In this case at least let us raise our voices in polite, but firm expressions of concern.

**Literature Cited**


**Appendix**

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**ZOO VIEW**

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**Amphibian Conservation Efforts at the Smithsonian’s National Zoological Park and Conservation Biology Institute**

In response to the global extinction crisis, the Smithsonian’s National Zoological Park (NZP) and Conservation Biology Institute (SCBI) launched a major new amphibian conservation effort in 2008, to coincide with the American Association of Zoos and Aquarium (AZA) “Year of the Frog” campaign (Mendelson et al. 2006). A full-time interdisciplinary research position was created (and filled by BG) to focus exclusively on amphibian conservation, with a mandate from NZP to leverage existing internal Smithsonian resources to help implement the global amphibian conservation action plan (Gascon et al. 2007). We reviewed our existing global research capacity and identified two key areas of overlap with amphibian biodiversity hotspots. Specifically, the SCBI headquarters (Front Royal, Virginia) includes a 3200-acre research facility in the heart of Appalachia, a region with more than 70 salamander species, mostly endemic to the region (Stuart et al. 2008), and the Smithsonian Tropical Research Institute (Panama City, Panama) where an exceptionally diverse group of anurans has declined precipitously due to the pathogenic fungus *Batrachochytrium dendrobatidis* (Bd), the causative agent of chytridiomycosis (Lips et al. 2006). In addition to field conservation work, the Smithsonian’s National Zoological Park is a tremendous resource for disseminating amphibian science and conservation messages, with more than 2 million people each year visiting our herpetological exhibits. JBM served as the curator of herpetology from 2009–2014 and worked to shift the management paradigm from traditional animal exhibitions to herpetological conservation by focusing limited space and keeper resources on species of conservation concern, encouraging staff to participate in field research efforts, and becoming more engaged in conservation breeding programs (see Murphy and Xanten 2007 for a detailed account of the history of the department). This paper outlines the contributions of these centers (SCBI, STRI, and NZP) to amphibian conservation from 2008–2016. Our goals are to promote a culture of amphibian conservation within the AZA and to acknowledge the tireless contributions of our conservation colleagues to these important efforts.

**Panamanian Frogs**

*Building Capacity in Panama.*—Despite volumes of research, few effective conservation actions have been effectively implemented to mitigate threats to amphibians. Between 2005–2015, over 12,000 papers were published on amphibian conservation, of which about 1000 focused specifically on

*Biologist Jorge Guerrel tends to a captive assurance colony of Atelopus inside a modified shipping container at the Smithsonian Tropical Research Institute’s new Gamboa Amphibian Research and Conservation Center in Panama.*

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chydridiomycosis (Thompson Reuters 2016). While this research greatly advanced our understanding of the threats to amphibian survival, a lack of funding and capacity for applied conservation seriously limit our ability to save these species from extinction (Gratwicke et al. 2012). We found that a lack of facilities and full-time dedicated professionals focused on amphibian conservation in Panama was a major limiting factor. As such, the primary goal of our work in Panama has been to build a team of talented herpetologists and adequate facilities at STRI to implement conservation actions at a meaningful scale.

Panama is home to 214 amphibians, and about 36 species are thought to be highly susceptible to the amphibian chytrid fungus, of which, nine have disappeared completely (Gratwicke et al. 2015). About 100 species continue to persist—even in Bd positive situations and the remainder were too rare to evaluate (Gratwicke et al. 2015). To counter the loss of amphibians due to chytridiomycosis, we launched the Panama Amphibian Rescue and Conservation Project in 2009. The project is a consortium of zoos and aquariums whose founders included Africam Safari (Mexico), Cheyenne Mountain Zoo (Colorado), Defenders of Wildlife (DC), Houston Zoo (Texas), Summit Municipal Park (Panama), Zoo New England (Massachusetts), NZP (Washington, D.C.), and STRI (Panama). Our project is directed by prominent Panamanian herpetologist Roberto Ibáñez with a primary goal to build additional facilities in Panama to house and grow captive populations of endangered amphibians threatened by Bd. Our new 5000-square-foot Gamboa Amphibian Research and Conservation Center opened in 2015 (Figs. 1, 2). It is a major new resource for on-the-ground conservation that complements the Houston Zoo's El Valle Amphibian Conservation Center (EVACC) (Gagliardo et al. 2008). Prior to opening the Gamboa center to the public, we conducted expeditions to collect priority species, treated Bd-infected frogs gathered in the field, and worked to resolve veterinary issues associated with the captive collection (Baitchman and Pessier 2013; Pessier et al. 2014). The combined facilities currently employ 12 people and house 12 species of priority frogs (Fig. 3), six of which were successfully bred in captivity for the first time as part of this effort (PARC 2015). One of our primary focal species for research and conservation has been the Panamanian Golden Frog (Atelopus zeteki) that was named for STRI's first director, James Zetek, who collected the type specimen in 1929 (Dunn 1933). The captive population of Golden Frogs in the US is managed by the American Zoo and Aquarium Association's Golden Frog Species Survival Plan (SSP) at the Maryland Zoo in Baltimore, which has greatly facilitated research on this species in captivity. In 2013 we worked with the IUCN Species Survival Commission to develop a stakeholder-based conservation plan aimed at the restoration of wild Panamanian Golden Frog populations (Estrada et al. 2013).

