

Virginia's Amphibians: Status, Threats and Conservation

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ABSTRACT

Virginia's diverse environments support 84 amphibian species (anurans and caudates), making it the third highest state in terms of species richness. However, the Commonwealth matches the global trend in declining amphibian populations with over one-third of its amphibian species in conservation need. The Species of Greatest Conservation Need included in the most recent Virginia Wildlife Action Plan cut across amphibian families and ecoregions. It is challenging to ascertain the exact cause of most of the population declines. In one degree or another, all of the global threats to amphibians exist within Virginia's borders. While an active research program on amphibians exists in the Commonwealth, there are an abundance of data deficient topics where research can help detect and inform the cause of these declines, as well as evaluate management efforts. On a positive note, there are a large number of existing conservation efforts being undertaken across Virginia that directly or indirectly benefit local amphibians.

"These foal and loathsome animals are abhorrent because of their cold body, pale color, cartilaginous skeleton, filthy skin, fierce aspect, calculating eye, offensive smell, harsh voice, squalid habitation, and terrible venom; and so their Creator has not exerted his powers to make many of them." Carolus Linnaeus 1758

INTRODUCTION

Some people would likely still describe amphibians as Linnaeus once did, but today we know they are a diverse class of vertebrates, many in number and integral components of ecosystems (Hocking and Babbitt 2014). They are ecologically recognized for their energy efficiency and nutrient cycling. Amphibians serve as prey to many different organisms and as predators consuming vast numbers of insects, including those species that are vectors for diseases or cause agricultural damage.

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People have, and continue, to use amphibians for a variety of purposes, including as food, pets and cultural icons. Additionally, the applications of amphibians for human health are wide ranging, including serving as important research subjects and for the treatment of all kinds of ailments (Burggren and Warburton 2007, O'Rourke 2007, Hocking and Babbitt 2014).

Amphibians are the earliest terrestrial Tetrapods, first appearing during the late Devonian Period about 360 million years ago. Their physiological, biological, behavioral and ecological adaptations have enabled them to inhabit every continent except Antarctica. Over 7,400 species of amphibians have been described globally across three orders: Anura (frogs and toads), Caudata (salamanders and newts) and Gymnophiona (caecilians) (refer to www.amhibiaweb.org for the most up to date species list). Amphibians are ectothermic organisms mostly known for their permeable skin, complex life cycles, limited mobility, and strong site fidelity. They have anamniotic (jelly-like) eggs with dozens of reproductive modes, ranging from internal to external fertilization, and small clutches of guarded eggs on land to thousands of eggs deposited in standing water. The same characteristics which make amphibians unique are the very attributes which also make them susceptible to changes in the environment. For these reasons, amphibians are considered good indicator species of ecosystem health (Blaustein et al. 1994, Welsh and Droege 2001, Davic and Welsh 2004, Hopkins 2007). However, if the responses of these organisms are truly indicative of what is happening in the environment, there is great cause for continued concern. Over the past few decades, amphibian populations across the globe have experienced declines, local extirpations and species extinctions (Blaustein and Wake 1990, Gibbons et al. 2000, Stuart et al. 2004, Lannoo 2005, Bishop et al. 2012). Amphibians are now considered one of the most threatened groups of organisms globally, with approximately 40% of species threatened (Stuart et al. 2004, Bishop et al. 2012). This paper explores the status of amphibian populations across Virginia, their potential threats, and actions taken to conserve them.

VIRGINIA'S AMPHIBIANS

Noted for their loud calls, the first printed record of frogs in Virginia is from Robert Beverley's *The History and Present State of Virginia* in 1705 (Mitchell 2013). However, it was not until the early 1900s when Emmet Reid Dunn conducted his seminal work on Virginia's amphibians that the true diversity was realized (Mitchell 2013). Even today, studies using genetic techniques are describing new species (Tilley et al. 2008, Fienberg et al. 2014).

There are currently 84 documented species of anurans (referred to as frogs throughout the remainder of paper) and caudates (referred to as salamanders throughout the remainder of paper) in the Commonwealth of Virginia (Appendix). The Big Levels Salamander (*Plethodon sherando*), Shenandoah Salamander (*P. shenandoah*) and Peaks of Otter Salamander (*P. hubrichti*) are endemic to the Commonwealth; meaning they are found only in Virginia and nowhere else in the world. The other 81 species are found in at least one other adjacent state. The most recent addition to Virginia's species

list came in 2015, with the Atlantic Coast Leopard Frog (*Rana kauffeldi*) (Feinberg et al. 2014).

Virginia has the third highest amphibian diversity of the states (Stein 2002). Supported by a diverse array of habitats, these amphibians span the Commonwealth, from coastal wetlands to mountain top ridgelines. Some species of amphibians are habitat generalists, such as the ubiquitous American Bullfrog (*Lithobates catesbeianus*) which occupies every county in Virginia and a variety of freshwater aquatic habitats. Other species are habitat specialists, such as the rock outcrop residing Green salamander (*Aneides aeneus*).

Virginia has six main ecoregions as described by The Nature Conservancy, including the Cumberland and Southern Ridge and Valley, Southern Blue Ridge, Central Appalachian Forest, Piedmont, Mid-Atlantic Coastal Plain, and the Chesapeake Bay Lowlands. Each region differs in topography, geology, climate and vegetation. Both frogs and salamanders occupy each ecoregion, but they display different patterns of species richness (Figure 1). In general, frogs predominate in the eastern ecoregions, while more salamanders reside in the western ecoregions.

STATUS OF VIRGINIA'S AMPHIBIANS

Species assessments are conducted by multiple organizations and for a variety of purposes. This paper uses established rating systems in discussing the status of Virginia's amphibians (Appendix), including the IUCN Red List, NatureServe Conservation Status (global=GRank and state=SRank), U.S. Fish and Wildlife Service endangered species listing (ESA), State of Virginia endangered species listing (State) and the Virginia Wildlife Action Plan (2005 and 2015 WAP). The authors consider a species of concern to be one that has been ranked as imperiled by at least one of the known ranking systems.

All 28 species of frogs found in Virginia also occur in at least one other state. According to the range-wide assessments (i.e. IUCN, NatureServe GRank and ESA), none of these species are imperiled. On the local level, eight of the 28 species (29% of total frogs) are of conservation concern in Virginia. Five species are listed by both NatureServe SRank and Virginia's WAP, while an additional three species are listed only on the WAP. The Barking Treefrog (*Hyla gratiosa*), listed as State Threatened, is the only State listed species. The Atlantic Coast Leopard Frog is not considered in any ranking system because it is newly described (Feinberg et al. 2014) and therefore no previous data for comparison are available for assessment purposes.

