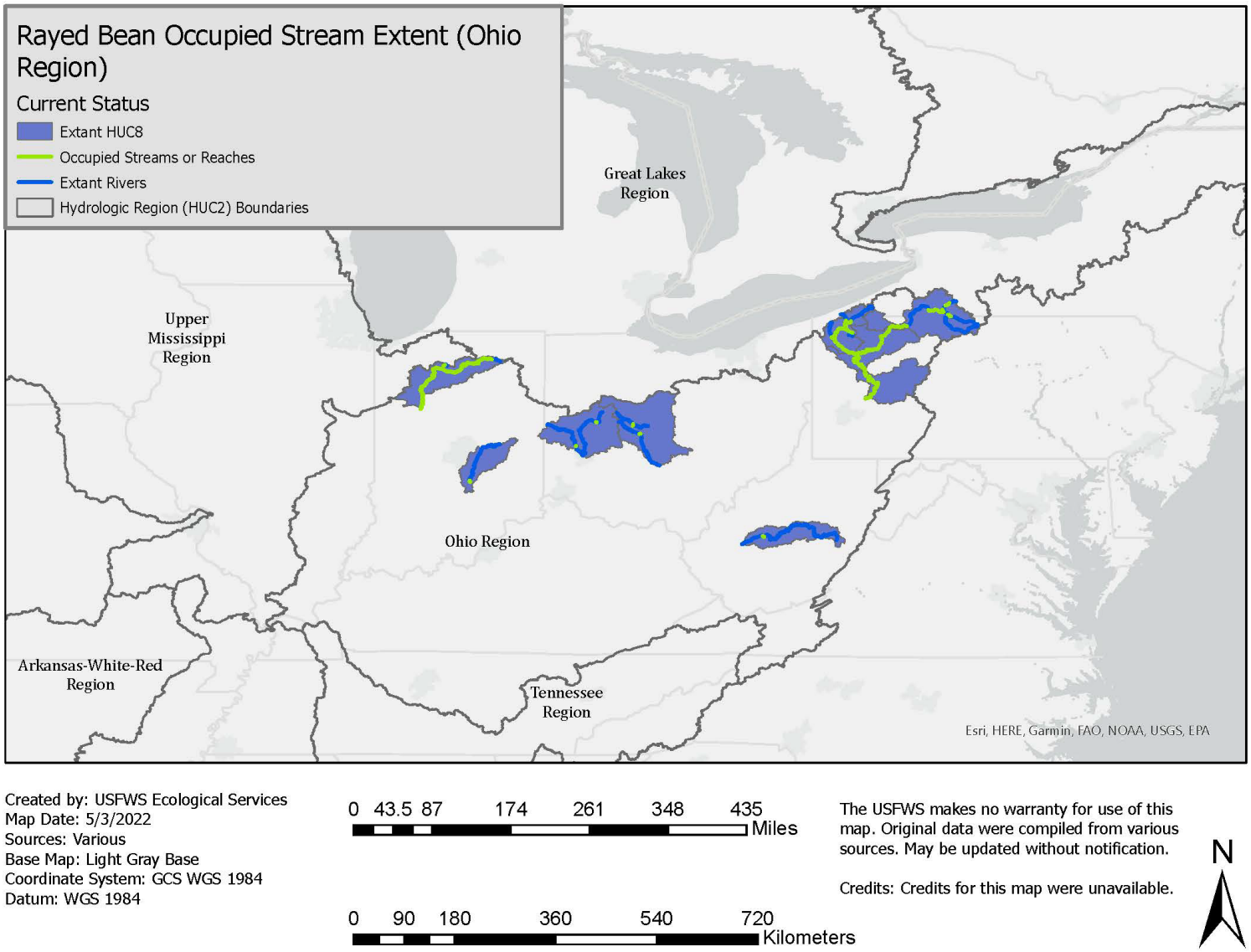


Figure 4.5. Occupied stream extent within the Ohio basin for the rayed bean.

Tennessee Basin Representation Unit

Demographic condition – The Tennessee basin has one rayed bean population in Tennessee (Figure 4.6). This single extant population is the result of a 2008 reintroduction effort and has been categorized as having a low demographic condition (Figure 4.6, Table E.6).

Risk factors – The single population is in overall high-level risk condition (Figure 4.7; Table E.5). The population is at high risk for water quality (Table E.5) with no catastrophic risk from coal mining or oil and natural gas (Table E.6). For detailed information on risks for each population, see Tables G.11, G.12, G.13, G.14, G.15, and G.16.

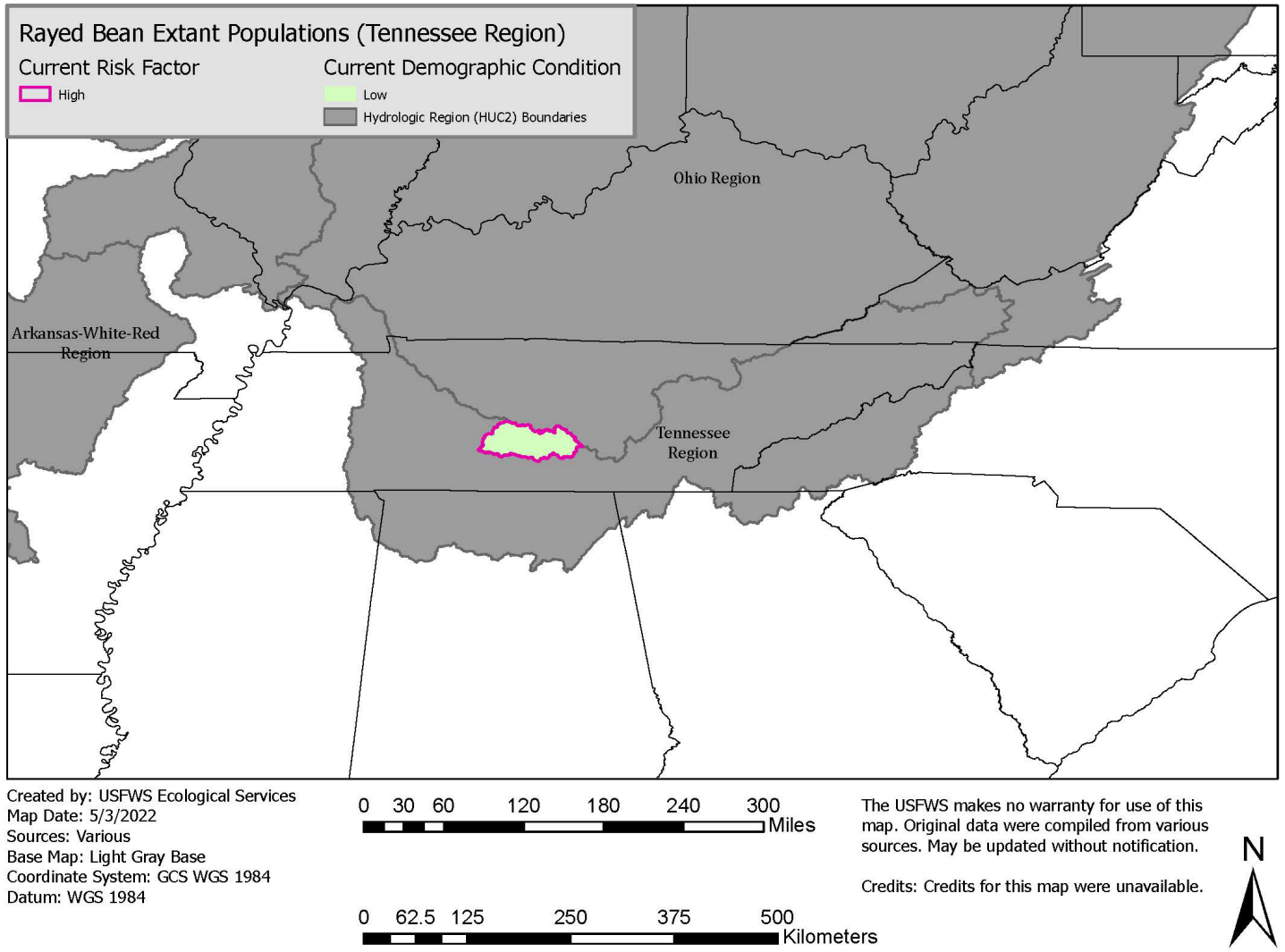


Figure 4.6. Extant population within the Tennessee basin for the rayed bean.

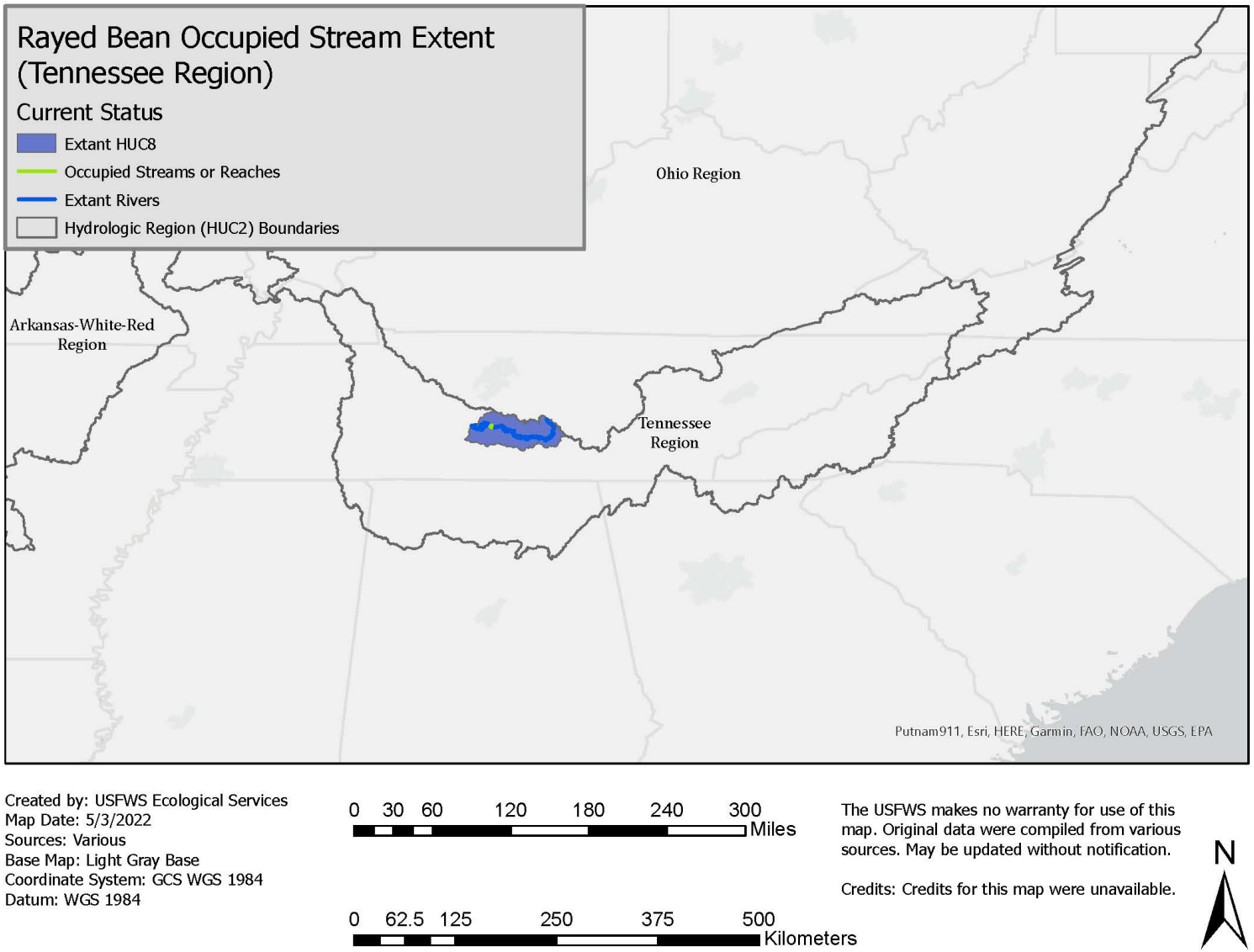


Figure 4.7. Occupied stream extent within the Tennessee basin for the rayed bean.

4.2.4 Population Resiliency Summary

Demographic condition – Of the 19 populations, more than half (N=10; 53%) are in a low demographic condition (Table 4.3). Approximately one-third (N=7; 37%) of the current populations are in a high demographic condition (Table E.6).

Risk factors – Of the 19 populations, the majority (N=14; 74%) are at high risk (Table 4.4, Table E.5). Only one population (5%) is at low risk, and three (16%) are at moderate risk. Risk factors could not be assessed for one population that occurs in Canada based on the availability of datasets for risk metrics. Of the populations at high risk, 79% (N=11) are at risk due to contaminants and 57% (N=8) are at high risk for landscape threats. For detailed information on risks for each population, see Tables G.11, G.12, G.13, G.14, G.15, and G.16.

Table 4.3. Summary of demographic condition for rayed bean populations across the range.

Demographic Condition	Number of Populations
High	7
Moderate	2
Low	10
Functionally Extirpated	0
Total	**19**

Table 4.4. Summary of risk factor condition for rayed bean populations across the range.

Risk Factor Condition	Number of Populations
High Risk	14
Moderate Risk	3
Low Risk	1
Unknown	1
Total	**19**

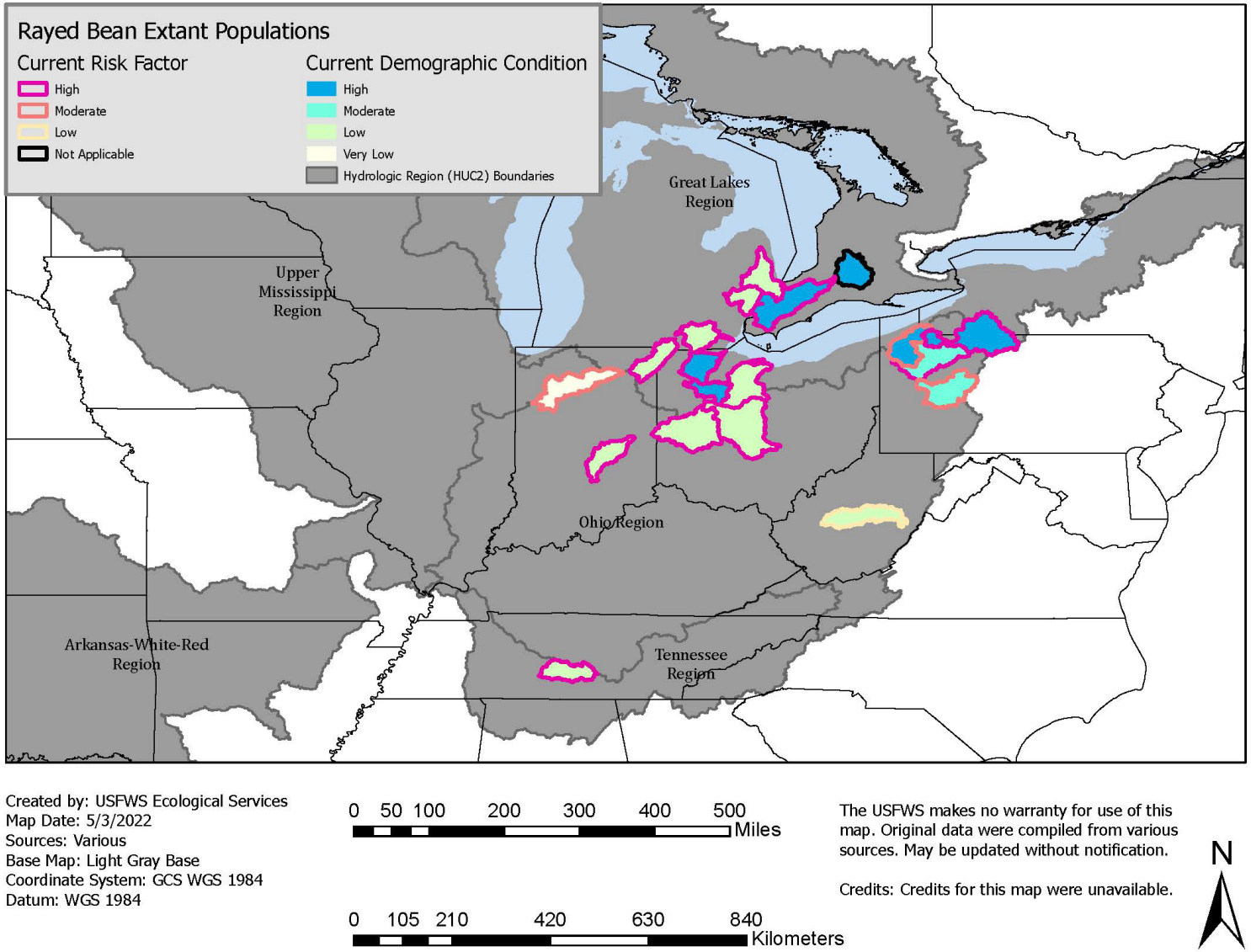


Figure 4.8. Map of rayed bean population status, risk factor condition, and demographic condition range wide.

4.3 Species Representation

We used HUC2 river basins to delineate three representation units for rayed bean: Great Lakes, Ohio, and Tennessee basins (refer to section 2.5.2). The species currently ranges across all three representation units (Figure 4.1). The Great Lakes basin has nine populations; of these, eight are at high risk and five have a low current demographic condition (Table 4.5). We did not have information to complete the risk analysis for one population in Canada (Table 4.5). The Ohio basin has nine populations, of which five are at high risk and four have a low current demographic condition (Table 4.5). The Tennessee basin has one population, which is at high risk and low current demographic condition (Table 4.5). Furthermore, the Tennessee basin population was reintroduced with adults translocated from the Allegheny River in the Ohio basin. Therefore, the Tennessee basin population is not genetically different from the same as the Ohio basin rayed bean.

4.4 Species Redundancy

There are 19 extant populations of the rayed bean. These populations are spread across the representation units unevenly. The Great Lakes basin contains nine populations; the Ohio basin contains nine populations; and the Tennessee basin contains one population (Figure 4.2, Figure 4.4, Figure 4.6). Historically, the rayed bean range only occurred in the Great Lakes, Ohio, and Tennessee basins. Given the current status encompasses 19 populations throughout its range and 2 of the 3 basins 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 (74%) of populations are currently at high risk.

*Table 4.5. Summary of rayed bean demographic condition and risk category for the representation units. (*one population in Canada and does not have an overall risk category assigned.) (L = Low, M = Moderate, H = High)*

								Total
Demographic Condition	H	H	H	M	M	L	L	
Risk Condition	H	M	U	H	M	H	L	
Great Lakes	3		*1			5		9
Ohio	1	2		1	1	3	1	9
Tennessee						1		1
Total	5	1	*1	1	1	9	1	19

CHAPTER 5. FUTURE CONDITION

5.1 Future Projections of Influences on Viability

5.1.1 Demographic Factors Methodology Overview

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 a population is projected to be at high risk into the future, the demographic condition will decline two levels (in other words, high current demographic condition is projected to decline to a low demographic condition into the future). If a population is projected to be at moderate risk into the future, the demographic condition is 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 improve demographically.

Table 5.1 Rule set for projected future demographic condition 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 Risk Factors Methodology Overview

There is substantial uncertainty regarding the magnitude, duration, and location of effects related to hydrological regime, habitat degradation, contaminants, connectivity, and invasive species into the future. Because of this, we forecasted future viability for the rayed bean under two future scenarios that represent the range of plausible environmental conditions and the projected consequences on the species' viability (Table 5.2, Table 5.3). We projected out 50 years when information was available (2070; approximately 3-4 life spans). 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 species' range.

Scenario 1 is the upper plausible-limit scenario reflecting the most favorable, plausible future conditions for the species; Scenario 2 is the lower plausible-limit scenario reflecting the least favorable, but still plausible, future condition for the species (see Table 5.2). Together, these two scenarios provide the upper and lower bounds of the plausible future condition of the species - - the actual future condition likely falls somewhere between these two scenarios.

