

**Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*)**  
**Species Status Assessment Report**

**Version 2.1**  
**November 2024**



Photo Credit: Ryan Wolfe

**Prepared by:**  
**U.S. Fish and Wildlife Service**  
**Midwest Region**

Version 2.1 (November 2024) of the SSA corrects minor typographical errors and inadvertent omissions from the Literature Cited in Version 2.0.

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## Executive Summary

This report summarizes the results of a species status assessment (SSA) completed for the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) to assess the species' overall viability. The Eastern Hellbender is a large, aquatic salamander found in perennial streams. Historically, the species was widespread across 15 states from northeastern Mississippi, northern Alabama, and northern Georgia northeast to southern New York, with disjunct populations occurring in east-central Missouri.

To assess the biological status of the Eastern Hellbender across its range, we used the best available information, including peer-reviewed scientific literature, academic reports, and survey data provided by State and Federal agencies across the range. We also elicited input from species experts to inform our analyses.

The Eastern Hellbender is a long-lived species that inhabits fast-flowing, cool, and highly oxygenated streams with unembedded boulder, cobble, and gravel substrates. Eastern Hellbenders reproduce via external fertilization (females deposit eggs under a nest rock and males fertilize the egg clutch) after which a single male defends the nest from other hellbenders. Survival and successful recruitment require abundant prey (primarily crayfish but also small fish, insects, and frogs) and large (greater than 30 cm), flat rocks, partially embedded with a single opening usually facing downstream, for nests and shelter.

The primary stressor to Eastern Hellbender is sedimentation caused by multiple sources, which is occurring throughout much of the species' range. As documented in literature, other major stressors include water quality degradation, habitat destruction and modification, disease, and direct mortality or removal of hellbenders from a population by collection, persecution, recreation, or gravel mining. Additional risk factors include climate change, small population effects, and increased abundance of native and non-native predators. Conservation measures for the species include habitat restoration and management, and captive propagation, augmentation, and reintroduction.

To assess the species' viability, we assessed the change in the number, health, and distribution of Eastern Hellbender populations over time and evaluated how these changes have and are predicted to affect the viability of the species. We used the three conservation biology principles of resiliency, redundancy, and representation (together, the 3Rs) to characterize Eastern Hellbender viability.

Resiliency is the ability to withstand variability in the environment, periodic disturbances, and anthropogenic stressors, and is a function of the number of healthy populations and the distribution of these populations. To assess resiliency, we developed status and trend categories that define a population's status as extant, functionally extirpated (FX), presumed extirpated (PX), or unknown (US) and its trend (health) as stable recruiting (SR), recruiting with unknown trend (UR), declining (D), or unknown trend (UT). Redundancy is the ability to withstand catastrophes and is best achieved by having multiple, widely distributed healthy populations relative to the spatial occurrence of catastrophic events. We identified disease and chemical spill events as the most likely catastrophes for Eastern Hellbender. Representation is the ability to

adapt to novel changes in the environment and is a function of a species' breadth of adaptive diversity. We delineated four geographical units (referred to as adaptive capacity units, ACUs) to delineate variation in genetic and ecological traits across the Eastern Hellbender's historical range (i.e., evolutionary lineages). The units are: 1) Missouri (MACU), 2) Ohio River-Susquehanna River drainages (OACU), 3) Tennessee River drainage (TACU), and 4) Kanawha River drainage (KACU).

Data show that 626 Eastern Hellbender populations existed across 15 states, and we assumed all historical populations were healthy. Currently, 153 populations (24%) are considered extirpated or functionally extirpated (PX or FX), 373 (60%) are extant, and 100 (16%) are unknown status (US). Of the 373 extant populations, 57 (15%) are declining (D), 42 (11%) are stable and recruiting (SR), 54 (14%) are recruiting with unknown trend (UR), and 220 (59%) have unknown trend (UT). The experts provided their judgments to the likely status of the 320 populations with unknown status or trends. Incorporating the experts' estimates, 255 populations are believed to be extirpated (41%) and 371 (59%) populations are believed extant; of these extant populations, 45 (12%) are believed to be SR, 108 (29%) UR, and 218 (59%) D. Overall, 76% of all historical Eastern Hellbender populations are thought to be either extirpated or declining.

The Eastern Hellbender's geographical extent within the MACU is and always has been limited, with five occupied streams in a small region of one state. The OACU is geographically large, with 138 occupied and widely dispersed streams across nine states (i.e., high redundancy). The TACU is also geographically large, with 182 occupied and widely dispersed streams across six states (i.e., high redundancy). The healthy streams in the TACU are concentrated in western North Carolina, eastern Tennessee, and northern Georgia but are still dispersed over a fairly large area. The KACU is geographically small, with 46 occupied streams distributed among three states.

To assess the future number, health, and distribution of Eastern Hellbender populations, we elicited from species experts the anticipated change in the number of SR, R, D, FX, and PX populations at 10-year and 25-year timeframes under three future scenarios: their reasonable worst plausible (RWP), reasonable best plausible (RBP), and their most likely (ML) value. Population declines are expected to continue over the next 25 years under both the RWP and RBP scenarios. Under these scenarios, there is a projected 3% to 40% decrease in the number of extant populations over the current condition, representing extirpation of 42% to 64% of the total number of historical populations within the next 25 years. The number of SR and UR populations is predicted to increase by 9% (total n=167) over the next 25 years under the RBP scenario, while the number decreases by 52% (total n=73) under the RWP scenario.

Under the RWP scenario, populations will persist in each of the ACUs through year 25; however, the number of SR and UR populations in the OACU, KACU, and MACU will be 4, 4, and 0, respectively. In the TACU, 65 populations (65% of current) are anticipated to be SR or UR.

Under the RBP scenario, each of the four ACUs will contain SR and/or UR populations. Although still below historical levels, widely dispersed SR and UR populations will remain

within the OACU and TACU (57 and 94 populations, respectively). Within the MACU and KACU, far fewer populations will still be SR or UR by year 25 (1 and 15, respectively).

Although healthy populations are predicted to persist, a continued reduction in geographic range is expected. These reductions have and will continue to impair the species' ability to withstand environmental stochasticity and periodic disturbances and increase its vulnerability to catastrophes. The predicted losses include the potential loss of the OACU, KACU, and MACU. Loss of these units will lead to reductions in genetic and ecological diversity, both of which are potential sources of adaptive diversity. The loss of adaptive diversity, in turn, will render Eastern Hellbender less able to adapt to novel changes (e.g., new predators, pathogens, climate conditions, etc.) in its environment.

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## Chapter 1. Analytical Framework and Methods

This report summarizes the results of a species status assessment (SSA) conducted for the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*). The SSA report, the product of conducting a SSA, is intended to be a concise review of the species' biology 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.

### 1.1 Analytical Framework

For the purpose of this SSA, we define viability as the ability of a species to maintain populations in the wild over a biologically meaningful timeframe. To assess viability, we use the conservation biology principles of resiliency, redundancy, and representation (Shaffer and Stein 2000, pp. 308–311). To sustain populations over time, a species must have a sufficient number and distribution of healthy populations to withstand:

- (1) Annual variation in its environment (Resiliency);
- (2) Catastrophes (Redundancy); and
- (3) Novel changes in its biological and physical environment (Representation).

Viability is a continuous measure of the likelihood that the species will sustain populations over time and can be defined in relative terms, such as “low” or “high” viability. A species with a high degree of resiliency, representation, and redundancy (the 3Rs) is generally better able to adapt to future changes and to tolerate stressors (factors that cause a negative effect to a species or its habitat), and thus, typically has a high viability.

Resiliency means having populations robust to environmental stochasticity (normal, year-to-year variations in environmental conditions such as temperature, rainfall), periodic disturbances (e.g., fire, floods, storms), and anthropogenic stressors (Redford et al. 2011, p. 40). Simply stated, resiliency refers to a species' ability to sustain populations through bad and good years.

Species resiliency is a function of having demographically healthy populations, meaning that they are able to persist and recover from unfavorable conditions (“bad years”) and disturbances. Demographically healthy means having robust population size and vital rates (e.g., survival, reproductive, and growth rates). In addition to demographically healthy populations, species resiliency is also a function of having populations distributed across areas with varying environmental conditions. Environmental stochasticity operates at both local (within populations) and regional scales (across populations), i.e., spatially correlated environmental stochasticity (Hanski and Gilpin 1997, p. 372), and as such, populations can fluctuate in synchrony over broad geographical areas (Kindavall 1996, pp. 207, 212; Oliver et al. 2010, pp. 480–482). Additionally, over longer periods, landscape and habitat changes can be synchronized over large areas, leading to correlated extinction risks among populations at a larger regional scale (Hanski 1999, pp. 381–382). Thus, having populations distributed across a diversity of environmental conditions helps guard against concurrent losses of populations at local and regional scales by inducing asynchronous fluctuations among populations. The greater degree of

spatial heterogeneity (specifically, the diversity of temperature and precipitation conditions occupied), the greater resiliency the species will possess. Lastly, resiliency may be influenced by the degree of connectivity among populations, which may be important for genetic health via gene flow and demographic rescue. Maintaining gene flow among populations promotes genetic variability (heterozygosity) within populations. Connectivity also provides for supplementing or recolonizing populations that have suffered declines or extirpation due to stochastic events.

Redundancy is the ability of a species to withstand catastrophic events, those infrequent but highly consequential events for which adaptation is unlikely. This provides a margin of safety to reduce the risk of losing substantial portions of adaptive diversity or the species to a single or series of catastrophic events. Redundancy is best achieved by having multiple populations widely distributed across the species' range, thereby reducing the likelihood that all populations are exposed simultaneously and possess similar vulnerabilities to catastrophes. A minimal level of redundancy is essential for long-term viability (Shaffer and Stein 2000, pp. 307, 309–310; Groves et al. 2002, p. 506), and greater redundancy likely results in higher viability for a species. In short, redundancy is about spreading the risk and can be measured by the number and distribution of resilient populations across a species' range.

Representation is the ability of a species to adapt to both near-term and long-term novel changes in the physical (e.g., climate conditions, habitat conditions or structure across large areas) and biological (e.g., novel diseases, pathogens, predators) conditions of its environment. Simply stated, representation is the evolutionary or adaptive capacity of the species (Beever et al. 2015, p. 132; Nicotra et al. 2015, p. 2), and its ability to persist in the face of multiple threats (Lankau et al. 2011, p. 323). Thus, it is essential for species viability (Lankau et al. 2011, p. 316).

### *Sources of Adaptive Capacity*

Species respond to novel changes in their environment by altering their physical or behavioral traits (phenotypes) to match the new environmental conditions either through plasticity or genetic change (Chevin et al. 2010, p. 2; Hendry et al. 2011, p. 162; Nicotra et al. 2015, p. 3). For adaptation to occur, there must be variation upon which to act (Lankau et al. 2011, p. 320). Because phenotypes are determined by genes or sets of genes (genotypes), genetic diversity is crucial for adapting to changing environmental conditions (Hendry et al. 2011, pp. 164–165; Sgro et al. 2011, p. 326).

There are two types of intraspecific genetic diversity: adaptive and neutral (Sgro et al. 2011, p. 328; Holderegger et al. 2006, p. 797). Both are important for preserving the adaptive potential of a species (Moritz 2002, p. 243) but in different ways. Adaptive diversity is the variation in traits that control fitness (Holderegger et al. 2006, pp. 801, 803; Lankau et al. 2011, p. 316); thus, it is the variation that influences evolution (Sgro et al. 2011, p. 328). The more adaptive diversity a species harbors, the more capacity it has to adapt to changing environmental conditions. Adaptive diversity is difficult to measure because evolutionary response is controlled by a complex interaction among multiple traits (Hendry et al. 2011, p. 162; Teplitsky et al. 2014, p. 190) and, most often, involves both plastic and genetic components (Hendry et al. 2011, p. 163; Lankau et al. 2011, p. 316). Accordingly, variation in biological characteristics and ecological conditions are used as indicators of adaptive diversity. Variation in biological traits (e.g.,

physiological, morphological, and life history characteristics, collectively referred to as phenotypic plasticity), will preserve important adaptive traits and their underlying genetic variation (Crandall et al. 2000, p. 291; Forsman 2014, p. 304; Nicotra et al. 2015, p. 3). Maintaining populations across an array of environments (Hoffmann and Sgro 2011, p. 484; Lankau et al. 2011, p. 320; Sgro et al. 2011, p. 332; Shafer and Stein 2000, p. 308) and on the periphery of its distribution (Ruckelshaus et al. 2002, p. 322) helps preserve the breadth of a species' adaptive diversity.

Conversely, neutral genetic diversity is the variation in genotypes that have no direct effect on fitness (i.e., selectively neutral; Sgro et al. 2011, p. 328). This type of genetic diversity arises from historical isolation and gives rise to evolutionary lineages (Moritz 2002, p. 239). The longer the history of isolation, the more likely it is that the populations within each lineage harbor unique genetic variation, including adaptive traits (Hendry et al. 2011, p. 167). Hence, populations that are phylogenetically (molecularly or morphologically) divergent can serve as indicators of underlying adaptive diversity.

### *Evolutionary Process and Forces*

Maintaining the adaptive capacity of a species also requires preserving the processes that allow for evolution to occur (Crandall et al. 2000, p. 290; Sgro et al. 2011, p. 327). The key evolutionary forces are natural selection, gene flow, mutations, and genetic drift (Zackay 2007, p. 1; Crandall et al. 2000, p. 291). Natural selection is the process by which heritable traits can become more (selected for) or less (not selected for) common in a population by favoring those traits that enhance survival (Hendry et al. 2011, p. 169). To preserve natural selection as a functional evolutionary force, it is necessary to maintain populations across the breadth of biological and ecological conditions. Gene flow influences genetic diversity by introducing new alleles into a population, and hence, increasing the gene pool size. Genetic drift influences the frequency of alleles in a population via random, stochastic events. Genetic drift is most influential in isolated populations or those with small effective population sizes (Zackay 2007, p. 4). Preserving historical movement among populations is necessary to maintain gene flow and reduce the risk of genetic drift in isolated populations (Crandall 2000, p. 293).

## **1.2 Methods**

To assess Eastern Hellbender viability over time, we: 1) gathered occurrence data, 2) described the species' ecological requirements, 3) assessed the historical and current conditions, i.e., the number, health, and distribution of populations, 4) identified the substantive factors leading to the species' current condition and the magnitude and extent of future influences, 5) forecasted the future number, health, and distribution of populations given these influences, and 6) assessed the resulting change in resiliency, redundancy, and representation over time and the implications of this change for the species' viability. We describe these analytical steps below.

### *Occurrence Data*

There is no systematic sampling regime to monitor Eastern Hellbender distribution and status across its range. We acquired Eastern Hellbender occurrence data from multiple sources,

including State Natural Heritage Database queries, survey reports, environmental DNA (eDNA) survey results, published literature, state status assessments, and species experts. We grouped all occurrence data by named stream and organized it by 8-digit hydrologic unit code watershed (HUC8), state, and adaptive capacity unit (ACU). ACUs are geographic units that represent distinct evolutionary lineages of the Eastern Hellbender.

### *Defining Population Units*

The smallest Eastern Hellbender population unit is an occupied patch of suitable habitat (habitat patch), which may vary in size/length. Occasional or regular interaction among individual Eastern Hellbenders in different habitat patches likely occurs and is influenced by habitat fragmentation and distance among habitat patches. In some cases (e.g., close proximity and little fragmentation), multiple habitat patches may constitute a single population, while in other cases (one highly isolated habitat patch), a single habitat patch may constitute a single population. There may be multiple populations in a single stream, or a single population occupying multiple streams. Further, some movement likely occurs among HUC8 watersheds, although the frequency of movement among these watersheds is not known. See Figure 1.1 for a conceptual representation of Eastern Hellbender population structure.

Given these variables, it is impractical to delineate individual Eastern Hellbender populations (i.e., “interacting” habitat patches) throughout its entire range. Because our available data are organized by named stream and named streams often contain one or multiple interacting habitat patches, we used named stream as the unit with which to delineate an individual population. In this context, “stream” and “population” are used synonymously herein. In addition, Eastern Hellbender range includes very long streams (e.g., Ohio River, Allegheny River), which likely include multiple populations that rarely interact. Therefore, for long streams, we designated a separate population for each HUC8 through which the stream flows (if there was an occurrence record for the stream in that watershed). For example, in the Ohio River, there are occurrence records in eight of the 12 HUC8 watersheds through which it flows, and hence, our analyses assume that there are eight separate Eastern Hellbender populations in the Ohio River.

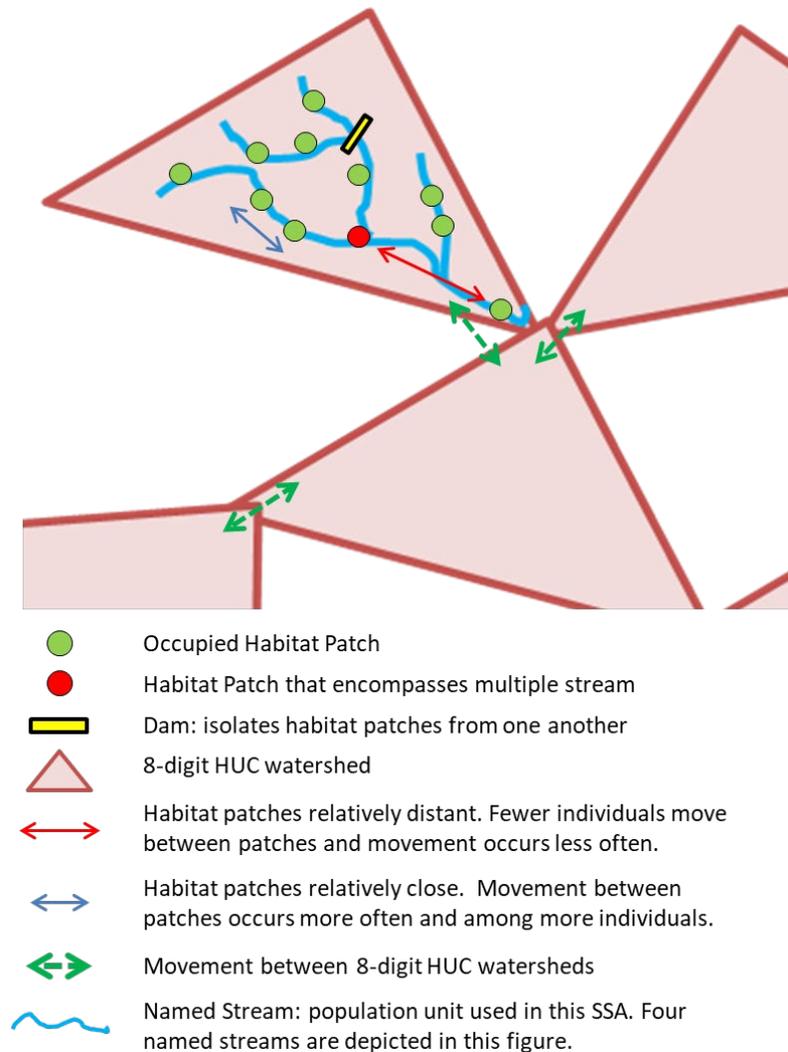


Figure 1.1. Schematic representation of population structure of the Eastern Hellbender, showing individual habitat patches, movement among patches, 8-digit HUC watersheds, and barriers to movement.

### *Status and Trend Categories*

To assess the health, number, and distribution of populations through time, we first developed status and trend categories that define a population’s status as extant, extirpated, or unknown (US), and its trend (health) as stable recruiting (SR), unknown recruiting (UR), declining (D), or unknown trend (UT) (Table 1.1). We developed two categories for extirpated. Presumed extirpated (PX) is assigned to a population for which no individuals have been found, despite substantive survey effort. We use the descriptor “presumed” to acknowledge that absolute extinction is difficult, if not impossible, to prove. A functionally extirpated (FX) population is one for which only older individuals have been found and there is no evidence of reproduction, despite significant survey effort. Although not extirpated in the strictest sense of the term, extirpation is likely inevitable for these populations without substantial intervention and augmentation (Pitt et al. 2017, p. 973).

Table 1.1. Definitions of status and trend categories assigned to the Eastern Hellbender populations. Status: extant, extirpated, or unknown. Trend: stable recruiting (SR), recruiting with unknown trend (UR), declining (D), extant with unknown trend (UT), unknown status and trend (US), functionally extirpated (FX), and presumed extirpated (PX).

Status	Trend	Definition
Extant	SR	Evidence of recruitment as demonstrated by a range of post-metamorphic juveniles and adults since 2000, <b>and</b> no documentation of declines
	UR	Evidence of recruitment ( $\geq 1$ juvenile including subadults) since 2000, but insufficient data to document a trend
	D	Observations since 2000 <b>and</b> evidence of a decline in abundance or recruitment (e.g., shift in size classes or biased sex ratio) as demonstrated by survey data
	UT	Observation(s) since 2000 but insufficient data to document a trend (R or D)
Unknown	US	No observation since 2000 <b>and</b> insufficient survey effort to indicate absence or presence
Extirpated	FX	Despite sufficient survey effort, few individuals in adult age classes found <b>and</b> no evidence of reproduction
	PX	Either 1) despite sufficient effort (multiple survey methods or survey events), only negative observations since 2000 <b>or</b> 2) no suitable habitat available (includes water quality)

### *Influences*

We searched published and unpublished literature and queried species experts to identify past and current negative and beneficial factors that have influenced the status of Eastern Hellbender populations across its historical range. Factors having a negative impact on Eastern Hellbender individuals are referred to as risk factors (also as stressors) while factors having a beneficial effect are referred to as conservation factors. We referred to risk and conservation factors collectively as “influences.”

We elicited input from species experts on the accuracy of our list of influences and the relative magnitude of impact such influences had on Eastern Hellbender status. We asked the experts to review our list of influences and to identify additional influences. After grouping influences into similar categories, we elicited estimates of the relative magnitude of impact each of the categories has had to date on Eastern Hellbender population health, as well as the future cumulative impact of the influences. Experts provided judgments based on their geographical area of expertise. Only one expert assigned points for the Missouri populations. For all other river drainages (Ohio River-Susquehanna River, Tennessee River, and Kanawha River), multiple experts assigned scores to the categories of influences. In areas with multiple experts, we compiled the experts’ responses and calculated the mean relative weight for each category. Influences are discussed in more detail in Chapter 5.

### *Future Condition*

To assess the future number, health, and distribution of Eastern Hellbender populations, we elicited from experts the anticipated change in the number of SR, UR, D, FX, and PX populations at 10-year and 25-year timeframes. Using the 4-Step elicitation method, we elicited the experts' individual estimates of reasonable worst plausible (RWP), reasonable best plausible (RBP), and most likely (ML) estimates within their geographical area (State) of expertise for each of the two timeframes. Experts based their estimates on the predicted change in influences (see *Influences* above). When geographical areas overlapped, we used the mean values of the experts to forecast the status of populations. Most experts had little confidence in predictions beyond 25 years, but using these expert-elicited estimates, we were able to forecast the health and distribution of populations into the future at 10- and 25-year increments.

To assess the species' ability to sustain populations over time, we analyzed the Eastern Hellbender's representation, resiliency and redundancy at historical, current, and future time periods.

Resiliency – We analyzed the health of populations over time by tallying the number of populations in the SR, UR, D, FX, and PX categories for historical, current, and future time periods. Given these results, we evaluated the change in the ability of Eastern Hellbender to withstand environmental stochasticity and periodic disturbances over time.

Redundancy – To assess the species' ability to withstand catastrophic events, we assessed the likelihood of catastrophic events occurring across the Eastern Hellbender's range. We defined a catastrophe as an event that would cause complete population failure irrespective of population health, and we considered whether one or more catastrophic events could result in the loss of an entire ACU. We first identified potential sources for catastrophic events and sought expert input on our list. We then sought data on the frequency of occurrence for each of these events. We were unable to find specific frequency estimates for the catastrophic events, so we devised, drawing from the IPCC (2014) classification system, three broad categories or bins of likelihoods. The three bins are:

1. Unlikely - a less than 33% chance of occurring
2. About As Likely As Not - a 33%–66% chance of occurring
3. Likely - a greater than 66% chance of occurring

We initially evaluated the following events: (1) extreme weather, (2) altered temperature regimes, (3) collection, (4) chemical pollution, and (5) disease. Of these five, we concluded that only disease and chemical pollution have the potential to cause catastrophic losses at the ACU scale. Based on available data and number and distribution of populations over time, we developed best and worst-case scenarios for both sources of catastrophes. Using these results, we determined the relative risk of extirpation over time at the ACU level.

Representation – To assess change in representation over time, we spatially partitioned Eastern Hellbender diversity into four geographical units (referred to as adaptive capacity units, ACU). As explained in Chapter 2, the delineation of the ACUs was primarily based on genetic variation

across the Eastern Hellbender's historical range. We evaluated the relative change in representation over time by analyzing the change in the distribution of healthy populations over time.

Uncertainty – Our analyses are based on the best data currently available to us and rely on expert judgments about the future, and thus, are necessarily predicated upon numerous assumptions, which could lead to over- and underestimates of viability. We identify the fundamental assumptions used and the implications of these assumptions in Chapter 7.

## Chapter 2. Species Ecology

In this chapter, we briefly describe the Eastern Hellbender taxonomy and discuss the species' life history characteristics at the individual, population, and species levels. This is not an exhaustive review of the species' natural history; rather, it provides the ecological basis for the SSA analyses conducted in Chapters 3–7.

### 2.1 Species Description and Taxonomy

The Eastern Hellbender is a large, entirely aquatic salamander that commonly exceeds 50 centimeters (cm) (20 inches (in)) in length (Green and Pauley 1987, p. 45). Large adults may grow up to 74 cm (29 in) (Fitch 1947, p. 210; Petranka 1998, p. 140). Eastern Hellbenders have a large, flat head; small, lidless eyes; a wide neck; heavily wrinkled body; and a keeled tail (Green and Pauley 1987, pp. 45–46). Their short limbs have four toes on the front feet and five toes on the hind feet (Green and Pauley 1987, p. 46). A fold of skin extends along the side of the body between the fore and hind limbs (Green and Pauley 1987, p. 45; Petranka 1998, p. 140). Coloration is variable but is generally dark green, olive, or gray. Irregular, dark spots, brownish or black in color, are often present on the dorsal surface (Cope 1889, p. 41; Bishop 1941, pp. 41, 49; Green and Pauley 1987, p. 46; Petranka 1998, p. 140).

The Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) belongs to the Order Caudata, family Cryptobranchidae. This family contains three extant species belonging to two living genera of salamanders: *Andrias*, which occurs in Japan and China, and *Cryptobranchus*, which occurs in parts of the eastern United States. The genus *Cryptobranchus* is monotypic and currently contains two recognized subspecies: *C. alleganiensis alleganiensis* and *C. alleganiensis bishopi*. However, the taxonomic differentiation between hellbender subspecies is not well agreed upon by experts and discussion continues on whether *C. a. alleganiensis* and *C. a. bishopi* are distinct species or subspecies.

### 2.2 Individual-Level Ecology and Requirements

Eastern Hellbenders are commonly found in perennial streams described as fast-flowing, cool, and highly oxygenated (Green 1934, p. 28; Bishop 1941, pp. 50–51; Green and Pauley 1987, p. 46). Some notable exceptions to fast flow include an Ohio stream with a yearly low flow of 0.04 cubic meters per second ( $m^3/s$ ) (1.31 cubic feet per second ( $ft^3/s$ )) (Pfungsten 1988, p. 12), and a Kentucky stream with little to no surface flow in some portions during low-flow periods (Lipps 2009b, p. 9). Exceptions to cool water include a recorded daytime stream temperature of 33° Celsius (C) (91° Fahrenheit (F)) in an Ohio stream (Pfungsten 1988, pp. 9–10), although some streams in North Carolina may not exceed 20° C during the summer (Nickerson et al. 2003, p. 622). In addition, recent research suggests that water conductivity may be a limiting factor, and that low conductivity is an important habitat requirement (Bodinof Jachowski and Hopkins 2018, pp. 220–221).

Eastern Hellbenders respire cutaneously (through the skin), aided by prominent, highly vascularized lateral skin folds (Guimond 1970, pp. 287–288; Nickerson and Mays 1973, pp. 26–27), but also have lungs and can use them for respiration under certain conditions (Guimond

1970, p. 108). Eastern Hellbenders are not well adapted to low-oxygen conditions (Ultsch and Duke 1990, p. 255). Harlan and Wilkinson (1981, p. 386) concluded that rocking motions, observed of captive Eastern Hellbenders held in a low-oxygen environment, increased oxygen transfer across the skin. Others have observed rocking behavior in both wild and captive Eastern Hellbenders under low-oxygen conditions or other stressors (e.g., fungal infection, capture, and blood draw) (Terrell 2012, pers. comm.; Williams 2012, pers. comm.) and in individuals under no obvious stressor (Petokas 2012, pers. comm.; Terrell 2012, pers. comm.).

Eastern Hellbenders are covered in mucus that may provide protection from abrasion and parasites (Smith 1907, p. 13) and may have antibiotic properties (Nickerson and Mays 1973, p. 35). Stress stimulates secretion of a milky, gelatinous substance (Smith 1907, p. 13), which is probably unpalatable to some predators (Brodie 1971, p. 8). Nickerson and Mays (1973, p. 34) observed obvious irritation in channel catfish (*Ictalurus punctatus*) placed in water with Ozark Hellbender skin secretions. Juveniles can produce a protective slime at least 25 weeks post-hatching, which is unusual for most larvae of aquatic salamanders (Gall et al. 2010, p. 59).

Eastern Hellbenders generally breed between late August and early October (Smith 1907, p. 15; Bishop 1941, p. 42; Peterson et al. 1983, p. 226; Humphries and Pauley 2000, p. 605). Nests have been found in bedrock fissures (Nickerson and Tohulka 1986, p. 66) but are typically excavations beneath partially embedded large flat rocks.

Eastern Hellbenders reproduce via external fertilization (Nickerson and Mays 1973, p. 45) and multiple individuals (both males and females) have been observed under Ozark and Eastern Hellbender nest rocks during the breeding season (Bishop 1941, pp. 43–44; Nickerson and Tohulka 1986, p. 66). This is consistent with genetic analyses indicating that multiple females may deposit eggs under one nest rock and an egg clutch may be fertilized by multiple males (Chudyk 2013, pp. 53–55; Unger and Williams 2015, p. 536). After fertilization, a single male typically cares for the eggs (O'Brien 2023, p. 99) and defends the nest from both male and female Eastern Hellbenders, sometimes violently (Smith 1907, pp. 24–25). Egg counts in nests include an average of approximately 400–450 eggs (Peterson et al. 1988, p. 299), 138 eggs (Dundee and Dundee 1965, p. 369), and 300–350 eggs (Williams and Groves 2012, pers. comm.). Eggs hatch in approximately 45 to 75 days (Green and Pauley 1987, p. 46; Petranka 1998, p. 143). Larvae lose their gills about 1.5 to 2 years after hatching (Bishop 1941, p. 49; Nickerson and Mays 1973, p. 53). Bishop (1941, p. 50) believed that Eastern Hellbenders sexually mature at an age of approximately 5 or 6 years.

Boulders, especially large slab rocks, act as cover and are consistently identified as the most important indicator of adult Eastern Hellbender habitat (Lipps 2009c, p. 9; Humphries 2005, p. 10; Bothner and Gottlieb 1991, p. 45). Shelter rocks are typically partially embedded with a single opening usually facing downstream (Smith 1907, p. 7). Other shelter types, such as fissures in bedrock, are sometimes used (Nickerson and Tohulka 1986, p. 66; Peterson 1987, p. 199; Williams 2012, pers. comm.). Adult Eastern Hellbenders are typically found singly under shelter rocks, which they defend from other Eastern Hellbenders (Smith 1907, pp. 24–25; Swanson 1948, p. 362; Hillis and Bellis 1971, p. 125; Humphries and Pauley 2005, p. 138). Juveniles have been found in the interstices of cobble piles (Foster 2006, pp. 73–74) and under large rocks (Williams 2012, pers. comm.; Foster 2006, pp. 73–74). Larvae can be found under

large rocks (Groves et al. 2015, p. 70; Nickerson et al. 2003, p. 624; Hect-Kardasz et al. 2012, p. 232) but are more often associated with the interstices of cobble and gravel (Nickerson et al. 2003, p. 624; Keitzer 2007, pp. 16–17; Foster et al. 2008, p. 184), which may be due to the increased presence of macroinvertebrates that provide a food source (Keitzer 2007, pp. 16–17).

Adult Eastern Hellbenders eat crayfish and, to a lesser degree, small fish (Smith 1907, p. 12; Swanson 1948, p. 363; Peterson et al. 1989, p. 440). Other occasional food items include insects, frogs, and water snakes (Green 1935, p. 36; Pfingsten 1990, p. 49; Foster 2006, p. 74) and hellbenders have also been observed scavenging dead fish (Unger 2018, p. 1). Diet of larval Eastern Hellbenders consists mainly of aquatic insects (Pitt and Nickerson 2005, p. 69; Hecht et al. 2017, p.159; Unger et al. 2020a, p. 3). Cannibalism of all life stages has been documented (Smith 1907, pp. 18, 36; Nickerson and Mays 1973, p. 22; Humphries et al. 2005, p. 428; Phillips and Humphries 2005, p. 649, Groves and Williams 2014, p. 109; Unger et al. 2020b, p. 169).