A different story holds true for the salamanders. According to the NatureServe GRank, ten species are of conservation concern across their entire range. The IUCN ranking is in agreement with the NatureServe GRank on eight of these species. According to the NatureServe SRank and Virginia's WAP an additional 19 species are of conservation concern within Virginia. Four of these species, however, are only listed by NatureServe and one additional species only by the WAP. Including all listings, the total salamander species of conservation concern in Virginia is 29 (52% of total). Three of these species are listed as State Threatened or Endangered, including the Mabee's Salamander (*Ambystoma mabeei*) (ST), Eastern Tiger Salamander (*A.*

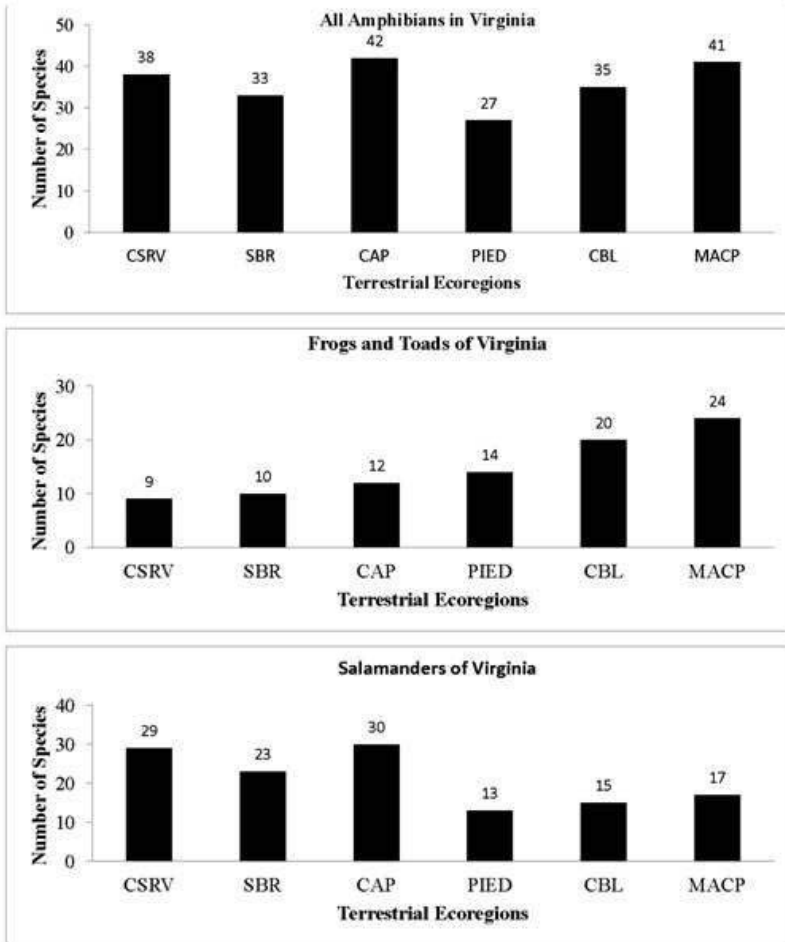


FIGURE 1. Number of Virginia amphibians based on the six terrestrial ecoregions designated by The Nature Conservancy. A species may be represented in more than one ecoregion. Ecoregions include CSRV = Cumberland and Southern Ridge and Valley, SBR = Southern Blue Ridge, CAP = Central Appalachian Forest, PIED = Piedmont, CBL = Chesapeake Bay Lowlands and MACP = Mid-Atlantic Coastal Plain.

tigrinum) (SE) and Shenandoah Salamander (SE)). The Shenandoah Salamander is the only amphibian in Virginia listed by the U.S. Fish and Wildlife Service as Federally Endangered.

Of note are the discrepancies in the different assessments. There may be a number of contributing factors for the differences, but two considerations are worth mentioning in relation to rankings in Virginia. The NatureServe listings were last reviewed on average 11 years ago and in some cases may be outdated. In addition, useful information about species on a state or more local level are not always published in the peer-reviewed literature used in conducting the larger assessments. Considering government biologists and local researchers are consulted in drafting state WAPs, heavier weight on these rankings may be warranted.

Directed by the U.S. Fish and Wildlife Service, the first Virginia WAP was developed in 2005 with 38% of Virginia's amphibians listed as Species of Greatest Conservation Need (SGCN). During the recent 2015 review of the WAP, none of the species on the 2005 list were removed or downgraded in ranking. Although no additional species were added to the list either, the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*), Peaks of Otter Salamander, Cow Knob Salamander (*P. punctatus*) and Weller's Salamander (*P. welleri*) were moved up in ranking to Tier 1 species. The Big Levels Salamander was omitted from consideration in the 2005 WAP because it had just been identified and its status in the revised 2015 WAP is being reviewed. The 2015 WAP is meant to be more detailed than that from 2005. It includes an additional conservation opportunity ranking for listed species, emphasizes habitats, and provides for local action plans with effectiveness measures.

The SGCN listed in the 2015 WAP cut across orders, families and ecoregions. Four of the five families of frogs and five of the six families of salamanders contain species listed in the WAP. When taking into account ecoregions, those frogs found within the Mid-Atlantic Coastal Plain and Chesapeake Bay Lowlands ecoregions appear to be more imperiled than those species farther inland (Table 1). The imperiled salamanders, however, are distributed more broadly across the different ecoregions. For most of these species, the exact cause of decline is unknown. What is known is that amphibians face many threats across the Commonwealth.

ENVIRONMENTAL THREATS AND IMPACTS TO VIRGINIA'S AMPHIBIANS

Virginia's amphibians follow the global trend with nearly 40% of all species listed as SGCN. An abundance of threats are present across the Commonwealth and it is challenging to ascertain the direct cause(s) of decline for each of the amphibian species. Amphibians may be affected directly or indirectly from the threats, which could be acute or chronic. Impacts may manifest in lethal or sublethal manners and affect each species, and even life stage, differently. Furthermore, amphibians are affected not only by individual threats, but likely also by multiple threats acting synergistically. The information presented here is not meant to be an exhaustive review or indicative of all known or possible environmental threats to amphibians. The intent is to highlight the leading threats to amphibians globally and put them in the context of Virginia.