Table 5.2. Description of Future Scenarios

	Method/Data Source	Scenario 1	Scenario 2
Contaminants	USGS FORE-SCE land cover change model to project how land cover types associated with the contaminants of interest will change. The FORE-SCE model uses IPCC Special Report on Emissions Scenarios (SRES) to develop different scenarios for land cover change under various emissions scenarios. The IPCC SRES A2 and B1 scenarios are roughly analogous to the updated IPCC Representative Concentration Pathway scenarios 8.5 and 4.5, respectfully, and were chosen to bookend climate scenarios in accordance with internal SSA guidance (FWS 2021).	High carbon emissions (IPCC SRES A2). The A2 storyline and scenario family describes a very heterogeneous world. The underlying theme is self-reliance and preservation of local identities. Fertility patterns across regions converge very slowly, which results in continuously increasing global population. Economic development is primarily regionally oriented and per capita economic growth and technological change are more fragmented and slower than in other storylines.	Medium-low carbon emissions (IPCC SRES B1) The B1 storyline and scenario family describes a convergent world with the same global population that peaks in midcentury and declines thereafter, as in the A1 storyline, but with rapid changes in economic structures toward a service and information economy, with reductions in material intensity, and the introduction of clean and resource-efficient technologies. The emphasis is on global solutions to economic, social, and environmental sustainability, including

			improved equity, but without additional climate initiatives.
Landscape	Used the USGS FORE-SCE land cover change model to project how land cover types of interest may change in the future. Projected changes in urban and forest land cover types were used as proxies to project percent changes in imperviousness and vegetative/canopy cover, respectively.	High carbon emissions (IPCC SRES A2) (See Contaminants for more details)	Medium-low carbon emissions (IPCC SRES B1) (See Contaminants for more details)
Hydrological Regime	USFS Cumulative Severe Drought Index projections which predict severe drought based on climate models representing warm/wet; hot/wet; hot/slightly dry; and hot/dry conditions.	Warm/wet scenario for drought severity	Hot/dry scenario for drought severity
Connectivity	Unpaved road crossings: U.S. Global Roads Inventory Project (GRIP) Shared Socioeconomic Pathway (SSP) scenarios. Assumed that future unpaved road length would increase commensurately with overall projected changes in road length in the GRIP model, which did not	Dams: No change Unpaved road crossings: High-end projection of road length in the U.S. (SSP5)	Dams: American Rivers dam removal database to predict where dams may be removed based on recent (last two decades) dam removals Unpaved road crossings: Low-end projection of road length in the U.S. (SSP3)

	distinguish between road surface types.		
Invasive species	Optimized Hotspot Analysis	Increased risk from invasive species if there was an invasive species hotspot in an adjacent watershed. If currently an invasive species hotspot, no change.	No change (due to difficulty to eradicate established invasive species).

Table 5.3. 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

Table 5.3.(continued) 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))

Risk Factor Metrics	Scenario 1	Scenario 2
Catastrohpic Events		
Oil and Natural Gas	U.S. Energy Information Administration (EIA) Annual Energy Outlook 2021 - High oil and gas supply case, we assume production and consumption increase where infrastructure is present	U.S. Energy Information Administration (EIA) Annual Energy Outlook 2021 - Low oil and gas supply case, we assume production and consumption decrease
Coal	U.S. Energy Information Administration (EIA) Annual Energy Outlook 2021, Electricity generation from coal expected to decrease, we assume no change in risk	U.S. Energy Information Administration (EIA) Annual Energy Outlook 2021, Electricity generation from coal expected to decrease, we assume reduction in risk where activities are currently present

5.2 Population Resiliency Future Assessment

For 83% (N=15) of the populations that could be analyzed, the risk did not change from the current condition risk under Scenario 1 or Scenario 2 (Table 5.4, Table 5.5, Table 5.6). In the Ohio basin, the French and Tippecanoe populations changed from a current moderate risk to a high risk and the Elk population changed from a current low risk to moderate risk under Scenario 1 (Figure 4.8, Figure 5.1, Figure 5.2). There were no changes to the risk condition of the Great Lakes and Tennessee basin populations under either scenario (Figure 4.8, Figure 5.1, Figure 5.2).

Based on the projected overall risk factor, 12 populations are projected to be extirpated or functionally extirpated under Scenario 1 and 10 populations are projected to be extirpated or functionally extirpated under Scenario 2 (Figure 5.1, Figure 5.2; See Appendix F for more detail). Under Scenario 1, five populations are projected to be in low demographic condition (Lake St. Clair, Blanchard, Lower Maumee, Upper Allegheny, Middle Allegheny – Redbank) into the future and one population is projected to be in moderate condition (French). No populations are projected to be in high demographic condition. Under Scenario 2, two populations are projected to be in moderate demographic condition (French and Tippecanoe) and six populations are projected to be in low demographic condition (Lake St. Clair, Blanchard, Lower Maumee, Upper Allegheny, Middle Allegheny - Redbank, Elk).

Approximately 89% (N=16) of the 18 populations analyzed experience high risk under Scenario 1 and 78% (N=14) of the 18 populations analyzed experience high risk under Scenario 2 (Table 5.4, Table 5.5, Table 5.6), meaning there is <60% probability of persistence over 50 years for 89% of the populations under Scenario 1 and 78% of the populations under Scenario 2 (Table 4.2). Approximately 11% (N=2) of the 18 populations analyzed experience moderate risk under Scenario 1 and 17% (N=3) of the 18 populations analyzed experience moderate risk under Scenario 2 (Table 5.4, Table 5.5, Table 5.6), meaning there is between 60-90% probability of persistence over 50 years for 11% of the populations under Scenario 1 and 17% of the populations under Scenario 2 (Table 4.2). None of the 18 populations analyzed experience low risk under Scenario 1 and 6% (N=1) of the 18 populations analyzed experience low risk under Scenario 2 (Table 5.4, Table 5.5, Table 5.6), meaning there is >90% probability of persistence over 50 years for none of the populations under Scenario 1 and 6% of the populations under Scenario 2 (Table 4.2). There is one population (Upper Thames River) that is not evaluated for risk factor condition because it occurs in Canada and the analysis could not be completed for the population area (Table 5.4, Table 5.5, Table 5.6). Based on Scenario 1 and 2, rayed bean 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 projected to be either lost or reduced in the future regardless of scenario. The Upper Duck population is currently the only remaining population left in the Tennessee basin. The Upper Duck population is projected to be extirpated into the future under

both scenarios. Under both future scenarios, the Great Lakes basin would have three remaining populations, all in low condition. In the future, five of currently extant Great Lakes basin populations are projected to be extirpated. The Ohio basin is projected to have five populations remaining under Scenario 1, with one in moderate condition, two in low condition and two functionally extirpated. The Ohio basin is expected to have six populations remaining under Scenario 2, with two populations in moderate condition, three in low condition, one functionally extirpated population, and three extirpated populations. In the future, six of currently extant Ohio basin populations are projected to be extirpated or functionally extirpated under Scenario 1 and four of the currently extant Ohio basin populations are projected to be extirpated or functionally extirpated under Scenario 2. It is clear that future projections result in reduced representation (by loss of populations) of rayed bean across the range of the species.

5.4 Species Redundancy Future Assessment

The rayed bean populations are spread across the representation units unevenly. The core area of the rayed bean range remains the Great Lakes and Ohio basins. The Great Lakes and Ohio basins are projected to have all of the remaining populations as the one population in Tennessee basin is projected to be extirpated under both scenarios (Figure 5.1, Figure 5.2). The Great Lakes basin would have three extant populations in low condition under both scenarios (Table 5.4, Table 5.5, Table 5.6, Figure 5.1, Figure 5.2). The Ohio basin would have one extant population in moderate condition, two extant populations in low condition, and two functionally extirpated populations under Scenario 1 and two extant populations in moderate condition, three extant populations in low condition, and one functionally extirpated population under Scenario 2. The redundancy within the core basins for the populations was cut by half or more under either scenario. The Great Lakes basin lost 56% (N=5) of the populations in both Scenarios (Table 5.4, Table 5.5, Table 5.6, Figure 5.1, Figure 5.2). The Ohio basin was projected to have six populations (67%) extirpated or functionally extirpated under Scenario 1 and four populations (44%) extirpated or functionally extirpated under Scenario 2 (Table 5.4, Table 5.5, Table 5.6, Figure 5.1, Figure 5.3). In addition, all of the remaining Great Lakes basin populations, 60% of the remaining Ohio basin populations under Scenario 1 and 33% of the remaining Ohio basin populations under Scenario 2 will be experiencing high risk (Table 5.4, Table 5.5, Table 5.6, Figure 5.1, Figure 5.3).

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

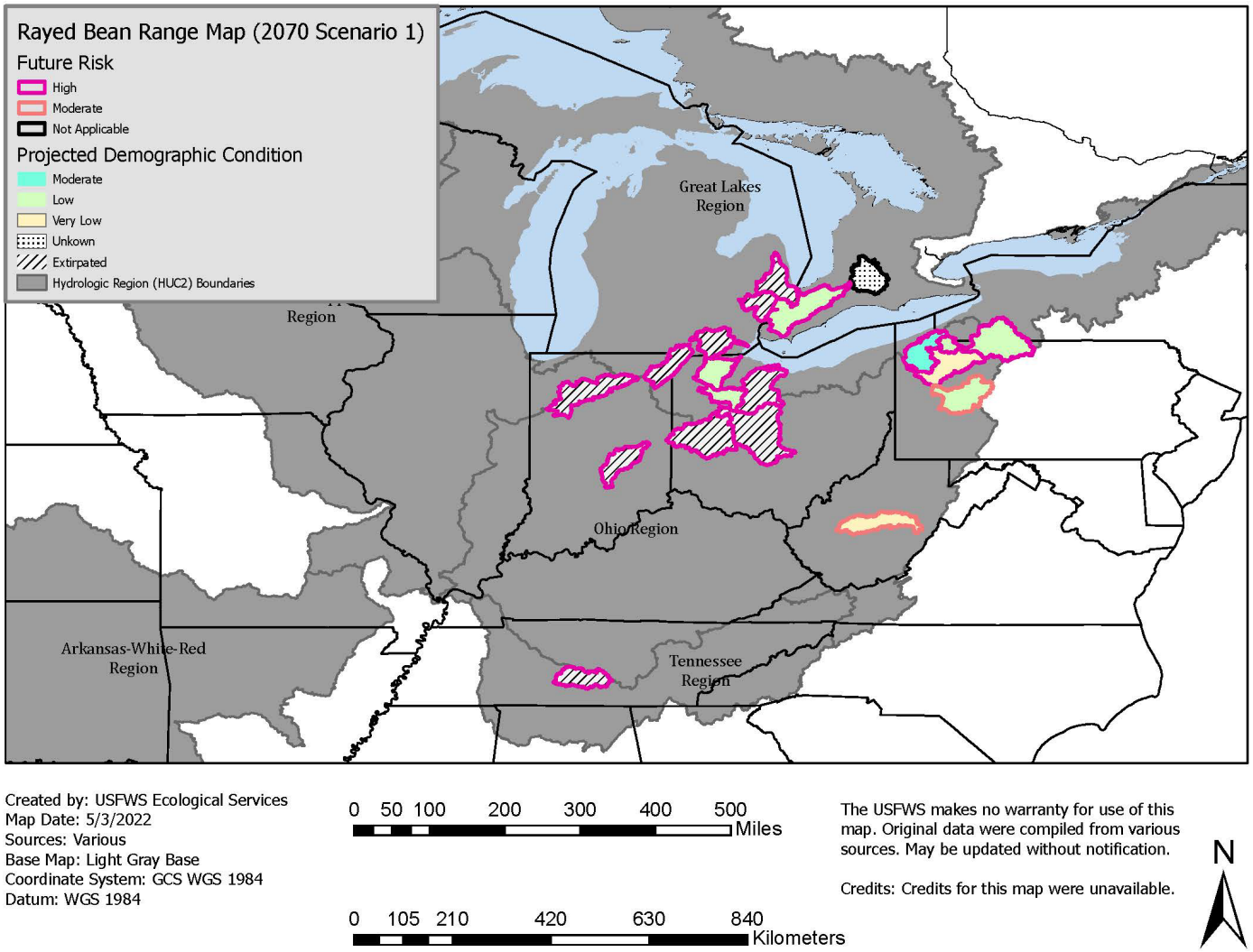


Figure 5.1. Rayed bean Future Scenario 1 population projected status, risk factor condition, and demographic condition range wide.

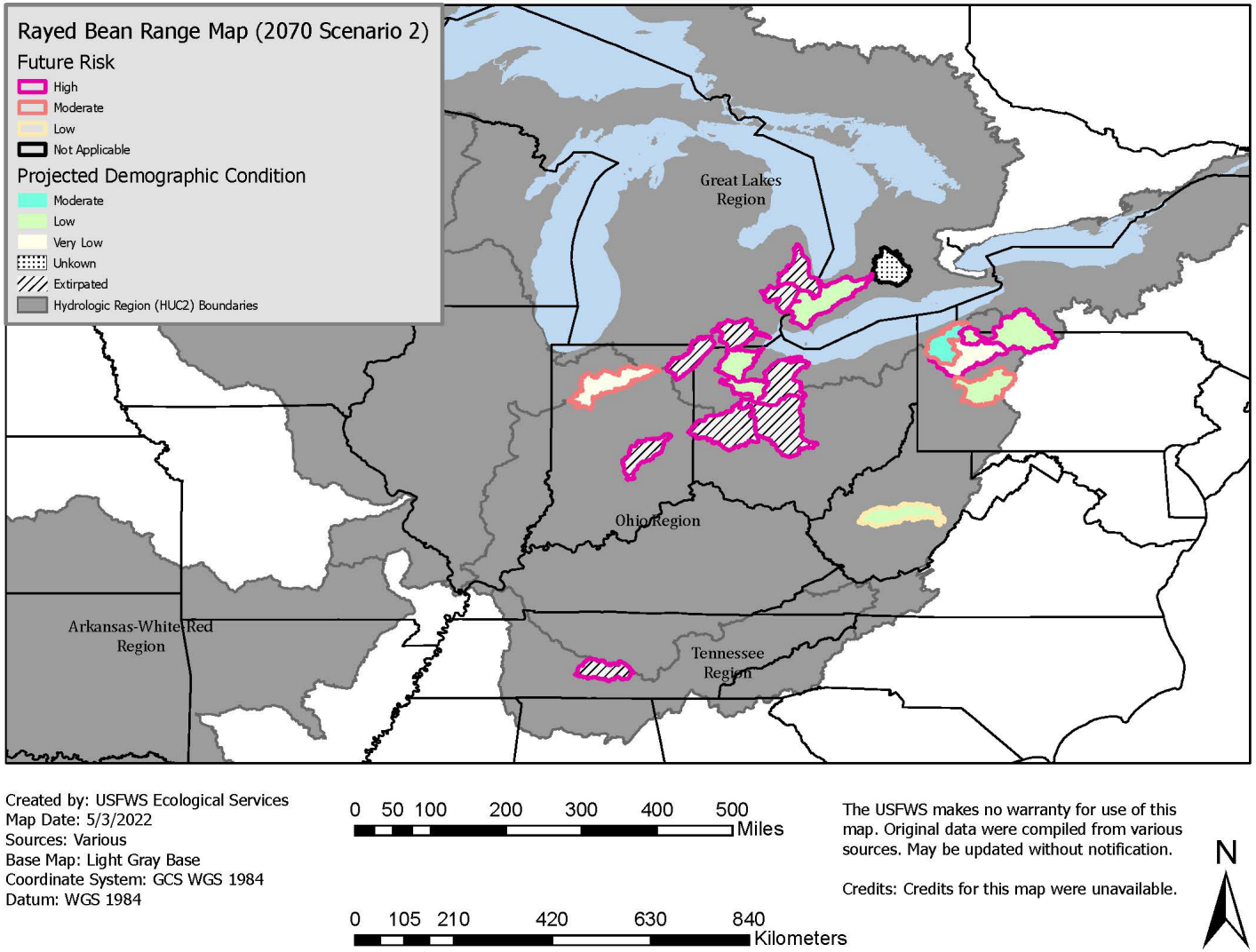


Figure 5.2. Rayed bean Future Scenario 2 population projected status, risk factor condition, and demographic condition range wide.

*Table 5.4. Summary of rayed bean population overall risk category by status and representation unit for Future Scenario 1. *One population in Canada does not have an overall risk category assigned.*

Risk Category	High	Moderate	Low	Total Population Count
Representation Unit				
Great Lakes*	8	0	0	**8**
Ohio	7	2	0	**9**
Tennessee	1	0	0	**1**
Species Range Total	**16**	**2**	**0**	**18***

*Table 5.5. Summary of rayed bean population overall risk category by status and representation unit for Future Scenario 2. *One population in Canada does not have an overall risk category assigned.*

Risk Category	High	Moderate	Low	Total Population Count
Representation Unit				
Great Lakes*	8	0	0	**8**
Ohio	5	3	1	9
Tennessee	1	0	0	**1**
Species Range Total	**15**	**2**	**1**	**18***

Table 5.6. Current, and Future (Scenario 1 and Scenario 2) demographic condition, risk, and status.

HUC2 Representation Unit	HUC8 Population	Current Demographic Condition	Current Risk	Scenario 1 Status	Scenario 1 Demographic Condition	Scenario 1 Risk	Scenario 2 Status	Scenario 2 Demographic Condition	Scenario 2 Risk
Great Lakes	St. Clair	Low	High	Extirpated	Extirpated	High	Extirpated	Extirpated	High
	Lake St. Clair	High	High	Extant	Low	High	Extant	Low	High
	Clinton	Low	High	Extirpated	Extirpated	High	Extirpated	Extirpated	High
	Upper Thames River	High	NA	UNK	UNK	NA	UNK	UNK	NA
	Raisin	Low	High	Extirpated	Extirpated	High	Extirpated	Extirpated	High
	St. Joseph	Low	High	Extirpated	Extirpated	High	Extirpated	Extirpated	High
	Blanchard	High	High	Extant	Low	High	Extant	Low	High
	Lower Maumee	High	High	Extant	Low	High	Extant	Low	High
	Sandusky	Low	High	Extirpated	Extirpated	High	Extirpated	Extirpated	High
Ohio	Upper Allegheny	High	High	Extant	Low	High	Extant	Low	High
	Middle Allegheny-Tionesta	Moderate	High	Extant	Functionally Extirpated	High	Extant	Functionally Extirpated	High
	French	High	Moderate	Extant	Moderate	High	Extant	Moderate	Moderate
	Middle Allegheny-Redbank	Moderate	Moderate	Extant	Low	Moderate	Extant	Low	Moderate
	Elk	Low	Low	Extant	Functionally Extirpated	Moderate	Extant	Low	Low
HUC2 Representation Unit	HUC8 Population	Current Demographic Condition	Current Risk	Scenario 1 Status	Scenario 1 Demographic Condition	Scenario 1 Risk	Scenario 2 Status	Scenario 2 Demographic Condition	Scenario 2 Risk

Ohio	Upper Scioto	Low	High	Extirpated	Extirpated	High	Extirpated	Extirpated	High
	Upper Great Miami	Low	High	Extirpated	Extirpated	High	Extirpated	Extirpated	High
	Tippecanoe	High	Moderate	Extirpated	Extirpated	High	Extant	Moderate	Moderate
	Driftwood	Low	High	Extirpated	Extirpated	High	Extirpated	Extirpated	High
Tennessee	Upper Duck	Low	High	Extirpated	Extirpated	High	Extirpated	Extirpated	High

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APPENDIX A. ECOLOGY BACKGROUND

A.1 Taxonomy and Genetics

The rayed bean belongs to the family Unionidae, also known as the naiads or pearly mussels. The rayed bean 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 rayed bean was originally described as *Unio fabalis* by Lea in 1831 (pp. 86–87). The type locality (the location of the first identified specimen) is the Ohio River (Parmalee and Bogan 1998, p. 244), probably in the vicinity of Cincinnati, Ohio. Over the years, the rayed bean has been placed in the genera *Unio*, *Margarita*, *Margaron*, *Eurynia*, *Micromya*, and *Lemiox*. It was ultimately placed in the genus *Villosa* by Stein (1963, p. 19), where it remains today (Turgeon *et al*. 1998, p. 33). We recognize *Unio capillus*, *U. lapillus*, and *U. donacopsis* as synonyms of *Villosa fabalis*.

The currently accepted classification is (Turgeon et al. 1998, p. 33; Williams et al. 2017, p. 44):

- Phylum: Mollusca
- Class: Bivalvia
- Subclass: Palaeoheterodonta
- Order: Unionoida
- Family: Unionidae
- Subfamily: Ambleminae
- Genus: *Villosa*
- Species: *Villosa fabalis*

It is also important to note that genetic analysis of rayed bean populations has not been conducted. Therefore, no data exists on the genetic diversity of these species rangewide.