Adults are primarily nocturnal (Green 1934, p. 28; Ball 2001, p. 53), remaining beneath cover during the day although some diurnal activity has been observed (Nickerson and Mays 1973, pp. 40–41; Noeske and Nickerson 1979, p. 94), especially during the breeding season (Smith 1907, p. 6; Foster 2006, p. 25). Ball (2001, p. 55) found that Eastern Hellbenders were more active in winter and spring in a North Carolina stream than they were in summer and fall. The Eastern Hellbender moves by walking on stream bottoms (Smith 1907, p. 9) but can swim short distances quickly, presumably to avoid predators (Nickerson and Mays 1973, p. 41). The extended time required to recover from lactic acidosis (lactic acid buildup in tissues) caused by exercise restricts them to a lifestyle of minimal activity (Ultsch and Duke 1990, pp. 256–257). Studies have documented relatively small home ranges, with estimates ranging from approximately 30 square meters (m<sup>2</sup>) (322 square feet (ft<sup>2</sup>)) to approximately 2,212 m<sup>2</sup> (23,810 ft<sup>2</sup>) (Hillis and Bellis 1971, p. 124; Coatney 1982, p. 23; Peterson and Wilkinson 1996, p. 126; Humphries and Pauley 2005, p. 137; Burgmeier et al. 2011a, p.139; Nissen et al. 2023, p. 15). Despite having generally restricted home ranges, hellbenders are capable of long-distance movements and have been documented moving up to 12.9 kilometers (km) (8 miles (mi)) (Petokas 2011, pers. comm.; Foster 2012, pers. comm.).

Eastern Hellbender maximum age is not known with certainty. Longevity records in captivity include 29 years (Nigrelli 1954, p. 297) and 30 years (Groves 2012, pers. comm.). Some estimates suggest that they can live at least 25–30 years in the wild (Taber et al. 1975, p. 635; Peterson et al. 1988, p. 298). However, the longest study of growth to date (11 years) suggests that many adults captured during the study were at least 50 years old (Horchler 2010, p. 19). Eastern Hellbenders exhibit indeterminate growth, and growth slows to less than 5 millimeters (mm) (0.2 in) per year as individuals approach 25–30 years of age (Peterson et al. 1983, p. 227; Horchler 2010, p. 19). Table 2.1 summarizes ecological requirements at the individual level.

Table 2.1. The requisites needed throughout the year for individuals.

<b>Life Stage</b>	<b>Requirements</b>	<b>Description</b>
All stages	Perennial streams	Inhabited streams must have continuous flow of water throughout the year
All stages	Good water conditions	Stream current should be swift flowing, have relatively cool temperatures, and be highly oxygenated
Eggs, Juveniles, Adults	Presence of suitable habitat for breeding, shelter	Presence of large ( $\geq 30$ cm) flat rocks; rocks should be partially embedded to allow a single opening for males to guard eggs underneath; or presence of rock shelves
Larvae, Juveniles	Presence of suitable habitat for shelter, foraging	Substrate should consist of unembedded cobble and coarse gravel material where interstitial spaces are present for individuals, especially larvae, to seek shelter and feed
Larvae, Juveniles, Adults	Abundant prey availability	Individuals primarily feed on crayfish, but will occasionally consume small fish, insects and frogs

### 2.3 Population-Level Ecology

The population-level ecological requirements of a healthy (stable recruiting) Eastern Hellbender population are discussed below and summarized in (Table 2.2).

#### *Demography*

For Eastern Hellbender populations to have a healthy demography, the population growth rate ( $\lambda$ , or  $\lambda$ ) must be sufficient to withstand natural environmental fluctuations. At a minimum,  $\lambda$  must be at least 1 for a population to remain stable over time. Given that environmental fluctuations vary spatially, healthy growth rates likely vary across populations. Rangewide estimates are lacking. Based on expert input, 1.05 (1.0–1.2) is needed for a stable and recruiting population growth rate. This expert-elicited range encompasses the  $\lambda$  value (1.028) used to simulate stable conditions for Eastern Hellbender in a Population and Habitat Viability Assessment (Briggler et al. 2007, p. 88). In the absence of population growth rates, survivorship and recruitment rates also can be used to represent healthy demography. Though these rates likely also vary among populations, the following rates have been used to represent annual survivorship in modelling a stable hellbender population: 70–85% for adults, 67–75% for subadults, and 10% for early life stages (eggs and larvae) (Briggler et al. 2007, p. 82; Unger et al. 2013, p. 425).

Eastern Hellbender populations also require a population size large enough to be resilient to environmental fluctuations. Similar to population growth rate, the minimum population size to be healthy likely varies among populations. The expert-elicited minimum population size ranged from 45 to 1050, with a median most likely value of 160.

*Habitat Quality and Quantity*

Healthy Eastern Hellbender populations need to have habitat of sufficient quality and quantity to support all life stages. The required habitat quality is described under *Individual-Level Ecology and Requirements*. The quantity of habitat likely varies among populations. The expert-elicited minimum number of suitable habitat patches ranged from 3 to 15, with a median most likely value of 4. Patch sizes reportedly vary from 1,150 to 21,400 m<sup>2</sup> (0.3–5.3 acres) (Peterson 1985, p. 46; Humphries and Pauley 2005, p. 136; Foster et al. 2009, p. 582; Burgmeier et al. 2011c, p. 196). The minimum patch size required to support a healthy population likely depends upon the number of suitable habitat patches and on the degree of connectivity between those patches.

*Movement Among Habitat Patches*

Eastern Hellbender populations typically consist of individuals dispersed among multiple patches of suitable habitat within a stream or a portion of a stream. For these populations, movement among habitat patches is needed to maintain genetic diversity and to allow recolonization of patches in the event of local extirpation. For movement to occur, the patches must be in sufficient proximity of each other to allow at least occasional interaction among individuals. Based on radio telemetry and mark-recapture studies to date, we believe patches should generally be no more than 1 km (0.6 mi) apart for this movement to occur (Nickerson and Mays 1973, pp. 14–15; Blais 1996, p. 30; Burgmeier et al. 2011a, p. 138). In addition, movement between patches must not be restricted. Thus, barriers such as dams or large stretches of unsuitable habitat, must not be present.

Table 2.2. Requisites for population-level viability.

Requisite		Description
Healthy demography	Population growth ( $\lambda$ )	A positive $\lambda$ sufficient to withstand natural environmental fluctuations; 1.0 to 1.2
	Population size	Minimum N= 45 to 1050
Habitat to support a healthy demography	Habitat quality	See Table 2.1 for specifics
	Habitat quantity	At least 3 to 15 habitat patches
Movement	Configuration	Interpatch distances less than 1 km
	Connectivity	Unrestricted movement of individuals among patches

## 2.4 Species-level Ecology

The species level ecological requirements are discussed below and summarized in Table 2.3.

### *Resiliency*

Eastern Hellbender resiliency is a function of the number of healthy populations and the distribution of these populations. A healthy population is defined above under “Population-level Ecology”. The required number and distribution of populations is influenced by the degree and spatial extent of environmental stochasticity. Generally speaking, the greater the number of healthy populations and spatial heterogeneity occupied by the species, the greater likelihood of sustaining populations through time. Healthy populations are better able to recover from stochastic events and withstand variation in the environment. Thus, the greater the number of healthy populations, the more resiliency the species possesses.

Environmental stochasticity acts at local and regional scales, and hence, populations can fluctuate in synchrony over broad geographical areas (Hanski 1999, p. 372), which can lead to contemporaneous population losses across broad areas. Thus, populations distributed across a diversity of environmental conditions help guard against concurrent losses of populations by inducing asynchronous fluctuations among populations. Similarly, landscape and habitat changes can be synchronized over large areas, leading to correlated extinction risks among populations at a regional scale (Hanski 1999, pp. 381–382). Thus, generally speaking, the greater degree of spatial heterogeneity occupied by Eastern Hellbender and the more widely distributed, the more resiliency the species possesses.

For many species, resiliency also requires connectivity among populations for gene flow and demographic rescue. However, as explained further below under *Evolutionary Processes: Gene Flow, Genetic Drift, and Natural Selection*, gene flow among major river drainages was limited historically (e.g., Tennessee River, Ohio River, etc.) (Sabatino and Routman 2009, p. 1241; Tonione et al. 2011, pp. 214–215; Hime et al. 2016, p. 12). Thus, connectivity among major river drainages does not influence Eastern Hellbender resiliency.

### *Redundancy*

Species-level redundancy is best achieved by having multiple, widely distributed populations of Eastern Hellbenders relative to the spatial occurrence of catastrophic events. As further explained in Chapter 5, we identified disease and chemical spill events as the most likely catastrophic factors. Although a species’ ability to withstand catastrophic events can be influenced by its health (i.e., a demographically robust population is more likely to withstand disease), it is most strongly influenced by exposure to such events. Exposure is a function of both the number of populations (the more populations, the less likely all will be exposed) and the distribution of populations (the more widely distributed, the less likely all will be exposed). Thus, generally speaking, the greater the number of populations and the more widely distributed, the more redundancy the species possesses.

In addition to guarding against a single or series of catastrophic events extirpating all populations of the Eastern Hellbender, redundancy is important to protect against losing irreplaceable sources

of genetic and adaptive diversity. Having multiple Eastern Hellbender populations within each evolutionary lineage (see “Representation” section below) will guard against losses of adaptive diversity due to catastrophic events. Thus, Eastern Hellbender redundancy is described as having multiple, healthy populations widely distributed across the breadth of genetic and adaptive diversity relative to the spatial occurrence of catastrophic events.

### *Representation*

Eastern Hellbender representation is a function of both genetic and adaptive diversity. As described in Chapter 1, genetic diversity is important because it can delineate evolutionary lineages that may harbor unique genetic variation, including adaptive traits, and can also indicate gene flow, migration, and dispersal. Adaptive diversity is important because it provides the variation in phenotypes and ecological settings on which natural selection acts. By maintaining these two sources of diversity across the species’ range, as well as the processes that drive evolution (gene flow, natural selection, mutations, and genetic drift), the responsiveness and adaptability of the Eastern Hellbender over time is preserved.

### Genetic Diversity

Hime et al. (2016, p. 14) evaluated samples from 228 individual hellbenders collected from 96 sites across 14 states using a novel set of genetic markers spread throughout the *Cryptobranchus* genome. Phylogenetic relationships among individuals were estimated using a species tree analysis as well as an independent discriminate analysis of genetic variation (Hime et al. 2016, p. 4). Preliminary results from the two independent methods are highly concordant and indicate that the Eastern Hellbender subspecies consists of four evolutionary lineages that are distinct from each other (Hime et al. 2016, pp. 4–13): the Ohio River drainage, the Kanawha River drainage, the Tennessee River drainage, and the Missouri populations (Figure 2.1). These groupings largely coincide with results from previous phylogenetic assessments (Sabatino and Routman 2009, pp. 1,239–1,241; Tonione et al. 2011, pp. 212–213; Unger et al. 2013, pp. 5–8).

### Ecological Diversity

We assessed whether potential adaptive variation in phenotypes or ecological settings exists across the range of the Eastern Hellbender. We evaluated potential differences in body size, color pattern, and diurnal activity as proxies for underlying variation in adaptive diversity. We also evaluated potential differences throughout the species’ range in stream temperature and stability of stream temperature regime, stream order, ecoregions in which the populations occur, and physiographic regions in which populations occur. We then sought input from species experts on these potential types of diversity throughout the Eastern Hellbender range and whether any of the diversity might provide meaningful adaptive diversity. The two types of variation that experts thought would best represent underlying adaptive diversity are stream temperature regime and stream order.

Variation in mean annual stream temperature or the annual fluctuation in stream temperature likely results in ecological differences among hellbender populations in movement patterns (e.g., seasonal movements due to extreme temperatures), physiological tolerances, and naturally-occurring microbes. Pfingsten (1988, p. 49) recorded daytime stream temperature of 33°C (91°F) in a stream that currently harbors the best remaining Eastern Hellbender population in Ohio.

Petokas (2012, pers. comm.) also reports summer temperatures commonly reaching 32°C (90°F) in one of the best remaining Eastern Hellbender populations in Pennsylvania. In other streams, however, such as those with significant groundwater influence (e.g., portions of the Green River system in Kentucky and many streams in Missouri), water temperatures may not exceed 25°C (77°F) throughout the summer (Briggler 2012, pers. comm.).

Stream order is used to define stream size based on a hierarchy of tributaries and can be used to characterize a number of physical conditions, such as hydrological patterns. Variation in these characteristics influences the diversity and abundance of predators and prey (Vannote et al. 1980, pp. 132–135). Stream order is often also correlated with stream gradient, which influences stream velocity, discharge rates and patterns (i.e., “flashiness”), and sediment transport. Differences in these conditions may influence hellbender behavior during flood events, foraging behavior (e.g., in high- vs. low-velocity water or turbid vs. clear water), when or how individuals move among sites, and habitat selection (e.g., available cover likely differs in headwater streams compared to large rivers), among other aspects. Eastern Hellbenders occupy streams of orders 2 to 8, and thus, stream order may represent a range of hellbender adaptive diversity.

#### Evolutionary Processes: Gene Flow, Genetic Drift, and Natural Selection

As explained in the genetics studies previously described, the Eastern Hellbender exhibits low levels of gene flow among populations. Hime et al. (2016, p. 12) found that genetic variation within the separate lineages is up to four orders of magnitude lower than the variation among the lineages and that three of the four Eastern Hellbender lineages represent “hotspots” of genetic diversity, with the Missouri lineage having reduced genetic variation. Although there is still some ongoing gene flow within the lineages, the researchers’ analyses of spatial distribution of migration rates suggests that significant barriers to gene flow exist between the lineages (Hime et al. 2016, pp. 7, 12).

Sabatino and Routman’s (2009, p. 1,241) mitochondrial DNA (mtDNA) analysis also indicated that female gene flow among hellbender populations is restricted. They noted this is consistent with results from mark-recapture studies showing low within-river movement and philopatry (the tendency to stay or habitually return to a particular area) observed in both genders of the Eastern Hellbender (Nickerson and Mays 1973, p. 14; Peterson 1987, pp. 199–201; Routman et al. 1994, p. 1,802). These researchers hypothesize that the rarity of the Eastern Hellbender’s specific habitat requirements (streams with clean, clear, cold, well-oxygenated water and large, flat rocks), especially at low elevations, may limit migration between rivers in this species and result in natural fragmentation (Sabatino and Routman 2009, p. 1,241). They further attribute restricted gene flow to the life history trait of external fertilization in the species, which reduces the colonization of new populations due to flooding since this would require at least a breeding pair, as opposed to a single inseminated female, to be moved to a new location (Sabatino and Routman 2009, p. 1,242).

The genetic divergences within hellbender lineages may be millions of years old (Hime et al. 2016, p. 12) and are likely the result of ancient geologic and climatic events (Sabatino and Routman 2009, p. 1,242). For example, the formation and dissipation of Pleistocene glaciations would have created and destroyed river habitats and migratory routes available to hellbenders (Sabatino and Routman 2009, p. 1,242). Therefore, geologic and climatic changes are likely to

have played a significant role in shaping the distribution of mtDNA variation observed in hellbenders today, and successful migration and colonization may occur only when these processes result in the formation of migratory paths suitable to hellbenders (Sabatino and Routman 2009, p. 1,242).

Since Eastern Hellbender gene flow is limited and existing genetic divergences are likely the result of ancient events, it appears likely that natural selection and genetic drift are more important drivers of evolutionary change than gene flow in this species. This was noted by Hime et al. (2016, p. 12) in their assessment that large portions of the hellbender genome have differentiated between the distinct lineages, suggesting that the forces of natural selection and genetic drift are driving divergence in hellbenders.

In summary, the available data indicate low levels of genetic variation within the four distinct Eastern Hellbender lineages with higher genetic variation between these lineages (Hime et al. 2016, p. 12). Restricted gene flow within these lineages is likely due to limited migration of hellbenders and other life history factors (Sabatino and Routman 2009, pp. 1,241–1,242). The major sources of genetic diversity in this species are likely due to natural selection and genetic drift with gene flow playing a minor role in driving evolutionary processes. Thus, conserving the full breadth of representation for the Eastern Hellbender should involve maintaining populations across and within the four distinct lineages.

## **2.5 Adaptive Capacity Units**

Given the information described above, the breadth of genetic diversity can likely be captured by a wide distribution of populations within the four genetic groupings identified by Hime et al. (2016, entire). Thus, to facilitate our analyses, we used these four groupings as our adaptive capacity units (ACUs) to evaluate past, current, and future representation of the Eastern Hellbender. The four units are: 1) Missouri (MACU), 2) Ohio River-Susquehanna River drainages (OACU), 3) Tennessee River drainage (TACU), and 4) Kanawha River drainage (KACU) (Figure 2.1).

In addition to the four adaptive capacity units, Eastern Hellbender representation is also a function of adaptive diversity in the form of ecological variation within these units. Based on available information and input from species experts, we believe the species requires populations distributed across a diversity of ecological and physical conditions across the Eastern Hellbender range to preserve the full breadth of Eastern Hellbender adaptive diversity. To ensure adaptive diversity of each ACU, populations should also be distributed across a range of ecological and physical conditions within each ACU. Species experts recommended using stream temperature regime and stream order as proxies for ecological variation indicating underlying adaptive diversity.

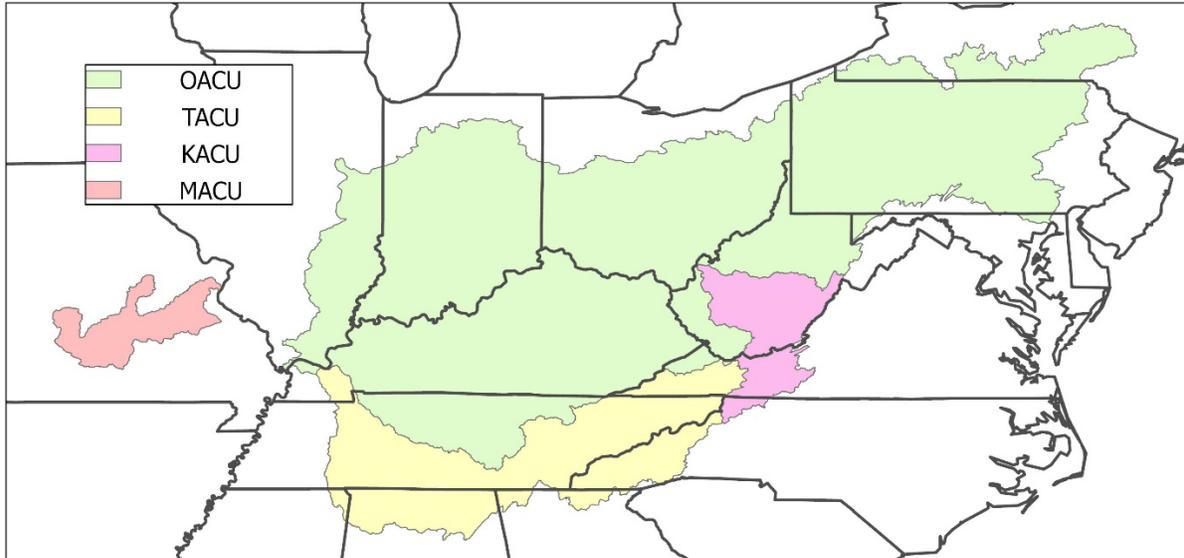


Figure 2.1. Evolutionary distinct lineages identified within the Eastern Hellbender subspecies by Hime et al. (2016, pp. 4–13). We delineated each of the four lineages as an adaptive capacity unit (ACU): Missouri (MACU), 2) Ohio River-Susquehanna River drainages (OACU), 3) Tennessee River drainage (TACU), and 4) Kanawha River drainage (KACU).

Table 2.3. The requisites for species-level viability.

<b>3Rs</b>	<b>Requirements</b>
<b>Resiliency</b>	Healthy populations (see Table 2.1) widely distributed across spatially heterogenous conditions (temperature and precipitation gradients)
<b>Redundancy</b>	Numerous healthy populations widely-distributed to minimize the likelihood of exposure to catastrophic disease and chemical spill events across the species range and within ACUs
<b>Representation</b>	Healthy populations across the breadth of evolutionary lineages and distributed across temperature regimes and stream orders within each evolutionary lineages (ACUs)

## Chapter 3. Analysis of Historical Condition

### 3.1 Distribution and Number of Populations

Historically, the Eastern Hellbender was widespread across 15 states from northeastern Mississippi, northern Alabama, and northern Georgia northeast to southern New York, with disjunct populations occurring in east-central Missouri (Figure 3.1). Early population studies (e.g., from the 1970's) estimate densities ranging from 1.0 to 6.3 hellbenders per 100 m<sup>2</sup> (328 ft<sup>2</sup>) (Hillis and Bellis 1971, pp. 121, 223; Peterson 1985, p. 46), although these numbers were likely less than what was present prior to European settlement (Foster 2018, pp. 37–38). Many populations were skewed towards larger, mature individuals (Taber et al. 1975, p. 636; Peterson 1985, p. 47; Wheeler et al. 2003, p. 155). Larvae and smaller individuals (e.g., total length < 20 cm (7.8 in)) were not captured frequently, presumably due to their low detectability. However, these smaller size classes did comprise a portion of the samples in many studies (Taber et al. 1975, p. 636; Peterson 1985, p. 47; Pfingsten 1988, p. 9; Wheeler et al. 2003, p. 155) and outnumbered adults in one Appalachian stream (Hecht-Kardasz et al. 2012, p. 232).

Throughout its range, 626 populations have been documented in four ACUs and 15 states (Figure 3.1). The spatial arrangement of the populations varies across the range (Figure 3.1), with 1% of the populations occurring in the MACU, 41% in the OACU, 48% in the TACU, and 10% in the KACU. We assume that all historical populations were healthy at one time.

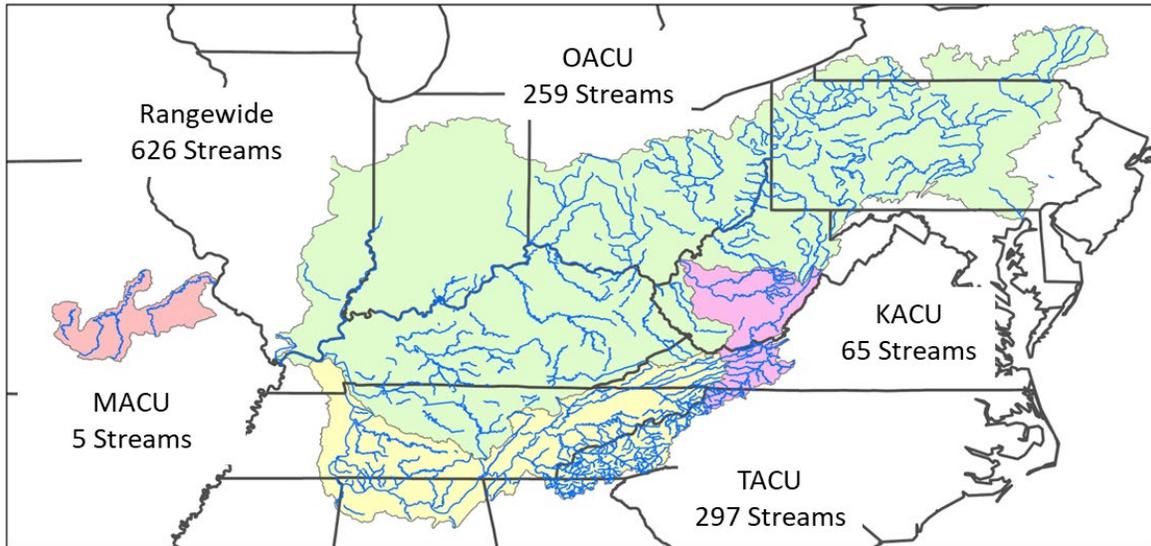


Figure 3.1. Historical range and distribution of the Eastern Hellbender. The number of streams in which the species has been documented is provided rangewide and for each adaptive capacity unit (ACU).

## Chapter 4. Analysis of Current Condition

Eastern Hellbender abundance has decreased in many parts of the range, with reduced numbers observed as early as 1948 (Swanson 1948, p. 363). Population declines have subsequently been documented in several states throughout the range (Gates et al. 1985, p. 4; Gottlieb 1991, p. 47; Wheeler et al. 2003, p. 153; Burgmeier et al. 2011c, pp. 198–200), with declines often characterized as severe or drastic (Wheeler et al. 2003, p. 155; Briggler et al. 2007, p. 85; Burgmeier et al. 2011c, p. 198). Density estimates since 2000 range from 0.06 to 1.2 hellbenders per 100 m<sup>2</sup> (328 ft<sup>2</sup>) in areas where declines have been documented (Humphries and Pauley 2005, p. 137; Foster et al. 2009, p. 583; Burgmeier et al. 2011c, p. 196). Declines in density are often accompanied by a shift to older individuals, with young (small) individuals making up a significantly smaller proportion of the samples (Gottlieb 1991, p. 47; Wheeler et al. 2003, p. 155). This shift to older individuals indicates poor recruitment in these populations. In some areas, however, Eastern Hellbender appears abundant with a size class structure indicative of successfully recruiting populations (Horchler 2010, p. 20; Hecht-Kardasz et al. 2012, pp. 231, 238; Freake and DePerno 2017, pp. 6–7). New populations have also been discovered since 2000 (Gowins et al. 2014, p. 12; Wethington 2017, pers. comm.; Williams 2016, pers. comm.; Lipps 2010; Chapman 2017, pers. comm.; Godwin 2016, pers. comm.). However, most of these discoveries were observations of a single individual or detection via eDNA. A lack of data regarding abundance or size class structure in these populations precludes assessments of population trends.

Since 2000, the Eastern Hellbender has been documented from the four ACUs across 15 states (Figure 4.1). The number of populations varies among ACUs, with 1% of the extant populations occurring in the MACU, 41% in OACU, 48% in TACU, and 10% in KACU (Figure 4.1, Table 4.1).

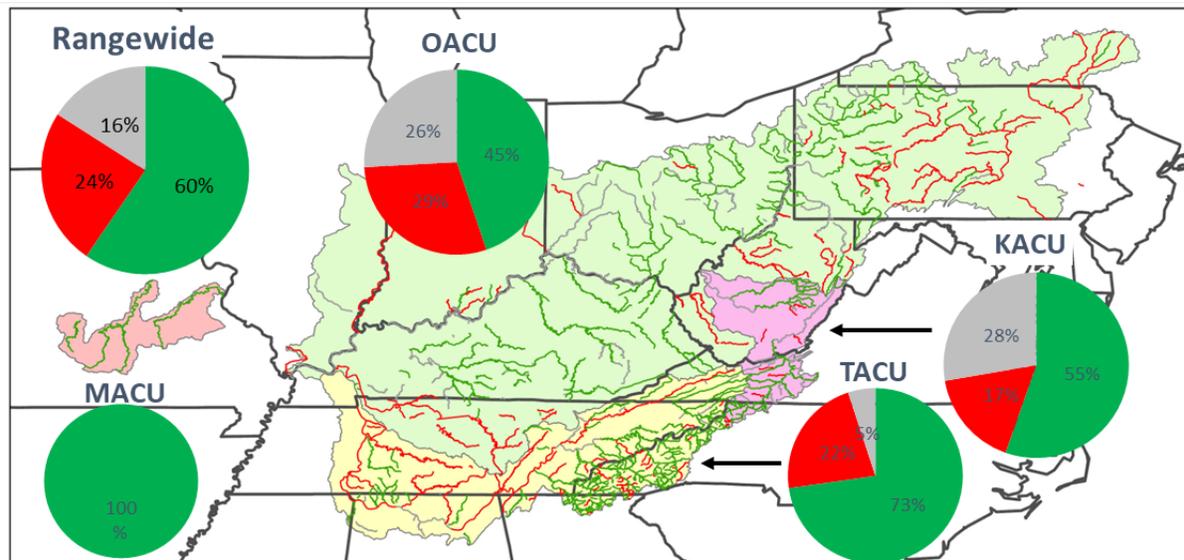


Figure 4.1. Current distribution and status of historical (n=626) Eastern Hellbender populations rangewide and per adaptive capacity unit (ACU). Green = extant, Gray = Unknown, Red = functionally or presumed extirpated.

Currently, there are 373 extant populations, 153 extirpated populations, and 100 populations with unknown status (Table 4.1). Of the extant populations, 11% are considered stable and recruiting (SR), 14% are recruiting but have no trend data (UR), 15% are declining (D), and 59% have an unknown trend (UT) (Table 4.2). Within the ACUs, the number of SR populations ranges from 0 (MACU) to 42 (TACU) and the number of UR populations ranges from 0 (MACU) to 54 (TACU). The number of extant populations with unknown trend (UT) ranges from 0% in the MACU to 70% in the OACU (Table 4.1, Figure 4.2). Eastern Hellbender survey effort has increased substantially over the last decade. The number of known populations has increased by 181 since 2012. However, we lack the data on many newly discovered populations to determine whether they are SR, UR, D, or FX.

Table 4.1. The number of populations by status and trend category range wide and in each adaptive capacity unit (ACU). Status categories: Extant, Extirpated and Unknown Status; Trend categories: US = unknown status; UT = extant, unknown trend; SR = stable recruiting; UR = recruiting, unknown trend; D = declining; and FX and PX = functionally and presumed extirpated, respectively.

ACU	Total # of Historical Streams	Status			Trend						
		Extant	Extirpated	Unknown	US	UT	SR	UR	D	FX	PX
Rangewide	626	373	153	100	100	220	42	54	57	57	96
OACU	259	116	76	67	67	81	10	10	15	18	58
TACU	297	216	66	15	15	120	26	38	32	31	35
KACU	65	36	11	18	18	19	6	6	5	8	3
MACU	5	5	0	0	0	0	0	0	5	0	0

#### *Extrapolating to Populations with Unknown Status and Trends*

Of the 626 populations range wide, there are 320 populations with either unknown status (US) or trend (UT). To garner insights on the distribution, number, and health of these populations, we asked the experts, based on their knowledge of the environmental conditions and status of known populations within their areas of expertise, for the number of populations with unknown status or trends (US, UT) that they believe are SR, UR, D, FX, and PX.

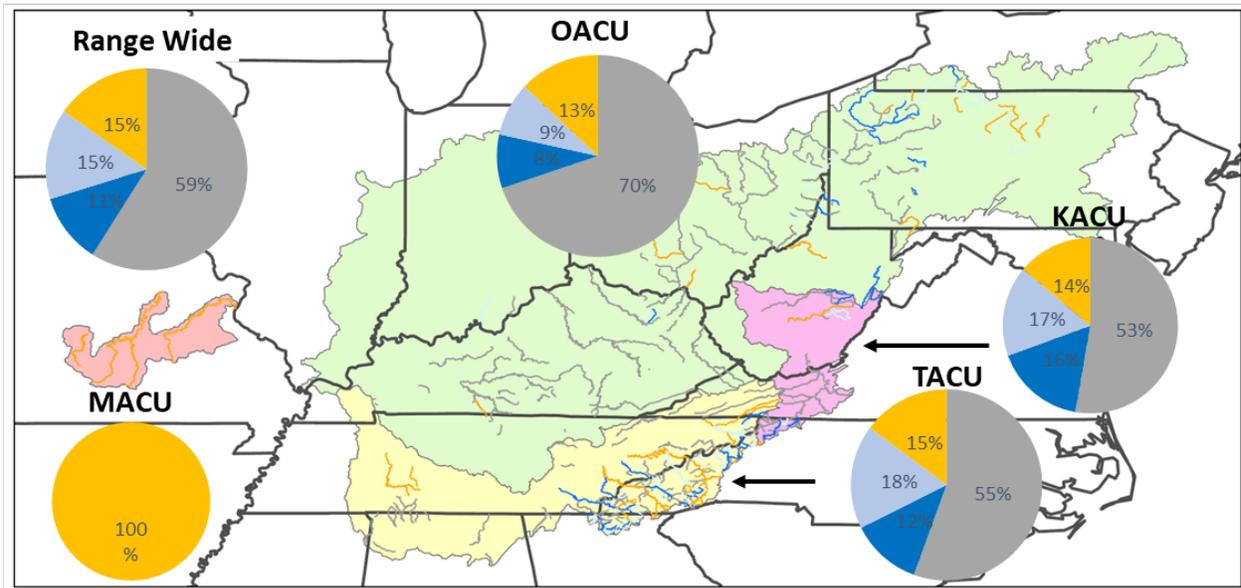


Figure 4.2. The proportion of known extant populations by trend category rangewide (RW) and in each adaptive capacity unit (ACU): Dark Blue = SR (stable recruiting); Light Blue = UR (recruiting, unknown trend); Orange = D (declining); Gray = UT (extant, unknown trend).

When including all 626 populations in the analysis of current condition by incorporating expert judgments of the unknown status and trend streams, 255 (41%) populations are FX or PX and 371 (59%) are extant. Of the 371 extant populations, 45 (12 %) are SR, 108 (29%) are UR, and 218 (59%) are D (Table 4.2, Figure 4.3).