TABLE 1. Distribution of amphibian species by number and percentage (in parentheses) listed as Species of Greatest Conservation Need on the 2015 Virginia Wildlife Action Plan. Some species are listed in more than one ecoregion. Ecoregions include CSRV = Cumberland and Southern Ridge and Valley, SBR = Southern Blue Ridge, CAP = Central Appalachian Forest, PIED = Piedmont, CBL = Chesapeake Bay Lowlands and MACP = Mid-Atlantic Coastal Plain.

	CSRV	SBR	CAP	PIED	CBL	MACP
Anurans	1 (11)	0 (0)	1 (8)	1 (7)	4 (20)	6 (25)
Caudates	11 (38)	7 (30)	9 (30)	2 (15)	4 (27)	7 (41)

Habitat Loss

Changes in habitats are credited with being the largest threat to amphibians globally (Bishop et al. 2012). Maintaining large patches of undisturbed forests, wetlands and other habitats is challenging anywhere where there is a large demand on natural resources and human population. Farming, the leading economic industry in Virginia, covers approximately 32% of Virginia's land (VDACS 2016). The leading factor in loss of forest land, however, is urbanization and development (VDOF 2016). According to the U.S. Census Bureau, Virginia's population is over 8 million, an increase of 5 million since 1950. Since 1977, Virginia has lost over a half million acres of forest land (VDOF 2014) and over 40% of the Commonwealth's wetlands have been lost since colonial times (Booth 2012). Despite loss of habitat, co-author J. D. Kleopfer believes many species listed in the WAP have abundant habitat to maintain viable populations. He believes their limited distribution and dispersal ability within the Commonwealth makes them vulnerable, particularly to stochastic events and climate change.

Roads

Roads as a form of infrastructure are a threat to amphibians globally (Andrews et al. 2008). Amphibians are often seen crossing Virginia's roads during the breeding season, rains, or when roads are adjacent to wetlands or other prime habitat. The vast network of roads across the landscape coupled with the inability of amphibians to quickly escape automobile traffic results in direct impacts (i.e. injury or mortality), as well as indirect affects (i.e. barriers to gene flow, introduction of pollution) (Andrews et al. 2008, Beebee 2013, Cosentino et al. 2014). Despite the prevalence of amphibians on roads in Virginia, there is only one known study which looked at road mortality over five visits in three years of juvenile red-spotted newts (red eft; *Notophthalmus viridescens*) on a portion of Country Road 629 (Mitchell 2000). The highest observed mortality was 182 dead individuals over a 0.25 km distance, but other visits only found 10 or less dead salamanders (Mitchell 2000). Actions have been taken to reduce mass mortality of explosive breeders when they migrate to breeding sites. For instance, Riverside Drive has been closed to facilitate the migration of Spotted Salamanders (*A.*

maculatum) to and from Richmond's James River State Park during spring rains. In addition, the Loudoun Wildlife Conservancy and other organizations have a brigade of citizens who help amphibians cross roads safely during breeding events.

Less evident and harder to mitigate are the indirect impacts of roads (Andrews et al. 2008). Even unpaved roads can create forest-edge effects and negatively impact salamander abundance adjacent to them (Marsh and Beckman 2004, Marsh 2007). Wide paved roads are considered a possible barrier to gene flow of Red-backed Salamanders (*P. cinereus*) (Marsh et al. 2008), although this species also shows genetic differentiation over areas 200 meters or more in contiguous habitat (Cabe et al. 2007). Additionally, pollution originating from roads is a threat to amphibians. Stormwater runoff from roads and paved surfaces is a source of pollution into amphibian habitats. American Bullfrog and Green Frog (*L. clamitans*) tadpoles in highway drainages in Virginia had higher concentrations of heavy metals than their counterparts (Birdsall et al. 1986). Litter (solid waste) along roadsides may also pose a challenge to amphibians. A study conducted to quantify impacts of littered bottles on small mammals along Virginia roads documented trapped amphibians (Benedict and Billester 2004). Nylon landscaping netting often used on roadsides to control erosion has also been observed to entangle and kill frogs (J. D. Kleopfer, pers. obs. 2016).

Pollution

Pollution, whether from a specific episodic event, or chronic input over an extended period of time, can negatively influence amphibians in lethal and sub-lethal ways. There is a breadth of information globally on pollution impacting amphibians, ranging from pesticides as endocrine disruptors to nutrients increasing the prevalence of deformities in amphibians (Karraker 2009, Mann et al. 2009, Bishop et al. 2012, Egea Serrano et al. 2012, Baker et al. 2013). However, there is a great need for researching the potential impacts of pollution on amphibians from agriculture, road deicing and other sources in Virginia. Locally, acidity and mercury are the two pollutants investigated most frequently.

Acid pollution in Virginia originates from both non-point sources, such as acid rain, and point sources, including acid mine drainage. The characteristics of a particular habitat, the species and species' life stages all play roles in the response of amphibians to acidic inputs (for laboratory levels of acidity impacting amphibians refer to Freda et al. 1991, Green and Peloquin 2008). The acid neutralizing capacity (ANC) of a habitat is an important predictor of how a particular system will respond to inputs of acid. In Virginia, habitats with little or no ANC, such as ephemeral ponds in York County, are becoming more acidic over time (Fairman et al. 2013). Many amphibians rely on these types of habitats for breeding. The breeding of Spotted Salamanders decreased over an eight year period in ephemeral ponds with higher acidity and concentrations of aluminum, copper, silicon, and zinc than in other ponds (Blem and Blem 1991). Fairman et al. (2013) also documented a decrease in pond occupancy by Mabee's Salamanders in highly acidic ponds, but it was difficult to make a direct causal relationship to acidity.

In other cases where the measured pH of aquatic habitats is decreasing, the impact on amphibian populations is not evident. In Shenandoah National Park, where stream

acidity has been increasing, amphibian species richness and abundance have remained stable (Mitchell 1999, Grant et al. 2005). The acidity of these streams, however, has not dropped below pH 4.9 (Grant et al. 2005) and laboratory studies indicate Virginia stream salamanders show susceptibility starting around pH 4.2 (Green and Peloquin 2008). In models predicting occupancy of plethodontid salamanders, pH is a covariate with low level support in candidate models (Grant et al. 2014). The acidity levels in the streams included in the models were quite low (avg. pH 5.5) but a stronger negative association may become evident at higher acidity levels (Grant et al. 2014).