A.2 Species Description

The rayed bean is a small mussel, usually less than 1.5 inches (in) (3.8 centimeters (cm)) long (Cummings and Mayer 1992, p. 142; Parmalee and Bogan 1998, p. 244; West et al. 2000, p. 248). The shell outline is elongate or ovate in males and elliptical in females, and moderately inflated in both sexes, but more so in females (Parmalee and Bogan 1998, p. 244). The valves are thick and solid. The anterior end is rounded in females and bluntly pointed in males (Cummings and Mayer 1992, p. 142). Females are generally smaller than males (Parmalee and Bogan 1998, p. 244). Dorsally, the shell margin is straight, while the ventral margin is straight to slightly curved (Cummings and Mayer 1992, p. 142). The beaks are slightly elevated above the hingeline (West et al. 2000, p. 248), with sculpture consisting of double loops with some nodules (Parmalee and Bogan 1998, p. 244). No posterior ridge is evident. Surface texture is smooth and sub-shiny, and green, yellowish-green, or brown, with numerous, wavy, dark-green rays of various widths (sometimes obscure in older, blackened specimens) (Cummings and Mayer 1992,

p. 142; West et al. 2000, p. 248). Internally, the left valve has two pseudocardinal teeth (tooth-like structures along the hingeline of the internal portion of the shell) that are triangular, relatively heavy, and large, and two short, heavy lateral teeth (Cummings and Mayer 1992, p. 142). The right valve has a low, triangular pseudocardinal tooth, with possibly smaller secondary teeth anteriorly and posteriorly, and a short, heavy, and somewhat elevated lateral tooth (Parmalee and Bogan 1998, p. 244). The color of the nacre (mother-of-pearl) is silvery white or bluish and iridescent posteriorly. Key characters useful for distinguishing the rayed bean from other mussels are its small size, thick valves, unusually heavy teeth for a small mussel, and color pattern (Cummings and Mayer 1992, p. 142).

APPENDIX B. PRIMARY INFLUENCES ON VIABILITY

B.1. Contaminants

B.1.1. 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 and Zam 1991, pp. 539–546; Naimo 1995, pp. 341–362; Valenti et al. 2005, pp. 1242–1246; Wang et al. 2007, 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) suggest 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; the revised 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 2021, pers. comm.). 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 bioavailability 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).

B.1.2. 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, pp. 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 complete 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 species declines.

B.1.3. 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 and 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, logperch).

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 2020, pers. comm.). 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 them especially 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 (host fish), 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, as well as the adult stage of the ellipse 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 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.

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 averaged 42% in sub-adults (range, 23–54%) and 20% in adults (range, 3–44%) 21 days after exposure, over all exposure durations. 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).

Fisheries and Oceans Canada (DFO) has completed a risk assessment of the effects of granular Bayluscide assessments on freshwater species at risk, including snuffbox (DFO 2021, entire). The study found that the risk to the snuffbox are of moderate concern given that there is a poor match between Snuffbox preferred habitat and larval lamprey habitat. However, any changes in current application patterns could result in very different impacts due to observed toxicity (DFO 2021, entire; T. Morris 2022, pers. comm.)

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 et al. (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 (*Obliquaria reflexa*) and Wabash pigtoe (*Fusconaia flava*) 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 2020, pers. comm.).

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 80° F (27 °C). They stated that the species can often experience a natural die off during mid to late summer (Boogaard et al. 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 2020, pers. comm.). 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 (55°F (13 °C) versus 70° (21 °C)) resulted in higher mussel survival.

B.2 Sedimentation

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). 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 and 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).

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 the 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).

Increased sedimentation may also 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 due to concentrations of montmorillonite clay ranging from 1,250 to 5,000 mg/L (Beussink et al. 2007, pp. 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 use 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). Impacts to fish population performance (growth, reproduction, and survival) were observed between 20 to 5000 mg/L, however, depending on factors such as testing a species ability to resurface after burial, clearance rate, and filtration rate. (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 and 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 and Widdows 1978, p. 137; Madon et al. 1998, p. 401). Clearance rates were negatively impacted when TSS concentrations were >8 mg/L (Tuttle-Raycraft et al. 2017, pp. 1161-1167), and respiratory stress was prevalent when TSS was about 600 mg/L (Goldsmith et al. 2021, pp. 102 and 104). Tuttle-Raycraft et al. (2017) found that TSS reduced suspension feeding rates in freshwater mussels, with decreases in feeding five-times greater in juveniles compared to adults, indicating how vulnerability differs across live stages. 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 and 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 (*P. grandis* [least sensitive], *Ligumia recta* [second sensitive], and *F. 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 and 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 and 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, pp. 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 and 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 and Lauzon-Guay 2005, p. 420; Roberts and 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 in interstitial waters (waters between sand particles, sediment, gravel) (Sparks and Strayer 1998, p. 129). Low dissolved oxygen can be caused by excess sedimentation, nutrient loading, organic inputs, changes in flow, and higher temperatures (Sparks and 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 and 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 and 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 and 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 and Strayer 1998, pp. 131–133).

Stegmann (2020, pp. 1–55) used hypoxia (oxygen deficiency) trials to evaluate the behavioral response of salamander mussel (*Simpsonaias ambigua*) 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).

B.5.1. 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 (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. 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.

B.5.2. 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.

B.5.3. 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 and Taylor 1999, pp. 915–916).

B.5.4. 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 and 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. 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 host fish 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). Barriers to host fish movement can also cause changes to genetic diversity for most mussel species (Hoffman et al. 2017, p. 9617).

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 (2014, 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.

B.7. Invasive Species

*B.7.1. 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 in the 1980s 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 inhabits all the Great Lakes, all large navigable rivers within the eastern United States, and many lakes within the Great Lakes region. 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.

Zebra mussels have a profound effect on the ecosystems they invade through biofouling (accumulating on surfaces, including native mussels) and significantly reducing the amount of phytoplankton that native mussels need for food (Holland 1993, p. 622; Fahnenstiel et al. 1993, p. 471; Caraco et al. 1997, p. 597). With a 90% filter efficiency rate and the ability to filter particles less than 1 micrometer in diameter (with preference for larger particles), zebra mussels have been found to be more efficient at filtration than unionids (Sprung and 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 zebra mussels negatively impacts the native species' locomotion, valve-movement, and 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 zebra mussels 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 pseudofeces (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).

While it is well documented that the zebra mussels have significant negative effects on freshwater mussels in lakes and large rivers, it should be noted that zebra mussels appear to coexist with mussels in small and medium sized streams (for example, the Clinton, Huron, and Grand River watersheds in Michigan) (D. Zanatta 2022, pers. comm.). Therefore, it is unclear how much of a deleterious effect zebra mussels pose to mussels in small and medium sized streams (D. Zanatta 2022, pers. comm.).

B.7.2. Asian Clam (Corbicula fluminea)

The Asian clam (*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 the Asian clam 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 and Widlak 1987, p. 6). Additionally, it has been suggested Asian clam 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). Asian clam actively disturb sediment altering benthic substrates and ultimately reduce habitat for juvenile native mussels (Strayer 1999, p. 82).

Research suggests invasion of Asian clam tends to occur in areas where native freshwater mussel density is low or declining (Strayer 1999, pp. 82–83; Vaughn and Spooner 2006, pp. 332–336). It appears Asian clam cannot successfully invade dense, healthy mussel beds in small-scale habitats (Vaughn and Spooner 2006, pp. 334–335). However, while Asian clam 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 and Spooner 2006, pp. 335–336).

B.7.3. Invasive Carps

"Invasive carp" typically refers to five invasive fish species originating from Asia and Europe: the black carp (*Mylopharyngodon piceus*), grass carp (*Ctenopharyngodon Idella*), bighead carp (*Hypophthalmichthys nobilis*), common carp (*Cyprinus carpio*), and silver carp (*Hypophthalmichthys molitrix*) (Berg 1949, entire; Lee et al. 1980, entire; Shireman and Smith 1983, entire; Li and Fang 1990, pp. 244–250; Page and Burr 1991, entire; Robins et al. 1991, p. 243; Balon 1995, p. 5.; Nico et al. 2005, p. 337). As a group, invasive carps were introduced to the United States via stocking for fishing and control purposes (such as biological control of vegetation and phytoplankton, and other organisms) in the 1960s and 1970s, and escaped into freshwater systems (DeKay 1842, Part IV; Cole 1905, entire; Guillory and Gasaway 1978, p. 105; Freeze and Henderson 1982, p. 197; Li and Fang 1990, pp. 244–250; Robins et al. 1991, p. 243; Nico et al. 2005, p. 337). Currently these species are reported in many states across the American Midwest, though the Great Lakes Basin and the Mississippi and Missouri Rivers are particularly impacted (Bailey and Smith 1981, pp. 1539–1561; Pflieger 1997, p. 372; Burr et al. 1996, entire; Nico et al. 2005, p. 337).

Invasive carps negatively impact native aquatic ecosystems by a) 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) and/or preying on the eggs of other fish species, negatively impacting species recruitment (Shireman and Smith 1983, entire; Chilton and Muoneke 1992, pp. 284–287; Miller and Beckman 1996, pp. 338–340); b) uprooting and destroying native vegetation, resulting in increased turbidity and deterioration of habitat

(Shireman and Smith 1983, entire; Bain et al. 1990, p. 553; Chilton and Muoneke 1992, pp. 294-298; Laird and Page 1996, pp. 13–14); and c) outcompeting native fish and mussel populations that rely on the same food sources, particularly plankton and plant foods (Shireman and Smith 1983, entire; Chilton and Muoneke 1992, pp. 294-298; Laird and Page 1996, pp. 13–14). Predation and feeding habits of these carp species have the potential to restructure benthic communities.

B.7.4. 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 to areas outside its native range 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 and Strayer 2004, pp. 174–175). Currently, the species is found in 20 states and can live at high densities (Klocker and 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 and Strayer 2004, pp. 174–175).

B.7.5. 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). 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 and Branstrator 2004, pp. 1–8).

The species is responsible for significant declines and shifts in plankton communities and directly competes with small fish and bivalves that rely on these food stocks (USEPA 2008, p. 37). 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.

B.7.6. Brown Trout (Salmo trutta)

The brown trout (*Salmo trutta*) is a fish species native to Europe, northern Africa, and western Asia (Page and 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. Due to mussel's unique reproductive strategy, without the presence of host, 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.

B.7.7. 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). Currently, the quagga mussel is found within the lower Great Lakes and harbor and nearshore areas of Lake Superior.

Similar to zebra mussels, the quagga mussel can be harmful to aquatic ecosystems through biofouling and use 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 et al. 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 and Mackie 1998, p. 2010). Because quagga mussels filter high concentrations of phytoplankton, the quality of habitat will likely degrade due to an increase in pseudofeces. 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 (the family of mussels that includes zebra mussels) 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). 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).

B.8. Mussel Disease

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.

B.9. Potential Catastrophic Events

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 and Beer 2000, 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 (1,247 km) 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 and 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.

B.10. Mussel Conservation Programs and Efforts

Mussel augmentation and translocation is an important tool that is being used to reintroduce rayed bean populations in West Virginia and Tennessee. Rayed bean were translocated from the Allegheny River in Pennsylvania in 2006 and reintroduced into the Elk River in West Virginia. In 2007, additional rayed bean were collected from the Allegheny River to augment the Elk River. In 2013, additional rayed bean were collected from French Creek in Pennsylvania for a subsequent augmentation into the Elk River. In 2008, rayed bean were collected from the Allegheny River and were released into the Duck River in Tennessee. All of the rayed bean

collected for these translocation efforts were obtained during salvage activities for bridge replacement projects. The success of these translocations has not yet been determined. However, live rayed bean were observed at both reintroduction sites several years after the translocations occurred.

APPENDIX C. METHODS FOR CURRENT CONDITION DEMOGRAPHIC FACTOR ANALYSIS

The following sections describe the metrics and scoring methodology used to analyze the species current demographic condition.

C.1. Cumulative Population Size Estimate

The Cumulative Population Size takes into account all live or fresh dead specimens collected from a population within the last 20 years (2000-2020), with an exception for two-pre-year 2000 occurrences (Stillwater River and Lake Maxinkuckee) where post-year 2000 survey data are lacking. It is important to note a single observation of an individual noted as live or fresh dead is considered an extant population. We carried forward and analyzed current condition for only the extant populations.

As such, this metric is highly dependent on the number and frequency of search efforts conducted and survey methodologies used. Further, this metric may be skewed by intensive search efforts, such as relocation events, and regular monitoring of the same areas where the same individual(s) may be encountered multiple times. It was assumed that some mixture of survey types and methodologies have been conducted within most (if not all) extant rayed bean populations within the last two decades, therefore, lessening the effects of these events on the overall assessment results.

Due to the limitations of the Cumulative Population Size metric described above, we considered estimating or categorizing population size based on available density estimates, the average number of individuals collected per survey effort, or population trend overtime. However, limitations associated with varying survey methodologies and level of effort, differing data collected per survey event, and the lack of data for surveys where rayed bean were not detected precluded further consideration of these approaches.

C.2. Reproduction and Recruitment

For the purposes of this assessment, evidence of recruitment was defined as the collection of individuals estimated to be 5 years of age or younger or gravid adult females. The amount of information collected on recruitment and methodology used to determine age of individuals varied extensively amongst the data reviewed, with these differences likely resulting in some level of variation in age estimation. It is common for malacologists to consider specimens of 5-years of age or younger as “juveniles” when reporting findings. This is often estimated in-field by means of counting external annuli, resulting in an approximate age determination. Age of sexual maturity for the rayed bean has not been determined though it likely occurs around age 4 or 5. We consider the collection of individuals estimated to be ≤ 5 years of age or identified as a “juvenile” as an indicator of recent recruitment. In some instances, specimen shell lengths were

reported in-place of age estimations. We assessed available records where both an estimated age and shell length were reported to define a range of shell lengths within each age class.

We considered a population to have a high Reproduction and Recruitment condition if evidence of reproduction (presence of juveniles or gravid females) had been observed within the population at least once per decade over the past 20 years (2000-2020), indicating persistent and on-going reproduction. Further, at least one juvenile had to be detected within a population within the most recent five years (2015-2020) for a population to be considered actively recruiting and assigned “High” condition. Although detectability of juveniles five years of age or younger is low, all of the populations currently considered in “High” condition met this criteria. In some instances, a population did not meet an individual condition category criteria due to lack of survey data gathered and/or reported. In these instances, available information and expert opinion were used to assign a condition category with an associated justification.

C.3. Population Extent and Distribution

The Population Extent is defined as the stream segment length between the upstream-most and downstream-most live or fresh dead specimen collection locations, taking into account all occurrence records between the years 2000 and 2020. In some instances, local resource managers provided estimations of upper and lower bounds of the occupied reaches and in other instances, available information for a population was limited to point location occurrence data. While estimates of each population’s occupied reach are helpful to understand the full extent of a waterbody the species is believed to occupy, in an effort to standardize what is considered a population's occupied reach for the purposes of this analysis, this metric was informed solely by occurrence records dated 2000 through 2020 within each HUC8. Once mapped, the upper and lower occupied reach bounds were determined by buffering the upstream- and downstream-most point location records by approximately 0.5 miles in an attempt to account for potential unsearched areas beyond known occurrence locations. Limited exceptions to this rule were made when additional information was available documenting the search and lack of detection of rayed bean beyond areas of documented occurrence. If uncertainty was present for an occurrence record location to the extent that it would influence the assigned condition category, the location uncertainty was quantified (estimated) and described. If only a single specimen had been collected from a population between 2000 and 2020, the population was automatically assigned to the "<1 mile" category and a 0.5-mile buffer was applied upstream and downstream of the occurrence location.

One or more large impoundments bisect occupied reaches within a subset of the extant HUC8s. In instances where an impoundment was present within the 0.5-mile buffer, but no additional occurrence records were available beyond the impoundment, the impoundment was considered the upstream or downstream extent of the occupied reach regardless of the 0.5-mile buffer. However, if one or more large impoundments separated multiple occurrence records

within a HUC8, the full extent between the upstream-most and downstream-most record was included within the population's defined occupied reach. Although impoundments present within a HUC and bisecting known occupied reaches may affect the long-term persistence of a population, these effects were not accounted for within the demographic current condition assessment but were captured within the habitat risk portion of this assessment.

C.4. Scoring

We defined a total of three demographic categories (Cumulative Population Size Estimate, Reproduction/ Recruitment, and Population Extent/ Distribution) to assess the demographic condition of each population, as described above and with Table 4.1. Additive scoring was used across the three categories to generate an overall demographic score for each population.

Definitions associated with high, medium, low, or very low (functionally extirpated) conditions are provided for each of these three condition categories used within Table 4.1. Populations were assigned 3 points for each category meeting the "high" condition definition, 2 points for "medium" condition, 1 point for "low" condition, and 0 points for a condition of "very low (functionally extirpated)." Points across the three demographic condition categories were summed for each population with an additive score of 0 representing an overall condition of "very low (functionally extirpated)," a score of 1-3 representing an overall "low" demographic condition, a score of 4-6 representing an overall "medium" condition, and a score of 7-9 representing an overall "high" condition (Table C.1).

Table C.1. Overall demographic condition cumulative scoring.

Cumulative Score	Overall Demographic Condition
7-9	High
4-6	Medium
1-3	Low
0\	Very Low (Functionally Extirpated)

APPENDIX D. METHODS FOR CURRENT CONDITION RISK FACTOR ANALYSIS

We developed a rule set to guide how to assess overall current condition for the five 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 11-15, the overall population condition is categorized as high risk, scores 8–10, the overall population condition is categorized as moderate risk and for additive scores 5–7, the overall population condition is categorized as low risk (Table D.9). Refer to Table D.1 for a description of how individual risk factors were assigned points.

D.1 Water Quality/Contaminants

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 the rayed bean, we used toxicity studies from other freshwater mussel species as a surrogate, with the assumption that the rayed bean would be either equally or more sensitive than the species tested. The majority of species tested (largely non-listed) were found to have similar sensitivities. However, in at least one case a listed mussel species has shown increased sensitivity to a primary contaminant. 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 D.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. If none of the risk metrics were high, then 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 limited research covering relatively few species of mussels indicates these contaminants may not be as lethal to mussels as other aquatic organisms. Therefore, in our assessments, secondary contaminants alone do not present as high a risk to freshwater mussels as the primary contaminants for which mussels are the most sensitive (Table D.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 15–18 across all secondary

contaminant risk metrics (lead, potassium, sulfate, zinc, aluminum, and cadmium) results in an overall contaminant risk of high; a score of 9–14 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 were based on where the majority of contaminants fall within that risk category.

We also qualitatively analyzed chemicals that pose a risk to freshwater mussels (for example, Contaminants of Emerging Concern), but for which we do not have acute toxicity data that establish thresholds for quantitative evaluation (See Appendix B.1.2).

To evaluate the risk posed by primary and secondary contaminants, we began by obtaining ambient water quality data from 2000–2020 available from the National Water Quality Monitoring Council's Water Quality Portal and filtered results to include only samples collected from surface waters of reservoirs, streams, rivers, impoundments, and ditches to focus on samples from possible mussel habitat. We further filtered the data to include only samples collected within 12-digit HUC watersheds immediately draining into extant rivers. There was not enough data to make meaningful assessments of occupied reaches within extant streams and rivers. We then established thresholds (that is, LD50, or the dose at which 50% of mussels died during laboratory tests) for high, moderate, and low risk for each contaminant based on a review of the literature, input from contaminant experts within the Service, and toxicity studies on aquatic organisms (Table D.1) and compared the Water Quality Portal data to the risk thresholds.

Since the toxicity of certain contaminants are influenced by water chemistry, we also queried the Water Quality Portal for measurements of hardness, pH, and temperature that were collected concurrently with samples analyzed for concentrations of primary and secondary contaminants to adjust for watershed and site-specific conditions. For example, the toxicity of metals such as copper are influenced by hardness, which impacts the bioavailability of metals in water. Ammonia toxicity, on the other hand, 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 HUC8 scale. For watersheds in which pH and temperature data collected concurrently with ammonia samples were available, we calculated 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 to compare to ambient water quality data in Table D.2.

Limitations of the data

Some uncertainty is associated with assessing contaminants risks to the rayed bean; 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. Assessments were limited largely to acute LD50s 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 chronic exposure and of sublethal effects (that is, reproductive, behavioral) would also 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 rayed bean 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 adults 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.

Temperature and Dissolved Oxygen

Suitable water temperature and dissolved oxygen levels are essential to rayed bean 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. The salamander mussel appears to not be sensitive to thermal changes within propagation facilities (M. Bradley 2021, pers. comm.). Sand and muck occupied by the 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 2021, pers. comm.).

While we do not know the thermal lethal temperature for rayed bean, there has been extensive research on other species of mussel across life stages. This research indicates there is likely a thermal lethal limit for rayed bean. Ganser et al. (2013, entire) found negative impacts to survival, heart rate, and growth of juvenile freshwater mussels when exposed to elevated temperatures over time. Survival of fatmucket 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 that because no research has been completed for the thermal sensitivity or DO limits of rayed bean or 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.

