4-22-2018

Linking Husbandry and Behavior to Enhance Amphibian Reintroduction Success

Luke Jack Linhoff
llinh001@fiu.edu

DOI: 10.25148/etd.FIDC006549

Recommended Citation

Linhoff, Luke Jack, "Linking Husbandry and Behavior to Enhance Amphibian Reintroduction Success" (2018). *FIU Electronic Theses and Dissertations*. 3688.
https://digitalcommons.fiu.edu/etd/3688

FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

LINKING HUSBANDRY AND BEHAVIOR TO ENHANCE AMPHIBIAN REINTRODUCTION SUCCESS

A dissertation submitted in partial fulfillment of the
requirements for the degree of
DOCTOR OF PHILOSOPHY
in
BIOLOGY
by
Luke Jack Linhoff

2018

To: Dean Michael R. Heithaus
College of Arts, Sciences and Education

This dissertation, written by Luke Jack Linhoff, and entitled Linking Husbandry and Behavior to Enhance Amphibian Reintroduction Success, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

Joel Heinen

Steven Oberbauer

Joseph Mendelson

Yannis Papastamatiou

Maureen Donnelly, Major Professor

Date of Defense: 22 March 2018

The dissertation of Luke Jack Linhoff is approved.

Dean Michael R. Heithaus
College of Arts, Sciences and Education

Andrés G. Gil
Vice President for Research and Economic Development
and Dean of the University Graduate School

Florida International University, 2018

DEDICATION

I dedicate this dissertation to coffee, Wyoming Toad IPA, spicy Asian food, my trusty Aztek, and the Gods of Space Herpetology.

ACKNOWLEDGMENTS

Throughout my dissertation, I have been fortunate to receive help and support from numerous people. In no particular order, I want to acknowledge many of them, though I am doubtlessly missing some. First, my lab mates have provided me with countless hours of emotional, intellectual and logistical support, namely Michael Britton, Michelle Thompson, Kelsey Reider, Lilly Eluvithingal, Vivian Maccachero, Monica Isola, and Ed Metzger. While spending countless hours and late nights working in the lab and field, they have always been supportive. Previous Donnelly Lab graduates James Watling, Steven Whitfield, Alessandro Catenazzi and Ralph Saporito have also given me great encouragement and have become lasting friends. Numerous FIU graduate students were wonderful friends during my time in Miami, and there are too many people to list. However, I want to thank James Stroud who was both a wonderful source of inspiration, a great friend, and solid roommate.

My committee members have greatly helped me on my journey to complete my dissertation, namely Mike Heithaus, Joel Heinen, Joseph Mendelson, and Yannis Papastamatiou. Thanks to Steve Oberbauer for huge help at the end. I thank Michael Maunder and John Withey for serving on my committee while they were at FIU. Their advice, encouragement and trust allowed me to undertake research I truly enjoyed. In particular, I want to thank Michael Maunder for opening doors to work on the IUCN Amphibian Reintroduction Guidelines project. His help allowed me to work with numerous leaders in the field of amphibian reintroductions who I would have otherwise not had a chance to meet. Other researchers who have made a lasting impression and helped me include Jennifer Germano, Brian Gratwick, & Kevin Donmoyer.

My friends and colleagues who helped with my research on the Wyoming Toad include Melanie Murphy, Doug Keinath, Jason Palmer, and Elizabeth (Lizzy) Mack. They were all instrumental in helping me through two difficult field seasons. The 2015 field season field technicians who helped on my project include Melissa Herbert, Nick Buss, Brandon Zinman, Jaina Gandhi, and Kim Goehring. I want to give a special thanks to Julia (Julie) Polasik and Jessica Sellers for help in the field, advice on working with the Wyoming Toad, and most importantly, letting me stay in their home once the weather turned for the worst. The staff of Cold Creek Coffee graciously hosted many Wyoming Toad meetings. The campground hosts, Mike and Kate, at Vedawooo campground welcomed me as a frequent guest as well.

A special thanks goes to David Bickford. His support and willingness to welcome me into his home and lab in Singapore provided me with some of the best years of my life. Being able to work with a second lab of talented graduate students in the Southeast Asian tropics was a wonderful opportunity that introduced me to many lasting friends and collaborators. All the members of the National University of Singapore's Ecology Group were wonderful to have worked with. In particular, I want to thank Francesca Mcgrath, Louis DeFalco, Laura Billings, Minko Posa, Banjo and all my other friends and collaborators in Singapore who are too many to list here. Additionally, my close friends and collaborates William Sigourney Symes and Oliver Worsley were always there with a joke and time for a beer. My field assistants Tan Hui Ting Robyn and Jie En Tham were skilled, curious and hardworking students while they pursued their senior projects. In Costa Rica, the staff and other researchers at La Selva Biological Station were a wonderful help. In particular I want to thank Chelsea Robinson for her encouragement

and help doing fieldwork. For work on captive frogs, Doug, Dale, and Zach were instrumental in helping me complete my data collection.

My parents and siblings provided a great deal of support throughout my studies. My brother, Ben Linhoff, provided guidance navigating nearly every aspect of working towards a PhD. To get into graduate school, I want to thank Jesse Krebs at Omaha’s Henry Doorly Zoo who supported my dream of pursuing a PhD. Joe Mendelson’s thoughtful recommendation to contact Mo as an advisor might be one of the greatest, life altering sentences I have ever received.

Most importantly, I want to thank my advisor Mo Donnelly who took a chance on accepting me into her lab. I am greatly indebted to her for unyielding support, patience, encouragement, and making me feel like I was part of a family. Her help and patience improving my writing, navigating graduate school, and magically making so many problems and snags go away with a phone call were invaluable. Hiking in the Snowy Range in Wyoming, traveling through Borneo, and driving back from New Orleans with Mo are some of my favorite memories of graduate school. Throughout my time as a graduate student, I have always felt that I had chosen the best lab in the world. I have been truly lucky to have such a great advisor and friend.

This dissertation was completed with funding from numerous sources including GPSC FIU travel awards, US Department of Fish and Wildlife Research Support grant, National University of Singapore EAPSI Fellowship, National Science Foundation EAPSI Fellowship, FIU Department of Biological Science Travel/Research Funds, Federal Graduate Needs Grant, and finally a National Science Foundation, Graduate Research Fellowship.

ABSTRACT OF THE DISSERTATION

LINKING HUSBANDRY AND BEHAVIOR TO ENHANCE AMPHIBIAN REINTRODUCTION SUCCESS

by

Luke Jack Linhoff

Florida International University, 2018

Miami, Florida

Professor Maureen Donnelly, Major Professor

Wildlife in captivity has a long history of benefiting global conservation goals. Captive animals can raise awareness and appreciation for the conservation of endangered species. Additionally, captive animals can be used as source populations to reintroduce animals back to the wild or to supplement existing wild populations. The rapid increase in amphibian species threatened with imminent extinction has necessitated the creation of dozens of captive-breeding programs. The focus of this dissertation has integrated topics across the spectrum of animals in captivity and the wild, and the results provide useful recommendations for conservation action. First, I describe how market pressures over a 28-year period are causing meteoric increases in the prices of amphibians sold in the pet trade, indicating a high risk of overexploitation. Pet amphibians may facilitate greater understanding and appreciation of amphibians, but the pet trade must be sustainable. Improving amphibian husbandry will increase the number of captive-bred animals available in the pet trade, and it will allow greater production of threatened species for reintroductions. Secondly, by performing a systematic review of husbandry for 289 amphibian species native to the US, I identified a critical lack in taxon-specific husbandry and developed husbandry research prioritizations. Next, I used a combination of laboratory and field studies to examine domestication processes in amphibians by comparing defensive behaviors in two species of captive-bred and wild poison frog. Captive-bred amphibians had significantly reduced defensive behaviors compared to wild conspecifics, likely resulting from habitation processes related to their husbandry. Finally, I performed three reintroductions of the critically endangered Wyoming Toad (*Anaxyrus baxteri*) in Wyoming, US. I demonstrated how providing a transitionary period, called a soft-release, to captive-bred toads moving to a novel, wild environment can improve reintroduction success. My work illustrates how improving our understanding of the nexus between captivity and the wild can improve conservation action for endangered species.

TABLE OF CONTENTS

TABLE OF TABLES

TABLE PAGE

TABLE OF FIGURES

ABBREVIATIONS AND ACRONYMS

ACAP	Amphibian Conservation Action Plan
ASA	Amphibian Survival Alliance
AZA	Association of Zoos and Aquariums
Bd	*Batrachochytrium dendrobatidis*
Bsal	*Batrachochytrium salamandrivorans*
CB	captive-bred
ED	Evolutionarily Distinct
EDGE	Evolutionarily Distinct and Globally Endangered
IUCN	International Union for the Conservation of Nature
MLNWR	Mortenson Lake National Wildlife Refuge
REBL	Red Biological Laboratory
WAZA	World Association of Zoos and Aquariums
WC	wild-caught

INTRODUCTION

Reintroduction as a conservation tool

A wildlife translocation is defined as human-mediated movement of an organism from one area to another (IUCN/SSC 2013). A reintroduction is a type of translocation, wherein an organism is released within its indigenous range. Reintroduced animals may be either wild animals or those born in captivity. Hundreds of threatened animals are currently bred in captivity for conservation-related purposes, such as reintroducing them back into nature to reestablish populations or supplement existing wild populations (Magin et al. 1994; Balmford et al. 1996). Captive rearing and reintroductions have become a deeply entrenched and necessary practice utilized by a variety of conservation practitioners for both flora and fauna. Criticism of reintroductions have focused on their low success rates (e.g., Griffith et al. 1989; Fischer & Lindenmayer 2000; Germano & Bishop 2009). Low success rates of translocations utilizing captive-bred animals have significant conservation and economic implications (Seddon et al. 2012). Translocations are costly and if unsuccessful they may result in negative impacts to the conservation status of a species. However, numerous successful conservation reintroduction case-studies exist, and reintroductions may be the only feasible option to save some species from extinction (Soorae 2013, 2016). Estimates indicate that 2000-3000 species may need some form of *ex situ* support to avoid extinction in the foreseeable future (Seal 1991; Tudge 1992). It is clear that captive-breeding with conservation translocations is a critical tool for global conservation that appears to be increasing in its importance.

The recent establishment of reintroduction biology as a distinct sub-discipline of conservation biology is an important development for creating skilled scientists and managers to work on current and future conservation translocations (Seddon 2007, Seddon et al. 2012). Reintroduction biology encompasses several biological sub-disciplines, such as behavior, genetics, husbandry, reproductive biology, dispersal ecology, and evolution. All of these aspects of biology play unique and overlapping roles in translocating threatened organisms (Seddon et al. 2012). Although many factors are involved in a successful conservation reintroduction, it appears that many past unsuccessful reintroductions were destined for failure because of poor design and implementation of the reintroduction project (Price and Soorae 2003). In a recent review, Pérez et al. (2012) examined several hundred translocations with a range of purposes. They found that less the half of programs met best-practices criteria outlined by the IUCN (1998) for translocation planning and management. Using the author's criteria, 65% were deemed unnecessary, 90% were not technically well advised, and 79% may have risks of causing more harm than good. Unsuccessful conservation translocations are a waste of limited conservation resources. Additionally, ill-conceived reintroductions may cause significant damage to ecosystems via disease (Viggers et al. 1993), introduction of invasive species (Lowe et al. 2000), or provide an ethical dilemma of animal welfare issues (Harrington et al. 2013). Fischer & Lindenmayer (2000) concluded that 27% of reviewed translocations were failures and 47% had unknown results. Although, translocations are clearly difficult and challenging, they may still be the best option for the conservation of some species. Reintroductions have saved numerous, and often charismatic megafaunal species, species from near extinction (e.g., the Arabian

Oryx [Spalton et al. 1999], Whooping Cranes [Johns 2005], and the California Condor [Toone and Wallace 1994]). Rapid developments occurring within the field of reintroduction biology (e.g., Armstrong et al. 2015) show great promise for improving reintroductions. I believe the current high failure rate, which persists under the lens of widespread acceptance how difficult reintroduction can be, is the failure to utilize knowledge founded in ecological and evolutionary theory to both justify and plan a successful project. A broad, multidisciplinary view should be taken when considerating each major stage of a reintroduction. The low success rates of reintroductions, combined with their urgent necessity, highlights the importance of research pertaining to all aspects of reintroductions, from capture to release.

Amphibian declines

The vertebrate class Amphibia contains approximately 7800 described species (Frost 2017). Currently, amphibians are experiencing unprecedented declines globally (Scheele et al. 2017), thus requiring an unprecedented conservation response (Mendelson et al. 2006). Descriptions of amphibian declines have been intermittently described for about 50 years (e.g., Conant 1958; Bragg 1960). However, most descriptions of declines were anecdotal. In 1989, scientific researchers at the First World Congress of Herpetology in Kent, England realized the extent of amphibian declines around the world (Bishop et al. 2012). Following the congress, publications documenting declines of amphibian populations started to rapidly appear in the literature (e.g., Czechura & Ingrem 1990; Rabb 1990).

The cause of amphibian declines can be understood from the viewpoint of their unique physiology, behavior, ecology, and life history, all of which combine to make them susceptible to a broad spectrum of potential anthropogenic threats (Blaustein et al. 2011). The small body size seen in most amphibian species, their reliance on moist habitats, and their permeable skin make them susceptible to desiccation. Limited dispersal ability and population persistence is closely tied to metapopulation dynamics. The low dispersal rate exacerbates the effects of habitat fragmentation for amphibians because it is difficult for population "rescue" seen in more vagile species like birds or mammals (Cushman 2006). In other cases where taxa have declined (e.g., corals, migratory songbirds), diseases may interact synergistically with other drivers to affect populations (Grant et al. 2016).

There is no doubt that amphibians are rapidly declining faster than any other vertebrate class (Stuart et al. 2004). Additionally, in the midst of these declines, new amphibian species continue to be described (Frost 2017). The substantial number of amphibian communities, populations, and species needing interventionist conservation methods to prevent their extirpation has created a global conservation emergency. Amphibian conservation research has continued to develop in novel directions, and the conservation community's response to rapid global declines has been multifaceted.

Amphibian reintroductions

To combat global amphibian declines, the Amphibian Conservation Action Plan (ACAP) was created to stimulate conservation actions to protect imperiled amphibians (Gascon et al. 2007). Additionally, the increasing importance and need for *ex situ* conservation methods to protect many amphibian species, necessitated the creation of the Amphibian Ark organization (Zipple et al. 2011).

Amphibian Ark filled a void to provide organization and develop guidelines for other institutions and conservation organizations working with or planning amphibian *ex situ* conservation. The Amphibian Ark has fostered collaboration and communication among disparate sectors including research universities, conservation non-governmental agencies (NGOs), zoos, parks, public education agencies, as well as the sources of funding. Dozens of new *ex situ* conservation facilities have been established around the world (Zipple et al. 2011; Harding et al. 2016). In spite of the rapid pace of conservation action for amphibians, amphibian translocations are still considered to be highly experimental and fraught with problems. Germano & Bishop (2009) determined only 52% of amphibian translocations were considered successful. It is clear that amphibian reintroductions are an area fertile for study. Hundreds of amphibian species may need drastic interventionist conservation to prevent extinction, but basic research urgently needs to be done.

Dissertation Overview

Herein I present four studies related to amphibian *ex situ* conservation and reintroductions. These studies fall along a spectrum of captivity to wild including: amphibians being collected in the wild and sold, their husbandry in captivity, the changes amphibians undergo in a captive environment, and finally how amphibians can be reintroduced back into the wild using a case study of the Wyoming Toad (*Anaxyrus baxteri*).

In Chapter 1, I provide a background of how amphibians are collected and sold for the pet trade. Market fluctuations in the amphibian trade may directly impact conservation goals with potential global consequences. I examined changes in a 28-year time period in the market values of 58 species of amphibians sold in the United States. All but two species dramatically increased in price during the study period. The mean percentage adjusted market price of U.S. amphibians rose 822% and indicates a serious risk for over-exploitation of many species. I also compared these price trends with various metrics such as range size, taxonomic order and population trend. The results indicated an urgent need for increased monitoring of the amphibian trade. The collection of wild amphibians for human consumption and the pet trade may directly reduce populations through overexploitation. Additionally, trade and movement of amphibians is a facilitating vector for diseases and invasive species.

In Chapter 2, I performed a thorough systematic review of amphibian husbandry for all 289 native amphibian species found in the United States. Our ability to care for amphibians in captivity is limited by our knowledge and experience. A lack of taxon-specific amphibian husbandry knowledge has limited the effectiveness of many amphibian conservation programs resulting in several near-extinctions events related to poor husbandry. My review collated and ranked hundreds of primary and gray literature sources in the first systematic review of amphibian husbandry. I found that 55.5% of all US amphibian species had no taxon-specific amphibian husbandry information. Furthermore, my results were used to perform a gap-analysis to identify what species in the United States are in the greatest need of husbandry research. These results indicate an urgent and critical need for further amphibian husbandry research. The collated list of husbandry sources produced though this research will also provide a much needed resource for amphibian husbandry practitioners globally.

In Chapter 3, I describe direct evidence of behavioral domestication in captive-bred amphibians with major implications for conservation programs keeping amphibians in captivity. In my experimental study, I utilized two poison frog species and tested wild frogs in Costa Rica and captive-reared frogs at two facilities in the United States. I found that captive-bred *Dedrobates auratus* had reduced flight responses to a simulated looming predator. A reduced tendency to flee predators in captive-bred animals might indicate that these animals would have increased predation rates compared to wild animals. Additionally, I tested the tonic immobility response of both species of captive-bred and wild individuals. I found that the captive-bred frogs in my study entered tonic immobility faster and spent a significantly longer time in tonic immobility length. Modifications to husbandry practices could reduce some of these maladaptive behaviors from developing in the future. My results show that we may require a paradigm shift for how amphibians are held in captivity.

In Chapter 4, I focus on reintroductions of the critically endangered Wyoming Toads in Wyoming, USA. The species is bred in captivity, and I performed three experimental reintroductions to study the spatial ecology of Wyoming Toads (*Anaxyrus baxteri*) under different release treatments. I tracked captive-bred, adult toads (N = 46) and field collected, overwintered adult toads (N = 12) using a harmonic tracking system. Toads were tracked using harmonic telemetry tags in the summers of 2014 and 2015. I soft released three groups of captive-bred toads using a delayed release strategy. Delayed released toads were kept in an outdoor enclosure at the release site for 14 days to acclimate them more natural conditions prior to release. I compared the spatial ecology of delayed released toads, hard released toads, and overwintered, adult toads tagged *in situ*. Toads in the delayed release group moved significantly shorter distances in total, utilized a smaller area, and stayed closer to the released site than hard-released toads. The results suggest that the initial spatial ecology of captive-bred adult toads after reintroduction are more similar to overwintered toads if they are delayed released than if hard released.

The Conclusion will show how these four chapters inform our conservation actions moving forward. We can alter our strategies for maintaining animals in captivity to reduce domestication effects. I will provide suggestions for future research that uses the scientific method to develop best conservation practices in each chapter.

LITERATURE CITED

Armstrong, D., Hayward, M., Moro, D., & Seddon, P. (Eds.). (2015). *Advances in reintroduction biology of Australian and New Zealand fauna*. CSIRO Publishing, Clayton South, Australia.

Balmford, A., Mace, G. M., & Leader-Williams, N. (1996). Designing the ark: setting priorities for captive breeding. *Conservation Biology*, *10*(3), 719-727.

Bishop, P. J., Angulo, A., Lewis, J. P., Moore, R. D., Rabb, G. B., & Moreno, J. G. (2012). The Amphibian Extinction Crisis-what will it take to put the action into the Amphibian Conservation Action Plan?. *SAPIENS. Surveys and Perspectives Integrating Environment and Society*, (5.2).

Blaustein, A. R., Han, B. A., Relyea, R. A., Johnson, P. T., Buck, J. C., Gervasi, S. S., & Kats, L. B. (2011). The complexity of amphibian population declines: understanding the role of cofactors in driving amphibian losses. *Annals of the New York Academy of Sciences*, *1223*(1), 108-119.

Bragg, A. N. (1960). Population fluctuation in the amphibian fauna of Cleveland County, Oklahoma during the past twenty-five years. *The Southwestern Naturalist*, 165-169.

Conant, R. (1958). *A field guide to reptiles and amphibians of the United States and Canada East of the 100th Meridian*. Houghton Mifflin Company, Boston, Massachusetts.

Cushman, S. A. (2006). Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological Conservation*, *128*(2), 231-240.

Czechura, G. and Ingram, G.J. (1990) *Taudactylus diurnus* and the case of the disappearing frogs. Mere. Queensland Mus. 29, 361-5.

Fischer, J., & Lindenmayer, D. B. (2000). An assessment of the published results of animal relocations. *Biological Conservation*, *96*(1), 1-11.

Frost, D. R. 2017. *Amphibian Species of the World: an Online Reference*. Version 6.0 (28 Dec 2017*)* Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/index.html. American Museum of Natural History, New York, USA.

Gascon, C., Collins, J. P., Moore, R. D., Church, D. R., McKay, J. E. and Mendelson, J. R. III (eds). 2007. *Amphibian Conservation Action Plan*. IUCN/SSC Amphibian Specialist Group. Gland, Switzerland and Cambridge, UK.

Germano, J. M., & Bishop, P. J. (2009). Suitability of amphibians and reptiles for translocation. *Conservation Biology*, *23*(1), 7-15.

Grant, E. H. C., Miller, D. A. W., Schmidt, B. R., Adams, M. J., Amburgey, S. M., Chambert, T., … Muths, E. (2016). Quantitative evidence for the effects of multiple drivers on continental-scale amphibian declines. *Scientific Reports*, *6*, 25625.

Griffith, B., Scott, J.M., Carpenter, J.W., Reed, C., (1989). Translocation as a species tool: status and strategy. *Science* 245, 477–480.

Griffiths, R. A., & Pavajeau, L. (2008). Captive breeding, reintroduction, and the conservation of amphibians. *Conservation Biology*, *22*(4), 852-861.

Harding, G., Griffiths, R. A., & Pavajeau, L. (2016). Developments in amphibian captive breeding and reintroduction programs. *Conservation Biology*, *30*(2), 340-349.

Harrington, L. A., Moehrenschlager, A., Gelling, M., Atkinson, R. P., Hughes, J., & Macdonald, D. W. (2013). Conflicting and complementary ethics of animal welfare considerations in reintroductions. *Conservation Biology*, *27*(3), 486-500.

IUCN/SSC (1998). Guidelines for Reintroductions and Other Conservation Translocations. 0. Gland, Switzerland: IUCN Species Survival Commission.

IUCN/SSC (2013). Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0. Gland, Switzerland: IUCN Species Survival Commission.

Johns, B. (2005). Whooping Crane recovery-A North American success story. *Biodiversity*, *6*(3), 2-6.

Lowe, S., Browne, M., Boudjelas, S., & De Poorter, M. (2000). 100 of the world's worst invasive alien species: a selection from the global invasive species database (Vol. 12). Auckland: Invasive Species Specialist Group.

Magin, C. D., Johnson, T. H., Groombridge, B., Jenkins, M., & Smith, H. (1994). Species extinctions, endangerment and captive breeding. In *Creative Conservation* (pp. 3-31). Springer, Netherlands.

Mendelson, J. R., Lips, K. R., Gagliardo, R. W., Rabb, G. B., Collins, J. P., Diffendorfer, J. E., ... & Wright, K. M. (2006). Confronting amphibian declines and extinctions. *Science*, *313*(5783), 48-48.

Pérez, I., Anadón, J. D., Díaz, M., Nicola, G. G., Tella, J. L., & Giménez, A. (2012). What is wrong with current translocations? A review and a decision-making proposal. *Frontiers in Ecology and the Environment*, *10*(9), 494-501.

Price, M. S., & Soorae, P. S. (2003). Reintroductions: whence and whither? *International Zoo Yearbook*, *38*(1), 61-75.

Rabb, G. B. (1990). Declining amphibian populations. *Species*, *13*, 33-34.

Scheele, B. C., Skerratt, L. F., Grogan, L. F., Hunter, D. A., Clemann, N., McFadden, M., ... & Brannelly, L. (2017). After the epidemic: Ongoing declines, stabilizations and recoveries in amphibians afflicted by chytridiomycosis. *Biological Conservation*, *206*, 37-46.

Seal US (1991) Life after extinction. In: Beyond Captive Breeding: Re-Introducing Endangered Mammals to the Wild (ed. Gipps JHW), pp. 39–55. Oxford, UK: Zoological Society of London.

Seddon, P. J., Armstrong, D. P., & Maloney, R. F. (2007). Developing the science of reintroduction biology. *Conservation biology*, *21*(2), 303-312.

Seddon, P. J., Strauss, W. M., & Innes, J. (2012). Animal translocations: what are they and why do we do them. *Reintroduction Biology: integrating science and management*, *12*(1).

Soorae, P. S. (ed.) (2016). Global Re-introduction Perspectives: 2016. Case-studies from around the globe. Gland, Switzerland: IUCN/SSC Reintroduction Specialist Group and Abu Dhabi, UAE: Environment AgencyAbu Dhabi.

Soorae, P. S. (Ed.). (2013). *Global Re-introduction Perspectives, 2013: Further Case Studies from Around the Globe* IUCN IUCN/SSC Re-introduction Specialist Group & Environment Agency-Abu Dhabi.

Spalton, J. A., Brend, S. A., & Lawrence, M. W. (1999). Arabian Oryx reintroduction in Oman: successes and setbacks. *Oryx*, *33*(2), 168-175.

Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S., Fischman, D. L., & Waller, R. W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science*, *306*(5702), 1783-1786.

Toone, W. D., & Wallace, M. P. (1994). The extinction in the wild and reintroduction of the California condor (*Gymnogyps californianus*). In *Creative conservation* (pp. 411-419). Springer, Dordrecht.

Tudge, C. 1992. Last animals at the zoo: How mass extinction can be stopped. Island Press, Washington, D.C.

Viggers, K.L., Lindenmeyer, D.B. & Speatt, D.M. (1993). The importance of disease in reintroduction programmes. *Wildlife Research* 20: 697–698.

Zippel, K., Johnson, K., Gagliardo, R., Gibson, R., McFadden, M., Browne, R., ... & Townsend, E. (2011). The Amphibian Ark: a global community for *ex situ* conservation of amphibians. *Herpetological Conservation and Biology*, *6*(3), 340-35.

CHAPTER 1: DRAMATIC INCREASES IN THE VALUES OF TRADED U.S.A. AMPHIBIANS INDICATES INCREASED RISK OF OVEREXPLOITATION

ABSTRACT

The collection of wild amphibians for human consumption and the pet trade may directly reduce populations through overexploitation. Additionally, human mediated movement of amphibians facilitates the spread of wildlife diseases and invasive species. Market fluctuations in the amphibian trade may directly impact conservation goals with potential global consequences. We examined changes in the market values of 52 species of amphibians in the United States in a 28-year time period by carrying out systematic surveys of U.S. based sellers of live amphibians. All but two species had increased in price dramatically during the study period. The mean percentage adjusted market price of U.S. amphibians rose 822%. The mean price for anuran species increased from US$4.26 to US$15.56 and caudates from US$5.32 to US$33.60. We found the market value is significantly higher for caudates with declining populations than other groups. We also found price increase to be positively correlated to range size from IUCN maps, indicating these maps may be insensitive to underlying range changes. Additionally, there was no influence on the prices of U.S. caudate species that were recently affected by a contentious 2016 emergency trade ban. The results provide valuable information on price trends of commonly traded North American amphibian species. We urge increased monitoring of amphibian market pressures and price dynamics to ensure the pet trade is sustainable.

INTRODUCTION

Amphibians are the most endangered class of vertebrates with hundreds of species threatened with extinction (Stuart et al., 2008). The interlinked and complex causes of worldwide amphibian declines include habitat loss, infectious diseases, pollution, introduced species, climate change, and overexploitation (Stuart et al. 2008; Blaustein et al. 2011; Li and Rohr 2013). Amphibians have been bought, sold, and traded throughout human history. Archaeological evidence indicates Neolithic people regularly ate frogs over 5000 years ago (e.g., Kyselý 2008). Furthermore, the cultivation of frogs has taken place for several hundred years. For example, the Grand Dictionnaire de Cuisine written in the 1600s, describes a man who became wealthy from fattening up frogs and selling them to Parisian restaurants (Dumas 1873). Currently, some amphibian species are farmed or wild-collected in large numbers for human consumption, such as American bullfrogs (*Lithobates catesbeianus*) and the Chinese Giant Salamander (*Andrias davidianus*) (Schloegel et al. 2009; Carpenter et al. 2014). Estimates of the international commercial trade in amphibians for food are roughly 10,000 tonnes/year, with the greatest local consumption in Asia and Africa (Warkentin et al. 2009). Other than consumption, amphibians are used for a variety of additional purposes including scientific research, pets, medicine, and religious ceremonies (Schlaepfer et al. 2005, Carpenter et al. 2014). As such, captive-bred and wild caught amphibians from a range of species are available for purchase worldwide at markets, pet stores, and Internet websites (Carpenter et al. 2014).

The local, regional, and international trade in amphibians may be amplifying some causes of decline, via overexploitation and the spread of disease (Fisher and Garner

2007; Gilbert et al. 2013, Rowley et al. 2016). The overexploitation of wild amphibians directly reduces population numbers and may contribute to an increased extinction risk (Warkentin et al. 2009, Chan et al. 2014). The United States (U.S.) imports large numbers of wild-caught amphibians from around the world. Schlaepfer et al. (2005) reported that between 1998 and 2002 14.7 million wild-caught amphibians (intact animals) were imported into the U.S. Included in that total, were 2,611,251 wild-caught individuals not identified to species. Within the U.S., large numbers of amphibians are also collected from the wild for local and international markets. In the State of Florida, Enge (2005) reported that 88,096 anurans (17 species) and 5,683 caudates (13 species) were wild collected for commercial trade over a four-year period. Other than a few large bodied ranids, such as *L. catesbeianus*, most U.S. species are not collected for human consumption, but are used for other purposes, mainly the pet trade. Direct harvesting pressure may cause significant reductions in abundance and increased risk of population extirpation for wild amphibian populations (Chan et al. 2014).