Mercury is another pollutant which enters the environment from both point and non-point sources. Widespread emissions result in wet and dry deposition of mercury across the landscape. The mercury then becomes available for uptake by amphibians through their skin or by ingesting contaminated prey. Hamed (2014) found Black-bellied Salamanders (*Desmognathus quadramaculatus*) on Whitetop Mountain to contain extremely high levels of mercury. Furthermore, salamanders from higher elevations and north facing slopes, where deposition was greater, had higher levels of mercury than other salamanders. Interestingly, mercury levels in salamanders from museum samples taken in the 1950s from the same location were found to have even higher levels of mercury, indicating that regulations have been successful in reducing levels of mercury pollution (Hamed 2014).

Old manufacturing factories in Virginia, such as those on the South River and North Fork of the Holston River, are sources of point source mercury inputs. Amphibians from downstream sites have higher mercury levels than reference sites above the pollution source (Bergeron et al. 2010, Bergeron et al. 2011, Burke et al. 2010). Contaminated Two-lined Salamanders (*Eurycea* spp.) from the study area demonstrated altered locomotor performance and prey capture (Burke et al. 2010). American Toads (*Anaxyrus americanus*) transferred mercury to eggs, although this did not appear to impact population numbers (Bergeron et al. 2011).

Introduced, Naturalized and Invasive Species

Often considered a type of biological pollution, invasive species have been linked to declines in native amphibian populations around the globe. There are no known naturalized or invasive amphibian species in Virginia (VDGIF 2014), although there are records of some non-native amphibian species being introduced into the Commonwealth without establishing naturalized populations. The U.S. Geological Service Aquatic Nuisance Database (2015) documents introductions of Cuban Tree Frogs (*Osteopilus septentrionalis*) and African Clawed Frogs (*Xenopus laevis*). The Cuban Treefrogs were not expected to be able to survive the mid-Atlantic winter and the African Clawed Frogs are believed to have been eradicated during two collection events (USGS 2012). At least one known native Virginia amphibian was introduced outside its range. Between 1935-1945 the Northern Gray-Cheeked Salamander (*P. montanus*, formerly *P. jordani*) was introduced to Mountain Lake Biological Station for research purposes. A breeding population was established, but the addition of this species to the community has no apparent impact on the local salamander species, particularly its congener the Slimy Salamander (*P. glutinosus*) (Cunningham and Rissler 2013).

Unfortunately, naturalized and invasive species of other taxa are present in Virginia. No study has linked any one of these species to declines in local amphibians, but the threat is possible. For instance, the invasive Snakehead Fish (*Channidae* spp.) are known to consume amphibians (Courtenay and Williams 2004), as are the introduced Brown Trout (*Salmo trutta*) (Gratwicke 2008). Feral and pet cats, believed to be killing millions of birds a year, are also known to kill and eat amphibians (Mitchell and Beck 1992). Beyond predation, invasive species can also alter amphibian habitat, such as the Hemlock Woolly Adelgid (*Adelges tsugae*), an insect which kills hemlock trees (*Tsuga* spp.) causing an increase in soil erosion and stream sedimentation (VISWG 2012). Feral Hogs (*Sus scrofa*) are a growing issue in Virginia (VDGIF 2016). Their impacts on local amphibian populations are unknown, but among eating amphibians as prey, they have also been known to destroy wetlands used as amphibian breeding sites (Jolley et al. 2010).

Emerging Infectious Diseases

A number of pathogens and parasites, including trematodes, fungi, bacteria, and viruses have been associated with declines and deformities of amphibian species globally (Daszak et al. 2003). While malformations of amphibians have been documented in Virginia, the numbers are not high enough to warrant concern (J.D. Kleopfer, pers. Obs. 2015). The discussion here only focuses on a few higher profile diseases.

Ranavirus is a genera of DNA-based iridoviruses found in amphibians, reptiles and fish. Each taxon experiences different symptoms, but often the disease becomes apparent only when a mass die-off occurs. Die-offs occur suddenly and, at least in most amphibians, are usually associated with the metamorphosing stages. There are unpublished data and anecdotal evidence of mass amphibian die-offs occurring in Virginia, but no published records. One author (J. Sevin, unpublished) knows of multi-year die offs of Wood Frog (*L. sylvaticus*) metamorphosing tadpoles in two artificial ponds in Warren County.

Infection by *Ranavirus*, however, does not always result in disease and mortality. The presence of *Ranavirus* has been detected in the wild in a number of salamander species in Virginia without any known mortality (Davidson and Chambers 2011, Hamed et al. 2013, Blackburn et al. 2015). Nelson (2010) documented the presence of *Ranavirus* in anuran tadpoles associated with some Virginia fish-hatcheries. It is possible some amphibians testing positive for *Ranavirus* may be asymptomatic and serve as reservoirs for the pathogens (Goodman and Ararso 2012). Likewise, *Ranavirus* is not ubiquitous in amphibian communities. Muletz et al. (2014) did not find *Ranavirus* across an array of amphibian species. Surprisingly, Goodman and Ararso (2012) did not find *Ranavirus* in frogs in Prince Edward County, even when it was detected in syntopic aquatic turtles.

Much about *Ranavirus* is still unknown, including the best method to use in its detection. Virginia is one of several states taking part in a Regional Conservation Need project to document the extent of *Ranavirus*. Preliminary results of this study indicate that 11 of 25 breeding ponds surveyed in Virginia would have tested positive for *Ranavirus* using older analytical techniques (S. Smith, MDNR, pers. comm.).

However, use of a new stricter protocol would only classify one of these sites as positive.

Another notorious amphibian disease is the fungus *Batrachochytrium dendrobatidis* (*Bd*), which causes chytridiomycosis infections. *Bd* has been linked to amphibian mass mortalities globally (Bishop et al. 2012). In Virginia, there have been multiple studies to investigate the prevalence of *Bd* both in frogs and salamanders. Despite being detected in various counties in the Commonwealth and across a diversity of amphibian species, the prevalence of *Bd* has been relatively low (Rothermel et al. 2008, Hossack et al. 2010, Davidson and Chambers 2011, Gratwicke et al. 2011, Eskew et al. 2014, Goodman and Ararso 2012, Hughey et al. 2014, Muletz et al. 2014, Bales et al. 2015). A recent study of Green Salamanders found the highest prevalence of *Bd* in the Commonwealth at 15% of the sample (6 of 41 individuals) (Blackburn et al. 2015). There are no published records of high levels of zoospore infections or observations of dead or dying amphibians attributed to chytridiomycosis in Virginia. Furthermore, Muletz et al. (2014) found no evidence of *Bd* in museum specimens of salamanders from Virginia in the 1970s-1980s, when Highton (2005) noted many population declines in the area. Unlike many other areas around the world, it appears *Bd* has not resulted in population declines of amphibian species in Virginia. The Commonwealth also initiated early measures to reduce the spread of amphibian related diseases by prohibiting the sale or possession of African Clawed Frogs and the African Dwarf Frogs (*Hymenochirus* spp.), believed to be carriers and transmitters of the disease, unless a permit is issued.