Table D.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 rayed bean populations. ([3] See EPA Aquatic Life Ambient Water Quality Criteria for Ammonia - Freshwater 2013, Tables 5b and 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 - 2020.	Chloride concentration in surface water within HUC12s draining into extant rivers from 2000 - 2020.	Nitrate concentration in surface water within HUC12s draining into extant rivers from 2000 - 2020.	Copper concentration in surface water within HUC12s draining into extant rivers from 2000 - 2020.
High Risk (3 points)	Water quality concentration exceeds acute toxicity levels >2% of samples (2000 - 2020).	Water quality concentration exceeds acute toxicity levels >2% of samples (2000 - 2020).	Water quality concentration exceeds acute toxicity levels >2% of samples (2000 - 2020).	Water quality concentration exceeds acute toxicity levels >2% of samples (2000 - 2020).
Moderate Risk (2 points)	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2000 - 2020).	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2000 - 2020).	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2000 - 2020.	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2000 - 2020).
Low Risk (1 point)	Water quality concentrations at levels below acute toxicity to mussels (2000 - 2020).[3]	Water quality concentrations at levels below acute toxicity to mussels (2000 - 2020).	Water quality concentrations at levels below acute toxicity to mussels (2000 - 2020).	Water quality concentrations at levels below acute toxicity to mussels (2000 - 2020).

Table D.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 rayed bean 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 - 2020.	Potassium concentration in surface water within HUC12s draining into extant rivers from 2000 - 2020.	Sulfate concentration in surface water within HUC12s draining into extant rivers from 2000 - 2020.
High Risk (3 points)	Water quality concentration exceeds acute toxicity levels >2% of samples (2000 - 2020).	Water quality concentration exceeds acute toxicity levels >2% of samples (2000 - 2020).	Water quality concentration exceeds acute toxicity levels >2% of samples (2000 - 2020).
Moderate Risk (2 points)	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2000 - 2020).	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2000 - 2020).	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2000 - 2020).
Low Risk (1 point)	Water quality concentrations at levels below acute toxicity to mussels (2000 - 2020).	Water quality concentrations at levels below acute toxicity to mussels (2000 - 2020).	Water quality concentrations at levels below acute toxicity to mussels (2000 - 2020).

Table D.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 rayed bean 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 - 2020.	Aluminum concentration in surface water within HUC12s draining into extant rivers from 2000 - 2020.	Hardness normalized cadmium concentration in surface water within HUC12s draining into extant rivers from 2000 - 2020.
High Risk (3 points)	Water quality concentration exceeds acute toxicity levels >2% of samples (2000 - 2020).	Water quality concentration exceeds acute toxicity levels >2% of samples (2000 - 2020).	Water quality concentration exceeds acute toxicity levels >2% of samples (2000 - 2020).
Moderate Risk (2 points)	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2000 - 2020).	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2000 - 2020).	Water quality concentration exceeds acute toxicity levels to mussels <2% of samples (2000 - 2020).
Low Risk (1 point)	Water quality concentrations at levels below acute toxicity to mussels (2000 - 2020).	Water quality concentration at levels below acute toxicity to mussels (2000 - 2020).	Water quality concentrations at levels below acute toxicity to mussels (2000 - 2020).

Table D.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 (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: *Epioblasma capsaeformis*, *Lampsilis rafinesqueana*, *Lampsilis higginsii*, and *Lampsilis abrupta*)	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 siliquoidea*	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 (*Lampsilis siliquodea)* 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 (*Lampsilis siliquoidea*) listed in EPA 2016.
Chloride	244 mg/L	9 genera representing 12 species of freshwater mussels (including 1 federally listed species: *Epioblasma torulosa rangiana*)	Gillis 2011	Mussels are sensitive to chloride and as a result, we used the lowest acute value for freshwater mussels from Gillis 2011.

Table D.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 (that is, 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 (*Actinonaias pectorosa* and *Utterbackia imbecillis*)	EPA 2007	Mussels are not among the most sensitive species (top 4) upon which EPA water quality criteria are based, but are nonetheless sensitive to copper. Water chemistry data needed to use the Biotic Ligand Model to calculate site-specific thresholds for aquatic organisms was limited. 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 Department of Environment, Great Lakes, and Energy (EGLE) formula using average hardness across each HUC8.
Nitrate	41 mg/L	*Lampsilis siliquoidea*	Monson 2010	EPA and Michigan EGLE acute values were not available, so we used the draft criteria from Minnesota Pollution Control Agency.
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 (*Margaritifera falcata*, *Amblema plicata*, *Utterbackia imbecillis*, *Lampsilis siliquoidea*, and *Megalonaias nervosa*)	Wang et al. 2017	EPA acute values were not available, so we used acute values derived for freshwater mussels in Wang et al. 2017.

Table D.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 (that is 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.

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.

D.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 (NLCD) (Jin et al. 2019, entire). 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. Specific metrics were selected to determine overall landscape risk: mean imperviousness and urban and agriculture cover within the HUC8, and percent vegetative and canopy cover remaining within a riparian buffer; (Table D.3). These categories and the criteria for risk scoring were derived from USEPA's Health Watersheds Assessment and existing species status assessment for other freshwater mussel species (Josh Hundley, USFWS, pers. Comm. October 13, 2021). Vegetive cover refers to the area on the ground that is comprised of vegetation while canopy cover refers to the area of the landscape that is shaded by vegetation. 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.

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 and 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 and other 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 trees and other riparian vegetation to make room for more crops (decreases vegetative cover and allows for more runoff; Broadmeadow and Nisbet 2004, p. 286; Nolte et al. 2013, p. 296).

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 current landscape condition has a score of 5–7; a population that is at overall moderate risk due to landscape condition has a score of 8–10; and a population that is at an overall high risk due to

landscape condition has a score of 11–15. 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 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 ArcGIS Pro 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 riparian zone of extant river for each population by using the USEPA's Watershed Index Online Riparian Zone Mask as a mask to extract NLCD 2016 raster cells within the riparian zone (USEPA 2016). This dataset buffers aquatic features by approximately 100m to obtain a mask of the riparian zone. 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 HUC8 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 within the riparian buffer of 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 Zonal Statistics as Table tool to calculate the average value of all Tree Canopy Cover raster cells to find the average tree canopy in the riparian buffer using the USEPA's Watershed Index Online Riparian Zone Mask as the zone representing the riparian buffer (USEPA 2016).

Table D.3. Indicator descriptions for the five landscape risk metrics used to evaluate the overall landscape risk to rayed bean 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

D.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 D.1.). Per our assessment of risk to the rayed bean (see 3.1.4), categories with USGS weekly streamflow below 5% of the median of "daily" percentiles for 7-day average flow of the weekly median stream flow 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 D.4.

			Ranges				
Category	Description	Possible Impacts	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 D.1. U.S. Drought Monitor severity classification system.

Table D.4. Indicator descriptions for the drought risk metric used to evaluate the overall hydrological regime risk to rayed bean populations.

Current Condition Indicator – Hydrological Regime	**Drought**
Description of Indicator	Consecutive weeks of extreme to exceptional drought (below 5% of the median of "daily" percentiles for the 7-day average flow) and multi-year droughts classified as extreme or 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

D.4 Connectivity

We evaluated the number of dams and the density of unpaved road stream crossings to evaluate connectivity within each population (Table D.5). The number of dams within a population was evaluated using the 2012 National Anthropogenic Barrier Dataset (Ostroff et al. 2013, entire). We then used ArcGIS Pro to count the number of dams within each population.

Unpaved road stream crossings impact ecosystems 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. Most states had comprehensive road data while others only contained state-maintained roads. We filtered each state's data to include only unpaved roads as we assumed many unpaved stream crossings are barriers to fish passage in other freshwater mussel status assessments and merged the data to create a single unpaved road layer (USFWS 2020). Next, we filtered the NHD Flow Line shapefile 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 calculated the total kilometers NHD features in each watershed. Then, we identified all crossings of unpaved roads and NHD features, summed the number of crossings in each watershed, and divided the number of crossings by the kilometers of named stream in the watershed to calculate the density of unpaved road crossings.

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 D.5. Indicator descriptions for the risk metrics used to evaluate the overall connectivity risk to rayed bean populations.

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

D.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, Asian clam, five species of invasive carps (silver, bighead, black, grass, common), rusty crayfish, spiny waterflea, brown trout, quagga mussel, 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 rayed bean where they occur (Table D.6). We prepared the downloaded data by first merging invasive species into categories (Table D.6), then aggregating the positive (the species is present) occurrence records by occupied HUC12 watersheds for each HUC8 occupied by freshwater mussel species. Afterwards, we tested each aggregated invasive species category for significant clustering using Spatial Autocorrelation (Table D.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 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 D.7). Risk levels were based on the presences of hotspots in the analysis (Table D.8, See Section 3.3 for additional information).

Table D.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; Asian clam; spiny waterflea
Reduction of reproductive potential	Displaces host species via competition and predation (including eggs)	invasive carps; 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; Asian clam; invasive carps; brown trout; rusty crayfish; hydrilla
Direct harm/predation	Includes smothering and predation	zebra mussel; invasive carps

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

Category	Index	ZScore	PValue
Direct competition	0.127125	11.101837	0
Reduction of reproductive potential	0.100776	7.789087	0
Disturbance to ecosystems and/or reduction of habitat quality	0.124509	11.156017	0
Direct harm/predation	0.077981	5.874854	40

Table D.8. Indicator descriptions for the risk metric used to evaluate the overall invasive species risk to rayed bean *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	Hot spots were identified in HUC regardless of number or confidence levels
Moderate Risk	Invasive species incidences were identified to occur in HUC, though no hot spots were identified AND/OR cold spots were identified in HUC regardless of number or confidence levels
Low Risk	No invasive species incidences were identified to occur in HUC; there are no hot or cold spots identified

D.6 Catastrophic Events

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.

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

Similarly, to evaluate the risk posed by oil and gas pipelines, we analyzed the presence of pipeline stream crossings within each HUC8. If no pipeline crossings were present in the HUC, we considered that HUC8 at low risk for a catastrophic event due to pipeline spills; if there were crossings present, the population was considered at high risk.

D.7. Scoring

Table D.9. Overall risk factor scoring

Risk Factor 1	Risk Factor 2	Risk Factor 3	Risk Factor 4	Risk Factor 5	Total Score	Total Risk
1	1	1	1	1	5	Low
1	1	1	1	2	6	Low
1	1	1	2	2	7	Low
1	1	2	2	2	8	Moderate
1	2	2	2	2	9	Moderate
2	2	2	2	2	10	Moderate
2	2	2	2	3	11	High
2	2	2	3	3	12	High
2	2	3	3	3	13	High
2	3	3	3	3	14	High
3	3	3	3	3	15	High

APPENDIX E. CURRENT CONDITION

E.1. Great Lakes Basin Representation Unit

The **St. Clair population** in Michigan includes extant occurrences in the Black River, Mill Creek, Pine River, and Belle River. This population is experiencing high risk and has a current demographic condition that is low (Table E.1, Table E.2).

Black River **-** A tributary of the St. Clair River, linking Lakes Huron and St. Clair, the Black River is located in southeastern Michigan. Hoeh and Trdan (1985, entire) surveyed 17 sites in the Black River system, including 12 main stem sites, but failed to find the rayed bean. The rayed bean was not discovered there until the summer of 2001. A single live specimen was found in the lower river in the Port Huron State Game Area (Butler 2002, p. 8).

Mill Creek - Mill Creek is a tributary of the Black River, St. Clair County, in southeastern Michigan. The rayed bean was not discovered in Mill Creek until August 2002. Five presumably FD specimens were found approximately 0.5 miles above its confluence with the Black in the Port Huron State Game Area (Butler 2002, p. 8).

Pine River - Another tributary of the St. Clair River, the Pine River is located in southeastern Michigan. The rayed bean was apparently not collected in the Pine until 1982 when specimens were found at three sites (Hoeh and Trdan 1985, pp. 115-116). These collections included 5 live, 18 whole fresh dead, and 5 unmatched fresh dead shells (Butler 2002, p. 8). Hoeh and Trdan considered it to be rare, semi-quantitatively defined as occurring at a rate of <1 specimen per person hour sampling effort. At one of the four known sites, notes in the MNFI database state that the 1984 sampling estimated rayed bean density at 0.7/foot2 or an estimated 350-500 specimens from the locality. In 1985 samples, 198 specimens of the rayed bean were found at this site, and the species was common in pea gravel and sand at another site sampled that same year. Another site had an estimated 1.1/foot2 in the 1984 sampling. Evidence of recruitment was found during the mid-1980s at three of the four sites. Two live specimens were found in 1997 and the last survey in the Pine River occurred in 2002 (Badra 2008, pers. comm.), and one live rayed bean was documented (Badra and Goforth 2003, p. 6).

Belle River - The Belle River is a third tributary of the St. Clair River harboring an extant population of the rayed bean. This species was first collected from the Belle in 1965, when 17 fresh dead specimens were collected (OSUM 1965:0106). The same site (Wadhams Road Bridge) was revisited in 1978, but only one fresh dead shell is represented in OSUM 1978:0013. Since that time, live or fresh dead specimens have been found in 1983 and 1992, while only relic shells were found in 1994 (Butler 2002, p. 8). In 1992, nine whole and two unmatched valves, all fresh dead, were collected from the same site as those from 1965 and 1978 (Butler 2002, p. 8). Owing to recent rains, turbid conditions prevented Layzer from actively searching for live specimens, but he noted that the shells were found on a bar immediately downstream of the

bridge. During summer 2002 sampling, single live specimens were found at two new sites, with an additional four and two fresh dead specimens also found from these sites (Butler 2002, p. 8).

The **Lake St. Clair population** currently only occurs in the Sydenham River in Ontario, Canada. This population is experiencing high risk but also has a current demographic condition that is high (Table E.1, Table E.2).

Sydenham River - The rayed bean in the Sydenham River represents one of the largest remaining rayed bean occurrences. West et al. (2000, pp. 252-253) presented a highly detailed collection history of the rayed bean in the Sydenham River. The rayed bean was first collected there by H.D. Athearn in 1963, with subsequent collections in the 1960s from other sites in the stream. However, surveys in 1971 (11 sites) and 1985 (20 sites) failed to find the rayed bean. Time efforts at individual sites were limited to approximately 1 person hour per site during these surveys, much shorter than time spent in the 1960s records.

Not until a 1991 survey (16 sites) was the rayed bean rediscovered in the system, represented by a single specimen near Alvinston (Butler 2002, p. 9). West et al. (2000, p. 252) reported sampling conducted by Metcalfe-Smith et al. (1998, 1999) during 1997-98. They sampled 4.5 person hours per site from at least 17 sites, including all 5 sites where the rayed bean had previously been recorded. Live or fresh dead shells were found at 9 sites, including 15 live specimens from 5 sites. Average numbers of live or whole fresh dead specimens per site (n = 8) were 3.6/site (the 37 single fresh dead valves they reported would increase this number considerably). The sex ratio was skewed towards females (62:38), and size ranged from 0.6-1.5 inches.

Additional sampling in 2001 resurveyed sites reported by West et al. (2000), plus several addition localities (Woolnough 2002, p. 50). The rayed bean was located live from 15 sites, including most of the sites where West et al. (2000) reported it as live or fresh dead, in addition to several previously unsampled sites. A total of 522 specimens were found live, with many more fresh dead shells discovered. Average densities per site in the 2001 survey for live specimens were 34.8/site. More than 100 live specimens were located at 2 sites. Sampling effort averaged approximately 6.4 person hours per site. Size range was from 0.5-1.8 inches. The data gathered from the two surveys clearly indicates the importance of very intensive sampling efforts for this diminutive species.

The rayed bean is currently thought to exist in an approximately 57-mile reach of the East Sydenham, from the general vicinity of Napier, Ontario, downstream to Dawn Mills (Fisheries and Oceans Canada 2016, p. 29). The species appears to be most abundant in the lower half of this river reach. Although the range has remained relatively consistent over time, abundance data at repeatedly sampled sites from the 1960s to the late 1990s indicate a general decline of the

rayed bean. Based on the range of sizes and roughly equal number of specimens in various size classes of the live and fresh dead material they gathered, West et al. (2000, p. 253) considered the rayed bean to be "healthy" and "reproducing" (recruiting) in this system based on 1997-1998 data. Data from sampling in 2001 show evidence of recruitment and variable size classes for both sexes from most of the sites (Woolnough 2002, p. 50). Woolnough and Morris (2009, p. 12) estimate that there were 1.5 million mature rayed bean in the Sydenham River living in the 57-mile stretch between Napier Road near Alvinston, Ontario, and Dawn Mills, Ontario. In 2015, Morris (2015, pers. comm.) reported that there are still abundant signs of reproduction in this system; however, repeated sampling at nine monitoring stations throughout the occupied reach in the East Sydenham River has shown a decline from an average density of 1.76 animals/m2 in 1999–2003 to 0.39 animals/m2 in 2013–2015 (Morris 2017, pers. comm.).

The **Clinton population** in Michigan only currently occurs in the Clinton River. This population is experiencing high risk. The population also has a current demographic condition that is low (Table E.1, Table E.2).

Clinton River - The rayed bean was first recorded from the Clinton River by H. van der Schalie in 1933 (Butler 2002, p. 9).

Van der Schalie (1975) found the rayed bean at 4 of 11 sites surveyed, including 3 sites on the main stem Clinton and 1 in the lower North Branch Clinton River (Strayer 1980, p. 145). Main stem sites were widely distributed with one on the lowermost Clinton (Mt. Clemons) and two in the river from Pontiac upstream in the headwaters of the Clinton. In 1977-78, Strayer (1980) surveyed 76 sites in the drainage and found the rayed bean to be uncommon at four main stem sites, all in areas that are now highly developed west of Pontiac. He located no live specimens, but fresh dead shells were found at three of his sites.

Eight live specimens were found at a single site in 1991, and the species was recorded there in 1995 (Butler 2002, p. 9). In 1992, Trdan and Hoeh (Butler 2002, p. 9) found 26 live specimens using a suction dredge from a bridge site slated for widening where Strayer (1980, p. 145) found only relic shells. They represented 1.2% relative abundance of the 10 species collected at the site. The rayed bean appears to be currently restricted to about three miles of stream in the western suburbs of Pontiac.

The **Upper Thames River population** currently only occurs in the Thames River in Ontario, Canada. No data was available to analyze risk to this population. The current demographic condition of this population is high (Table E.1, Table E.2).

Thames River - The Thames River flows west through southwestern Ontario, Canada. The rayed bean occupies approximately an eight mile stretch of the North Thames River upstream of Fanshawe Reservoir and appears to have historically occupied a small stretch of the South Thames River near Dorchester, Ontario (McNichols-O'Rourke 2022, pers. comm). The first North Thames record is of a single live individual collected in 2004. In July 2008, six gravid

females were collected at two North Branch sites (Woolnough 2008, pers. comm.). In September 2008, four live females and two live males were collected at two different North Thames sites (Woolnough 2008, pers. comm.).

In 2013, a single live individual was collected at the Elginfield Bridge, representing the farthest up stream record within the system (Morris 2017, pers. comm.). An additional 16 individuals were collected in 2015 at the same North Thames site where the species was first collected in 2004 (Morris 2015, pers. comm.). A few scattered shells have been found downstream of the confluence of the North and South Thames Rivers. Additional collections include single individuals from seven sites and five individuals from one site within the North Branch. Woolnough and Morris (2009, p. 13) estimate that there are 4,300 mature rayed bean in the Thames River.

The **Raisin population** occurs in only the River Raisin in Michigan. The risk to this population is high and the current demographic condition is low (Table E.1, Table E.2).

River Raisin - The River Raisin is a Lake Erie tributary in southeastern Michigan. The rayed bean was thought to be extirpated from the River Raisin until a survey in 2015 found seven live individuals (Spaeth 2015, pers. comm). Another 2015 survey found eight live rayed bean at a proposed pipeline crossing (TRC Environmental Corporation 2015, p. 4-1). A survey in 2018 found one live rayed bean in Lenawee County, Michigan (TRC Environmental Corporation 2018, p. 2). There is no additional information on the size or distribution of the rayed bean in this stream.

The **St. Joseph population** in Indiana and Ohio includes extant occurrences in the St. Joseph River and Fish Creek. This population is experiencing high risk and also has a current demographic condition that is low (Table E.1, Table E.2).

St. Joseph River - The St. Joseph River is one of the two major headwater tributaries to the Maumee, with a drainage area in southeastern Michigan, northwestern Ohio, and northeastern Indiana. The main stem flows in a southwesterly direction to its confluence with the St. Marys River to form the Maumee in Ft. Wayne, Indiana. Rayed bean was historically known from numerous sites on the river. Watters (1988a, pp. 39-79) reported it from eight sites in the 1980s and 1990s, with fresh dead at only two of them. After sampling at 85 sites in the entire St. Joseph River system in 1988, he considered the rayed bean to be very uncommon and of sporadic occurrence in the drainage.