Limiting local and international trade in amphibians has been suggested as an important step in the conservation of many species (Carpenter et al. 2014; Natusch and Lyons 2012). In the U.S., local, interstate, and international trade in amphibians is a facilitating vector for the spread of pathogens, such as *Batrachochytrium dendrobatidis* (*B.d.*), which has caused declines, extirpations, and extinctions of several wild populations (e.g., Gratwicke et al. 2010; Kolby et al. 2014). The emergence of a new infectious fungus, *B. salamandrivorans* (*B. sal.*), has already caused declines in European salamanders, and the disease has the potential to spread to North American salamander populations with devastating consequences (Martel et al 2014; Stokstad 2014; Yap et al.

2015; Stegen et al. 2017). In response to the threat posed by *B. sal.,* in January 2016, the U.S. Fish and Wildlife issued an emergency interim rule to restrict importation, exportation, and interstate movement of 201 species of salamanders in the U.S. (U.S. Fish & Wildlife Service 2016). The restricted salamander species are not typically traded for human consumption, but are widely available as pets. The U.S. salamander trade restriction was a highly contentious issue among multiple stakeholders, most notably between the pet trade and conservationists (e.g., Church et al. 2014; Caudata.org 2016; Goss et al. 2016).

It is clear that a detailed understanding of the pet trade in amphibians, including the monetary value and species commercially available, is critically important for conservation and developing effective public policy. Furthermore, quantifying market value of amphibians is important for monitoring price trends, conservation planning and decision-making. Management decisions may incur conservation trade-offs with direct economic consequences for commercially traded species, the magnitude of which depends on market fluctuations. The economic value of species is estimated using various methods, such as replacement cost and existence cost (de Groot et al., 2002). The value of an organism can also be calculated at an ecosystem-level by estimating importance of a species to ecosystem functioning (i.e., with keystone species). However, such estimation is challenging because of the difficulties in establishing the worth of the direct ecosystem services supplied by amphibians as well as their intrinsic value (Burton and Likens 1975; Stevens et al. 1991; Searcy and Shaffer 2008). Furthermore, knowing the monetary value of amphibians is necessary to accurately calculate potential mitigation cost and price of conservation offsets (Searcy and Shaffer 2008), if they are chosen as suitable conservation mechanisms. When direct market prices are available, they can provide a useful valuation as they reveal the consumers' willingness to pay. Furthermore, a consumer's revealed willingness to pay and fluctuations in value may be directly linked to the increased collection of rare amphibians and subsequent decline of wild populations (Andreone et al. 2006; Tournant et al. 2012).

Herein we assess how the market price for North American amphibians has changed over a 28-year period, from 1989 to 2016. We then use the current price and change in price to explore how the monetary value varies between orders (Caudata and Anura), species affected and not affected by the 2016 caudate trade ban, and with the IUCN Red List conservation listing status (e.g., least concern, endangered, etc.), population trend, and range size proved by Red List species assessments. Our results provide valuable information on price trends of commonly traded North American amphibian species and provide insights into the relationship between conservation status and market price of U.S. amphibians with potentially important global consequences.

METHODS

Data collection of amphibian prices

Historical prices for 148 North American amphibian species were taken from a 1989 report produced by a special sub-committee in the Society for the Study of Reptiles and Amphibians (SSAR Monetary Value of Amphibians Subcommittee, 1989). These prices were produced by examining price lists of amphibian and reptile dealers. The committee subtracted 40% of the listed value for individual animals to produce wholesale prices. If prices for some species were not available from price lists, the committee estimated their price by using values from closely related species, and by expert consensus as to their monetary value. Because the 1989 prices were converted to wholesale, we increased their prices uniformly to the original price to represent the re-adjusted actual individual market prices in 1989. These data were then adjusted for inflation using the consumer price index for direct comparison to prices in 2016.

Current prices were collected in April 2016 from U.S. amphibian sellers by obtaining physical pricelists, online price lists, and calling sellers to ask for prices. Price lists were systematically searched, and individual market prices for North American species that also matched the species list from 1989 were recorded. We found 24 vendors with current prices for species that had paired data from 1989. Several dozen other amphibian sellers surveyed did not have any species on the 1989 price list because they only sold non-native species or the species listed could not be identified. Prices for 58 species in 2016 were collated for the final dataset. Prices for the 58 species were recorded from multiple venders and then averaged for each species. We recorded if the species was affected by the 2016 caudate import/export ban (U.S. Fish & Wildlife Service 2016). Prices for animals that were unusual color morphs (e.g., albino) were only found in two species and ignored as they often are worth substantially more in the pet trade than normal color morphs (Tapely et al. 2011). Multiple individuals listed for a single price (bulk prices) were ignored. Prices specifically listed as wholesale and prices that did not specifically list the genus and species were also ignored. As a consequence of changes in taxonomic nomenclature for some amphibian species since 1989, and the fact that sellers do not all use current nomenclature, we matched prices listed under older nomenclature to the correct species nomenclature by using the Amphibian Species of the World database (Frost 2016).

Explanatory Variables

For 58 species, with both 1989 and 2016 price data, we obtained each species' IUCN Red List status, and population trend, and range data provided by the IUCN Red List (IUCN 2016). Population trend was categorical with each species having an increasing, decreasing, stable, or unknown trend. The range for each species was calculated from the area of occupancy polygons provided by IUCN Red List species assessments using ArcGIS 10.3 software (in km^2).

Data analysis

The percentage price change was calculated for all 58 species. We used generalized linear models with Gaussian errors to assess the correlation of three different dependent variables including percentage price change between 1989 and 2016 (model 1), absolute difference in price (in USD) between 1989 and 2016 (model 2), and the 2016 price of each species (model 3). Each of these models were run against the independent variables species range size (km^2), population trend (increasing, decreasing, stable, and unknown), listing status (least concern, near threatened, threatened, and endangered), and taxonomic group (Caudata and Anura). We transformed dependent variables by taking their natural logs to meet assumptions of normality for the model. We handled negative values by adding a constant to the dependent variables such that the lowest value was set to one. Models were simplified using stepwise deletion and likelihood ratio tests (Zuur

2009). Finally, we repeated this analysis for both Caudata and Anura separately. All analyses were completed using R 3.3.0 statistical software (R Development Core Team 2016).

RESULTS

Descriptive statistics

A total of 58 species with price data from both 1989 and 2016 were found (Appendix 1). We identified a large increase in the retail prices of North American amphibians since 1989. The average price of anurans and caudates in 1989 was $4.26 and $5.32 (inflation adjusted), respectively (Table 1.1). In 2016, the average price of anurans and caudates rose to $15.56 and $33.60, respectively. Average price for both orders were significantly different between 1989 and 2016 ($p < 0.01$) using a Wilcoxon test. The average percentage price increase for amphibians in our dataset was 822%. Some traded species experienced a percentage increase in trade in the thousands (Table 1.2). For example, the Cave Salamander's (*Eurycea lucifuga*) price increased 7,191% from 1989 to 2016. The species that held the highest market value was the Colorado River toad (*Incilius alvarius*), with the average price (for eight sellers sampled) of $93.12 per individual (Figure 1.1). Two species prices declined during the study period, namely the Sonoran Green Toad *(Anaxyrus retiformis)* and the Mexican Narrow-mouthed Toad (*Hypopachus variolosus*). Although not included in the price analysis, the only unusual color morphs encountered during 2016 data collection were albino variants of two anuran species representing 3% of the 38 US species encountered during data collection.

Of the 58 species for which we had data, two species found for sale in 2016 were listed as near threatened by the IUCN Red List: the Greater Siren (*Siren lacertina*) and the Western Spadefoot Toad (*Spea hammondi*). Eight species were listed as having a decreasing population trend and nine species were listed as unknown. The range sizes of species studied varied from 49,305 km^2 to 9,819,086 km^2.

The interim ban on interstate trade, and import of some caudates (Fish and Wildlife 2016) affected six species in our data set four months prior to our data collection. The average price in 2016 was very similar for caudates affected by the ban (\$33.66, N = 6) and those that were not (\$33.58, N = 23). The average percentage price change was lower for restricted caudates (589%) compared to non-restricted caudates (1353%).

3.2 Model results

Our model 1 results indicate that percentage price change between 1989 and 2016 was significantly different between the two orders, with Caudata having a higher rate of price change (0.85, SE 0.33, $P < 0.05$). The absolute difference in price between 1989 and 2016 (model 2) also showed a statistically significant difference between the orders, with Caudata having a more positive effect than Anura (0.72, SE 0.15, $P < 0.001$), indicating Caudata had a higher absolute change in price. Model 3, comparing only the current 2016 market price yielded the same result, with caudates having a significantly higher price than anurans (0.82, SE 0.13, $P < 0.001$). Our results indicate that both caudates and anurans have greatly increased in price although caudates increased in price more than did anurans.

Our GLM analysis of the orders separately showed caudates with a decreasing population status (per IUCN) had a significantly higher monetary value in 2016 (-0.77, SE 0.21, $P < 0.001$) and smaller changes in price from 1989 to 2016 (-1.21, SE 0.48, $P < 0.05$) than caudates with a stable population status. Anurans showed a positive correlation between IUCN range size and percentage price increase (0.5, SE 0.2, $P > 0.05$) as well as absolute price increase (0.21, SE 0.1, $P > 0.05$). Therefore, anurans with large ranges have experienced larger price increases since 1989 than those with smaller ranges, an unexpected result.

DISCUSSION

Patterns of the amphibian trade

The average percentage price increase of 822% of the 58-studied species indicates a major shift in market prices and pet trade dynamics between 1989 and 2016. Our model results indicate that caudates have a higher current market value and underwent larger price increases than anurans. Our analysis of the Caudata alone show that species with declining populations have higher monetary values than non-declining species. This result is expected as declining populations likely indicate decreasing supply (resulting from increasing difficulty of collection), and under conditions of stable demand (or increasing demand) would lead to an increase in price, suggesting that many native North American caudates are at risk of overexploitation. As a result of market trends, rare species may experience an anthropogenic Allee Effect that would accelerate the effects of overexploitation on native populations (Tournant et al. 2012). Furthermore, individual desires for wanting rare species, with a higher perceived value, may accelerate declines (Gault. et al 2008; Natusch and Lyons 2012). However, the desirability of species varies. For example, reasons for the inflated price of *I. alvarius* are unclear, but people seeking the toad for its hallucinogenic properties (Weil and Davis 1994), could contribute to its high price.

It is possible that the combination of an increase in demand and a stable or decreasing supply of native species have driven the price increases for widely traded native amphibians. Additionally, increased restrictions on the collection and interstate trade of many amphibian species during the intervening 28 years may have affected values. However, laws regarding collection and trade of native amphibian species vary widely among states and species making rigorous analyses a challenge. Without a strong legal framework for protection throughout species ranges, many native amphibian species are at risk of overexploitation driven by their high market value.

Our model for the change in price of anurans found it positively correlated with range size, a somewhat counter-intuitive result. We would expect to see price increasing with decreasing range size, given the effect of supply constraints on price seen in Caudata. Our result, therefore, is likely because of the insensitivity of IUCN ranges to changes in the underlying distribution of amphibians, the greater proliferation of omission, commission errors in larger IUCN ranges (Rondinini et al. 2006), and other factors influencing the supply and demand of anurans that we were not able to capture in our model. Despite this, the large relative price increases of Anura throughout our dataset are indicative of an underlying shortfall in supply from either restriction on collection or decreasing populations over the last 28 years.

Interim ban

We did not find a significant difference between prices of caudates affected by the interim ban (N = 6) and non-restricted species (N = 23). Our result tentatively supports the conclusion that price rises have been driven by decreasing supply rather than restriction on trade and collection. However, the small sample size and limited timespan of our dataset reduce our ability to detect the market impacts of the ban, hence we are unwilling to draw strong conclusions from our single case study. More restricted species may be available on the black market and were not found for sale using our data collection methodology. But the study of the short-term dynamic impacts of this ban are essential for evaluating both amphibian overexploitations and the efficacy of the controversial trade restriction for fighting the spread of pathogens.

Conservation implications of amphibian trade

Few native U.S. species found for sale in our study are of conservation concern. We found only two U.S. species that were listed as Near Threatened by the IUCN Red List. The majority of species in our dataset were listed as Least Concern (Table 1.3), showing internationally and federally listed species are not generally traded openly in the US. However, the regulatory environment for amphibians in the U.S. is complicated, with some states providing protection to species not listed under the US Endangered Species Act (e.g., Fish and Game Commission 2016). Despite this, amphibians are the most under-listed taxon in the U.S., with 82% of at risk species on NatureServe not listed as threatened or endangered by the federal Endangered Species Act (Gratwicke et al. 2010).

Many states have increased restrictions on the collection and trade of native amphibians, which are not federally protected, to limit exploitation during the 28-year period of our study (e.g., Fish and Game Commission 2016). However, those same states allow keeping species that are native to other states, which paradoxically may both suppress intrastate trade while promoting interstate trade. Additionally, captive-bred animals may be exempt from trade restrictions making enforcement difficult resulting from the necessity to demonstrate that traded animals were not wild collected. The period of our study also coincided with the invention of the World Wide Web and its use as a platform for the trade of amphibians which has likely profoundly altered the way amphibians are bought and sold in the U.S. Given the ability of online marketplaces to bring together disparate consumers and suppliers, the ease which consumers may obtain a wide variety of species has likely increased. The current system of state level regulation likely is ineffective at curbing the trade nationally, without rigorous enforcement of online commerce.

The black-market trade in amphibians is a global problem (Garner et al. 2009; Schlaepfer et al. 2005) where seizures of CITES listed amphibian species are well documented by organizations such as TRAFFIC (http://www.traffic.org/reptiles-amphibians). The demand for keeping amphibians as pets is unlikely to end, and full restrictions on the trade and collection of amphibians would be difficult to enforce (Garner et al. 2009). Price increases identified by our study (several thousand percent for many species) suggest growing incentive for black market or illegal trade in amphibians. Illegal interstate trade of native amphibians may also damage wild populations similar to international trade (e.g., Huss et al. 2013).

Animals produced through captive breeding for trade may provide significant conservation benefits (Garner et al. 2009; Tensen 2016). High prices should incentivize captive breeding efforts. It is not known if captive breeding amphibians can reduce negative impacts associated with the collection of wild specimens (but see Matolli et al. 2006). If local captive-bred species are not available in the pet trade, responsible collection of common and local amphibian species may circumvent potential problems associated with trade in non-native species, and increase pet owner's familiarity and willingness to conserve local amphibian populations.

Policy recommendations

Conservationists should aim to balance responsible amphibian trade with potential overexploitation, spread of disease, and the risk of escaped animals becoming invasive. To improve our understanding of the amphibian trade and associated market dynamics, we suggest researchers (a) collect market data regularly for a wide range of species, particularly before and after changes to trade policy, (b) monitor prices of declining species that may be impacted by an anthropogenic Allee Effect, and (c) increase collection of data on interstate and international trade in the U.S. The complex and inconsistent amphibian trade regulatory environment in the U.S. may hamper potential amphibian trade enforcement efforts. The looming dangers of emerging infectious diseases spread through trade combined with the increasing monetary value of amphibians highlights the urgency and importance of understanding the trade (Yap et al. 2015; Spitzen-van der Sluijs et al. 2016). Conservationists should carefully analyze how market pressures may interact with conservation goals.

ACKNOWLEDGMENTS

We would like to acknowledge David Bickford, Joseph Mendelson, Joel Heinen and Maureen Donnelly for comments and revisions to improve early versions of this manuscript. We also thank James Stroud for advice, positive encouragement and early data entry. We would like to thank Paper-Jam 2016 supported by Emerald Gardens. Partial funding and support for this project was provided by a U.S. National Science Foundation Graduate Research Fellowship to L.J.L. (grant #2013172527).

LITERATURE CITED

Andreone F, Mercurio V, & Mattioli F. 2006. Between environmental degradation and international pet trade: conservation strategies for the threatened amphibians of Madagascar. Natura, *95*(2), 81-96.

Bartlett RD, & Bartlett PP. 2002. Designer Reptiles and Amphibians. Barron's Educational Series, Inc. Hauppauge, NY.

Blaustein AR, Han BA, Relyea RA, Johnson PT, Buck JC, Gervasi SS, Kats LB. 2011. The complexity of amphibian population declines: understanding the role of cofactors in driving amphibian losses. Annals of the New York Academy of Sciences, 1223(1), 108-119.

Burton TM, & Likens GE. 1975. Salamander populations and biomass in the Hubbard Brook experimental forest, New Hampshire. Copeia 1954 (3), 541-546.

Carpenter AI, Andreone F, Moore RD, Griffiths RA. 2014. A review of the international trade in amphibians: the types, levels and dynamics of trade in CITES-listed species. Oryx, 48(04), 565-574.

Caudata.org. 2016. Official Caudata.org Comment on the Federal Register. Availible from http://www.caudata.org/forum/f1173-advanced-newt-salamander-topics/f1175-herpetological-science-politics/f1176-laws-legality-ethics/105645-official-caudata-org-comment-federal-register.html (accessed April 2016).

Chan HK, Shoemaker KT, Karraker NE. 2014. Demography of Quasipaa frogs in China reveals high vulnerability to widespread harvest pressure. Biological Conservation, *170*, 3-9.

Church D, Baker A, Gei C, Clark J, Jenkins P, Cleary R, 2014. Urgent – protecting native salamnders; Meeting request. Available from http://www.amphibianark.org/pdf/lt-Ashe-Bsal-final.pdf (Accessed May 2016).

de Groot, RS, Wilson MA, Boumans RM. 2002. A typology for the classification, description and valuation of ecosystem functions, goods and services. Ecological Economics, 41(3), 393-408.

Dumas A. 1873. Le grand dictionnaire de cuisine. H. Veyrier, Torino, 1978.

Enge, KM. 2005. Commercial harvest of amphibians and reptiles in Florida for the pet trade. Amphibians and reptiles: status and conservation in Florida. Krieger, Malabar, Florida, USA, 198-211.

Fish and Game Commission. 2016. California Freshwater Sport Fishing Regulations 2016-2017. Available from https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=117095&inline (accessed March 2016)

Fisher MC, Garner TW. 2007). The relationship between the emergence of *Batrachochytrium dendrobatidis*, the international trade in amphibians and introduced amphibian species. Fungal Biology Reviews, 21(1), 2-9.

Frost DR. 2016. Amphibian Species of the World: an Online Reference. Version 6.0 (accessed January 2016). Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/index.html. American Museum of Natural History, New York, USA.

Garner TW, Stephen I, Wombwell E, Fisher MC. 2009. The amphibian trade: bans or best practice? EcoHealth, 6(1), 148-151.

Gault A, Meinard Y, Courchamp F. 2008. Consumers' taste for rarity drives sturgeons to extinction. Conservation Letters, *1*(5), 199-207.

Gilbert M, et al. 2012. Amphibian pathogens in Southeast Asian frog trade. EcoHealth, 9(4), 386-398.

Goss P, Galvin J, Hawkins A. 2016 Re: Interim Rule: Listing Salamanders Due to Risk of Salamander Chytrid Fungus, Docket No. FWS–HQ–FAC–2015–0005. Available from http://usark.org/wp-content/uploads/2016/04/USARK-Salamander-Lacey-Act-comment-final.pdf (accessed July 2016)

Gratwicke B, Evans MJ, Jenkins PT, Kusrini MD, Moore RD, Sevin J, Wildt DE. 2010. Is the international frog legs trade a potential vector for deadly amphibian pathogens? Frontiers in Ecology and the Environment, 8(8), 438-442.

Huss M, Huntley L, Vredenburg V, Johns J, Green S. 2013. Prevalence of *Batrachochytrium dendrobatidis* in 120 archived specimens of *Lithobates catesbeianus* (American bullfrog) collected in California, 1924–2007. EcoHealth, 10(4), 339-343.

IUCN 2016. The IUCN Red List of Threatened Species. Version 2016-2. Available from http://www.iucnredlist.org. Downloaded on June 2016.

Kolby JE, Smith KM, Berger L, Karesh WB, Preston A, Pessier AP, Skerratt LF. 2014. First evidence of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) and ranavirus in Hong Kong amphibian trade. PLoS One, 9(3), e90750.

Kyselý R. 2008. Frogs as a part of the Eneolithic diet. Archaeozoological records from the Czech Republic (Kutná Hora-Denemark site, Řivnáč Culture). Journal of Archaeological Science, 35(1), 143-157.

Li Y, Cohen JM, Rohr JR. 2013. Review and synthesis of the effects of climate change on amphibians. Integrative Zoology, 8(2), 145-161.

Martel A, et al. 2014. Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. Science, 346(6209), 630-631

Mattioli F, Gili C, Andreone F. 2006. Economics of captive breeding applied to the conservation of selected amphibian and reptile species from Madagascar. Natura–Società italiana di Scienze naturali e Museo civico di Storia Naturale di Milano, 95(2), 67-80.

Natusch DJ, Lyons JA. 2012. Exploited for pets: the harvest and trade of amphibians and reptiles from Indonesian New Guinea. Biodiversity and Conservation, 21(11), 2899-2911.

R Core Team 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.R-project.org/ (Accessed July 2016)

Rondinini C, Wilson KA, Boitani L, Grantham H, Possingham HP, 2006. Trade-offs of different types of species occurrence data for use in systematic conservation planning. Ecology Letters, 9, 1136–1145.

Rowley JJ, et al. 2016. Estimating the global trade in Southeast Asian newts. Biological

Conservation, 199, 96-100.

Schlaepfer MA, Hoover C, Dodd CK. 2005. Challenges in evaluating the impact of the trade in amphibians and reptiles on wild populations. BioScience, *55*(3), 256-264.

Schloegel LM, Picco AM, Kilpatrick AM, Davies AJ, Hyatt AD, Daszak P. 2009. Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidis* and ranavirus infection in imported North American bullfrogs (*Rana catesbeiana*). Biological Conservation, 142(7), 1420-1426.

Searcy CA, Shaffer HB. 2008. Calculating biologically accurate mitigation credits: insights from the California tiger salamander. Conservation Biology, 22(4), 997-1005.

Spitzen-van der Sluijs A, et al. 2016. Expanding Distribution of Lethal Amphibian Fungus *Batrachochytrium salamandrivorans* in Europe. Emerging Infectious Diseases, *22*(7).

Stegen G, et al. 2017. Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. Nature, 544(7650), 353-356.

Stevens TH, Echeverria J, Glass RJ, Hager T, More TA. 1991. Measuring the existence value of wildlife: what do CVM estimates really show? Land Economics, *67*(4), 390-400.

Stokstad E. 2014. The coming salamander plague. Science, *346*(6209), 530-531.

Stuart SN, Hoffman M, Chanson JS, Cox NA, Berridge RJ, Ramani P, Young BE, editors. 2008. Threatened Amphibians of the World. Lynx Edicions, Barcelona, IUCN, Gland, Switzerland, and Conservation International, Arlington, VA.

Tapley B, Griffiths RA, Bride I. 2011. Dynamics of the trade in reptiles and amphibians within the United Kingdom over a ten-year period. *The Herpetological Journal*, *21*(1), 27-34.

Tensen L. 2016. Under what circumstances can wildlife farming benefit species conservation? Global Ecology and Conservation, *6*, 286-298.

Tournant P, Joseph L, Goka K, Courchamp F. 2012. The rarity and overexploitation paradox: stag beetle collections in Japan. Biodiversity and Conservation, *21*(6), 1425-1440.

U.S Fish & Wildlife Services 2016. Injurious Wildlife Species; Listing Salamanders due to the risk to salamander chytrid fungus. Interim rule 50 CFR Part 16. Available from https://www.fws.gov/policy/library/2016/2016-00452.pdf (Accessed April 28, 2016)

Warkentin IG, Bickford D, Sodhi NS, Bradshaw CJ. 2009. Eating frogs to extinction. Conservation Biology, 23(4), 1056-1059.

Weil AT, Davis W. 1994. *Bufo alvarius*: a potent hallucinogen of animal origin. Journal of Ethnopharmacology, 41(1), 1-8.

Yap TA, Koo MS, Ambrose RF, Wake DB, Vredenburg VT. 2015. Averting a North American biodiversity crisis: a newly described pathogen poses a major threat to salamanders via trade. Science, 349 (62-47).

Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM. 2009. Mixed effects models and extensions in ecology with R, Statistics for Biology and Health. Springer New York, New York, NY. doi:10.1007/978-0-387-87458-6

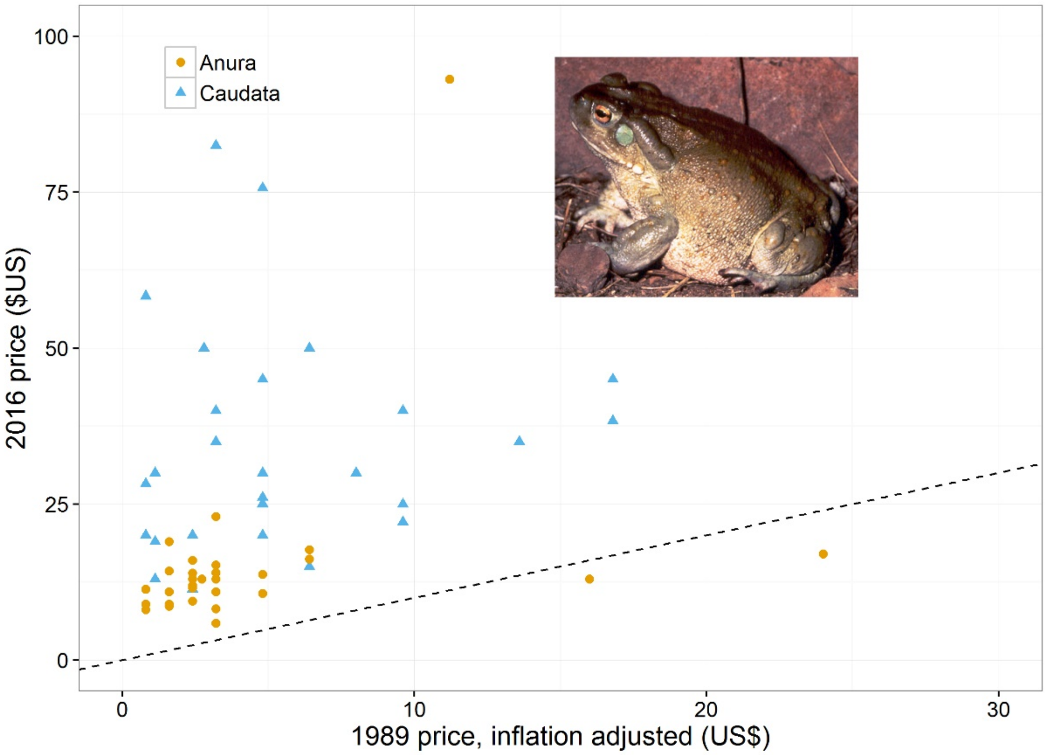

Figure 1.1. Scatter plot of 2016 prices against 1989 prices. The dashed black line corresponds to x=y, all the points above the line represent species which have increased in price since 1989. The inset is a picture (credit: Wikimedia Commons) of the most valuable species in our dataset, *Incilius alvarius,* the Colorado River Toad.

	Number of species with both prices	Average inflation adjusted price in 1989 (USD)	Average Price in 2016 (USD)	Percentage change of average price	Average percentage increase in price per species
Anura	29	4.26	15.56	365.28%	448.945%
Caudata	29	5.32	33.60	532.16%	1158.06%
All species	58	4.79	24.58	413.44%	822.01%

Table 1.1. Breakdown of all species, anuran and caudate average percentage price change

Top five species	Red List status	IUCN population trend	Range Size*	Inflation adjusted price in 1989 (USD)	Price in 2016 (USD)	Relative price change 1989-2016
Greatest price change						
Eurycea lucifuga	LC	Unknown	449,641	0.8	58.33	7,190.8%
Eurycea longicauda	LC	Stable	848,917	0.8	28.25	3,430.6%
Desmognathus quadramaculatus	LC	Stable	84,423	1.12	29.99	2,577.7%
Pseudotriton ruber	LC	Decreasing	1,068,840	3.2	82.50	2,478.0%
Desmognathus fuscus	LC	Stable	1,548,640	0.8	19.99	2,398.8%
Lowest price change						
Amphiuma tridactylum	NT	Decreasing	477,336	16.8	38.32	128.1%
Scaphiopus holbrookii	LC	Unknown	1,122,190	4.8	10.66	122.1%
Hyla cinerea	LC	Stable	1,290,870	3.2	5.91	84.8%
Hypopachus variolosus	LC	Stable	727,037	16	12.99	-18.8%
Anaxyrus retiformis	LC	Stable	52,061	24	16.99	-29.2%

Table 1.2. Price changes for the top five species with the highest and lowest price changes. 2016 prices are averages across all suppliers.

	Population trend			
	Increasing	Stable	Decreasing	Unknown
Anura	2	22	3	2
Caudata	0	18	4	7
Both	2	40	7	9

Table 1.3. The number of species in each population trend categories as assessed by the International Union for the Conservation of Nature (IUCN) Red List.for all amphibians' species found for sale.