It is worth noting that a novel fungal species, *B. salamandrivorans* (*Bsal*), was first linked to die offs of Fire Salamanders (*Salamandra salamandra*) in Europe (Martel et al. 2014). In laboratory experiments, salamander species found to occur in Virginia, such as the Red-spotted Newt, were deemed to be highly susceptible to *Bsal* (Martel et al. 2014). Early studies have not detected the presence of *Bsal* in wild salamander species from the central Appalachians (Muletz et al. 2014, Bales et al. 2015). However, the potential transmission of *Bsal* or similar novel pathogens to the United States through the amphibian pet trade is a great concern. Recently, the U.S. Fish and Wildlife Service took measures to reduce potential *Bsal* transmission by publishing an interim report to prevent the trade of 201 species of salamanders (USFWS 2016).

The bacterium *Salmonella* is an infectious disease associated with herpetofauna and is briefly mentioned here because it is a zoonotic disease (transfers between wildlife and humans). *Salmonella* poisoning can result in human illness or death. Regulations already existed in Virginia limiting the sale of small turtles, but in 2009, 11 cases of *Salmonella* were reported in Virginia from aquatic pet frogs (VDH 2011). Individuals in contact with amphibians should wash their hands with soap and water following contact.

Climate Change

Climate change is a global threat that will have local repercussions with higher sea levels, increasing temperatures and storm events, as well as seasonal changes in precipitation. The 2015 WAP seeks consideration of climate change as a threat to species and the inclusion of related management efforts in action plans. Virginia's

Climate Modeling and Species Vulnerability Assessment used three amphibians as candidate species in their models: the Oak Toad (*A. quercicus*), Cope's Gray Treefrog (*Hyla chrysoscelis*) and Wood Frog (*L. sylvaticus*) (Kane et al. 2013). The particular models, which used 40 variables, indicate that expected conditions in Virginia with climate change may actually be favorable to these three species (Kane et al. 2013). The report cautioned that having more favorable conditions for amphibians does not mean their distribution will increase, since they have limited dispersal ability and a number of anthropogenic barriers exist. It should also be mentioned that no salamanders were included in this particular study.

Recent climate models conducted by Milanovich et al. (2010) indicate all Appalachian salamanders will experience range contractions in the future. This is supported by the models of Sutton et al. (2015) which estimated climate niches for salamanders would decrease by 2050. Some species, including the State threatened Mabee's Salamander, are predicted to lose most of their climate niches. Furthermore, Hamed (2014) also used down scaled climate models to predict that three of 12 salamanders on Whitetop Mountain will likely become extirpated by 2070, while the other nine will have to move up in elevation to survive the changing conditions. In addition, stream temperature has been shown to be an important variable in salamander models (Grant et al. 2014).

Current evidence linking existing changes in amphibian populations to climate change are few and far between. Some papers have documented how unseasonable changes in precipitation and temperature in Virginia, such as warm fronts occurring during January, affected the breeding phenology of amphibians and survival of individuals (Briggs 1994, Bulmer and Cherok 1998, Gibson et al. 2008). Surveys conducted on Whitetop Mountain show at least some salamander species have either expanded or contracted their ranges compared to 1950s values of the mean elevations (Hamed 2014). However, these changes were not able to be correlated with changes in temperature as no temperature readings were available for Whitetop Mountain and the closest measurements showed no temperature changes over that time period. Another recent study documented the body size of plethodontid salamanders has decreased over the last 55 years and attributed the decrease to an increased metabolism due to climate change (Caruso et al. 2014). However, Connette et al. (2015) cautioned that other factors could have produced the change in body size.

CONSERVATION INITIATIVES

Numerous conservation actions are underway across Virginia to conserve amphibians, as well as to directly address many of the threats. The information below is meant to illustrate the breadth of efforts and highlight some of the initiatives being undertaken.

Habitat Preservation, Acquisition and Restoration

Acquiring, preserving and restoring amphibian habitats are priority conservation actions. Land is preserved in Virginia by State agencies as wildlife management areas, parks, forests and more. No land acquisitions or preservation, however, have been directly attributed to amphibians, but they are secondary benefactors of these actions.

According to Virginia Department of Conservation and Recreation's Conservation Lands Database, over 16,000 square kilometers (~16% of the Commonwealth's land) is preserved by private owners, organizations and government agencies. State laws also protect certain types of habitat that are privately owned, such as the commitment for "no net loss" to the amount and function of wetlands. Permits for impacts on surface waters, including wetlands, must be obtained by the Virginia Department of Environmental Quality. Financial and technical resources are also made available through the State and Federal government to help private land owners place their property under conservation easements and implement actions, such as creating stream buffers.

Amphibians in Virginia benefit from small and large scale practices, such as installation of backyard ponds and restoration of large wetland areas. Multiple agencies in the Commonwealth undertake initiatives that help improve amphibian habitat. The Virginia Aquatic Resources Trust Fund is an example of one such program. The Trust Fund, a collaboration of the Army Corps of Engineers, the Virginia Department of Environmental Quality and The Nature Conservancy, is a mitigation project for streams and wetlands where the government has permitted certain impacts to take place. Over \$42 million has been invested in 121 mitigation projects. Another example comes from the Virginia Department of Mines, Minerals and Energy Orphaned Land Program where land undergoes reclamation. For instance, acid mine drainage from a lead-zinc mine and an adjacent gold mine resulted in such poor water quality of Knights Branch that no flora or fauna could survive. A reclamation process begun in 2001 and ending in 2005 resulted in decreased acidity and metals. As the habitat was restored Southern Leopard Frogs (*L. sphenoccephalus*) and Pickerel Frogs (*L. palustris*) were observed (Sobeck et al. 2008). While State and Federal agencies have supported many initiatives across Virginia, various non-profit organizations and many individuals have also protected and restored habitat.