The rayed bean occurs in the lower portion of the river in Allen and DeKalb Counties, Indiana and Williams County, Ohio. One old, large female was found in the river at the mouth of Fish Creek in Ohio in 2012. The rayed bean was historically known from numerous sites on the river, but now apparently persists only at a couple of sites in the lower river (Watters 1988a, p. 15; 1998, Appendix C); a few fresh dead specimens were found in both studies, but no live

individuals were found. Grabarkiewicz and Crail (2008, p. 13) surveyed six sites on the West Branch St. Joseph River in 2007 but did not encounter any rayed bean.

Fish Creek – A tributary of the St. Joseph River, Fish Creek drains primarily Indiana, but eventually flows eastward into Ohio before joining the St. Joseph at Edgerton, Ohio. Overlooked by Clark (1977, p. 21), Hoggarth (1987, p. 7) reported the rayed bean from one site in Ohio. It persists in Williams County, Ohio, and possibly DeKalb County, Indiana. Based on the appearance of fresh dead shells, it inhabits the lower 10 miles or less of the stream (Watters 1988a, pp. 40-76).

Watters (1988a, p. ii) considered Fish Creek to be "the most pristine tributary of the St. Joseph system." A major diesel fuel spill from a ruptured pipeline in DeKalb County in 1993 resulted in a mussel kill in the lower portion of the stream (Sparks et al. 1999, p. 12). It is not known if the rayed bean was affected by the spill. Surveys in 2004 (at 64 qualitative sites) and 2005 (at 11 quantitative sites) failed to detect the species (Brady et al. 2004, p. 2; 2005, p. 3). However, Grabarkiewicz (2009, pers. comm.) reported finding two live and three fresh dead rayed bean in 2005 at the County Road 3 bridge in Ohio. In 2009, two fresh dead rayed bean were found in lower Fish Creek in Ohio. Fresh dead rayed bean were found at three sites in Williams County in 2011 (Ahlstedt 2011, pp. 4-6). In 2012, live and fresh dead rayed bean were found at two sites (Enviroscience 2012, pp. 5-9).

The **Blanchard population** only currently includes the Blanchard River in Ohio. This population is experiencing high risk but also has a high current demographic condition (Table E.1, Table E.2).

Blanchard River - The Blanchard River is a tributary of the Auglaize River in the Maumee River system, in northwestern Ohio. The Blanchard River occurrence was first discovered in 1946. The rayed bean in the Blanchard River is restricted to a 25–30-river-mile stretch in the upper portion of the stream in Hardin and Hancock Counties upstream of Findlay (Hoggarth et al. 2000, p. 22). Hoggarth et al. (2000, p. 1) reported the rayed bean to be the fourth most common species in the drainage. Grabarkiewicz and Gotten's data (2011, p. 24) indicate that the rayed bean numbers in the Blanchard River have been relatively stable since it was surveyed by Hoggarth in 1994-1996 (Hoggarth et al. 2000, p. 1). Grabarkiewicz and Gottgens (2011) conducted a study in the middle and upper Blanchard River in 2010 to establish baseline occurrence estimates, document age class diversity and find evidence of recent recruitment of mussels. Rayed bean comprised 6.5 percent of mussels found in the study in the upper river and juveniles were noted, indicating recruitment in the stream (Grabarkiewicz and Gottgens 2011, p. 26).

The **Lower Maumee population** only occurs in Swan Creek in Ohio. The risk to this population is high while the current demographic condition is also high (Table E.1, Table E.2).

Swan Creek - Swan Creek is a tributary of the lower Maumee River in northwestern Ohio. The rayed bean was discovered in Swan Creek in 2005. The rayed bean appears to be limited to a 5-river mile reach.

The rayed bean was the fourth most abundant unionid present within a 2006-2008 sample area between river miles 15.3 and 18.3, reaching densities of eight individuals per square meter in some areas and comprising about 14.1 percent of the total mussel community (Grabarkiewicz 2008, p. 10).

Grabarkiewicz and Gottgens (2011, entire) conducted a study in middle and upper Swan Creek in 2010 to establish baseline population estimates, document age class diversity and find evidence of recent recruitment of mussels. In the study, rayed bean accounted for 10.2 percent of mussels found in the middle creek and juveniles were noted, indicating recent recruitment in the stream. The study found an overall rayed bean density estimate of 0.13 rayed bean per square meter in the middle reach of Swan Creek.

In 2015, a bridge scour repair project required the relocation of five rayed bean upstream of the project in Swan Creek (ODOT 2015, p. 8).

In 2016, Woolnough (2017, pers. comm.) found 122 live rayed bean during timed searches and quadrat excavations in middle Swan Creek.

The total area of Swan Creek that supports rayed bean has not been determined so a total estimate of rayed bean cannot be determined at this time.

The **Sandusky population** includes only Tymochtee Creek in Ohio. The risk to this population is high and the current demographic condition is low (Table E.1, Table E.2).

Tymochtee Creek - Tymochtee Creek is a tributary to the upper Sandusky River in north-central Ohio, which flows into the southwestern portion of Lake Erie.

Prior to listing, all collections of the rayed bean were small, with not more than five fresh dead shells found in any one collection effort. At the time of listing in 2012, the rayed bean was known from three sites in a reach of Tymochtee Creek in Wyandot County and was first collected in 1970.

In 2013, two surveys were performed at bridge crossings in Wyandot County for bridge replacement projects. No living or dead rayed bean were found (Enviroscience 2013a, p. 10; Enviroscience 2013b, p. 11). During a 2014 Wyandot County survey for a pipeline repair project, 72 live rayed bean were found (Environmental Solutions & Innovations 2014, p. 2). This new

information confirms that the species is present in the stream and that reproduction is likely occurring; however, nothing else is known about the size and distribution of the species in this stream.

Table E.1. Current condition risk factor analysis for rayed bean populations within the Great Lakes basin representation unit. (CN = Canada)

State	HUC8 Name	HUC8 Code	Contaminant	Landscape	Hydrological Regime	Connectivity	Invasive Species	Overall Current Risk
MI	St. Clair	04090001	High	Moderate	Low	Low	Moderate	High
CN	Lake St. Clair	04090002	Low	High	Low	Low	Unknown	High
MI	Clinton	04090003	High	High	Low	Moderate	Moderate	High
CN	Upper Thames River	04090006	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
MI	Raisin	04100002	Moderate	High	Low	Moderate	Moderate	High
IN, OH	St. Joseph	04100003	High	High	Moderate	Moderate	Moderate	High
OH	Blanchard	04100008	High	Moderate	Low	Low	Moderate	High
OH	Lower Maumee	04100009	High	High	Low	Low	Moderate	High
OH	Sandusky	04100011	High	Moderate	Low	Moderate	Moderate	High

Table E.2. Current condition summary of demographic, risk factor, catastrophic event analysis for rayed bean populations within the Great Lakes basin representation unit. (CN = Canada)

State	HUC8 Name	HUC8 Code	Demographic Condition	Overall Current Risk	Risk of Catastrophic event - Coal	Risk of Catastrophic event - Oil and Gas
MI	St. Clair	04090001	Low	High	Low	High
CN	Lake St. Clair	04090002	High	High	Low	High
MI	Clinton	04090003	Low	High	Low	High
CN	Upper Thames River	04090006	High	Unknown	Low	Low
MI	Raisin	04100002	Low	High	Low	High
IN, OH	St. Joseph	04100003	Low	High	Low	High
OH	Blanchard	04100008	High	High	Low	High
OH	Lower Maumee	04100009	High	High	Low	High
OH	Sandusky	04100011	Low	High	Low	High

E.2. Ohio Basin Representation Unit

The **Upper Allegheny population** includes extant occurrences in the Allegheny River in New York and Pennsylvania and Olean Creek, Cassadaga Creek, Oswayo Creek and Oil Creek in New York. This population is experiencing high risk and has a high current demographic condition (Table E.3, Table E.4).

Allegheny River - The Allegheny River drains northwestern Pennsylvania and western New York, joining the Monongahela River at Pittsburgh, Pennsylvania, to form the Ohio River. At the time of listing, no recent data existed for the rayed bean in the New York portion of the Allegheny River. New data documents that the rayed bean occurs between the City of Olean and the Allegheny Reservoir. Three live rayed bean were found in the Allegheny River near the confluence of Kings Brook and Olean Creek in 2013 and two live rayed bean were documented in 2015 during a bridge survey in Carrollton, New York (EcoLogic 2014a, p. 8; Lord 2015, p. 5). In 2017, 439 rayed bean were found in the City of Olean during an initial survey for a petroleum

clean-up project (Environmental Solutions & Innovations 2017, p. 2). All of these rayed bean were relocated upstream of the project area to suitable habitat. Thousands of additional rayed bean were salvaged and relocated for this clean-up project (USFWS 2019, p. 20). The overall effects of the petroleum spill on the rayed bean in this reach are unknown. Following the removal of the petroleum-impacted sediments, the river bottom will be restored to provide non-impacted suitable habitat for the rayed bean and other mussels.

Olean Creek - Olean Creek is a tributary of the Allegheny River in western New York. Rayed bean only occurs in the lower portion of Olean Creek, likely no more than two miles upstream of the mouth. The rayed bean occurs at a low density from the lower portions of the stream. Strayer et al. (1991, p. 67) reported the rayed bean from three sites during 1987–90 sampling, although just one live individual was located with relic shells from the other two sites. Only relic shells were found in Olean Creek in 1994, but three live individuals were found in 2000, at the proposed construction site of the City of Olean Water Treatment Plant (ESI 2000, p. 8). Collected only during their quantitative sampling effort, the rayed bean represented a relative abundance of 11.5 percent of the seven live species sampled. Relic specimens are known from an 8-mi reach of stream, with live individuals known from less than 1.5 mi of the lower creek.

Cassadaga Creek - Cassadaga Creek is a tributary of Conewango Creek in the Allegheny River system, in western New York. Rayed bean may be restricted to mid-reaches of the stream in less than one mile, but 75+ rayed bean were observed during a 2015 survey. At the time of listing, the rayed bean was known from a single riffle (Ross Mills) in the lower creek north of Jamestown, New York. Four live specimens were found in 1994 (Butler 2015, p. 15). Muskrat middens (a pile of shells) collected during the winter of 2002 produced 38 fresh dead rayed bean with a size range of 0.8–1.7 in, representing multiple year classes and indicating that the species was recruiting (Clapsadl 2002, pers. comm.). In 2015, 216 live rayed bean were found at the Ross Mills site during a bridge project survey (Environmental Solutions & Innovations 2015, p. i). The bridge project was completed with no instream work to be protective of mussels, including the rayed bean (Doran 2018, pers. comm.). Although the rayed bean is not known from other sites in the stream, the large number of rayed bean found at the site in 2015 indicated that the species is healthy at least in the vicinity of this site.

Oswayo Creek – Oswayo Creek is a tributary to the Allegheny River in Cattaraugus County, New York that flows into the Allegheny River in Portville, New York. The rayed bean occurs in less than one mile of the creek. In 2014, only a single dead shell was found in this creek (EcoLogic 2014b, p. 3). In a 2015 survey at the Route 305 Bridge for a maintenance project, about 0.5 mile upstream of the mouth, two live rayed bean were found and relocated in the stream out of harm's way of the project (Doran 2016, pers. comm.). In 2017, two live and three fresh dead rayed bean were found during a survey at a proposed pipeline crossing in McKean

County, Pennsylvania (Enviroscience 2017a, p. iii). Nothing else is currently known about the rayed bean occurrence in this stream.

Oil Creek - Oil Creek and Ischua Creek join in Hinsdale, New York to form Olean Creek. The rayed bean was thought to be extirpated from this stream at the time of listing. In 2015, four live rayed bean was found in Oil Creek near the confluence with Ischua Creek (EcoLogic 2015, p. 3). Nothing else is currently known about the rayed bean in this stream.

The **Middle Allegheny – Tionesta population** currently is comprised of only the Allegheny River in Pennsylvania. The risk to this population is high with a moderate current demographic condition (Table E.3, Table E.4).

Allegheny River – The Allegheny River drains northwestern Pennsylvania and western New York, joining the Monongahela River at Pittsburgh, Pennsylvania, to form the Ohio River. The rayed bean occurs from the Kinzua Dam to the mouth of the Clarion River. In 2015, four live rayed bean were salvaged and relocated within the river for a bridge replacement project at Hunters Station in Forest County, Pennsylvania (Enviroscience 2018, p. 15). Live rayed bean were found at multiple survey locations in 2015.

The **French population** includes four occupied streams in Pennsylvania: French Creek, LeBoeuf Creek, Muddy Creek, and Cussawago Creek. The risk to this population is moderate with a high current demographic condition (Table E.3, Table E.4).

French Creek – French Creek is a major tributary of the middle Allegheny River, in western New York and northwestern Pennsylvania. The rayed bean occurs from the Union City Dam to the Allegheny River confluence. The rayed bean is not known from the New York portion of stream. Ortmann (1909a, p. 188; 1919, pp. 262-264) reported the species from two Pennsylvania counties, Crawford and Venango. Not until circa 1970 did the status of the rayed bean in this stream become more thoroughly known, with museum lot sizes indicating sizable numbers at several sites, particularly in the lower reaches of the stream.

During a 2013 mussel salvage at State Route 1015 (Carlton Bridge) in Pennsylvania, 162 live rayed bean were collected (Enviroscience 2013c, p .4). All of these individuals were translocated to Queen Shoals in the Elk River, West Virginia (Elk HUC8 population) (Enviroscience 2013c, p. 5). Recent collections indicate that numbers remain high and the rayed bean occurs throughout the mainstem (Huehner 2016, pers. comm.; Enviroscience 2015a, p. 8; Enviroscience 2013c, p. 4).

In 2017, a survey and salvage/relocation effort found 396 live rayed bean during the survey in Carlton, Pennsylvania (Enviroscience 2017b, p. 18-19). Salvaged rayed bean were relocated into

French Creek upstream of the project area. A 2017 survey found 33 live rayed bean in Meadsville, Pennsylvania (Enviroscience 2017c, p. 9).

LeBoeuf Creek – Le Boeuf Creek is a small western tributary of upper French Creek, flowing in a southerly direction just west of West Branch French Creek in Erie County, Pennsylvania. A total of five live individuals were collected at two out of five sites during a 2006 survey (Smith *et al.* 2009, pp. 68–76; Welte 2011, pers. comm.). The rayed bean occurs from LeBoeuf Lake outfall to the French Creek confluence. No other information is available on the status in this stream

Muddy Creek – Muddy Creek is an eastern tributary of upper French Creek in Crawford County, Pennsylvania. The rayed bean was not discovered until the summer of 2006. Live and fresh dead rayed bean were reported from 2 of 20 lower river sites (Mohler et al. 2006, pp. 581–582). No live juveniles were found during the 2006 survey (Mohler et al. 2006, p. 576). The rayed bean occurs from the Route 77 Bridge to the French Creek confluence. In 2015, 20 live rayed bean were found at eight sites in the Erie National Wildlife Refuge in Crawford County, Pennsylvania (Enviroscience 2015b, p. 27).

Cussawago Creek – Cussewago Creek is a tributary of lower French Creek, with its confluence at Meadville, Crawford County, Pennsylvania. A small population was reported in 1991 from the creek in 1991 (Proch 2001, pers. comm.). Welte (pers. comm. 2011) found several fresh dead rayed bean immediately downstream of the Spring Street Bridge in 2011. The rayed bean occurs from the Interstate 79 Bridge to the French Creek confluence.

The **Middle Allegheny – Redbank population** is currently comprised of only the Allegheny River in Pennsylvania. This population is at moderate risk with a moderate current demographic condition (Table E.3, Table E.4).

Allegheny River – The Allegheny River drains northwestern Pennsylvania and western New York, joining the Monongahela River at Pittsburgh, Pennsylvania, to form the Ohio River. The rayed bean occurs from the mouth of the Clarion River to downstream of Lock and Dam No. 5 (~84 river miles). Twenty-one live rayed bean were found in 2019 during salvage efforts downstream of Lock and Dam No. 5 (Lewis Environmental Consulting 2019, p. 6). Salvaged rayed bean were moved to an areas within the same mussel consentrtion outside of the navigation approach (Lewis Environmental Consulting 2019, p. 8). Ortmann (1909a, p. 188) was the first to report the rayed bean from the Allegheny. Based on historical collections, it appears that the rayed bean is more abundant now than it was historically in the Allegheny River. This may indicate that the rayed bean in the Allegheny has expanded in the past 100 years. Many streams in western Pennsylvania have improved water quality since Ortmann's time, when he reported on the wholesale destruction of mussels from pollution in several streams (Ortmann

1909b, p. 11-12). In 2016, one live rayed bean was found just downstream of Lock and Dam No. 5 (Lewis 2016, pers. comm.). Huehner (2016, pers. comm.) also reported finding three live rayed bean in Pool 6 approximately four miles upstream of Lock and Dam No. 6. The rayed bean is now known to occur in an additional 22 miles (35 km) farther downstream than previously documented at the time of listing.

The **Elk population** is currently comprised of only the Elk River in West Virginia. The risk to this population is low and this reintroduced population has a low demographic condition (Table E.3, Table E.4).

Elk River – The Elk River is a major 181-river-mi tributary in the lower Kanawha River system draining central West Virginia and flowing west to the Kanawaha River at Charleston. The rayed bean was extirpated from the Elk River sometime in the 1990s. In 2006 and 2007, 684 adults were reintroduced (translocated from French Creek in the French HUC8 population) into the Elk River above Clendenin. In 2008, an effort was made to monitor the reintroduction. A 30-minute search yielded two live individuals, but efforts were discontinued due to high water and excessive habitat disturbance caused by the search effort (Clayton 2008, pers. comm.).

In 2010, none of the individuals released in 2006 were found, but an additional 189 individuals were released (Clayton 2010, pers. comm.). The translocated adults are thought to persist in the stream, but it is unknown if this new population is reproducing. Subsequent translocations occurred 2013 (n =162), and 2014 (n = 81) from the Middle Allegheny – Tionesta and Middle Allegheny – Redbank populations. All of the translocated rayed bean were collected during salvage operations for bridge projects (Eliason 2022a, pers. comm.). The last reported sighting of the stocked animals was in 2015 and no specific search efforts has taken place since 2017 (Eliason 2022b, pers. comm.).

The **Upper Scioto population** includes two occupied streams in Ohio: Big Darby Creek and Mill Creek. This population is experiencing high risk with a low demographic condition (Table E.3, Table E.4).

Big Darby Creek – Big Darby Creek is one of the major tributaries draining the northwestern portion of the Scioto River system in central Ohio. A sizable rayed bean population was noted in Big Darby Creek from OSUM collections, primarily from the 1960s. Watters (1994, p. 105) reported finding a few fresh dead specimens in 1986, but none in 1990, and indicated that the rayed bean was probably extirpated from Big Darby Creek.

In 2006, one live individual was found at the U.S. Highway 42 bridge replacement project site (Hoggarth 2006, p. 6). This individual was relocated to a site upstream out of the impact zone of the bridge project, and nine additional live individuals were subsequently found at the relocation

site (Hoggarth 2006, p. 6). In 2007, three live rayed bean were found at the relocation site (Hoggarth 2007b, p. 9). Hoggarth (2008, pers. comm.) visited the same relocation site in 2008 and reported finding ‘‘numerous living specimens’’ of rayed bean. Surveys have occurred in the river, but none have documented any live rayed bean since 2008.

Mill Creek – Mill Creek is a tributary of the Scioto River in central Ohio that joins the Scioto River at the O’Shaughnessy Reservoir northwest of the City of Columbus. In 2004, seven fresh dead specimens were found during a survey in the City of Marysville (Hoggarth 2005, p. 7). In 2007, Hoggarth (2007a, pp. 5–6) found two live rayed bean at the same site and one live individual at an additional site. In 2019, one live and one fresh dead rayed bean were found near the same 2007 site (Wells 2019, p. 5).

The **Upper Great Miami population** occurs in the Great Miami River and Stillwater River in Ohio. The risk to this population is high and the current demographic condition is low (Table E.3, Table E.4).

Great Miami River – The Great Miami River is a major northern tributary of the Ohio River in southwestern Ohio that originates from Indian Lake in west-central Ohio and flows into the Ohio River west of Cincinnati. The occurrence of the rayed bean in the Great Miami River was discovered in August 2009, during a mussel survey for a bridge project in Logan County, Ohio. Only one individual was documented, a male approximately seven to eight years of age (Hoggarth 2009, pers. comm.). The following year, Hoggarth (2010, p. 5) found a juvenile rayed bean.