Searching for a Cure.—Multiple approaches have been proposed for mitigating the effects of chytridiomycosis in nature (Woodhams et al. 2011) and promising advances have been made in the area of climate refuges (Puschendorf et al. 2011), application of antifungal agents to habitat (Bosch et al. 2015), and vaccines (McMahon et al. 2014). Our research has focused on the use of probiotics—beneficial bacteria that produce antifungal agents when applied to frog skin. Harris et al. (2009) demonstrated that probiotic bacteria could protect Mountain Yellow-legged Frogs (Rana muscosa) from Bd mortality, highlighting the potential of this strategy for applied conservation efforts. We attempted to apply this strategy to Bd-infected Atelopus (using surplus frogs), but the beneficial bacteria failed to persist on the frogs, which succumbed to chytridiomycosis (Becker et al. 2011; Becker et al. 2015). In the process of this research, we discovered a great deal about the microbiome of Golden Frogs both in the wild and in captivity (Becker et al. 2014), and found that the ability to clear a Bd infection was associated with both unique skin microbes and differential gene expression (Ellison et al. 2014; Becker et al. 2015) (Fig. 5).

Understanding the mechanisms of natural resistance to Bd is another strategy to developing a cure for chytridiomycosis in Atelopus and other susceptible species. In partnership with the Smithsonian's Center for Conservation Genetics, we studied the relationship between gene expression (i.e., transcriptomics) and disease outcomes in the Lowland Leopard Frog, Lithobates yavapaiensis. This frog is from the southwestern U.S., where populations naturally differ in their susceptibility to chytridiomycosis (Savage and Zamudio 2011). In recent studies, we determined that frogs with lower levels of immune proteins in the blood are more susceptible to Bd (Savage et al. 2016). However, specific genes could not be associated with a particular disease outcome, instead, an overactive immune response was associated with chytridiomycosis susceptibility (Anna Savage, pers. comm). These studies highlight the complexity of Bd pathology and the need for further research to inform potential solutions.

Sustaining Captive Populations in the U.S. and Panama.—Sustaining and managing captive collections of amphibians over the longer term can be greatly facilitated by active genome resource banking and assisted reproduction. These tools can preserve genetic diversity, enhance reproductive success, and reduce overall numbers of live animals required to meet population goals (Wildt et al. 1997). We developed a comprehensive biobanking protocol and in 2015 and began systematically cryopreserving tissues and living amphibian sperm using methods developed by Gina DellaTogna as part of her PhD studying a population of male captive Panamanian Golden Frogs (DellaTogna 2015). DellaTogna is the first Panamanian student to earn her PhD studying this emblematic animal, and is continuing this research in partnership with Maryland Zoo, with the goal of producing Atelopus tadpoles from in-vitro fertilization of eggs using cryopreserved spermatozoa samples, and applying the methods to other priority species (Fig. 4).
In addition to developing these tools we have used the scientific method to address other problems associated with captive management issues. Spindly leg syndrome is an issue associated with captive care of amphibians. We have characterized the problem in Golden Mantellas (*Mantella aurantiaca*) from Madagascar but its causes remain unclear (Claunch and Augustine 2015). Our initial experiments on *Atelopus* in Panama showed that prevalence of spindly leg syndrome was not related to tadpole dietary protein and we will continue to design experiments that systematically evaluate...
other potential causes (J. Guerrel, pers. comm.). Lack of space is another factor limiting captive amphibian collections so we studied the physiological and behavioral consequences of housing *Atelopus* in groups of 2–8 individuals. Group housing improves space efficiency and expands conservation capacity, but may cause increased disease susceptibility related to physiological stress. In our study of co-housed *Atelopus* males, we found that they fought vigorously in the first two weeks of being placed together, as measured by aggressive interactions and elevated stress hormones in their feces. However, after the third week, both the aggressive interactions and fecal glucocorticoids subsided to baseline levels in small and large groups, and we concluded that we could safely co-house larger groups of males over the longer term (Cikanek et al. 2014).