Table 4.2. The number of populations by status and trend category range wide and within each adaptive capacity unit (ACU), including unknown populations (US – unknown status and UT – extant, unknown trend) for which experts used their best professional judgement to assign trends (SR – stable recruiting, UR – recruiting, unknown trend, D – Declining, FX – functionally extirpated, and PX – presumed extirpated).

	Total number of Historical Streams	SR	UR	D	FX	PX
Range Wide	626	45	108	218	133	122
OACU	259	12	25	101	45	76
TACU	297	26	74	82	73	42
KACU	65	7	9	30	15	4
MACU	5	0	0	5	0	0

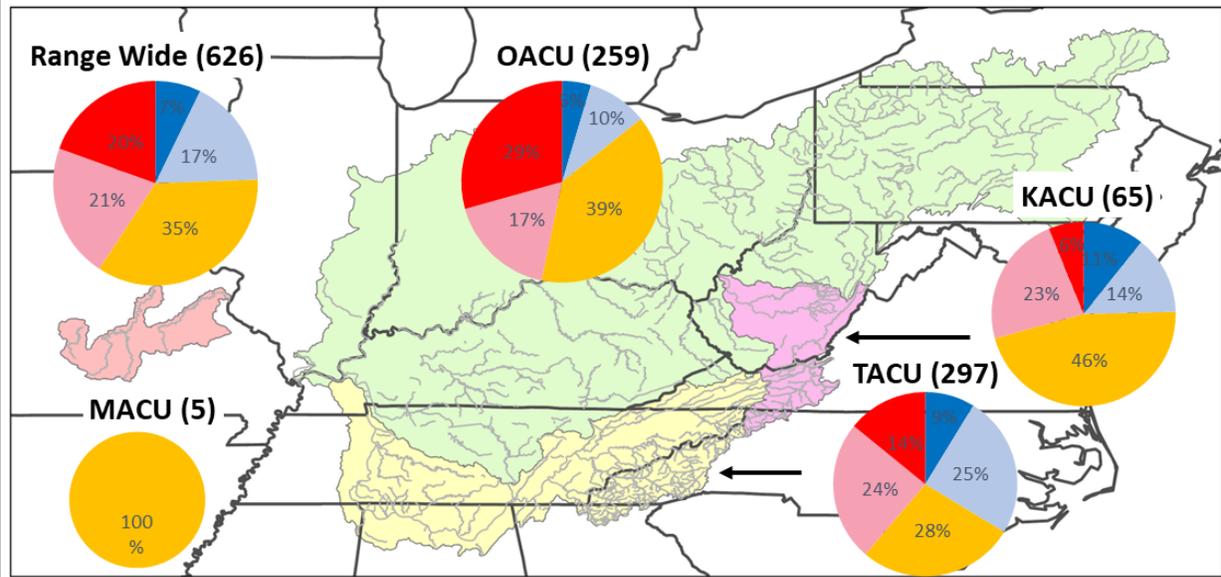


Figure 4.3. The proportion of all known populations by status category range wide and in each adaptive capacity unit (ACU), including unknown populations (US and UT) for which experts used their best professional judgement to assign trends (SR, UR, D, FX, and PX): dark blue = SR (stable recruiting), light blue = UR (recruiting, unknown trend), orange = D (declining), light red = FX (functionally extirpated), and dark red = PX (presumed extirpated). Number of populations is indicated in parentheses.

## Chapter 5. Risk and Conservation Factors

In this chapter, we describe both risk and conservation factors (or influences) that have led to the Eastern Hellbender’s current conditions and which may influence population dynamics into the future. We identified primary factors likely influencing the species’ status (Table 5.1) and then elicited input from species experts on the relative weight of each factor (Table 5.2).

Across the range, sedimentation was identified by experts as the factor most impacting the status of the species. It has specifically been implicated as a cause of Eastern Hellbender declines and as a continuing threat throughout much of the species’ range. Degraded water quality (from development, chemical pollution, etc.), which can cause direct mortality and increase vulnerability to other risk factors, was estimated as having the second highest impact on the Eastern Hellbender status in all ACUs. Destruction of habitat (from activities such as gravel mining and impoundments) was also ranked relatively high. Other factors experts identified include disease, habitat disturbance that causes direct impacts to individuals (recreation, off road vehicles, etc.), activities resulting in mortality (illegal collection, persecution, etc.), climate change, and increased abundance of native and non-native predators. Two of these factors, disease and chemical pollution, also have the potential to rise to the level of catastrophic events and could cause complete loss of some of the ACUs. All of these factors are discussed below in further detail.

Table 5.1. List of the primary influences.

<b>Influence</b>	<b>Description</b>
Sedimentation	off-road vehicles, deforestation, development, gravel mining, impoundments
Climate change	extreme droughts, floods, water temp
Mortality	collection, persecution, anglers, recreationists, gravel mining
Water quality degradation	development, deforestation
Overabundance of predators	trout, walleye, river otters
Disease & pathogens	Bd, ranavirus, leeches, trypanosomes
Habitat disturbance	scientific collection, recreationists, off-road vehicles
Invasive species	rusty crayfish
Small population effects/isolation	environmental and demographic stochasticity
Destruction of habitat	gravel minining, rock removal, impoundments

Table 5.2. The current relative weight (%) of the primary factors. For OACU, TACU, and KACU, the values represent the median, minimum, and maximum among ACU-specific experts; there was only one expert for the MACU.

Influences	MACU	OACU			TACU			KACU		
	Med	Med	Min	Max	Med	Min	Max	Med	Min	Max
Sedimentation	17	24	13	25	21	10	23	20	10	23
Climate change	3	7	5	10	7	2	10	7	5	10
Mortality	5	2	1	10	11	1	15	12	11	15
Water quality degradation	15	17	6	22	20	10	23	19	10	23
Overabundance predators	8	6	2	11	8	2	11	8	5	11
Disease & pathogens	13	2	1	13	5	4	11	6	5	10
Habitat disturbance	11	6	1	12	4	2	6	5	2	10
Invasive species	0	0	0	7	0	0	1	0	0	0
Small population effects/isolation	9	10	8	24	7	2	16	10	5	16
Destruction of habitat	3	7	7	12	6	5	22	5	5	10

## 5.1 Sedimentation

For all ACUs, sedimentation was identified by experts as the factor most impacting the status of the Eastern Hellbender and has been identified as an ongoing threat in every major river system in the range of the species. It has specifically been implicated as a cause of Eastern Hellbender declines and emanates from multiple sources, including agriculture, silviculture, oil and gas development, residential development, off-road vehicles, impoundments, instream gravel mining, and road construction (Briggler 2012, pers. comm.; Chapman 2009, pers. comm.; Conrad 2012, pers. comm.; Feller and Thompson 2011, p. 3; Gates 1983, pp. 5, 17; Greathouse 2007, p. 45; Hauswald 2008, pers. comm.; Hopkins and Durant 2011, p. 108; Hopkins et al. 2011, pp. 21, 22; Horschler 2010, p. 21; Humphries 2005, entire; Jensen 2009, pers. comm.; Kaunert 2011, p. 22; Keitzer 2007, pp. 15, 23; Lawson 2009, pers. comm.; Lipps 2009a, pers. comm.; Lipps 2009b, p. 7; Lipps 2010, pers. comm.; MDEQ 2005, p. 2; Nickerson and Mays 1973, p. 64; Rayman 2012, pers. comm.; Scott 2009, pers. comm.; Wheeler et al. 2003, p. 155; Williams 2011a, pers. comm.). Though sedimentation is a source of both habitat and water quality degradation, we analyzed it separately due to the magnitude and severity of the threat it poses to the Eastern Hellbender.

Sedimentation is the addition of fine soil particles (e.g., sands, silts, clays) to streams, and it modifies aquatic habitats by increasing stream turbidity, reducing light penetration, reducing water depth, increasing temperature, and reducing the complexity and abundance of interstitial spaces among coarse substrates important to the Eastern Hellbender (i.e., gravel, cobble, and boulder) (Ellis 1936, p. 41; Waters 1995, pp. 67–69, 118). The reduction of interstitial spaces among coarse substrates can degrade habitat for larval and juvenile hellbenders, as well as habitat for macroinvertebrates, which are an important food source for larval hellbenders (Cobb and Flannagan 1990, pp. 35–37; Nickerson et al. 2003, p. 624; Brooks et al. 2023, p. 3). Unger et

al. (2020c, p. 3) found that juveniles were unable to burrow under cobble that was 50% imbedded with fine sediments and Unger et al. (2021a, p. 163) showed that occupancy of larval hellbenders is significantly negatively correlated with substrate embeddedness. Excessive sedimentation can also affect adult hellbenders by burying shelter and nest rocks (Blais 1996, p. 11; Lipps 2009b, p. 10; Hopkins and DuRant 2011, p. 112; Brooks et al. 2023, p. 2) and by affecting habitat for crayfish, the primary food source of adult Eastern Hellbenders (Santucci et al. 2005, pp. 986–987; Kaunert 2011, p. 23). In addition, sedimentation can suffocate eggs, thereby reducing their viability (Nickerson and Mays 1973, pp. 55–56).

Further effects of excessive sedimentation include increased water temperature and lower dissolved oxygen levels (Allan and Castillo 2007, pp. 323–324), as well as increased exposure to chemical pollutants (see *5.2 Water Quality Degradation*). Various chemicals, such as some pesticides, bind to silt particles and become suspended in the water column when flushed into a stream. The hellbender's permeable skin can allow direct exposure to these chemicals, which can be toxic (Wheeler 1999, pp. 1–2).

## 5.2 Water Quality Degradation

Compared to other influences, degraded water quality was estimated as having the second highest impact on the status of the Eastern Hellbender. Degraded water quality can cause direct mortality to sensitive species, such as the Eastern Hellbender and, at sub-lethal levels, can alter physiological processes and increase vulnerability to other threats (Maitland 1995, p. 260; see also *Synergistic Effects*). Major sources of aquatic pollutants include domestic wastes, agricultural runoff, coal mining activities, and unpermitted industrial discharges, all of which have been identified as threats to Eastern Hellbenders. Additionally, chemical spills can extirpate populations. There are a few documented cases of Eastern Hellbender kills (Williams, Chapman, and Floyd 2017, pers. comm.; Feller and Thompson 2011, entire) and many examples of fish and mussel kills from chemical pollution within the Eastern Hellbender range (USFWS 2013, pp. 59279–59284; Henley et al. 2002, entire). However, there is no information available to estimate how frequently chemical pollution events occur or the likelihood of this causing catastrophic decline in an ACU. There are several databases tracking reported chemical spill events, Clean Water Act section 303(d) listed streams<sup>1</sup>, and chemical pollution; however, the effects of chemicals on Eastern Hellbender remain largely unknown (Burgmeier et al. 2011b, p. 836; Pugh et al. 2015, pp. 105–6). While it is unlikely that a chemical spill could cause catastrophic loss of an entire ACU, it is possible if multiple spills occur in an ACU with low redundancy.

### *Nutrient Pollution*

Untreated and poorly treated municipal wastewater (sewage) from wastewater treatment plants (WWTP) and livestock waste, especially where livestock have unrestricted stream access, are common sources of chemical pollution. Sewage and livestock waste contain chemical contaminants that include ammonia, pathogenic bacteria, nutrients (e.g., phosphorus and nitrogen), and organic matter that increases biological oxygen demand (BOD) (Cooper 1993, p. 405). BOD is a measure of the oxygen consumed through aerobic respiration of microorganisms that break down organic matter in the sewage or livestock waste. Nutrients also enter streams

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<sup>1</sup> 303(d) listed streams are streams, rivers or lakes identified by each state as impaired or threatened.

from agricultural and lawn-care fertilizers. Nutrients and BOD may have the greatest impact on Eastern Hellbenders because they decrease dissolved oxygen (DO) levels. Elevated levels of nutrients lead to excess algal growth, which can lead to physical alterations of habitat when it covers bottom substrates (Cooper 1993, p. 405). Nocturnal respiration of live algae and decomposition of dead algae consumes oxygen (Cooper 1993, p. 405). As noted previously, low DO may be particularly harmful to Eastern Hellbenders, which are not well adapted to low DO conditions (Harlan and Wilkinson 1981, p. 383). Da Silva Neto et al. (2023, p. 354) found that extirpated and functionally extirpated populations were associated with relatively lower DO than in streams with recruiting populations.

Nutrient and organic enrichment are common in some parts of the Eastern Hellbender's range. For example, almost 6,437 km (4,000 mi) of stream in Pennsylvania (PDEP 2016, p. 52) and over 3,219 km (2,000 mi) each in Kentucky (KDEP 2014, p. 62) and Tennessee (TDEC 2014, p. 60) are impaired because of nutrient enrichment, organic enrichment, and/or low DO. Sources of these impairments include livestock, municipal WWTPs, urban runoff, and improper application of fertilizers (TDEC 2014, p. 50). Feller and Thompson (2011, p. 4) note that an overloaded WWTP currently empties into an Eastern Hellbender stream in the Monongahela River system in Maryland. Pinder (2009, pers. comm.) and Williams (2012, pers. comm.) state that new vacation homes along many rivers in the Kanawha and Tennessee river systems in Virginia and North Carolina will contribute additional nutrient and organic enrichment due to increased numbers of septic systems. Williams (2012, pers. comm.) also reports increased golf course development in the French Broad and Little Tennessee river basins and speculates that they will result in increased fertilizer runoff to streams in these systems. Hauswald (2008, pers. comm.) reports that failing septic systems impact water quality in the Blue River system in Indiana and that new residential development has the potential to contribute additional impacts. Burgmeier et al. (2011b, p. 845) found nutrient levels (orthophosphate and nitrate) exceeding USEPA recommended criteria in the Blue River system, Indiana; however, they did not observe noticeable signs of nutrient enrichment (e.g., high density of submerged plants) and suggested that nutrient enrichment was not a cause of the observed Eastern Hellbender declines there. Straight pipes discharge untreated domestic sewage into some streams in North Carolina (Williams 2012, pers. comm.) and in the Green River system in Kentucky (KDEP 2008, p. 8).

### *Endocrine Disrupting Compounds*

Endocrine disrupting compounds (EDCs) are chemicals that interfere with an organism's normal endocrine or reproductive functions by mimicking natural hormones or stopping the production or function of hormones. Endocrine disrupting compounds encompass a variety of chemical classes (e.g., pharmaceutical drugs, pesticides, nonionic surfactants, environmental pollutants, plastics, and some naturally produced botanical chemicals) and can enter aquatic systems through WWTP effluents, runoff from livestock operations, industrial discharges, and runoff or leaching of pesticides into groundwater or surface waters. In amphibians, EDCs have caused male feminization (Hayes et al. 2002, pp. 5477–5478), decreased survival (Storrs and Kiesecker 2004, pp. 1056–1057), and increased susceptibility to disease (Forson and Storfer 2006b, pp. 170–171). In addition, EDCs may affect salamanders indirectly by impairing immune function and increasing their susceptibility to disease (Kiesecker 2002, pp. 9902–9903; Forson and Storfer 2006a, pp. 2328–2329; Hayes et al. 2006, pp. 29–30; Brodtkin et al. 2007, pp. 81–82) (also see

*Synergistic Effects*). Eastern Hellbenders may be at an increased risk given their entirely aquatic life cycle, frequent contact with stream substrates, and long life span. Increased contact with stream substrates increases risk of exposure because EDCs accumulate in stream sediments (White et al. 1994, p. 176). A long life span increases risk because EDCs accumulate in fatty tissues, and therefore can accumulate in higher concentrations in long-lived species, like the Eastern Hellbender (Diamanti-Kandarakis et al. 2009, pp. 3–4).

Information on the prevalence of EDCs within Eastern Hellbender streams is limited. Tests of organic compounds in 12 Ohio streams of historical or current Eastern Hellbender occurrence documented EDCs at multiple sites, but concentrations in streams with extant populations were equal to or higher than those with declining or presumed extirpated populations (Lipps and Pflingsten 2010, p. 10). EDCs were also present in an Indiana river occupied by Eastern Hellbenders, but sperm appeared healthy and no vitellogenin (a biomarker used to indicate exposure to estrogenic chemicals in the environment) was detected in males (Burgmeier et al. 2011b, p. 840–841). Kolpin et al. (2002, p. 1208) found the presence of organic wastewater-derived contaminants, many of which are known EDCs, in 80% of the 139 streams sampled throughout the U.S. (within and outside of the Eastern Hellbender range) from 1999–2000. Individually, compounds were generally detected at low levels, but total concentrations commonly exceeded 1 microgram per liter ( $\mu\text{g/L}$ ) (1 part per billion (ppb)). Concentrations less than  $0.001\mu\text{g/L}$  (ppb) can cause adverse effects in fish (Routledge et al. 1999, pp. 1561–1563; Sedlak et al. 2000, pp. 508A–515A). Moreover, WWTP effluents are prevalent throughout much of the Eastern Hellbender range.

### *Conductivity*

Conductivity, the ability of water to pass an electrical current, is an indirect measure of dissolved salts and the resulting ion concentration in the water. Conductivity in streams is dependent on geology of the surrounding area (USEPA 2017) but is also affected by erosion, addition of pollutants (Allan and Castillo 2007, pp. 61–62), and exposure of underlying geology (e.g., from mining and road cuts). Although elements comprising common mineral salts are essential nutrients, aquatic organisms are adapted to specific ranges of salinity and experience toxic effects from excess salinity (USEPA 2011). The impacts of dissolved salts on stream biota depend on both quantity (total conductivity) and the specific ions present and relative amounts of each. Wineland et al. (2018, p. 216) found that conductivity was negatively correlated with Eastern Hellbender eDNA detection. Pitt et al. (2017, p. 972) found that conductivity below 278 milli Siemens per cm (ms/cm) was the strongest predictor of Eastern Hellbender persistence at 24 historical sites and that conductivity was negatively correlated to tree canopy cover. Similar results were found by Bodinof Jachowski and Hopkins (2018, p. 23) and Da Silva Neto et al. (2023, p. 354). However, Brown (2022, pp. 25–28) showed only minimal effects on egg hatching rates in a laboratory experiment. The effects of dissolved salts on stream biota are complex and varied. Ultimately, the effects of conductivity on Eastern Hellbenders are not known with certainty.

## *Coal Mining and Road Construction*

Activities associated with coal mining and road construction can also contribute chemical pollutants to streams. Acid mine drainage (AMD) is created from the formation of sulfuric acid in the oxidation of iron-sulfide minerals, such as pyrite (Sams and Beer 2000, pp. 3–5), and may produce high concentrations of aluminum, manganese, zinc, and other constituents (TDEC 2014, p. 72). These metals, and the high acidity typically associated with AMD, can be acutely and chronically toxic to aquatic life (Erichsen and Jones 1962, pp. 258–259). In amphibians, AMD can inhibit amphibian egg development and larval feeding and can result in indirect effects that kill eggs, larvae, and adults (Dodd 1997, p. 181). Implementation of the Surface Mining Control and Reclamation Act of 1977 (SMCRA) has significantly reduced AMD from new coal mines; however, unreclaimed areas mined prior to SMCRA continue to generate AMD in portions of the Eastern Hellbender’s range. In addition, road construction that exposes acid-producing geology can result in acidic drainage with properties similar to AMD (TDEC 2014, p. 53). Huckabee et al. (1975, pp. 677–678) documented the absence of fish and salamanders in Great Smoky Mountain streams following exposure of acid-forming rock during road construction and attributed their absence to lowered pH and raised dissolved metal concentrations. Since Eastern Hellbenders’ primary means of respiration is cutaneous, introduced toxins are readily absorbed through the skin (Jensen 1999, p. 99), and they are likely affected by AMD similarly to other amphibians. Because Eastern Hellbenders are long-lived, they may also be at higher risk of bioaccumulation of some pollutants (Peterson et al. 1998, pp. 12–13).

Acidic drainage from coal mines and road construction has been identified as a threat to Eastern Hellbenders (Nickerson and Mays 1973, p. 56; Feller and Thompson 2011, pp. 3–4; Petokas 2011, pers. comm.; L. Williams 2011b, pers. comm.) and is present in many parts of the Appalachian region in the Eastern Hellbender’s range. Abandoned mine drainage is the source of pollution in more than 9,102 km (5,600 mi) of impaired streams in Pennsylvania (PDEP 2016, p. 51). Mine drainage affects 17% of stream miles in West Virginia (WVDEP 2014, p. 20), and surface mining has been identified as a source of impairment for approximately 1,247 km (775 mi) of stream in Kentucky (KDEP 2014, p. 66), including in the upper Kentucky River watershed where historical unreclaimed mines and active coal mines are prevalent (Kentucky Geological Survey 2008). Mining continues to impair streams in the Cumberland Plateau and Central Appalachian regions of Tennessee (upper Cumberland River system and upper Tennessee River system) (TDEC 2014, p. 62) and is the primary source of low pH impairment of 605 km (376 mi) of stream in the state (TDEC 2014, p. 53). In Maryland, a 1929 coal mine fire was extinguished by diverting water from the Youghiogheny River. The resulting acidic return water then killed aquatic biota, including Eastern Hellbenders, for many miles downstream (Feller and Thompson 2011, p. 4). Williams (2012, pers. comm.) also reports that pending road construction projects in the Little Tennessee and Hiwassee river basins in North Carolina in areas of acid-producing geology could result in significant acidification to some streams in those systems.

## *Chemical Spills*

Other sources of chemical pollutants include unpermitted releases of contaminants into streams (i.e., spills). Deep horizontal drilling for oil and gas in shale formations in Pennsylvania, Ohio,

West Virginia, and Kentucky has accelerated significantly in the last 5 years and presents a greater water quality risk than traditional shallow wells. Approximately 1,892 cubic meters (m<sup>3</sup>) (500,000 gallons (gal)) to more than 11,356 m<sup>3</sup> (3,000,000 gal) of water are needed for bit cooling, rock cutting removal, and creation of permeable flow paths for natural gas movement using hydraulic fracturing techniques (Harper 2008, pp. 11–12; Soeder and Kappel 2009, pp. 2, 4). Water withdrawals, especially from small streams during drought conditions, have the potential to negatively affect hellbenders. In addition, drilling wastewater may contain contaminants and is not easily treated (Soeder and Kappel 2009, pp. 4–5). Accidental spills of drilling wastewater have occurred in the range of the Eastern Hellbender in Pennsylvania and West Virginia, sometimes resulting in significant levels of mortality of fishes, salamanders, mussels, and crayfishes (Greathouse 2009, pers. comm.; Renner 2009, p. 9046; Marcellus-shale.us 2012).

Unpermitted contaminant releases from other sources include a 2008 coal slurry release at a major underground mine in a tributary to a high-quality Eastern Hellbender stream in eastern Ohio. The release resulted in acutely toxic concentrations of polycyclic aromatic hydrocarbons in stream sediments (OEPA 2010, p. 25). Multiple contaminant releases have been documented in the Tennessee River and New River systems in North Carolina, including a WWTP failure in 2003 in the New River system that resulted in mortality of over 50 Eastern Hellbenders; follow up studies showed no recolonization by Eastern Hellbenders three years after the event (Williams 2011b, pers. comm.). In the French Broad River system, a wastewater treatment plant failure in 2008 resulted in chemicals and sewage release to the river for several months. Several dead and dying Eastern Hellbenders were reported (Williams 2011b, pers. comm.). At least five other spills of a variety of pollutants (e.g., fungicide, acid, chlorine, chemicals used in paper processing, and fire retardants) have occurred in French Broad River since 2000; and although dead Eastern Hellbenders were not documented, each spill resulted in a fish and/or amphibian kill (Williams 2011b, pers. comm.). Four dead Eastern Hellbenders were found in a West Branch Susquehanna River tributary in 2006 following a chemical spill from a railway container (Hartle 2016, pp. 54–55).

### **5.3 Habitat Destruction and Modification**

#### *Impoundments*

Construction of artificial impoundments (dams) modifies Eastern Hellbender habitat in multiple ways. Impoundments reduce upstream streamflow, increasing sedimentation in the impounded reaches (Baxter 1977, p. 260; Bhowmik and Adams 1989, pp. 17–18) and subsequently lowering dissolved oxygen. Sedimentation from reduced stream flow also reduces available substrate for both hellbenders and their prey (Williams et al. 1981a, p. 99; Santucci et al. 2005, pp. 986–987). In some cases, impoundments can create unsuitable conditions for Eastern Hellbenders downstream due to low DO, cold hypolimnion releases, and variable flow rates. In addition, dams can create a barrier to Eastern Hellbender movement by isolating populations, and limiting gene flow and recolonization of formerly occupied habitat, thereby exacerbating local population declines and extirpations. Dams have been constructed in every major stream system in the range of the Eastern Hellbender and have contributed to population declines and local extirpations, especially in large streams used for navigation (e.g., Ohio, Cumberland, and Tennessee rivers),

and are currently restricting movement among some populations and into some previously occupied habitats.

Dams are pervasive throughout much of the range of the Eastern Hellbender and include major water supply, flood control, and hydroelectric dams (e.g., in the Susquehanna, Allegheny, Loyalhanna, Shenango, Kanawha, Scioto, New, Cumberland, and Tennessee river watersheds), navigational locks and dams (e.g., Ohio, Allegheny, Muskingum, Kentucky, Green, Cumberland, and Tennessee rivers), and hundreds of low-head dams in nearly every major river system in the range of the species. In the Scioto River system in Ohio, there are six major water supply or flood control reservoirs and multiple low-head dams (18 in Franklin County alone) (ODNR 2010). Approximately 90% of the 904-km (562-mile) length of Cumberland River downstream of Cumberland Falls is either directly impounded by dams or otherwise impacted by cold tailwater releases from dams on tributaries (Butler 2007, p. 75). Impoundments on Cumberland River tributaries (e.g., Obey and Caney Fork rivers) have inundated approximately 161 additional river km (100 mi) of historical or potential Eastern Hellbender habitat (Butler 2007, p. 75). In the Tennessee River system, which includes approximately 35% of the Eastern Hellbender streams of record, there are over 36 major dams that impound 3,700 km (2,300 mi) of stream (approximately 20% of all stream miles in the system) (TVA 1971). These include nine mainstem dams and several others on tributary streams with current or historical populations of Eastern Hellbenders (e.g., Holston, South Fork Holston, Clinch, Elk, and Duck rivers; and Bear and Cedar creeks). Further, mainstem impoundments have created reservoir conditions in the lower portions of many tributary streams (e.g., Powell, Little, Sequatchie, Paint Rock, Flint, Elk, and Duck rivers; and South Chickamauga and Bear creeks). Duck River (Tennessee River system) alone has had at least 25 mill dams constructed (Ahlstedt et al. 2004, p. 6 [citing LaForest and Oliveira 1979]).

Impoundments have specifically been implicated in population declines and local extirpations throughout much of the range of the Eastern Hellbender, including in Youghiogheny River in Pennsylvania (Nickerson and Mays 1973, p. 61; Gates 1983, pp. 4–5; Gates et al. 1985, p. 17); the Susquehanna River system in Maryland (Gates 1983, p. 5; Gates et al. 1985, p. 18), Pennsylvania, and New York (Blais 1996, p. 11); the Allegheny River system in New York (Bothner and Gottlieb 1991, p. 45; Roblee 2012, pers. comm.); the mainstem of Ohio River throughout its entire length (Pfingsten 1990, p. 49); the Little Kanawha River system in West Virginia (Greathouse 2009, pers. comm.); and the Tennessee River system in Kentucky, Mississippi (Nickerson and Mays 1973, p. 63), Alabama (Nickerson and Mays 1973, p. 58; Mount 1975, p. 109; Graham et al. 2011, p. 246), Georgia, Tennessee (Gentry 1955, p. 169; Nickerson and Mays 1973, p. 66; Echternacht 2009, pers. comm.), and North Carolina (Williams 2012, pers. comm.). The continued presence of dams results in restricted movement of the Eastern Hellbender and an inability of individuals to repopulate formerly occupied habitat.

#### *Channelization and Removal of Riparian Vegetation*

Typically conducted for drainage improvements, channelization is any combination of widening, straightening, and deepening of streams and often includes removal of riparian vegetation (Brooker 1985, p. 63). Channelization results in accelerated erosion, decreased habitat diversity, including loss of boulder habitat, and channel instability (Hubbard et al. 1993, pp. 136–145). The

effects of channelization and riparian vegetation removal on stream biota are well documented. Etnier (1972, pp. 373–375) attributed the loss of fish diversity and abundance in a Tennessee stream following channelization to a loss of macroinvertebrates caused by substrate instability and habitat homogeneity. Ebert and Filipek (1988, p. 29) documented smaller substrates and decreased biodiversity in channelized reaches of an Ozark stream. Channelization also typically results in the loss of stream length, which contributes to flashier hydrographs (higher peak flows during rain events and lower base flows during dry periods) (Brooker 1985, p. 63). Flashier hydrographs can also be caused by a high rate of wetland loss and a high density of impervious surfaces (e.g., parking lots, roads, roofs) in the watershed (Paul and Meyer 2001, p. 335; Booth and Jackson 1997, p. 1080; USEPA 1997, p. 2). As little as 10% impervious cover in a watershed can modify hydrographs, resulting in erosion, channel instability and widening, substrate alteration, and in-stream and riparian habitat loss (Booth and Jackson 1997, p. 1084). Da Silva Neto et al. (2023, p. 354) found that health of Eastern Hellbender populations was greatest when development in the watershed was less than 25%.

Eastern Hellbenders depend heavily on stable, coarse substrates (gravel, cobble, boulder). Physical habitat modifications that reduce the abundance and quality of this habitat adversely affect Eastern Hellbenders. Nickerson et al. (2007, pp. 115–116) hypothesize that flood disturbance of benthic structure in streams, especially smaller streams, can lead to reduced recruitment because larval Eastern Hellbenders are dependent on gravel and cobble substrates. Keitzer (2007, p. 23) rarely found Eastern Hellbenders in areas without intact forested buffers in the French Creek system in Pennsylvania. In Georgia (Humphries 2005, p. 10) and North Carolina (Williams 2012, pers. comm.; Unger et al. 2021a, p. 163), the highest Eastern Hellbender densities have been found in streams with wide, undisturbed forested buffers. Bodinof Jachowski and Hopkins (2018, p. 220–221) found that riparian forest cover within the entire stream catchment upstream of a population was the best predictor of Eastern Hellbender density and recruitment in the Virginia streams they studied and Hopkins et al. (2023, p. 96) documented much higher whole clutch filial cannibalism (when the male eats all the eggs in a nest) in areas with low riparian forest cover. They hypothesized that this may be due to changes in water chemistry in streams with low riparian forest cover, which led to a reduction in viability of eggs. However, Bodinof Jachowski et al. (2024, pp. 8–9) found that Eastern Hellbenders in areas with high riparian forest cover had higher parasite loads and poorer body condition than those in areas with low riparian forest cover, potentially because denser populations in these streams led to intraspecific competition.

Altered stream and riparian habitats occur throughout many parts of the Eastern Hellbender range. Aquatic habitat modifications, including channelization and bank modification, account for more than 1,754 km (1,090 mi) of stream impairment in Pennsylvania (PDEP 2016, p. 51). Flow modification is a cause of approximately 2,939 km (1,826 mi) of stream impairment in Pennsylvania (PDEP 2016, p. 52) and runoff from urban areas is the third largest source of stream impairment in Pennsylvania (PDEP 2016, p. 51). Stream channel alterations have been described as one of two main anthropogenic threats to aquatic biota in the French Creek system, Pennsylvania (Bowers et al. 1992, p. 22). Heavy channelization and dredging has also been documented in some streams in the Elk River system, West Virginia (WVDEP 1997, p. 59). In Kentucky, channelization has been identified as a source of impairment for 1,112 km (691 mi) of stream and loss of riparian habitat has been identified as a source of impairment for almost 2,837

km (1,763 mi) (KDEP 2014, p. 66). In Tennessee, 5,686 km (3,533 mi) are impaired because of channelization, 4,865 km (3,023 mi) are impaired due to riparian habitat removal, and 10,029 km (6,232 mi) are impaired due to grazing in riparian areas (TDEC 2014, pp. 63–64). In parts of the Tennessee and Cumberland River watersheds, rapid commercial and residential development is leading to increased areas of impervious surfaces (TDEC 2014, p. 62).

Stream and riparian habitat alterations have specifically been implicated as causes for Eastern Hellbender declines and are continuing threats. These include in the upper Allegheny River system in New York (Foster et al. 2009, p. 586); French Creek system in Pennsylvania (Keitzer 2007, p. 23); Youghiogheny River system in Maryland (Feller and Thompson 2011, p. 4); many river systems in Ohio (Nickerson and Mays 1973, p. 64); Licking River system and Kentucky River system in Kentucky (Lipps 2009b, pp. 7–8); and Tennessee River system in Alabama (Nickerson and Mays 1973, p. 58; Mount 1975, p. 109; Cline and Rayburn 2008, p. 7; Graham et al. 2011, p. 247), Georgia (Humphries 2005, p. 10; Jensen 2009, pers. comm.), and North Carolina (Williams 2011b, pers. comm.).