Stillwater River - The Stillwater River is a western tributary of the middle Great Miami River in southwestern Ohio. The rayed bean is only known from two specimens, one fresh dead and one relic, collected in 1987 at two sites spanning the Miami–Montgomery County line (OSUM records). Both sites occur in the impounded area of Englewood Reservoir (constructed circa 1920). Post-year 2000 mussel data for this stream is limited.

The **Tippecanoe population** in Indiana consists of one currently occupied stream, the Tippecanoe River, and one lake, Lake Maxinkuckee. The risk to this population is moderate and the demographic condition is very low/functionally extirpated (Table E.3, Table E.4).

Tippecanoe River – The Tippecanoe River is a large northern tributary of the middle Wabash River in north-central Indiana. The rayed bean occurs in records that exist in Carroll, Kosciusko, Pulaski, and Tippecanoe Counties, Indiana. The species may also occur in Fulton, Starke and White Counties, though there are no recent records of rayed bean in these counties. The first records for the rayed bean date to circa 1900 (Daniels 1903, p. 646). Historically, this species was known from numerous sites in six counties. A total of 12 fresh dead specimens from 5 of 30

sites were found when sampled in 1992. The last documented occurrence is at the Pretty Prairie Road Bridge in 2014 (Pruitt 2016, pers. comm.).

Lake Maxinkuckee – Lake Maxinkuckee is a glacial lake in the headwaters of the Tippecanoe River in north-central Indiana. The rayed bean has been known from the lake for more than a century (Blatchley 1901, p. 3). A 1997 Ohio State University Museum record included seven fresh dead specimens collected at the Wilson Ditch outlet to the Tippecanoe River. No post-year 2000 mussel surveys have been reported for this lake.

The **Driftwood population** includes only one currently occupied stream, Sugar Creek, in Indiana. This population is experiencing high risk with a low demographic condition (Table E.3, Table E.4).

Sugar Creek - Sugar Creek is a tributary of the East Fork White River, in the lower Wabash River system in south-central Indiana. The rayed bean was first reported in Sugar Creek in 1930 (Butler 2002, p. 19). Harmon (1992, p. 33) sampled 27 mainstem and 16 tributary sites, finding fresh dead specimens at 3 mainstem sites and relic specimens from 2 other sites. The sites with fresh dead material were found in the lowermost 6 mi (9.7 km) of stream.

Table E.3. Current condition risk factor analysis for rayed bean populations within the Ohio basin representation unit.

State	HUC8 Name	HUC8 Code	Contaminant	Landscape	Hydrological Regime	Connectivity	Invasive Species	Overall Current Risk
NY, PA	Upper Allegheny	05010001	High	Low	Low	Moderate	Moderate	High
PA	Middle Allegheny - Tionesta	05010003	High	Moderate	Low	Moderate	Moderate	High
PA	French	05010004	Moderate	Moderate	Low	Moderate	Moderate	Moderate
PA	Middle Allegheny-Redbank	05010006	Moderate	Moderate	Low	Moderate	Moderate	Moderate
WV	Elk	05050007	Moderate	Low	Low	Low	Moderate	Low
OH	Upper Scioto	05060001	High	High	Low	Moderate	Moderate	High
OH	Upper Great Miami	05080001	High	High	Low	Moderate	Moderate	High
IN	Tippecanoe	05120106	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
IN	Driftwood	05120204	Moderate	High	Moderate	Moderate	Moderate	High

Table E.4. Current condition summary of demographic, risk factor, catastrophic event analysis for rayed bean populations within the Ohio basin representation unit.

State	HUC8 Name	HUC8 Code	Demographic Condition	Overall Current Risk	Risk of Catastrophic event - Coal	Risk of Catastrophic event - Oil and Gas
NY, PA	Upper Allegheny	05010001	High	High	Low	High
PA	Middle Allegheny - Tionesta	05010003	Moderate	High	High	High
PA	French	05010004	High	Moderate	Low	High
PA	Middle Allegheny-Redbank	05010006	Moderate	Moderate	High	High
WV	Elk	05050007	Low	Low	High	Low
OH	Upper Scioto	05060001	Low	High	Low	High
OH	Upper Great Miami	05080001	Low	High	Low	High
IN	Tippecanoe	05120106	High	Moderate	Low	High
IN	Driftwood	05120204	Low	High	Low	High

E.3. Tennessee Basin Representation Unit

The **Upper Duck population** includes only one extant stream, the Duck River, in Tennessee. This population is at high risk and has a low demographic condition (Table E.5, Table E.6).

Duck River - The Duck River is the downstream-most large tributary of the Tennessee River draining south-central Tennessee and flowing 285 river miles (459 river km) west to its confluence near the head of Kentucky Reservoir. The rayed bean was considered to be extirpated from the river until a reintroduction took place in September 2008. A total of 969 adults were collected from the Allegheny River at East Brady, Pennsylvania (Middle Allegheny – Redbank population), in 2008 (Welte 2011, pers. comm.). Following quarantine and retention of several

individuals for propagation efforts, a total of 681 rayed bean were translocated to the Duck River near Lillard Mill, Tennessee (Urban 2010, pers. comm.; Moles and Layzer 2009, pp. 2–3; Welte 2011, pers. comm.). The status of the reintroduced population was assessed in 2009. Rayed bean survival rates from three plots and downstream dispersal areas ranged from 38 to 62 percent (Moles and Layzer 2009, pp. 4–7). Live rayed bean were also noted at the reintroduction site in 2012 (Hubbs 2017, pers. comm.).

Table E.5. Current condition risk factor analysis for rayed bean populations within the Tennessee basin representation unit.

State	HUC8 Name	HUC8 Code	Contaminant	Landscape	Hydrological Regime	Connectivity	Invasive Species	Overall Current Risk
TN	Upper Duck	06040002	High	Moderate	Moderate	Moderate	Moderate	High

Table E.6. Current condition summary of demographic, risk factor, catastrophic event analysis for rayed bean populations within the Tennessee basin representation unit.

State	HUC8 Name	HUC8 Code	Demographic Condition	Overall Current Risk	Risk of Catastrophic event - Coal	Risk of Catastrophic event - Oil and Gas
TN	Upper Duck	06040002	Low	High	Low	Low

APPENDIX F. METHODS FOR FUTURE CONDITION RISK FACTORS

F.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 5.3). 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, pp. 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, pp. 3–18).

F.1.a. Water Quality and Climate Change

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 and 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 stream 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. Based 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 rayed bean 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.

F.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 and development in HUC8s, 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 commensurate rates. 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.

F.3 Hydrological Regime

Although we used U.S. Drought Monitor data to quantitatively evaluate current condition, we could not use these 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 and 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 and 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 as consistent as possible with our other current and future condition analyses – data was not available that matched our current and future time periods exactly. 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 modeled future (2040–2069), averaged CDSI (drought severity index) 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. These watershed-scale results comport with the regional-scale results presented in Peters and Iverson (2019, entire) which suggest that drought severity may decrease relative to current conditions 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 results of the models for 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 and Iverson 2019, entire; Mishra and Cherkauer 2010, entire; Cook et al. 2014, entire; Wehner et al. 2011, entire; Zhao and Dai 2017, entire; Cook et al. 2020, entire) all indicate a tendency towards increasing drought relative to current conditions after mid-century.

F.4 Connectivity

In some areas of rayed bean, 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 Global biodiversity model for policy support's U.S. Global Roads Inventory Project's (GRIP). For Future Scenario 1 we used socio-economic pathway 5 (SSP5: 27.3%; projections for increases in all road type 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).

F.5 Invasive Species

We assessed the risk of negative impacts because of invaders worsening in the future by identifying the number of hot spots that occur in neighboring HUC8 watersheds (HUC8 watershed was the level considered for populations and associated analyses) directly adjacent to each HUC8 population included in the current condition risk assessment (Table 5.3). We included neighboring watersheds 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 HUC8 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, or from moderate risk to high risk) sometime in the future (Table F.1).

Table F.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)	Increases from Moderate Risk; 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
Moderate Risk (2 points)	Increases from Low Risk; 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 5.3). If there is a hotspot neighbor to any occupied HUC8 population, we are making the assumption the frequency and abundance necessary to cause that hotspot would mean the dispersal into these populations is sufficient to move them into a moderate or high risk category.

Future Scenario 2 is the same invasive rate as it is now (Table 5.3).

For the HUC8 populations that remained unchanged from the current condition risk assessment, 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.

F.6 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 decreases 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 G. FUTURE CONDITION

G.1. Scenario 1

Great Lakes Basin Representation Unit

Based on the overall risk levels in the Great Lakes basin, we project five populations will be extirpated – St. Clair, Clinton, Raisin, St. Joseph, and Sandusky (Figure 5.1, Table G.1) – all of which are currently in low demographic condition. One population that had demographic data evaluated overlaps with Canada and was not fully assessed by projecting risk levels. The remaining three populations in the Great Lakes basin will be in low demographic condition and will be experiencing high risk under Scenario 1 (Figure 5.1, Table G.1).

Ammonia, chloride, copper, nitrate, and metal levels are projected to increase for all populations in the Great Lakes basin in Scenario 1. Six populations remain at a high level of risk for contaminants (Table G.2, Table G.11.).

The St. Clair, Blanchard, and Sandusky populations have a projected increase to the landscape risk factor, with the risk level increasing from moderate to high. (Table G.2).

Seven of the populations experience low risk for drought, but the CDSI is projected to increase, ranging from 77.2–127% and averaging 109% increase. The other population analyzed for risk, St. Joseph, is experiencing moderate risk, and the CDSI is projected to increase 112% (Table G.2).

All populations in the Great Lakes basin except for the Upper Thames population 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 G.2).

*Table G.1. Summary of projected demographic condition for rayed bean populations given Scenario 1 and Scenario 2 risk levels within the Great Lakes basin. (*one population within Canada was 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 - Scenario 1	Projected HUC8 Count – Scenario 2
High	4		
Moderate			
Low	5	3	3
Functionally Extirpated			
Extirpated		5	5
Total	9	8*	8*

Table G.2. Scenario 1 future projections for risk factors for populations of rayed bean in the Great Lakes basin. Bold text indicates changes from the current condition risk factor condition. (CN = Canada).

State	HUC8 Name	Current Demographic Condition	Contaminant [6]	Landscape	Hydrological Regime (% change in CDSI)	Connectivity	Invasive Species	Overall Current Risk
MI	St. Clair	Low	High ↑	**High**	Low (127.57)	Low	Moderate	High
CA, MI	Lake St. Clair	High	Low ↑	High	Low (126.42)	Low	Unknown	High
MI	Clinton	Low	High ↑	High	Low (123.09)	Moderate	Moderate	High
CN	Upper Thames River	High	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
MI	Raisin	Low	Moderate ↑	High	Low (107.93)	Moderate	Moderate	High
IN, OH	St. Joseph	Low	High ↑	High	Moderate (112.94)	Moderate	High	High
OH	Blanchard	High	High ↑	**High**	Low (77.24)	Low	Moderate	High
OH	Lower Maumee	High	High ↑	High	Low (101.95)	Low	Moderate	High
OH	Sandusky	Low	High ↑	**High**	Low (98.93)	Moderate	Moderate	High

[6] ↑ = Increased percentage of urban and/or agricultural landcover projected under Scenario 1, indicating an increased risk associated with primary contaminants (ammonia, chloride, copper, nitrate)

Ohio Basin Representation Unit

We project the Middle Allegheny-Tionesta and Elk populations will be functionally extirpated in the future under Scenario 1 (Figure 5.1, Table G.3). We expect the Upper Scioto, Upper Great Miami, Tippecanoe, and Driftwood populations will be extirpated under Scenario 1 (Figure 5.1, Table 5.6). Under Scenario 1, the Upper Allegheny population will be in low condition experiencing high risk, the French population will be in moderate condition experiencing high risk, and the Middle Allegheny-Redbank population will be in low condition experiencing moderate risk. (Figure 5.1, Table G.4).

The Ohio basin is projected in Scenario 1 to have an increase of ammonia, chloride, copper, and nitrate levels in all populations. The Upper Allegheny, Middle Allegheny-Tionesta, Upper Scioto, and Upper Great Miami populations continue to be at high risk for contaminants under Scenario 1. The following populations are at moderate risk for contaminants in Scenario 1: French, Middle Allegheny-Redbank, Elk, Tippecanoe, and Driftwood (Table G.4, Table G.11).

Seven of the populations currently experience low risk from drought, but the CDSI is projected to increase by 77.5–241%. The other two populations currently experience moderate risk from drought, with projected increases in CDSI ranging from 77.5–110% (Table G.13).

Oil and gas supply are projected to increase in areas that already have infrastructure. 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 G.16).

Table G.3. Summary of projected demographic condition for rayed bean 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 - Scenario 1	Projected HUC8 Count – Scenario 2
High	3		
Moderate	2	1	2
Low	4	2	3
Functionally Extirpated		2	1
Extirpated		4	3
Total	9	9	9

Table G.4. Scenario 1 future projections for risk factors for populations of rayed bean in the Ohio basin. Bold text indicates changes from the current condition risk factor condition.

State	HUC8 Name	Current Demographic Condition	Contaminant [7]	Landscape	Hydrological Regime (% change in CDSI)	Connectivity	Invasive Species	Overall Current Risk
PA, NY	Upper Allegheny	High	High ↑	**Moderate**	Low (241.76)	Moderate	**High**	High
PA	Middle Allegheny-Tionesta	Moderate	High ↑	**Moderate**	Low (171.83)	Moderate	**High**	High
PA	French	High	Moderate ↑	**High**	Low (146.83)	Moderate	High	Moderate
PA	Middle Allegheny-Redbank	Moderate	Moderate ↑	**Moderate**	Low (193.68)	Moderate	Moderate	Moderate
WV	Elk	Low	Moderate ↑	**Moderate**	Low (77.60)	Low	Moderate	Low
OH	Upper Scioto	Low	High ↑	High	Low (113.85)	Moderate	Moderate	High
OH	Upper Great Miami	Low	High↑	High	Low (110.29)	Moderate	Moderate	High
IN	Tippecanoe	High	Moderate ↑	**High**	Moderate (54.70)	Moderate	High	Moderate

[7] ↑ = Increased percentage of urban and/or agricultural landcover projected under Scenario 1, indicating an increased risk associated with primary contaminants (ammonia, chloride, copper, nitrate)

IN	Driftwood	Low	Moderate ↑	High	Moderate (60.84)	Moderate	Moderate	High

Tennessee Basin Representation Unit

The one population in the Tennessee basin, the Upper Duck, is currently in a low demographic condition with high risk (Figure 5.1, Table G.5). Under Scenario 1, this population is predicted to be extirpated.

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 (Table G.11).

Projected landscape changes for the Upper Duck population increase the risk level of this populations from moderate to high. The Upper Duck population is projected to have a decrease in vegetative cover in the riparian area (Table G.12).

The Upper Duck populations experiences moderate risk for hydrological regime with a CDSI increase of 82.71% (Table G.13).

There was no change in the projected risk resulting from invasive species, connectivity, or host species vulnerability (Table G. 15).

Oil and gas supply are projected to increase in areas that already have infrastructure. The Upper Duck population is 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 the population remains at a low risk (Table G.16).

Table G.5. Summary of projected demographic condition for rayed bean 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 - Scenario 1	Projected HUC8 Count – Scenario 2
High			
Moderate			
Low	1		
Functionally Extirpated			
Extirpated		1	1
Total	1	1	1

Table G.6. Scenario 1 future projections for risk factors for populations of rayed bean in the Tennessee basin. Bold text indicates changes from the current condition risk factor condition.

State	HUC8 Name	Current Demographic Condition	Contaminant [8]	Landscape	Hydrological Regime (% change in CDSI)	Connectivity	Invasive Species	Overall Current Risk
TN	Upper Duck	Low	High ↑	**High**	Moderate	**High**	Moderate	High

[8] ↑ = Increased percentage of urban and/or agricultural landcover projected under Scenario 1, indicating an increased risk associated with primary contaminants (ammonia, chloride, copper, nitrate)

G.2. 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 for all populations except the Sandusky population. Under Scenario 1, this population is predicted to be extirpated, but under Scenario 2 it is predicted to be functionally extirpated (Figure 5.2, Table G.1, Table G.7).

Ammonia, chloride, copper, nitrate, and metal levels are projected to increase in all populations in the Great Lakes basin in Scenario 2. Seven populations remain at a high level of risk for contaminants (Table G.7). Lake St. Clair and Raisin populations remain at a moderate level of risk (Table G.11).

All but one population is projected to be at high risk for landscape factors under Scenario 2, with the Sandusky population remaining at moderate risk (Table G.12).

Seven of the populations experience low risk for drought and two experience moderate risk, with the CDSI actually projected to have a decreased drought severity for Scenario 2 for four of the nine populations. These four populations have a projected percent decrease ranging from -9.7% to -29%. The population experiencing an increase in risk have a CDSI percent increase ranging from 2.46% to 104% (Table G.13).

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 G.16).

Table G.7. Scenario 2 future projections for risk factors for populations of rayed bean in the Great Lakes basin. Bold text indicates changes from the current condition risk factor condition. (CN = Canada).

State	HUC8 Name	Current Demographic Condition	Contaminant [9]	Landscape	Hydrological Regime (% change in CDSI)	Connectivity	Invasive Species	Overall Current Risk
MI	St. Clair	Low	High ↑	**High**	Low (-13.83)	Low	Moderate	High
CA, MI	Lake St. Clair	High	Low ↑	High	Low (-36.78)	Low	Unknown	High
MI	Clinton	Low	High ↑	High	Low (-24.48)	Moderate	Moderate	High
CN	Upper Thames River	High	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
MI	Raisin	Low	Moderate ↑	High	Low (-27.12)	Moderate	Moderate	High
IN, OH	St. Joseph	Low	High ↑	High	Moderate (-40.72)	Moderate	Moderate	High
OH	Blanchard	High	High ↑	**High**	Low (-39.00)	Low	Moderate	High
OH	Lower Maumee	High	High ↑	High	Low (-33.91)	Low	Moderate	High
OH	Sandusky	Low	High ↑	Moderate	Low (-43.65)	Moderate	Moderate	High

[9] ↑ = Increased percentage of urban and/or agricultural landcover projected under Scenario 2, indicating an increased risk associated with primary contaminants (ammonia, chloride, copper, nitrate)

Ohio Basin Representation Unit

Based on the overall risk levels in the Ohio basin, we project the same projected demographic conditions as Scenario 1 for all populations except the Elk and Tippecanoe populations (Figure 5.2, Table G.3). Under Scenario 2, the Elk population is predicted to be in low condition with low risk whereas under Scenario 1 it is predicted to be in functionally extirpated with moderate risk. Under Scenario 2, the Elk population is predicted to be in moderate condition with moderate risk whereas under Scenario 1 it is predicted to be extirpated.

In the Ohio basin in Scenario 2, ammonia, chloride, copper, nitrate, and metal levels are projected to increase in all populations. Four of the populations continue to be at high risk for contaminants (Upper Allegheny, Middle Allegheny-Tionesta, Upper Scioto, Upper Great Miami). The following populations are projected to be at moderate risk for contaminants: the French, Middle Allegheny-Redbank, Elk, Tippecanoe, and Driftwood populations (Table G.8, Table G.11). The projected risk in Scenario 2 for all other populations in the Ohio basin stayed the same as the risk for current condition for all other factors.

Seven of the populations experience low risk for drought. Of these seven populations, the Upper Scioto and Upper Great Miami populations had a decrease ranging between -26% and -29% in the drought severity index. The range of the increase in drought severity index for the low-risk populations was between 2.46% and 104%. The other two populations experience moderate risk with a CDSI ranging from a decrease of -9.7% to -22% (Table G.13).

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. All but three 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 G.16).

Table G.8. Scenario 2 future projections for risk factors for populations of rayed bean in the Ohio basin. Bold text indicates changes from the current condition risk factor condition.