The saying "if you build it, they will come" easily applies to amphibians as they are known to use created and restored habitats (Brown et al. 2012). Non-profit organizations play a significant role in facilitating habitat conservation through providing funding and expertise, as well as recruiting a large volunteer work force. For instance, the Chesapeake Bay Foundation has a number of initiatives, including planting riparian buffers along streams and rivers. Other organizations initiate efforts that remove invasive species from habitats. Individuals are taking action on their own properties to enhance amphibian habitat, such as blocking cattle from streams to minimize erosion, building ephemeral ponds, leaving rocks and woody debris on lawns, and much more.

Outreach and Education

Amphibians may not be considered charismatic megafauna, but they certainly do have a following in Virginia. Many of Virginia's zoological facilities have exhibits on herpetofauna that draw large crowds. While many exhibits include non-native species, groups such as the National Zoo are highlighting more of the areas unique amphibian diversity. The National Zoo's Reptile Discovery Center includes a salamander lab for

the public to learn about ongoing research on local amphibians and a newly opened *Jewels of the Appalachia* exhibit that includes 10 native species of salamanders.

Herpetologists affiliated with government agencies, academic institutions and non-profit organizations often conduct lectures for the public on topics related to amphibian conservation. County, State and National Parks also offer a wide variety of interpretive signage, classes, camps and public events related to amphibians. Fairfax County Park Authority, for example, offers programs for scouts where they can earn their Reptile and Amphibian Merit Badge. In addition to informal opportunities, the Virginia Aquarium and Marine Science Center and the Virginia Living Museum are among the groups providing lessons correlated to Virginia's standards of learning for teachers to easily integrate amphibian education into their curricula.

A number of organizations and agencies provide resources for youth and adults on amphibians. Online videos and other social media offer a plethora of information on amphibians at anyone's fingertips. The topics range from identifying species to creating a backyard habitat. VDGIF's designation of 2015 as the *Year of the Frog*, which was promoted through public events and social media outlets, was extremely popular. The Virginia Herpetological Society's (VHS) website, which is a great resource for disseminating information on local amphibians, receives on average 46,000 visitors per month. Professionals also have the opportunity to share and gain knowledge through VHS and the regional Partners in Amphibian and Reptile Conservation (PARC) groups.

Citizen Science

Individuals interested in using their education to take a more active role in amphibian conservation can engage in various citizen science initiatives being offered in Virginia. Since 1999 VDGIF's Frog and Toad Calling Survey has been part of the North American Amphibian Monitoring Program (NAAMP) which uses volunteers from across the Commonwealth to survey various wetland habitats for frogs and toads. There are 53 designated routes ensuring a wide variety of habitats are covered. The NAAMP data are then used by the U.S. Geological Survey and other researchers to study trends. A similar initiative offered through the Association of Zoos and Aquariums is the nationally run FrogWatch USA program. Volunteers from local chapters document frog and toad calls. There are currently eight chapters across the Commonwealth.

Not limiting citizen science to just frogs, VHS organizes various surveys each year where amateur and professional herpetologists help inventory the diversity of amphibians in various locations across Virginia. Citizens can also be engaged in collecting important data on habitat, such as vernal pools. Virginia Commonwealth University, Virginia Master Naturalists and a number of state agencies are collaborating to locate, characterize and monitor vernal pools on public lands (S. Watson, VDGIF, pers. comm.) A number of other habitat-related initiatives are available as well, such as the VA Department of Environmental Quality's Citizen Water Quality Monitoring Program.

Research

Having the proper information to make conservation decisions is critical. Unfortunately it is challenging to have up-to-date and scientifically robust information

on every species across Virginia. Over the last ten years, VDGIF has issued research permits for almost every species of amphibian (S. Dressler, VDGIF, pers. comm.). Research is also conducted through State agencies, such as DCR's Natural Heritage Program. Despite this, there still remains a large research gap. Approximately 45% of Virginia's frogs and less than 20% of salamander species have had at least one population monitored for four or more years (Walls 2014). The type of research needing to be conducted to gain a holistic understanding of a species requires time, funding and people with considerable expertise.

State funding for amphibian research is almost exclusively limited to State Wildlife Grants and the Virginia Nongame Fund. The State Wildlife Grants Program provides funds for developing and implementing programs that move species towards recovery and eventual delisting or preclude the need for federal listing under the Endangered Species Act. Its primary focus is on those species identified as Species of Greatest Conservation Need listed in the WAP. The Virginia Nongame Fund's revenue source is through the Virginia Tax Check-Off Program. The Federal government also supports research in the Commonwealth through its various agencies, including the National Park Service, National Science Foundation, U.S. Fish and Wildlife Service, and the U.S. Geological Survey. Non-governmental organizations, such as VHS, provide small research grants. Additionally, research is supported through funding mechanisms within academic institutions, zoos and aquaria and foundations. Fortunately, many conservation programs not specifically focused on amphibians also indirectly benefit them.

Virginia Laws, Regulations and Enforcement

Establishing and enforcing policies are important conservation mechanisms. Regulations regarding Virginia's amphibians are clearly stated on the VDGIF website (<http://www.dgif.virginia.gov/wildlife/laws/>). In summary, it is unlawful to take, possess, import, cause to be imported, export, cause to be exported, buy, sell, offer for sale or liberate within the Commonwealth any wild animal unless otherwise specifically permitted by law or regulation. No threatened or endangered amphibian species or the Eastern Hellbender may be possessed. Otherwise, individuals may possess up to five amphibians listed on the VDGIF Native and Naturalized Fauna of Virginia list, with the exception of the American Bullfrog which has a 15 per day bag-limit. Only under specific conditions are native amphibians allowed to be released, but naturalized species may never be released. No salamanders can be taken from Grayson Highlands State Park or on parts of the Jefferson National Forest.

No amphibian species native or naturalized to Virginia may be bought or sold, except the American Bullfrog, Green Frog, Southern Leopard Frog and Green Treefrog, which can only be bought for educational or researcher purposes and must be purchased from a permitted captive breeder in Virginia or from a properly permitted business out-of-state. Non-native (exotic) amphibian species may be possessed, bought or sold, as long as it is in compliance with all other Local, State, Federal and International laws and regulations. However, special permits are needed for the following species: Giant or Marine Toad (*Rhinella marina*), African Clawed Frog and Barred Tiger Salamander (Gray Tiger Salamander and Blotched Tiger Salamander - *A.*

mavortium). Special permits are required for exhibiting or conducting research on amphibians.