### *Instream Gravel Mining*

Instream gravel mining results in stream channel modifications similar to channelization, including channel incision and headcutting, increased sediment transport, altered habitat, and altered water quality (e.g., increased turbidity, increased temperature), and reduction in macroinvertebrate diversity and abundance (Kanehl and Lyons 1992, pp. 26–27; Roell 1999, p. 5). Sand and gravel mining are also common activities in many Kentucky streams (Lipps 2009a, pers. comm.). Substrate alterations, including those resulting from gravel mining, are the source of impairment to 7,149 km (4,442 mi) of stream in Tennessee (TDEC 2014, p. 48).

## **5.4 Disease**

Disease can act as a stressor on Eastern Hellbender populations and has the potential to cause catastrophic loss of ACUs. Based on current information, the diseases that could impact hellbenders are described below. There are two avenues by which disease could result in a catastrophic event for Eastern Hellbender. The first is through the introduction of novel pathogens and the second is through mortality events caused by existing pathogens and triggered by additional stressors. While it is difficult to predict the likelihood that existing pathogens will lead to catastrophic losses in the ACUs within the next 25 years, it does seem likely that the introduction of novel pathogens could result in catastrophic losses in one or more ACUs in that time frame because emerging infectious diseases (EIDs), especially fungal EIDs in wildlife, are on the rise and salamanders are especially susceptible given the high magnitude of legal and illegal trade in herpetofauna. Given the long-lived environmental stages of fungi, a novel fungal pathogen could cause mass mortality in Eastern Hellbenders if it is introduced and spread rapidly through the stream environment (as demonstrated by *Batrachochytrium dendrobatidis* (*Bd*)). Additionally, the ability to treat fungal pathogens is limited. Thus an EID could cause catastrophic loss of the species on a broad scale (i.e., the ACU scale).

EIDs have been increasing in large part because of globalization and the increased frequency and rapidity of international travel and trade (McLean 2007, p. 262; Brand 2013, p. 447; Smith et al.

2017, pp. 30–31). Global wildlife trade is a significant contributor and occurs mostly through uncontrolled or illegal networks, and involves millions of birds, mammals, reptiles, amphibians, and fish every year (Karesh et al. 2005, p. 1000; Smith et al. 2017, pp. 30–31). Increasingly, disease is being recognized as a driver of population declines and extinctions (Brand 2013, p. 447; McPhee and Greenwood 2013, p. 6), and amphibians are one of the vertebrate groups most negatively impacted by the introduction of emerging diseases world-wide (Martel et al. 2014; Brunner et al. 2015; Chambouvet et al. 2015; Berger et al. 2016 as in Garcia-Diaz et al. 2016, p. 235). Additionally, the past two decades have seen the arrival of novel fungal diseases that are causing extirpations and extinctions. These diseases are emerging at rates not seen with bacterial or viral-borne diseases and this threat is increasing (Fisher et al. 2012, p. 188). While we have made progress in recognizing disease as a potential driver of extirpations and even extinctions, effective surveillance for EIDs is lacking (Grogan et al. 2014, p. 2; Smith et al. 2017, p. 37). The rapid and data-driven response system for human, livestock, and crop disease does not exist for wildlife disease (Fisher et al. 2012, p. 192; Cunningham et al. 2017, p. 3), and it is very difficult or even impossible to prevent catastrophic losses after an EID has been introduced (Fisher et al. 2012, p. 192).

#### *Batrachochytrium dendrobatidis* (*Bd*)

*Batrachochytrium dendrobatidis* is a fungal pathogen which can cause chytridiomycosis, a highly infectious amphibian disease associated with mass die-offs, population declines and extirpations, and potentially species extinctions on multiple continents (Berger et al. 1998, pp. 9031–9036; Bosch et al. 2001, pp. 331–337; Lips et al. 2006, pp. 3165–3166). *Bd* attacks the keratinized tissue of amphibian skin and can lead to thickened epidermis, lesions, body swelling, lethargy, loss of righting reflex, and death in all life stages (Berger et al. 1998, pp. 9031–9036; Bosch et al. 2001, p. 331; Carey et al. 2003, p. 130). Chytridiomycosis infection rates among amphibians exposed to *Bd* vary by species (Woodhams et al. 2007, p. 4), and resistance to *Bd* infection in some amphibians is likely related to levels of antimicrobial peptides found in skin secretions (Woodhams et al. 2007, p. 4), beneficial skin bacteria (Harris et al. 2006, p. 55), and possibly frequent skin shedding (Woodhams et al. 2007, p. 6). The earliest known record of an infected Eastern Hellbender is from Missouri in 1975 (Bodinof et al. 2011, p. 3).

*Bd* infection on Eastern Hellbenders has been confirmed in every state where testing has occurred (i.e., New York, Pennsylvania, West Virginia, Ohio, Kentucky, Indiana, North Carolina, Tennessee, Georgia, and Missouri) (Greathouse 2007, p. 42; Briggler et al. 2008, p. 444; Burgmeier et al. 2011b, p. 845; Gonynor et al. 2011, pp. 58–59; Regester et al. 2012, p. 20; Roblee 2012, pers. comm.; Souza et al. 2012, p. 562; Williams and Groves 2014, p. 457; Wolfe 2012, pers. comm.). Prevalence rates in streams where *Bd* has been detected have varied from <1% (1 of 230 individuals) in Virginia in 2011–2012 (Eskew et al. 2014, p. 426) to 50% (15 of 30 individuals) in two streams in Tennessee (Nolan et al. 2021, p. 151).

The specific effects of *Bd* infection in wild Eastern Hellbenders are not clear. Hardman et al. (2020a, p. 5) found that Ozark Hellbender toe lesion severity was associated with *Bd* infection. Burgmeier et al. (2011b, p. 845) believed that it was unlikely that chytrid fungus is the major cause of observed Eastern Hellbender declines in Indiana but stated that it would be important to monitor its presence to prevent or mitigate a future outbreak. Nolan et al. (2021, p. 152) did not

observe signs of disease in *Bd*-positive Eastern Hellbenders in Tennessee and Williams and Groves (2014, p. 457) did not note any obvious symptoms in *Bd*-positive Eastern Hellbenders in North Carolina. Bodinof et al. (2011, p. 3) found *Bd* infection in 5.4% of Eastern Hellbenders collected in Missouri between 1896 and 1994. They note that infections were generally light (p. 3) and determined that they lacked the data to determine the role that *Bd* may have played in Eastern Hellbender declines in Missouri (p. 6). However, they noted that even mild chronic *Bd* infections may negatively impact Eastern Hellbenders. As an example, they point out that saprolegniosis, a common and sometimes lethal secondary infection to cutaneous injury or immunocompromised individuals, was present on multiple Eastern Hellbenders infected with *Bd* but was not present on *Bd*-free individuals. They suggest that the co-occurrence of *Saprolegnia* and *Bd* may indicate that *Bd* is more common in immunocompromised Eastern Hellbenders, or that *Bd* infection may increase susceptibility of Eastern Hellbenders to other infection (Bodinof et al. 2011, p. 6). Similarly, Regester et al. (2012, p. 19) point out that *Bd* pathogenicity may interact with numerous other threats. *Bd* has caused disease in captive Ozark Hellbenders at the St. Louis Zoo (Junge 2007, pers. comm.) and mortality in captive raised Eastern Hellbenders in the Allegheny River watershed in New York (Bell 2013, pers.comm.).

While *Bd* currently does not appear to be causing large-scale mortality events in wild populations, there is concern that other stressors that can weaken animals' immune systems, such as environmental contaminants or rising water temperatures, could lead to outbreaks of clinical disease and cause mortality events in the future (Briggler et al. 2007, p. 18; Regester et al. 2012, p. 19). Bales et al. (2015, p. 4) concluded more empirical research is needed to determine the consequences of *Bd* infection in Eastern Hellbenders.

### *Batrachochytrium salamandrivorans*

Another fungal pathogen, *B. salamandrivorans* (*Bsal*), invaded Europe from Asia around 2010 and is responsible for causing mass die-offs of fire salamanders (*Salamandra salamandra*) in northern Europe (Martel et al. 2014, p. 631; Fisher 2017, p. 300–301). Given extensive unregulated trade and the recent discovery of *Bsal* in amphibians, there is concern about the introduction of a novel pathogen causing extirpations of naive salamander populations in North America (Yap et al. 2017, entire). While we still do not have a clear understanding of all the salamander species that will be susceptible to *Bsal*, there is concern that Eastern Hellbenders could be impacted. Gray et al. (2023, pp. 2–3) found that 75% of 35 test species of frogs and salamanders exposed to *Bsal* became infected, and 35% experienced mortality. The degree to which Eastern Hellbenders are susceptible to *Bsal* is unknown. However, Hellbender skin peptides have been shown to reduce growth of *Bsal* in a laboratory setting (Hardman et al. 2023, p. 5), suggesting some possible resistance.

Regions with a high risk of introduction of *Bsal* include portions of the southeastern and northeastern United States (Richgels et al. 2016, p. 5; Yap et al. 2017, pp. 857–858), two regions that comprise a substantial portion of the Eastern Hellbender range. The Appalachian Mountains, a region containing some of the best remaining Eastern Hellbender populations, was identified as a region most likely to have salamander declines from *Bsal* based on environmental suitability and species richness (Richgels et al. 2016, p. 4). Additionally, Grisnik et al. (2023, p. 9) and Moubarak et al. (2022, p. 2326) identified the northeast and southeast portions of the Eastern

Hellbender range as areas with relatively high overall risk of *Bsal* emergence. Since *Bsal* can be transmitted via environmentally-resistant zoospores and encysted spores that can float at the water-air interface (Stegen et al. 2017, pp. 354–355) in addition to direct contact, it is expected to spread readily in stream environments.

Given the high risk of *Bsal* invasion, the USFWS recently listed 20 amphibian genera known to carry *Bsal* as injurious under the Lacey Act to limit importation into the United States (USFWS 2016, entire). Despite this protection, it is possible that an unknown carrier or illegal import could introduce this pathogen into Eastern Hellbender populations. The likelihood of introduction of *Bsal* or another EID remains high because incoming amphibians are not monitored/tested for amphibian diseases; wildlife trade is prevalent and increasing (the number of declared shipments doubling since 2000 (Smith et al. 2017, p. 35)); some of the species that carry *Bsal* could still be entering the country illegally; international wildlife trade is commonly plagued by misidentification of animals and their origins (Gerson 2012, pp. 104, 106); and there are other ways that pathogens and especially fungal pathogens could enter the United States (e.g., some species of toads) could be carriers (Stegen et al. 2017, p. 356)).

### *Ranaviruses*

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

Amphibian larvae seem to be the developmental stage most susceptible to ranaviruses (Daszak et al. 1999, p. 742), with physical characteristics of infections in larval stages including skin hemorrhages, ulcers, and bloating (Marschang and Miller 2012, p. 1). Overt signs of infection may not be exhibited in juvenile and adult stages (Daszak et al. 1999, p. 742), but when present typically include skin abnormalities (e.g., sloughing, hemorrhaging) and sometimes necrosis (tissue death) of digits and limbs (Cunningham et al. 1996, pp. 1539, 1541; Jancovich et al. 1997, p. 163). The exact mechanism by which *Ranavirus* infections cause amphibian mortalities remains unclear, but hemorrhaging in skeletal tissue (Daszak et al. 1999, p. 743) and extensive necrosis in the liver, spleen, kidneys, and digestive tract have been observed in infected individuals (Gray et al. 2009, p. 253). It is also postulated that viral infections may suppress the immune system, resulting in secondary invasion by opportunistic pathogens (Miller et al. 2008, p. 448). In a laboratory experiment, the mortality rate of juvenile Eastern Hellbenders exposed to *Ranavirus* was 80% to 100% (Cusaac et al. 2020, p. 27).

Although broad scale *Ranavirus* outbreaks among Eastern Hellbenders have not been documented, presence of the virus has been detected on individuals. In the French Broad River system in North Carolina, 1 out of nearly 100 Eastern Hellbenders tested positive for *Ranavirus*

although the animal showed no physical signs of infection or illness (Williams and Groves 2012, pers. comm.). Souza et al. (2012, p. 562) found *Ranavirus* DNA in skin swab samples from 40% of 45 individuals captured from the Hiwassee River and Little River systems in Tennessee in 2009. Interestingly, none of the 52 individuals collected in 2010 tested positive for *Ranavirus*, including a recaptured individual that was positive in 2009. These results suggest that Eastern Hellbenders may be able to rid themselves of the virus. Souza et al. (2012, p. 564) did not observe obvious symptoms of *Ranavirus* infection in any of the infected animals. Testing for *Ranavirus* in Eastern Hellbenders in Missouri in 2010 via internal cloacal and buccal swabs, and external swabs of skin hemorrhages and ulcers yielded no positive results (Briggler 2012, pers. comm.). Souza et al. (2012, p. 564) noted that prevalence of the virus in the study was likely underestimated as non-lethal testing procedures can underestimate prevalence compared to testing samples from internal organs (Gray et al. 2012, pp. 3–4). Other factors likely influencing the degree of threat posed by ranaviruses are virulence of the *Ranavirus* strain individuals are exposed to, and if exposed individuals are already compromised by immunosuppression (Gray 2012, pers. comm.). Although acute *Ranavirus* pathogenicity in wild Eastern Hellbenders has not been demonstrated, Hardman et al. (2020b, p. 811) found a negative correlation between Hellbender body condition and *Ranavirus* infection. Additionally, outbreaks causing over 60% mortality have occurred among captive Chinese giant salamanders (*Andrias davidianus*) (Geng et al. 2011, pp. 97–100), a close relative to Eastern Hellbenders. The role that *Ranavirus* may play in declines of Eastern Hellbenders, and the threat it poses to this species, is unclear.

## 5.5 Direct Mortality or Permanent Removal of Animals

Direct mortality or removal of Eastern Hellbenders from a population can be caused by various practices, including collection, persecution, recreation, and gravel mining.

### *Authorized and Unauthorized Collection*

Eastern Hellbenders were historically collected extensively as educational specimens and for the pet trade. Swanson (1948, p. 362) reported that he commercially collected over 750 Eastern Hellbenders from an Allegheny River tributary in Pennsylvania between 1932 and 1948. He notes, “This commercial collecting has apparently diminished their numbers considerably, as they are much more difficult to collect on that stretch of the stream at present.” Originally one could find an individual under almost every suitable rock (Swanson 1948, p. 362). Foster (2018, pp. 32–34) examined historical records and documented that from 1750 to 1940, in the Susquehanna River system and Allegheny River system in New York and Pennsylvania, 3,443 Eastern Hellbenders were captured and killed, commercially collected, collected for scientific use, or held in captivity. Nickerson and Mays (1973, p. 57) report that preserved Eastern Hellbenders sold for \$4 each and live Eastern Hellbenders sold for \$15–35 each from 1969 to 1972. Nickerson and Briggler (2007, p. 208) documented the collection of 558 Ozark Hellbenders from the North Fork of White River between 1969 and 1989, primarily for scientific study (50%) and the pet trade (46%). Those taken for the pet trade were transported to facilities in Michigan, New Jersey, and Japan (Nickerson and Briggler 2007, p. 208). They noted that this number is likely a modest percentage of the actual number of Eastern Hellbenders removed during that time period and attribute the drastic decline of Eastern Hellbenders at removal locations, in part, to such collections for the scientific trade. Collectors were aided in locating

significant populations of Eastern Hellbenders by the technical literature (Nickerson and Briggler 2007, p. 214).

Collection and sale of Eastern Hellbenders continues to be a threat. In 2001, an advertisement in a Buffalo, New York newspaper was selling Eastern Hellbenders for \$50 each (Mayasich et al. 2003, p. 20). In 2003, a pet dealer in Florida posted an internet advertisement that offered “top dollar” for large numbers of Eastern Hellbenders (wanted in groups of at least 100) (Briggler 2007, pers. comm.). In 2010, an internet advertisement solicited purchase of wholesale lots of Eastern Hellbenders (Briggler 2010, pers. comm.). At the 2005 Eastern Hellbender Symposium, it was announced that Eastern Hellbenders collected in the U.S. were found for sale in Japanese pet stores, which is likely the largest market for this species (Briggler, pers. comm. with Okada 2005). In Japan, the majority of Eastern Hellbenders are sought for pets rather than for food (Briggler, pers. comm. with Okada 2005). Roblee (2012, pers. comm.) reports that adult Eastern Hellbenders have been removed from Allegheny River system streams in New York and suspects that this may contribute to the observed reduction in adults in this system.

Given their large size, novel appearance, and relative ease of capture, Eastern Hellbenders are a popular target for nature enthusiasts. Some individuals captured are kept for a personal collection (Briggler et al. 2007, p. 18). Williams (2012, pers. comm.) reports a growing concern with nature enthusiasts capturing and photographing Eastern Hellbenders in North Carolina and credits the growth of social media websites, where posting photos of Eastern Hellbenders is popular, as a contributing factor.

Eastern Hellbenders are also popular research subjects, and many have been collected and permanently removed from streams for research. Merkle et al. (1977, p. 551) collected 105 Eastern Hellbenders from throughout the range for genetics research. Peterson (1985, p. 59) collected 54 Eastern Hellbenders in two tributaries to Missouri River in a study of fecundity. Ingersol et al. (1991, pp. 61, 63) collected 118 Eastern Hellbenders during a study of Eastern Hellbender reproduction from a third Missouri River tributary. The total population size of that stream was estimated to be 400 animals as of 2007 (Briggler et al. 2007, p. 84). Currently, most Eastern Hellbender research is conducted without sacrificing wild-captured individuals.

Even though many Eastern Hellbenders targeted by scientists and nature enthusiasts are returned to the stream, the act of searching for Eastern Hellbenders can result in increased egg and larval mortality. Eastern Hellbenders are typically captured by lifting large shelter rocks and catching individuals by hand. Many researchers have speculated that rock lifting to collect Eastern Hellbenders results in adverse impacts, especially when done during the breeding season. Williams et al. (1981b, p. 26) stated that “Habitat disruption during sampling could be an important consideration, especially during the breeding season. It could result in increased egg and larval mortality, cannibalism or predation.” Lindberg and Soule (1991, p. 8) and Williams (2012, pers. comm.) observed that eggs washed away with the current when nest rocks were disturbed. However, they stated that researchers could replace the eggs under the rock and that the “rebuilt” nests were accepted by the brooding male when one was present. Foster et al. (2008, p. 182) found that several nests in the upper Allegheny River system, discovered by rock turning, were later found to be destroyed. They believed destruction may have been a result of the disturbance caused during the initial discovery. More recently, however, alternative survey

methods are increasingly being used, including nighttime dive lighting surveys (Ortega 2020, entire), use of artificial nest boxes (Bodinof Jachowski et al. 2020, entire) and flexible rods to probe animals from underneath shelter rocks. Lids on the nest boxes can be removed to observe inside, without disturbing eggs or injuring larvae and adults.

Large numbers of Eastern Hellbenders have historically been removed from some streams for scientific and educational purposes, for the pet trade, and for eradication efforts. These removals likely contributed to the population declines seen in some streams. The current rate of permanent removal of Eastern Hellbenders is likely significantly lower than it was historically. However, killing of Eastern Hellbenders by some anglers and the removal of individuals for personal use and the pet trade continues in some areas. As a long-lived species, removing adult Eastern Hellbenders from stream populations may be particularly detrimental, as stable populations of long-lived species typically have high adult survival rates, which compensates for correspondingly low rates of recruitment into the adult populations (Miller 1976, p. 2). Congdon et al. (1994, p. 406) stated that populations of long-lived organisms with low fecundity are severely limited in their ability to respond to chronic increases in mortality of adults. Consequently, the naturally low levels of Eastern Hellbender recruitment may not adequately compensate for increased loss of adults caused by removal of adults from the populations. Pflingsten (1988, p. 16) noted that in Eastern Hellbender populations with low densities and little evidence of recent recruitment into the adult population, the removal of any individuals from a population may be deleterious. Because many Eastern Hellbender populations are already stressed by habitat degradation, compensation for high adult mortality through high recruitment of juveniles is even less likely. Although the magnitude of this threat is not known with certainty, its occurrence is commonly noted by field researchers, suggesting that it is a relatively common occurrence in some portions of the species range. Furthermore, as the number of populations decline and become concentrated on public lands, locations and animals might be easier to find, especially if artificial nest box use increases in the future. Permanent removal of adult Eastern Hellbenders from stream populations remains a threat to the species.

### *Angling and Persecution*

Eastern Hellbenders are often caught on rod and reel, trotline, and by gigging (spearing) (Green 1934, p. 29; Ferguson 1961, p. 392; Nickerson and Mays 1973, p. 56; Green and Pauley 1987, p. 47; Nickerson and Briggler 2007, pp. 209, 212; Foster et al. 2009, p. 586; Nichols 2012, pers. comm.). They have also historically been used as bait for large game fish (Nickerson and Mays 1973, p. 58), and occasionally for human consumption (Swanson 1948, p. 364; Minton 1972, p. 27; Greathouse 2009, pers. comm.). In the past, some Eastern Hellbender populations were targeted for eradication by sportsmen's groups (through night-time gigging) because they were thought to decimate fish populations (despite the fact that they prey predominantly on crayfish) (Green 1934, p. 28; Nickerson and Mays 1973, p. 57). Green (1934, p. 28) remarked "...it is despised by sportsmen and fishermen" and there is misconception among some anglers that Eastern Hellbenders are poisonous (Reese 1903, p. 526; Gentry 1955, p. 169; Green and Pauley 1987, p. 47; Jensen 2009, pers. comm.). Pflingsten (1988, p. 18) stated, "There is almost universal dislike and prejudice against these 'big ugly waterdogs' on the part of fishermen that we talked with [in Ohio]. It seemed clear that they would destroy an animal rather than return it to the stream." However, Unger and Hickman (2020, p. 4) found generally more positive perceptions

of hellbenders in print media after 1980 than before, suggesting that negative attitudes toward the species are less common now than in the past. Improved angler perceptions of Eastern Hellbenders may be due, in part, to outreach efforts like those conducted from 2007 – 2017 by the North Carolina Wildlife Resources Commission and others to engage trout anglers and other stakeholders to help conserve the species (Williams et al. 2019, entire).

Although there is no evidence of current widespread systematic eradication efforts, killing by anglers still occurs in portions of the Eastern Hellbender range (Williams et al. 2019, p. 167). Jensen (2009, pers. comm.;;) states that Eastern Hellbenders are often caught on baited hooks and killed by anglers who mistakenly believe their bite is venomous. Field researchers, especially in the New River system and upper Tennessee River tributary systems, commonly report dead Eastern Hellbenders on stream banks, sometimes multiple individuals at a single location, with evidence of blunt force trauma (Humphries 2005, p. 17; Echternacht 2009, pers. comm.;; Horchler 2010, p. 15; Williams 2011a, pers. comm.). They attribute their death to killing by anglers. Field researchers also report personal communications with anglers who describe killing Eastern Hellbenders they catch (Humphries 2005, p. 45; Lipps 2009b, p. 6; Greathouse 2009, pers. comm.;; Williams 2011a, pers. comm.;; Hopkins 2012, pers. comm.). Some researchers, however, report documentation of intentional safe release of Eastern Hellbenders following accidental capture by hook and line anglers (Williams 2012, pers. comm.). Even so, unintentional mortality following capture on hook and line sometimes occurs, due to trauma from being hooked, fought, and released (Unger et al. 2016, p. 639)

In addition to hook and line angling, there are reports from Missouri of Eastern Hellbenders accidentally killed during frog or fish gigging (Nickerson and Briggler 2007, pp. 209, 212). In Missouri, the opening of fish-gigging season spans the peak of the Eastern Hellbender breeding season when Eastern Hellbenders tend to move greater distances and congregate in small groups, thus making them more susceptible to gigging (Nickerson and Briggler 2007, p. 212).

### *Recreational Activities*

Anthropogenic disturbance in the form of rock-moving by people recreating on rivers is becoming an increasing stressor on hellbenders and can cause mortality. Rocks are moved to construct dams, cairns, tubing shoots, and wading pools. In some streams in North Carolina (Unger et al. 2017, entire, Unger et al. 2020b, pp. 259–260) and in the Missouri River system in Missouri (Nickerson and Mays 1973, p. 56), large shelter rocks are removed to reduce obstructions to recreational canoeing or tubing. In the Licking River system and Kentucky River system in Kentucky, Lipps (2009a, pers. comm.) reports Off Highway Vehicle (OHV) use of streams, which crushes and imbeds Eastern Hellbender rocks. In some streams in North Carolina (Unger et al. 2017, entire) and an Ohio River tributary in West Virginia, shelter rocks are moved to create swimming areas (Greathouse 2011, pers. comm.). Unger et al. (2017, p. N11) speculate that this could be a widespread occurrence in streams across the range of the species, particularly in easy-to-access recreational areas on National Forests and other public lands. This seems likely given visitation to these areas is increasing (USFS 2016, p. 3; NPS 2017). Additionally, collection of boulders, rocks, and cobble for landscaping has been suspected in some areas (Briggler et al. 2007, p. 62), and this activity was captured on a wildlife camera in North Carolina (Williams 2017, pers. comm., Unger et al. 2020b, pp. 258-259). Because large rocks

serve as shelter and nesting habitat for adults and smaller rocks and cobble provide larval and juvenile habitat, moving rocks of any size has the potential to lead to mortality of some life stage. Unger et al. (2017, entire) documented direct mortality to Eastern Hellbenders as a result of shelter rock disturbance.

### *Gravel Mining*

The effects of gravel mining on habitat quality are discussed under *Other Physical Habitat Modifications*. However, direct mortality of Eastern Hellbenders can also occur from instream gravel mining activities. Gravel mining physically disturbs Eastern Hellbender habitat in dredged areas, and dredging equipment can crush and embed cover rocks (Lipps 2009b, p. 8), potentially killing Eastern Hellbenders in the process. The removal of gravel and cobble substrate also reduces larval habitat. Commercial gravel mining is still legal in some states within the Eastern Hellbender range and some state highway departments use gravel from streams. Non-commercial gravel mining is not regulated by the states or U.S. Army Corps of Engineers. Further, the court's decision in *American Mining Congress v. USACE* (D.D.C. 1997) resulted in the deregulation of many gravel mining operations under the Clean Water Act. Gravel mining continues to be a threat to some populations of Eastern Hellbenders, including in the densest remaining known Eastern Hellbender population of the Licking River system in Kentucky (Lipps 2009b, p. 8). Sand and gravel mining are also common activities in other Kentucky streams (Lipps 2009a, pers. comm.).

## **5.6 Other Risk Factors**

### *Climate Change*

Warming of the climate system since the 1950s has been well documented (IPCC 2013, p. 4). As a result, changes in many extreme weather and climate events have been observed since circa 1950 (IPCC 2013, p. 7). The Intergovernmental Panel on Climate Change (IPCC) assigns a likelihood to these events. For example, it is *very likely* that the number of cold days and nights has decreased and the number of warm days and nights has increased on the global scale, and the frequency or intensity of heavy precipitation events has *likely* increased in North America (IPCC 2013, p. 5).

In order to predict future changes to the climate, scientists rely on climate model simulations that use Representative Concentration Pathways (RCPs), which describe four different 21<sup>st</sup> century pathways of greenhouse gas emissions and atmospheric concentrations, air pollutant emissions, and land use (IPCC 2014, p. 57). The four scenarios include a stringent mitigation scenario (RCP2.6), two intermediate scenarios (RCP4.5 and RCP6.0), and one scenario with very high greenhouse gas emissions (RCP8.5) (IPCC 2014, p. 57). The global mean surface temperature change for the period 2016–2035 relative to 1986–2005 is similar for the four RCPs, and will likely be in the range 0.3°C to 0.7°C (medium confidence) (IPCC 2014, p. 58).

Climate change is expected to result in rising average temperatures throughout the range of the Eastern Hellbender, along with more frequent heat waves and increased periods of drought punctuated by intense rainstorms, likely resulting in elevated stream temperature regimes and

lower summer base-flows (Karl et al. 2009, pp. 44, 107, 111–112, 117–118). Higher stream temperatures will result in lower levels of dissolved oxygen, which could negatively impact growth, immune function, survival, and reproductive success. Higher stream temperatures may also reduce the prevalence of *Bd*, which is adapted to cool water. Low base flows may reduce available instream habitat and increased flashiness from more intense rain events may increase down-cutting and substrate instability. Low base flows may also increase the risk of predation by terrestrial predators. Increased flashiness may also cause greater variability in stream temperatures, which is known to negatively impact immune function in other salamander species (Raffel et al. 2006, pp. 823–826). An increase in summer water temperatures has also been shown to reduce immune function and cause loss of body mass leading into the breeding season, which may affect reproductive success (Terrell et al. 2021, pp. 7, 9).

Furthermore, migration of Eastern Hellbenders as an adaptation to climate change is unlikely, due to their limited mobility, high site fidelity, restriction to defined stream systems, and the extensive network of impoundments throughout their range. Sutton et al. (2023, p. 11) modelled climate suitability in the OACU, TACU, and KACU in 2050 and 2070, and predicted relatively stable average climatic suitability in the OACU (1.6% decrease) to an average decrease of 5.8% in the TACU. According to the NatureServe Climate Change Vulnerability Index, release 2.1 (<http://www.natureserve.org/prodServices/climatechange/ccvi.jsp>), the Eastern Hellbender is highly vulnerable to climate change.

#### *Small Populations, Population Fragmentation and Isolation*

Many Eastern Hellbender populations are small and isolated from one another by impoundments and large reaches of unsuitable habitat. This isolation restricts movement among populations and precludes natural recolonization from source populations (Dodd 1997, p. 178; Benstead et al. 1999, pp. 662–664; Poff and Hart 2002, p. 660). As a result, recolonization may be unavailable as a buffer to counter local extirpations caused by environmental perturbations (e.g., acute pollution event), demographic stochasticity, or human harvest or persecution (Lande 1988, p. 1458). Risk of local extirpations is further exacerbated by lack of genetic flow, and genetic drift can lead to over-representation of deleterious alleles which could impact viability of populations. Individuals in small populations are more likely to suffer from decreased fitness (ability to produce viable offspring) as inbreeding among close relatives occurs, resulting in greater expression of deleterious recessive genes (Allendorf and Luikart 2007, pp. 306, 315). With small populations, genetic drift (random change in gene frequencies) is also more likely to result in reduced genetic diversity, which may cause the loss of genes that help allow populations to adapt to environmental change. These factors can increase the likelihood of extirpation (Allendorf and Luikart 2007, p. 355).

#### *Increased Abundance of Predators*

Some native predators of the Eastern Hellbender, such as raccoons, have increased in abundance due to anthropogenic influences (e.g., elimination of top predators, reduction in trapping, habitat changes), while others have recently been reintroduced into hellbender streams (e.g., river otters). Though research is limited on the relationship between Eastern Hellbenders and the increased abundance of predators, predation by river otters, mink, raccoons, and bald eagles is

suspected to be particularly intense during periods of drought when water levels are low (Briggler et al. 2007, p. 17, Williams 2024, pers. comm.). Nissen et al. (2023, p. 10) documented significant predation by river otters in a translocated population of Eastern Hellbenders.

Non-native predators are also present within a large portion of the Eastern Hellbender range and include predatory fish stocked for recreation, such as rainbow trout (*Oncorhynchus mykiss*) (Unger et al. 2021b, p. 817) and brown trout (*Salmo trutta*) and are often stocked at a time when Eastern Hellbender larvae are leaving the protection of the nest. Stocking of these species is widespread throughout much of the range of the Eastern Hellbender, and optimal habitat for trout and Eastern Hellbenders often overlap (i.e., cool, clear, high-gradient streams with coarse substrates). Non-native trout species are thought to directly impact Eastern Hellbenders by preying on eggs, larvae, sub-adults, and adults and by impacting hellbenders indirectly through competition for resources. Though the exact effects of non-native trout on Eastern Hellbender populations are unclear, research suggests that non-native salmonids may present a higher predation risk to larval Eastern Hellbenders than do native fishes because larvae may not recognize non-native trout as predators (Gall and Mathis 2010, pp. 51–54).

### *Invasive Species*

Invasive species are a significant threat to amphibians worldwide. Approximately one-third of amphibian extinctions have been caused by invasive species (Falaschi et al. 2020, p. 216). Invasive species impact amphibians through predation, competition, hybridization, habitat alteration, and spread of disease (Falaschi et al. 2020, p. 216). The Rusty Crayfish (*Faxonius rusticus*) has been identified as an invasive species that is a potential threat to Eastern Hellbenders.

Rusty Crayfish are a stream-dwelling crayfish native to Western Ohio, Indiana, Kentucky, and Northern Tennessee. They are also found outside of their native range in 12 of 14 states within the range of the Eastern Hellbender (all of Indiana is in the native range of Rusty Crayfish) (Durland et al. 2024, p. 1). Angler bait bucket release is thought to be the main cause of introduction and spread throughout its non-native range (Durland et al. 2024, p. 1). Rusty Crayfish are often larger and more aggressive than native crayfishes and can outcompete them. Quin et al. (2013, p. 83) found that rusty crayfish far outnumbered native crayfish in their study streams in the Susquehanna River system in New York, and suspected that they may prey on hellbender eggs and larvae, and may be unpalatable to adults. Hartsell et al. (2022, pp. 25–26), however found that in a laboratory setting, Eastern Hellbenders ate both Rusty Crayfish and native crayfish, and Rusty Crayfish did not displace Eastern Hellbenders from shelter rocks. The overall effect of Rusty Crayfish on Eastern Hellbenders is unclear.