**Building a Constituency.**—Given the central role of human values in conservation, our approach to amphibian outreach has focused on engaging people with nature to foster inspiration and a desire to protect the natural world (Gratwicke et al. 2016).

The products of our Panamanian amphibian outreach program include numerous news articles and television segments, a bilingual website, a feature-length documentary, and a total of five frog-themed exhibitions at Smithsonian’s facilities in Panama and the National Zoo. The El Valle Amphibian Conservation Center is the only place in Panama where the public can see the endemic Golden Frog, and the exhibit is visited by about 100,000 people each year. In 2014, we opened a new bilingual “Fabulous Frogs of Panama” exhibition at the Punta Culebra Nature Center in Panama City. It is visited by about 60,000 people per year, about 15,000 of whom are Panamanian students on school field trips. The “Fabulous Frogs of Panama” exhibit features the diversity of common frogs in Panama and species of conservation concern, while interpreters share the cultural significance of frogs in Panama and tell the story of the chytrid threat to amphibians (Fig. 6). At the National Zoo, we tell our amphibian conservation story to our 2 million annual visitors primarily at the “Amphibian Alert!” exhibit in the Amazonia Science Gallery. This exhibit explains amphibian diversity, the chytrid fungus, and amphibian arks, connecting the visitor to our Panama field work and research efforts though a looping documentary entitled “Mission Critical: Amphibian Rescue,” produced and aired by Smithsonian Networks. A second smaller display at the Reptile Discovery Center specifically showcases the Panama Amphibian Rescue and Conservation Project and our participation in the Golden Frog SSP program (Evans et al. 2012).

We do not have a full time amphibian conservation education staff member, but all of our staff pull together to help volunteers with educational activities for the Golden Frog Festival which takes place in Panama during the week of August 14, which is officially legislated as “Día de la Rana Dorada.” The festival has grown in popularity since its first incarnation in 2010. Now, thousands of people participate in multiple events around the country, including the Golden Frog Day parade coordinated by the Ministry of the Environment where hundreds of school children dress up like Golden Frogs and march in the streets of El Valle de Anton (PARC 2015). For teachers, we developed an informal curriculum with age-appropriate student activities that align with the national curriculum, to be used in association with one of the exhibits or educational posters (PARC 2014). Each curriculum topic incorporates a conservation action for students to help achieve our conservation goals such as fundraising, volunteering or participating in the Global Amphibian Bioblitz citizen-science survey of wildlife (Loarie et al. 2011). In the US, we participate in the AZA Frogwatch program to engage and train zooogoers to observe and listen to frog calls in their own back yards. While it is challenging to evaluate the effects of any outreach program, we feel that the significant efforts by AZA members, scientists, and the popular press since the 2008 Year of the Frog has led to a shift in public discourse about amphibian conservation. The tone has changed from a more defensive position on “Why frogs matter,” to one where amphibians are a more central part of the conversation about biodiversity and environmental stewardship both in Panama and the US.

**Appalachian Salamanders**

Almost half of all salamander species are listed as threatened or endangered (Stuart et al. 2008). The Appalachian region is home to more salamander species than anywhere else in the world, making it a true hotspot for salamander biodiversity (Stuart et al. 2008). Several populations of Appalachian salamanders...
living in apparently pristine habitat have experienced enigmatic declines (Highton 2005; Caruso and Lips 2013). We convened an Appalachian salamander workshop in 2008 at SCBI in Front Royal, Virginia to evaluate conservation threats to salamanders using IUCN threat categories. Attendees ranked 1) climate change, 2) pollution, 3) residential development, 4) energy production and mining, and 5) invasive species and disease as the top potential threats to salamanders, but acknowledged that much more systematic research was needed to understand the scope and nature of the problems (Gratwicke 2008).

Evaluating the Threat of Climate Change.—As a result of this prioritization, our focus at the Smithsonian Conservation Biology Institute has been working to develop a better understanding how climate change might affect salamanders. Mountaintop endemic species such as the Shenandoah Salamander (Plethodon Shenandoah) have been a primary concern. We evaluated climate risks to P. shenandoah by developing systematic baseline occupancy survey methods (Sevin 2014) and manipulating climate in an experimental mesocosm approach with US Geological Survey colleagues. We found that under future climate scenarios, P. shenandoah was able to compete favorably with Red-backed Salamanders (P. cinereus; Dallaiolo 2013). One recent study suggested that warmer, drier climatic conditions were responsible for observed reductions of adult Plethodon salamander body size (Caruso et al. 2014). However, we examined museum collections and found the opposite relationship in P. cinereus. Specifically, body size in museum specimens increased slightly in association with warmer, drier conditions (McCarthy et al., in prep).