APPENDIX 1.1

Latin name	IUCN listing	IUCN pop. trend	IUCN Range Size	1989 retail price	1989 Inflation adjusted price	2016 average price	% change
Acris crepitans	LC	stable	3,209,406	0.42	0.80	8.99	1,023.8
Ambystoma gracile	LC	stable	350,891	1.67	3.20	34.99	993.4
Ambystoma jeffersonianum	LC	stable	470,696	2.50	4.80	19.99	316.5
Ambystoma laterale	LC	stable	1,977,215	5.00	9.60	24.99	160.3
Ambystoma maculatum	LC	stable	3,105,941	2.50	4.80	25.08	422.5
Ambystoma opacum	LC	stable	1,683,647	5.00	9.60	22.14	130.6
Ambystoma tigrinum	LC	stable	3,680,960	2.50	4.80	26.10	443.8
Amphiuma macrodacatylum	LC	stable	1,443,333	2.50	4.80	24.99	420.6
Amphiuma means	LC	unknown	491,482	8.75	16.80	44.99	167.8
Amphiuma tridactylum	NT	decreasing	477,336	8.75	16.80	38.32	128.1
Anaxyrus americanus	LC	stable	5,225,512	0.83	1.60	14.33	795.4
Anaxyrus cognatus	LC	unknown	2,795,074	1.67	3.20	12.99	305.9
Anaxyrus debilis	LC	stable	1,460,542	3.33	6.40	16.23	153.6
Anaxyrus punctatus	LC	stable	2,436,579	1.25	2.40	12.99	441.3
Anaxyrus quercicus	LC	stable	450,930	0.42	0.80	11.37	1,320.9
Anaxyrus retiformis	LC	stable	52,061	12.50	24.00	16.99	-29.2
Anaxyrus speciosus	LC	stable	1,063,680	1.25	2.40	12.00	400.0

Latin name	IUCN listing	IUCN pop. trend	IUCN Range Size	1989 retail price	1989 Inflation adjusted price	2016 average price	% change
Anaxyrus terrestris	LC	stable	545,693	1.25	2.40	9.43	293.0
Anaxyrus woodhousii	LC	stable	3,242,458	0.83	1.60	18.99	1,086.9
Desmognathus auriculatus	LC	stable	572,601	0.58	1.12	12.99	1,059.8
Desmognathus Fuscus	LC	stable	1,548,640	0.42	0.80	19.99	2,398.8
Desmognathus monitcola	LC	stable	324,005	0.58	1.12	18.99	1,595.5
Desmognathus quadramaculatus	LC	stable	84,423	0.58	1.12	29.99	2,577.7
Eurycea bislineata	LC	stable	1,198,567	0.42	0.80	19.99	2,398.8
Eurycea longicauda	LC	stable	848,917	0.42	0.80	28.25	3,430.6
Eurycea lucifuga	LC	unknown	449,641	0.42	0.80	58.33	7,190.8
Gastrophryne carolinensis	LC	stable	1,576,038	0.83	1.60	8.99	461.9
Gryinophilus porphyriticus	LC	stable	773,390	3.33	6.40	49.99	681.1
Hemidacylium scutatum	LC	stable	1,539,052	3.33	6.40	14.99	134.2
Hyla cinerea	LC	stable	1,290,870	1.67	3.20	5.91	84.8
Hyla gratiosa	LC	stable	593,264	1.67	3.20	15.24	376.1
Hyla squirella	LC	stable	772,872	0.42	0.80	8.09	911.8
Hyla versicolor	LC	stable	2,278,080	1.67	3.20	10.99	243.5
Hypopachus variolosus	LC	stable	727,037	8.33	16.00	12.99	-18.8
Incilius alvarius	LC	stable	364,920	5.83	11.20	93.12	731.4

Latin name	IUCN listing	IUCN pop. trend	IUCN Range Size	1989 retail price	1989 Inflation adjusted price	2016 average price	% change
Incilius valliceps	LC	stable	563,334	1.25	2.40	13.99	482.9
Lithobates catesbeianus	LC	Increasing	6,320,278	3.33	6.40	17.68	176.2
Lithobates grylio	LC	decreasing	357,550	1.67	3.20	14.00	337.3
Lithobates pipiens	LC	decreasing	6,021,922	0.83	1.60	8.67	441.8
Lithobates sphenocephalus	LC	stable	2,004,554	1.67	3.20	8.24	157.6
Lithobates sylvaticus	LC	stable	9,135,390	1.67	3.20	22.99	618.4
Necturus maculosus	LC	stable	1,884,283	2.50	4.80	44.99	837.3
Necturus viridescens	LC	stable	6,957,280	1.25	2.40	11.34	372.7
Pseudacris crucifer	LC	stable	4,302,852	0.83	1.60	10.99	586.9
Pseudobranchus striatus	LC	decreasing	102,698	1.46	2.80	49.99	1,685.4
Pseudotriton montanus	LC	unknown	758,984	5.00	9.60	39.99	316.6
Pseudotriton ruber	LC	decreasing	1,068,840	1.67	3.20	82.50	2,478.0
Pseudotriton ruber	LC	decreasing	1,068,840	1.67	3.20	40.00	1,149.8
Rhinella marina	LC	Increasing	9,818,086	2.50	4.80	13.77	186.9
Scaphiopus bombifrons	LC	stable	2,525,430	1.25	2.40	15.99	566.3
Siren intermedia	LC	unknown	961,432	4.17	8.00	30.00	275.0
Sirena lacertina	LC	unknown	418,249	7.08	13.60	34.99	157.3
Spea couchii	LC	stable	1,954,143	1.25	2.40	11.60	383.2

Latin name	IUCN listing	IUCN pop. trend	IUCN Range Size	1989 retail price	1989 Inflation adjusted price	2016 average price	% change
Spea hammondii	NT	decreasing	122,002	1.42	2.72	12.99	377.6
Spea holbrookii	LC	unknown	1,122,190	2.50	4.80	10.66	122.1
Taricha granulosa	LC	stable	372,158	1.25	2.40	19.99	732.9
Taricha torosa	LC	unknown	49,305	2.50	4.80	30.00	525.0

CHAPTER II: HUSBANDRY OF AMPHIBIANS IN THE UNITED STATES: IDENTIFICATION OF RESEARCH PRIORITIZATIONS FOR *EX SITU* CONSERVATION ACTIONS

ABSTRACT

In response to global amphibian declines, over 100 amphibian species are currently kept in captivity for *ex situ* conservation, and hundreds of other amphibian species may require interventionist conservation actions requiring captivity to prevent their extinction in the future. However, our ability to care for amphibians in captivity is limited, and a lack of taxon-specific amphibian husbandry knowledge has limited the effectiveness of many amphibian conservation programs because of high mortality and low reproductive output of captive animals resulting in several near-extinctions events related to husbandry. To assess whether the perceived lack of amphibian husbandry knowledge is limited to a few species or a broader systemic problem within amphibian conservation, I performed a systematic review of amphibian husbandry for all 289 native amphibian species found in the United States. The availability, quality and type of husbandry literature for each species was recorded. I then performed a taxonomic gap-analysis to identify amphibian groups with poorly understood husbandry. I found that 55.5% of all US amphibian species had no taxon-specific amphibian husbandry information, and only 12.5% of species had a *high* husbandry knowledge ranking. Species and their associated husbandry ranks were also compared to conservation needs indices including each species' IUCN's Red List status, the U.S. list of federally endangered species status, the Evolutionarily Distinct and Globally Endangered (EDGE) species rating, and the Evolutionary Distinctiveness (ED) rating produced by the EDGE Programme to identify species and taxonomic groups for amphibian husbandry research prioritization. The

results of the review indicate that amphibian husbandry information is critically lacking for the majority of amphibian species in the US. The development of taxon-specific amphibian husbandry is an area largely forgotten in amphibian conservation and research, and warrants increased research.

INTRODUCTION

In contrast to keeping domesticated species, husbandry of wildlife species represents unique challenges inherent with an organism poorly adapted to a novel, captive environment. Although a rich animal husbandry literature exists, most husbandry publications are skewed towards domesticated species, and husbandry literature for wildlife is largely lacking (Fa et al. 2011). Wildlife husbandry for many groups of taxa is poorly known, with little to no peer-reviewed literature available (Wildt et al. 2003). Furthermore, there is a strong publication bias within wildlife husbandry towards animals used for research, or large-bodied mammals (Balmford et al. 1996; Wildt et al. 2003; Zimmerman et al. 2007).

Animal husbandry is a discrete scientific discipline with peer-reviewed journals focused on hypothesis driven research (e.g., *Journal of Veterinary Science & Animal Husbandry*, *Journal of Animal Science and Biotechnoalogy,* and *Zoo Biology*). However, animal husbandry is often still considered experience-driven rather than evidence-based, and may be perceived as a craft or art rather than science (Marantelli 1999). For poorly studied wildlife species, unsubstantiated husbandry practices without scientific backing, known as “folklore husbandry,” may be commonplace, and can negatively impact the quality of life of captive animals. For example, folklore husbandry is described as pervasive for reptile and amphibian species (Arbuckle 2013), and low husbandry knowledge may negatively impact conservation efforts.

Poor knowledge of husbandry may limit our capacity to utilize *ex situ* conservation methods (e.g., captive breeding animals to produce stock for reintroduction or supplementing existing populations; Baker 2007; Michaels et al. 2014). The difficulties and risks associated with employing *ex situ* conservation for the last few individuals of a species can be compounded by unknown husbandry, leaving little room for error or experimentation that may result in additional stress for declining populations (e.g., Channing et al. 2006; Gagliardo et al. 2008).

Both short-term and long-term captivity of wildlife has been linked to negative behavioral and physiological changes, such as depressed immune system, loss of the “fight or flight” response, reduced reproductive capacity, and increased risk of predation (see reviews by Moore & Jessop [2003], McDougall et al. [2006], Morgan & Tromborg [2007], Dickens et al. [2010], and Parker et al. [2012]). Evolutionary pressures are likely different in captivity than in the wild (Ford 2002), and poor husbandry practices (e.g., inadequate caging, nutrition, or lighting) may exacerbate changes tied to domestication processes. Phenotypic variation within a captive population may result in some animals being predisposed to survive in captivity over others. Animals poorly adapted to captivity will be filtered out of the captive population and can result in domestication (McDougall et al. 2006). Thus, husbandry that mimics what a wild animal would naturally experience is preferable to help keep wild animals “wild” (Price 1999). Even a single generation in captivity may result in heritable reductions in fitness of captive bred animals upon return to the wild (Araki et al. 2007).

Regardless of moral and ethical questions relating to keeping wild animals in captivity (reviewed by Harrington et al. 2013), there is a long history of captive animals benefitting from *ex situ* conservation efforts. Baker (2007) describes six ways in which *ex situ* programs that hold animals in captivity can contribute to conservation: (1) demographic reservoirs for *in situ* populations, (2) production of *ex situ* animals used for translocations, (3) conducting research not easily accomplished on animals *in situ,* (4) development of technologies relevant to *in situ* conservation, (5) conservation education, and finally (6) as animal fund-raising ambassadors. Our ability to breed animals in captivity is a critical component for *ex situ* conservation, and is required to maintain captive populations, reduce collections of wild animals, and allow for reintroductions to take place if necessary. All *ex situ* conservation programs require a solid understanding of proper husbandry methodology to be successful and ethically responsible regardless of reason, species, or length of time that an animal is kept in captivity (Harrington et al. 2013).

Husbandry as a Cornerstone of Modern Amphibian Conservation

Among vertebrates, amphibians have risen to prominence in *ex situ* conservation resulting from estimations of how many species may require interventionist conservation methods requiring captivity (Zippel et al. 2011; Harding et al. 2016). Currently, 30.8% of amphibian species evaluated by the International Union for the Conservation of Nature (IUCN) Red List are threatened with extinction (IUCN 2014), and Zippel et al. (2011)

estimated that 943 different amphibian species may need *ex situ* conservation to safeguard against extinction in the immediate future. The organization Amphibian Ark (AArk), a coalition between the World Association of Zoos and Aquariums and the IUCN, was specifically created in 2007 to foster development of amphibian *ex situ* conservation programs (Amphibian Ark 2015). Thus, facilities for amphibian captive assurance colonies have been created in over 20 countries currently experiencing amphibian declines (Amphibian Ark 2015). These programs are considered a translocation-focused initiative in which amphibians are taken from the wild to act as assurance against extinction, and if necessary, captive colonies may provide a source of animals that can be used for reintroductions (reviewed by Tapley et al. 2015). Currently, more than one hundred species of amphibian reside in *ex situ* conservation programs (Amphibian Ark 2016). Many amphibian *ex situ* conservation programs have struggled with husbandry-associated difficulties (e.g., Channing et a. 2006; Galliardo et al. 2008; Soorae 2010, 2011, 2016; Pessier et al. 2014). The husbandry techniques used to keep amphibians in captivity have become critical tools for successful conservation of many species (Harding et al. 2015). How how much do we know about amphibian husbandry?

Publications detailing generalized or multi-species amphibian husbandry methods have been produced (e.g., Wright & Whitaker 2001; Browne et al. 2007; Pough 2007; Poole and Grow 2008; Pessier and Mendelson 2017. These published resources provide a good starting point for many aspects of husbandry such as lighting, feeding, plumbing, and water filtration. However, generalized amphibian husbandry is not specific enough to apply to every amphibian species brought to captive settings.

The assumption that amphibian species are easy to maintain and breed in captivity is an over-generalization (Kouba et al. 2009; Michaels et al. 2014). For example, Smith and Sutherland (2014) found that of amphibian captive breeding programs they reviewed, only 55% could produce tadpoles, metamorphs, or juveniles. Numerous publications have suggested the importance of developing taxon-specific amphibian husbandry practices (e.g., Mattison 1992; Staniszewski 1995; Holt et al. 2003; Grow and Poole 2007; Michaels 2014; Tapley et al. 2015). Why does taxon-specific husbandry knowledge matter? The extreme diversity of behaviors, ecology, physiology, and reproductive modes found within the amphibian clade containing over 7500 species makes generalized husbandry methods essentially impossible (Michaels et al. 2014; Tapley et al. 2015). Substantial variation in husbandry requirements may exist between species in the same genus (Staniszewski 1995; Zimmerman 1993; Grenard 1999: Poole and Grow 2008) and between populations of the same species (Räsänen et al. 2003; Michaels et al. 2014). Husbandry differences among species often varies as a function of breeding requirements. For example, anurans have dozens of reproductive modes (Crump 2015), and each mode may have differences in husbandry requirements for breeding triggers, behaviors, and potentially involve parental care of young. Captive breeding presents difficulties for many current *ex situ* amphibian conservation programs (Kouba et al. 2009; Smith and Sutherland 2014). Using amphibian *ex situ* populations as conservation tools, as outlined by Baker (2007), cannot work without the ability to reliably breed and maintain a diverse group of amphibians in captivity. If husbandry is one of the cornerstones of modern amphibian conservation, yet is understudied, how many amphibian species can we competently care for in captivity? Furthermore, what kind of published taxon-specific husbandry information exists for amphibians and where can it be found? Where are the gaps in our amphibian husbandry knowledge across taxonomic groupings, and what are their conservation needs? Can we prioritize which species need husbandry research to meet future amphibian conservation challenges?

In this study, I systematically reviewed and quantified information about taxon-specific amphibian husbandry for all native amphibian species found in the United States of America (US) (excluding Puerto Rico and outlying territories). The information was derived from peer-reviewed literature and gray literature. I then (1) compare quantity and quality of husbandry publications among media types; (2) identify taxonomic groups lacking taxon-specific husbandry information; (3) compare availability of husbandry information for each species against the conservation metrics of IUCN's Red List status, the U.S. list of federally endangered species status, the Evolutionarily Distinct and Globally Endangered (EDGE) species rating, and the Evolutionary Distinctiveness (ED) rating produced by the EDGE Programme; (4) and finally, I discuss conservation implications of the findings.

METHODS

Target species reviewed

To assess the amount and quality of husbandry literature available for amphibians, a subsample of the approximately 7500 described amphibian species were assessed. I reviewed all native amphibians found within the US: I gathered information for 289 species using the names list produced by Crother (2012). Non-native amphibian species and those in Puerto Rico or US territories were excluded. Species newly described during data collection were not included in the analysis (e.g., *Lithobates kauffeldi*). Amphibian species in the US were chosen for several reasons. First, a single country makes comparison between available husbandry information and existing legal protection status uniform. Second, amphibian species in the US are likely well studied compared to other parts of the world, and current conservation status relatively well understood for all species (e.g., Lannoo 2005). Third, the US has a substantial community of amphibian hobbyists, dozens of amateur herpetological societies, and over 200 accredited zoos and aquariums, leading to the assumption that there is a substantial amount of available husbandry literature on local amphibian taxa written in English. Fourth, species known from the US represent a diverse array of species including 18 families and 47 genera (Frost 2016). Eighty-eight species are of conservation concern as listed by the IUCN Red List in the data set, and 206 species are endemic to the US (AmphibiaWeb Online Database 2015). Except for pantropical caecilians, the amphibian fauna of the US provides a valuable case study to review for husbandry knowledge.

Data Collection and Analyses

The inclusion of gray literature in conservation reviews is recommended to avoid publication bias and increase reliability and coverage (Pullin and Stewert 2006; Haddaway and Baliss 2015). Gray literature may provide a substantial portion of available documentation available to conservation managers (Corlett 2011; Haddaway and Bayliss 2015). Because of these recommendations, my systematic review included various forms of gray literature in addition to traditional peer-reviewed literature to review taxon-specific husbandry information for amphibians in the US. The types of literature included were peer-reviewed journals, published books, published organizational reports (produced by or for conservation programs and typically published online), and online sources such as husbandry care-sheets and other well-known herpetological websites with dedicated husbandry information pages (e.g., www.caudata.org). Some types of gray literature were not assessed. Magazine articles were not reviewed because of difficulty locating issues. Online social media, blog, and forum posts were not included. Only taxon-specific information was reviewed; thus, a husbandry source must address a specific species. To maximize husbandry information for inclusion in the review, this research project was described at the Association of Zoos and Aquariums 2013 Amphibian Taxon Advisory Group meeting in Detroit, Michigan and the 2014 Joint Meeting of Ichthyologists and Herpetologists in Chattanooga, Tennessee to solicit information from attendees, to find husbandry accounts, and to solicit comments on existing data.

Books and reports were located though internet searches, library databases, and by examining the personal collections of several professional amphibian husbandry workers. There are several academic journals that contain husbandry information, but did not appear in online searches during data collection because articles may take the form of notes or short entries. Thus, physical copies of every published issue from the peer-reviewed journals *Zoo Biology*, *Herpetological Review*, and *Herpetological Bulletins* were manually searched. For peer-reviewed literature and government-reports, keyword searches on the electronic databases Web of Science (www.webofknowledge.com) and Google Scholar (https://scholar.google.com) were completed. Sources containing at least two of the following terms, one from each category: (1.) each individual species' name (e.g., *Ambystoma tigrinum*), amphibian*, frog*, toad*, salamander*, anuran*, or caudate*, and (2.) husbandry, captive*, *ex situ*, pet*. I searched the first 20 entries returned for each electronic database search.

Nomenclature problems (e.g., the true frog genus *Rana* versus *Lithobates*) were solved by matching older taxonomy in the reviewed literature to the most current taxonomy (Frost 2016). Husbandry accounts in which the species was not identified and used only a general term, (e.g., ‘frog’, ‘salamander’, or ‘toad') were not included. If a common name was used, such as 'bullfrog', it was matched as closely as possible to the appropriate species via common name listed on AmphibiaWeb Online Database (2015). If only a common name was available and it could be attributed to multiple species, such as 'dusky salamander', it was recorded but not used for analysis at the species level, but included in the analysis at the Genus and Family levels. If a husbandry account met these initial criteria, it was graded on a scale of 1–4 using a simple grading rubric corresponding to a *minimal* (1), *low* (2), *medium* (3), and *high* (4) quality ranking (Appendix 1). A quality rank of *minimal* (1) would indicate a very short taxon-specific entry (one or two sentences) of negligible utility to a husbandry worker. A quality ranking of *high* includes in-depth husbandry information and information on captive breeding. The ranking applied via the grading rubric purposefully favors captive breeding methodology over other knowledge areas (e.g., lighting requirements or diet) because successful reproduction in captivity is an essential outcome for conservation efforts requiring the production of animals (e.g., reintroductions). The rated husbandry account citation was then placed into a database linking publications to the appropriate species (Appendix 2).

RESULTS

Literature

I identified 420 taxon-specific husbandry accounts (hereafter “husbandry accounts”) pertaining to species included in the analysis. An additional 42 husbandry accounts were found that could only be applied to the Genus category (e.g., husbandry listed for “Florida siren”, “*Scaphiopus*”, or “dusky salamander” could each apply to several species). Publication dates of husbandry accounts ranged from 1909 to 2016. I found husbandry accounts satisfying criteria to be given a quality index (462 accounts) in each of four categories of literature: 89 in peer-reviewed journals (19.3%), 304 in books (65.9%), 17 published reports (3.7%), and 51 internet resources (11.1%) (Figure 2.1). Anurans (101 species) and caudates (188 species) had 220 and 241 husbandry accounts, respectively (for Species or Genus). Most of the husbandry information was of *minimal* (94 accounts) or *low* (176 accounts) quality. Husbandry accounts with a *medium* (205 accounts) and *high* (49 accounts) quality ranking favored caudates over amphibians (Figure 2.2).

Gaps in Husbandry Knowledge

To find gaps in husbandry knowledge, I examined the quality of husbandry accounts for each taxonomic Family (19), Genus (40), and all amphibian species (289). There were 101 anuran species and 188 caudate species in the data set. Only the family Craugastoridae had no husbandry accounts of any rank, but it only includes one native US species. However, five families (21%) had no husbandry accounts with a quality of *low*, *medium*, or *high* (Figure 2.3). Thirteen families contained at least one species with a husbandry rank of *high*. Seven families lack detailed husbandry information for any species with a *high* husbandry ranking: Craugastoridae, Leptodactylidae, Rhinophrynidae, Rhyacotritonidae, Microhylidae, Scaphiopodidae, and Amphiumidae.

Nine genera in my analysis had no husbandry accounts for any species (Figure 2.4). Thirteen genera contained no species with a husbandry rank of *low*, *medium*, or *high*. Twenty genera contained at least one species with a husbandry account of rank *high*. The caudate genus *Plethodon* contained the greatest number of species with no husbandry information of any quality (49 of 54 spp.), followed by *Batrachoseps* (17 of 21 spp.) and *Eurycea* (16 of 27 spp.). The anuran genus containing the most species with no husbandry rank was *Lithobates* (nine of 22 spp.), followed by *Pseudacris* (six of 16 spp.) and *Hyla* (six of 11 spp.)

Of 289 species analyzed, 161 species (55.7%) had no taxon-specific husbandry information. The four husbandry knowledge ranks for all species included: *minimal* (11 spp., 3.8%), *low* (35 spp., 12.1%), *medium* (47 spp., 16.3%), and *high* (35 spp., 12.1%), and were analyzed by order (Figure 2.5). A higher percentage of caudate species had no husbandry (66.4%) compared to anuran species (40.6%).

Conservation Status

There were 88 species in the dataset listed by the IUCN Red List (2016) with a status of conservation concern (Figure 2.6), and 71.6% (63 spp.) had no husbandry accounts ranked *low* or higher. Species with a *none* or *minimal* husbandry ranking were found in the IUCN Red List categories *Near Threatened* (21 spp.), *Vulnerable* (28 spp.), and *Endangered* (14 spp.). Species in the data set listed as *Least Concern* (172 spp.)

included 82 species with husbandry rank of *none* or *minimal.* The data set included 14 species listed as *Data Deficient*, which included 12 species with no available husbandry data. An additional 15 species in the dataset had not been assessed by the IUCN Red List, which all had no available husbandry information.

Species in the data set and their associated husbandry ranks were compared against the Evolutionary Distinct and Globally Endangered (EDGE) ranking as well as their Evolutionary Distinct ranking (ED), which were both produced by the EDGE of Existence Programme (www.edgeofexistence.org, 2017). Sixteen species in the dataset had an EDGE ranking in the top 250 species of 4339 assessed globally (Table 2.1). The Alabama Waterdog (*Necturus alabamensis*) had the highest global ranking as the 27th EDGE species globally and notably, *N. alabamensis* has no published taxon-specific husbandry information. Twenty-seven species in the dataset had an ED ranking within the top 100 of 4399 amphibian species assessed by the EDGE programme. Only three species of the top 15 ED ranked species did not have husbandry information valued at two or above (Table 2.2). Both EDGE and ED rankings and their associated husbandry level may help prioritizing species specific husbandry research.

The United States Endangered Species Act (ESA) is used to legally protect species, subspecies or distinct populations of flora and fauna. Thirty-three amphibian species or subspecies are listed in the US (U.S. Fish & Wildlife Service 2016), representing twenty-nine full species in the data set listed as *Threatened* (12 spp.) or *Endangered* (17 spp.) under the ESA (Figure 2.7). Seven *Endangered* species and five *Threatened* species did not have any available husbandry information. Sixteen listed species had husbandry ranked two or above, including nine species with a *high* husbandry knowledge ranking.

DISCUSSION

Discussion of the main results

My study has shown that most amphibian species in the US have no taxon-specific husbandry information of any kind (55.6%). The lack of information means that husbandry related problems will impact most species if they are kept in captivity. Furthermore, the high number of species with no husbandry information ranked *low*, *medium*, or *high* (171 spp.) was surprising considering how well-studied amphibians are in the US. Only 12.5% of US amphibian species have established husbandry methodology with thorough descriptions and protocol for captive breeding. Several dozen species threatened with extinction in the US are also lacking husbandry information of any kind. Thus, numerous species most likely to require *ex situ* conservation efforts are lacking husbandry that will allow for both long-term maintenance of captive populations and production of captive-bred animals for conservation. The results emphasize that conservationists should focus on *in situ* conservation of amphibians, and when deciding if *ex situ* conservation methods are necessary, conservationist should consider the challenges and limitations imposed by husbandry in conservation planning processes. The results of the present study will improve conservation planning, husbandry research prioritization, and inform conservation managers of the current state of husbandry knowledge for any native US amphibian species.

Only 10.6% of husbandry accounts received the highest quality rank of *high*. Indicating that, in a relatively large body of literature, few accounts are specific or detailed enough to provide thorough guidance for captive breeding and/or long-term captive management. Furthermore, 20 species accounted for 48.3% of all husbandry accounts; a disproportionate amount of husbandry information. These well studied species with large numbers of husbandry accounts appear to be common, widely distributed species frequently kept as pets (e.g., *Lithobates pipiens* [17 accounts], *Notophthalmus viridescens* [15 accounts]) or species frequently utilized in research (e.g., *Ambystoma tigrinum* [19 accounts]). Most husbandry accounts were in books (65.9%), and most were written for hobbyist interested in keeping amphibians as pets (Appendix 3). Many peer-reviewed papers containing husbandry information were not specifically written to describe or study aspects of husbandry of the focal species, rather they included information on how animals were kept in captivity during experimental trials for other purposes. My results highlight the value of gray literature in animal husbandry because peer-reviewed literature contributed only 19.3% to the total husbandry accounts (Haddaway and Bayliss 2015). These results show that the bulk of husbandry information is developed and published for hobbyists keeping amphibians.

My systematic literature review represents the taxon-specific husbandry accounts which are easily accessible for all included species, however some limitations may have existed in the ability to locate some husbandry accounts. For example, some peer-reviewed laboratory studies utilizing amphibians may have husbandry information in the study's methods section detailing how animals were kept (e.g., what feeding, housing, or lighting was utilized). These may have been missed using this study's search methodology. For example, amphibians have been common subjects in embryological studies for almost 100 years. Although embryological literature is immense, only a very small number of species have been used (e.g., *Ambystoma tigrinum*) (DeNardo 1995). Although literature may have been missed for highly studied, model research species such as *A. tigrinum*, four ranked husbandry accounts were already included in analysis for these species and additional sources of repetitive information of similar or lower rank would not impact results. Some sources of potential information were not included. For example, internet sources such as forums, blog post, and social media posts were also ignored because of search constraints, their highly variable quality, questionable reliability, and many posts may be ephemeral and not permanently available. Magazines targeted at hobbyists were also not searched because of difficulty in finding out-of-print back issues. Several obscure or rare books that appeared in keyword searches of library and internet databases were not able to be obtained after considerable effort resulting from their extreme rarity.

As a result of large variation in quality, format, length, and publication types of husbandry accounts, utilizing a standardized quality rating system was a challenge. The ranking rubric helped standardize and eliminate many accounts, particularly husbandry information that only listed natural history information of animals *in situ*. To make the ranking method as consistent as possible, it was conducted by a single individual (LJL). However, the included literature represents what is readily accessible to amphibian husbandry practitioners, and provides the first ever husbandry gap-analysis to provide better conservation planning for each species. The literature list for each species (Appendix 2) will provide amphibian keepers with a expansive reference list for species that have this information. Hopefully, the publication of this list will stimulate husbandry workers to fill in gaps for species that do not have published husbandry care.

Importance and challenges of studying amphibian husbandry

When working with a new amphibian species in captivity, the initial husbandry learning period may vary greatly in difficulty, time, and necessary resources (Synder et al. 1996). The challenge in achieving both long-term survival in captivity that is similar to or greater than that of wild individuals and reliably breeding an amphibian species in captivity should not be underestimated. Captive populations may decline from a difficult initial learning curve and a failure to identify appropriate husbandry needs, thus jeopardizing species recovery efforts. For example, creation of the Olduvai dam in Tanzania threatened the only known population of the Kihansi Spray Toad (KST) (*Nectophrynoides asperginis*). Individuals were brought to the US to establish a captive assurance population. By 2004, the KST was considered extinct in the wild, and it nearly went extinct in captivity as well. The KST conservation program had a long initial husbandry learning period characterized by high mortality rates. Of the 499 animals originally brought into captivity, the population crashed to only 38 individuals. Successful husbandry techniques were developed at the Toledo Zoo, and the population has rebounded to several thousand animals kept at multiple institutions (Channing et al. 2006; Lee et al. 2006).

If animals do not experience high mortality in captivity, getting a species to breed in captivity can take years of research and effort, much of it trial-and-error. The critically-endangered, Dusky Gopher Frog (*Lithobates sevosus*) was reduced to one wild population near two ponds in Harrison County, Mississippi, which contained roughly 100–200 adults (Lannoo 2005). A small captive population of approximately 60 adults existed in captivity, and intensive multi-year efforts at Omaha’s Henry Doorly Zoo & Aquarium and the Memphis Zoo to captive breed *L. sevosus* were unsuccessful. After years of effort, in February 2011, a breakthrough in husbandry protocols resulted in the first production captive-bred frogs Omaha’s Henry Doorly Zoo & Aquarium (Aaltonen 2011; Kouba et al. 2011). The captive breeding methodology has resulted in several new assurance colonies, surplus animals for conservation research, and the possibility of translocating animals back to the wild.

Because of complexities associated with captive breeding amphibians and maintaining genetic diversity in captive populations, artificial reproductive technologies (ARTs) and cryopreservation of genetic material is increasingly important in modern amphibian conservation (Kouba et al. 2009; Clulow et al. 2014). However, utilization of cryopreserved spermatozoa and other ARTs to produce captive-bred progeny will still rely on a foundation of traditional amphibian husbandry methods (e.g., nutrition, lighting, caging, and disease management) to be successful. A working knowledge of specific amphibian husbandry practices and methodologies for endangered species will likely allow *ex situ* conservation programs to be more cost effective in keeping and producing animals through reduced mortality, and increase their chances of meeting program goals.