### *Synergistic Effects*

In some instances, effects from one threat may increase effects of another threat, resulting in what is referred to as synergistic effects. Synergistic effects have been well documented in amphibians and, in the presence of one or more stressors, often include an increased susceptibility to predation (Moore and Townsend 1998, pp. 332–333), disease (Kiesecker and Blaustein 1995, pp. 11050–11051; Taylor et al. 1999, pp. 539–540), or parasites (Kiesecker

2002, pp. 9902–9903; Gendron et al. 2003, pp. 472–473). One mechanism by which this synergism can occur is through modification or suppression of normal immune functions (Gilbertson et al. 2003, pp. 104–107). Two stressors that have been demonstrated to modify immune response in amphibians and are relevant to Eastern Hellbenders are pesticides (Christin et al. 2003, pp. 1129–1130; Rohr et al. 2008, pp. 1236–1237) and heavy metals (Goulet and Hontela 2003, pp. 2108–2111). Cusaac et al. (2020, p. 27) found that Eastern Hellbender mortality was greater in animals exposed to both *Ranavirus* and the pesticide glyphosate than in hellbenders exposed to *Ranavirus* alone. Additionally, because chronic, increased levels of stress hormones have been shown to inhibit immune response (Rollins-Smith and Blair 1993, pp. 156–159; Romero and Butler 2007, pp. 93–94), while other stressors present in the Eastern Hellbender’s environment (e.g., habitat modification, degraded water quality, non-native predators, electrofishing) could also reduce immune response and thereby increase vulnerability to disease and parasites. Boerner (2014, p. 26) hypothesized that Bd infection in captive bred and released hellbenders decreased their ability to absorb oxygen through their skin, thereby causing them to move closer to shore to absorb air into their lungs, which increased their vulnerability to terrestrial predators like racoons.

## **Conservation Efforts**

Hellbender conservation efforts occur in every state in the range, but these efforts vary widely by state. Some states have developed and follow conservation plans specific to the Eastern Hellbender, including Ohio, Indiana, Missouri, and New York. Other states conduct conservation at a more case-by-case level.

### **5.7 Habitat Restoration, Management, and Preservation**

Stream habitat restoration includes streambank stabilization, natural channel restoration, riparian buffer plantings, livestock exclusion, dam removal, and rock shelter placement. Habitat improvements help reduce the species’ stressors related to sedimentation, water quality, and habitat degradation and fragmentation.

Habitat management includes placement of artificial nest boxes in streams to provide additional nesting habitat and cover for adult hellbenders. Artificial nest boxes have been successfully used by hellbenders in Ohio, West Virginia, Missouri, Virginia, and New York for reproduction. However, the survival of fertilized eggs and larvae from these nest boxes is unknown. In addition to providing nesting habitat, boxes may allow for improved research on reproduction and early life stages, since they include a lid which gives access inside the nest. However, because nest boxes may present a curiosity to stream recreationists, hellbenders occupying the nests are susceptible to disturbance, persecution, and collection if the nest boxes are not properly camouflaged.

Habitat preservation includes land acquisition and land protection instruments (e.g., conservation easements) in stream riparian zones and the broader watershed. Although there is no central database of stream restoration and preservation activities for the range of the Eastern Hellbender, stream habitat restoration and preservation occur in every state in the range. However, because stream habitats generally flow through multiple land ownership parcels and stream quality is

affected by land-uses throughout the entire watershed, individual stream restoration and preservation actions have only a limited benefit to hellbenders. Activities are most beneficial where they occur throughout the watershed, as in preservation and management of large areas of Federal and State-owned land. The overall benefit of habitat restoration, management, and preservation range-wide is unknown.

## **5.8 Captive Propagation**

Captive rearing is a strategy used to increase the survival rate of young individuals. Captive propagation efforts for the Eastern Hellbender have increased in recent years and either have occurred or are occurring in Missouri, Indiana, Ohio, Kentucky, West Virginia, Tennessee, and New York (Briggler 2017, pers. comm.; Greathouse 2015, pp. 2–3; Lipps 2018, pers. comm.; McGinnity 2017, pers. comm.; Krauss et al. 2017, entire). Efforts have focused on collecting fertilized eggs from the wild and head-starting young by raising them in captivity to 2–4 years of age. Once reared, young are released into the wild to augment existing populations or reintroduced into areas in which the species has been extirpated. In Ohio, for example, there have been 1,837 head-started individuals (mostly 3-year old animals) released at 27 sites in 10 waterways from 2012–2023. As of March 2024, there are 1,026 juveniles being reared for release in Ohio. (Lipps 2024, pers. comm.). Additionally, translocation of adult hellbenders from sustainable populations to declining or extirpated populations can also increase population sizes in these streams (Nissen et al. 2023, pp. 19-20). Though success of Eastern Hellbender translocations is still being studied (Boerner 2014, entire; Kraus et al. 2017, p. 275; McCallen et al. 2018, entire; Kocher 2019, entire; Burgmeier et al. 2022, entire), captive propagation has the potential to increase the number of reproductive adults in streams with low densities or where the Eastern Hellbender is extirpated (Kaunert et al. 2023, p. 12). Although released individuals have been observed guarding fertilized eggs and larvae, we have no data on the survival rates of offspring.

## **5.9 Other Beneficial Factors**

### *Monitoring and Research*

Since 2003, hellbender researchers and managers throughout the range have met biannually to discuss and collaborate on hellbender research and conservation. Since then, and more recently with the advent of eDNA surveys, hellbender monitoring and research has expanded greatly, including presence/absence surveys, health assessments and disease monitoring, and research on temporal trends, life history, genetics, land-use effects, and captive propagation. Purdue University maintains the most comprehensive up-to-date list of hellbender research publications (<https://ag.purdue.edu/departments/extension/hellbender/publications.html>). Of the approximately 200 hellbender-specific publications listed (dating back to 1812), more than half have been published since 2003.

### *Public Outreach*

Public outreach occurs throughout portions of the Eastern Hellbender range. Examples include Purdue University College of Agriculture’s “Help the Hellbender” website, North Carolina

Zoological Society's hellbender costume ("Snotty the Snot Otter"), North Carolina Wildlife Resources Commission's captive-raised, ambassador hellbender, "Rocky", as well as informational signs on the National Forest in North Carolina. Additionally, in North Carolina, Eastern Hellbenders are featured in the state's fishing regulations booklet, magazines, posters, pamphlets, etc. (Williams et al. 2019, entire). Purdue's outreach program has shown at least some effectiveness in changing the attitudes of landowners along Blue River in Indiana (Mullendore et al. 2014, pp. 172–175). However, public outreach in itself does not mitigate the often complex stressors of sedimentation, habitat degradation, disease, etc.

### *State and Federal Laws*

All states within the range of the Eastern Hellbender have enacted legislation to protect rare or non-game animals, including the Eastern Hellbender. In some states, these laws prohibit killing, sale, and/or possession of any Eastern Hellbenders (e.g., Ohio, Indiana, Missouri, New York).

It is also unlawful under section 3372(a)(2)(A) of the Lacey Act Amendments of 1981 (16 U.S.C. 3371–3378) to import, export, transport, sell, receive, acquire, or purchase in interstate or foreign commerce any wildlife taken, possessed, transported, or sold in violation of any law or regulation of any State. Because sale of Eastern Hellbenders is illegal in all states within the species' range, interstate or international sale of Eastern Hellbenders collected in those states is prohibited by the Lacey Act.

In addition, the Eastern Hellbender is listed on Appendix III of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). CITES is an international agreement among governments with the purpose of ensuring that international trade in wild animals and plants does not threaten their survival. Appendix III includes native species that at least one Party country (i.e., a country that is part of CITES) has identified as requiring regulation to prevent or restrict exploitation. Under Appendix III, that Party country requests the help of other Parties to monitor and control the trade of that species.

## Chapter 6. Analysis of Future Condition

Below we describe the forecasted future condition of the Eastern Hellbender given the spectrum of future influences over the next 10 and 25 years.

### 6.1 Future Influences

Species experts ranked the influences affecting Eastern Hellbender populations over the next 10 and 25 years and assigned a relative weight to each stressor (Table 6.1). Conservation measures such as augmentation were not included in future scenarios because their long-term efficacy is unknown. Rangewide, sedimentation had the highest average ranking at both 10 years and 25 years. Other highly ranked stressors at 10 years and 25 years included water quality degradation, climate change, and small population effects. Climate change moved up two places in ranking from year 10 to year 25 and its relative weight score changed from 59 to 80 (Table 6.1).

In the OACU, sedimentation was the highest ranked influence, followed by water quality degradation, climate change, and small population sizes (Table 6.1). Relative weight scores for sedimentation and water quality degradation were above 80 at year 10 and scores for sedimentation, water quality degradation, and climate change were above 80 at year 25. One expert in the Tennessee portion of the OACU stated, “Habitats in which we know hellbenders still exist have declined in quality and population status over the past three decades. There is no evidence that these habitats will improve in the near future” (Sutton 2024, pers. comm.). An expert in Kentucky stated, “Sedimentation and habitat destruction is likely to affect Eastern Hellbender over the next 25 years” (Hayes 2024, pers. comm.). An expert in Ohio stated “The effects of climate change, particularly precipitation patterns, and - even more important - human caused changes to the landscape in response - is likely to increasingly threaten habitats and populations, particularly in Ohio's smaller waterways” (Lipps 2024, pers. comm.).

In the TACU, sedimentation was the highest ranked influence, followed by water quality degradation, climate change, and small population sizes (Table 6.1). Relative weight scores for sedimentation and water quality degradation were above 80 at year 10 and scores for sedimentation, water quality degradation, and climate change were above 80 at year 25. An expert in the North Carolina portion of the TACU noted that “We continue to see more deforestation in watersheds, an increasing amount of impervious surfaces, and more water quality and sedimentation issues with storm water. Those threats will only grow in magnitude in the future” (Williams 2024, pers. comm.). A Tennessee expert noted, however, that “My best case scenario populations that will remain in SR or UR status are those that have watersheds contained in protected areas such as National Forests or National Parks” (Sutton 2024, pers. comm.).

In the KACU, sedimentation was the highest ranked influence, followed by water quality degradation, climate change, and destruction of habitat (Table 6.1). Relative weight scores for sedimentation and water quality degradation were above 80 at year 10 and scores for sedimentation, water quality degradation, and climate change were above 80 at year 25. An expert in the North Carolina portion of the KACU noted that “Threats are becoming magnified as more of western North Carolina gets developed, primarily for vacation (or 2nd/3rd+) homes

and to build short-term rentals (Air BnB, VRBO, tiny houses, other rental properties), and with those high-end developments come more roads, more driveways, more infrastructure, etc.” (Williams 2024, pers. comm.). An expert in West Virginia, however, stated, “Best case scenarios are that strong populations, particularly those located within National Forest boundaries, continue to maintain and perhaps even increase with growing populations” (Greathouse 2024, pers. comm.).

In the MACU, sedimentation was the highest influence, followed by disease and pathogens, climate change, and small population sizes (Table 6.1). Relative weight scores for sedimentation and disease and pathogens were above 80 at year 10 and scores for sedimentation, disease and pathogens, and climate change were above 80 at year 25. The expert in the MACU stated, “...Eastern Hellbender populations in Missouri are expected to continue to decline into the future due to impacts from threats, particularly sedimentation, amphibian chytrid fungus, extreme flooding, non-native species (primarily stocked trout), and illegal collection. From 2034 to 2050, sedimentation will likely continue to increase, as will the frequency and severity of severe flood events which can mobilize large rocks and also degrade habitat due to fine and coarse sedimentation. With ongoing population declines, effects from small populations size and isolated habitat patches (due to a lower number of occupied sites) will likely increase” (Briggler 2024, pers. comm.).

Table 6.1. Expert forecasted rankings and relative weights of influences, 10 years and 25 years into the future. Experts assigned 100 points to their top (1<sup>st</sup>) ranked influence. Then, they assigned relative weights to the remaining influences based on each one's influence relative to the top rank. For example, a stressor that is half as influential as the top ranked stressor is assigned 50 points.

	Sedimentation		Climate Change		Direct Mortality Permanent Removal		Water Quality Degradation		Over-abundance of Predators		Disease and Pathogens		Habitat Disturbance (e.g., Rock Moving)		Invasive Species		Small Population Effects Isolation		Destruction of Habitat	
	Rank	Rel. Wgt.	Rank	Rel. Wgt.	Rank	Rel. Wgt.	Rank	Rel. Wgt.	Rank	Rel. Wgt.	Rank	Rel. Wgt.	Rank	Rel. Wgt.	Rank	Rel. Wgt.	Rank	Rel. Wgt.	Rank	Rel. Wgt.
	<b>10 years</b>																			
Average Rangewide	1	96	5	59	7	29	3	84	6	43	6	42	7	37	8	22	4	64	5	61
Average OACU	2	93	5	60	8	21	3	86	6	44	6	46	6	38	8	26	4	65	4	73
Average TACU	1	100	6	48	7	37	3	94	6	43	7	32	7	47	9	14	4	68	5	69
Average KACU	1	99	3	74	6	38	2	82	6	50	7	30	8	22	7	15	4	52	8	25
Average MACU	1	100	3	75	6	30	8	8	7	18	2	93	10	6	5	50	4	70	9	7
	<b>25 Years</b>																			
Average Rangewide	2	97	3	80	7	30	3	83	6	42	6	48	7	37	7	32	4	66	5	63
Average OACU	2	94	3	81	8	20	3	85	7	39	5	52	7	35	8	30	4	67	4	72
Average TACU	1	100	4	69	6	40	3	94	7	44	7	41	7	50	8	28	4	69	5	73
Average KACU	1	99	1	98	5	48	2	83	6	55	7	40	8	28	5	43	4	53	7	35
Average MACU	1	100	2	85	7	20	8	5	6	25	3	80	10	3	5	45	4	75	9	4

## 6.2 Forecasted number, health, and distribution of populations

To assess the future status and trend for all 626 populations, we asked the experts to predict the status and trends (SR, UR, D, FX, or PX) of Eastern Hellbender populations in their geographic area of expertise at years 10 and 25, under the RBP, ML, and RWP scenarios. Projections varied among the three scenarios and among ACUs. Under the RBP scenario, 80 SR populations and 87 UR are expected to persist range wide at year 25 (Table 6.2, Figure 6.1). Twenty-four SR populations and 49 UR populations are expected to persist at year 25 under the RWP scenario. From year 10 to year 25, there is a decrease in the number of SR and UR populations in all three scenarios. There is also an increase in the number of extirpated populations from year 10 to year 25 in all three scenarios (Table 6.2, Figure 6.1). At year 25, 64% of historical populations are extirpated under the RWP scenario and 42% are extirpated under the RBP scenario (Figure 6.1).

In the OACU, under the RBP scenario, 28 SR populations and 29 UR populations are projected to persist at year 25. Only two SR populations and two UR populations are projected to persist at year 25 under the RWP scenario. (Table 6.2, Figure 6.1). From year 10 to year 25, there is a decrease in the number of the total number of SR and UR in the RWP scenario from 12 to 4, while the number of these populations in the RBP scenario remains unchanged. There is an increase in the number of extirpated populations from year 10 to year 25 in all three scenarios (Table 6.2, Figure 6.1). At year 25, 82% of historical populations are extirpated under the RWP scenario and 47% are extirpated under the RBP scenario (Figure 6.1).

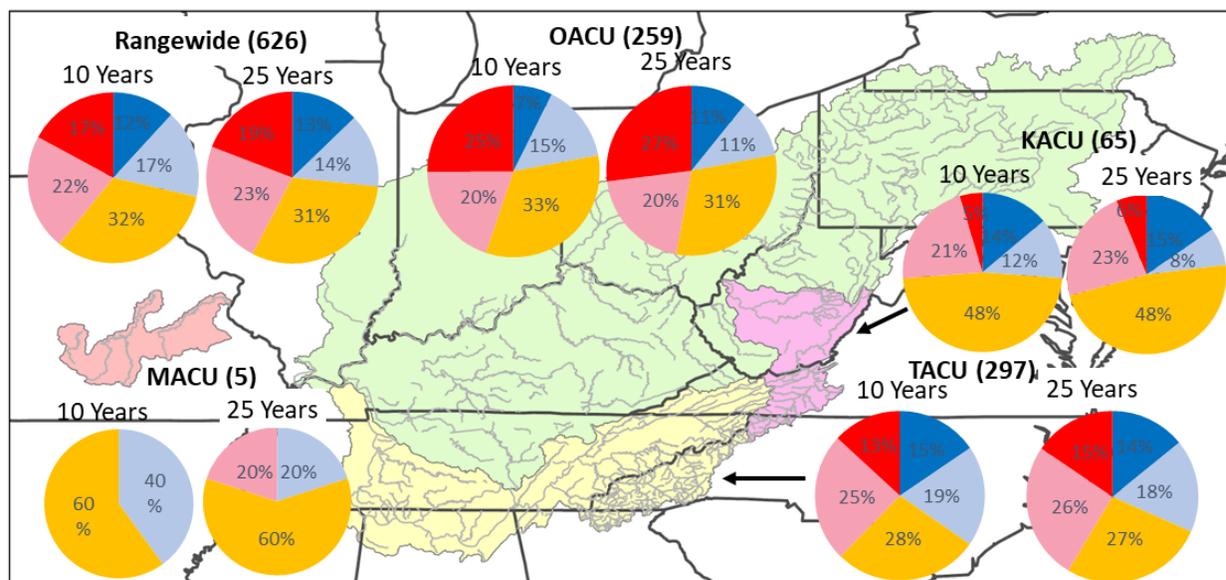
In the TACU, 42 SR and 52 UR populations persist under the RBP scenario at year 25. Under the RWP scenario, 20 SR and 45 UR populations persist at year 25 (Table 6.2, Figure 6.1). From year 10 to year 25, the number of SR and R populations decreases by three under the RWP scenario and by nine under the RBP scenario. There is an increase of 11 extirpated populations in both the RWP and RBP scenarios from year 10 to year 25 (Table 6.2, Figure 6.1). At year 25, 50% of historical populations are extirpated under the RWP scenario and 41% are extirpated under the RBP scenario (Figure 6.1).

In the KACU, at year 25, 10 SR and 5 UR populations are expected to persist under the RBP scenario. Under the RWP scenario, only two SR populations and two UR populations are expected to persist at year 25 (Table 6.2, Figure 6.1). From year 10 to year 25, the number of SR and UR populations decreases by two in both the RWP and RBP scenarios. There is an increase of six extirpated populations from year 10 to year 25 in the RWP scenario and an increase of two in the RBP scenario. At year 25, 57% of historical populations are extirpated under the RWP scenario and 29% are extirpated under the RBP scenario (Figure 6.1).

In the MACU, no SR and one UR population is projected to persist at year 25 under the RBP scenario. Under the RWP scenario, no SR or UR populations are projected to persist at year 25 (Table 6.2, Figure 6.1). From year 10 to year 25, one declining population becomes extirpated under the RWP scenario, while in the RBP scenario, one declining population is extirpated and one UR population becomes D (Table 6.2, Figure 6.1).

Table 6.2. The predicted number of stable recruiting (SR), recruiting with unknown trend (UR), declining (D), and functionally or presumed extirpated (FX and PX) populations, range wide and by ACU, at 10 and 25 years in the future. The predictions include all historical populations. The predicted number is given for the reasonable best plausible, most likely, and reasonable worst plausible scenarios.

	Reasonable Best					Most Likely					Reasonable Worst				
	SR	UR	D	FX	PX	SR	UR	D	FX	PX	SR	UR	D	FX	PX
<b>Range Wide</b>															
10 Years	74	105	202	138	107	56	79	219	136	136	29	57	170	180	190
25 Years	80	87	194	145	120	52	73	205	123	173	24	49	152	188	213
<b>OACU</b>															
10 Years	19	38	86	51	65	11	21	104	43	80	4	8	47	79	121
25 Years	28	29	80	52	70	9	19	95	30	106	2	2	42	83	130
<b>TACU</b>															
10 Years	46	57	82	73	39	39	51	83	79	45	22	46	92	87	50
25 Years	42	52	80	77	46	37	48	80	77	55	20	45	84	88	60
<b>KACU</b>															
10 Years	9	8	31	14	3	6	6	29	13	11	3	3	28	12	19
25 Years	10	5	31	15	4	6	5	28	15	11	2	2	24	15	22
<b>MACU</b>															
10 Years	0	2	3	0	0	0	1	3	1	0	0	0	3	2	0
25 Years	0	1	3	1	0	0	1	2	1	1	0	0	2	2	1



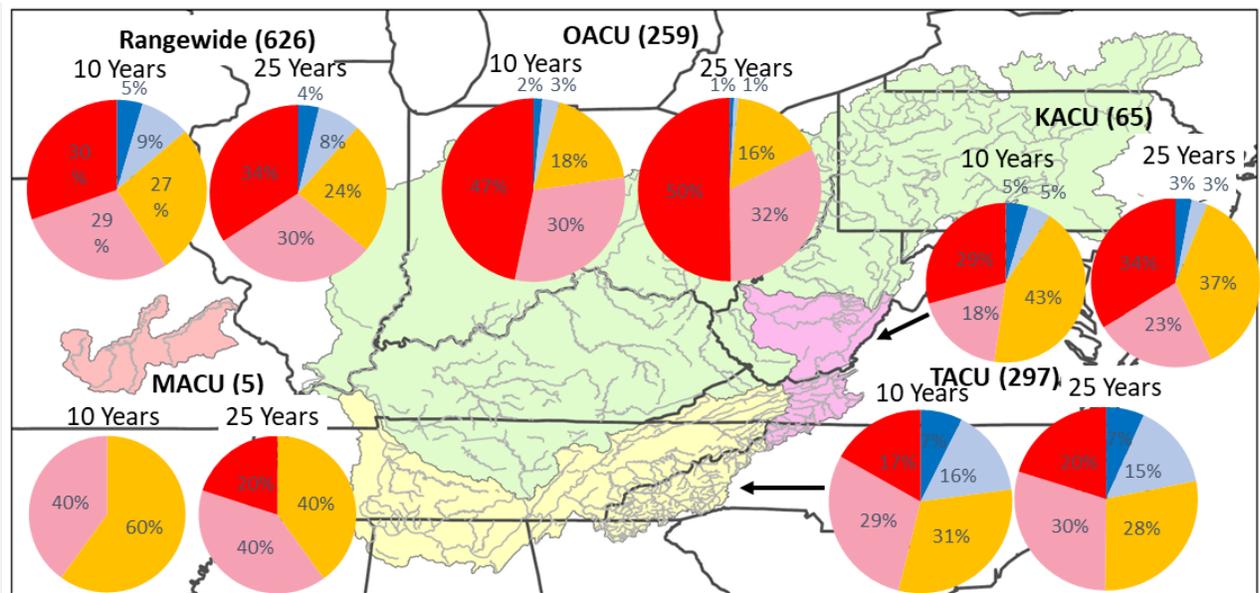
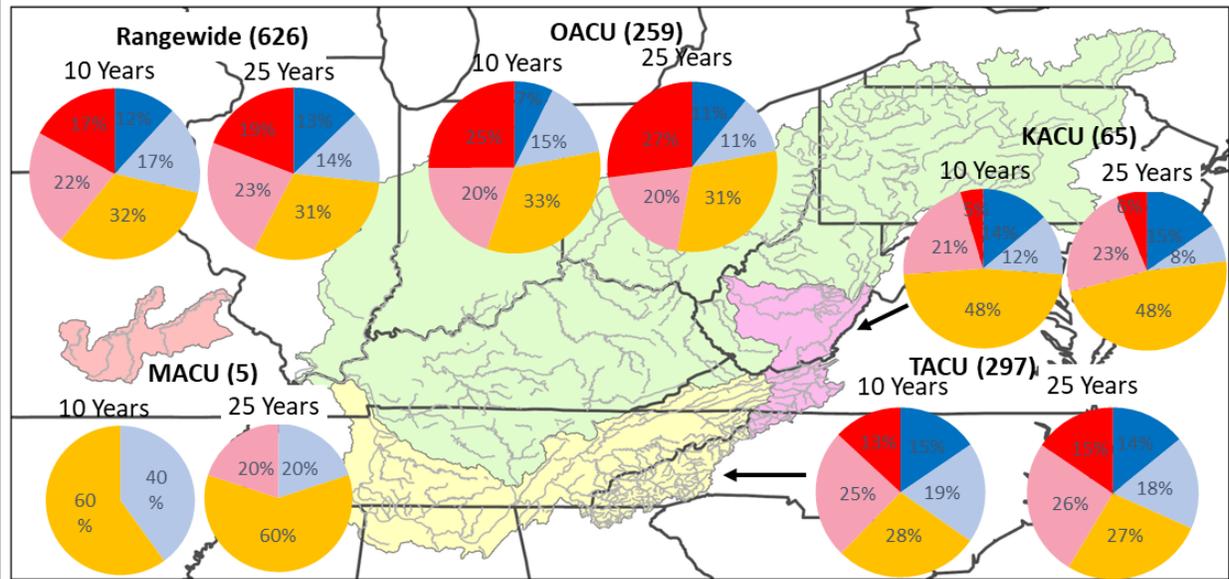


Figure 6.1. Predicted reasonable best plausible (top), most likely (middle) and reasonable worst plausible (bottom) status and trends at 10 and 25 years. Dark blue = SR (stable recruiting), light blue = UR (recruiting, trend unknown), orange = D (declining), light red = FX (functionally extirpated), and dark red = PX (presumed extirpated). Number of populations is indicated in parentheses.

## Chapter 7. Synthesis

This Chapter synthesizes the results from our historical, current, and future analyses and discusses the consequences for the future viability of the Eastern Hellbender. We first compile the changes in the number, health, and distribution of Eastern Hellbender populations over time given predictions about future influences. We then describe the consequences of these changes to the viability of Eastern Hellbender into the future and evaluate the likelihood of catastrophes causing ACU-wide extirpations.

### 7.1 Trends over time

#### *Rangewide trend in the number, health, and distribution of populations*

There has been a decline in the number of extant populations over time (Figure 7.1). Historically, 626 healthy Eastern Hellbender populations are known to have existed across 15 states. Currently, 255 populations (41%) are FX or PX, and another 218 populations (35%) are D. The reasons for the declines are multi-factored and vary by ACU, but the primary drivers are sedimentation (via development of forest land adjacent to streams, silviculture, row crops, and livestock entering streams), water quality degradation, habitat destruction (via flooding, gravel mining, impoundments), disease, direct mortality (via illegal collection, persecution), climate change, and small population effects. Of the 371 currently extant populations across the range, 45 are SR (12%), 108 (29%) are UR, and 218 (59%) are D (Table 7.1).

Population declines are expected to continue over the next 25 years under both the RWP and RBP scenarios. Under these scenarios, there is a projected 3%–40% decrease in the number of extant populations over the current condition (Figure 7.1), representing extirpation of 42%–64% of the total number of historical populations within the next 25 years. Future projections for the number of SR and UR range from an increase of 9% (14 more than current) to a decrease of 52% (80 fewer than current) over the next 25 years (Table 7.1, Figure 7.1).

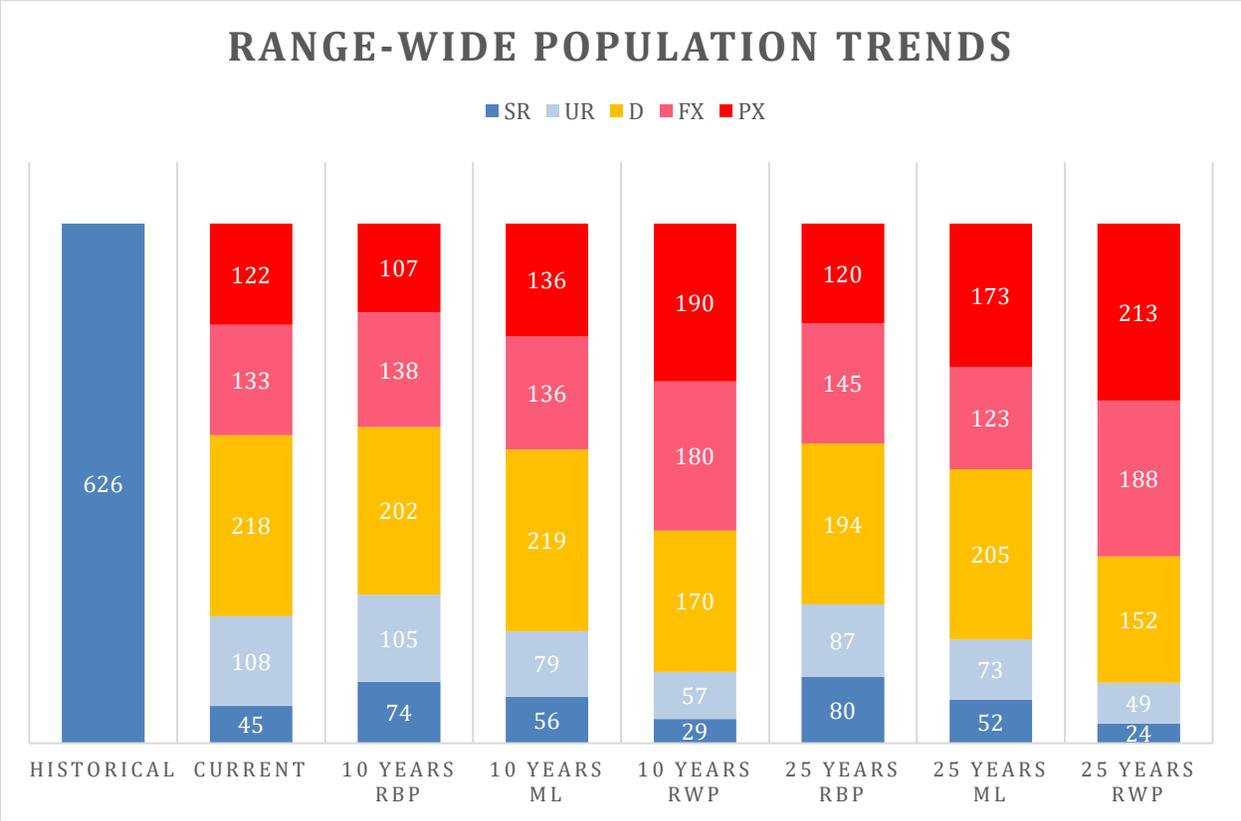


Figure 7.1. The range wide number of SR, UR, D, FX and PX over time under the reasonable best case (RBP), most likely (ML), and reasonable worst case (RWP) scenarios.

Table 7.1. Number of stable recruiting (SR), recruiting with unknown trend (UR), declining (D), functionally extirpated (FX) and presumed extirpated (PX). The predicted number is given for the reasonable best-case, most likely, and reasonable worst-case scenarios..

	Reasonable Best					Most Likely					Reasonable Worst				
	SR	UR	D	FX	PX	SR	UR	D	FX	PX	SR	UR	D	FX	PX
<b>Range Wide</b>															
Historical	626	0	0	0	0	626	0	0	0	0	626	0	0	0	0
Current	45	108	218	133	122	45	108	218	133	122	45	108	218	133	122
10 Years	74	105	202	138	107	56	79	219	136	136	29	57	170	180	190
25 Years	80	87	194	145	120	52	73	205	123	173	24	49	152	188	213
<b>OACU</b>															
Historical	259	0	0	0	0	259	0	0	0	0	259	0	0	0	0
Current	12	25	101	45	76	12	25	101	45	76	12	25	101	45	76
10 Years	19	38	86	51	65	11	21	104	43	80	4	8	47	79	121
25 Years	28	29	80	52	70	9	19	95	30	106	2	2	42	83	130
<b>TACU</b>															
Historical	297	0	0	0	0	297	0	0	0	0	297	0	0	0	0
Current	26	74	82	73	42	26	74	82	73	42	26	74	82	73	42
10 Years	46	57	82	73	39	39	51	83	79	45	22	46	92	87	50
25 Years	42	52	80	77	46	37	48	80	77	55	20	45	84	88	60
<b>KACU</b>															
Historical	65	0	0	0	0	65	0	0	0	0	65	0	0	0	0
Current	7	9	30	15	4	7	9	30	15	4	7	9	30	15	4
10 Years	9	8	31	14	3	6	6	29	13	11	3	3	28	12	19
25 Years	10	5	31	15	4	6	5	28	15	11	2	2	24	15	22
<b>MACU</b>															
Historical	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0
Current	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0
10 Years	0	2	3	0	0	0	1	3	1	0	0	0	3	2	0
25 Years	0	1	3	1	0	0	1	2	1	1	0	0	2	2	1

## 7.2 Viability

Eastern Hellbender viability is dependent on its ability to withstand environmental stochasticity, periodic disturbances, anthropogenic stressors, catastrophes, and novel changes to its environment (see Chapter 2 for additional discussion). This ability requires multiple healthy populations widely distributed across spatially heterogeneous conditions and across the breadth of adaptive diversity. Healthy populations have positive population growth rates, stable age structure, and large population sizes (Chapter 2). The breadth of adaptive diversity—ecological and genetic diversity—is preserved by ensuring population persistence within the four distinct phylogenetic lineages (i.e., ACUs, Chapter 2).