In addition to potential effects on terrestrial salamanders, changing rainfall patterns will likely lead to more extreme variation in stream flow and temperatures. This is likely to affect aquatic species like Hellbenders (Cryptobranchus alleganiensis) and exacerbate existing threats to wild populations (Wheeler et al. 2002; Burgmeier et al. 2011). To gain a better understanding of this species’ current distribution, we have been collaborating with a larger regional citizen-science initiative to detect Hellbenders using environmental DNA from 180 sites in the Northeast region (K. Terrell, pers. comm). We are conducting experimental research at the National Zoo’s Appalachian Salamander Lab to better understand how naturalistic patterns of temperature change influence various indicators of in C. alleganiensis (Fig. 7). We were surprised to find that Hellbenders were quite resilient to these fluctuations and even had improved immune function (Terrell et al. 2013).

Emerging Diseases in US Salamanders.—At the time of our 2008 salamander workshop it was unclear whether Bd may have been responsible for unexplained plethodontid salamander declines. Subsequent surveys in the wild and of museum specimens only detected Bd on wild plethodontids at very low frequencies, along with low zoospore loads indicating that Bd is unlikely to be playing a major role in shaping contemporary or historical salamander populations (Gratwicke et al. 2011; Caruso and Lips 2013; Mulet et al. 2014). The disease has higher prevalence rates in aquatic salamanders like Hellbenders, but it is unclear to what extent it has affected their wild populations (Bales et al. 2015).

A recent cause for concern is the new salamander-specific species of chytrid fungus Batrachochytrium salamandrivorans (Bsal) that was discovered recently in Europe but has not been detected on US salamanders (Martel et al. 2014; Bales et al. 2015). Just as the international trade in amphibians for pets and food may have been a vector for Bd (Gratwicke et al. 2010), more than 100,000 potential carriers of Bsal are imported into the US as pets each year, which is a substantial risk for the accidental introduction of Bsal to the United States (Gray et al. 2015). The legal trade in these species has been temporarily halted with a salamander trade moratorium issued by the U.S. Fish and Wildlife Service (USFWS 2016), but it is unclear whether the pathogen is already in the country. We partnered with the Amphibian Survival Alliance to engage hobbyists to screen their pets for Bsal and obtained more than 600 citizen-science-collected swabs from pet salamanders that will be tested for Bsal at the SCBI genetics lab.

Appalachian Salamander Lab Exhibit.—One of the top-level conservation actions identified at the 2008 Salamander Workshop was educating local residents of Appalachia about the importance and uniqueness of salamanders. Andy Odum had already been leading a successful effort to exhibit and breed native salamanders at the Toledo Zoo, but the National Zoo was...
and active research and field conservation. Our future goals are to optimize breeding and husbandry protocols for understudied amphibian species and to continue to facilitate research and education efforts. Our programs provide the next generation of frog conservationists with opportunities to gain skills and inspiration, while contributing to the global efforts to protect and understand threatened amphibians.

**Acknowledgments.**—This paper is dedicated to the National Zoo’s Senior Curator Ed Bronikowski for staunch support of the Smithsonian’s amphibian program. His efforts include identifying potential financial support sources, exhibit design, interacting with contractors and reviewing construction documents, developing husbandry protocols, presenting public lectures, and successfully carrying the Japanese Giant Salamanders from Asa Zoo to NZP. The herpetological staff at Reptile Discovery Center has been directly involved in public lectures and programs, captive management, and have participated in fieldwork and research: Lauren Augustine, Matt Evans, Kyle Miller, Michael Miller, Matt Neff, and Robin Saunders. Steve Monfort and Dave Wildt (Smithsonian Conservation Biology Institute) and George Rabb (President Emeritus of the Chicago Zoological Society) were instrumental in conceptualizing, creating, and sustaining the SCBI amphibian conservation program. Rob Fleischer and Pierre Comizzoli (SCBI) led the genetics and assisted reproduction work respectively. Sharon Ryan (STRI) was instrumental in developing our public programs and exhibits. Pamela Baker Masson (NZP) directed public affairs. Lisa Belden, Reid Harris, and Kevin Minbiole are key academic collaborators in the probiotics studies. Roberto Ibáñez, Jorge Guerrero, Matthew Evans, Liza Dadone, Eric Baitchman, Brad Wilson, and Heidi Ross form the implementation team of the Panama Amphibian Rescue and Conservation Project. Other valued students and collaborators are credited in the relevant citations.