Fostering research on amphibian husbandry

If amphibian husbandry is a field fertile for study, why is there a profound lack of available information? How do we foster increased research on amphibian husbandry?

The lack of accessible information on amphibian husbandry is likely the result of multiple drivers. Some commercial amphibian breeders may not want to share husbandry information. Certain amphibian species are worth large sums of money to collectors, and hobbyists may wish to protect such "trade secrets." Unsuccessful attempts at keeping animals in captivity are rarely reported, resulting in repeated mistakes by different amphibian keepers and slowing progress on protocol development. Additionally, there are few venues to publish unsuccessful, anecdotal information which may be useful in amphibian husbandry. Furthermore, many peer-reviewed journal's publication requirements may be beyond the ability or interests of hobbyist or professional amphibian keepers. Amphibian keepers may assume their knowledge is redundant, not useful, and would not be accepted for publication. The present study highlights the importance of husbandry information contained in gray literature formats, often by hobbyists, which may be the highest quality husbandry information available for a species. Prior to the present study, locating amphibian husbandry was also difficult. Because most amphibian husbandry mostly found in printed gray literature (notably books), and finding this information (Appendix 2) was problematic and required manually searching numerous books (prior to this study) to locate information that may or may not exist.

But, how do conservationists foster increased research and publication of amphibian husbandry-related topics? Increasing awareness among amphibian keepers that descriptive husbandry is publishable and important is critical. Developing or improving amphibian husbandry through hypothesis driven experiments is a valid avenue of research with both animal welfare and conservation benefits. Researchers doing amphibian studies involving captivity should provide details in the methods section outlining the husbandry utilized. Including “husbandry” as a keyword can improve search indexing to help amphibian keepers find husbandry information in experimental studies.

Conservationists should attempt to tap into the large knowledge reservoir within the amphibian hobbyist community by promoting non-professionals to publish or share their experiences with captive amphibians. Captive breeding has been suggested as a tool to reduce collection of wildlife (Tensen 2016), however few case studies exist studying amphibians (but see Mattioli et al. [2006]). Collection of wild individuals for the pet trade has been linked to declines of amphibian populations (Rabemananjara et al. 2008; Carpenter et al. 2014), and many US species could potentially be exploited in the pet trade (Schlaepfer et al. 2005). Rare species in the pet trade may also be subjected to an anthropogenic Allee Effect, which may accelerate effects of overexploitation (Tournant et al. 2012).

When selecting a species to work with, amphibian husbandry practitioners should prioritize developing husbandry protocols for common, local species in taxonomic groups with little published husbandry information. Transporting amphibians outside of a species native range for *ex situ* collections increases the risk of spreading novel diseases that may spread to wild populations (Schloegel et al. 2009), thus working with local species is preferable. Hopefully, Appendix 2 will help select amphibian species for husbandry research, promote amphibian keepers to publish their experience and protocols, and allow for easy location of valuable amphibian husbandry information that is not indexed on online searches.

Future Research and Recommendations for Amphibian Husbandry

The natural history of most US amphibian species has been well studied (e.g., Petranka 2010), which provides significant advantages when developing husbandry protocols (Michaels et al. 2014). Generalized amphibian husbandry resources may provide a good starting point, even if no taxon-specific information exists for other species in a focal Genus or Family. For example, the Association of Zoos and Aquariums has produced and distributed the Amphibian Husbandry Resource Guide (Poole and Grow 2008). Additionally, Amphibian Ark curates a small bibliography of amphibian husbandry related publications (available at http://www.amphibianark.org/resources/amphibian-husbandry/husbandry-documents).

Preemptive development of husbandry protocols prior to a species become endangered is preferred. A husbandry paradox exists for endangered species with unknown husbandry requirements. Developing captivity protocols inherently requires collection of wild individuals, directly reducing size of the wild population. Additionally, high mortality associated with developing husbandry protocols may further reduce the global population (e.g., Channing et al. 2006) or cause concern to government agencies and other stakeholders, and it highlights the importance of working with non-threatened species that are closely related to species already threatened with extinction. For example, Stoops et al. (2014) developed husbandry protocol for the common Gulf Coast waterdog (*Necturus beyeri*) as a surrogate species specifically to gain experience that could be applied to the highly-endangered Alabama Waterdog (*Necturus alabamensis*).

Conservationists deciding if *ex situ* conservation strategies are appropriate for an amphibian species should consider the challenges and limitations which husbandry may impose (Snyder et al. 1996). While taxon-specific husbandry is lacking for most US species, husbandry for amphibian species in other geographical regions (e.g., Latin America, South East Asia, or Africa) is likely less understood than for US species. Research and developments covering a variety of amphibian husbandry aspects, such as lighting, nutrition, housing, water quality, and captive population management, will improve our chances for success utilizing *ex situ* conservation methods and benefit the welfare of all captive amphibians. Amphibian species that are currently common may drastically decline in the future and require *ex situ* conservation many years from now. Thus, developing, preserving and making husbandry knowledge publically accessible for the foreseeable future is necessary.

ACKNOWLEDGEMENTS

Funding and support for my project was provided by a National Science Foundation Graduate Research Fellowship to LL (grant #2013172527). I would like to thank M. Donnelly, B. Linhoff, K. Reider and W. Symes for feedback on early drafts of the manuscript.

LITERATURE CITED

Aaltonen, J. T., Bohlender, T., Snyder, W., Krebs, J., Linhoff, L., Snoza, M., ... & Loskutoff, N. M. 2011. The developmental competence of tadpoles produced in vitro from the endangerd dusky gopher frog (*Rana sevosa*). *Reproduction, Fertility and Development*, 24(1), 172-172.

Amphibian Ark. 2016. Frightening statistics Available online, http://www.amphibianark.org/the-crisis/frightening-statistics/ (accessed 14 Oct 2016).

AmphibiaWeb Online Database. 2015. Available online, http://amphibiaweb.org (accessed 15 Sept. 2015).

Araki, H., Cooper, B., & Blouin, M. S. 2007. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. *Science*, 318(5847), 100-103.

Arbuckle, K. 2013. Folklore husbandry and a philosophical model for the design of captive management regimes. *Herpetol. Rev*, 44, 44.

Baker A. 2007, “Animal ambassadors: an analysis of the effectiveness and conservation impact of ex situ breeding efforts,” in: Zimmerman A., Hatchwell M., West C., and Lattis R. (eds.), *Zoos in the 21 Century: Catalysts for Conservation?* Cambridge Univ. Press, Cambridge, UK.

Balmford, A.*,* G. M. Mace*, and* N. Leader-Williams*.* 1996*.* Designing the ark: setting priorities for captive breeding*. Conservation Biology* 10*:*719–727*.*

Browne, R. K., R. A. Odum, T. Herman, and K. Zippel. 2007. Facility design and associated services for the study of amphibians*. ILAR Journal* 48(3):188-202.

Carpenter, A. I., Andreone, F., Moore, R. D., & Griffiths, R. A. 2014. A review of the international trade in amphibians: the types, levels and dynamics of trade in CITES-listed species. *Oryx*, *48*(04), 565-574.

Channing, A., Finlow-Bates, K. S., Haarklau, S. E., & Hawkes, P. G. 2006. The biology and recent history of the critically endangered Kihansi spray toad *Nectophrynoides asperginis* in Tanzania. *Journal of East African Natural History*, 95(2), 117-138.

Clulow, J., Trudeau, V. L., & Kouba, A. J. 2014. Amphibian declines in the twenty-first century: why we need assisted reproductive technologies. *In Reproductive Sciences in Animal Conservation* (pp. 275-316). Springer, New York.

Corlett, R. T. 2011. Trouble with the gray literature. *Biotropica*, 43(1), 3-5.

Crump, M. L. 2015. Anuran reproductive modes: evolving perspectives. *Journal of Herpetology*, 49(1), 1-16.4

DeNardo, D. 1995. Amphibians as laboratory animals. *ILAR Journal*, 37(4), 173-181.

Dickens, M. J., Delehanty, D. J., & Romero, L. M. 2010. Stress: an inevitable component of animal translocation. *Biological Conservation*, 143(6), 1329-1341.

Fa, J. E., Funk, S. M., & O'Connell, D. 2011. *Zoo Conservation Biology*. Cambridge University Press. Cambridge, UK.

Ford, M. J. 2002. Selection in captivity during supportive breeding may reduce fitness in the wild. *Conservation Biology,* 16(3), 815-825.

Frost, D R. 2016. *Amphibian Species of the World: an Online Reference*. Version 6.0 (Accessed 4 Febuary 2016). Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/index.html. American Museum of Natural History, New York, USA.

Gagliardo, R., Crump, P., Griffith, E., Mendelson, J., Ross, H., & Zippel, K. 2008. The principles of rapid response for amphibian conservation, using the programmes in Panama as an example. *International Zoo Yearbook*, *42*(1), 125-135.

Grenard S. 1999 *Amphibians: Their Care and Keeping*. Howell Book House. Nashville, TN.

Grow, S., & Poole, V. A. 2007. *Amphibian Conservation Resource Manual*. Association of Zoos & Aquariums. A publication of AZA's Amphibian Taxon Advisory Group.

Haddaway, N. R., & Bayliss, H. R. 2015. Shades of grey: two forms of grey literature important for reviews in conservation. *Biological Conservation*, 191, 827-829.

Harding, G., Griffiths, R. A., & Pavajeau, L. 2016. Developments in amphibian captive breeding and reintroduction programs. *Conservation Biology*. 30: 340–349. Harrington, L. A., Moehrenschlager, A., Gelling, M., Atkinson, R. P., Hughes, J., & Macdonald, D. W. 2013. Conflicting and complementary ethics of animal welfare considerations in reintroductions. *Conservation Biology*, 27(3), 486-500.

Holt, W., A. Pickard, J. Rodger, and D. Wildt, editors. 2003. *Reproductive Science and Integrated Conservation*. Cambridge University Press, Cambridge, UK.

IUCN 2014. *Amphibians Initiative*. Online. Available from (http://www.iucnredlist.org/initiatives/amphibians). Accessed Oct 15 2014.

Kouba, A. J., C. K. Vance, and E. L. Willis. 2009. Artificial fertilization for amphibian conservation: Current knowledge and future considerations. *Theriogenology* 71(1):214-227.

Kouba, A., Willis, E., Vance, C., Hasenstab, S., Reichling, S., Krebs, J., … & Germano, J. 2011. Development of assisted reproduction technologies for the endangered Mississippi gopher frog (*Rana sevosa*) and sperm transfer for in vitro fertilization. *Reproduction, Fertility and Development,* 24(1), 170-170.

Lannoo, M. J. (ed.) 2005. *Amphibian Declines: The Conservation Status of United States Species*. University of California Press, Berkeley, CA.

Lee, S., Zippel, K., Ramos, L., & Searle, J. 2006. Captive breeding programme for the Kihansi spray toad *Nectophrynoides asperginis* at the Wildlife Conservation Society, Bronx, New York. *International Zoo Yearbook*, *40*(1), 241-253.

Marantelli, G.1999. Husbandry: science or art? Are captive technologies ready to contribute to recovery processes for Australian frogs? In. Campbell, A. (Ed.), *Declines and disappearances of Australian frogs*. pp. 166-178. Biodiversity Group, Environment Australia.

Mattioli, F., Gili, C., & Andreone, F. 2006. Economics of captive breeding applied to the conservation of selected amphibian and reptile species from Madagascar. *Natura–Società italiana di Scienze naturali e Museo civico di Storia Naturale di Milano*, *95*(2), 67-80.

Mattison, C. 1992. The care of reptiles and amphibians in captivity. Blandford Press. London, UK.

McDougall, P. T., Réale, D., Sol, D., & Reader, S. M. 2006. Wildlife conservation and animal temperament: causes and consequences of evolutionary change for captive, reintroduced, and wild populations. *Animal Conservation*, 9(1), 39-48.

Michaels, C. J., Gini, B. F., & Preziosi, R. F. 2014. The importance of natural history and species-specific approaches in amphibian ex-situ conservation. *The Herpetological Journal*, *24*(3), 135-145.

Moore, I. T., & Jessop, T. S. 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. *Hormones and Behavior*, 43(1), 39-47.

Morgan, K. N., & Tromborg, C. T. 2007. Sources of stress in captivity. *Applied Animal Behaviour Science,* 102(3), 262-302.

Parker, K. A., Dickens, M. J., Clarke, R. H., & Lovegrove, T. G. 2012. The theory and practice of catching, holding, moving and releasing animals. In. Ewen, J. G., Armstrong, D. P., Parker, K. A., & Seddon, P. J. (Eds.). *Reintroduction Biology: Integrating Science and Management*. John Wiley & Sons.

Pessier, A. P. *&* Mendelson III, J. R. *(Eds.)* 2017. A manual for control of infectious diseases in amphibian survival assurance colonies and reintroduction programs, Version 2.0. Apple Valley, MN: IUCN/SSC Conservation Breeding Specialist Group.

Pessier, A. P., Baitchman, E. J., Crump, P., Wilson, B., Griffith, E., & Ross, H. 2014. Causes of mortality in anuran amphibians from an ex situ survival assurance colony in Panama. *Zoo Biology*, 33(6), 516-526.

Petranka, J. W. 2010. Salamanders of the United States and Canada. Smithsonian Institution Press. Washington, DC.

Poole, V. A., & Grow, S. 2008. Amphibian husbandry resource guide. Association of Zoos & Aquariums. A publication of AZA's Amphibian Taxon Advisory Group.

Pough, F. H. 2007. Amphibian biology and husbandry. *ILAR Journal*, 48(3), 203-213.

Price, E. O. 1999. Behavioral development in animals undergoing domestication. *Applied Animal Behaviour Science*, 65(3), 245-271.

Pullin, A. S., & Stewart, G. B. 2006. Guidelines for systematic review in conservation and environmental management. *Conservation Biology*, 20(6), 1647-1656.

Rabemananjara, F. C., Rasoamampionona Raminosoa, N., Ravoahangimalala Ramilijaona, O., Andreone, F., Bora, P., Carpenter, A. I., … & Vences, M. 2008. Malagasy poison frogs in the pet trade: a survey of levels of exploitation of species in the genus *Mantella*. *Amphibian and Reptile Conservation*, 5, 3-16.

Räsänen, K., Laurila, A., & Merilä, J. 2003. Geographic variation in acid stress tolerance of the moor frog, *Rana arvalis*. I. Local adaptation. *Evolution*, 57(2), 352-362.

Schlaepfer, M. A., Hoover, C., & Dodd Jr, C. K. 2005. Challenges in evaluating the impact of the trade in amphibians and reptiles on wild populations. *BioScience*, 55(3), 256-264.

Schloegel, L. M., Picco, A. M., Kilpatrick, A. M., Davies, A. J., Hyatt, A. D., & Daszak, P. 2009. Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidis* and *Ranavirus* infection in imported North American bullfrogs (*Rana catesbeiana*). *Biological Conservation*, 142(7), 1420-1426.

Smith, R. K., & Sutherland, W. J. 2014. *Amphibian conservation: Global Evidence for the Effects of Interventions* (Vol. 4). Pelagic Publishing Ltd.

Snyder, N. Derrickson, S. R. Beissinger, J. W. Wiley, T.B. Smith, W. D. Toone, and B. Miller. 1996. Limitations of captive breeding in endangered species recovery. *Conservation Biology,* 10:338–348.

Society for the Study of Amphibians and Reptiles. Committee on Standard English and Scientific Names, & Crother, B. I. 2012. *Scientific and Standard English Names of Amphibians and Reptiles of North America North of Mexico*. Kansas: Society for the Study of Amphibians and Reptiles. Herpetological Circular.

Soorae, P. S. (ed.) 2010 *Global Re-introduction Perspectives: Additional Case-studies from around the Globe*. IUCN/ SSC Re-introduction Specialist Group, Abu Dhabi, UAE,

Soorae, P. S. (ed.) 2011. *Global Re-introduction Perspectives: 2011. More Case-studies from around the Globe*. Gland, Switzerland: IUCN/SSC Re-introduction Specialist Group and Abu Dhabi, UAE: Environment Agency-Abu Dhabi.

Soorae, P. S. (ed.) 2016. *Global Re-introduction Perspectives: 2016. Case-studies from around the Globe*. Gland, Switzerland: IUCN/SSC Reintroduction Specialist Group and Abu Dhabi, UAE: Environment Agency, Abu Dhabi.

Staniszewski, M. 1995. *Amphibians in Captivity*. 1st edition. TFH Publications, New York, NY.

Stoops, M. A., Campbell, M. K., & Dechant, C. J. 2014. Successful captive breeding of *Necturus beyeri* through manipulation of environmental cues and exogenous hormone administration: a model for endangered *Necturus*. *Herpetological Review*, 45(2), 251-256.

Tapley, B., Bradfield, K. S., Michaels, C., & Bungard, M. 2015. Amphibians and conservation breeding programmes: do all threatened amphibians belong on the ark?. *Biodiversity and Conservation*, *24*(11), 2625-2646.

Tensen, L. 2016. Under what circumstances can wildlife farming benefit species conservation?. *Global Ecology and Conservation*, *6*, 286-298.

Tournant, P., Joseph, L., Goka, K., & Courchamp, F. 2012. The rarity and overexploitation paradox: stag beetle collections in Japan. *Biodiversity and Conservation*, *21*(6), 1425-1440.

U.S. Fish & Wildlife Service 2016. U.S. Species. Online. Retrieved from https://www.fws.gov/endangered/species/us-species.html.

Wildt. D, Ellis, S., Janssen, D., Buff, J. 2003. Toward more effective reproductive science for conservation. In: *Reproductive Science and Integrated Conservation*. Cambridge University Press. Cambridge, UK.

Wright, K. M., & Whitaker, B. R. 2001. *Amphibian Medicine and Captive Husbandry*. Krieger Publishing Company. Malabar, FL.

Zimmerman, E. 1993. *Reptiles and Amphibians: Care Behavior and Reproduction*. TFH Publishers Inc., Walnut Creek, CA.

Zimmermann, A., M. Hatchwell, L. Dickie, and C. West. editors. 2007. *Zoos in the 21st Century: Catalysts for Conservation?* Cambridge University Press, New York.

Zippel, K., Johnson, K., Gagliardo, R., Gibson, R., McFadden, M., Browne, R. … & Townsend, E. 2011. The Amphibian Ark: A global community for ex situ conservation of amphibians. *Herpetological Conservation and Biology*, 6(3), 340-352.

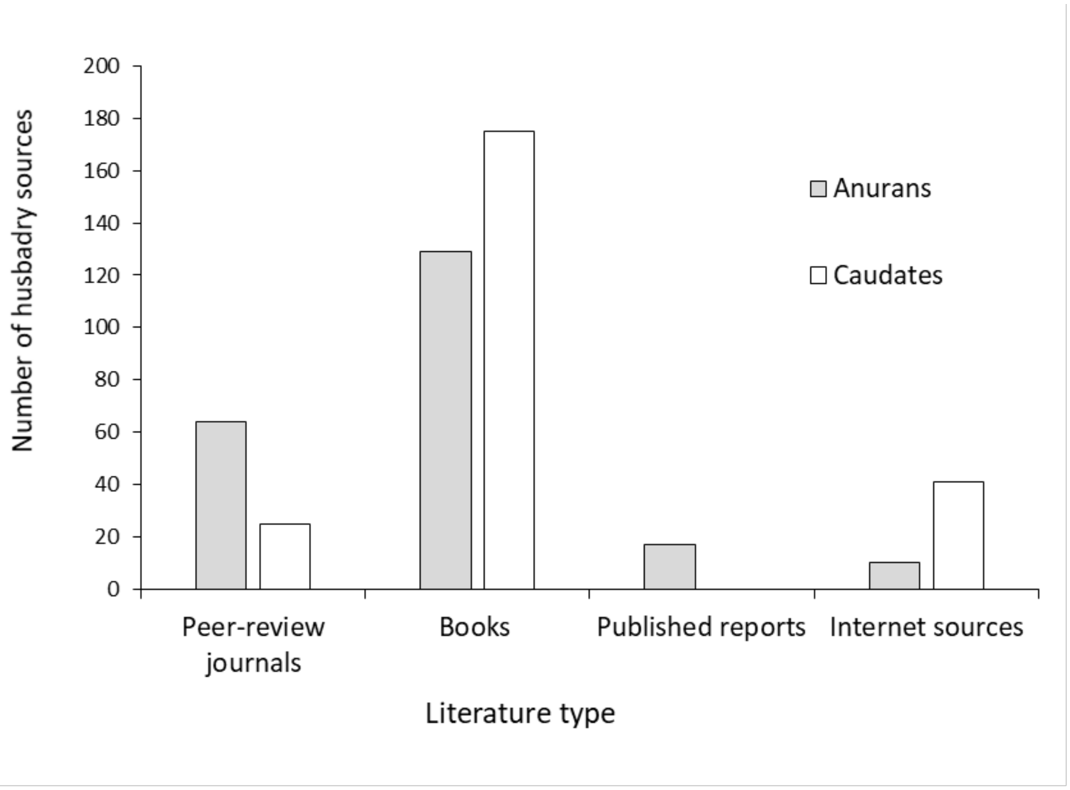

Figure 2.1. The number of husbandry resources in each literature type: peer-reviewed journals, books, published reports, internet sources.

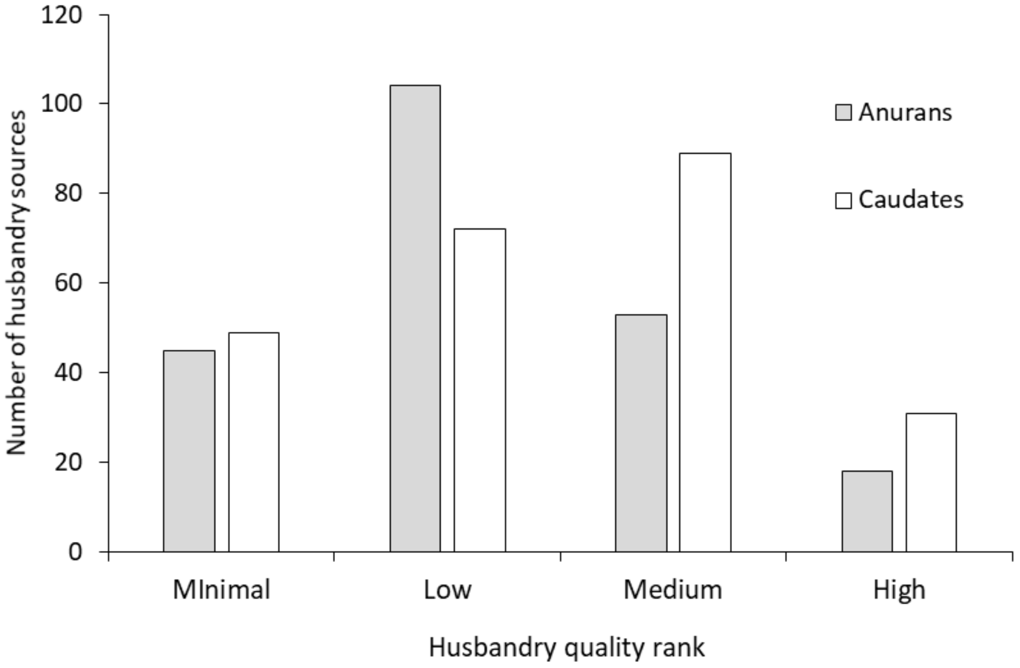

Figure 2.2. The number of publications of each quality rank (minimal, low, medium, and high) reviewed in the analysis for anurans and caudates.

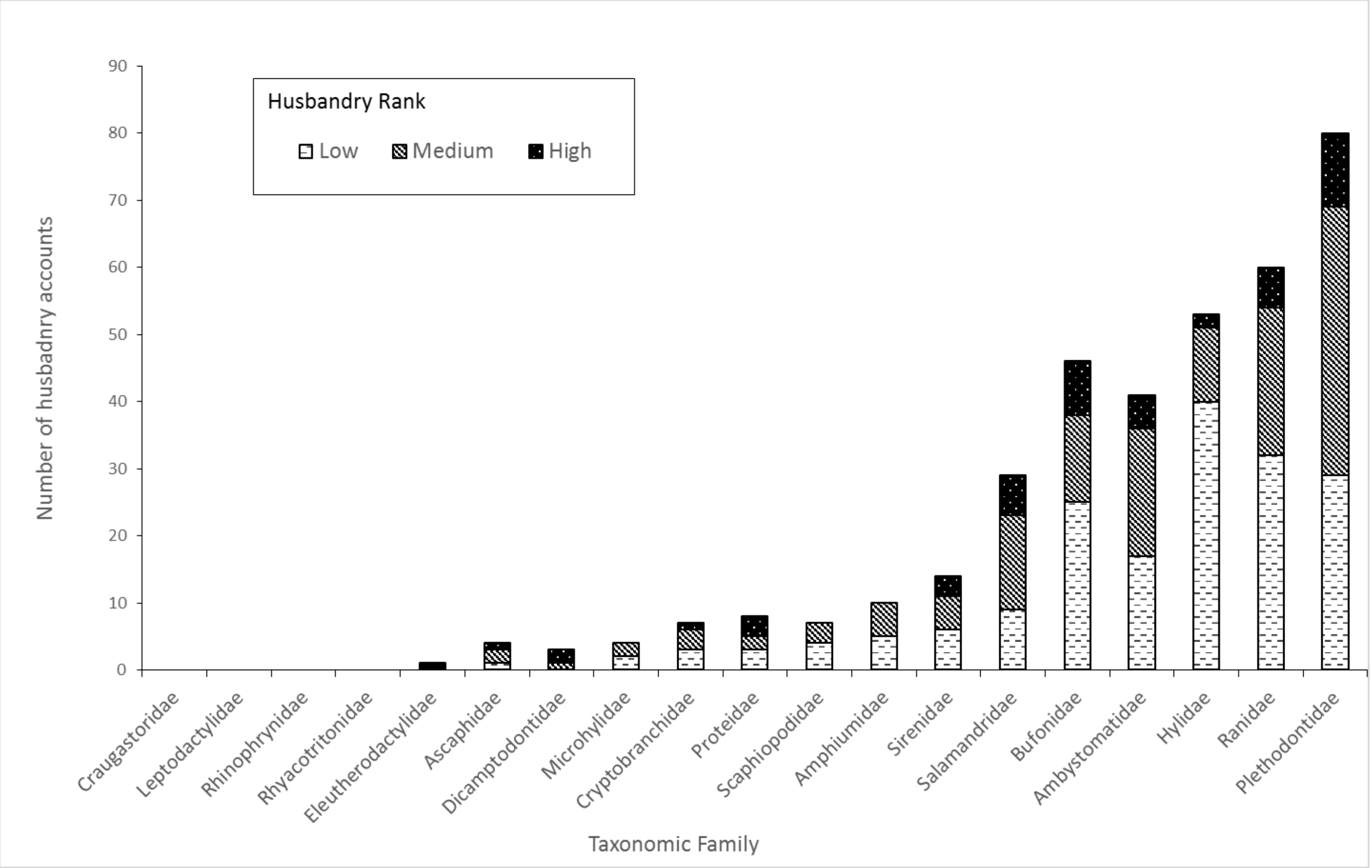

Figure 2.3. The amount of published husbandry information for each amphibian taxonomic family in the United States. Literature with a quality rank of one was not included because of its limited utility to husbandry practitioner.

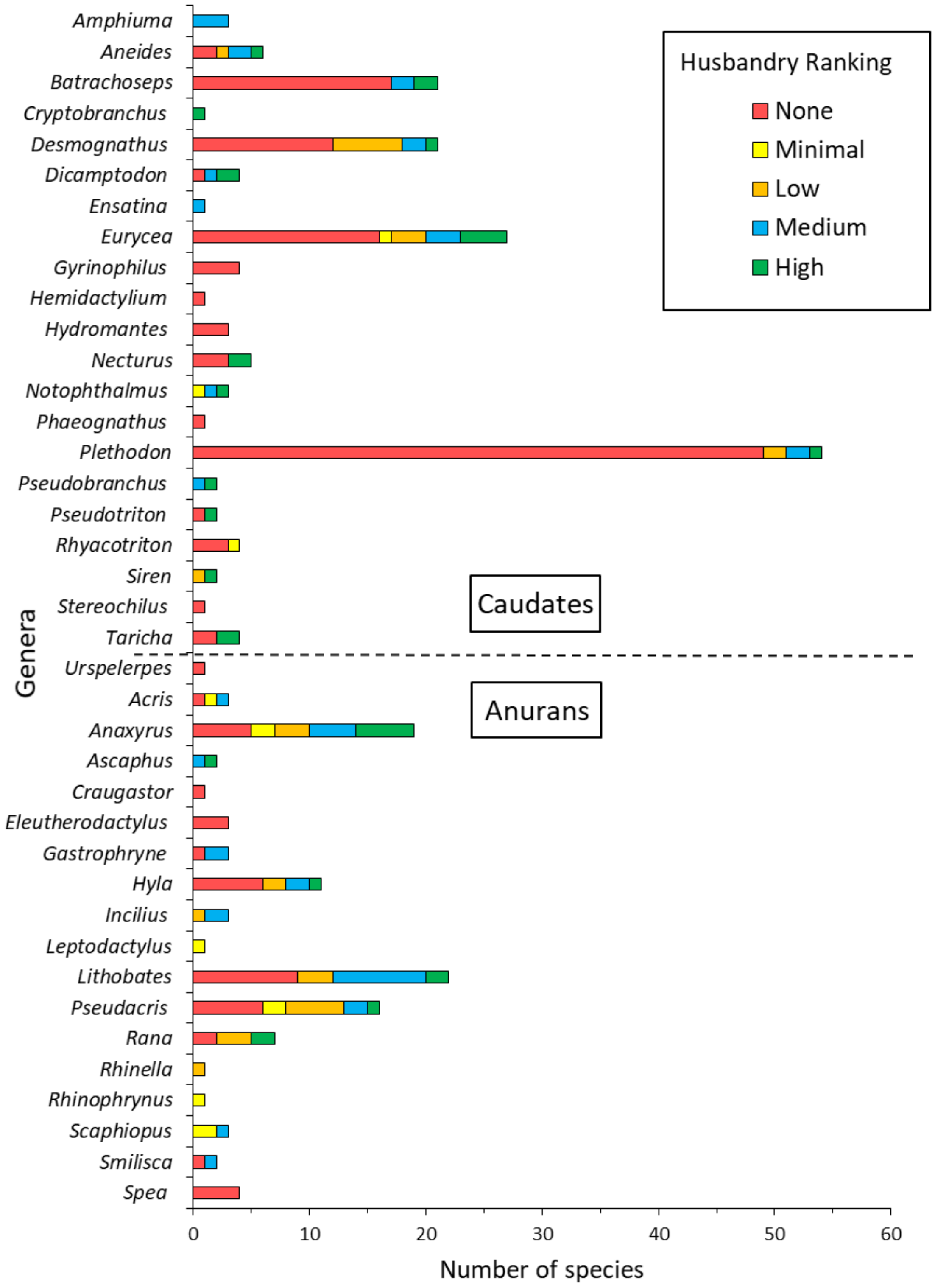

Figure 2.4. The number of species in each genera and their associated husbandry rank.