### *Ohio River- Susquehanna River ACU (OACU)*

Of the 259 historically documented populations, 12 (5%) are currently SR, 25 are UR (10%), 121 (47%) are FX or PX, and 101 (39%) are D (Table 7.2, Figure 7.2). The most important influences affecting Eastern Hellbender's current and future status and trends in the OACU are sedimentation, water quality degradation, small population sizes, and climate change (refer to Table 5.2 and 6.1). Considering these influences, future projections indicate that the OACU will have between 46 and 137 extant populations (0.7%–67% decrease from current) and between 4 and 57 SR and UR populations (54% increase to 89% decrease from current) by year 25 (Table 7.1, Figure 7.2). The number of SR populations is expected to remain below historical conditions under both scenarios (90%–99% loss) (Table 7.1, Figure 7.2). The forecasted number of FX and PX populations by year 25 ranges from 122 to 213, representing 47%–82% of all historical populations in the OACU.

The OACU is geographically large, with 138 occupied and widely dispersed streams across 9 states (i.e., high redundancy), making it less vulnerable to catastrophic events than other ACUs. Given the current and predicted future geographic spread of populations within the OACU, disease is the only reasonably foreseeable catastrophic event. The OACU is at moderate risk of introduction of *Bsal* because of proximity to areas with high levels of imports (Atlanta and New York) and high numbers of pet trade establishments (Richgels et al. 2016, p. 5). It is assumed that risk would be moderate in these areas for other introduced EIDs as well. Although the OACU falls within the high suitability region for *Bsal*, the number and spatial extent of populations under the RBP scenario likely provide sufficient redundancy to protect against ACU-wide extirpation over the next 25 years (Table 7.2). However, under the RWP scenario, the number and spatial extent of populations are predicted to decline substantially, and thus, the risk of ACU-wide extirpation is likely (Table 7.2).

Given the current and projected loss of healthy populations, Eastern Hellbender resiliency in the OACU is substantially lower than historical conditions and may remain so in the future. Under the RBP scenario the current and projected geographic spread of populations is such that we expect 57 SR and UR populations to persist across spatially heterogeneous environmental conditions and a diversity of stream orders and temperature regimes. Under the RWP scenario, however, only four SR and UR populations are expected to persist, resulting in greatly reduced ability of Eastern Hellbenders in the OACU to withstand normal environmental variation, periodic disturbances, and catastrophes. OACU-wide extirpation is likely within the next 25 years due to the threat from a disease epidemic under the RWP scenario.

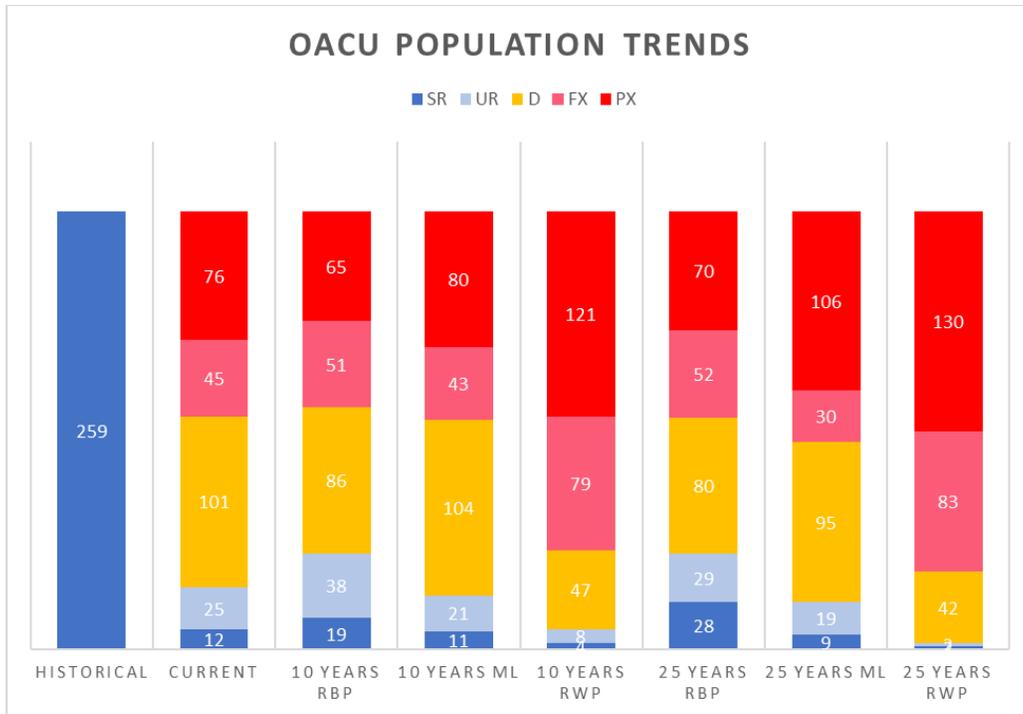


Figure 7.2. The number of SR, UR, D, FX and PX populations over time in the OACU under the reasonable best case (RBP), most likely (ML), and reasonable worst case (RWP) scenarios.

### Tennessee River ACU

Currently, of the 297 historically documented populations, 26 (9%) are SR, 74 (25%) are UR, 82 (27%) are D, and 115 (39%) are FX or PX (Figure 7.3). The most important influences affecting Eastern Hellbender’s current and future status and trends in the TACU are sedimentation, water quality degradation, climate change, and small population sizes (refer to Table 5.2 and 6.1). Considering these influences, future projections indicate that the TACU may have between 174 and 149 extant populations (4%–18% decrease from current) and between 65 to 94 SR and UR populations (6%–35% decrease from current) by year 25 (Table 7.1, Figure 7.3). The forecasted number of functionally and presumed extirpated populations at year 25 ranges from 123–148, representing 41%–50% of all historical populations in the TACU.

The TACU is geographically large, with 182 currently occupied and widely dispersed streams across 6 states (i.e., high redundancy). However, redundancy is expected to decrease in the next 25 years, as the number of extant streams decreases from 4%–18%. The healthy populations are concentrated in western North Carolina, eastern Tennessee, and northern Georgia, but are still dispersed over a fairly large area. Given the current and predicted future geographic extent of populations within the TACU, disease is the only reasonably foreseeable catastrophic event. As with other portions of the range, the TACU is at moderate risk of introduction of *Bsal* and other potential EIDs. Although the TACU falls within the high suitability region for *Bsal*, the number and spatial extent of populations under the RBP scenario likely provide sufficient redundancy to protect against ACU-wide extirpation over the next 25 years (Table 7.2). However, under the

RWP scenario, the number and spatial extent of populations are predicted to decline, and thus, the risk of ACU-wide extirpation is likely (Table 7.2).

Given the current and projected loss of healthy populations, Eastern Hellbender resiliency in the TACU is substantially lower than historical conditions and will likely become less resilient in the future. Despite these losses, because of the current and projected geographic spread of populations, we expect healthy populations to persist across spatially heterogeneous environmental conditions and a diversity of stream orders and temperature regimes. TACU-wide extirpation is still plausible within the next 25 years due to the threat from a disease epidemic, though this is unlikely under the RBP scenario.

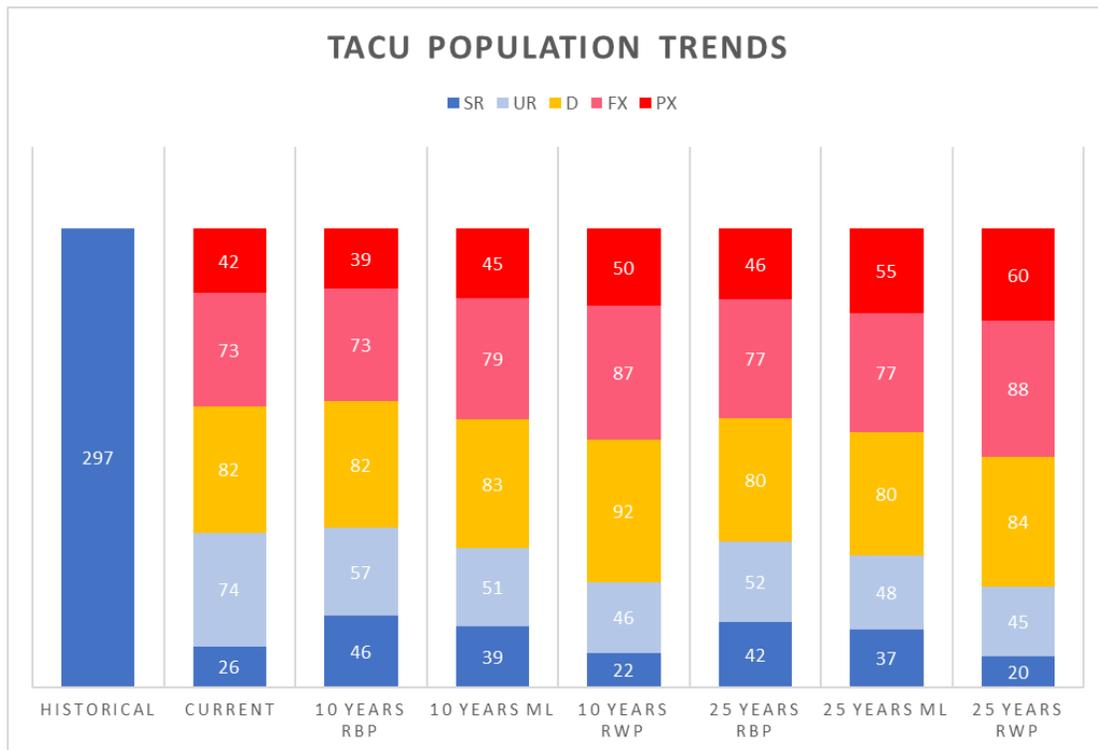


Figure 7.3. The number of SR, UR, D, FX and PX populations over time in the TACU under the reasonable best case (RBP), most likely (ML), and reasonable worst case (RWP) scenarios.

#### *Kanawha River ACU (KACU)*

Of the 65 historically documented populations, 7 (10%) are currently SR, 9 (14%) are UR, 30 (46%) are D, and 19 (29%) are FX or PX (Figure 7.4). The most important influences affecting Eastern Hellbender current and future status and trends in the KACU are sedimentation, water quality degradation, climate change, and destruction of habitat (refer to Table 5.2 and 6.1). Considering these influences, future projections indicate that the KACU will have between 28 and 46 extant populations (0%–39% decrease from current) and between 4 and 15 SR and UR populations (6%–75% less than current) at year 25 (Table 7.1, Figure 7.4). The number of SR populations is predicted to remain below historical conditions under both scenarios (85%–97%

loss) (Table 7.1, Figure 7.4). The forecasted number of functionally and presumed extirpated populations by year 25 ranges from 19 to 37, representing 29%–57% of all historical populations in the KACU (Table 7.1, Figure 7.4).

The KACU is geographically small, with 46 currently occupied streams distributed among 3 states, making it vulnerable to catastrophic losses. Redundancy may decrease further in 25 years, as the number of extant streams is expected to be reduced by 0%–39%. Given the small historical distribution of the KACU, with the exception of ACU-wide extirpation, losses of stream order and temperature regime diversity seem unlikely. While the KACU has low to moderate risk of *Bsal* introduction (Richgels et al. 2016, p. 5) and other potential EIDs, introduction of infectious disease is likely to spread rapidly throughout the KACU. This, coupled with the small number of healthy populations, leads us to believe the potential for ACU-wide extirpation due to a disease outbreak is likely under the RWP scenario (Table 7.2). The risk of catastrophic loss under the RBP scenario is potentially lower as there is a greater number and spatial extent of populations predicted (Table 7.2). The risk due to chemical pollution is unlikely under the RWP and RBP scenarios (Table 7.2).

Given the current and projected loss of healthy populations, Eastern Hellbender resiliency in the KACU is substantially lower than historical conditions and will likely become less resilient in the future. Under the RWP scenario, only 4 SR and UR populations remain, resulting in greatly reduced ability of Eastern Hellbenders in the KACU to withstand normal environmental variation, periodic disturbances, and catastrophes. Under the RBP scenario, several SR and UR populations persist but a declining trend is predicted. Thus, it is likely that the ability of Eastern Hellbenders to withstand normal environmental variation, periodic disturbances, and catastrophes has and will continue to decrease into the future. As with the other ACUs, the risk of ACU-wide extirpation due to catastrophic events is plausible within the next 25 years.

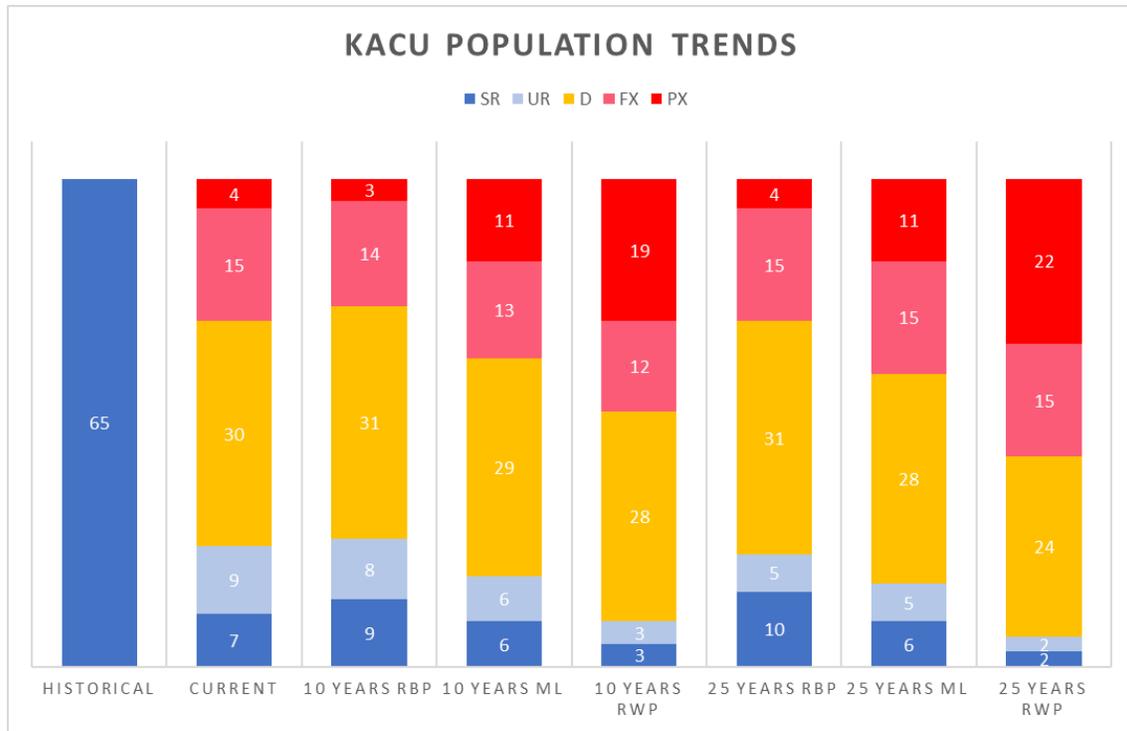


Figure 7.4. The number of SR, UR, D, FX and PX populations over time in the KACU under the reasonable best case (RBP), most likely (ML), and reasonable worst case (RWP) scenarios.

### Missouri ACU (MACU)

Of the five historical populations, all 5 (100%) are declining (Table 7.1, Figure 7.5). The most important influences affecting Eastern Hellbender’s current and future status and trends in the MACU are sedimentation, disease and pathogens, climate change, and small population sizes (refer to Table 5.2 and 6.1). Considering these influences, future projections indicate that the MACU will have between 2 and 4 extant populations and between 0 and 1 UR population by year 25 (Table 7.1, Figure 7.5). No SR populations are expected in 25 years under either scenario. The forecasted number of FX and PX populations by year 25 ranges from 0 to 2, representing 0%–40% of all historical populations in the MACU (Table 7.1).

The geographical extent within the MACU has always been limited, with only 5 streams closely located to one another (i.e., low redundancy). With the exception of ACU-wide extirpation, population losses within the MACU are not likely to lead to losses of adaptive diversity, despite being vulnerable to catastrophic events. The MACU has a low to moderate risk of *Bsal* introduction (Richgels et al. 2016, p. 5) and other potential EIDs; but if introduced, the spread is likely to be rapid and widespread. Given this, coupled with the lack of healthy populations, the potential for ACU-wide extirpation due to a disease outbreak is likely under the RWP scenario and is about as likely as not under the RBP scenario (Table 7.2). The potential for ACU-wide extirpation is predicted to be unlikely due to one or more catastrophic chemical pollution events under the RWP and RBP scenarios (Table 7.2).

The lack of healthy populations and the limited spatial extent of the MACU greatly reduce the ability of Eastern Hellbenders to withstand normal environmental variation, periodic disturbances, stressors, and catastrophes currently and into the future.

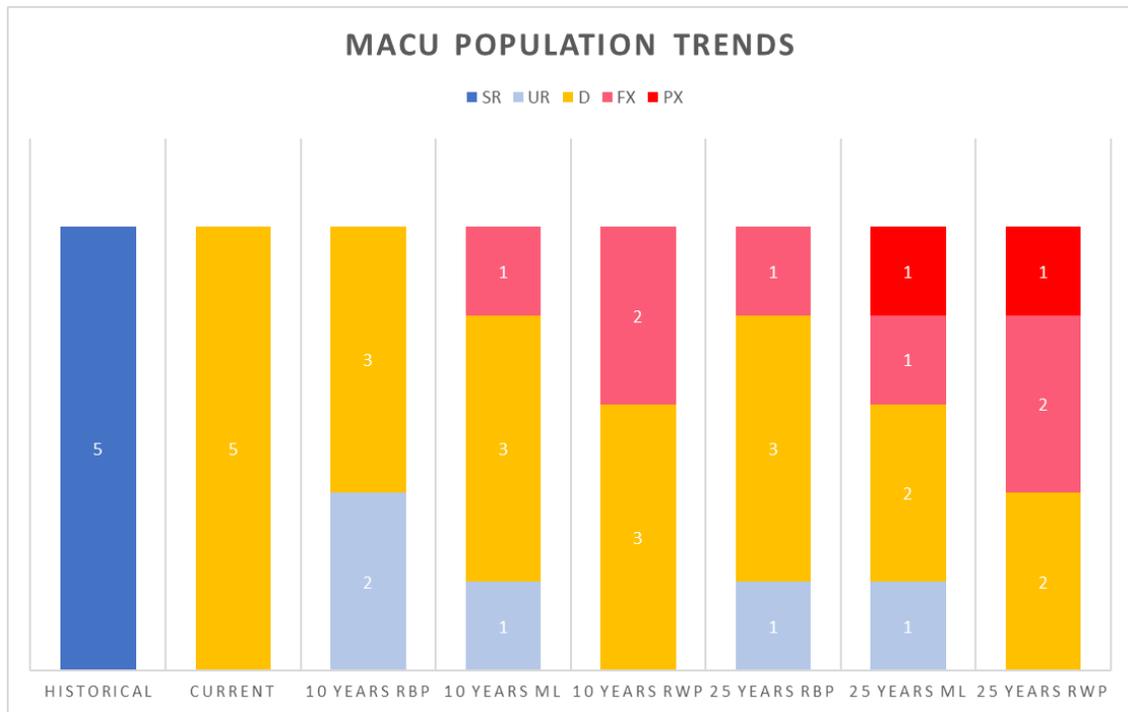


Figure 7.5. The number of SR, UR, D, FX and PX populations over time in the KACU under the reasonable best case (RBP), most likely (ML), and reasonable worst case (RWP) scenarios.

Table 7.2. Likelihood of loss of ACUs from two catastrophic events under current conditions and predicted future conditions. Note: Unlikely (U, green) = 0–33% probability; About As Likely As Not (A, yellow) = 33–66% probability; Likely (L, red) = 66–100% probability (IPCC 2014).

Current Conditions								
	MACU		OACU		TACU		KACU	
	RWP	RBP	RWP	RBP	RWP	RBP	RWP	RBP
Disease	L	A	L	U	A	U	L	A
Chemical Pollution	U	U	N/A	N/A	N/A	N/A	U	U
10-Year Predictions								
	MACU		OACU		TACU		KACU	
	RWP	RBP	RWP	RBP	RWP	RBP	RWP	RBP
Disease	L	A	L	U	A	U	L	A
Chemical Pollution	U	U	N/A	N/A	N/A	N/A	U	U
25-Year Predictions								
	MACU		OACU		TACU		KACU	
	RWP	RBP	RWP	RBP	RWP	RBP	RWP	RBP
Disease	L	A	L	U	L	U	L	A
Chemical Pollution	U	U	N/A	N/A	N/A	N/A	U	U

*Synopsis*

Eastern Hellbender viability requires healthy, resilient populations distributed across spatially heterogeneous conditions to ensure asynchronous population fluctuations in the face of environmental stochasticity and periodic disturbances as well as being widely dispersed to guard against catastrophes. Additionally, these populations should be distributed across the breadth of genetic and ecological diversity (i.e., populations occurring in a range of ecological and physical conditions within the 4 genetically distinct ACUs) to allow for adaptation to novel changes in its biological and physical environment.

Historically, the Eastern Hellbender was broadly distributed with 626 populations occurring in 15 eastern U.S. states and across spatially heterogeneous environments spanning the 4 ACUs. Today, the species has undergone a widespread decline with 41% of the populations believed extirpated or functionally extirpated and another 35% declining. Myriad factors contributed to the decline, including sedimentation, water quality degradation, habitat destruction and disturbance, disease, direct mortality, climate change, and small population effects. Although the reduced number of populations and the health of the remaining populations has rendered the Eastern Hellbender less able to cope with stressors and environmental fluctuations, impaired its ability to adapt to novel changes, and increased its vulnerability to catastrophes, widely distributed healthy populations continue to persist across heterogeneous conditions and within 3 of the 4 ACUs.

Looking into the future, the specific trajectory is unknowable, and hence, we looked to those with extensive knowledge of Eastern Hellbender biology, status, and trends to garner insights. Using these experts' judgments, Eastern Hellbender is predicted to continue to decline over the next 25 years under both reasonable best and worst-case scenarios, with the number of extant populations declining by 3% to 40%. These losses will likely lead to further reductions in resiliency and redundancy. However, multiple SR and UR populations over a broad range are predicted to persist over the next 25 years (73 (RWP) to 167 (RBP) SR and UR populations, representing a 52% decrease to a 9% increase from current conditions).

The predicted magnitude of decline varies among the ACUs. In all ACUs except the KACU, the number of extant populations is expected to decline under both the best and worst-case scenarios (the RBP scenario in the KACU predicts the same number of extant populations in 25 years). The range of predicted SR and UR populations in the OACU at 25 years is wide, from 4 to 57. The number of SR and UR populations in the TACU is expected decline from 6%–35% in the next 25 years, although it will still harbor the greatest number of SR and UR populations, with between 65 and 94 expected to persist. In the KACU, declines are expected to continue, with 4 to 15 SR and UR populations persisting. Similarly, within the MACU, between 0 and 1 UR population is predicted to persist and no SR populations are expected to persist. Thus, the predictions for TACU indicate that healthy populations will persist over the next 25 years, while in the OACU, MACU, and KACU, poor redundancy and resiliency may lead to ACU-wide extirpations. The OACU, MACU, and KACU comprise genetically distinct lineages and provide a diversity of ecological and physical conditions, both of which may provide important sources of adaptive diversity for Eastern Hellbender.

Although healthy populations are predicted to persist, a continued reduction in geographic range is expected. These reductions have and will continue to impair the Eastern Hellbender's ability to withstand environmental stochasticity and periodic disturbances and increase its vulnerability to catastrophes. The predicted losses include the potential loss of the OACU, KACU, and MACU. Loss of these units would lead to reductions in genetic and ecological diversity, both of which are potential sources of adaptive diversity. The loss of adaptive diversity, in turn, will render Eastern Hellbender less able to adapt to novel changes (e.g., new predators, pathogens, climate conditions, etc.) in its environment.

The synopsis above is predicated on various assumptions, which are listed in the "Uncertainty" section below. There are a few key assumptions that are particularly important to bear in mind. The current condition of the species is not precisely known. Of the 626 historical populations, the status of 100 (16%) is unknown and the population trend of another 220 (35%) populations is unknown. These status uncertainties are largely due to survey and detectability challenges, the difficulty in determining population health (in part because of the longevity of the Eastern Hellbender, at least 30 years), and logistical issues such as lack of resources and standard protocols. Also, although inherently uncertain, the experts' confidence levels in their future predictions averaged 83%. Some of the uncertainty is owed to the two previous uncertainties, but much of it is due to the uncertainty of the magnitude and extent of influences into the future. Many of the future best-case scenario predictions are contingent on threats being reduced and habitat conditions improving. Little data exist that provide evidence of reduced negative influences, such as sedimentation, water quality degradation and improved stream conditions,

over the next 25 years. Similarly, although it seems plausible that the conditions underlying the worst-case scenario could occur, there is uncertainty regarding the rate of decline that has occurred and will occur over the next 25 years due to ongoing stressors.

### 7.3 Uncertainty

Inherently, predicting the future condition requires us to make plausible assumptions. Our analyses are predicated on multiple assumptions, which could lead to over- and underestimates of viability. In Table 7.3, we identify the key sources of uncertainty and indicate the potential effect of our assumptions on the viability assessment. The uncertainty associated with determining current status and trend underpins all of our analyses, and thus, warrants further explanation.

Assessing current status and trends of Eastern Hellbender populations is challenging because of the difficulty detecting the animals in some stream environments, the long-lived demography of the species, and the lack of comprehensive surveys in much of the range.

The most common Eastern Hellbender survey technique—using log cant hooks to lift potential shelter rocks and capturing individuals by hand (Nickerson and Krysko 2003, p. 38)—can be effective in detecting adults, especially in shallow streams (<1.0 m (3.3 ft)) (Foster et al. 2008, p. 184) with good water clarity. This technique has also been effective (along with searching cobble beds) in detecting juveniles and larvae in these habitats (Nickerson and Krysko 2003, pp. 38–39, Foster et al. 2008, p. 184), especially in high-density populations (Williams and Groves 2012, pers. comm.). However, at depths greater than 1 meter, in streams with rocks too large to lift, and in turbid streams, the efficacy of this technique decreases as depth, turbidity, and rock size increase (Nickerson and Krysko 2003, p. 38; Foster et al. 2008, p. 184; Pflingsten 1988, p. 3; Burgmeier et al. 2011a, p. 144).

Scuba diving, diving with surface-supplied air, and traps have also occasionally been used in deeper water (Foster et al. 2008, p. 183; Petokas 2011, pers. comm.). In addition, hellbenders have been found using other shelters such as undercut banks and cavities in banks (Wethington 2017, pers. comm; Greathouse 2015, p. 99), which are rarely targeted during surveys. Recently, eDNA has emerged as an effective survey technique; it has detected hellbender presence where rock-lifting surveys did not and in locations where rock-lifting surveys were impractical (Lipps 2012, p. 4). However, eDNA is limited to detecting hellbender presence and cannot determine population density or age structure. Lastly, some states (e.g., Ohio, Kentucky, North Carolina) conduct outreach to anglers and request notification of hellbender sightings, thereby receiving occasional photographic records of hellbenders.

Because Eastern Hellbenders are long-lived (at least 30 years), adults may persist in a population for decades after recruitment has ceased. Therefore, the presence of adult Eastern Hellbenders in a stream does not indicate a viable population (Williams et al. 1981b, p. 99). A population decline may not be evident until the adults begin dying and numbers begin declining from lack of recruitment (Gates 1983, p. 7; Bodinof Jachowski and Hopkins 2018, p. 224). Monitoring of populations over many years may be necessary to assess viability (Williams et al. 1981b, p. 99), and monitoring of changes in demography may be especially important (Bodinof Jachowski and

Hopkins 2018, p. 224).

There is no systematic survey effort throughout the range. Survey effort has increased significantly since 2003 (following the first biennial hellbender symposium) and since the USFWS’s tally of historically occupied streams in 2012, 181 new populations have been discovered, largely due to this increased survey effort. Many of these new discoveries are represented by only a single adult animal or a positive eDNA result, neither of which provides demographic information to determine population trend. Because of all of these challenges, accurately determining that status and trends of the majority of Eastern Hellbender populations is exceptionally difficult.

Table 7.3. Key assumptions made in the analysis and the potential impact on our viability assessment if such assumptions are incorrect. “Overestimates” means the viability of the species may be optimistic; “Underestimates” means the viability of the species may be pessimistic. “Either” means the impact could lead to over- or underestimates if our assumption is incorrect.

<b>Assumption</b>	<b>Impact on Viability Assessment</b>
Adaptive capacity units capture the full breadth of adaptive diversity	Overestimates
Populations categorized as healthy (based on definitions in the SSA) are actually healthy, even when data are lacking on temporal trends	Overestimates
Habitat patches within a stream represent a single population; the status and trend assigned to the population apply to all habitat patches	Either
Trends were correctly assigned by experts to populations with unknown status or trends	Either
SR populations in future scenarios are genetically and demographically robust populations (i.e., sufficient size and connectivity)	Overestimates
Regardless of length of stream occupied by hellbenders, all streams contribute equally to the species’ status	Either
The current known range accurately represents the number of streams occupied by Eastern Hellbenders (low detectability, as evidenced by the number of new populations found in recent years)	Underestimates
The extent and magnitude of future influences is accurately predicted	Either
The future response of populations is within the range of RWP and RBP scenarios	Either
Populations categorized as FX will eventually become PX, without intervention.	Underestimates

## LITERATURE CITED

- Ahlstedt, S.A., P.D. Johnson, J.R. Powell, R.S. Butler, M.T. Fagg, D.W. Hubbs, S.F. Novak, and S.R. Palmer. 2004. Historical and current examination of freshwater mussels (Bivalvia: Margaritiferidae, Unionidae) in the Duck River basin, Tennessee. Tennessee Wildlife Resources Agency, Nashville, 213 pp.
- Allen, J.D. and M.M. Castillo. 2007. Stream Ecology: structure and function of running waters. Springer, Dordrecht, Netherlands, 436 pp. as cited in USFWS 2016, p. 61966.
- Allendorf, F.W. and G. Luikart. 2007. Conservation and the genetics of populations. Blackwell Publishing, Malden, MA, 642 pages.
- Bales, E.K., O.J. Hyman, A.H. Loudon, R.N. Harris, G. Lipps, E. Chapman, K. Roblee, J.D. Kleopfer, and K.A. Terrell. 2015. Pathogenic chytrid fungus *Batrachochytrium dendrobatidis*, but not *B. salamandrivorans*, detected on Eastern Hellbenders. PLoS ONE 10(2): e0116405. doi:10.1371/journal.pone.0116405.
- Baxter, R.M. 1977. Environmental effects of dams and impoundments. Annual Review of Ecology and Systematics 8:255–283.
- Beever, E.A., O’Leary, J., Mengelt, C., West, J.M., Julius, S., Green, N., Magness, D., Petes, L., Stein, B., Nicotra, A.B. and Hellmann, J.J., 2015. Improving conservation outcomes with a new paradigm for understanding species’ fundamental and realized adaptive capacity. *Conservation Letters*, 9(2), pp. 131–137.
- Bell, T. 2013. Email from Thomas Bell, Senior Biologist, New York Department of Environmental Conservation, Cortland, NY to Jeromy Applegate, USFWS, Columbus, OH (January 7, 2013).
- Benstead, J.P., J.G. March, C.M. Pringle, and F.N. Scatena. 1999. Effects of a low-head dam and water abstraction on migratory tropical stream biota. *Ecological Applications* 9:656–668.
- Berger, L., A.A. Roberts, J. Voyles, J.E. Longcore, K.A. Murray, L.R. Skerratt. 2016. History and recent progress on chytridiomycosis in amphibians. *Fungal Ecology* 19:89–99.
- Berger, L., R. Speare, R. P. Daszak, D.E. Green, A.A. Cunningham, C. L . Goggin, R. Slocombe, M. Ragan, A.D. Hyatt, K.R. McDonald, et al. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences* 95:9031–9036.
- Bhowmik, N.G. and J.R. Adams. 1989. Successional changes in habitat caused by sedimentation in navigation pools. *Hydrobiologia* 176/177:17–27.
- Bishop, S. C. 1941. Salamanders of New York. *New York State Museum Bulletin* 324:1–365.