State	HUC8 Name	Current Demographic Condition	Contaminant [10]	Landscape	Hydrological Regime (% change in CDSI)	Connectivity	Invasive Species	Overall Current Risk
PA, NY	Upper Allegheny	High	High ↑	Low	Low (50.18)	Moderate	Moderate	High
PA	Middle Allegheny-Tionesta	Moderate	High ↑	Moderate	Low (17.47)	Moderate	Moderate	High
PA	French	High	Moderate ↑	Moderate	Low (2.47)	Moderate	Moderate	Moderate
PA	Middle Allegheny-Redbank	Moderate	Moderate ↑	Moderate	Low (104.90)	Moderate	Moderate	Moderate
WV	Elk	Low	Moderate ↑	Low	Low (79.18)	Low	Moderate	Low
OH	Upper Scioto	Low	High ↑	High	Low (-26.46)	Moderate	Moderate	High
OH	Upper Great Miami	Low	High ↑	High	Low (-29.12)	Moderate	Moderate	High
IN	Tippecanoe	High	Moderate ↑	Moderate	Moderate (-9.76)	Moderate	Moderate	Moderate
IN	Driftwood	Low	Moderate ↑	High	Moderate (-22.22)	Moderate	Moderate	High

[10] ↑ = Increased percentage of urban and/or agricultural landcover projected under Scenario 2, indicating an increased risk associated with primary contaminants (ammonia, chloride, copper, nitrate)

Tennessee Basin Representation Unit

The one population in the Tennessee basin, the Upper Duck, is currently in a low demographic condition with high risk (Figure 5.1, Table G.5). Under Scenario 2, this population is predicted to be extirpated.

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, copper, nitrate, and metals (Table G.11).

Projected landscape changes for the Upper Duck population increase the risk level of this populations from moderate to high (Table G.2.).

The Upper Duck populations experiences moderate risk for hydrological regime with a CDSI increase of 82.71% (Table G.13).

There was no change in the projected risk resulting from invasive species, connectivity, or host species vulnerability (Table G.14).

Oil and gas supply are projected to increase in areas that already have infrastructure. The Upper Duck population is at high risk for catastrophic events for oil and gas. Scenario 2 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 the population remains at a low risk (Table G.16).

Table G.9. Scenario 2 future projections for risk factors for populations of rayed bean in the Tennessee basin. Bold text indicates changes from the current condition risk factor condition.

State	HUC8 Name	Current Demographic Condition	Contaminant [11]	Landscape	Hydrological Regime (% change in CDSI)	Connectivity	Invasive Species	Overall Current Risk
TN	Upper Duck	Low	High ↑	Moderate	Moderate (91.11)	High	Moderate	High

G.3. Summary of Scenarios 1 and 2.

Table G.10 Summary of current demographic population condition, with the future risk factors for Scenarios 1 and 2 and the predicted demographic population conditions (CN=Canada).

State	HUC2 Representation Unit	HUC8 Name	HUC8 Code	Current Demographic Condition	Scenario 1 Overall Risk Factor	Scenario 1 Predicted Demographic Condition	Scenario 2 Overall Risk Factor	Scenario 2 Predicted Demographic Condition
MI	Great Lakes	St. Clair	04090001	Low	High	Extirpated	High	Extirpated
CN	Great Lakes	Lake St. Clair	04090002	High	High	Low	High	Low
MI	Great Lakes	Clinton	04090003	Low	High	Extirpated	High	Extirpated
CN	Great Lakes	Upper Thames River	04090006	High	NA	Unknown	NA	Unknown
MI	Great Lakes	Raisin	04100002	Low	High	Extirpated	High	Extirpated

[11] ↑ = Increased percentage of urban and/or agricultural landcover projected under Scenario 2, indicating an increased risk associated with primary contaminants (ammonia, chloride, copper, nitrate)

IN, OH	Great Lakes	St. Joseph	04100003	Low	High	Extirpated	High	Extirpated
OH	Great Lakes	Blanchard	04100008	High	High	Low	High	Low
OH	Great Lakes	Lower Maumee	04100009	High	High	Low	High	Low
OH	Great Lakes	Sandusky	04100011	Low	High	Extirpated	High	Extirpated
PA, NY	Ohio	Upper Allegheny	05010001	High	High	Low	High	Low
PA	Ohio	Middle Allegheny-Tionesta	05010003	Moderate	High	Functionally Extirpated	High	Functionally Extirpated
PA	Ohio	French	05010004	High	High	Moderate	Moderate	Moderate
PA	Ohio	Middle Allegheny-Redbank	05010006	Moderate	Moderate	Low	Moderate	Low
WV	Ohio	Elk	05050007	Low	Moderate	Functionally Extirpated	Low	Low
OH	Ohio	Upper Scioto	05060001	Low	High	Extirpated	High	Extirpated
OH	Ohio	Upper Great Miami	05080001	Low	High	Extirpated	High	Extirpated
IN	Ohio	Tippecanoe	05120106	High	High	Extirpated	Moderate	Moderate
IN	Ohio	Driftwood	05120204	Low	High	Extirpated	High	Extirpated
TN	Tennessee	Upper Duck	06040002	Low	High	Extirpated	High	Extirpated

Table G.11. Water Quality/ Contaminants Risk Factors - Metrics Summary

HUC2 Name	HUC8 Name	HUC8 ID	Current Condition											
			Individual Contaminant Risk Category										Overall Score	Overall Risk
			Ammonia	Chloride	Copper	Nitrate	Aluminum	Cadmium	Lead	Potassium	Sulfate	Zinc		
Great Lakes	St. Clair	04090001	Low	High	Low	Low	----	Low	Low	Low	Low	Low	3	High
	Lake St. Clair	04090002	Low	Low	Low	Low	----	Low	Low	Low	Low	Low	1	Low
	Clinton	04090003	Low	High	High	Low	Low	Low	Low	Low	Low	Low	3	High
	Upper Thames River	04090006												
	Raisin	04100002	Low	Low	Low	Moderate	----	Low	Low	Low	Low	Low	2	Moderate
	St. Joseph	04100003	Moderate	High	Moderate	Low	Low	Moderate	Moderate	Low	Low	Moderate	3	High
	Blanchard	04100008	Moderate	Moderate	High	Low	High	High	Moderate	High	Low	High	3	High
	Lower Maumee	04100009	Moderate	Moderate	High	----	High	High	Moderate	Moderate	Low	High	3	High
	Sandusky	04100011	Moderate	High	Moderate	Low	Low	Low	Low	Low	Low	Low	3	High
Ohio	Upper Allegheny	05010001	Low	Moderate	High	Low	Low	Low	Low	High	Low	Moderate	3	High
	Middle Allegheny-Tionesta	05010003	Low	Low	High	Low	Low	Low	Moderate	Low	Low	Moderate	3	High
	French	05010004	Low	Low	Moderate	Low	Low	Low	Low	Low	Low	Moderate	2	Moderate
	Middle Allegheny-Redbank	05010006	Low	Low	Moderate	Low	Low	Low	Low	Low	Low	Moderate	2	Moderate
	Elk	05050007	Moderate	Low	Moderate	#DIV/0!	Low	Low	Low	Low	Low	Low	2	Moderate
	Upper Scioto	05060001	Moderate	Moderate	High	Low	Moderate	High	Moderate	Moderate	Moderate	High	3	High
	Upper Great Miami	05080001	High	Moderate	High	Low	High	High	High	Moderate	Low	High	3	High
	Tippecanoe	05120106	Low	Low	Moderate	Low	Low	Low	Low	High	Low	Moderate	2	Moderate
	Driftwood	05120204	Low	Moderate	Moderate	Low	Low	Low	Low	Low	Low	Low	2	Moderate
Tennessee	Upper Duck	06040002	Low	Low	High	Low	Low	High	Low	Low	Low	Low	3	High

HUC2 Name	HUC8 Name	HUC8 ID	Scenario 1									
			Percent Change Urban Development Land Cover	Urban Land Cover Trend	Percent Change Agricultural Land Cover	Ag Land Cover Trend	Trends of Primary Contaminants (Ag and/or Urban Land Cover)**					Number of Primary Contaminants with Increasing Trends*
							Ammonia	Chloride	Copper	Nitrate	Metals	
							Urban and Ag	Urban and Ag	Urban	Urban and Ag	Urban	
Great Lakes	St. Clair	04090001	86.15	Increasing	8.80	Increasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Lake St. Clair	04090002	26.64	Increasing	12.22	Increasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Clinton	04090003	22.35	Increasing	1.75	Increasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Upper Thames River	04090006	----	----	----	----	----	----	----	----	----	----
	Raisin	04100002	89.47	Increasing	4.36	Increasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	St. Joseph	04100003	100.31	Increasing	5.36	Increasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Blanchard	04100008	87.84	Increasing	1.45	Increasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Lower Maumee	04100009	57.68	Increasing	-0.73	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Sandusky	04100011	91.75	Increasing	2.64	Increasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
Ohio	Upper Allegheny	05010001	69.75	Increasing	97.67	Increasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Middle Allegheny-Tionesta	05010003	73.56	Increasing	116.85	Increasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	French	05010004	126.37	Increasing	40.71	Increasing	Increasing	Increasing	Increasing	Increasing	Increasing	4

HUC2 Name	HUC8 Name	HUC8 ID	Scenario 1									
			Percent Change Urban Development Land Cover	Urban Land Cover Trend	Percent Change Agricultural Land Cover	Ag Land Cover Trend	Trends of Primary Contaminants (Ag and/or Urban Land Cover)**					Number of Primary Contaminants with Increasing Trends*
							Ammonia	Chloride	Copper	Nitrate	Metals	
							Urban and Ag	Urban and Ag	Urban	Urban and Ag	Urban	
	Middle Allegheny-Redbank	05010006	14.21	Increasing	48.51	Increasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Elk	05050007	147.25	Increasing	71.63	Increasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Upper Scioto	05060001	59.83	Increasing	-2.18	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Upper Great Miami	05080001	119.50	Increasing	-6.44	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Tippecanoe	05120106	74.87	Increasing	2.83	Increasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Driftwood	05120204	142.76	Increasing	-9.38	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
Tennessee	Upper Duck	06040002	149.92	Increasing	39.58	Increasing	Increasing	Increasing	Increasing	Increasing	Increasing	4

HUC2 Name	HUC8 Name	HUC8 ID	Scenario 2									
			Percent Change Urban Development Land Cover	Urban Land Cover Trend	Percent Change Agricultural Landcover	Ag Land Cover Trend	Trends of Primary Contaminants by Source (Ag and/or Urban Land Cover)**					Number of Primary Contaminants with Increasing Trends*
							Ammonia	Chloride	Copper	Nitrate	Metals	
							Urban and Ag	Urban and Ag	Urban	Urban and Ag	Urban	
Great Lakes	St. Clair	04090001	45.49	Increasing	-3.94	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Lake St. Clair	04090002	15.68	Increasing	-11.60	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Clinton	04090003	13.50	Increasing	-10.94	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Upper Thames River	04090006	----	----	----	----	----	----	----	----	----	----
	Raisin	04100002	18.58	Increasing	-3.48	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	St. Joseph	04100003	41.20	Increasing	-4.00	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Blanchard	04100008	17.60	Increasing	-0.08	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Lower Maumee	04100009	24.59	Increasing	0.22	Increasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Sandusky	04100011	13.70	Increasing	-0.29	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
Ohio	Upper Allegheny	05010001	36.11	Increasing	-46.38	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Middle Allegheny-Tionesta	05010003	21.74	Increasing	-31.20	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	French	05010004	38.45	Increasing	-17.13	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Middle Allegheny-Redbank	05010006	8.94	Increasing	-13.86	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Elk	05050007	85.20	Increasing	-32.36	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Upper Scioto	05060001	24.29	Increasing	-0.28	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Upper Great Miami	05080001	39.77	Increasing	0.46	Increasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Tippecanoe	05120106	54.01	Increasing	-1.95	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
	Driftwood	05120204	55.66	Increasing	1.76	Increasing	Increasing	Increasing	Increasing	Increasing	Increasing	4
Tennessee	Upper Duck	06040002	142.21	Increasing	-17.77	Decreasing	Increasing	Increasing	Increasing	Increasing	Increasing	4

Primary Contaminants = Ammonia, Chloride, Copper, Nitrate

*Trend projected based on percent change for agriculture and/or urban development in FORE-SCE land cover change model SRES A2.

**Trend projected based on percent change for agriculture and/or urban development in FORE-SCE land cover change model SRES B1.

Table G.12. Landscape Risk Factors - Metrics Summary

HUC2 Name	HUC8 Name	HUC8 ID	Current Condition																
			Mean Impervious Surface Value	Impervious Surface Risk Score	Impervious Surface Risk Category	Percent Urban Land Cover	Urban Land Cover Risk Score	Urban Land Cover Risk Category	Percent Ag Land Cover	Ag Risk Score	Ag Land Cover Risk Category	Percent Riparian Buffer Veg	Veg Risk Score	Riparian Veg Cover Risk Category	Mean Canopy Cover Value	Canopy Cover Risk Score	Canopy Cover Risk Category	Overall Risk Score	Overall Risk Category
Great Lakes	St. Clair	04090001	11.98	2	Moderate	9.86	2	Moderate	61.03	3	High	57.98	2	Moderate	39.10	3	High	2	Moderate
	Lake St. Clair	04090002	106.54	3	High	46.59	3	High	19.11	1	Low	25.13	3	High	5.37	3	High	3	High
	Clinton	04090003	23.24	3	High	56.74	3	High	17.98	1	Low	46.00	3	High	31.88	3	High	3	High
	Upper Thames River	04090006	127.00	----	----	----	----	----	----	----	----	50.46	2	Moderate	25.36	3	High	----	----
	Raisin	04100002	2.71	1	Low	10.97	3	High	67.72	3	High	45.13	3	High	29.10	3	High	3	High
	St. Joseph	04100003	2.56	1	Low	10.03	3	High	68.28	3	High	24.91	3	High	12.23	3	High	3	High
	Blanchard	04100008	2.27	1	Low	9.50	2	Moderate	83.32	3	High	41.34	3	High	27.70	3	High	2	Moderate
	Lower Maumee	04100009	4.26	1	Low	14.26	3	High	76.75	3	High	37.36	3	High	20.55	3	High	3	High
	Sandusky	04100011	2.30	1	Low	9.01	2	Moderate	79.19	3	High	68.48	2	Moderate	33.79	3	High	2	Moderate
Ohio	Upper Allegheny	05010001	0.58	1	Low	4.74	1	Low	10.27	1	Low	81.31	1	Low	50.74	2	Moderate	1	Low
	Middle Allegheny-Tionesta	05010003	0.54	1	Low	5.20	2	Moderate	10.25	1	Low	79.32	1	Low	42.99	3	High	2	Moderate
	French	05010004	1.10	1	Low	7.20	2	Moderate	29.59	2	Moderate	72.31	2	Moderate	44.73	3	High	2	Moderate
	Middle Allegheny-Redbank	05010006	1.18	1	Low	8.02	2	Moderate	21.64	1	Low	75.24	1	Low	36.88	3	High	2	Moderate
	Elk	05050007	0.54	1	Low	4.28	1	Low	1.99	1	Low	80.13	1	Low	57.32	2	High	1	Low
	Upper Scioto	05060001	6.71	1	Low	19.79	3	High	67.09	3	High	42.89	3	High	27.72	3	High	3	High
	Upper Great Miami	05080001	3.34	1	Low	13.39	3	High	75.09	3	High	42.30	3	High	24.19	3	High	3	High
	Tippecanoe	05120106	1.39	1	Low	6.95	2	Moderate	80.52	3	High	56.22	2	Moderate	29.24	3	High	2	Moderate
	Driftwood	05140206	3.01	1	Low	12.02	3	High	73.20	3	High	48.69	3	High	26.55	3	High	3	High
Tennessee	Upper Duck	06040002	1.48	1	Low	8.38	2	Moderate	46.85	3	High	57.21	2	Moderate	37.61	3	High	2	Moderate

HUC2 Name	HUC8 Name	HUC8 ID	Scenario 1*																	
			% Imperv. Surface	Imperv. Risk Score	Imperv. Surface Risk Category	% Urban Land Cover	% Change Urban Devel. Land Cover	Urban Land Cover Risk Score	Urban Land Cover Risk Category	% Ag Land Cover	% Change Ag Land Cover	Ag Land Cover Risk Score	Ag Land Cover Risk Category	% Riparian Buffer Veg Land Cover	% Change Riparian Buffer Veg Land Cover	Riparian Buffer Veg Risk Score	Riparian Buffer Veg Risk Category	% Canopy Cover	Canopy Cover Risk Score	Canopy Cover Risk Category	Overall Risk Score	Overall Risk Category
Great Lakes	St. Clair	04090001	22.29	3	High	18.36	86.15	3	High	66.40	8.80	3	High	32.98	-43.11	3	High	22.24	3	High	3	High
	Lake St. Clair	04090002	134.92	3	High	59.00	26.64	3	High	21.45	12.22	1	Low	14.26	-43.25	3	High	3.05	3	High	3	High
	Clinton	04090003	28.43	3	High	69.42	22.35	3	High	18.30	1.75	1	Low	26.08	-43.30	3	High	18.08	3	High	3	High
	Upper Thames River	04090006	----	----	----	----	----	----	----	----	----	NA	----	----	----	----	----	----	----	----	----	----
	Raisin	04100002	5.13	1	Low	20.79	89.47	3	High	70.68	4.36	3	High	27.17	-39.79	3	High	17.52	3	High	3	High
	St. Joseph	04100003	5.13	1	Low	20.08	100.31	3	High	71.94	5.36	3	High	12.69	-49.05	3	High	6.23	3	High	3	High

HUC2 Name	HUC8 Name	HUC8 ID	Scenario 1*																	
			% Imperv. Surface	Imperv. Risk Score	Imperv. Surface Risk Category	% Urban Land Cover	% Change Urban Devel. Land Cover	Urban Land Cover Risk Score	Urban Land Cover Risk Category	% Ag Land Cover	% Change Ag Land Cover	Ag Land Cover Risk Score	Ag Land Cover Risk Category	% Riparian Buffer Veg Land Cover	% Change Riparian Buffer Veg Land Cover	Riparian Buffer Veg Risk Score	Riparian Buffer Veg Risk Category	% Canopy Cover	Canopy Cover Risk Score	Canopy Cover Risk Category	Overall Risk Score	Overall Risk Category
	Blanchard	04100008	4.26	1	Low	17.85	87.84	3	High	84.53	1.45	3	High	14.71	-64.40	3	High	9.86	3	High	3	High
	Lower Maumee	04100009	6.72	1	Low	22.48	57.68	3	High	76.19	-0.73	3	High	24.59	-34.17	3	High	13.53	3	High	3	High
	Sandusky	04100011	4.40	1	Low	17.27	91.75	3	High	81.28	2.64	3	High	34.89	-49.05	3	High	17.22	3	High	3	High
Ohio	Upper Allegheny	05010001	0.98	1	Low	8.04	69.75	2	Moderate	20.30	97.67	1	Low	66.30	-18.45	2	Moderate	41.38	3	High	2	Moderate
	Middle Allegheny-Tionesta	05010003	0.94	1	Low	9.02	73.56	2	Moderate	22.22	116.85	1	Low	63.71	-19.67	2	Moderate	34.53	3	High	2	Moderate
	French	05010004	2.48	1	Low	16.29	126.37	3	High	41.63	40.71	3	High	49.85	-31.05	3	High	30.84	3	High	3	High
	Middle Allegheny-Redbank	05010006	1.34	1	Low	9.16	14.21	2	Moderate	32.14	48.51	2	Moderate	58.89	-21.74	2	Moderate	28.86	3	High	2	Moderate
	Elk	05050007	1.34	1	Low	10.58	147.25	3	High	3.41	71.63	1	Low	74.75	-6.72	2	Moderate	53.47	2	Moderate	2	Moderate
	Upper Scioto	05060001	10.73	2	Moderate	31.63	59.83	3	High	65.63	-2.18	3	High	21.54	-49.79	3	High	13.92	3	High	3	High
	Upper Great Miami	05080001	7.34	1	Low	29.39	119.50	3	High	70.26	-6.44	3	High	20.82	-50.77	3	High	11.91	3	High	3	High
	Tippecanoe	05120106	2.43	1	Low	12.15	74.87	3	High	82.79	2.83	3	High	37.30	-33.64	3	High	19.40	3	High	3	High
	Driftwood	05120204	7.30	1	Low	29.19	142.76	3	High	66.34	-9.38	3	High	34.66	-28.80	3	High	18.90	3	High	3	High
Tennessee	Upper Duck	06040002	3.70	1	Low	20.94	149.92	3	High	65.39	39.58	3	High	34.97	-38.89	3	High	22.98	3	High	3	High