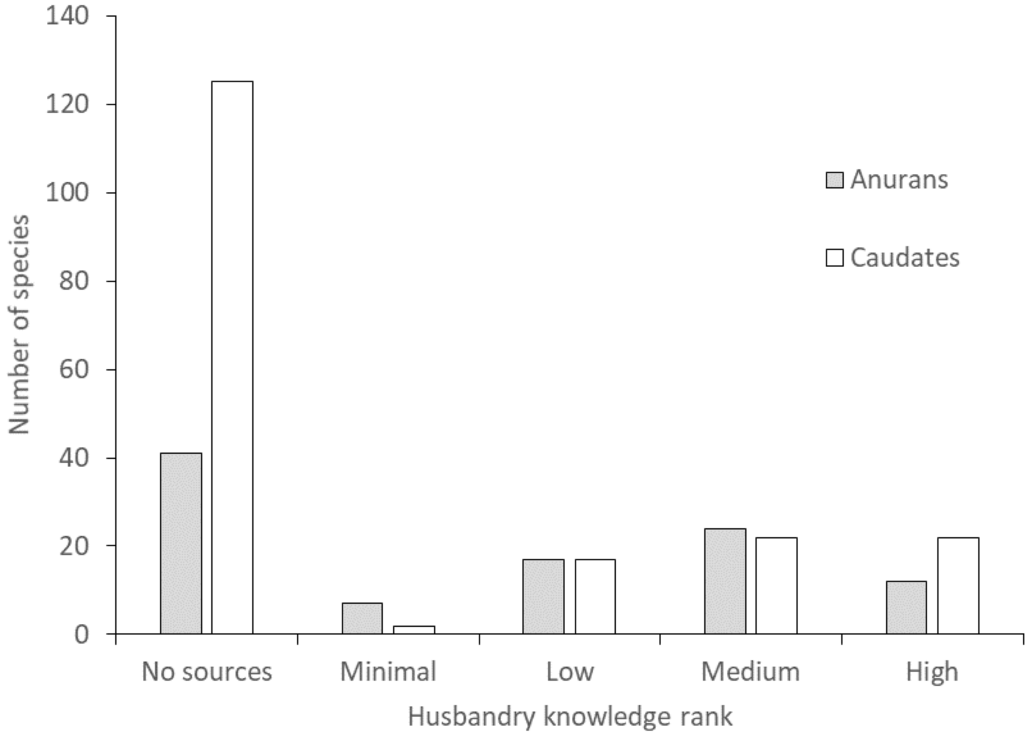

Figure 2.5. The number of species within each husbandry knowledge rank for anurans and caudates.

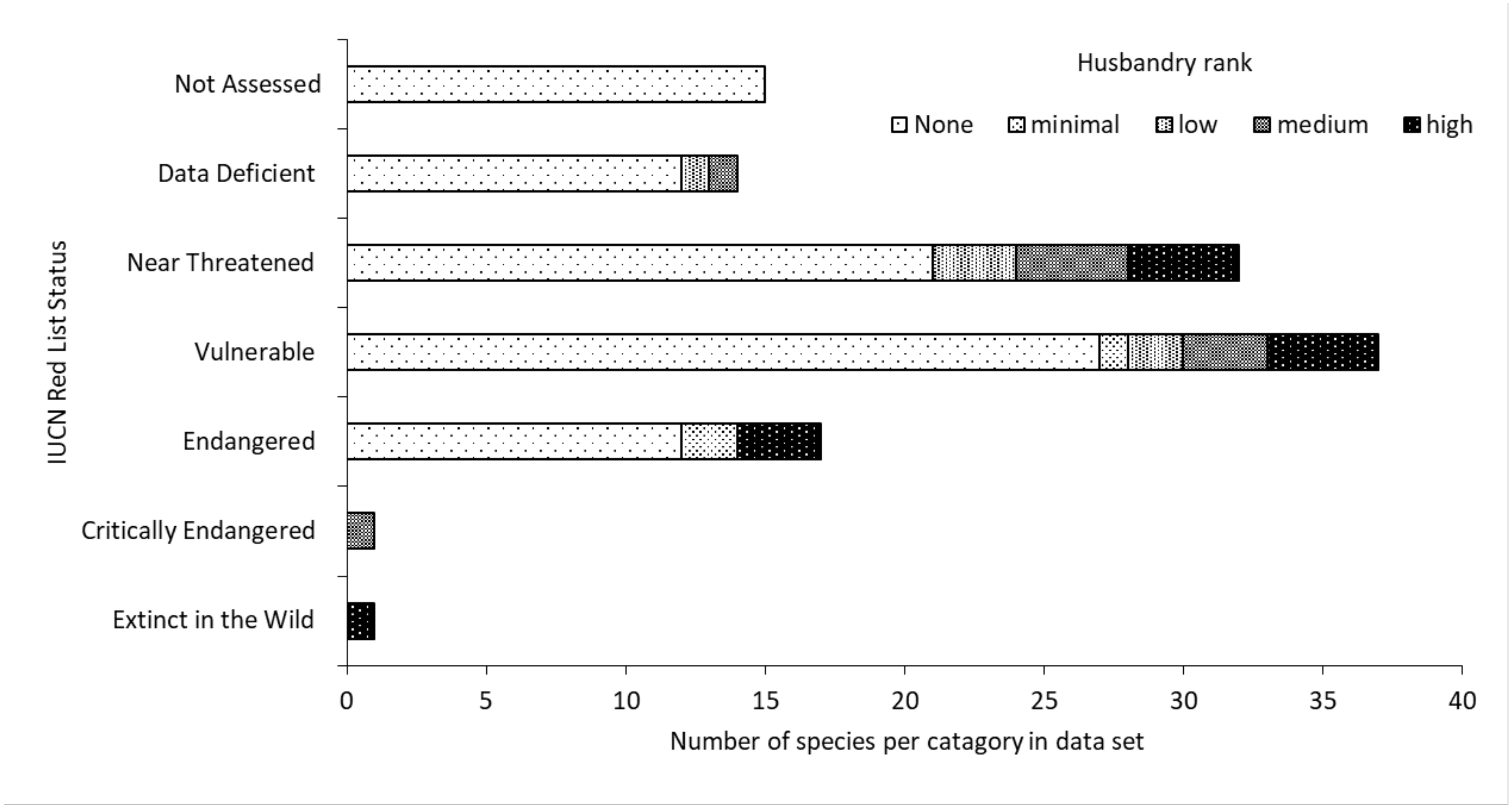

Figure 2.6. The number of species in the data set and their associated husbandry rank, listed by the IUCN Red List categories of conservation concern, species listed as data deficient and species that have not been assessed.

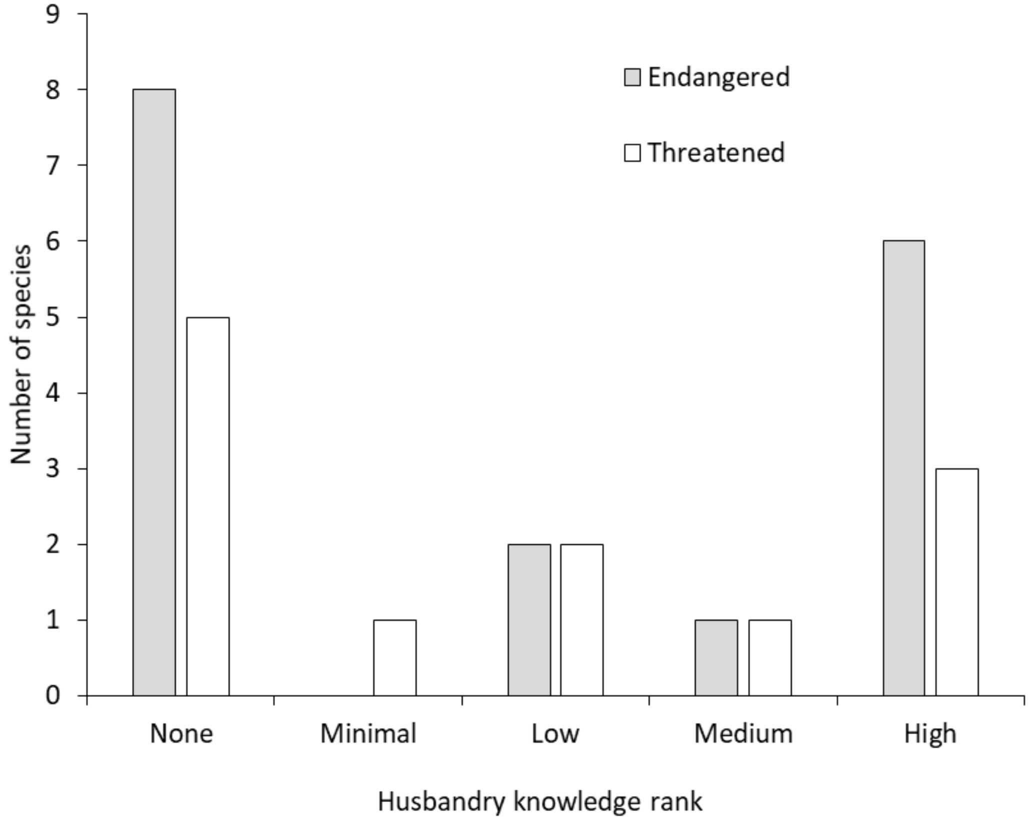

Figure 2.7. The number of native US amphibian species listed as endangered or threatened by the US Endangered Species Act and their associated husband rank.

Scientific Name	Vernacular Name	EDGE ranking	Husbandry level	IUCN Red List Status
Necturus alabamensis	Alabama Waterdog	27	0	Endangered
Phaeognathus hubrichti	Red Hills Salamander	43	0	Endangered
Rhyacotriton olympicus	Olympic Torrent Salamander	127	1	Vulnerable
Necturus lewisi	Neuse River waterdog	165	0	Near Threatened
Rhinophrynus dorsalis	Mexican Burrowing Toad	196	1	Least Concern
Gyrinophilus gulolineatus	Berry Cave Salamander	207	0	Endangered
Gyrinophilus subterraneus	West Virginia Spring Salamander	207	0	Endangered
Cryptobranchus alleganiensis	Hellbender	208	4	Near Threatened
Ambystoma californiense	California Tiger Salamander	212	2	Vulnerable
Ambystoma cingulatum	Frosted Flatwoods Salamander	212	0	Vulnerable
Plethodon stormi	Siskiyou Mountains salamander	214	0	Endangered
Batrachoseps campi	Inyo Mountains Salamander	223	0	Endangered
Plethodon welleri	Weller's Salamander	229	0	Endangered
Eurycea naufragia	Georgetown Salamander	236	0	Endangered

Table 2.1. The 15 species with the highest Evolutionary Distinct and Globally Endangered (EDGE) rating and associated husbandry level in the data set corresponding to none (0), minimal (1), low (2), medium (3), and high (4) husbandry ranking. The EDGE ranking is out of 4339 assessed amphibian species, and lower EDGE rankings indicate greater conservation concern and evolutionary distinctiveness.

Scientific name	Vernacular name	ED ranking	Husbandry level	IUCN Red List Status
Ascaphus truei	Pacific Tailed Frog	5	4	Least Concern
Ascaphus montanus	Rocky Mountain Tailed Frog	4	3	Least Concern
Pseudobranchus striatus	Northern Dwarf Siren	8	3	Least Concern
Pseudobranchus axanthus	Southern Dwarf Siren	7	4	Least Concern
Necturus maculosus	Mudpuppy	10	4	Least Concern
Necturus lewisi	Neuse River Waterdog	9	0	Near Threatened
Cryptobranchus alleganiensis	Hellbender	12	4	Near Threatened
Hemidactylium scutatum	Four-toed Salamander	20	0	Least Concern
Siren intermedia	Lesser Siren	32	4	Least Concern
Siren lacertina	Greater Siren	31	2	Least Concern
Amphiuma tridactylum	Three-toed Amphiuma	53	3	Least Concern
Amphiuma means	Two-toed Amphiuma	52	3	Least Concern
Amphiuma pholeter	One-toed Amphiuma	51	3	Near Threatened
Necturus punctatus	Dwarf Waterdog	56	0	Least Concern
Necturus beyeri	Gulf Coast Waterdog	55	4	Least Concern

Table 2.2. The 15 species with highest Evolutionary Distinct (ED) rating and husbandry level in the data set corresponding to none (0), minimal (1), low (2), medium (3), and high (4) husbandry ranking. The ED ranking is out of 4399 assessed amphibian specie by the Evolutionary Distinct and Globally Endangered program, and the lower the number, the greater the evolutionary distinctivenes

APPENDIX 2.1. A list of the species used in the data set, and their associated ranked husbandry citations. THE IUCN Red List Status (RLS) status include least concern (LC), vulnerable (VU), endangered (EN), critically endangered (CR), extinct in the wild (EX), and data deficient (DD. The species organized Anura and Caudata, and then sorted alphabetically by taxonomic genera.

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
Ascaphus montanus	Rocky Mountain Tailed Frog	LC	Essner, R. & Suffian, D. 2010 (3)
Ascaphus truei	Pacific Tailed Frog	LC	Stanly, H. 1985 (4); Coborn, J. 1992 (3); Mara, W.P. 1994 (2); Altig, R. & Brodie, E. (2); Brown, H. 1975 (3); Noble, G. & Putnam, G. 1931 (3) Stephenson, B. & Verrell, P. 2003 (3)
Anaxyrus spp. (specific at genus level)	Toad		New England Herpetological Society 2012 (2)
Anaxyrus americanus	American Toad	LC	Obringer et al. 2000 (4); Johnson et al. 2002 (3); Buck, M. 1960 (2); Kramer, J. 1973 (1); Grenard, S. 1999 (2); Alderton, D. 2007 (2); Staniszewski, M. 1995 (2); Mattison, C. 1993 (3); Odum et al. (n.d.) (2); Edmonds. 2005a (2)
Anaxyrus baxteri	Wyoming Toad	EW	Browne, R.et al. 2006 (4); Li, H. et al. (2); Perpinan D. et al. 2010 (2); Taylor, SK. 1999b (2); Obringer et al. 2000 (4); Polasik et al. 2015 (3); Odum et al. (n.a.) (2)
Anaxyrus boreas	Western Toad	NT	Scherff-Norris, K et al. 2002 (4); Staniszewski, M. 1995 (2); Mattison, C. 1993 (1)
Anaxyrus californicus	Arroyo Toad	EN	
Anaxyrus canorus	Yosemite Toad	EN	Martin, D. 1991 (4)
Anaxyrus cognatus	Great Plains Toad	LC	Kramer, J. 1973 (1); Alderton, D. 2007 (3); Flowers, S.S. 1925 (1)
Anaxyrus debilis	Green Toad	LC	Kramer, J. 1973 (1); Mattison, C. 1993 (1)
Anaxyrus exsul	Black Toad	VU	
Anaxyrus fowleri	Fowler's Toad	LC	Browne, R. et al. 2006 (3); Buck, M. 1960 (2)
Anaxyrus hemiophrys	Canadian Toad	LC	Taylor, S. 1999 (2)
Anaxyrus houstonensis	Houston Toad	EN	Quinn, H. R., & Mengden, G. 1984 (3); Quinn, H. R. 1980 (3); Kennedy1994, J. P. 1962 (3)
Rhinella marina	Cane Toad	LC	Narayan, E. et al. 2011 (2); Mattison, C. 1993 (2); Davis, R. & Davis, V. 1997 (2)

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
Anaxyrus microscaphus	Arizona Toad	LC	
Anaxyrus nelsoni	Amargosa Toad	EN	
Anaxyrus punctatus	Red-Spotted Toad	LC	Mattison, C. 1993 (2)
Anaxyrus quercicus	Oak Toad	LC	Kramer, J. 1973 (1); Alderton, D. 2007 (3); Staniszewski, M. 1995 (3); Davis, R. & Davis, V. 1997 (3); Coborn, J., J. 1992 (1)
Anaxyrus retiformis	Sonoran Green Toad	LC	
Anaxyrus speciosus	Texas Toad	LC	Kramer, J. 1973 (1); Quinn, H. & Mengden, G. (3)
Anaxyrus woodhousii	Woodhouse's Toad	LC	Frost, J.S. 1982 (2); Kramer, J. 1973 (1); Grenard, S. 1999 (2); Mara, W.P. 1994 (2)
Anaxyrus terrestris	Southern Toad	LC	Searle, C. L. et al. 2011 (2); Grenard, S. 1999 (2)
Incilius alvarius	Sonoran Desert Toad	LC	Alderton, D. 2007 (3); Staniszewski, M. 1995 (3); Mara, W.P. 1994 (2)
Incilius valliceps	Gulf Coast Toad	LC	Alderton, D. 2007 (2)
Incilius nebulifer	Gulf Coast Toad	LC	Rowson, A. et al. 2001 (3)
Craugastor augusti	Barking Frog	LC	
Eleutherodactylus spp. (specific at genus level)	Chirping Frog		Mattison, C. 1993 (4)
Eleutherodactylus cystignathoides	Rio Grande Chirping Frog	LC	
Eleutherodactylus guttilatus	Spotted Chirping Frog	LC	
Eleutherodactylus marnockii	Cliff Chirping Frog	LC	
Acris spp. (specific at genus level)	Cricket Frog		Buck, M. 1960 (2); Grenard, S. 1999 (2)
Acris blanchardi	Blanchard's Cricket Frog	LC	
Acris crepitans	Northern Cricket Frog	LC	McCallum, M. & Trauth, S. 2007 (3); Coborn, J. 1992 (1)
Acris gryllus	Southern Cricket Frog	LC	Coborn, J. 1992 (1)
Hyla andersonii	Pine Barrens Treefrog	NT	
Hyla arenicolor	Canyon Treefrog	LC	

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
Hyla avivoca	Bird-voiced Treefrog	LC	
Hyla chrysoscelis	Cope's Gray Treefrog	LC	Frost, J.S. 1982 (2); Haislip, N. et al. (2) 2012; Hoverman, J. T. et al. 2010 (2); Buck, M. 1960 (2); Buck, M. 1958 (1); Kramer, J. 1973 (1); Grenard, S. 1999 (2); Alderton, D. 2007 (2); Leutscher, A. 1976 (2); Staniszewski, M. 1995 (2); Mattison, C. 1993 (2); Edmonds, D. 2005e (2)
Hyla cinerea	Green Treefrog	LC	Brannelly, et al. 2012 (2); Blouin, M. 1992 (3); Zimmerman, E. 1983 (3); Kramer, J. 1973 (1); Alderton, D. 2007 (2); Staniszewski, M. 1995 (3); Mattison, C. 1993 (2); Coborn, J. 1992 (3); Edmonds, D. 2007 (3); McLeod, L. 2017b (2); “Green tree frogs” (n.d.) (2); Edmonds, D. 2005c (2)
Hyla eximia	Mountain Treefrog	LC	
Hyla femoralis	Pine Woods Treefrog	LC	Wilbur, H. M. 1982 (3)
Hyla gratiosa	Barking Treefrog	LC	Alderton, D. 2007 (2); Staniszewski, M. 1995 (2); Coborn, J. 1992 (1); Mara, W.P. 1994 (2)
Hyla squirella	Squirrel Treefrog	LC	
Hyla versicolor	Eastern Gray Treefrog	LC	Welch et al. 1998 (4); Searle, C. L. et al. 2011(2); Buck, M. 1960 (2); Buck, M. 1958 (1); Kramer, J. 1973 (1); Grenard, S. 1999 (2); Alderton, D. 2007 (2); Leutscher, A. 1976 (2); Staniszewski, M. 1995 (2); Mattison, C. 1993 (2); Edmonds, D. 2005e (2)
Hyla wrightorum	Arizona Treefrog	LC	
Pseudacris brachyphona	Mountain Chorus Frog	LC	
Pseudacris brimleyi	Brimley's Chorus Frog	LC	Mara, W.P. 1994 (1)
Pseudacris cadaverina	California Treefrog	LC	
Pseudacris clarkii	Spotted Chorus Frog	LC	Whitehurst, P. & Pierce, B. (2) 1991;
Pseudacris crucifer	Spring Peeper	LC	Stewart, K. & Lougheed, S. 2013 (4); Buck, M. 1960 (2); Buck, M. 1958 (1); Greenberg, S. & Raskin, E. 1952 (3); Grenard, S. 1999 (3); Staniszewski, M. 1995 (2); Coborn, J. 1992 (1); Mara, W.P. 1994 (2)
Pseudacris feriarum	Upland Chorus Frog	LC	Haislip, N. A. et al. 2012 (2)
Pseudacris fouquettei	Cajun Chorus Frog	LC	
Pseudacris illinoensis	Illinois Chorus Frog		
Pseudacris kalmi	New Jersey Chorus Frog	LC	Welch et al. 1998 (4); Searle, C. L. et al. 2011(2); Buck, M. 1960 (2); Buck, M. 1958 (1); Kramer, J. 1973 (1); Grenard, S. 1999 (2); Alderton, D. 2007 (2); Leutscher, A. 1976 (2); Staniszewski, M. 1995 (2); Mattison, C. 1993 (2)

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
Pseudacris maculata	Boreal Chorus Frog	LC	Amburgey et al. 2012 (2)
Pseudacris nigrita	Southern Chorus Frog	LC	Martof, B. & Thompson, E. 1958 (3); Kramer, J. 1973 (1)
Pseudacris ocularis	Little Grass Frog	LC	
Pseudacris ornata	Ornate Chorus Frog	LC	Kramer, J. 1973 (1); Coborn, J. 1992 (2)
Pseudacris regilla	Pacific Treefrog	LC	Kramer, J. 1973 (1); Alderton, D. 2007 (3); Coborn, J. 1992 (2)
Pseudacris streckeri	Strecker's Chorus Frog	LC	Burt, C. E. 1936 (1)
Pseudacris triseriata	Midland Chorus Frog	LC	Hoppe, D. 1979 (1); Searle, C. L. et al. 2011(2)
Smilisca baudinii	Mexican Treefrog	LC	Coborn, J. 1992 (3)
Smilisca fodiens	Northern Casquehead Frog	LC	
Leptodactylus fragilis	Mexican White-lipped Frog	LC	Coborn, J. 1992 (1)
Gastrophryne carolinensis	Eastern Narrow-mouthed Toad	LC	Hoverman, J. T. et al. 2010 (2) Mattison, C. 1993 (3); Coborn, J. 1992 (1)
Gastrophryne mazatlanensis	Great Plains Narrow-mouthed Toad	LC	Mattison, C. 1993 (3); Coborn, J. 1992 (1); Mara, W.P. 1994 (2)
Gastrophryne olivacea	Sheep Frog	LC	
Lithobates areolata	Crawfish Frog	NT	Stiles, R. et al. 2016 (3)
Rana aurora	Red-legged Frog	LC	Gregory, P. T. 1979 (2)
Lithobates berlandieri	Rio Grande Leopard Frog	LC	Frost, J.S. 1982 (3)
Lithobates blairi	Plains Leopard Frog	LC	Frost, J.S. 1982 (3)
Rana boylii	Foothill Yellow-legged Frog	NT	Sparling, D. & Fellers, G. 2007 (2)
Lithobates capito	Carolina Gopher Frog	NT	
Rana cascadae	Cascades Frog	NT	
Lithobates catesbeiana	Bullfrog	LC	Alworth, L. & Vazquez, V. 2009 (3); Emmerson, F. & Kay, F. 1971 (2); Buck, M. 1958 (1); Kramer, J. 1973 (1); Greenberg, S. & Raskin, E. 1952 (3); Grenard, S. 1999 (2); Leutscher, A. 1976 (2); Staniszewski, M. 1995 (3); Mattison, C. 1993 (2); Coborn, J. 1992 (3); Mara, W.P. 1994 (2); Flowers, S.S. 1925 (1)

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
Lithobates chiricahuensis	Chiricahua Leopard Frog	VU	Fernandez, P. & Rosen, P. 1996 (3); Sredl, M. & Healy, B. L. 1999 (3); Frost, J.S. 1982 (3); Sredl, M. 2014 (2); US Fish & Wildlife Service 2007 (4)
Lithobates clamitans	Bronze/Green Frog	LC	Emmerson, F. & Kay, F. 1971 (2); Mara, W.P. 1994 (2); Haislip, N. A. et al. 2012 (2); Greenberg, Sylvia, 1952 (3); Grenard, S. 1999 (2); Staniszewski, M. 1995 (2); Staniszewski, M. 1995 (3); Flowers, S.S. 1925 (1)
Rana draytonii	California Red-legged Frog	VU	Padgett-Flohr, G. 2008 (2)
Lithobates grylio	Pig Frog	LC	Grenard, S. 1999 (2)
Lithobates heckscheri	River Frog	LC	
Rana luteiventris	Columbia Spotted Frog	LC	
Rana muscosa	Southern Mountain Yellow-legged Frog	EN	Andre, S. et al. 2008 (2); Lovich, K., 2007 (4)
Lithobates okaloosae	Florida Bog Frog	VU	
Lithobates onca	Relict Leopard Frog	EN	
Lithobates palustris	Pickerel Frog	LC	Hoverman, J. T. et al. 2010 (2) Buck, M. 1960 (2); Buck, M. 1958 (1); Greenberg, S. & Raskin, E., 1952 (3); Kramer, J. 1973 (1); Grenard, S. 1999 (2); Mara, W.P. 1994 (2)
Lithobates pipiens	Northern Leopard Frog	LC	Frost, J.S. 1982 (3); Glennemeier, K. & Denver, R. 2002a (2); Glennemeier, K. & Denver, R. 2002b (2); Buck, M. 1960 (2); Buck, M. 1958 (1); Greenberg, Sylvia, 1952 (3); Lane-Petter, W. 1963 (4); Grenard, S. 1999 (2); Leutscher, A.1976 (1); Staniszewski, M. 1995 (2); Mattison, C. 1993 (3); Coborn, J. 1992 (3); Flowers, S. S. 1925 (1); Wind, E. 2002 (3); Adama, D.B. et al. 2003 (3); Lansley 2004 (4); McLeod, L. 2017 (2); Edmonds, D. 2005b (2)
Rana pretiosa	Oregon Spotted Frog	VU	Reinking, L. N. et al. 1980 (2); Padgett-Florh, G. & Hayes P. 2011 (2); Plomski, L. 2011 (3)
Lithobates septentrionalis	Mink Frog	LC	
Lithobates sevosa	Dusky Gopher Frog	CR	Kouba et al. 2014 (3); Sutten et al. 2015 (1); Graham et al. 2016 (2); Aaltonen et al. 2011 (3)
Lithobates sierrae	Sierra Nevada Yellow-legged Frog	EN	
Lithobates sphenocephala	Florida Leopard Frog	LC	Frost, J.S. 1982 (3); Edmonds, D 2005b (2)

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
Lithobates sylvatica	Wood Frog	LC	McCallum, M. & Trauth, S. 2002 (2); Searle, C. L., C. L. et al. 2011(2); Buck, M. 1960 (2); Buck, M. 1958 (1); Greenberg & Raskin 1952 (3); Kramer, J. 1973 (1); Mattison, C. 1993 (1);
Lithobates tarahumarae	Tarahumara Frog	VU	
Lithobates virgatipes	Carpenter Frog	LC	Kramer, J. 1973 (1); Mara, W.P. 1994 (2)
Lithobates yavapaiensis	Lowland Leopard Frog	LC	
Rhinophrynus dorsalis	Mexican Burrowing Toad	LC	Coborn, J. 1992 (1)
Scaphiopus spp. (specific at genus level)	Spadefoot toad		Buck, M. 1960 (2);Vogel, Z. 1964 (2); Grenard, S. 1999 (2); Staniszewski, M. 1995 (3); Davis, R. & Davis, V. 1997 (3)
Scaphiopus couchii	Couch's Spadefoot	LC	Mattison, C. 1993 (3); Coborn, J. 1992 (1); Mara, W.P. 1994 (2)
Scaphiopus holbrookii	Eastern Spadefoot	LC	Kramer, J. 1973 (1); Flowers, S.S. 1925 (1)
Scaphiopus hurterii	Hurter's Spadefoot	LC	
Spea bombifrons	Plains Spadefoot	LC	
Spea hammondii	Western Spadefoot	NT	
Spea intermontana	Great Basin Spadefoot	LC	
Spea multiplicata	Mexican Spadefoot	LC	
Ambystoma spp. (specific at genus level)	Mole Salamanders		New England Herpetological Society 2012 (2)
Ambystoma annulatum	Ringed Salamander	LC	Grenard, S. 1999 (3); Staniszewski, M. 1995 (4); Coborn, J., J. 1993 (1); Coborn, J. 1992 (1)
Ambystoma barbouri	Streamside Salamander	NT	
Ambystoma bishopi	Reticulated Flatwoods Salamander	VU	
Ambystoma californiense	California Tiger Salamander	VU	Padgett-Flohr, G. (2)
Ambystoma cingulatum	Frosted Flatwoods Salamander	VU	
Ambystoma gracile	Northwestern Salamander	LC	

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
Ambystoma jeffersonianum	Jefferson Salamander	LC	Buck, M. 1960 (2); Staniszewski, M. (3); Coborn, J. 1992 (1)
Ambystoma laterale	Blue-spotted Salamander	LC	Grenard, S. 1999 (3); Staniszewski, M. 1995 (2); Coborn, J. 1992 (1)
Ambystoma mabeei	Mabee's Salamander	LC	
Ambystoma macrodactylum	Long-toed Salamander	LC	Kramer, J. 1973 (1); Staniszewski, M. 1995 (3)
Ambystoma maculatum	Spotted Salamander	LC	Buck, M. 1958 (1); Buck, M. 1960 (2); Lane-Petter, W. 1963 (4); Grenard, S. 1999 (3); Alderton, D. 2007 (3); Staniszewski, M. 1995 (3); Mattison, C. 1993 (1); Edmonds, D. 2009 (2); Coborn, J., J. 1993 (1); Coborn, J. 1992 (3); Spinner, L. 2005 (3); Davis, A. 2012 (3)
Ambystoma mavortium	Barred Tiger Salamander	LC	Edmonds, D. 2009 (2); Edmonds. D. 2005d (2)
Ambystoma opacum	Marbled Salamander	LC	Smyers, S. & Rubbo, M. 2001 (3); Alderton, D. 2007 (3); Bartlett, P. 2003 (2); Staniszewski, M. 1995 (2); Mattison, C. 1993 (3); Edmonds, D. 2009 (2); Coborn, J., J. 1993 (1); Kowalski, E 2002 (4)
Ambystoma talpoideum	Mole Salamander	LC	Davis, A. & Maerz, J. 2008 (2); Kramer, J. 1973 (1); Staniszewski, M. 1995 (2)
Ambystoma texanum	Small-Mouthed Salamander	LC	
Ambystoma tigrinum	Eastern Tiger Salamander	LC	Emmerson, F. & Kay, F. 1971 (1); Buck, M. 1958 (1); Zimmerman, E., E. 1983 (2); Buck, M. 1960 (2); Greenberg, S. & Raskin, E. 1952 (3); Lane-Petter, W. 1963 (4); Kramer, J. 1973 (1); Grenard, S. 1999 (3); Alderton, D. 2007 (3); Bartlett, P. 2003 (2); Leutscher, A.1976 (1); Staniszewski, M. 1995 (4); Mattison, C. 1993 (3); Edmonds, D. 2009 (1); Davis, R. & Davis, V. 1997 (3); Flowers, S.S. 1925 (1); Kowalski, E. 2001 (3); Williams, J. 2011 (3); Edmonds, D. 2005d (2)
Amphiuma spp. (specific at genus level)			Vogel, Z. 1964 (2); Grenard, S. 1999 (2); Edmonds, D. 2009 (3); Coborn, J., J. 1993 (2)
Amphiuma means	Two-toed Amphiuma	LC	Alderton, D. 2007 (2); Staniszewski, M. 1995 (3); Coborn, J. 1992 (2); Flowers, S.S. 1925 (1); Kowalski, E. & Watkins-Colwel, G. 2004 (3)
Amphiuma pholeter	One-toed Amphiuma	NT	Kowalski, E. & Watkins-Colwel, G. 2004 (3)
Amphiuma tridactylum	Three-toed Amphiuma	LC	Kowalski, E. & Watkins-Colwel, G. 2004 (3)
Cryptobranchus alleganiensis	Hellbender	NT	Dierenfeld, E. et al. 2009 (2); Ettling et al. 2013 (4); Vogel, Z. 1964 (2); Grenard, S. 1999 (3); Leutscher, A.1976 (2); Staniszewski, M. 1995 (3); Coborn, J. 1992 (1); Flowers, S.S.