- Blais, D. P. 1996. Movement, home range, and other aspects of the biology of the Eastern Hellbender *Cryptobranchus alleganiensis alleganiensis*: a radiotelemetric study. Unpublished master's thesis, State University of New York at Binghamton.
- Bodinof, C. M., J. T. Briggler, M. Duncan, J. Beringer, and J. J. Millspaugh. 2011. Historic occurrence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in hellbender *Cryptobranchus alleganiensis* populations from Missouri. *Diseases of Aquatic Organisms* 96:1–11.
- Bodinof Jachowski, C. M., V. Alaasam, A Blumenthal, A. K. Davis, and W. A. Hopkins. 2024. The habitat quality paradox: loss of riparian forest cover leads to decreased risk of parasitism and improved body condition in an imperiled amphibian. *Conservation Physiology* 12:1–21.
- Bodinof Jachowski, C. M. and W. A. Hopkins. 2018. Loss of catchment-wide riparian forest cover is associated with reduced recruitment in a long-lived amphibian. *Biological Conservation* 220:215–227.
- Bodinof Jachowski, C. M., B. E. Ross, and W. A. Hopkins. 2020. Evaluating artificial shelter arrays as a minimally invasive monitoring tool for the hellbender *Cryptobranchus alleganiensis*. *Endangered Species Research* 41:197–181.
- Boerner, J. A. 2014. Comparison of movement patterns in captive–released Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) using three different release methods. Master's thesis, State University of New York Buffalo State.
- Booth, D. B. and C. J. Jackson, 1997. Urbanization of aquatic systems - degradation thresholds, stormwater detention, and the limits of mitigation. *Water Resources Bulletin* 33:1077–1090.
- Bosch J., I. Martinez-Solano, and M. Garcia-Paris. 2001. Evidence of a chytrid fungus infection involved in the decline of the common mid-wife toad (*Alytes obstetricans*) in protected areas of central Spain. *Biological Conservation* 97:331–337.
- Bothner, R. C., and J. A. Gottlieb. 1991. A study of the New York State populations of the hellbender, *Cryptobranchus alleganiensis alleganiensis*. *Proceedings of the Rochester Academy of Science* 17:41–54.
- Bowers, N. J., J. R. Stauffer, and J. R. Pratt. 1992. The distribution, population status and ecology of *Etheostoma maculatum* Kirtland in upper French Creek, New York, final report to Kate Hubbs, The Nature Conservancy New York Field Office. School of Forest Resources, The Pennsylvania State University, University Park, PA, 52 pp.
- Brand, C.J. 2013. Wildlife mortality investigation and disease research: contributions of the USGS National Wildlife Health Center to endangered species management and recovery. *EcoHealth* 10:446–454.
- Briggler, J. 2017. Email from Jeff Briggler, Herpetologist, Missouri Department of Conservation, Jefferson City, MO to Jeromy Applegate, USFWS, Columbus, OH (May 15, 2017).

Briggler J. 2012. Email from Jeff Briggler, Herpetologist, Missouri Department of Conservation, Jefferson City, MO to Jeromy Applegate, USFWS, Columbus, OH (August 3, 2012).

Briggler, J. 2010. Email from Jeff Briggler, Herpetologist, Missouri Department of Conservation, Jefferson City, MO to Jeromy Applegate, USFWS, Columbus, OH (May 15, 2010).

Briggler, J.T., K.A. Larson and K.J. Irwin. 2008. Presence of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) on hellbenders (*Cryptobranchus alleganiensis*) in the Ozark Highlands. *Herpetological Review* 39:443–444.

Briggler, J., J. Utrup, C. Davidson, J. Humphries, J. Groves, T. Johnson, J. Ettl, M. Wanner, K. Traylor-Holzer, D. Reed, V. Lindgren, O. Byers (eds.) 2007. Hellbender Population and Habitat Viability Assessment: Final Report. IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, MN.

Brodie, E. D., Jr. 1971. Two more toxic salamanders, *Ambystoma maculatum* and *Cryptobranchus alleganiensis*. *Herpetological Review* 3:8.

Brooker, M.P. 1985. The ecological effects of channelization. *The Geographic Journal* 151:63–69.

Brooks, G. C., W. A. Hopkins, and H. K. Kindsvater. 2023. Concurrent threats and extinction risk in a long-lived, highly fecund vertebrate with parental care. *Ecological Applications* e2946:1–14.

Brown, R. K. 2022. Identifying factors for successful recruitment in Eastern Hellbender (*Cryptobranchus a. alleganiensis*) populations. Unpublished Master's Thesis, Ohio University, Athens.

Burgmeier, N. G., E. B. McCallen, E. K. Kenison, and R. N. Williams. 2022. Comparing the effects of environmental enrichment, seasonality, and soft release on site retention and survivorship of captive-reared Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*). *Herpetologica* 78:225–234.

Burgmeier, N.G., T. M. Sutton, and R.N. Williams. 2011a. Spatial ecology of the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana. *Herpetologica* 67:135–145.

Burgmeier, N.G., S.D. Unger, J.L. Meyer, T.M. Sutton, and R.N. Williams. 2011b. Health and habitat quality assessment for the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana, USA. *Journal of Wildlife Diseases* 47:836–848.

Burgmeier, N.G., S.D. Unger, T.M. Sutton, and R.N. Williams. 2011c. Population status of the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana. *Journal of Herpetology* 45:195–201.

Butler, R.S. 2007. Status assessment report for the snuffbox, *Epioblasma triquetra*, a freshwater mussel occurring in the Mississippi River and Great Lakes Basins. U.S. Fish and Wildlife Service, Asheville, NC, 188 pp.

Carey C., Pessier A.P., Peace A.D. (2003) Pathogens, infectious disease, and immune defenses. In: Amphibian Conservation, Semlitsch R. (editor), Washington DC: Smithsonian Institution Press, pp. 127–136.

Chambouvet, A., D.J. Gower, M. Jirku, M.J. Yabsley, A.K. Davis, G. Leonard, F. Maguire, T.M. Doherty-Bone, G.B. Bittencourt-Silva, M. Wilkinson, and T.A. Richards. 2015. Cryptic infection of a broad taxonomic and geographic diversity of tadpoles by Perkinsea protists. *Proceedings of the National Academy of Sciences of the United States of America* 112:E4743–E4751. <https://doi.org/10.1073/pnas.1500163112>

Chapman, E.J. 2009. Email from Eric Chapman, Director of Aquatic Science, Western Pennsylvania Conservancy, Blairsville, PA to Jeromy Applegate, USFWS, Columbus, OH (March 3, 2009).

Chapman, E.J. 2017. Email from Eric Chapman, Director of Aquatic Science, Western Pennsylvania Conservancy, Blairsville, PA to Jeromy Applegate, USFWS, Columbus, OH (January 16, 2017).

Chevin, L.M., Lande, R. and Mace, G.M., 2010. Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS biology*, 8(4), p.e1000357.

Christin, M.S., A.D. Gendron, P. Brousseau, L. Menard, D.J. Marcogliese, D. Cyr, S. Ruby, and M. Fournier. 2003. Effects of agricultural pesticides on the immune system of *Rana pipiens* and on its resistance to parasitic infection. *Environmental Toxicology and Chemistry* 22:1127–1133.

Chudyk, S.A. 2013. Parentage analysis and conservation genetics educational material for the Eastern Hellbender salamander, *Cryptobranchus alleganiensis alleganiensis*. M.S. Ed. Project, SUNY Buffalo State, Buffalo, NY. 196 pp.

Cline, G.R. and J.R. Rayburn. 2008. Survey of the distribution of Eastern Hellbenders (*Cryptobranchus alleganiensis*) in Alabama. Final Report to the Alabama Department of Natural Resources, Division of Wildlife and Freshwater Fisheries, Montgomery, AL, 10 pp.

Coatney, C. E., Jr. 1982. Home range and nocturnal activity of the Ozark hellbender. Unpublished master's thesis. Southwest Missouri State University, Springfield.

Cobb, D.G. and J.F. Flannagan. 1990. Trichoptera and substrate stability in the Ochre River, Manitoba. *Hydrobiologia* 206:29–38.

Congdon, J.D., A.E. Dunham, and R.C. Van Loben Sels. 1993. Delayed sexual maturity and demographics of Blanding's Turtles (*Emydoidea blandingii*): implications for conservation and management of long-lived organisms. *Conservation Biology* 7:826–833.

Conrad, N. 2012. Email from Nicholas Conrad, Information Resources Coordinator, New York Natural Heritage Program, Albany, NY to Jeromy Applegate, USFWS, Columbus, OH (March 29, 2012).

Cooper, C.M. 1993. Biological effects of agriculturally derived surface water pollutants on aquatic systems. *Journal of Environmental Quality* 22:402–408.

Cope, E. D. 1889. The batrachia of North America. *U.S. National Museum Bulletin* 34:1–525.

Crandall, K.A., Bininda-Emonds, O.R., Mace, G.M. and Wayne, R.K., 2000. Considering evolutionary processes in conservation biology. *Trends in ecology and evolution* 15:290–295.

Cunningham, A.A., T.E.S. Langton, P.M. Bennett, J.F. Ledwin, S.E.N. Drury, R.E. Gough, and S.K. MacGregor. 1996. Pathological and microbial findings from incidents of unusual mortality of the common frog (*Rana temporaria*). *Philosophical Transactions: Biological Sciences* 351:1539–1557.

Cunningham, A.A., P. Daszak, and J. Wood. 2017. One health, emerging infectious diseases and wildlife: two decades of progress? *Phil. Trans. R. Soc. B* 372:20160167

Cusaac, J. P. W., E. D. Carter, D. C. Woodhams, J. Robert, J. A. Spatz, J. L. Howard, C. Lillard, A. W. Graham, R. D. Hill, S. Reinsch, D. McGinnity, B. Reeves, and D. Bemis. 2020. Emerging pathogens and a current-use pesticide: potential impacts on Eastern Hellbenders. *Journal of Aquatic Animal Health* 33:24–32.

Da Silva Neto, J. G., L. A. Williams, C. R. Lawson, J. D. Groves, T. D. Byl, C. M. Gibson, C. R. Perkins, and W. B. Sutton. 2023. Land-use and water-quality threats to current and historical *Cryptobranchus alleganiensis* streams across multiple ecoregions. *Freshwater Science* 42:347–362.

Daszak, P., L. Berger, A.A. Cunningham, A.D. Hyatt, D.E. Green, R. Speare. 1999. Emerging infectious diseases and amphibian population declines. *Emerging Infectious Diseases* 5:735–748.

Diamanti-Kandarakis, E., J. Bourguignon, L.C. Giudice, R. Hauser, G.S. Prins, A.M. Soto, R.T. Zoeller, and A.C. Gore. 2009. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocrine Reviews* 30:293–342.

Diaz, L., S. D. Unger, L. A. Williams, and C. M. Bodinof Jachowski. 2022. Resource selection patterns of immature Eastern Hellbenders in North Carolina, USA. *Ichthyology and Herpetology* 110:398–408.

Dodd, Jr., C.K. 1997. Imperiled amphibians: a historical perspective. In: G.W. Benz and D.E. Collins (Eds.). *Aquatic Fauna in Peril: The Southeastern Perspective*. Southeast Aquatic Research Institute, Lenz Design and Communications, Decatur, Georgia, Special Publication 1, pp. 165–200.

- Dundee, H. A., and D. S. Dundee. 1965. Observations on the systematics and ecology of *Cryptobranchus* from the Ozark Plateaus of Missouri and Arkansas. *Copeia* 3:369–370.
- Ebert, D.J. and S.P. Filipek. 1988. Response of fish communities to habitat alteration in a small Ozark stream. *Proceedings Arkansas Academy of Science* 42:28–32.
- Echternacht, A.C. 2009. Email from Arthur Echternacht, Professor, University of Tennessee, Knoxville, TN to Jeromy Applegate, USFWS, Columbus, Ohio (June 16, 2009).
- Ellis, M.M. 1936. Erosion silt as a factor in aquatic environments. *Ecology* 17:29–42.
- Erichsen Jones, J.R. 1962. Fish and river pollution. in *River pollution II: causes and effects*, L. Klein (ed.) pp. 254–310. Butterworth and Co., London.
- Eskew, E., A. Todd, and W. A. Hopkins. 2014. Extremely low prevalence of *Batrachochytrium dendrobatidis* infection in Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) in Southwest Virginia, USA. *Herpetological Review*, 45:425–427.
- Etnier, D.A. 1972. The effect of annual rechanneling on a stream fish population. *Transactions of the American Fisheries Society* 1972:372–375.
- Falaschi, M., A. Melotto, R. Manenti, and G. F. Ficetola. 2020. Invasive species and amphibian conservation. *Herpetologica* 2020:216–227.
- Feller, D. and E. Thompson. 2011. Hellbender, *Cryptobranchus alleganiensis*, status summary for Maryland. Unpublished status summary report by the Maryland Department of Natural Resources, Natural Heritage Program, 4 pp.
- Ferguson, D.E. 1961. The herpetofauna of Tishomingo County, Mississippi with comments on its zoogeographic affinities. *Copeia* 1961:391–396.
- Fisher, M.C. 2017. In peril from a perfect pathogen. *Nature* 544:300–301.
- Fisher, M.C., D.A. Henk, C.J. Briggs, J.S. Brownstein, L.C. Madoff, S.L. McCraw and S.J. Gurr. 2012. Emerging fungal threats to animal, plant and ecosystem health. *Nature* 484:186–194.
- Fitch, F. W. 1947. A record *Cryptobranchus alleganiensis*. *Copeia* 1974:210.
- Forsman, A., 2014. Effects of genotypic and phenotypic variation on establishment are important for conservation, invasion, and infection biology. *Proceedings of the National Academy of Sciences* 111:302–307.
- Forson, D.D. and A. Storfer. 2006a. Atrazine increases ranavirus susceptibility in the tiger salamander, *Ambystoma tigrinum*. *Ecological Applications* 16:2325–2332.
- Forson, D.D. and A. Storfer. 2006b. Effects of atrazine and Iridovirus infection on survival and life-history traits of the long-toed salamander (*Ambystoma macrodactylum*). *Environmental Toxicology and Chemistry* 25:168–173.

Foster, R. L. 2006. A study of the hellbender salamander (*Cryptobranchus alleganiensis*) in the Allegheny River drainage of New York State: Examination of population trends, assessment of capture methods, and development of genetic techniques. Unpublished master's thesis, State University College at Buffalo, Buffalo, New York, 117 pp.

Foster, R. L. 2012. Email from Robin Foster, PhD Student, State University of New York at Buffalo, Buffalo, NY to Jeromy Applegate, USFWS, Columbus, OH (August 11, 2012).

Foster, R. 2018. Lessons from the past: A historical approach to conservation of the Eastern Hellbender (*Cryptobranchus alleganiensis*). Ph.D Dissertation, State University of New York at Buffalo.

Foster, R. L., A. M. McMillan, A. R. Breisch, K. J. Roblee, and D. Schranz. 2008. Analysis and comparison of three capture methods for the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*). Herpetological Review 39:181–186.

Foster, R. L., A. M. McMillan, and K. J. Roblee. 2009. Population status of hellbender salamanders (*Cryptobranchus alleganiensis*) in the Allegheny River drainage of New York State. Journal of Herpetology 43: 579–588.

Freake, M.J., and C.S. DePerno. 2017. Importance of demographic surveys and public lands for the conservation of Eastern Hellbenders *Cryptobranchus alleganiensis alleganiensis* in the southeast USA. PLoS One 12:e0179153.

Gall, B. G., A. L. Crane, and A. Mathis. 2010. *Cryptobranchus alleganiensis alleganiensis* (Eastern Hellbender) secretion production. Herpetological Review 41:59.

Gall, B.G. and A. Mathis. 2010. Innate predator recognition and the problem of introduced trout. Ethology 116:47–58.

Gates, J.E. 1983. The distribution and status of the hellbender (*Cryptobranchus alleganiensis*) in Maryland: I. The distribution and status of *Cryptobranchus alleganiensis* in Maryland. II. Movement patterns of translocated *Cryptobranchus alleganiensis* in a Maryland stream. Maryland Wildlife Administration.

Gates, J.E., C.H. Hocutt, J.R. Stauffer, Jr., and G.J. Taylor. 1985. The distribution and status of *Cryptobranchus alleganiensis* in Maryland. Herpetological Review 16:17–18.

Gendron, A.D., D.J. Marcogliese, S. Barbeau, M.S. Christin. P. Brousseau, S. Ruby, D. Cyr, and M. Fournier. 2003. Exposure of leopard frogs to a pesticide mixture affects life history characteristics of the lungworm *Rhabdias ranae*. Oecologia 135:469–476.

Geng, Y., K.Y. Wang, Z.Y. Zhou, C.W. Li, J. Wang, M. He, Z.Q. Yin, and W.M. Lai. 2011. First report of ranavirus associated with morbidity and mortality in farmed Chinese giant salamanders (*Andrias davidianus*). Journal of Comparative Pathology 145:95–102.

Gentry, G. 1955. An annotated checklist of the amphibians and reptiles of Tennessee. Journal of the Tennessee Academy of Science 30:168–176.

- Gilbertson, M.K., D. Haffner, K.G. Drouillard, A. Albert, and B. Dixon. 2003. Immunosuppression in the northern leopard frog (*Rana pipiens*) induced by pesticide exposure. *Environmental Toxicology and Chemistry* 22:101–110.
- Godwin, J. 2016. Email from Jim Godwin, Alabama Natural Heritage Program, Auburn University, Auburn, AL to Jeromy Applegate, USFWS, Columbus, OH (November 7, 2016).
- Gonynor, J.L., M.J. Yabsley, and J.B. Jensen. 2011. A preliminary survey of *Batrachochytrium dendrobatidis* exposure in hellbenders from a stream in Georgia, USA. *Herpetological Review* 42:58–59.
- Gottlieb, J.A. 1991. A population study of the hellbender salamander, *Cryptobranchus alleganiensis*, in the Allegheny River drainage of New York State. Unpublished master's thesis, St. Bonaventure University, Olean, NY.
- Goulet, B.N. and A. Hontela. 2003. Toxicology of cadmium, endosulfan, and atrazine in adrenal steroidogenic cells of two amphibian species, *Xenopus laevis* and *Rana catesbeiana*. *Environmental Toxicology and Chemistry* 22(9): 2106–2113.
- Gowins, G, M. Clare, M. Colber, K Samborsky, C. Byrne, and J. Greathouse. 2014. eDNA Assessment of the Eastern Hellbender, *Cryptobranchus alleganiensis*, in Ohio. The Wilds, Department of Conservation Science, Cumberland, OH, 12 pp.
- Graham, S.P., E.C. Soehren, G.R. Cline, C.M. Schmidt, W.B. Sutton J.R. Rayburn, S.H. Stiles, J.A. Stiles. 2011. Conservation status of hellbenders (*Cryptobranchus alleganiensis*) in Alabama, USA. *Herpetological Conservation and Biology* 6:242–249.
- Granoff, A., P.E. Came, and K. A. Rafferty. 1965. The Isolation and Properties of Viruses from *Rana pipiens*: their possible relationship to the renal adenocarcinoma of the leopard frog. *Annals of the New York Academy of Sciences* 126:237–255.
- Gray, M. J., E. D. Carter, J. Piovia-Scott, J. P. W. Cusaac, A.C. Peterson, R. D. Whetstone, A. Hertz, A. Y. Muniz-Torres, M. C. Bletz, D. C. Woodhams, and J. M. Romansic. 2023. Broad host susceptibility of North American amphibian species to *Batrachochytrium salamandrivorans* suggests high invasion potential and biodiversity risk. *Nature Communications* 14:3270.
- Gray, M.J., D.L. Miller, and J.T. Hoverman. 2009. Ecology and pathology of amphibian ranaviruses. *Diseases of Aquatic Organisms* 87:243–266.
- Gray, M.J., D.L. Miller, and J.T. Hoverman. 2012. Reliability of non-lethal surveillance methods for detecting ranavirus infection. *Diseases of Aquatic Organisms* 99:1–6.
- Greathouse, J. 2007. Development of a surveillance and monitoring protocol for the Eastern Hellbender, *Cryptobranchus alleganiensis alleganiensis*, in the Ohio River Valley of West Virginia. West Virginia Wildlife Diversity Program 2007 Research Project Report, Wheeling WV, 54 pp.

Greathouse, J. 2009. Email from Joe Greathouse, Curator of Animals, Good Zoo at Oglebay, Wheeling, WV to Jeromy Applegate, USFWS, Columbus, OH (June 28, 2009).

Greathouse, J. 2011. Email from Joe Greathouse, Curator of Animals, Good Zoo at Oglebay, Wheeling, WV to Jeromy Applegate, USFWS, Columbus, OH (January 14, 2011).

Greathouse, J. 2024. Email from Joe Greathouse, Director, Good Zoo at Oglebay, Wheeling, WV to Jeromy Applegate, USFWS, Columbus, OH (March 21, 2024).

Greathouse, W.J. 2015. Development of a conservation action plan for the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) in West Virginia. Doctoral Dissertation. Davis College of Agriculture, Natural Resources, and Design at West Virginia University, Morgantown, West Virginia, USA.

Green, N. B. 1935. Further notes on the food habits of the water dog, *Cryptobranchus alleganiensis* (Daudin). Proceedings of the Academy of Science 9:36.

Green, N.B. 1934. *Cryptobranchus alleganiensis* in West Virginia. Proceedings of the West Virginia Academy of Science 7:28–30.

Green, N.B. and T.K. Pauley. 1987. Amphibians and reptiles in West Virginia. University of Pittsburgh Press. Pittsburgh, PA.

Grisnik, M., Gray, M.J., Piovia-Scott, J., Carter, E.D. and W. B. Sutton. 2023. Incorporating caudate species susceptibilities and climate change into models of *Batrachochytrium salamandrivorans* risk in the United States of America. Biological Conservation 284:110181.

Grogan L., L. Berger, K. Rose, V. Grillo, and S.D. Cashins. 2014. Surveillance for emerging biodiversity diseases of wildlife. PLoS Pathog 10:e1004015.

Groves, J. 2012. Email from John Groves, Curator of Reptiles and Amphibians, North Carolina Zoo, Asheboro, NC to Jeromy Applegate, USFWS, Columbus, OH (August 6, 2012).

Groves, J. D., and L. A. Williams. 2014. *Cryptobranchus alleganiensis alleganiensis* (Eastern Hellbender) cannibalism. Herpetological Review 45:108–109.

Groves, J. D., L. A. Williams, and S. P. Graham. 2015. *Cryptobranchus a. alleganiensis* (Eastern Hellbender) larval/adult association. Herpetological Review 46:70–71.

Guimond, R. W. 1970. Aerial and aquatic respiration in four species of paedomorphic salamanders: *Amphiuma means means*, *Cryptobranchus alleganiensis alleganiensis*, *Necturus maculosus maculosus*, and *Siren lacertina*. PhD Dissertation. University of Rhode Island.

Hanski I., and M. E. Gilpin. 1997. Metapopulation biology: ecology, genetics, and evolution. San Diego, Academic Press. 512 pp.

- Hardman, R. H., L. K. Reinert, K. J. Irwin, K. Oziminski, L. Rollins-Smith, and D. L. Miller. 2023. Disease state associated with chronic toe lesions in hellbenders may alter anti-chytrid skin defenses. *Scientific Reports*, 13:1982.
- Hardman, R. H., K. J. Irwin, W. B. Sutton, and D. L. Miller. 2020a. Evaluation of severity and factors contributing to foot lesions in endangered Ozark Hellbenders, *Cryptobranchus alleganiensis* bishop. *Frontiers in Veterinary Science* 7:34.
- Hardman, R. H., W. B. Sutton, K. J. Irwin, D. McGinnity, S. D. Reinsch, M. Freake, P. Coclough, B. T. Miller, J. G. Da Silva Neto, M. Souza, B. Fitzpatrick, and D. L. Miller. 2020b. 803-814. Geographic and individual determinants of important amphibian pathogens in Hellbenders (*Cryptobranchus alleganiensis*) in Tennessee and Arkansas. *Journal of Wildlife Diseases* 56:803–814.
- Harlan, R. A., and R. F. Wilkinson. 1981. The effects of progressive hypoxia and rocking activity on blood oxygen tension for hellbenders, *Cryptobranchus alleganiensis*. *Journal of Herpetology* 15:383–388.
- Harper, J.A. 2008. The Marcellus Shale-an old "new" gas reservoir, in *Pennsylvania Geology: Pennsylvania Department of Conservation and Natural Resources* 38:2–13.
- Harris, R.N., T.Y. James, A. Lauer, M.A. Simon, and A. Patel. 2006. Amphibian pathogen *Batrachochytrium dendrobatidis* is inhibited by the cutaneous bacteria of amphibian species. *EcoHealth* 3:53–56.
- Hartle, M.A. 2016. What happens after a train wreck? Death and renewal in the Sinnemahoning Creek watershed. *Pennsylvania Angler and Boater*. July/August 2016:54–55.
- Hartzell, S. M., A. L. Pitt, and S. M. Davis. 2022. Invasive Rusty Crayfish (*Faxonius rusticus*) can serve as prey of Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*). *Herpetological Conservation and Biology* 17:22-30.
- Hauswald, C. 2008. Email from Cassie Hauswald, Conservation Assistant, TNC Blue River Project, Laconia, IN to Jeromy Applegate, USFWS, Reynoldsburg, OH (Oct. 3, 2008).
- Hayes, C. 2024. Email from Courtney Hayes, Wildlife Biologist, Kentucky Department of Fish and Wildlife Resources, Frankfort, KY to Jeromy Applegate, USFWS, Columbus, OH (April 1, 2024).
- Hayes, T.B., P. Case, S. Chui, D. Chung, C. Haeffele, K. Haston, M. Lee, V.P. Mai, Y. Marjua, J. Parker, and M. Tsui. 2006. Pesticide mixtures, endocrine disruption, and amphibian declines: are we underestimating the impact? *Environmental Health Perspectives* 114:40–50.
- Hayes, T. B., A. Collins, M. Lee, M. Mendoza, N. Noriega, A. A. Stuart, and A. Vonk. 2002. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. *Proceedings of the National Academy of Sciences* 99:5476–5480.

- Hecht, K. A., M. A. Nickerson, and P. B. Colclough. 2017. Hellbenders (*Cryptobranchus alleganiensis*) may exhibit an ontogenetic dietary shift. *Southeastern Naturalist* 16:157–162
- Hecht-Kardasz, K. A., M. A. Nickerson, M. Freake, and P. Colclough. 2012. Population structure of the hellbender (*Cryptobranchus alleganiensis*) in a Great Smoky Mountains stream. *Bulletin of the Florida Museum of Natural History* 51:227–241.
- Hendry, A. P., Kinnison, M. T., Heino, M., Day, T. , Smith, T. B., Fitt, G., Bergstrom, C. T., Oakeshott, J., Jørgensen, P. S., Zalucki, M. P., Gilchrist, G., Southerton, S., Sih, A., Strauss, S., Denison, R. F. and Carroll, S. P. 2011. Evolutionary principles and their practical application. *Evolutionary Applications* 4:159–183.
- Henley, W.F., J.W. Jones, J.L. Boyles, J.M. McLeod, and R.J. Neves. 2002. An evaluation of the suitability of the Cedar Bluff Spill Site for recovery of freshwater mussels, Clinch River, Tazewell County, Virginia. Final Report to the U.S. Fish and Wildlife Service, Gloucester, Virginia by the Virginia Cooperative Fish and Wildlife Research Unit, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. 25 pp.
- Hillis, R. E., and E. D. Bellis. 1971. Some aspects of the ecology of the hellbender *Cryptobranchus alleganiensis alleganiensis*, in a Pennsylvania stream. *Journal of Herpetology* 5:121–126.
- Hime, P. M., S. D. Unger, S. J. Price, J. T. Briggler, L. Williams, M. J. Freake, A. N. Drayer, A. M. McMillan, M. E. Foley, J. Groves, E. M. Lemmon, and D. W. Weisrock. 2016 (In prep.) Cryptic branches in the *Cryptobranchus* tree: Genomic data reveal an underestimation of North American aquatic salamander diversity. Draft report. 19 pp.
- Hoffmann AA and Sgro CM. 2011. Climate change and evolutionary adaptation. *Nature* 470:479–485.
- Holderegger R, Kamm U, and Gugerli F. 2006. Adaptive vs neutral genetic diversity: implications for landscape genetics. *Landscape Ecology* 21:797–807.
- Hopkins, W.A. 2012. Email from William Hopkins, Associate Professor, Virginia Tech University, Blacksburg, VA to Jeromy Applegate, USFWS, Columbus, OH (August 25, 2012).
- Hopkins, W. A., B. F. Case, J. Groffen, G. C. Brooks, C. M. Bodinof Jachowski, S. T. Button, J. J. Hallagan, R. S. M. O'Brien, and H. K. Kindsvater. 2023. Filial cannibalism leads to chronic nest failure of Eastern Hellbender Salamanders (*Cryptobranchus alleganiensis*). *The American Naturalist* 202:92–106.
- Hopkins, W.A. and S.E. DuRant. 2011. Innate immunity and stress physiology of Eastern Hellbenders (*Cryptobranchus alleganiensis*) from two stream reaches with differing habitat quality. *General and Comparative Endocrinology* 174:107–115.
- Horchler, D. C. 2010. Long-term growth and monitoring of the Eastern Hellbender (*Cryptobranchus a. alleganiensis*) in eastern West Virginia. Unpublished master's thesis, Marshall University, Huntington, WV, 48 pp.

- Hubbard, W.D., D.C. Jackson, and D.J. Ebert. 1993. Channelization. Pp. 135–155 in: E.F. Bryan and D.A. Rutherford, eds. Impacts on warm-water streams: guidelines for evaluation. Warm-water Stream Committee, Southern Division, American Fisheries Society, Little Rock, Arkansas.
- Huckabee, J.H., C.P. Goodyear, and R.D. Jones. 1975. Acid rock in the Great Smokies: unanticipated impact on aquatic biota of road construction in regions of sulfide mineralization. *Transactions of the American Fisheries Society* 104:677–684.
- Humphries, W. J. 2005. Georgia hellbender status assessment. Report to the Georgia Department of Natural Resources, Wildlife Resources Division, 77 pp.
- Humphries, W. J., and T. K. Pauley. 2000. Seasonal changes in nocturnal activity of the hellbender, *Cryptobranchus alleganiensis*, in West Virginia. *Journal of Herpetology* 34:604–607.
- Humphries, W. J., and T. K. Pauley. 2005. Life history of the hellbender *Cryptobranchus alleganiensis*, in a West Virginia stream. *American Midland Naturalist* 154:135–142.
- Humphries, W. J., M. Solis, and C. Cardwell. 2005. *Cryptobranchus alleganiensis* (hellbender). Cannibalism. *Herpetological Review* 36:428.
- Ingersol, C.A., R.F. Wilkinson, C.L. Peterson, and R.H. Ingersol. 1991. Histology of the reproductive organs of *Cryptobranchus alleganiensis* (Caudata: Cryptobranchidae) in Missouri. *The Southwestern Naturalist* 36:60–66.
- IPCC, 2013. Summary for Policymakers. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- IPCC, 2014. *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.
- Jancovich, J.K., E.W. Davidson, J.F. Morado, B.L. Jacobs, and J.P. Collins. 1997. Isolation of a lethal virus from the endangered salamander *Ambystoma tigrinum stebbinsi*. *Diseases of Aquatic Organisms* 31:161–167.
- Jensen, J.B. 1999. Hellbender *Cryptobranchus alleganiensis*. In *Protected animals of Georgia. Nongame-Endangered Wildlife Program*, Georgia Department of Natural Resources, Social Circle, GA. Pp. 98–99.
- Jensen, J. 2009. Email from John Jensen, Senior Wildlife Biologist, Georgia Department of Natural Resources, Forsyth, GA to Jeromy Applegate, USFWS, Columbus, OH (February 17, 2009).

- Kanehl, P. and J. Lyons. 1992. Impacts of in-stream sand and gravel mining on stream habitat and fish communities, including a survey on the Big Rib River, Marathon County, Wisconsin. Wisconsin Department of Natural Resources Research Report 155, Madison, 32 pp.
- Karl, T.R., J.M. Melillo, and T.C. Peterson. 2009. Global Climate Change Impacts in the United States. Cambridge University Press, New York, 196 pp.
- Karesh, W.B., R.A. Cook, E.L. Bennett, and J. Newcomb. 2005. Wildlife trade and global disease emergence. *Emerg. Infect. Dis.* 11:1000–1002.
- Kaunert, M.D. 2011. A historical assessment of the Eastern Hellbender (*Cryptobranchus a. alleganiensis*) in a Pennsylvania stream. Unpublished undergraduate thesis, Allegheny College, Meadville, PA, 33 pp.
- Kaunert, M. D., R. K. Brown, S. Spear, P. B. Johantgen, and V. D. Popescu. 2023. Restoring Eastern Hellbender (*Cryptobranchus a. alleganiensis*) populations through translocation of headstarted individuals. *Population Ecology*, 1–15.
- KDEP (Kentucky Department of Environmental Protection). 2008. Total maximum daily load for 15 fecal coliform impaired stream segments in the upper Green River USGS Hydrologic Unit 05110001, February 2008. KDEP, Division of Water, Frankfort.
- KDEP (Kentucky Department for Environmental Protection). 2014. Integrated Report to Congress on the condition of water resources in Kentucky, 2014, volume 1. Division of Water, Frankfort, KY, 104 pp. + appendices.
- Keitzer S. C. 2007. Habitat preferences of the Eastern Hellbender in West Virginia. Unpublished master's thesis, Marshall University, Huntington, West Virginia, 64 pp.
- Kentucky Geological Survey. 2008. Kentucky Geological Survey internet mapper. Kentucky Geological Survey, University of Kentucky, Lexington. Retrieved September 29, 2008 from Kentucky Geological Survey website: <http://kgsmmap.uky.edu/website/KGSGeology/viewer.asp#>
- Kiesecker, J.M. 2002. Synergism between trematode infection and pesticide exposure: a link to amphibian limb deformities in nature? *Proceedings of the National Academy of Sciences* 99:9900–9904.
- Kiesecker, J.M. and A.R. Blaustein. 1995. Synergism between UV-B radiation and a pathogen magnifies amphibian embryo mortality in nature. *Proceedings of the National Academy of Science* 92:11049–11052.
- Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. *Environmental Science and Technology* 36:1202–1211.