HUC2 Name	HUC8 Name	HUC8 ID	Scenario 2**																		
			% Imperv. Surface	Imperv. Surface Risk Score	Imperv. Surface Risk Category	% Urban Land Cover	% Change Urban Devel. Land Cover	Urban Land Cover Risk Score	Urban Land Cover Risk Category	% Ag Land Cover	% Change Ag Land Cover	Ag Land Cover Risk Score	Ag Land Cover Risk Category	% Riparian Buffer Veg Land Cover	% Change Riparian Buffer Veg Land Cover	Riparian Buffer Veg Risk Score	Riparian Buffer Veg Risk Category	% Canopy Cover	Canopy Cover Risk Score	Canopy Cover Risk Category	Overall Risk Score	Overall Risk Category
Great Lakes	St. Clair	04090001	17.42	3	High	14.35	45.49	3	High	58.62	-3.94	3	High	53.94	-6.97	2	Moderate	36.37	3	High	3	High
	Lake St. Clair	04090002	123.24	3	High	53.89	15.68	3	High	16.89	-11.60	1	Low	22.37	-10.97	3	High	4.78	3	High	3	High
	Clinton	04090003	26.37	3	High	64.39	13.50	3	High	16.02	-10.94	1	Low	41.99	-8.72	3	High	29.10	3	High	3	High
	Upper Thames River	04090006	----	----	----	----	----	NA	----	----	----	----	----	----	----	----	----	----	----	----	----	----
	Raisin	04100002	3.21	1	Low	13.01	18.58	3	High	65.37	-3.48	3	High	47.15	4.48	3	High	30.40	3	High	3	High
	St. Joseph	04100003	3.62	1	Low	14.16	41.20	3	High	65.55	-4.00	3	High	24.97	0.25	3	High	12.26	3	High	3	High
	Blanchard	04100008	2.67	1	Low	11.18	17.60	3	High	83.26	-0.08	3	High	31.70	-23.30	3	High	21.24	3	High	3	High
	Lower Maumee	04100009	5.31	1	Low	17.76	24.59	3	High	76.92	0.22	3	High	34.21	-8.42	3	High	18.82	3	High	3	High

HUC2 Name	HUC8 Name	HUC8 ID	Scenario 2**																	
			% Imperv. Surface	Imperv. Surface Risk Score	Imperv. Surface Risk Category	% Urban Land Cover	% Change Urban Devel. Land Cover	Urban Land Cover Risk Score	Urban Land Cover Risk Category	% Ag Land Cover	% Change Ag Land Cover	Ag Land Cover Risk Score	Ag Land Cover Risk Category	% Riparian Buffer Veg Land Cover	% Change Riparian Buffer Veg Land Cover	Riparian Buffer Veg Risk Score	Riparian Buffer Veg Risk Category	% Canopy Cover	Canopy Cover Risk Score	Canopy Cover Risk Category	Overall Risk Score	Overall Risk Category
	Sandusky	04100011	2.61	1	Low	10.24	13.70	3	High	78.96	-0.29	3	High	59.78	-12.69	2	Moderate	29.50	3	High	2	Moderate
Ohio	Upper Allegheny	05010001	0.79	1	Low	6.45	36.11	2	Moderate	5.51	-46.38	1	Low	84.27	3.64	1	Low	52.59	2	Moderate	1	Low
	Middle Allegheny-Tionesta	05010003	0.66	1	Low	6.33	21.74	2	Moderate	7.05	-31.20	1	Low	78.71	-0.77	1	Low	42.66	3	High	2	Moderate
	French	05010004	1.52	1	Low	9.96	38.45	2	Moderate	24.52	-17.13	1	Low	72.38	0.09	2	Moderate	44.77	3	High	2	Moderate
	Middle Allegheny-Redbank	05010006	1.28	1	Low	8.74	8.94	2	Moderate	18.64	-13.86	1	Low	75.59	0.46	1	Low	37.04	3	High	2	Moderate
	Elk	05050007	1.00	1	Low	7.92	85.20	2	Moderate	1.35	-32.36	1	Low	79.95	-0.23	1	Low	57.19	2	Moderate	1	Low
	Upper Scioto	05060001	8.35	1	Low	24.60	24.29	3	High	66.91	-0.28	3	High	39.72	-7.39	3	High	25.67	3	High	3	High
	Upper Great Miami	05080001	4.68	1	Low	18.71	39.77	3	High	75.44	0.46	3	High	42.70	0.95	3	High	24.42	3	High	3	High
	Tippecanoe	05120106	2.14	1	Low	10.70	54.01	3	High	78.95	-1.95	3	High	54.97	-2.21	2	Moderate	28.60	3	High	2	Moderate
	Driftwood	05120204	4.68	1	Low	18.72	55.66	3	High	74.49	1.76	3	High	47.87	-1.68	3	High	26.10	3	High	3	High
Tennessee	Upper Duck	06040002	3.59	1	Low	20.30	142.21	3	High	38.52	-17.77	2	Moderate	54.81	-4.20	2	Moderate	36.03	3	High	2	Moderate

*Percent change as modeled in FORE-SCE land cover change model SRES A2.

**Percent change as modeled in FORE-SCE land cover change model SRES B1.

Table G.13. Hydrological Regime Risk Factors – Metrics Summary

HUC2 Name	HUC8 Name	HUC8 ID	Current Condition						Scenario 1*			Scenario 2**		
			Number of Events	Min Cumulative Weeks	Max Cumulative Weeks	Mean Cumulative Weeks	Risk Score	Risk Category	% Change WW 1980-2009 vs 2040-2069	Risk Score (WW Scenario 1 CDSI Change 2040-2069)	Overall Risk Category	% Change HD 1980-2009 vs 2040-2069	Risk Score (HD, Scenario 2 CDSI Change 2040-2069)	Overall Risk Category
Great Lakes	St. Clair	04090001	none	none	none	none	Low	1	127.57	1 (127.%)	Low	-13.83	1 (-13.%)	Low
	Lake St. Clair	04090002	none	none	none	none	Low	1	126.42	1 (126.%)	Low	-36.78	1 (-36.%)	Low
	Clinton	04090003	none	none	none	none	Low	1	123.09	1 (123.%)	Low	-24.48	1 (-24.%)	Low
	Upper Thames River	04090006	----	----	----	----	----	----	----	NA (%)	----	----	----	----
	Raisin	04100002	none	none	none	none	Low	1	107.93	1 (107.%)	Low	-27.12	1 (-27.%)	Low
	St. Joseph	04100003	1	6	6	6	Moderate	2	112.94	2 (112.%)	Moderate	-40.72	2 (-40.%)	Moderate
	Blanchard	04100008	none	none	none	none	Low	1	77.24	1 (77.2%)	Low	-39.00	1 (-38.%)	Low
	Lower Maumee	04100009	none	none	none	none	Low	1	101.95	1 (101.%)	Low	-33.91	1 (-33.%)	Low
	Sandusky	04100011	none	none	none	none	Low	1	98.93	1 (98.9%)	Low	-43.65	1 (-43.%)	Low
Ohio	Upper Allegheny	05010001	none	none	none	none	Low	1	241.76	1 (241.%)	Low	50.18	1 (50.1%)	Low
	Middle Allegheny-Tionesta	05010003	none	none	none	none	Low	1	171.83	1 (171.%)	Low	17.47	1 (17.4%)	Low
	French	05010004	none	none	none	none	Low	1	146.83	1 (146.%)	Low	2.47	1 (2.46%)	Low
	Middle Allegheny-Redbank	05010006	none	none	none	none	Low	1	193.68	1 (193.%)	Low	104.90	1 (104.%)	Low
	Elk	05050007	1	2	2	2	Low	1	77.60	1 (77.5%)	Low	79.18	1 (79.1%)	Low
	Upper Scioto	05060001	none	none	none	none	Low	1	113.85	1 (113.%)	Low	-26.46	1 (-26.%)	Low
	Upper Great Miami	05080001	none	none	none	none	Low	1	110.29	1 (110.%)	Low	-29.12	1 (-29.%)	Low
	Tippecanoe	05120106	1	6	6	6	Moderate	2	54.70	2 (54.6%)	Moderate	-9.76	2 (-9.7%)	Moderate
	Driftwood	05120204	1	7	7	7	Moderate	2	60.84	2 (60.8%)	Moderate	-22.22	2 (-22.%)	Moderate
Tennessee	Upper Duck	06040002	5	1	46	12	Moderate	2	82.71	2 (82.7%)	Moderate	91.11	2 (91.1%)	Moderate

*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

Table G.14. Connectivity Risk Factors – Metrics Summary

HUC2 Name	HUC8 Name	HUC8 ID	Current Condition								
			Adjusted Dams	Adjusted Dam Risk Score	Adjusted Dam Risk Category	Unpaved Roads Stream Crossing Count	Unpaved Road Stream Crossings per KM	Unpaved Road Stream Crossing Risk Score	Unpaved Road Stream Crossing Risk Category	Overall Risk Score	Overall Risk Category
Great Lakes	St. Clair	04090001	7	1	Low	27	0.01	1	Low	1	Low
	Lake St. Clair	04090002	0	1	Low	1	0.00	1	Low	1	Low
	Clinton	04090003	21	2	Moderate	20	0.02	1	Low	2	Moderate
	Upper Thames River	04090006	0	1	Low	0	----	----	----	----	----
	Raisin	04100002	18	2	Moderate	18	0.01	1	Low	2	Moderate
	St. Joseph	04100003	21	2	Moderate	12	0.01	1	Low	2	Moderate
	Blanchard	04100008	0	1	Low	4	0.01	1	Low	1	Low
	Lower Maumee	04100009	7	1	Low	12	0.01	1	Low	1	Low
	Sandusky	04100011	14	2	Moderate	19	0.01	1	Low	2	Moderate
Ohio	Upper Allegheny	05010001	22	2	Moderate	45	0.01	1	Low	2	Moderate
	Middle Allegheny-Tionesta	05010003	13	2	Moderate	0	0.00	1	Low	2	Moderate
	French	05010004	24	2	Moderate	1	0.00	1	Low	2	Moderate
	Middle Allegheny-Redbank	05010006	31	3	High	2	0.00	1	Low	2	Moderate
	Elk	05050007	6	1	Low	507	0.16	1	Low	1	Low
	Upper Scioto	05060001	33	3	High	59	0.02	1	Low	2	Moderate
	Upper Great Miami	05080001	33	3	High	7	0.00	1	Low	2	Moderate
	Tippecanoe	05120106	42	3	High	9	0.00	1	Low	2	Moderate
	Driftwood	05120201	17	2	Moderate	0	0.00	1	Low	2	Moderate
Tennessee	Upper Duck	06040002	15	2	Moderate	1355	0.74	3	High	2	Moderate

HUC2 Name	HUC8 Name	HUC8 ID	Scenario 1								
			Number of Dams*	Adjusted Dam Risk Score	Adjusted Dam Risk Category	Percent Change Unpaved Road Crossing Density^	Unpaved Road Crossing Density	Unpaved Road Density Risk Score	Unpaved Road Density Risk Category	Overall Risk Score	Overall Risk Category
Great Lakes	St. Clair	04090001	7	1	Low	27.35	0.02	1	Low	1	Low
	Lake St. Clair	04090002	0	1	Low	27.35	0.00	1	Low	1	Low
	Clinton	04090003	21	2	Moderate	27.35	0.02	1	Low	2	Moderate
	Upper Thames River	04090006	0	1	Low	27.35	-----	NA	----	----	----
	Raisin	04100002	18	2	Moderate	27.35	0.02	1	Low	2	Moderate
	St. Joseph	04100003	21	2	Moderate	27.35	0.01	1	Low	2	Moderate
	Blanchard	04100008	0	1	Low	27.35	0.01	1	Low	1	Low
	Lower Maumee	04100009	7	1	Low	27.35	0.02	1	Low	1	Low
	Sandusky	04100011	14	2	Moderate	27.35	0.01	1	Low	2	Moderate
Ohio	Upper Allegheny	05010001	22	2	Moderate	27.35	0.02	1	Low	2	Moderate
	Middle Allegheny-Tionesta	05010003	13	2	Moderate	27.35	0.00	1	Low	2	Moderate
	French	05010004	24	2	Moderate	27.35	0.00	1	Low	2	Moderate
	Middle Allegheny-Redbank	05010006	31	3	High	27.35	0.00	1	Low	2	Moderate

	Elk	05050007	6	1	Low	27.35	0.20	1	Low	1	Low
	Upper Scioto	05060001	33	3	High	27.35	0.02	1	Low	2	Moderate
	Upper Great Miami	05080001	33	3	High	27.35	0.00	1	Low	2	Moderate
	Tippecanoe	05120106	42	3	High	27.35	0.00	1	Low	2	Moderate
	Driftwood	05120204	17	2	Moderate	27.35	0.00	1	Low	2	Moderate
Tennessee	Upper Duck	06040002	15	2	Moderate	27.35	0.95	3	High	3	High

HUC2 Name	HUC8 Name	HUC8 ID	Scenario 2									
			Number Dams Removed Post 2012	Projected Future Number of Dams**	Dam Risk Score	Dam Risk Category	Percent Change Unpaved Road Crossing Density^^	Unpaved Road Crossing Density	Unpaved Road Density Risk Score	Unpaved Road Density Risk Category	Overall Risk Score	Overall Risk Category
Great Lakes	St. Clair	04090001	0	7	1	Low	3.2	0.01	1	Low	1	Low
	Lake St. Clair	04090002	0	0	1	Low	3.2	0.00	1	Low	1	Low
	Clinton	04090003	1	20	2	Moderate	3.2	0.02	1	Low	2	Moderate
	Upper Thames River	04090006	0	0	1	Low	3.2	----	----	----	----	----
	Raisin	04100002	0	18	2	Moderate	3.2	0.01	1	Low	2	Moderate
	St. Joseph	04100003	1	20	2	Moderate	3.2	0.01	1	Low	2	Moderate
	Blanchard	04100008	0	0	1	Low	3.2	0.01	1	Low	1	Low
	Lower Maumee	04100009	0	7	1	Low	3.2	0.01	1	Low	1	Low
	Sandusky	04100011	3	11	2	Moderate	3.2	0.01	1	Low	2	Moderate
Ohio	Upper Allegheny	05010001	3	19	2	Moderate	3.2	0.01	1	Low	2	Moderate
	Middle Allegheny-Tionesta	05010003	1	12	2	High	3.2	0.00	1	Low	2	Moderate
	French	05010004	2	22	2	Moderate	3.2	0.00	1	Low	2	Moderate
	Middle Allegheny-Redbank	05010006	0	31	3	High	3.2	0.00	1	Low	1	Low
	Elk	05050007	0	6	1	Low	3.2	0.16	1	Low	2	Moderate
	Upper Scioto	05060001	1	32	3	High	3.2	0.02	1	Low	2	Moderate
	Upper Great Miami	05080001	4	29	2	Moderate	3.2	0.00	1	Low	2	Moderate
	Tippecanoe	05120106	1	41	3	High	3.2	0.00	1	Low	2	Moderate
	Driftwood	05120204	0	17	2	Moderate	3.2	0.00	1	Low	2	Moderate
Tennessee	Upper Duck	06040002	0	15	2	Moderate	3.2	0.77	3	High	3	High

*No change from Current Condition
**Dam removal based on 2000-2020 trends
^Increase density by 27.3% (GRIP Scenario SSP5)
^^Increase density by 3.2% (GRIP Scenario SSP3)

Table G.15. Invasive Species Risk Factors – Metrics Summary

HUC2 Name	HUC8 Name	HUC8 ID	Current Condition					Scenario 1*		Scenario 2**	
			Number of Hotspots	Number of Coldspots	Incidences (Y/N)	Risk Score	Risk Category^	Risk Score	Risk Category ^^	Risk Score	Risk Category^
Great Lakes	St. Clair	04090001	0	0	Y	2	Moderate	2	Moderate	2	Moderate
	Lake St. Clair	04090002	----	----	----	----	----	----	----	----	----
	Clinton	04090003	2	0	Y	2	Moderate	2	Moderate	2	Moderate
	Upper Thames River	04090006	----	----	----	----	----	----	----	----	----
	Raisin	04100002	0	0	Y	2	Moderate	2	Moderate	2	Moderate
	St. Joseph	04100003	0	0	Y	2	Moderate	3	High	2	Moderate
	Blanchard	04100008	0	0	Y	2	Moderate	2	Moderate	2	Moderate
	Lower Maumee	04100009	0	0	Y	2	Moderate	2	Moderate	2	Moderate
	Sandusky	04100011	0	0	Y	2	Moderate	2	Moderate	2	Moderate
Ohio	Upper Allegheny	05010001	0	0	Y	2	Moderate	3	High	2	Moderate
	Middle Allegheny-Tionesta	05010003	0	0	Y	2	Moderate	3	High	2	Moderate
	French	05010004	0	0	Y	2	Moderate	3	High	2	Moderate
	Middle Allegheny-Redbank	05010006	0	0	Y	2	Moderate	2	Moderate	2	Moderate
	Elk	05050007	0	0	Y	2	Moderate	2	Moderate	2	Moderate
	Upper Scioto	05060001	0	0	Y	2	Moderate	2	Moderate	2	Moderate
	Upper Great Miami	05080001	0	0	Y	2	Moderate	2	Moderate	2	Moderate
	Tippecanoe	05120106	0	7	Y	2	Moderate	3	High	2	Moderate
	Driftwood	05120204	0	2	Y	2	Moderate	2	Moderate	2	Moderate
Tennessee	Upper Duck	06040002	0	0	Y	2	Moderate	2	Moderate	2	Moderate

*Neighbor hotspot analysis

**No changes from current condition

^Based on December 16, 2021 data

^^Based on December 20, 2021 data

Table G.16. Catastrophic Events Risk Summary

HUC2 Name	HUC8 Name	HUC8 ID	Current Condition											Scenario 1		Scenario 2	
			Coal			Oil and Natural Gas							Coal*	Oil and Natural Gas^	Coal**	Oil and Natural Gas^^
			Coal Mines		Coal Overall Risk	All Pipe Crossings		Liquid Pipe Crossings		Oil and Natural Gas Wells		Oil and Natural Gas Overall Risk				
			Count	Present in HUC8 (Y/N)		Count	Present in HUC8 (Y/N)	Count	Present in HUC8 (Y/N)	Count	Present in HUC8 (Y/N)		Projected Overall Risk Trajectory	Projected Overall Risk Trajectory	Projected Overall Risk Trajectory	Projected Overall Risk Trajectory
Great Lakes	St. Clair	04090001	0	N	Low	237	Y	89	Y	266	Y	High	No Change	Increase	Decrease	Decrease
	Lake St. Clair	04090002	0	N	Low	80	Y	3	Y	107	Y	High	No Change	Increase	Decrease	Decrease
	Clinton	04090003	0	N	Low	227	Y	65	Y	94	Y	High	No Change	Increase	Decrease	Decrease
	Upper Thames River	04090006	0	N	Low	0	N	0	N	0	N	Low	No Change	No Change	Decrease	No Change
	Raisin	04100002	0	N	Low	203	Y	52	Y	89	Y	High	No Change	Increase	Decrease	Decrease
	St. Joseph	04100003	0	N	Low	44	Y	16	Y	14	Y	High	No Change	Increase	Decrease	Decrease
	Blanchard	04100008	0	N	Low	83	Y	69	Y	3484	Y	High	No Change	Increase	Decrease	Decrease
	Lower Maumee	04100009	0	N	Low	114	Y	24	Y	125	Y	High	No Change	Increase	Decrease	Decrease
	Sandusky	04100011	0	N	Low	284	Y	89	Y	1305	Y	High	No Change	Increase	Decrease	Decrease
Ohio	Upper Allegheny	05010001	0	N	Low	230	Y	8	Y	11030	Y	High	No Change	Increase	Decrease	Decrease
	Middle Allegheny-Tionesta	05010003	1	Y	High	127	Y	0	N	7996	Y	High	No Change	Increase	Decrease	No Change
	French	05010004	0	N	Low	49	Y	0	N	2948	Y	High	No Change	Increase	Decrease	No Change
	Middle Allegheny-Redbank	05010006	15	Y	High	214	Y	16	Y	15511	Y	High	No Change	Increase	Decrease	Decrease
	Elk	05050007	1	Y	High	253	Y	0	N	0	N	High	No Change	Increase	Decrease	No Change
	Upper Scioto	05060001	0	N	Low	341	Y	80	Y	550	Y	High	No Change	Increase	Decrease	Decrease
	Upper Great Miami	05080001	0	N	Low	145	Y	85	Y	188	Y	High	No Change	Increase	Decrease	Decrease
	Tippecanoe	05120106	0	N	Low	120	Y	35	Y	115	Y	High	No Change	Increase	Decrease	Decrease
	Driftwood	05120204	0	N	Low	114	Y	11	Y	62	Y	High	No Change	Increase	Decrease	Decrease
Tennessee	Upper Duck	06040002	0	N	Low	110	Y	20	Y	0	N	High	No Change	Increase	Decrease	Decrease

*We assume no change in risk.

**We assume a reduction in risk.

^ High Oil and Gas Supply Scenario: The U.S. Energy Information Administration (EIA) projects increased crude oil production; we assume production and supply will increase where infrastructure is already present, increasing the risk of a catastrophic event.

^^Low Oil and Gas Supply Scenario: Production and consumption decrease and the risk of a catastrophic event decrease