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
			1925 (1); Flanagan, W. 2002 (3)
Dicamptodon aterrimus	Idaho Giant Salamander	LC	
Dicamptodon copei	Cope's Giant Salamander	LC	Staniszewski, M. 1995 (3)
Dicamptodon ensatus	California Giant Salamander	NT	Staniszewski, M. 1995 (4); Coborn, J. 1992 (1)
Dicamptodon tenebrosus	Pacific Giant Salamander	LC	Staniszewski, M. 1995 (4)
Aneides spp. (specific at genus level)	Arboreal Salamander		Staniszewski, M. 2002a (3)
Aneides aeneus	Green Salamander	NT	Coborn, J. 1992 (1) Staniszewski, M. 2002a (2)
Aneides ferreus	Clouded Salamander	NT	Staniszewski, M. 1995 (4)
Aneides flavipunctatus	Black Salamander	NT	Staniszewski, M. 2002a (3)
Aneides hardii	Sacramento Mountains Salamander	LC	Staniszewski, M. 2002a (3)
Aneides lugubris	Arboreal Salamander	LC	Coborn, J. 1992 (1)
Aneides vagrans	Wandering Salamander	NT	
Batrachoseps spp. (specific at genus level)	Slender Salamander		Staniszewski, M. 2002c (3)
Batrachoseps altasierrae	Green Horn Mountains Slender Salamander		
Batrachoseps attenuatus	California Slender Salamander	LC	Staniszewski, M. 1995 (4); Coborn, J. 1992 (1)
Batrachoseps bramei	Fairview Slender Salamander		
Batrachoseps campi	Inyo Mountains Salamander	EN	
Batrachoseps diabolicus	Hell Hollow Slender Salamander	DD	
Batrachoseps gabrieli	San Gabriel Mountains Slender Salamander	DD	

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
Batrachoseps gavilanensis	Gabilan Mountains Slender Salamander	LC	
Batrachoseps gregarius	Gregarious Slender Salamander	LC	
Batrachoseps incognitus	San Simeon Slender Salamander	DD	
Batrachoseps kawia	Sequoia Slender Salamander	DD	
Batrachoseps luciae	Santa Lucia Mountains Slender Salamander	LC	
Batrachoseps major	Garden Slender Salamander	LC	
Batrachoseps minor	Lesser Slender Salamander	DD	
Batrachoseps nigriventris	Black-bellied Slender Salamander	LC	Staniszewski, M. 1995 (4)
Batrachoseps pacificus	Channel Islands Slender Salamander	LC	
Batrachoseps regius	Kings River Slender Salamander	VU	
Batrachoseps relictus	Relictual Slender Salamander	DD	Staniszewski, M. 2002c (3)
Batrachoseps robustus	Kern Plateau Salamander	NT	
Batrachoseps simatus	Kern Canyon Slender Salamander	VU	
Batrachoseps stebbinsi	Tehachapi Slender Salamander	VU	
Batrachoseps wrighti	Oregon Slender Salamander	VU	Staniszewski, M. 2002c (3)
Desmognathus spp. (specific at genus level)	Dusky Salamander		Buck, M. 1960 (2); Greenberg & Raskin 1952 (3); Staniszewski, M. 1995 (3); Edmonds, D. 2009 (3)

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
Desmognathus abditus	Cumberland Dusky Salamander	NT	
Desmognathus aeneus	Seepage Salamander	NT	
Desmognathus apalachicolae	Apalachicola Dusky Salamander	LC	
Desmognathus auriculatus	Southern Dusky Salamander	LC	Staniszewski, M. 1995 (2)
Desmognathus brimleyorum	Ouachita Dusky Salamander	LC	
Desmognathus carolinensis	Carolina Mountain Dusky Salamander	LC	
Desmognathus conanti	Spotted Dusky Salamander		
Desmognathus folkertsi	Dwarf Black-bellied Salamander	DD	Nelson, N. 2003 (2)
Desmognathus fuscus	Northern Dusky Salamander	LC	Zimmerman, E. 1983 (3); Grenard, S. 1999 (3); Grenard, S. 1999 (3); Alderton, D. 2007 (2); Staniszewski, M. 1995 (3)
Desmognathus imitator	Imitator Salamander	LC	
Desmognathus marmoratus	Shovel-nosed Salamander	LC	Nelson, N. 2003 (2)
Desmognathus monticola	Seal Salamander	LC	Staniszewski, M. 1995 (2); Kowalski, E. 2005 (2)
Desmognathus ochrophaeus	Allegheny Mountain Dusky Salamander	LC	Verrell, P. 1989 (3)
Desmognathus ocoee	Ocoee Salamander	LC	Bernardo, J. & Arnold, S. 1990 (4)
Desmognathus orestes	Blue Ridge Dusky Salamander	LC	
Desmognathus organi	Northern pygmy salamander		
Desmognathus planiceps	Flat-headed Salamander		

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
Desmognathus quadramaculatus	Black-bellied Salamander	LC	Staniszewski, M. 1995 (1); Nelson, N. 2003 (2)
Desmognathus santeetlah	Santeetlah Dusky Salamander	LC	
Desmognathus welteri	Black Mountain Dusky Salamander	LC	
Desmognathus wrighti	Pygmy Salamander	LC	Staniszewski, M. 1995 (2)
Ensatina eschscholtzii	Ensatina Salamander	LC	Staniszewski, M. 1995 (3); Coborn, J. 1992 (1) Staniszewski, M. 2002b (3
Eurycea spp. (specific at genus level)	Two Lined Salamander		Buck, M., 1958 (1); Greenberg & Raskin, 1952 (3); Nelson, N. 2003b (3)
Eurycea aquatica	Dark-sided Salamander		
Eurycea bislineata	Northern Two-lined Salamander	LC	Lane-Petter, W. 1963 (4); Alderton, D. 2007 (2); Staniszewski, M. 1995 (3); Edmonds, D. 2009 (3); Nelson, N. 2003b (2)
Eurycea chamberlaini	Chamberlain's Dwarf Salamander	DD	
Eurycea chisholmensis	Salado Salamander	VU	
Eurycea cirrigera	Southern Two-lined Salamander	LC	Edmonds, D. 2009 (3); Nelson, N. 2003b (2)
Eurycea guttolineata	Three-lined Salamander	LC	Alderton, D. 2007 (2)
Eurycea junaluska	Junaluska Salamander	VU	
Eurycea latitans	Cascade Caverns Salamander	VU	
Eurycea longicauda	Long-tailed Salamander	LC	Staniszewski, M. 1995 (2)
Eurycea lucifuga	Cave Salamander	LC	
Eurycea multiplicata	Many-ribbed Salamander	LC	
Eurycea nana	San Marcos Salamander	VU	Woods et al. 2010 (3); Gabor, C. et al. 2016 (2); Epp, K. & Gabor, K. 2008 (2); Najvar et al. 2001 (4)
Eurycea naufragia	Georgetown Salamander	EN	
Eurycea neotenes	Texas Salamander	VU	Roberts, D. et al. 1995 (4)

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
Eurycea pterophila	Fern Bank Salamander	DD	
Eurycea quadridigitata	Dwarf Salamander	LC	Staniszewski, M. 1995 (3)
Eurycea rathbuni	Texas Blind Salamander	VU	Epp, K. et al. 2010 (3); Fries, J. 2002 (4); Belcher, D. 1988 (3); Flowers, S.S. 1925 (1)
Eurycea robusta	Blanco Blind Salamander	DD	
Eurycea sosorum	Barton Springs Salamander	VU	DeSantis, D. et al. 2013 (3); Woods, H. A. et al. 2010 (3); Gabor, C. et al. 2016 (2); Cantu et al. (2016) (4)
Eurycea spelaea	Grotto Salamander	LC	
Eurycea tonkawae	Jollyville Plateau Salamander	EN	Gabor, C. et al. 2016 (2)
Eurycea tridentifera	Comal Blind Salamander	VU	
Eurycea troglodytes	Valdina Farms Salamander	DD	
Eurycea tynerensis	Oklahoma Salamander	NT	
Eurycea waterlooensis	Austin Blind Salamander	VU	
Eurycea wilderae	Blue Ridge Two-lined Salamander	LC	Nelson, N. 2003b (2)
Gyrinophilus gulolineatus	Berry Cave Salamander	EN	
Gyrinophilus palleucus	Tennessee Cave Salamander	VU	
Gyrinophilus porphyriticus	Spring Salamander	LC	
Gyrinophilus subterraneus	West Virginia Spring Salamander	EN	
Eurycea wallacei	Georgia Blind Salamander	VU	
Hemidactylium scutatum	Four-toed Salamander	LC	
Hydromantes brunus	Limestone Salamander	VU	
Hydromantes	Mount Lyell Salamander	LC	

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
platycephalus			
Hydromantes shastae	Shasta Salamander	VU	
Phaeognathus hubrichti	Red Hills Salamander	EN	
Plethodon: spp. (specific at genus level/common name)	Slimy Salamander		Buck, M. 1960 (2); Staniszewski, M. 1995 (3); Edmonds, D. 2009 (3); Kowalski, Ed. 2001 (3)
Plethodon: spp. (specific at genus level/common name)	Red Backed Salamander		Buck, M. 1960 (2); Greenberg, S. & Raskin, E. 1952 (3)
Plethodon albagula	Western Slimy Salamander	LC	
Plethodon amplus	Blue Ridge Gray-cheeked Salamander	VU	
Plethodon angusticlavius	Ozark Salamander	LC	
Plethodon asupak	Scott Bar Salamander	VU	
Plethodon aureolus	Tellico Salamander	DD	
Plethodon caddoensis	Caddo Mountain Salamander	NT	
Plethodon chattahoochee	Chattahoochee Slimy Salamander		
Plethodon cheoah	Cheoah Bald Salamander	VU	
Plethodon chlorobryonis	Atlantic Coast Slimy Salamander		
Plethodon cinereus	Eastern Red-backed Salamander	LC	Zimmerman, E. 1983 (3); Kramer, J. 1973 (1); Grenard, S. 1999 (3); Leutscher, A. 1976 (3); Staniszewski, M. 1995 (2); Mattison, C. 1993 (1); Edmonds, D. 2009 (3); Coborn, J. 1992 (1); Nelson, N. 2002 (4)
Plethodon cylindraceus	White-spotted Slimy Salamander	LC	
Plethodon dorsalis	Northern Zigzag	LC	

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
	Salamander		
Plethodon dunni	Dunn's Salamander	LC	
Plethodon electromorphus	Northern Ravine Salamander	LC	
Plethodon elongatus	Del Norte Salamander	NT	
Plethodon fourchensis	Fourche Mountain Salamander	VU	
Plethodon glutinosus	Northern Slimy Salamander	LC	Staniszewski, M. (2); Staniszewski, M. 1995 (2); Mattison, C. 1993 (2); Edmonds, D. 2009 (3); Coborn, J., J. 1993 (1); Coborn, J. 1992 (1)
Plethodon grobmani	Southeastern Slimy Salamander		
Plethodon hoffmani	Valley & Ridge Salamander	LC	
Plethodon hubrichti	Peaks of Otter Salamander	VU	
Plethodon idahoensis	Coeur d'Alene Salamander	LC	
Plethodon jordani	Jordan's Salamander	NT	Staniszewski, M. 1995 (2); Mattison, C. 1993 (1)
Plethodon kentucki	Cumberland Plateau Salamander	LC	
Plethodon kiamichi	Kiamichi Slimy Salamander	DD	
Plethodon kisatchie	Louisiana Slimy Salamander	LC	
Plethodon larselli	Larch Mountain salamander	NT	
Plethodon meridianus	Southern Gray-cheeked Salamander	VU	
Plethodon metcalfi	Southern Gray-cheeked Salamander	LC	

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
Plethodon mississippi	Mississippi Slimy Salamander		
Plethodon montanus	Northern Gray-cheeked Salamander	LC	
Plethodon neomexicanus	Jemez Mountains Salamander	NT	
Plethodon nettingi	Cheat Mountain Salamander	NT	
Plethodon ocmulgee	Ocmulgee Slimy Salamander		
Plethodon ouachitae	Rich Mountain Salamander	NT	
Plethodon petraeus	Pigeon Mountain Salamander	VU	
Plethodon punctatus	Cow Knob Salamander	NT	
Plethodon richmondi	Ravine Salamander	LC	
Plethodon savannah	Savannah Slimy Salamander		
Plethodon sequoyah	Sequoyah Slimy Salamander	DD	
Plethodon serratus	Southern Red-backed Salamander	LC	Edmonds, D. 2009 (3); Nelson, N. 2002 (3)
Plethodon shenandoah	Shenandoah Salamander	VU	
Plethodon sherando	Big Levels Salamander	VU	
Plethodon shermani	Red-legged Salamander	VU	
Plethodon stormi	Siskiyou Mountains salamander	EN	
Plethodon teyahalee	Southern Appalachian Salamander	LC	
Plethodon vandykei	Van Dyke's Salamander	LC	

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
Plethodon variolatus	South Carolina Slimy Salamander		
Plethodon vehiculum	Western Red-backed Salamander	LC	Staniszewski, M. 1995 (2)
Plethodon ventralis	Southern Zigzag Salamander	LC	
Plethodon virginia	Shenandoah Mountain Salamander	NT	
Plethodon websteri	Webster's Salamander	LC	
Plethodon wehrlei	Wehrle's Salamander	LC	
Plethodon welleri	Weller's Salamander	EN	
Plethodon yonahlossee	Yonahlossee Salamander	LC	
Pseudotriton montanus	Gulf Coast Mud Salamander	LC	
Pseudotriton ruber	Blue Ridge Red Salamander	LC	Greenberg & Raskins 1952 (3); Kramer, J. 1973 (1); Grenard, S. 1999 (3); Staniszewski, M. (2); Staniszewski, M. 1995 (4); Mattison, C. 1993 (2); Coborn, J., J. 1993 (1); Coborn, J. 1992 (1)
Stereochilus marginatus	Many-lined Salamander	LC	
Urspelerpes brucei	Patch-nosed Salamander	LC	
Necturus spp. (specific at genus level)	None		Grenard, S. 1999 (2)
Necturus alabamensis	Alabama Waterdog	EN	
Necturus beyeri	Gulf Coast waterdog	LC	Stoops et al. 2014 (4)
Necturus lewisi	Neuse River waterdog	NT	
Necturus maculosus	Mudpuppy	LC	Zimmerman, E., 1983 (3); Alderton, D. 2007 (3); Leutscher, A.1976 (2); Staniszewski, M. 1995 (4); Coborn, J., J. 1993 (2); Coborn, J. 1992 (1); Flowers, S.S. 1925 (1) Lembcke, P. 2005 (4)
Necturus punctatus	Dwarf waterdog	LC	
Rhyacotriton cascadae	Cascade torrent salamander	NT	

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
Rhyacotriton kezeri	Columbia torrent salamander	NT	
Rhyacotriton olympicus	Olympic Torrent Salamander	VU	Coborn, J. 1992 (1)
Rhyacotriton variegatus	Southern Torrent Salamander	LC	
Notophthalmus spp (specific at genus level)	Newt		Greenberg, S. & Raskin, E. 1952 (3); Macke, J. 2004 (3)
Notophthalmus meridionalis	Black-spotted newt	EN	Staniszewski, M. 1995 (1)
Notophthalmus perstriatus	Striped Newt	NT	Staniszewski, M. 1995 (1); Davis, R. & Davis, V. 1997 (3);
Notophthalmus viridescens	Eastern Newt	LC	Cameron, et al. 2004 (4); Khan, P. & Liversage, R.1995 (4); Lane-Petter, W. 1963 (4); Kramer, J. 1973 (1); Grenard, S. 1999 (3); Alderton, D. 2007 (3); Bartlett, P. 2003 (2); Leutscher, A1976 (2); Staniszewski, M. 1995 (4); Mattison, C. 1993 (3); Edmonds, D. 2009 (3); Coborn, J., J.1993 (3); Coborn, J. 1992 (1); Flowers, S.S. 1925 (1) Nelson, N. 2001 (3)
Taricha spp. (specific at genus level)	Newt, red eft		Grenard, S. 1999 (2); Buck, M. 1958 (2); Buck, M. 1960 (2);
Taricha granulosa	Rough-skinned newt	LC	Hanifin, C. et al. 2002 (2); Staniszewski, M. 1995 (3); Mattison, C. 1993 (3); Edmonds, D. 2009 (3); Nelson, N. 2001 (2); Gerlach, U. 2008 (4); Edmonds, D. 2005f (2)
Taricha rivularis	Red-bellied newt	LC	
Taricha sierrae	California Newt	LC	Lane-Petter, W. 1963 (4); Staniszewski, M. 1995 (3); Edmonds, D. 2009 (1); Coborn, J. 1992 (1); Flowers, S.S. 1925 (1); Wei, P. 2004 (3); Edmonds, D. 2005f (2)
Taricha torosa	Sierra Newt	LC	
Pseudobranchus spp. (specific at genus level)	Siren		Grenard, S. 1999 (2); Mattison, C. 1993 (2); Edmonds, D. 2009 (2)
Pseudobranchus axanthus	Southern Dwarf Siren	LC	Kowalski, E. 2004 (4)
Pseudobranchus striatus	Northern Dwarf Siren	LC	Pfaff, S. & Vause, K. 2002 (4); Coborn, J. 1992 (1); Kowalski, E. 2004 (3)
Siren spp. (specific at	Siren		Grenard, S. 1999 (2); Mattison, C. 1993 (3)

Species	Vernacular Name	RLS	Citations & quality rank: minimal (1), low (2), medium (3), high (4)
genus level)			
Siren intermedia	Lesser siren	LC	Zimmerman, E. 1983 (3); Alderton, D. 2007 (3); Staniszewski, M. 1995 (3) Kowalski, E. 2008 (4)
Siren lacertina	Greater siren	LC	Coborn, J., J. 1993 (2); Coborn, J. 1992 (1); Flowers, S.S. 1925 (2)

APPENDIX 2.2. Literature containing husbandry information referenced in appendix 2.1.

Aaltonen J. T., Bohlender T., Snyder W., Krebs J., Linhoff L., Snoza M., Plesuk S., Kouba A., Germano J., Reichling S., Loskutoff N. M. (2011) The developmental competence of tadpoles produced in vitro from the endangered Dusky Gopher Frog (*Rana sevosa*) using exogenous hormone treatment. *Reproduction, Fertility and Development* 24, 172-172.

Adama, D. B., Lansley, K., & Beaucher, M. A. (2003). Northern leopard frog (*Rana pipiens*) recovery: Captive rearing and reintroduction in southeast british columbia, *Report to the Columbia Basin Fish and Wildlife Compensation Program,* Nelson, BC*.,* 26. Retrieved from http://www.fwcpcolumbia.ca/version2/reports/pdfs/northern_leopard_frog.pdf

Alderton, D. (2007). *Firefly encyclopedia of the vivarium*. Firefly Books Ltd, New York, NY.

Altig, R., & Brodie Jr, E. D. (1972). Laboratory behavior of *Ascaphus truei* tadpoles. *Journal of Herpetology,* Vol. 6, No. 1. 21-24.

Alworth, L. C., & Vazquez, V. M. (2009). A novel system for individually housing bullfrogs. *Lab Animal, 38*(10), 329-333.

Amburgey, S., Funk, W. C., Murphy, M., & Muths, E. (2012). Effects of hydroperiod duration on survival, developmental rate, and size at metamorphosis in boreal chorus frog tadpoles (*Pseudacris maculata*). *Herpetologica, 68*(4), 456-467.

Andre, S. E., Parker, J., & Briggs, C. J. (2008). Effect of temperature on host response to *Batrachochytrium dendrobatidis* infection in the mountain yellow-legged frog (*Rana muscosa*). *Journal of Wildlife Diseases*, *44*(3), 716-720.

Bartlett, P. (2003). *Reptiles and amphibians for dummies* (1st ed.). New York, NY: Wiley Publishing, Inc.

Belcher, D.L. (1988). Courtship behavior and spermatophore deposition by the subterranean salamander, *Typhlomolge rathbuni* (Caudata, Plethodontidae). *Southwestern Naturalist* 33:124–126

Bernardo, J., & Arnold, S. J. (1990). Mass-rearing of plethodontid salamander eggs. *Amphibia-Reptilia,* (20), 219.

Blouin, M. S. (1992). Genetic correlations among morphometric traits and rates of growth and differentiation in the green tree frog, *Hyla cinerea*. *Evolution*, *46*(3), 735-744.

Brannelly, L. A., Chatfield, M. W., & Richards-Zawacki, C. L. (2012). Field and laboratory studies of the susceptibility of the green treefrog (*Hyla cinerea*) to *Batrachochytrium dendrobatidis* infection. *PloS one*, *7*(6), e38473.

Brown, H. A. (1975). Temperature and development of the tailed frog, *Ascaphus truei*. *Comparative Biochemistry and Physiology Part A: Physiology, 50*(2), 397-405.

Browne, R. K., Li, H., Seratt, J., & Kouba, A. (2006). Progesterone improves the number and quality of hormone induced fowler toad (*Bufo fowleri*) oocytes. *Reproductive Biology and Endocrinology, 4*(3), 1-7.

Browne, R. K., Seratt, J., Vance, C., & Kouba, A. (2006). Hormonal priming, induction of ovulation and in-vitro fertilization of the endangered Wyoming Toad (*Bufo baxteri*). *Reproductive Biology and Endocrinology, 4*, 34.

Buck, M. W. (1958). *Pets from the pond*. New York: Abingdon Press.

Buck, M. W. (1960). *Small pets from woods and fields*. New York: Abingdon Press.

Burt, C. E. (1936). Contributions to the herpetology of Texas I. frogs of the genus *Pseudacris*. *American Midland Naturalist, 17*(4), 770-775.

Cameron, C., Beug, S., & Tsilfidis, C. (2004). Captive breeding of *Notophthalmus viridesnens* through hormonal manipulation. *Herpetological Review, 35*(3), 257.

Cantu, V., Crow, J. C., & Ostrand, K. G. (2016). A comparison of two non-invasive spawning methods to genetically manage captive Barton Springs Salamanders, *Eurycea sosorum*. *Herpetological Review*, *47*(1), 59-63.

Coborn, J. (1992). *The proper care of amphibians*. Neptune City, NJ: T.F.H. Publications, Inc.

Coborn, J. (1993). *Salamanders and newts... as a hobby*. Neptune City, NJ: T.F.H.

Davis, A. K. (2012). Investigating the optimal rearing strategy for *Ambystoma* salamanders using a hematological stress index. *Herpetological Conservation and Biology, 7*(1), 95-100.

Davis, A. K., & Maerz, J. C. (2008). Comparison of hematological stress indicators in recently captured and captive paedomorphic mole salamanders, *Ambystoma talpoideum*. *Copeia,* (3), 613-617.

Davis, R., & and Davies, V. (1997). *The reptile and amphibian problem solver*. Minnesota: Voyageur Press.

DeSantis, D. L., Davis, D. R. Davis, and C. R. Gabor. (2013). Chemically mediated predator avoidance in the Barton Springs Salamander (*Eurycea sosorum*). Herpetologica 69:291-297.

Dierenfild, E., McGraw, K., Fritsche, K., Briggler, J., & Ettling, J. (2009). Nutrient composition composition of whole crayfish consumed by hellbener (*Cryptobranchus alleganiensis*). *Herpetological Review, 40*(3), 324.

Edmonds, D. (2005a). Amphibian Care, American Toad (*Bufo americanus*). Retrieved from http://www.amphibiancare.com/frogs/caresheets/americantoad.html

Edmonds, D. (2005b). Amphibian are, Leopard Frogs (*Rana pipiens* and *R. utricularia*). Retrieved from http://amphibiancare.com/frogs/caresheets/leopardfrog.html

Edmonds, D. (2005c). Amphibian are, Green Tree Frog (*Hyla cinerea*). Retrieved from http://amphibiancare.com/frogs/caresheets/greentreefrog.html.

Edmonds, D. (2005c). Amphibian are, Green Tree Frog (*Hyla cinerea*). Retrieved from http://amphibiancare.com/frogs/caresheets/californianewt.html

Edmonds, D. (2005e). Gray Tree Frogs (*Hyla chrysoscelis and H. versicolor*). Retrieved from http://amphibiancare.com/frogs/caresheets/graytreefrog.html

Edmonds, D. (2005f). California Newt (*Taricha torosa*) and Rough-skinned Newt (*T. granulosa*). Retrieved from http://amphibiancare.com/frogs/caresheets/tigersalamander.html

Edmonds, D. (2007). *Tree frogs: A complete guild to arboreal frogs* Neptune, NJ: TFH Publications.

Edmonds, D. (2009). *Newts and salamanders: A complete guide to caudate.* Neptune, NJ: TFH Publications.

Emmerson, F., & Kay, F. (1971). Agonistic behavior in some captive frogs (*Rana*) from nevada. *Herpetological Review, 3*(2), 39.

Epp, K. J., & Gabor, C. R. (2008). Innate and learned predator recognition mediated by chemical signals in *Eurycea nana*. *Ethology*, *114*(6), 607-615.

Epp, K. J., Gonzales Jr, R., & Gabor, C. R. (2010). The role of water-borne chemical cues in mediating social interactions of the Texas blind salamander, *Eurycea rathbuni*. *Amphibia-Reptilia*, *31*(2), 294-298.

Essner Jr, R. L., & Suffian, D. J. (2010). Herpetological Husbandry, Captive Husbandry in the Rocky Mountain Tailed Frog, *Ascaphus montanus*. *Herpetological Review*, *41*(2), 181.

Ettling, J. A., Wanner, M. D., Schuette, C. D., Armstrong, S. L., Pedigo, A. S., & Briggler, J. T. (2013). Captive reproduction and husbandry of adult Ozark hellbenders, *Cryptobranchus alleganiensis bishopi*. *Herpetological Review*, *44*(4), 605-610.

Fernandez, P. J. (1996). A facility for captive propagation of Chiricahua leopard frogs (*Rana chiricahuensis*). *Advances in Herpetoculture*, (1), 7.

Fernandez, P. J., & Rosen, P. C. (1996). Effects of an introduced crayfish (*Orconectes virilis*) on the stream habitat of the Chiricahua leopard frog (*Rana chiricahuensis*) at Three Forks, White Mountains, Arizona. *Final report to Arizona Game and Fish Heritage Program*.

Flanagan, W. (2002) Taxon Management Account Hellbender Salamander *Cryptobranchus alleganiensis alleganiensis* (Daudin). Retrieved from http://www.caudata.org/cig/taxon_management_account.html

Flower, S. S. (1925). Contributions to our knowledge of the duration of life in vertebrate animals.—II. Batrachians. *Proceedings of the Zoological Society of London, 95*(1) 269-289.

Fries, J. (2002). Upwelling flow velocity preferences of captive adult San Marcos salamanders. *North American Journal of Aquaculture* 64:113-116.

Frost, J. S. (1982). A time-efficient low cost method for the laboratory rearing of frogs. *Herpetological Review, 13*(3), 73.

Gabor, C. R., Zabierek, K. C., Kim, D. S., da Barbiano, L. A., Mondelli, M. J., Bendik, N. F., & Davis, D. R. (2016). A non-invasive water-borne assay of stress hormones in aquatic salamanders. *Copeia*, *104*(1), 172-181.