Kraus, B. T., E. B. McCallen, and R. N. Williams. 2017. Evaluating the survival of translocated adult and captive-reared, juvenile Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*). *Herpetologica* 73:271–276.

Lande, R. 1988. Genetics and demography in biological conservation. *Science* 241:1455–1461.

Lankau R, Jorgensen PS, Harris DJ. And Sih A. 2011. Incorporating evolutionary principles into environmental management and policy. *Evolutionary Applications* 4:315–325.

Lawson, C. 2009. Email from Charles Lawson, Biologist, Fish and Wildlife Associates, Inc., Whittier, NC to Jeromy Applegate, USFWS, Columbus, OH (February 26, 2009).

Lindberg, A.J. and N. Soule. 1991. Unpublished report to the New York State Department of Environmental Conservation Endangered Species Unit on the hellbender (*Cryptobranchus alleganiensis*), Survey of the Susquehanna and Unadilla Rivers 1991, 19 pp.

Lipps, G. 2009a. Email from Greg Lipps, Herpetologist contracted by the Kentucky Department of Fish and Wildlife Resources, Delta, OH to Jeromy Applegate, USFWS, Columbus, OH (March 5, 2009).

Lipps, G. 2009b. A survey of the Eastern Hellbender in the Licking and Kentucky River watersheds. Unpublished report to the Kentucky Department of Fish and Wildlife Resources, August 31, 2009, 35 pp.

Lipps, G. 2010. Email from Greg Lipps, Herpetologist contracted by the Ohio Department of Natural Resources, Delta, OH to Jeromy Applegate, USFWS, Columbus, OH (November 19, 2010).

Lipps, G. 2012. Status assessment and conservation of the Eastern Hellbender in Kentucky. Final report to the Kentucky Department of Fish and Wildlife Resources. July 15, 2012, 80 pp.

Lipps, G. 2018. Email from Greg Lipps, Amphibian and Reptile Conservation Coordinator, The Ohio State University, Columbus, OH to Jeromy Applegate, USFWS, Columbus, OH (January 29, 2018).

Lipps, G. 2024. Email from Greg Lipps, Amphibian and Reptile Conservation Coordinator, The Ohio State University, Columbus, OH to Jeromy Applegate, USFWS, Columbus, OH (March 26, 2024).

Lipps, G. 2024. Email from Greg Lipps, Amphibian and Reptile Conservation Coordinator, The Ohio State University, Columbus, OH to Jeromy Applegate, USFWS, Columbus, OH (April 8, 2024).

Lipps, G. and R. A. Pflingsten. 2010. Assessment and conservation of Ohio populations of the Eastern Hellbender. Unpublished Report to the Ohio Department of Natural Resources, Columbus, 22 pp + 1 appendix.

- Lips, K.R., F. Brem, R. Brenes, J.D. Reeve, R.A. Alford, J. Voyles, C. Carey, L. Livo, A.P. Pesser, and J.P. Collins. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences USA* 103:3165–3170.
- Maitland, P.S. 1995. The conservation of freshwater fish: past and present experience. *Biological Conservation* 72:259–270.
- Marcellus-shale.us. 2012. Our look at seeps, leaks, and spills: some negatives of drilling for natural gas. Internet resource located at [http://www.marcellus-shale.us/seeps\\_leaks\\_spills.htm](http://www.marcellus-shale.us/seeps_leaks_spills.htm) (accessed May 8, 2012).
- Marschang, E.E. and D. Miller. 2012. 2011 International Ranavirus Symposium. *Journal of Herpetological Medicine and Surgery* 21:1–2.
- Martel, A., M. Blooi, C. Adriaensen, P.V. Rooij, W. Beukema, M.C. Fisher, R.A. Farrer, B.R. Schmidt, U. Tobler, K. Goka, K.R. Lips, C. Muletz, K.R. Zamudio, J. Bosch, S. Lotters, E. Wombwell, T.W.J. Garner, A.A. Cunningham, A. Spitzen-van der Sluijs, S. Salvidio, R. Ducatelle, K. Nishikawa, T.T. Nguyen, J.E. Kolby, I. Van Bocxlaer, F. Bossuyt, and F. Pasmans. 2014. Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. *Science* 346:630–631.
- Mayasich, J., D. Grandmaison, and C. Phillips. 2003. Eastern Hellbender status assessment report. Report to the US Fish and Wildlife Service, NRRI/TR–2003/09. 66 pp.
- McCallen, E. B., B. T. Kraus, N. G. Burgmeier, Songlin Fei, and R. N. Williams. 2018. Movement and habitat use of Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) following population augmentation. *Herpetologica* 74:283–293.
- McLean, R.G. 2007. The Introduction and Emergence of Wildlife Diseases in North America. USDA National Wildlife Research Center – Staff Publications. 710. [https://digitalcommons.unl.edu/icwdm\\_usdanwrc/710](https://digitalcommons.unl.edu/icwdm_usdanwrc/710)
- McPhee, R.D.E. and A.D. Greenwood. 2013. Infectious disease, endangerment, and extinction. *International Journal of Evolutionary Biology* 2013:571939.
- MDEQ (Mississippi Department of Environmental Quality). 2005. Total maximum daily load, Bear Creek watershed, for biological impairment due to sediment. Office of Pollution Control, Jackson, MS, 32 pp.
- Merkle, D.A., S.I. Guttman, and M.A. Nickerson. 1977. Genetic uniformity throughout the range of the hellbender, *Cryptobranchus alleganiensis*. *Copeia* 3:549–553.
- Miller, R. 1976. Models, metaphysics, and long-lived species. *Bulletin of the Ecological Society of America* 57: 2–6.

- Miller, D.L., S. Rajeev, M. Brookins, J. Cook, L. Whittington, and C.A. Baldwin. 2008. Concurrent infection with Ranavirus, *Batrachochytrium dendrobatidis*, and *Aeromonas* in a captive anuran colony. *Journal of Zoo and Wildlife Medicine* 39:445–449.
- Minton, S. A., Jr. 1972. Amphibians and reptiles of Indiana. Indiana Academy of Science Monograph. 3:24–27.
- Moore, M.K. and V.R. Townsend. 1998. The interaction of temperature, dissolved oxygen and predation pressure in an aquatic predator-prey system. *Oikos* 81:329–336.
- Moritz, C., 2002. Strategies to protect biological diversity and the evolutionary processes that sustain it. *Systematic biology* 51:238–254.
- Mount, R.H. 1975. The reptiles and amphibians of Alabama. Auburn Printing Co., Auburn, Alabama, 347 pp.
- Mullendore, N., A.S. Mase, K Mulvaney, R. Perry-Hill, A Reimer, L. Gehbehani, R.N. Williams, and L.S. Prokopy. 2014. Conserving the Eastern Hellbender salamander. *Human Dimensions of Wildlife*, 19:166–178.
- National Park Service. 2017. NPS Stats - Park Reports. Internet resource located at <https://irma.nps.gov/Stats/Reports/Park/GRSM> (assessed November 20, 2017)
- Nichols, D. 2012. Email from David Nichols, Endangered Species Biologist, National Oceanic and Atmospheric Administration, Honolulu, HI to Jeromy Applegate, USFWS, Columbus, OH (April 17, 2012).
- Nickerson, M. A., and C. E. Mays. 1973. The hellbenders: North American giant salamanders. *Milwaukee Public Museum Publications in Biology and Geology* 1, 106 pp.
- Nickerson, M. A., and M. D. Tohulka. 1986. The nests and nest site selection by Ozark hellbenders, *Cryptobranchus alleganiensis bishopi* Grobman. *Transactions of the Kansas Academy of Science* 89:66–69.
- Nickerson, M. A., K. L. Krysko, and R. D. Owen. 2003. Habitat differences affecting age class distributions of the hellbender salamander, *Cryptobranchus alleganiensis*. *Southeastern Naturalist* 2:619–629.
- Nickerson, M.A. and J.T. Briggler. 2007. Harvesting as a factor in population decline of a long-lived salamander; the Ozark hellbender, *Cryptobranchus alleganiensis bishopi* Grobman. *Applied Herpetology* 4:207–216.
- Nickerson, M.A., A.L. Pitt, and M.D. Prysby. 2007. The effects of flooding on hellbender salamander, *Cryptobranchus alleganiensis* Daudin, 1803, populations. *Salamandra* 43:111–117.
- Nicotra, A.B., Beaver, E.A., Robertson, A.L., Hofmann, G.E. and O'leary, J., 2015. Assessing the components of adaptive capacity to improve conservation and management efforts under global change. *Conservation Biology*, 29:1268–1278.

Nigrelli, R. F. 1954. Some longevity records of vertebrates. *New York Academy of Sciences* 16:296–299.

Nissen, B. D., M. J. Freake, E. Nolan, R. H. Hardman, W. B. Sutton. 2023. Evaluating translocation success of wild Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) in Blue Ridge Ecoregion streams using pre- and post-translocation home range sizes and movement metrics. *Plos ONE* 18: e0283377.

Noeske, T. A., and M. A. Nickerson. 1979. Diel activity rhythms in the hellbender, *Cryptobranchus alleganiensis* (Caudata: Cryptobranchidae). *Copeia* 1979:92–95.

Nolan, E., B. Nissen, W. Sutton, M. Freake, R. Hardman. 2021. Translocation does not influence amphibian chytrid fungus prevalence among wild eastern hellbenders *Cryptobranchus alleganiensis*. *Diseases of Aquatic Organisms* 145:145–147.

O’Brien, R. 2023. Behavior and conservation: hellbender reproductive behavioral responses to poor water quality and the value of private lands research for conservation outreach. Unpublished Doctoral Dissertation, Virginia Tech., Blacksburg.

ODNR (Ohio Department of Natural Resources). 2010. Ohio low-head dam locations map and data. Internet resource located at [http://www.dnr.state.oh.us/water/dsafety/lowhead\\_dams/lowhead\\_locations\\_map/tabid/3361/Default.aspx](http://www.dnr.state.oh.us/water/dsafety/lowhead_dams/lowhead_locations_map/tabid/3361/Default.aspx) (accessed October 5, 2010).

OEPA (Ohio Environmental Protection Agency). 2010. Biological and water quality study of the Captina Creek watershed. Division of Surface Water, Columbus, OH, 42 pp. + appendices.

Ortega, F. J. 2020. Comparing the effectiveness of diurnal rock-lifting and nocturnal dive-lighting surveys for Eastern Hellbenders. Unpublished Master’s Thesis, Appalachian State University, Boone, North Carolina.

Paul, M.J. and J.L. Meyer. 2001. Streams in the urban landscape. *Annual Review in Ecology and Systematics* 32:333–365.

PDEP (Pennsylvania Department of Environmental Protection). 2016. Final 2016 Pennsylvania integrated water quality monitoring and assessment report. 87 pp.

Peterson, C.L. 1985. Comparative demography of four populations of the hellbender, *Cryptobranchus alleganiensis*, in the Ozarks. PhD Dissertation. University of Missouri, Columbia. 158 pp.

Peterson, C. L. 1987. Movement and catchability of the hellbender, *Cryptobranchus alleganiensis*. *Journal of Herpetology* 21:197–204.

Peterson, C. L., D. E. Metter, and B. T. Miller. 1988. Demography of the *Cryptobranchus alleganiensis* in the Ozarks. *American Midland Naturalist* 119:291–303.

- Peterson, C. L., J. W. Reed, and R. F. Wilkinson. 1989. Seasonal food habits of *Cryptobranchus alleganiensis* (Caudata: Cryptobranchidae). *The Southwestern Naturalist* 34:348–441.
- Peterson, C. L., and R. F. Wilkinson. 1996. Home range size of the hellbender (*Cryptobranchus alleganiensis*) in Missouri. *Herpetological Review* 27:126–127.
- Peterson, C. L., R. F. Wilkinson, Jr., M. S. Topping, and D. E. Metter. 1983. Age and growth of the Ozark hellbender (*Cryptobranchus alleganiensis bishopi*). *Copeia*. 1983:225–231.
- Petokas, P. 2011. Telephone interview with Peter Petokas, Research Associate, Lycoming College, Williamsport, PA by Jeromy Applegate, USFWS, Columbus, OH (March 25, 2011).
- Petokas, P. 2012. Email from Peter Petokas, Research Associate, Lycoming College, Williamsport, PA to Jeromy Applegate, USFWS, Columbus, OH (August 22, 2012).
- Petranka, J. W. 1998. Salamanders of the United States and Canada. Smithsonian Institution.
- Pfingsten, R. A. 1988. Unpublished field notes of hellbender surveys in Ohio, 1986–1988.
- Pfingsten, R. A. 1990. The status and distribution of the hellbender, *Cryptobranchus alleganiensis*, in Ohio. *Herpetological Review* 21:48–51.
- Phillips, C. A., and W. J. Humphries. 2005. *Cryptobranchus alleganiensis*; hellbender, pp. 648–651 in: M.J. Lannoo (eds.), *Amphibian declines; the conservation status of United States species*, University of California Press, Berkeley, California.
- Pitt AL, Shinskie JL, Tavano, JJ, Hartzell SM, Delahunty T, and Spear SF. 2017. Decline of a giant salamander assessed with historical records, environmental DNA and multi-scale habitat data. *Freshwater Biology* 62:967–976.
- Pitt, A. L. and M. A. Nickerson. 2005. *Cryptobranchus alleganiensis* larval diet. *Herpetological Review* 37:69.
- Poff, N.L. and D.D. Hart. 2002. How dams vary and why it matters for the emerging science of dam removal. *Bioscience* 52:659–668.
- Pugh, M.W., M. Hutchins, M. Madritch, L. Siefferman, and M.M. Gangloff. 2015. Land-use and local physical and chemical habitat parameters predict site occupancy by Hellbender salamanders. *Hydrobiologia* 770:105–116.
- Quinn, S. A., J. P. Gibbs, M. H. Hall, and P. J. Petokas. 2013. Multiscale factors influencing distribution of the Eastern Hellbender salamander (*Cryptobranchus alleganiensis alleganiensis*) in the northern segment of its range. *Journal of Herpetology* 47:78–84.
- Raffel, T.R., J.R. Rohr, J.M. Kieseckers, and P.J. Hudson. 2006. Negative effects of changing temperature on amphibian immunity under field conditions. *Functional Ecology* 20:819–828.

- Rafferty, K.A. 1965. The cultivation of inclusion-associated viruses from Lucké Tumor Frogs. *Annals of the New York Academy of Sciences* 126:3–21.
- Rayman, N.L. 2012. Email from Noelle Rayman, Endangered Species Biologist, USFWS, New York Field Office, Cortland NY to Jeromy Applegate, USFWS, Columbus, OH (August 14, 2012).
- Redford KH, Amato G, Baillie J, Beldomenico P, Bennett EL, Clum N, Cook R, Fonseca G, Hedges S, Launay F, Lieberman S, Mace GM, Murayama A, Putnam A, Robinson JG, Rosenbaum H, Sanderson EW, Stuart SN, Thomas P, Thorbjarnarson J. 2011. What does it mean to conserve a (vertebrate) species? *BioScience* 61:39–48.
- Reese, A. M. 1903. The habits of the giant salamander. *Popular Science Monthly* 62:526–531.
- Regeher, K.J., H. Simpson, E.J. Chapman, and P.J. Petokas. 2012. Occurrence of the fungal pathogen *Batrachochytrium dendrobatidis* among Eastern Hellbender populations (*Cryptobranchus a. alleganiensis*) within the Allegheny-Ohio and Susquehanna River drainages, Pennsylvania, USA. *Herpetological Review* 43:150–159.
- Renner, R. 2009. Salt-loving algae wipe out fish in Appalachian stream. *Environmental Science and Technology*, 43:9046–9047.
- Richgels, K.L.D, R.E. Russell, M.J. Adams, C.L. White, and E.H.C. Grant. 2016. Spatial variation in risk and consequence of *Batrachochytrium salamandrivorans* introduction in the USA. *R. Soc. open sci.* 3:150616.
- Roble, K. 2012. Email from Kenneth Roblee, Senior Wildlife Biologist, New York Department of Environmental Conservation, Buffalo, NY to Jeromy Applegate, USFWS, Columbus, OH (May 18, 2012).
- Roell, M.J. 1999. Sand and gravel mining in Missouri stream systems: aquatic resource effects and management alternatives. Missouri Department of Conservation, Columbia, 25 pp.
- Rohr, J.R., A.M. Schotthoefer, T.R. Raffel, H.J. Carrick, N. Halstead, J.T. Hoverman, C.M. Johnson, L.B. Johnson, C. Lieske, M.D. Piwoni, P.K. Schoff, and V.R. Beasley. 2008. Agrochemicals increase trematode infections in a declining amphibian species. *Nature* 455:1235–1240.
- Rollins-Smith, L.A. and P.J. Blair. 1993. The effects of corticosteroid hormones and thyroid hormones on lymphocyte viability and proliferation during development and metamorphosis of *Xenopus laevis*. *Differentiation* 54:155–160.
- Romero, L.M. and L.K. Butler. 2007. Endocrinology of Stress. *International Journal of Comparative Psychology* 20:89–95.
- Routman, E. J., R. Wu, and A.R. Templeton. 1994. Parsimony, molecular evolution, and biogeography: the case of the North American giant salamander. *Evolution* 48:1799–1809.

- Routledge, E.J., D. Sheahan, C. Desbrow, G.C. Brighty, M. Waldock, and J.P. Sumpter. 1998. Environmental Science and Technology 32:1559–1565.
- Ruckelhaus, M., P. McElhany and M.J. Ford. 2002. Recovering species of conservation concern: are populations expendable? In The Importance of Species: Perspectives on Expendability and Triage. Edited by P. Kareiva and S. Levin. Princeton University Press, Princeton, NJ. p. 305–329
- Sabatino, S. J., and E. J. Routman. 2009. Phylogeography and conservation genetics of the hellbender salamander (*Cryptobranchus alleganiensis*). Conservation Genetics 10:1235–1246.
- Sams, J.I., and K.M. Beer. 2000. Effects of coal-mine drainage on stream water quality in the Allegheny and Monongahela river basins - sulfate transport and trends. Water-Resources Investigations Report 99–4208. U.S. Geological Survey, National Water-Quality Assessment Program, Lemoyne, PA, 17 pp.
- Santucci, V.J., Gephard, S.R., and Pescitelli, S.M. 2005. Effects of multiple low-head dams on fish, macroinvertebrates, habitat, and water quality in the Fox River, Illinois. North American Journal of Fisheries Management 25:975–992.
- Scott, F. 2009. Email from Floyd Scott, Professor, Austin Peay State University, Clarksville, TN to Jeromy Applegate, USFWS, Columbus, OH (March 11, 2009).
- Sedlak, D.L., J.L. Gray, and K.E. Pinkston. 2000. Understanding microcontaminants in recycled water. Environmental Science and Technology 34:508A–515A.
- Sgro, C.M., Lowe, A.J. and Hoffmann, A.A., 2011. Building evolutionary resilience for conserving biodiversity under climate change. Evolutionary Applications 4:326–337.
- Shaffer ML, Stein MA. 2000. Safeguarding our precious heritage. Page 301–321 in Stein BA, Kutner LS, Adams JS, editors. Precious heritage: the status of biodiversity in the United States. New York: Oxford University Press.
- Smith, B. G. 1907. The life history and habits of *Cryptobranchus alleganiensis*. Biological Bulletin 13:5–39.
- Smith, K.M., C. Zambrana-Torrel, A. White, M. Asmussen, C. Machalaba, S. Kennedy, K. Lopez, T.M. Wolf, P. Daszak, D.A. Travis, and W.B. Karesh. 2017. Summarizing US wildlife trade with an eye toward assessing the risk of infectious disease introduction. EcoHealth 14:29–39.
- Soeder, D.J., and W.M. Kappel. 2009. Water resources and natural gas production from the Marcellus Shale. U.S. Geological Survey, Fact Sheet 2009–3032.
- Souza, M.J., M.J. Gray, P. Colclough, and D.L. Miller. 2012. Prevalence of infection by *Batrachochytrium dendrobatidis* and Ranavirus in Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) in Eastern Tennessee. Journal of Wildlife Diseases 48:560–566.

- Speirs-Bridge A, Fidler F, McBride M, Flander L, Cumming G, and Burgman M. 2010. Reducing overconfidence in the interval judgments of experts. *Risk Analysis* 30:512–523.
- Stegen, G., F. Pasmans, B.R. Schmidt, L.O. Rouffaer, S.V. Praet, M. Schaub, S. Canessa, A. Laudelout, T. Kinet, C. Adriaensen, F. Haesebrouck, W. Bert, F. Bossuyt, and A. Martel. 2017. Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. *Nature* 544:353–356.
- Storrs, S.I. and J.M. Kiesecker. 2004. Survivorship patterns of larval amphibians exposed to low concentrations of atrazine. *Environmental Health Perspectives* 112:1054–1057.
- Sutton, W. 2024. Email from William Sutton, Assistant Professor, Tennessee State University, Nashville, TN to Jeromy Applegate, USFWS, Columbus, OH (April 17, 2024).
- Sutton, W. B., M. Grisnik, L. A. Williams, and J. D. Groves. 2023. Climatic and landscape vulnerability of the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*). *Global Ecology and Conservation* 46:1–17.
- Swanson, P. L. 1948. Notes on the amphibians and reptiles of Venango County, Pennsylvania. *The American Midland Naturalist* 40:362–371.
- Taber, C. A., R. F. Wilkinson, Jr., and M. S. Topping. 1975. Age and growth of hellbenders in the Niangua River, Missouri. *Copeia* 1975:633–639.
- Taylor, S.E., R. Rummer, K.H. Yoo, R.A. Welch, and J.D. Thompson. 1999. What we know and don't know about water quality at stream crossings. *Journal of Forestry* 97:12–17.
- Teplitsky, C., Robinson, M.R. and Merilä, J., 2014. Evolutionary potential and constraints in wild populations. *Quantitative genetics in the wild*, pp. 190–208.
- Terrell, K. 2012. Email from Kimberly Terrell, Research Fellow, Smithsonian Conservation Biology Institute, Washington, DC to Jeromy Applegate, USFWS, Columbus, OH (August 5, 2012).
- Terrell, K. A., R. P. Quintero, V. A. Galicia, E. Bronikowski, M. Evans, J. D. Kleopfer, S. Murray, J. B. Murphy, B. D. Nissen, and B. Gratwicke. 2021. Physiological impacts of temperature variability and climate warming in hellbenders (*Cryptobranchus alleganiensis*). *Conservation Physiology* 9:1–11.
- Tonione, M., J. R. Johnson, and E. J. Routman. 2011. Microsatellite analysis supports mitochondrial phylogeography of the hellbender (*Cryptobranchus alleganiensis*). *Genetica* 139:209–219.
- TVA (Tennessee Valley Authority). 1971. Stream length in the Tennessee River Basin. Knoxville, Tennessee. 25 pp.
- Ultsch, G. R., and J. T. Duke. 1990. Gas exchange and habitat selection in the aquatic salamanders *Necturus maculosus* and *Cryptobranchus alleganiensis*. *Oecologia* 83:250–258.

- Unger, S. D. 2018. Scavenging behavior of the aquatic Eastern Hellbender salamander (*Cryptobranchus alleganiensis*) in North Carolina. *Journal of the North Carolina Academy of Science* 134:1–2.
- Unger, S. D., C. Bodinof Jachowski, L. Diaz, and L. Williams. 2020b. Adult female Eastern Hellbender *Cryptobranchus alleganiensis* (Cryptobranchidae) conspecific cannibalism confirmed via DNA barcoding. *Herpetology Notes* 13:169–170.
- Unger, S. D., C. Bodinof-Jachowski, L. Diaz, and L. A. Williams. 2020c. Observations on habitat preference of juvenile Eastern Hellbender salamanders (*Cryptobranchus alleganiensis*). *Acta Ethologica* 23:119–124.
- Unger, S., J. D. Groves, and L. A. Williams. 2021a. Factors influencing occupancy and detection probability of larval *Cryptobranchus a. alleganiensis* in North Carolina, USA. *Herpetological Conservation and Biology* 16:157–172.
- Unger, S. D. and C. R. Hickman. 2020. A content analysis from 153 years of print and online media shows positive perceptions of the hellbender salamander follow the conservation biology. *Biological Conservation* 246:1–10.
- Unger, S.D., C.R. Lawson, J.D. Groves, S.F. Spear, and C.E. Moore. 2016. *Cryptobranchus alleganiensis alleganiensis* (Eastern Hellbender): unusual mortality. *Herpetological Review* 47:639.
- Unger, S. D., O. E. Rhodes, Jr., T. M. Sutton, and R. N. Williams. 2013. Population genetics of the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) across multiple spatial scales. *PLOS ONE* 8:1–14.
- Unger, D. U., L. A. Williams, L. Diaz, and C. Bodinof Jachowski. 2020a. DNA barcoding to assess diet of larval Eastern Hellbenders in North Carolina. *Food Webs* 22:e00134.
- Unger, S. D., L. A. Williams, C. R. Lawson, and J. D. Groves. 2020b. Using trail cameras to assess recreation in Hellbender streams of North Carolina National Forest. *Journal of the Southeast Association of Fish and Wildlife Agencies* 7:255–262.
- Unger, S.D., L.A. Williams, J.D. Groves, C.R. Lawson, and W.J. Humphries. 2017. Anthropogenic associated mortality in the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*). *Notes of the Southeastern Naturalist* 16:N9–N13.
- Unger S. D. and R. N. Williams 2015. Genetic analysis reveals multiple parentage in captive reared Eastern Hellbender salamanders (*Cryptobranchus alleganiensis*). *Zoo Biol.* 34:535–537.
- Unger, S. D., L. A. Williams, J. Rash, L. Etchison, D. Owensby, and J. D. Groves. 2021b *Cryptobranchus alleganiensis alleganiensis* (Eastern Hellbender) larval predation. *Herpetological Review* 52:817.

United States Forest Service (USFS). 2016. National visitor use monitoring survey results: US Forest Service national summary report data collected FY 2011 through FY 2015. Available online at <https://www.fs.fed.us/recreation/programs/nvum> (accessed Nov. 20, 2017).

USEPA (U.S. Environmental Protection Agency). 1997. Urbanization and streams: studies of hydrologic impacts. EPA 841-R-97-009. Office of Water, Washington, D.C. 18 pp.

USEPA (U.S. Environmental Protection Agency). 2011. A Field-Based Aquatic Life Benchmark of Conductivity in Central Appalachian Streams. EPA-600-R-10-023F. Office of Research and Development, Washington, DC.

USEPA (U.S. Environmental Protection Agency). 2017. Ecoregions. <https://www.epa.gov/eco-research/ecoregions>. Accessed November 2, 2017.

USEPA (U.S. Environmental Protection Agency). 2017. Section 404 of the Clean Water Act - Exemptions to Permit Requirements. <https://www.epa.gov/cwa-404/exemptions-permit-requirements> (accessed 11/20/2017).

U.S. Fish and Wildlife Service. 2013. Endangered species status for the fluted kidneyshell and slabside pearl mussel, Final Rule. 78(187) FR 59269–59287.

U.S. Fish and Wildlife Service. 2016. Injurious wildlife species; listing salamanders due to risk of salamander chytrid fungus. 81(8) FR 1534–1556.

Durland Donahou, A., W. Conard, K. Dettloff, A. Fusaro, and R. Sturtevant. 2024. *Faxonius rusticus* (Girard, 1852): U.S. Geological Survey, Nonindigenous Aquatic Species Database, Gainesville, FL, <https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=214>, (accessed 8/15/2024).

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* 37:130–137.

Waters, T.F. 1995. Sediment in streams: sources, biological effects, and control: American Fisheries Society Monograph 7, 251 pp.

Wethington, K. 2017. Email from Keith Wethington, Fish and Wildlife Information System Supervisor, Kentucky Department of Fish and Wildlife Resources, Frankfort, KY, to Jeromy Applegate, USFWS, Columbus, OH (May 9, 2017).

Wheeler, B.A. 1999. Status of the Ozark Hellbender (*Cryptobranchus alleganiensis bishopi*): a long-term assessment. Unpublished master's thesis, Missouri State University, Springfield, 113 pp.

Wheeler, B.A., E. Prosen, A. Mathis, and R.F. Wilkinson. 2003. Population declines of a long-lived salamander: a 20+ year study of hellbenders, *Cryptobranchus alleganiensis*. *Biological Conservation* 109:151–156.

- White, R., S. Jobling, S.A. Hoare, J.P. Sumpter, and M.G. Parker. 1994. Environmentally persistent alkylphenolic compounds are estrogenic. *Endocrinology* 135:175–182.
- Williams, L. 2011a. Email from Lori Williams, Mountain Wildlife Diversity Biologist, North Carolina Wildlife Resources Commission, Fletcher, NC to Jeromy Applegate, USFWS, Columbus, OH (April 3, 2011).
- Williams, L. 2011b. Email from Lori Williams, Mountain Wildlife Diversity Biologist, North Carolina Wildlife Resources Commission, Fletcher, NC to Jeromy Applegate, USFWS, Columbus, OH (October 6, 2011).
- Williams, L. 2012. Email from Lori Williams, Mountain Wildlife Diversity Biologist, North Carolina Wildlife Resources Commission, Fletcher, NC to Jeromy Applegate, USFWS, Columbus, OH (August 6, 2012).
- Williams, L. 2016. Email from Lori Williams, Mountain Wildlife Diversity Biologist, North Carolina Wildlife Resources Commission, Fletcher, NC to Jeromy Applegate, USFWS, Columbus, OH (December 20, 2016).
- Williams, L. 2017. In-person discussion during August 29–31 Eastern Hellbender Expert Elicitation Meeting. Williamstown, WV.
- Williams, L. 2024. Email from Lori Williams, Wildlife Diversity Biologist, North Carolina Wildlife Resources Commission, Fletcher, NC to Jeromy Applegate, USFWS, Columbus, OH (March 28, 2024).
- Williams, L. A. and J. D. Groves. 2014. Prevalence of the amphibian pathogen *Batrachochytrium dendrobatidis* in Eastern hellbenders (*Cryptobranchus a. alleganiensis*) in Western North Carolina, USA. *Herpetological Conservation and Biology* 9:454–467.
- Williams, L.A. and J.D. Groves. 2012. Email from Lori Williams, Mountain Wildlife Diversity Biologist, North Carolina Wildlife Resources Commission, Fletcher, NC to Jeromy Applegate, USFWS, Columbus, OH (June 6, 2012).
- Williams R.D., J.E. Gates, C.H. Hocutt, and G.J. Taylor. 1981a. The hellbender: a nongame species in need of management. *Wildlife Society Bulletin* 9:94–100.
- Williams R.D., J.E. Gates and C.H. Hocutt. 1981b. An evaluation of known and potential sampling techniques for hellbender, *Cryptobranchus alleganiensis*. *Journal of Herpetology* 15:23–27.
- Williams, L. A., J. M. Rash, J. D. Groves, L. L. Stroup, and D. Blatny. 2019. Engaging North Carolina’s trout anglers and other stakeholders to help conserve Eastern Hellbenders. *Journal of the Southeastern Association of Fish and Wildlife Agencies* 6:166–174.
- Wineland, S. M., S. M. Welch, T. K. Pauley, J. J. Apodaca, M. Olszack, J. J. Mosher, J. N. Holmes, and J. L. Waldron. 2018. Using environmental DNA and occupancy modelling to

identify drivers of Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) extirpation. *Freshwater Biology* 64:208–221.

Wolfe, B. 2012. Email from Barbara Wolfe, Vice President of Animal Health, Columbus Zoo and the Wilds, Columbus, OH to Jeromy Applegate, USFWS, Columbus, OH (May 24, 2012).

Woodhams, D.C., K. Ardipradja, R.A. Alford, G. Marantelli, L.K. Reinert, and L.A. Rollins-Smith. 2007. Resistance to chytridiomycosis varies among amphibian species and is correlated with skin peptide defenses. *Animal Conservation* 2007:1–9.

WVDEP (West Virginia Department of Environmental Protection). 1997. An ecological assessment of the Elk River watershed, report number 05050007 - 1997. Watershed Assessment Program, Division of Water Resources, Charleston, WV, 122 pp.

WVDEP (West Virginia Department of Environmental Protection). 2014. West Virginia integrated water quality monitoring and assessment report 2014. Charleston, West Virginia, 291 pp.

Yap, T.A., N.T. Nguyen, M. Serr, A. Shepack, and V.T. Vredenburg. 2017. *Batrachochytrium salamandrivorans* and the risk of a second amphibian pandemic. *EcoHealth* 14:851–864.

Zackay, A., 2007. Random Genetic Drift and Gene Fixation.

(<https://pdfs.semanticscholar.org/1dcb/03aa10e74b1fc3d2dd1db06762cc19ce3f5a.pdf>)