VDGIF has been active in enforcing laws related to amphibians. However, there is no database containing documentation on the number of fines, confiscations and other related information. Biologists and law enforcement staff from VDGIF occasionally visit pet stores, trade shows, markets and other locations where amphibians may be sold illegally. Reports of violations from the public are also received and responded to by VDGIF.

Conservation Strategies and Collaborations

There are currently no captive breeding or rearing programs for any amphibian species in Virginia. The priority has always been, and will continue to be, to conserve all of Virginia's amphibian species in the wild. Success in these endeavors requires the collaboration among various organizations and agencies to inform, develop and implement conservation strategies. Several of the WAP listed species have management-related plans in place. For instance, Conservation Agreements were developed for the Cow Knob Salamander in 1994 and the Peaks of Otter Salamander in 1997. These agreements are signed by multiple agencies with the purpose and intent to prevent the need for federal listing of these species under the Endangered Species Act. In 1994, a Recovery Plan was developed for the endangered Shenandoah Salamander. Unlike other recovery plans which seek to increase population sizes, the Shenandoah Salamander plan highlights needs for research and ongoing monitoring. No Conservation Agreements exist for any frog species in Virginia.

Considering the range of most of Virginia's amphibians include other states and that threats to species also cut across boundaries, VDGIF actively collaborates with larger conservation initiatives. VDGIF is active in both the southeast and northeast chapters of PARC. PARC produces national and regional materials, such as the Habitat Management Guidelines, hosts meetings and facilitates working groups on issues which cut across State boundaries. VDGIF is also a member of both the Appalachian and North Atlantic Landscape Conservation Cooperative (LCC). The North Atlantic LCC is currently engaged in mapping all vernal ponds and identifying priority amphibian conservation areas. The U.S. Geological Survey's Amphibian Research and Monitoring Initiative (ARMI) is active in Shenandoah National Park and Prince William Forest Park.

RECOMMENDATIONS

There is much already being done in Virginia to address the declines in amphibian species, but there is still much more that needs to be achieved. Researchers can prioritize their efforts to gain a better understanding of local distributions of species and how these are changing over time, investigate potential causes of declines and study whether or not conservation actions are helping amphibians. Funding organizations can assist by recognizing the importance of these organisms and increase funding opportunities available for research, monitoring and conservation. Individuals, organizations and businesses can continue to highlight how critical this taxon is to

ecosystems and people. To maintain healthy populations of amphibians in Virginia, prevention and mitigation of threats must continue.

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APPENDIX. Status of Virginia's Amphibians

GR refers to NatureServe Global Ranking and SR refers to NatureServe State Ranking. NatureServe rankings include 1=critically imperiled, 2=imperiled, 3=vulnerable, 4=apparently secure, 5=secure and NR=no ranking; The 05 refers to 2005 Virginia Wildlife Action Plan and 15 refers to 2015 Virginia Wildlife Action Plan. Only Species of Greatest Conservation Need are issued a Tier level; 1=critical conservation need, 2=very high conservation need, 3=high conservation need and 4=moderate conservation need. In 2015 an additional Opportunity Ranking (OP) was added to the Wildlife Action Plan. These include A = managers have identified "on the ground" species or habitat management strategies expected to benefit the species, B = managers have only identified research needs for the species or managers have only identified "on the ground" conservation actions that cannot be implemented due to lack of personnel, funding, or other circumstance., C = managers have failed to identify "on the ground" actions or research needs that could benefit this species or its habitat or all identified conservation opportunities for a species have been exhausted. State (ST) refers to the listing of a species as threatened (T) or endangered (E). ESA refers to the Endangered Species Act and the federal listing of a species as threatened (T) or endangered (E). IUCN refers to the IUCN Redlist of species, with rankings of CR=critically endangered, EN= endangered, VU=vulnerable, NT= near threatened, LC=least concern and NE = not evaluated.

Species	Common name	GR	SR	05	15	OP	ST	ESA	IUCN
<i>Anaxyrus americanus</i>	American Toad	5	5						LC
<i>Anaxyrus fowleri</i>	Fowler's Toad	5	5						LC
<i>Anaxyrus quercicus</i>	Oak Toad	5	2	2	2	C			LC
<i>Anaxyrus terrestris</i>	Southern Toad	5	4						LC
<i>Acris crepitans</i>	Eastern Cricket Frog	5	4						LC

APPENDIX continued.

Species	Common name	GR	SR	05	15	OP	ST	ESA	IUCN
<i>Acris gryllus</i>	Southern Cricket Frog	5	4						LC
<i>Hyla crysoscelis</i>	Cope's Gray Treefrog	5	5						LC
<i>Hyla cinerea</i>	Green Treefrog	5	4						LC
<i>Hyla femoralis</i>	Pine Woods Treefrog	5	4						LC
<i>Hyla gratiosa</i>	Barking Treefrog	5	1	2	2	C	T		LC
<i>Hyla squirella</i>	Squirrel Treefrog	5	4						LC
<i>Hyla versicolor</i>	Gray Treefrog	5	5						LC
<i>Pseudacris brachyphona</i>	Mountain Chorus Frog	5	4	2	2	C			LC
<i>Pseudacris brimleyi</i>	Brimley's Chorus Frog	5	4						LC
<i>Pseudacris crucifer</i>	Spring Peeper	5	5						LC
<i>Pseudacris feriarum</i>	Upland Chorus Frog	5	5						LC
<i>Pseudacris kalmi</i>	Mew Jersey Chorus Frog	4	NR	4	4	C			LC
<i>Pseudacris nigrata</i>	Southern Chorus Frog	5	3	4	4	C			LC

APPENDIX continued.