Gerlach, U. (2008). Husbandry and breeding of *Taricha granulosa* Retrieved from http://www.caudata.org/cc/articles/Tgranulosa.shtml

Glennemeier, K. A., & Denver, R. J. (2002a). Developmental changes in interrenal responsiveness in anuran amphibians. *Integrative and Comparative Biology, 42*(3), 565-573.

Glennemeier, K. A., & Denver, R. J. (2002b). Small changes in whole-body corticosterone content affect larval *Rana pipiens* fitness components. *General and Comparative Endocrinology*, *127*(1), 16-25.

“Green tree frogs” (n.d.) Online. Retrieved from (www.froggie.info/green/index.htm)

Greenberg, S., & and Raskin, E. (1952). *Home-made zoo*. New York: David McKay Company, Inc.

Gregory, P. T. (1979). Predator avoidance behavior of the red-legged frog (*Rana aurora*). *Herpetologica, 32*(2). 175-184.

Grenard, S. (1999). *Amphibians: Their care and keeping*. New York, NY: Howell Book House.

Haislip, N. A., Hoverman, J. T., Miller, D. L., & Gray, M. J. (2012). Natural stressors and disease risk: Does the threat of predation increase amphibian susceptibility to ranavirus? *Canadian Journal of Zoology, 90*(7), 893-902.

Hanifin, C. T., Brodie, E. D., & Brodie, E. D. (2002). Tetrodotoxin levels of the rough-skin newt, *Taricha granulosa*, increase in long-term captivity. *Toxicon, 40*(8), 1149-1153.

Hoppe, D. (1979). Floating containers for rearing tadpoles. *Herpetological Review, 10*(1), 16.

Hoverman, J. T., Gray, M. J., & Miller, D. L. (2010). Anuran susceptibilities to ranaviruses: Role of species identity, exposure route, and a novel virus isolate. *Diseases of Aquatic Organisms, 89*(2), 97-107.

Johnson, C. J., Vance, C. K., Roth, T. L., & Kouba, A. J. (2002). Oviposition and ultrasound monitoring of American toads (*Bufo americanus*) treated with exogenous hormones. In *Proceedings of the American Association of Zoo Veteranarians* (pp. 299-301).

Kennedy, J. P. (1962). Spawning season and experimental hybridization of the Houston Toad, *Bufo houstonensis*. *Herpetologica, 17*(4), 239-245.

Khan, P., & Liversage, R. (1995). Spawning of *Notophthalmus viridescens* and rearing embryos under laboratory conditions. *Herpetological Review, 26*(2), 95.

Kouba, A., Willis, E., Vance, C., Hasenstab, S., Reichling, S., Krebs, J., ... & Germano, J. (2011). Development of assisted reproduction technologies for the endangered Mississippi Gopher Frog (*Rana sevosa*) and sperm transfer for in vitro fertilization. *Reproduction, Fertility and Development*, *24*(1), 170-170.

Kowalski, E. (2001). *Plethodon glutinosus* complex. Retrieved from http://www.caudata.org/cc/species/Plethodon/P_glutinosus.shtml

Kowalski, E. (2002). *Ambystoma opacum*. Retrieved from http://www.caudata.org/cc/species/Ambystoma/A_opacum.shtml

Kowalski, E. (2004). *Pseudobranchus axanthu, pseudobranchus striatus* Retrieved from http://www.caudata.org/cc/species/Pseudobranchus/Pseudobranchus.shtml

Kowalski, E. (2005). *Desmognathus monitcola*. Retrieved from http://www.caudata.org/cc/species/Amphiuma/Amphiuma.shtml

Kowalski, E. (2008). *Siren intermedia*. Retrieved from http://www.caudata.org/cc/species/Siren/S_intermedia.shtml

Kowalski, E. (2011). *Ambystoma mavortium, ambystoma tigrinum*. Retrieved from http://www.caudata.org/cc/species/Ambystoma/A_tigrinum.shtml

Kowalski, E., & Watkins-Colwell, G. (2004). *Amphiuma pholeter, amphiuma mean, amphiuma tridactylum.* Retrieved from http://www.caudata.org/cc/species/Amphiuma/Amphiuma.shtml

Kramer, J. (1973). *Pets and plants in miniature gardens*. Garden City, New York: Doubleday & Company, Inc.

Lane-Petter, W. (Ed.). (1963). *Animals for research, principles of breeding and managment.* New York: Academic Press.

Lembcke, P. (2005). *Necturus maculosus*. Retrieved from http://www.caudata.org/cc/species/Necturus/N_maculosus.shtml

Leutscher, A. (1976). *Keeping reptiles and amphibians*. New York: Charles Scribner's Sons.

Li, H., Vaughan, M. J., & Browne, R. K. (2009). A complex enrichment diet improves growth and health in the endangered Wyoming toad (*Bufo baxteri*). *Zoo Biology, 28*(3), 197-213.

Lovich, K. (2007). Husbandry Manual for *Rana Muscosa*. Final Version. *Amphibian Conservation Resource Manual*, 109.

Macke, J. (2004). Raising newt and salamander eggs. Retrieved from http://www.caudata.org/cc/articles/raising.shtml

Mara, W. P. (1994). *Frogs and toads: Breeding and keeping*. Neptune, NJ: TFH Publications.

Martin, D. L. (1991). Captive husbandry as a technique to conserve a species of special concern, the Yosemite toad. *North California Herpetological Society's Conference on Captive Propagation and Husbandry Conference,* 16-32.

Martof, B. S., & Thompson, E. F. (1958). Reproductive behavior of the chorus frog, *Pseudacris nigrita*. *Behaviour, 13*(3), 243-257.

Mattison, C. (1993). *Keeping and breeding amphibians* Bladford Press.

McCallum, M. L., & Trauth, S. E. (2002). Performance of Wood frog tadpoles (*Rana sylvatica*) on three soybean meal–corn meal rations. *Podarcis*, *3*, 78-85.

McCallum, M. L., & Trauth, S. E. (2007). Physiological trade-offs between immunity and reproduction in the northern cricket frog (*Acris crepitans). Herpetologica 63*(3), 269-274.

McLeod, L. (2017a) All About Leopard Frogs as Pets. Retrieved from https://www.thespruce.com/leopard-frogs-1236814.

McLeod, L. (2017b) American Green Tree Frogs as Pets. Retrieved from http://exoticpets.about.com/cs/frogsandtoads/a/amertreefrogs.htm.

Najvar, P. A., J. N. Fries, and J. T. Baccus. (2007). Fecundity of San Marcos salamanders in captivity. *The Southwestern Naturalist* 52: 145-147

Narayan, E. J., Cockrem, J. F., & Hero, J. (2011). Urinary corticosterone metabolite responses to capture and captivity in the cane toad (*Rhinella marina*). *General and Comparative Endocrinology, 173*(2), 371-377.

Nelson, N. (2001). *Taricha granulosa*. Retrieved from http://www.caudata.org/cc/species/Taricha/T_granulosa.shtml

Nelson, N. (2002). *Plethodon cinereus, Plethodon serratus.* Retrieved from http://www.caudata.org/cc/species/Plethodon/P_cinereus.shtml

Nelson, N. (2003a). *Desmognathus folkertsi, desmognathus marmoratus, desmognathus quadramaculatus*. Retrieved from http://www.caudata.org/cc/species/Desmognathus/Desmognathus_sp.shtml

Nelson, N. (2003b). Two-lined and junaluska salamanders. Retrieved from http://www.caudata.org/cc/species/Eurycea/Eurycea_sp.shtml

New England Herpetological Society, Inc. (2012). *Amphibian care sheets.* Retrieved from http://www.neherp.com/index.php/herptile-care-sheets/8-amphibian-care-sheets

Noble, G. K., & Putnam, P. G. (1931). Observations on the life history of *Ascaphus truei stejneger*. *Copeia, 1931*(3), 97-101.

Obringer, A. R., O'Brien, J. K., Saunders, R. L., Yamamoto, K., Kikuyama, S., & Roth, T. L. (2000). Characterization of the spermiation response, luteinizing hormone release and sperm quality in the American toad (*Bufo americanus*) and the endangered Wyoming toad (*Bufo baxteri*). *Reproduction, Fertility and Development*, *12*(2), 51-58.

Odum, R. A., Society, T. Z., Pramuk, J., Zoo, W. P., & Mendelson III, J. R. (n.a.) Hypovitaminosis A: Are captive-bred insect prey deficient in usable Vitamin A for *Anaxyrus* (Anura: Bufonidae)?. Retrieved from *http://popfrog.org/Documents/VitaminAstudyabstract%20final.pdf*

Padgett-Flohr, G. E. (2008). Pathogenicity of *Batrachochytrium dendrobatidis* in two threatened California amphibians: *Rana draytonii* and *Ambystoma californiense*. *Herpetological Conservation and Biology, 3*(2), 182-191.

Padgett-Flohr, G. E., & Hayes, M. P. (2011). Assessment of the vulnerability of the Oregon spottedfrog (*Rana pretiosa*) to the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*). *Herpetological Conservation and Biology*, *6*(2), 99-106.

Perpinan, D., Trupkiewicz, J. G., Armbrust, A. L., Geiser, D. M., Armstrong, S., Garner, M. M., & Armstrong, D. L. (2010). Dermatitis in captive Wyoming toads (*Bufo baxteri*) associated with *Fusarium* spp. *Journal of Wildlife Diseases, 46*(4), 1185-1195.

Pfaff, S., & Vause, K. (2002). Captive reproduction and growth of the broad striped dwarf siren (*Pseudobranchus s. striatus*). *Herpetological Review, 33*(1), 42.

Polasik, J. S., Murphy, M. A., Abbott, T., & Vincent, K. (2015). Factors limiting early life stage survival and growth during endangered Wyoming toad reintroductions. *The Journal of Wildlife Management,*

Quinn, H. (1980). Captive propagation of endangered Houston toads. *Herpetological Review* 11(4): 109.

Quinn, H. R., & Mengden, G. (1984). Reproduction and growth of *Bufo houstonensis* (Bufonidae). *The Southwestern Naturalist, 29*, 189-195.

Reinking, L. N., Daugherty, C. H., & Daugherty, L. B. (1980). Plasma aldosterone concentrations in wild and captive western spotted frogs (*Rana pretiosa). Comparative Biochemistry and Physiology Part A: Physiology, 65*(4), 517-518.

Roberts, D. T., D. M. Schleser, and T. L. Jordan. (1995). Notes on the captive husbandry and reproduction of the Texas Salamander *Eurycea neotenes* at the Dallas Aquarium. Herpetological Review 26: 23-25.

Rowson, A. D., Obringer, A. R., & Roth, T. L. (2001). Non- invasive treatments of luteinizing hormone-releasing hormone for inducing spermiation in American (*Bufo americanus*) and Gulf Coast (*Bufo valliceps*) toads. *Zoo Biology, 20*(2), 63-74.

Scherff-Norris, K. L., Livo, L. J., Pessier, A., Fetkavich, C., Jones, M., Kombert, M., ... & Spencer, B. (2002). Boreal toad husbandry manual. *Colorado Division of Wildlife*. Retrieved from http://hermes.cde.state.co.us/drupal/islandora/object/co%3A3371/datastream/OBJ/view

Searle, C. L., Gervasi, S. S., Hua, J., Hammond, J. I., Relyea, R. A., Olson, D. H., & Blaustein, A. R. (2011). Differential host susceptibility to *Batrachochytrium dendrobatidis*, an emerging amphibian pathogen. *Conservation Biology, 25*(5), 965-974.

Smyers, S. D., & Rubbo, M. J. (2001). Using a water bath to rear eggs of the marbled salamander, *Ambystoma opacum*. *Herpetological Review, 32*(2), 96.

Sparling, D. W., & Fellers, G. (2007). Comparative toxicity of chlorpyrifos, diazinon, malathion and their oxon derivatives to larval *Rana boylii*. *Environmental Pollution*, *147*(3), 535-539.

Spinner, L. (2005). Spotted salamander care sheet. Retrieved from http://www.reptilesmagazine.com/Care-Sheets/Spotted-Salamander/

Sredl, M. J. (2014). Chiricahua leopard frog (*Lithobates chiricahuensis*). *Sonoran Herpetologist, 27*(3), 61.

Sredl, M.S. and B.L. Healy. (1999). Conservation and Management Zones: Evaluating an approach to conserving Arizona populations of the Chiricahua leopard frog (*Rana chiricahuensis*). Nongame and Endangered Wildlife Program Technical Report 149. Arizona Game and Fish Department, Phoenix,

Staniszewski, M. (1995). *Amphibians in captivity* (1st ed.) TFH Publications.

Staniszewski, M. (2002a). Arboreal salamander (*Aneides*) care sheet. Retrieved from www.amphibian.co.uk/aneides.html

Staniszewski, M. (2002b). Monterey salamander (*Ensatina*) care sheet. Retrieved from http://www.amphibian.co.uk/ensatina.html

Staniszewski, M. (2002c). Slender slamander (*Batrachoseps*) care sheet. Retrieved from http://www.amphibian.co.uk/batrach.html

Stanley, H. (1985). Maintenance, exhibition and breeding of the tailed frog, *Ascaphus truei,* in a zoological park. *Herpetological Review, 16*(2), 48.

Stephenson, B., & Verrell, P. (2003). Courtship and mating of the tailed frog (*Ascaphus truei*). *Journal of Zoology, 259*(01), 15-22.

Stewart, K. A., & Lougheed, S. C. (2013). Testing for intraspecific postzygotic isolation between cryptic lineages of *Pseudacris crucifer*. *Ecology and Evolution, 3*(14), 4621-4630.

Stiles, R. M., Sieggreen, M. J., Johnson, R. A., Pratt, K., Vassallo, M., Andrus, M., . . . Lannoo, M. J. (2016). Captive-rearing state endangered crawfish frogs *Lithobates areolatus* from Indiana, USA. *Conservation Evidence, 13*, 7-11.

Stoops, M. A., Campbell, M. K., & Dechant, C. J. (2014). Successful Captive Breeding of *Necturus beyeri* through manipulation of environmental cues and exogenous hormone administration: a model for endangered *Necturus*. *Herpetological Review*, *45*(2), 251-256.

Taylor, S. K., Williams, E. S., & Mills, K. W. (1999). Mortality of captive Canadian toads from *Basidiobolus ranarum* mycotic dermatitis. *Journal of Wildlife Diseases, 35*(1), 64-69.

Taylor, S. K., Williams, E. S., Thorne, E. T., Mills, K. W., Withers, D. I., & Pier, A. C. (1999b). Causes of mortality of the Wyoming toad. *Journal of Wildlife Diseases, 35*(1), 49-57.

US Fish and Wildlife Service. (2007). Chiricahua leopard frog (*Rana chiricahuensis*) recovery plan. *US Fish and Wildlife Service, Southwest Region,* Albuquerque, NM,

Verrell, P. (1989). Hormonal induction of ovulation and oviposition in the salamander *Desmognathus ochrophaeus* (plethodontidae). *Herpetological Review, 20*(2), 42.

Vogel, Z. (1964). *Reptiles and amphibians: Their care and behavior* (G. Vevers Trans.). New York, NY: Viking Press.

Wei, P. (2004). *Taricha torosa*. Retrieved from http://www.caudata.org/cc/species/Taricha/T_torosa.shtml

Welch, A. M., Semlitsch, R. D., & Gerhardt, H. C. (1998). Call duration as an indicator of genetic quality in male gray tree frogs. *Science 280*(5371), 1928-1930.

Whitehurst, P. H., & Pierce, B. A. (1991). The relationship between allozyme variation and life-history traits of the spotted chorus frog, *Pseudacris clarkii*. *Copeia,* 1032-1039.

Wilbur, H. M. (1982). Competition between tadpoles of *Hyla femoralis* and *Hyla gratiosa* in laboratory experiments.*Ecology,* 278-282.

Williams, J. (2011). *Tiger salamander 101: Care guide for the beginner. Retrieved* from http://www.caudata.org/cc/articles/tiger_sal_101.shtml

Wind, E. (2002). *Northern leopard frog (Rana pipiens) husbandry manual.* Retrieved from http://www.caza.ca/media/Pdf/Conservation/member%20resource%20page/leopard_frog_Husbandry_Manual_.pdf

Plomski, L. (2011*) Inter-institutional evaluation of captive rearing for the Oregon Spotted Frog* (Rana pretiosa). Thesis. Retrieved from:

http://sustainabilityinprisons.org/wp-content/uploads/2011/06/Plomski_MES_Thesis1.pdf

Woods, H. A., Poteet, M. F., Hitchings, P. D., Brain, R. A., & Brooks, B. W. (2010). Conservation physiology of the Plethodontid salamanders *Eurycea nana* and *E. sosorum*: response to declining dissolved oxygen. *Copeia*, *2010*(4), 540-553.

Zimmermann, E. (1995) *Reptiles and amphibians: Care - behavior-reproduction* [Breeding Terrarium Animals] (E. Friese Trans.), 5th ed. Neptune City, NJ: TFH Publications.

CHAPTER III: BEHAVIORAL DOMESTICATION OF CAPTIVE AMPHIBIANS: ARE HUSBANDRY PRACTICES HARMING CONSERVATION EFFORTS?

ABSTRACT

The global amphibian conservation crisis has resulted in dozens of amphibian species relying on newly created captive-breeding programs to prevent their extinction. Although the effects of captivity on animal behavior is well studied in mammals, birds, and fish, domestication processes acting on amphibians have been ignored. In this study, we performed the first direct comparisons of wild and captive-bred, adult amphibians to detect evidence of domestication processes that may impact reintroduction success. We compared frogs in captive populations residing in the US with wild conspecifics in Costa Rica of two species of poison frog (*Oophaga pumilio* and *Dendrobates auratus*). First, their behavioral response to a simulated looming predator was experimentally assessed. Captive-bred *D. auratus* had significantly reduced defensive responses compared to wild conspecifics ($p < 0.05$). However, there was no difference between captive-bred and wild *O. pumilio* in the same test ($p = 0.340$). We hypothesize that habituation to various looming stimulus in captivity, such as a hand dropping food into an enclosure, may have resulted in the observed reduced defensive response in *D. auratus*. Secondly, the tonic immobility (TI) reaction was tested in captive and wild frogs. Amphibians may exhibit TI, wherein an animal loses their righting response, as a defensive reaction to physical manipulation. The length of TI was significantly longer in captive-bred *D. auratus* and *O. pumilio* than their respective wild conspecifics ($p < 0.01$). However, the cause of altered TI in captive frogs is unknown. Our results show the first evidence of behavioral

domestication processes occurring in adult captive-amphibians. Amphibian domestication processes are essentially ignored in current amphibian *ex situ* conservation projects. These results open a neglected field of study with broad conservation and husbandry implications for dozens of amphibian reintroduction programs.

INTRODUCTION

Reintroductions of captive-bred wildlife species is an increasingly popular conservation tool for the restoration of many endangered taxa. However, captive-bred animals reintroduced to the wild may have lower survival or fitness than translocated wild conspecifics (Jule et al. 2008). A variety of domestication processes acting on captive populations may cause captive-bred animals to be maladapted to natural conditions in the field, and it has been suggested as a primary factor for poor reintroduction outcomes with some taxa (e.g., Griffin et al. 2000; McPhee 2004; Frankhams 2008; Araki et al. 2009; Williams & Hoffman 2009, Christie et al. 2012). Domestication pressures on captive wildlife populations likely result from unique experiences in captivity and/or genetic changes from differential selection processes resulting in altered behavior, physiology, and anatomy between captive-bred and wild animals (Frankham 2008; Mason 2010). However, the timing and root causes of these changes in captivity are poorly understood, and domestication research on wildlife is strongly skewed towards the study of large-bodied mammals (Balmford et al. 1996; Zimmerman et al. 2007).

Captive-bred animals may never develop behaviors typically learned through experience in a captive environment (Murray et al. 2004). Alternatively, maladaptive learned behaviors not found in the wild can manifest in captive populations (Mason 2006). For example, a reduction in predator avoidance behaviors in captive-bred animals has been documented across a wide variety of taxa including snails (Turner et al. 2006), polecats (Miller et al. 1990), fish (Berejikain 1995; Johnsson et al. 2001), and others (reviewed by Griffin et al. 2000). Other differences between reintroduced captive-bred and wild animals may include ranging and dispersal behaviors (Bright and Morris 1994), social interactions (McDougall et al. 2006), and stress responses (Mason 2010; Dickens et al. 2010). While these behavioral changes from captivity are well documented in four classes of vertebrates (mammals, avian and non-avian reptiles, and fish, see Mason 2010; Griffin et al. 2000), research on how captivity may impact amphibians has not been thoroughly conducted, despite over 100 amphibian species currently residing in *ex situ* conservation programs resulting from recent, unprecedented amphibian declines (Griffiths & Pavajeu 2008; Harding et al. 2016). Amphibians have the capacity to learn and adapt (reviewed by Suboski [1992] and Burghardt [2013]), thus are likely undergoing behavioral domestication processes.

Captive breeding and reintroductions have become an integral part of the global conservation response to amphibian declines. Some literature suggests that their perceived lack of learned behaviors make them excellent candidate species for successful translocations (Bloxham & Tonge 1995; Seigal & Dodd 2001; Griffiths & Pavajeau 2008), but are supported by little empirical evidence. In amphibians, it is not clear what the potential differences are between captive-bred and wild individuals, and what impacts domestication effects may have on reintroduction programs and recovering populations.

The goal of the current study is to perform experimental tests to determine if different behaviors exist between captive-reared and wild-caught adult amphibians. To accomplish this goal, individuals of two frog species were tested in two behavioral experiments. We recorded reactions to simulated predation events using 1) a looming stimulus, and 2) handling to induce tonic immobility (TI). We tested the hypotheses that there is no difference in behavioral response to a looming stimulus, ease of TI induction, and the time spent in TI between captive-bred and wild frogs. Two poison-frog species that are abundant and accessible in the field in northeastern Costa Rica and in breeding facilities in the United States that use husbandry methods for the pet trade were used herein. We discuss how the results of the study have broad and immediate implications for amphibian *ex situ* conservation efforts. We urge additional research into how breeding facilities can minimize the impact of captivity on amphibian behaviour. We propose ways in which husbandry practices can be improved to reduce the effects of domestication on captive animals.

Looming stimulus: An object that approaches an animal quickly has been shown to elicit a collision-avoidance behavior in most vertebrates wherein the animal tries to move out the way. Collision-avoidance behaviors are antipredator defensive responses that occur as the animal tries to flee from a perceived approaching predator or object (Schiff 1965; Yilmaz and Meister 2013). An experimentally-produced looming visual stimuli has tested the defensive response in several taxa (e.g., Yamamoto et al. 2003 [bullfrog]; Yilmaz & Meister 2013 [mouse]; Temizer et al. 2015 [zebra fish]). In previous studies, the test animal is placed in an arena with a video screen facing the animal. After a pre-determined acclimation period, the image of a dark object on a white background appears on the video screen and grows in size quickly (Yamamoto et al. 2003). The stimulus triggers an avoidance or antipredator response from the animal because the animal perceives the black dot as a quickly approaching object or predator (Ingle and Hoff 1990). The rate at which the dark object grows can be altered to change the speed with which the simulated object approaches the animal. The looming response can be used to simulate a swooping predator such as a bird (Yilmaz & Meister 2013). Retention of avoidance behaviors to a looming object in nature, such as a predatory bird, might be very beneficial to reintroduced animals in the wild which are frequently prey to larger animals—especially for amphibians.

Tonic Immobility: Tonic immobility (TI) (also called thanatosis or death-feigning) is a defense behavior in which the animal becomes immobile to feign death such that a potential predator will lose interest (Gallup 1977; Toledo et al. 2010). Some amphibian species exhibit a TI response when handled by a predator (Brodie et al. 1974). Protocols to assess TI in various amphibian species have been utilized in multiple studies, and TI length has been found to correlate with temperature (e.g., Dodd 1990; Narayan et al. 2013). To assess the tonic immobility reaction in frogs, the animal is flipped onto its back repeatedly until it loses its righting response. Once the animal fails to exhibit a righting response, the amphibian is considered to have entered TI. After induction of TI, the animal may remain motionless on its back for several seconds to several minutes. We measured two aspects of TI: number of flips required to induce TI, and the time spent in TI.

MATERIALS AND METHODS

Study Species – We studied the behavior of two amphibian species: the Strawberry Poison Frog (*Oophaga pumilio*) and the Green and Black Poison Frog (*Dendrobates auratus*). Wild specimens were sampled with visual encounter surveys, and tested at the capture site in the field. We conducted the study on wild frogs at the Estación Biológica La Selva in the Sarapiquí region of Heredia Province, Costa Rica. Captive-bred frogs were tested at two private frog-breeding facilities in Owosso, Michigan, and Minneapolis, Minnesota, USA. All captive-bred frogs were 1–5 years of age and F1 (immediate offspring of wild-caught adults) or F2 (offspring of captive F1 individuals) captive animals. All captive frogs were maintained in vivaria with live plants, soil, and automatic misting systems. Most captive frogs were kept in male-female pairs in approximately 75-liter glass aquariums. Captive-bred animals were fed a diet of pin-head crickets and fruit flies, which were dropped by hand though the enclosure lid. All animals used in our study were handled while the investigator (LJL) wore nitrile gloves, and all equipment was sanitized between trials using Novlasan or Virkon disinfectants using protocol recommended by Phillott et al. (2010).

Looming stimulus experiment – To measure defensive behaviors to a looming predator, we designed and constructed a portable experimental arena that was used in all trials. The experimental arena consisted of a hard-sided, plastic box (approximately 20 L in volume) with white internal walls. A 44-cm diagonal, high-definition tablet computer (HP Slate 17-IO10, model: J4v73AA#ABA) was placed as the "ceiling" or lid of the arena with the backlit screen projecting downward into the arena. The screen was set to 127 ppi pixel density with a refresh rate of 120 Hz. A wide-angle video camera (GoPro Hero 3+ Black, model: CHDHX-302) was embedded onto the outside of the plastic box to record the trial. The experimental setup is portable (weighing less than 3 kilograms) so frogs can be tested near their capture point.

Each frog was placed in the arena and allowed to acclimate for five minutes while the overhead screen projected a white background. After the acclimation period, a black looming circle was projected on the monitor following Yamamoto et al. (2003) and Yilmaz & Meister (2013), while the subject's reactions were recorded by the video camera. The black circle simulated a retinal image of a 35-cm object approaching at a velocity of four m/s moving over a six-meter distance by expanding to full size in 0.85 seconds. The looming object appeared four times in a row with two seconds between each circle expansion. Fifteen seconds after the looming disk stimuli were projected, the frog was removed from the experimental arena, and the animal's weight, snout–vent length, and sex were recorded. Field collected animals were returned to the exact site where they were captured, and captive animals were returned to their enclosure. Response to the looming stimulus was recorded as either: 1. no reaction, 2. flight response, 3. raising response, or 4. shrinking response. A flight response was recorded if the animal jumps at the moment of the appearance looming stimulus (Yamamoto et al. 2003). A shrinking response occurred when the subject lowered the front of its body to press the ventral part of the body against the substrate, thus minimizing its body size (Toledo et al. 2010). A raising response was recorded when the frog lifted its body entirely off the substrate, raising behavior has been previously recorded in *D. auratus* (Blanchette and Saporito 2016).

Tonic immobility experiment - To induce a tonic immobility reaction, frogs were individually placed in a white, plastic, open container to standardize surrounding visual stimuli. Wild frogs were hand-captured and immediately tested. Captive frogs were removed from their enclosure and immediately tested. All frogs were tested in temperatures ranging from 25.5–30° C to minimize any impact temperature may have on length of time spent in TI (Dodd and Brodie 1976; Dabrowska and Manikowski 1982). Once the frog was placed in the plastic container it was flipped onto its dorsal surface by hand. Each subject was held in place by gently placing my index finger on the ventral surface for five seconds. After five seconds, we lifted the restraint and recorded the time it took for each subject to right itself. If the righting occurred within five seconds, the subject was recorded as not having entered TI and was flipped again for a second trial. Tonic immobility length and the associated number of flips to induce TI were recoded for each animal. If a frog remained in TI for over 300 seconds, the trial concluded and the animal was flipped onto its ventral surface.

Statistical analysis. - Data preparation and statistical analysis were performed using Excel 2010 (Microsoft) and R statistical program (R Core Team 2017). To determine if there was a difference in response to the looming stimulus we considered: flight, raising response, minimization response as yes/no variables indicating whether the animals displayed a defensive response or not. A Fisher's exact test was used to test the null hypothesis that the probability of exhibiting a defensive response to a looming stimulus is the same whether the animal is wild or captive-bred. For the TI experiment, the number of flips to induce TI and the time spent in TI (seconds) after TI induction were compared test the null hypothesis that there was no difference between wild and captive bred frogs using Mann-Whitney Tests.

RESULTS

Looming stimulus experiment. - The response to a looming stimulus was tested in N = 23 captive-bred and N = 20 wild *D. auratus* (Table 3.1). Captive-bred (N = 21) and wild (N = 24) *O. pumilio* were tested using the same protocol but postural changes were not observed so we recorded the presence or absence of a flight response for this species. Three of our *O. pumilio* trials were not recorded because individuals were not in view of the camera.

For *D. auratus*, the combined total of shrinking, flight or raising responses per treatment group were grouped together and categorized as a defensive response. The captive-bred *D. auratus* had a much lower rate of displaying defensive responses to the looming stimulus than did their wild counterpart (Fisher's exact test, $P = 0.013$; Table 3.2). However, the same comparison of captive-bred and wild *O. pumilio* revealed no difference (Fisher's exact test, P = 0.340).

Tonic Immobility experiment - The tonic immobility reaction was tested on captive-bred (N = 46) and wild (N = 38) *D. auratus,* and captive-bred (N = 72) and wild (N = 40) *O. pumilio*. In *D. auratus*, the mean TI length was 187.5 sec for captive-bred and 75.3 sec for wild individuals (Figure 3.1). The number of flips required to achieve TI were higher for wild caught frogs than captive bred *D. auratus* (Table 3.3). The mean TI length for *O. pumilio* was 86.7 sec for captive-bred and 47.3 sec for wild individuals, and the mean number of flips to achieve TI was 1.27 for captive-bred and 1.59 wild individuals. Captive-bred and wild *D. auratus* differed in the time they spent in TI (Mann-Whitney Test, P < .01; Table 3.3) and in number of flips required for the animal to enter TI, which were both higher in captive-bred than wild individuals (Mann-Whitney test, P < .05). The TI time for *O. pumilio* differed and captive-bred individuals have longer TI lengths than wild individuals (Mann-Whitney test, P < .01), but the number of flips to achieve TI were not different (Mann-Whitney, P = 0.238, NS).