This entire program has been made possible through generous donations and grants. We thank Susan and Frank Mars for making this project possible by supporting BG since the program’s inception. The Shared Earth Foundation and Anele Kolobe Foundation have generously provided key operational support for this program for the last eight years. We also thank the Wood Tiger Fund, the USFWS, Anne not exhibiting any native salamander species at the time. We agreed at that meeting that even though native salamanders are challenging to exhibit, we needed to showcase some of our nation’s incredible salamander diversity to the National Zoo’s 2 million annual visitors. We conceived a “salamander hall” in the Reptile Discovery Center that would have a custom-built multi-species *Plethodon* salamander wall on one side, and an active Appalachian salamander research lab on the other, where researchers and docents could engage visitors and promote our conservation message (Fig. 8). Using this space, we have conducted studies related to climate change (Terrell et al. 2013), olfaction (Kuppert 2013), and captive nutrition (Augustine et al. 2016).

The Appalachian salamander exhibit provides visitors with interesting context for the diversity of US salamanders by displaying our native Hellbenders in a salamander hallway alongside a Japanese Giant Salamander (*Andrias japonicas*) exhibit. This genus represents the largest amphibian species in the world (Fig. 9). Our goal is to inspire our zoo visitors with this superlative amphibian and to foster a sense of wonder about our local salamander biodiversity.

**Conclusion**

With interdisciplinary coordination and support from the highest levels of leadership, the Smithsonian’s amphibian conservation program has leveraged significant in-kind resources to support amphibian conservation. These resources include physical space and expertise from multiple departments, specifically, animal husbandry, research, veterinary science, public affairs, exhibits, and advancement. We currently employ 12 full-time conservation staff in Panama and have been assisted by a host of volunteers, interns, five PhD students, a DVM student, and four post-doctoral fellows. The active conservation research program is complimented by the captive collection which has shifted from one composed primarily of exotic least concern species, to one that is more focused on native species and species of conservation concern. Our conservation programs give us an engaging story to tell and our new amphibian exhibits all highlight the connections between the captive collections...
Keiser, Minera Panama, the Houston Zoo, The Cheyenne Mountain Zoo, and Zoo New England for their generous and sustained financial contributions over the course of this effort. Judith Block and Kim Terrell provided helpful comments on this manuscript. All photos were taken by BG.

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Vipera walser is the most recent addition to viperid taxonomy, having been “discovered” via molecular genetic analysis as distinct from nearby populations of the ecologically and morphologically similar *V. berus* (Ghielmi et al. 2016. Journal of Zoological Systematics and Evolutionary Research 54:161–173). It might seem surprising that new vertebrates—especially something like a viperid snake—continue to be found in relatively well-explored areas of Europe. In this case of *V. walser*, although specimens were present in museum collections dating from > 85 yrs ago, these were catalogued as range-margin *V. berus*. It was only until genetic sequencing was undertaken was this cryptic diversity brought to light. And indeed, not only are these allopatric species deeply differentiated and show no evidence of introgression, they are not even sister taxa. *Vipera walser* appears to be nested within a Caucasian clade rather than affiliated with the geographically proximate *V. berus* complex.

*Vipera walser* is a relict species that occurs in a small, high-elevation, high-rainfall region in the western Italian Alps. The new species appears to be quite common within its restricted range, occupying open, rock-strewn landscapes. Interestingly, it exhibits nearly the same range of pattern variation seen in populations of the widely distributed *V. berus*—a zigzag pattern on various ground colors, and including even a melanistic form. The species is named for the Walser people, who migrated from the north in the 12th and 13th centuries to settle in mountain valleys of the Alps.

The small and likely fragmented range of this species in alpine valleys potentially makes it vulnerable to extinction. Although loss of forest cover worldwide has strongly impacted the conservation of numerous amphibian and reptile species, one of the threats facing *V. walser* is the potential for return of forests to their currently open habitats. This is likely given the decline in agricultural practices that have historically maintained the open nature of alpine valleys in the Alps. An additional threat, based on experiences with other recently described viperids (e.g., *V. kaznakovi*), is collection for the pet trade.

The adult Walser Viper on our cover was photographed by Matteo Di Nicola. This “wide-angle macro” image was recorded using a Nikon D750 camera and a Tokina 10–17mm lens (f/2.8, ISO 320, 1/60 sec shutter speed, focal length 16mm), with fill flash provided by a Nikon R1 wireless dual flash system. Additional images by Di Nicola may be viewed at <www.matteodinicola.com>.