Species	Common name	GR	SR	05	15	OP	ST	ESA	IUCN
<i>Pseudacris ocularis</i>	Little Green Frog	5	3	4	4	C			LC
<i>Gastrophryne carolinensis</i>	Eastern Narrow-mouthed Toad	5	4						LC
<i>Lithobates catesbeianus</i>	American Bullfrog	5	5						LC
<i>Lithobates clamitans</i>	Green Frog	5	5						LC
<i>Lithobates kauffeldi</i>	Atlantic Coast Leopard Frog	NR	NR						NE
<i>Lithobates palustris</i>	Pickereel Frog	5	5						LC
<i>Lithobates sphenoccephalus</i>	Southern Leopard Frog	5	4						LC
<i>Lithobates sylvaticus</i>	Wood Frog	5	5						LC
<i>Lithobates virgatipes</i>	Carpenter Frog	4	3	3	3	C			LC
<i>Scaphiopus holbrookii</i>	Eastern Spadefoot	5	4	4	4	C			LC
<i>Ambystoma jeffersonianum</i>	Jefferson Salamander	4	3	4	4	C			LC
<i>Ambystoma mabeei</i>	Mabee's Salamander	4	1/2	2	2	C	T		LC

APPENDIX continued.

Species	Common name	GR	SR	05	15	OP	ST	ESA	IUCN
<i>Ambystoma maculatum</i>	Spotted Salamander	5	5						LC
<i>Ambystoma opacum</i>	Marbled Salamander	5	5						LC
<i>Ambystoma talpoideum</i>	Mole Salamander	5	2	2	2	C			LC
<i>Ambystoma tigrinum</i>	Eastern Tiger Salamander	5	1	2	2	C	E		NE
<i>Amphiuma means</i>	Two-toed Amphiuma	5	4						LC
<i>Cryptobranchus alleganiensis</i>	Eastern Hellbender	3	2/3	2	1	A			NT
<i>Aneides aeneus</i>	Green Salamander	3	3	2	2	C			NT
<i>Desmognathus auriculatus</i>	Southern Dusky Salamander	5	4						LC
<i>Desmognathus fuscus</i>	Northern Dusky Salamander	5	5						NE
<i>Desmognathus marmoratus</i>	Shovel-nosed Salamander	4	2	3	3	C			LC
<i>Desmognathus monticola</i>	Seal Salamander	5	5						LC

APPENDIX continued.

Species	Common name	GR	SR	05	15	OP	ST	ESA	IUCN
<i>Desmognathus ochrophaeus</i>	Alleghany Mountain Dusky Salamander	5	4						LC
<i>Desmognathus orestes</i>	Blue Ridge Dusky Salamander	4	3	4	4	C			LC
<i>Desmognathus wrightii</i>	Northern Pygmy Salamander	3	2	3	3	C			NE
<i>Desmognathus planiceps</i>	Flat-head Salamander	NR	NR						NE
<i>Desmognathus quadramaculatus</i>	Black-bellied Salamander	5	4						LC
<i>Desmognathus welteri</i>	Black Mountain Salamander	4	3						LC
<i>Eurycea bislineata</i>	Northern Two-lined Salamander	5	5						LC
<i>Eurycea cirrigera</i>	Southern Two-lined Salamander	5	5						LC
<i>Eurycea guttolineata</i>	Three-lined Salamander	5	4						LC

APPENDIX continued.

Species	Common name	GR	SR	05	15	OP	ST	ESA	IUCN
<i>Eurycea longicauda</i>	Long-tailed Salamander	5	5						LC
<i>Eurycea lucifuga</i>	Cave Salamander	5	4						LC
<i>Eurycea wilderae</i>	Blue Ridge Two-lined Salamander	5	2	3	3	C			LC
<i>Gyrinophilus porphyriticus danielsi</i>	Blue Ridge Spring Salamander	4	5						LC
<i>Gyrinophilus porphyriticus duryi</i>	Kentucky Spring Salamander	4	2						LC
<i>Gyrinophilus porphyriticus porphyriticus</i>	Northern Spring Salamander	5	5						LC
<i>Hemidactylium scutatum</i>	Four-toed Salamander	5	5						LC
<i>Plethodon chlorobryonis</i>	Atlantic Coast Slimy Salamander	5	NR						NE
<i>Plethodon cinereus</i>	Eastern Red-backed Salamander	5	5						LC

APPENDIX continued.

Species	Common name	GR	SR	05	15	OP	ST	ESA	IUCN
<i>Plethodon cylindraceus</i>	White-spotted Slimy Salamander	5	5						LC
<i>Plethodon glutinosus</i>	Northern Slimy Salamander	5	5						LC
<i>Plethodon hoffmani</i>	Valley and Ridge Salamander	5	4						LC
<i>Plethodon hubrichti</i>	Peaks of Otter Salamander	2	2	2	1	C			VU
<i>Plethodon kentucki</i>	Cumberland Plateau Salamander	4	3	4	4	C			LC
<i>Plethodon montanus</i>	Northern Gray-cheeked Salamander	4	3						LC
<i>Plethodon punctatus</i>	Cow Knob Salamander	3	2	2	1	C			NT
<i>Plethodon richmondi</i>	Southern Ravine Salamander	5	4						LC

APPENDIX continued.

Species	Common name	GR	SR	05	15	OP	ST	ESA	IUCN
<i>Plethodon sherando</i>	Big Levels Salamander	2	2						VU
<i>Plethodon ventralis</i>	Southern Zigzag Salamander	4	1	2	2	C			LC
<i>Plethodon virginia</i>	Shenandoah Mountain Salamander	2	2	3	3	C			NT
<i>Plethodon wehrlei</i>	Wehrle's Salamander	4	4						LC
<i>Plethodon welleri</i>	Weller's Salamander	3	2	2	1	C			EN
<i>Plethodon yonahlossee</i>	Yonahlossee Salamander	4	3	4	4	C			LC
<i>Pseudotriton montanus diastictus</i>	Midland Mud Salamander	5	NR						LC
<i>Pseudotriton montanus montanus</i>	Eastern Mud Salamander	5	5	4	4	C			LC
<i>Pseudotriton ruber nitidus</i>	Blue Ridge Red Salamander	3	NR						LC
<i>Pseudotriton ruber ruber</i>	Northern Red Salamander	5	5						LC

APPENDIX continued.

Species	Common name	GR	SR	05	15	OP	ST	ESA	IUCN
<i>Stereochilus marginatus</i>	Many-lined Salamander	5	3	4	4	C			LC
<i>Necturus maculatus</i>	Common mudpuppy	5	2	3	3	C			LC
<i>Necturus punctatus</i>	Dwarf Waterdog	5	2	3	3	C			LC
<i>Notophthalmus viridescens</i>	Eastern Newt	5	5						LC
<i>Siren intermedia</i>	Lesser Siren	5	2	3	3	C			LC
<i>Siren lacertina</i>	Greater Siren	5	3	4	4	C			LC