DISCUSSION

Our results support the contention that unintentional behavioral domestication processes occur in captive amphibian populations, and the observed changes vary between the two species we tested. We expect the domestication phenomenon is probably widespread and begs for additional research. The captive-reared *D. auratus* displayed reduced defensive responses compared to wild conspecifics when exposed to a simulated looming predator. The result supports the hypothesis that captive-bred individuals might be less successful at avoiding a predator if released in the wild than a wild conspecific. Furthermore, the captive-bred and wild *D. auratus* had different responses in the TI immobility trials, wherein the captive-reared animals entered TI more quickly and spent more time in TI than did wild frogs. Captive-bred *O. pumilio* spent more time in TI than wild individuals, but there was no difference flips to enter TI. Furthermore, *O. pumilo* did not exhibit any difference in the looming stimulus trails.

The differences between the two species we studied support the contention that an amphibian's response to captivity is species specific. The two taxa, although part of the same family, are in different clades within the poison frog family (Dendrobatidae) (Grant et al. 2017) The *O. pumilio* in our study is characterized by bright red dorsal colors whereas the *D. auratus* are black with green reticulations thus is likely more cryptic to predators. Although both species are toxic, their defensive chemistry differs considerably (Daly et al. 1987; Saporito et al. 2007), and they likely have different predator avoidance strategies. Interestingly, captive-bred *D. auratus* and *O. pumilio* are not toxic in comparison to wild individuals because their captive diet lacks the chemicals required for the accumulation of alkaloids (Santos et al. 2016). It might be hypothesized that a reduced fleeing response might correlate with higher toxicity, however this is unlikely as the non-toxic captive-bred frogs had reduced fleeing response in our study.

The only individuals that assumed an elevated posture in response to the looming stimulus were two captive-bred *D. auratus*. A raising, postural change that involves elevation from the substrate is a deimatic behavior hypothesized to be a defensive behavior wherein the animal exposes its toxic dorsal surface. The functional significance of the behavior is unclear (Blanchette & Saporito 2016). We are not aware of a raising behavior being documented in *O. pumilio.* Additionally, the lack of crouching behavior in *O. pumilio* in response to a looming predator was surprising. Both species were maintained in captivity using the same or very similar husbandry, of similar age, and the same number of generations in captivity indicating that behavioral changes resulting from captivity will likely vary by species.

We hypothesize that the reduction in defensive responses to a looming predator in *D. auratus* may have resulted from habituation to looming stimuli experienced in captivity. All captive animals in the study were typically fed on alternating days by a person opening the lid of the enclosure and dropping food in by hand. Additionally, the front of all enclosures are transparent, and any approaching person is in full view of the animal. This assumption is supported by other related work. For example, Van Bergeijk (1967) found that adult bullfrogs (*Lithobates catesbeianus*) developed anticipatory feeding behavior in a laboratory colony with the conditioned stimulus being the start of the work day (i.e., people working in the lab). The author found that that anticipatory behavior did not occur on holidays when people were not nearby. Anecdotal evidence indicates that multiple other captive amphibian species come out of hiding displaying anticipatory behavior when husbandry practitioners open the enclosure prior to prey items being released (LJL, unpublished data) further indicating animals anticipate feeding events. Additionally, Yamamoto et al. (2003) found that captive bullfrogs (*L. catesbeianus*) could become habituated to a looming stimulus similar to the one used in this study after repeated exposure and frogs would exhibit reduced tendencies to flee from the stimulus. Alternative to the habituation hypothesis, the number of generations in captivity may have played a role in reduced predator response in the studied species. In a study of captive-reared tadpoles of the Majorcan midwife toad (*Alytes muletensis*) Kraaijeveld-Smit (2006) found a reduction in predator avoidance behavior was correlated with the number of generations in captivity. Although all captive-bred animals in this study were only F1 or F2 captive generations the number of generations in captivity likely plays a role in the intensity of reduced predator avoidance behaviors. The possible mechanisms causing reduced predator avoidance behaviors and anticipatory feeding behavior in captive amphibians are unclear and should be considered to inform current and future amphibian reintroductions. Because of the evidence of reduced predator avoidance behaviors observed in our study, releasing animals in earlier life stages (e.g., eggs or larvae) my help avoid these types of issues. However it is important to note that some species have life histories making this impossible. For example, larvae of *O. pumilio* require parental care, negating the option of releasing animals prior to metamorphosis. This contention further promotes the practice of utilizing species-specific approaches designing amphibian conservation programs as suggested by other authors (e.g., Michaels et al. 2014)

The observed differences in tonic immobility between captive-bred and wild frogs may be caused by handling in captivity and subsequent habituation. However, the captive reared frogs in our study were typically handled twice annually. Visual stimuli experienced by captive-reared frogs prior to testing may have played a role in the altered TI response. Narayan et al. (2013) found that Fijian ground frogs (*Platymantis vitiana*) had an increased TI length after exposure to the sight of a predator and increased stress levels. It could be hypothesized that people walking past or removing an animal from an adjacent vivaria prior to an animal being tested may have elevated their stress levels. Furthermore, the length of tonic immobility has been found to positively correlate with stress level (tested through a hormonal stress marker) in amphibians, indicating a more intense acute stress response or an underlying level of chronic stress in the captive-bred animals (Narayan et al. 2013). If released to the wild, it is unclear if having a more sensitive reaction to handling would positively or negatively impact released animals. How amphibian's lineages may become changed across generations in captivity appears to be a forgotten area of research with immediate conservation implications for dozens of *ex situ* conservation programs.

Conservation implications

Rethinking how we view amphibians in captivity may be necessary to prevent captive breeding programs from failure. Our level of knowledge pertaining to how amphibians may change in captivity is clearly below that of other vertebrate classes. A wide variety of husbandry practices and techniques to retain wild-type behaviors in captive animals used for reintroductions have been experimentally tested in all vertebrate classes except amphibians. For example, the use of predator avoidance training (Moseby et al. 2015), toxic prey avoidance training (O'Donnell et al. 2010), conspecific imprinting (Alt and Beecham 1989), environmental enrichment (Shepherdson 1994; Roberts et al. 2011), and avoiding animal habituation to humans (Valutis and Marzluff 1999) previously explored in other vertebrate groups may benefit amphibian reintroductions. The view that amphibians do not have the cognitive and behavioral capacity to develop maladaptive behaviors in captivity and will never require some form of pre-release training prior to reintroduction is contrary to all current behavioral understanding of amphibians. Texiera and Young (2013) found that captive-bred *L. catesbeianus* could learn to avoid a model of avian predator, showing the potential for amphibians to be trained to avoid predators prior to reintroduction similarly to procedures used for mammals, birds, and fish (Griffin et al. 2000). However, the assumption appears to be a common in current amphibian *ex situ* conservation, and we are not aware of any amphibian reintroduction program implementing these practices.

Why have current amphibian reintroduction programs not documented maladaptive behaviors that could impede the survival and establishment of captive reared amphibians compared to wild conspecifics? Evidence of maladaptive behaviors manifesting from captivity in released animals is likely hard to detect in reintroduced amphibians. Monitoring amphibians post-release is difficult, with low detection rates, and requiring considerable time and effort. Monitoring protocols for reintroductions typically do not involve monitoring behavior, but rather survival, breeding activity, demography or population persistence (e.g., Muths & Drietz 2008). Identifying if reintroduced captive-bred amphibians have reduced survival or an increased rate of predation first requires data on wild populations for comparison. Accurate data for wild populations are typically absent for many amphibian reintroduction programs resulting from few or no wild populations left available for study. Alternatively, existing wild populations are currently declining and may not be useful for comparison as a natural, self-sustaining population. Furthermore, attributing low survival or fitness rates of captive-bred amphibians to altered behaviors may be hard to identify in the field as most post-release monitoring is limited to presence/absence and survival of animals. A more informative approach may consist of releasing captive-bred amphibians that have undergone behavioral conditioning or experienced altered husbandry methods, while simultaneously releasing captive-bred control groups for comparing survival and establishment between release treatments.

Daily husbandry practices such as feeding, cleaning enclosures, filling water bowls, and handling animals could induce habituation to looming stimuli or physical manipulation. Conservation practitioners keeping amphibians in captivity should be aware of this possibility and could implement husbandry methods to minimize the development of maladaptive behaviors. For example, preventive methods to limit looming habitation such as visual barriers or using a tube permanently attached to the side of the enclosure to funnel food into an enclosure would reduce looming stimuli associated with opening the lid of an enclosure to drop food in by hand should be further tested. Establishing methods to then test the retention of biologically relevant behavioral responses may help develop effective husbandry techniques experimentally and evaluate the suitability of individuals prior to release.

The observed behaviors recorded in this study occurred in largely naturalistic enclosures with limited contact with people throughout their life. In contrast, many amphibian programs use unnatural, sparse husbandry enclosures that may lack soil, plants, or hides to simplify husbandry and to produce large numbers of individuals for reintroduction (e.g., Scherff-Norris et al. 2014). Amphibian captive breeding programs that use less natural husbandry methods could hypothetically experience greater behavioral changes than those living in enhanced conditions that mimic natural microhabitats. The impacts of using high-density, non-naturalistic, and sparse enclosures for amphibians appears to be an area fertile for behavioral research. If required, integrating husbandry methods to retain wild-type behaviors in amphibian captive breeding programs is likely to be controversial and monetarily costly. However, the benefits that various husbandry methods may have on a specific amphibian likely are highly species-specific given the enormity of diversity found within class Amphibia. Making assumptions from studies of other species may be unreliable as our study has shown though the difference between two members of the same family: *D. auratus* and *O. pumilio*. Some amphibian species or taxonomic groups may exhibit either fewer or greater number and intensity of maladaptive behaviors and stress in a captive environment compared to some amphibians (Mason 2010). The importance of retaining any given behaviour in captive amphibians will be tied to a species' natural predators, evolutionary history, natural history, and biological relevance of the behaviour.

We have clarified that long-term captivity impacts amphibian behavior compared to wild conspecifics with potentially negative impacts on reintroduction success. Retaining natural behaviors may present unique challenges for current and future amphibian *ex situ* conservation, which is complicated by the incredible diversity within class Amphibia combined with the large number of diverse amphibian species requiring *ex situ* intervention. The alteration of predator defense behaviors we have described in captive amphibians opens a neglected area for further study. A paradigm shift in how amphibians are kept in captivity for reintroductions may be necessary as understanding of amphibian domestication processes increases.

ACKNOWLEDGEMENTS

Funding and support for this research was provided by a National Science Foundation Graduate Research Fellowship to LJL (grant #2013172527). This study was approved by the Florida International University Institutional Animal Care and Use Committee (Protocol numbers IACUC-14-004-CR02 and IACUC-16-070). We would like to thank Chelsea Robinson, Michelle Thompson, Tim Perez, and the staff at La Selva Biological Station for support during field work. Finally, we want to thank Joe Mendelson and Joel Heinen for feedback on early drafts of the manuscript.

LITERATURE CITED

Alt, G. L., & Beecham, J. J. (1984). Reintroduction of orphaned black bear cubs into the wild. *Wildlife Society Bulletin (1973-2006)*, *12*(2), 169-174.

Araki, H., Cooper, B., & Blouin, M. S. (2009). Carry-over effect of captive breeding reduces reproductive fitness of wild-born descendants in the wild. *Biology Letters*, *5*(5), 621-624.

Balmford, A., G. M. Mace, & N. Leader-Williams. (1996). Designing the ark: setting priorities for captive breeding. *Conservation Biology* 10*:*719–727.

Berejikian, B. A. (1995). The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (*Oncorhynchus mykiss*) to avoid a benthic predator. *Canadian Journal of Fisheries and Aquatic Sciences*, *52*(11), 2476-2482.

Blanchette, A. & Saporito, R. (2016). Defensive behaviour exhibited by the green and black poison frog (*Dendrobates auratus*) in response to simulated predation. Herpetological Bulletin 136, (2016) 39.

Bright, P.W. & Morris, P.A. (1994). Animal translocation for conservation: performance of dormice in relation to release methods, origin and season. *Journal of Applied Ecology*, *31, 699–708.*

Brodie Jr, E. D., Johnson, J. A., & Dodd Jr, C. K. (1974). Immobility as a defensive behavior in salamanders. *Herpetologica*, 30(1), 79-85.

Burghardt, G. M. (2013). Environmental enrichment and cognitive complexity in reptiles and amphibians: concepts, review, and implications for captive populations. *Applied Animal Behaviour Science*, *147*(3), 286-298.

Christie, M. R., Marine, M. L., French, R. A., & Blouin, M. S. (2012). Genetic adaptation to captivity can occur in a single generation. *Proceedings of the National Academy of Sciences*, *109*(1), 238-242

Dabrowska, B., & Manikowski, S. (1982). Temperature and immobility reaction in *Rana temporaria*. *Behavioural Processes*, *7*(2), 179-182.

Daly, J. W., Myers, C. W., & Whittaker, N. (1987). Further classification of skin alkaloids from neotropical poison frogs (Dendrobatidae), with a general survey of toxic/noxious substances in the amphibia. *Toxicon*, *25*(10), 1023-1095.

Dickens, M. J., Delehanty, D. J., & Michael Romero, L. (2010). Stress: An inevitable component of animal translocation. *Biological Conservation*, *143*(6), 1329-1341.

Dodd, C. K. (1990). The influence of temperature and body size on duration of immobility in salamanders of the genus *Desmognathus*. *Amphibia-Reptilia*, *11*(4), 401-410.

Dodd Jr, C. K., & Brodie Jr, E. D. (1976). Defensive mechanisms of neotropical salamanders with an experimental analysis of immobility and the effect of temperature on immobility. *Herpetologica*, 32(3), 269-290.

Frankham, R. (2008). Genetic adaptation to captivity in species conservation programs. *Molecular Ecology*, *17*(1), 325-333.

Gallup, G. G. (1977). Tonic immobility: the role of fear and predation. *The Psychological Record*, *27*(1), 41-61.

Grant, T., Rada, M., Anganoy-Criollo, M., Batista, A., Dias, P. H., Jeckel, A. M., ... & Rueda-Almonacid, J. V. (2017). Phylogenetic Systematics of Dart-Poison Frogs and Their Relatives Revisited (Anura: Dendrobatoidea). *South American Journal of Herpetology*, *12*(s1), S1-S90.

Griffin, A. S., Blumstein, D. T., & Evans, C. S. (2000). Training captive-bred or translocated animals to avoid predators. *Conservation Biology*, *14*(5), 1317-1326.

Griffiths, R. A., & L. Pavajeu. (2008). Captive breeding, reintroduction, and the conservation of amphibians; *Conservation Biology* 22(4):852-861.

Harding, G., Griffiths, R. A., & Pavajeau, L. (2016). Developments in amphibian captive breeding and reintroduction programs. *Conservation Biology*, *30*(2), 340-349.

Ingle, D., & Hoff, K. (1990). Visually elicited evasive behavior in frogs. *BioScience*, *40*(4), 284-291.

Johnsson, J. I., Höjesjö, J., & Fleming, I. A. (2001). Behavioural and heart rate responses to predation risk in wild and domesticated Atlantic salmon. *Canadian Journal of Fisheries and Aquatic Sciences*, *58*(4), 788-794.

Jule, K. R., Leaver, L. A., & Lea, S. E. (2008). The effects of captive experience on reintroduction survival in carnivores: a review and analysis. *Biological Conservation*, *141*(2), 355-363

Kraaijeveld-Smit, F. J., Griffiths, R. A., Moore, R. D., & Beebee, T. J. (2006). Captive breeding and the fitness of reintroduced species: A test of the responses to predators in a threatened amphibian. *Journal of Applied Ecology*, *43*(2), 360-365.

Mason, G. (2006). Stereotypic behaviour in captive animals: fundamentals and implications for welfare and beyond in *Stereotypic animal behaviour: fundamentals and applications to welfare* (pp. 325-356).

Mason, G. J. (2010). Species differences in responses to captivity: stress, welfare and the comparative method. *Trends in Ecology & Evolution*, *25*(12), 713-721.

McDougall, P. T., Réale, D., Sol, D., & Reader, S. M. (2006). Wildlife conservation and animal temperament: causes and consequences of evolutionary change for captive, reintroduced, and wild populations. *Animal Conservation*, *9*(1), 39-48.

McPhee, M. E. (2004). Generations in captivity increases behavioral variance: considerations for captive breeding and reintroduction programs. *Biological Conservation*, *115*(11), 71-77.

Michaels, C. J., Gini, B. F., & Preziosi, R. F. (2014). The importance of natural history and species-specific approaches in amphibian ex-situ conservation. *The Herpetological Journal*, *24*(3), 135-145.

Miller, B., Biggins, D., Wemmer, C., Powell, R., Calvo, L., Hanebury, L., & Wharton, T. (1990). Development of survival skills in captive-raised Siberian polecats (*Mustela eversmanni*) II: Predator avoidance. *Journal of Ethology*, *8*(2), 95-104.

Moseby, K., Carthey, A., & Schroeder, T. (2015). The influence of predators and prey naivety on reintroduction success: current and future directions. In Armstrong, D., Hayward, D. & Seddon, P (eds) *Advances in reintroduction biology of Australian and New Zealand fauna*, CSIRO Publishing, Clayton South, Australia.

Murray, D. L., Roth, J. D., & Wirsing, A. J. (2004). Predation risk avoidance by terrestrial amphibians: the role of prey experience and vulnerability to native and exotic predators. *Ethology*, *110*(8), 635-647.

Muths, E., & Dreitz, V. J. (2008). Monitoring programs to assess reintroduction efforts: A critical component in recovery. *Animal Biodiversity and Conservation*, *31*(1), 47.

O'Donnell, S., Webb, J. K., & Shine, R. (2010). Conditioned taste aversion enhances the survival of an endangered predator imperilled by a toxic invader. *Journal of Applied Ecology*, *47*(3), 558-565.

Phillott, A. D., Speare, R., Hines, H. B., Skerratt, L. F., Meyer, E., McDonald, K. R., ... & Berger, L. (2010). Minimising exposure of amphibians to pathogens during field studies. *Diseases of Aquatic Organisms*, *92*(2-3), 175-185.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Online: http://www.R-project.org/.

Roberts, L. J., Taylor, J., & de Leaniz, C. G. (2011). Environmental enrichment reduces maladaptive risk-taking behavior in salmon reared for conservation. *Biological Conservation*, *144*(7), 1972-1979.

Santos J.C., Tarvin R.D., O'Connell L.A. (2016) A review of chemical defense in poison frogs (Dendrobatidae): Ecology, pharmacokinetics, and autoresistance. In: Schulte B., Goodwin T., Ferkin M. (eds) *Chemical Signals in Vertebrates* 13. Springer

Saporito, R. A., Donnelly, M. A., Jain, P., Garraffo, H. M., Spande, T. F., & Daly, J. W. (2007). Spatial and temporal patterns of alkaloid variation in the poison frog *Oophaga pumilio* in Costa Rica and Panama over 30 years. *Toxicon*, *50*(6), 757-778.

Scherff-Norris, K. L., Livo, L. J., Pessier, A., Fetkavich, C., Jones, M., Kombert, M., ... & Spencer, B. (2002). Boreal toad husbandry manual. *Colorado Division of Wildlife*. Online http://cpw.state.co.us/Documents/Research/Aquatic/pdf/FinalHatcheryManual12-24-02.pdf (accessed Nov 10, 2017).

Schiff, W. (1965). Perception of impending collision: A study of visually directed avoidant behavior. *Psychological Monographs: General and Applied*, *79*(11), 1.

Seigel, R. A., & Dodd, C. K. (2002). Translocations of amphibians: proven management method or experimental technique?. *Conservation Biology*, *16*(2), 552-554.

Shepherdson, D. (1994). The role of environmental enrichment in the captive breeding and reintroduction of endangered species. In *Creative Conservation* (pp. 167-177). Springer Netherlands.

Suboski, M. D. (1992). Releaser-induced recognition learning by amphibians and reptiles. *Animal Learning & Behavior*, *20*(1), 63-82.

Teixeira, B., & Young, R. J. (2013). Can captive-bred American bullfrogs learn to avoid a model avian predator? *Acta Ethologica*, *17*(1), 15-22.

Temizer, I., Donovan, J. C., Baier, H., & Semmelhack, J. L. (2015). A visual pathway for looming-evoked escape in larval zebrafish. *Current Biology*, *25*(14), 1823-1834.

Toledo, L. F., Sazima, I., & Haddad, C. F. (2010). Is it all death feigning? Case in anurans. *Journal of Natural History*, *44*(31-32), 1979-1988.

Turner, A. M., Turner, S. E., & Lappi, H. M. (2006). Learning, memory and predator avoidance by freshwater snails: effects of experience on predator recognition and defensive strategy. *Animal Behaviour*, *72*(6), 1443-1450.

Van Bergeijk, W. A. (1967). Anticipatory feeding behaviour in the bullfrog (*Rana catesbeiana*). *Animal Behaviour*, 15(2), 231-238.

Valutis, L. L., & Marzluff, J. M. (1999). The Appropriateness of puppet-rearing birds for reintroduction. *Conservation Biology*, *13*(3), 584-591.

Williams, S. E., & Hoffman, E. A. (2009). Minimizing genetic adaptation in captive breeding programs: a review. *Biological conservation*, *142*(11), 2388-2400.

Yamamoto, K., Nakata, M., & Nakagawa, H. (2003). Input and output characteristics of collision avoidance behavior in the frog *Rana catesbeiana*. *Brain, Behavior and Evolution*, *62*(4), 201-211.

Yilmaz, M., & Meister, M. (2013). Rapid innate defensive responses of mice to looming visual stimuli. *Current Biology*, *23*(20), 2011-2015.

Zimmermann, A., M. Hatchwell, L. Dickie, & C. West. editors. (2007). *Zoos in the 21st Century: Catalysts for Conservation?* Cambridge University Press, New York, New York.

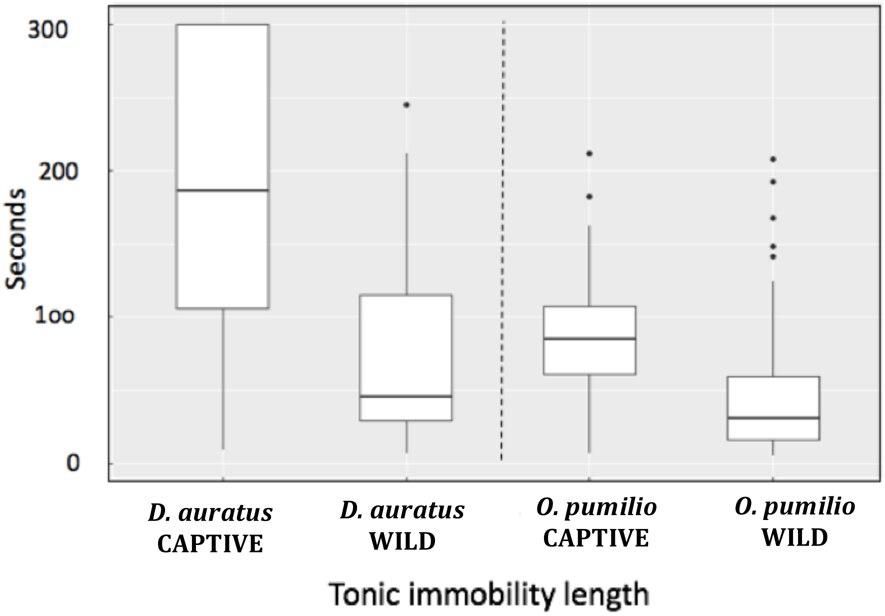

Figure 3.1. A box plot of tonic immobility length in wild and captive-bred treatments of *Dendrobates auratus* and *Oophaga pumilio*.

	Behavioral responses					*Total*
	no response	shrink	flee	raise	no visual	
D. auratus CB	15	2	4	2		23
D. auratus Wild	5	2	13			20
O. pumilio CB	9		11		1	21
O. pumilio Wild	13		7		3	24

Table 3.1. The behavioral responses to the looming stimulus experiment for captive bred (CB) and wild *Dendrobates auratus* and *Oophaga pumilio*.

Species - Group	*No response*	*Defensive response*	*Fisher's exact test*
D. auratus - CB	15	8	p = 0.013
D. auratus - Wild	5	15	
O. pumilio - CB	9	11	p = 0.340
O. pumilio - Wild	12	7	

Table 3.2. The number of combined defensive responses to the looming stimulus for each treatment group of captive-bred (CB) and wild frogs.

		Captive-bred	Wild	P-value
D. auratus	TI mean (sec)	187.5	75.3	$p < .01$
	mean flips to TI	1.09	1.36	$p < .05$
O. pumilio	TI mean (sec)	86.7	47.2	$p < .01$
	mean flips to TI	1.27	1.59	$p = 0.24$

Table 3.3. The mean lengths of tonic immobility and the number of flips to reach TI for both species. Captive-bred and wild individuals were compared with a Mann-Whitney test.

CHAPTER IV: REINTRODUCTIONS OF THE CRITICALLY ENDANGERED WYOMING TOAD: A COMPARISON OF RELEASE STRATEGIES

ABSTRACT

We performed three experimental reintroductions of the critically endangered Wyoming Toad (*Anaxyrus baxteri*). The species has been extinct in the wild since 1993. To study the toad's spatial ecology, behavior, and test the effectiveness of a soft release strategy designed to acclimate toads to the release site, we performed reintroductions at Mortenson Lake National Wildlife Refuge, Wyoming, USA. We tracked reintroduced captive-bred toads (n = 46) and field collected, toads that have been in the wild for at least one year (n = 12) using a harmonic tracking system in 2014 and 2015. We compared the spatial ecology and behavior of soft-released toads, hard-released toads and overwintered toads. Hard-released toads were simply transported to the reintroduction site and released. Soft-released toads were kept in an outdoor enclosure at the release site for 14 days prior to release. The soft release group moved significantly shorter distances, utilized a smaller area, and stayed closer to the release site than hard-released toads. The spatial ecology of soft-released reintroduced toads was more like overwintered toads than like hard-released toads. We suggest that soft-releases are an effective way to improve site fidelity and retain wild-type spatial ecology and behavior of reintroduced captive-bred amphibians.

INTRODUCTION

Wildlife translocations can be used to accomplish a variety of conservation goals, such as reintroducing animals to an area from which the animal has been extirpated, supplementing existing wild populations, or as part of various conservation mitigation strategies (Seddon et al. 2007). In any wildlife translocation, different release protocols may impact translocation success and establishment of animals. A translocation's release methodology typically falls into two categories, hard and soft release. A hard release strategy would mean the animal is not acclimated to the site before release, nor supported in any way following the release. The animal is simply brought to the new location and released. In contrast, for example, a soft-released animal may be maintained in on-site holding pens before release or receives support (e.g., food supplementation) after release, to improve its chance of establishment. A soft release may help acclimate an animal to a novel new environment and reduce stress associated with moving to a new environment (reviewed by Parker et al. 2012). However, soft and hard release options are not dichotomous, but lie along a spectrum of release strategies. Translocation release methods have been studied in a wide variety of taxa such as birds, reptiles, fish, and mammals (e.g., Bright & Morris 1994; Letty et al. 2000; Eastridge & Clark 2001; Clark et al. 2002), however release methods have not been well studied in amphibians.

For amphibians, release options vary widely depending on the life stage of the translocated animal. There are numerous types of soft releases that may be used separately or combined, including environmental enrichment, predator management, post-release disease management and delayed releases. 1) *Environmental enrichment* involves the practice of modifying the release environment to increase likelihood of establishment for translocated animals. For example, modifying the environment to provide breeding sites, shelter, water sources, or food supplementation may constitute environmental enrichment for an amphibian (e.g., Ting 2015). 2) Amphibians may be subjected to intense predation pressure after translocation, and *predator management* can reduce direct mortality of translocated animals. Predator-proof enclosures, removal of predators from the release site, or manipulating the environment to make it more difficult for predators to find or access translocated animals may reduce predation (e.g., Polasik et al. 2015). 3) *Post-release disease management* may be useful for many reintroduced amphibian species moving to an environment with an existing pathogen (e.g., infectious chytrid fungus or ranavirus). Eliminating disease vectors or removing the pathogen from the environment, may mitigate some disease concerns (e.g., Scheele et al. 2014; Bosch et al. 2015). Released individuals may also require monitoring for disease and/or medical treatment in the field post-release to prevent high mortality the animals (e.g., Hardy et al. 2015). 4) Finally, a *delayed release* involves holding the translocated animal in a pen or enclosure at the release site for a period prior to full release. The delayed release tactic may allow the animal to acclimate and get oriented to the release site and recover from stress associated with transport (Parker et al. 2012; Mendelson and Altig 2016). The delayed release may include predator management if predator-proof enclosures are used. Delayed release translocations have been shown to decrease animal stress, increase site fidelity, and increase survival in a wide variety of taxa—e.g., tortoises (Attum et al. 2011), snakes (Stiles 2013), rodents (Bright and Morris 1994), and birds (Maxwell & Jamieson 1997; Mitchell et al. 2011). However, a delayed release's effectiveness has never been studied in translocated adult amphibians. Delayed releases have been proposed for further study to increase success rates of amphibian translocations (Germano & Bishop 2009; Hall & Fleishman 2010). Many amphibian translocation programs typically utilize a *hard release* strategy for both translocation of larvae and adults. The dramatic conservation crisis facing amphibians has resulted in dozens of amphibian species residing in reintroduction programs (Harding et al. 2016), indicating an urgent need for studies of release methodology in amphibians.

1.2. The Wyoming Toad

The Wyoming Toad (*Anaxyrus baxteri*) is a federally-listed, critically endangered species endemic to the Laramie Basin in Wyoming, USA. The Wyoming Toad is brown, grey or yellowish in color with a rugose skin. Adults are approximately 47–60 mm snout-vent length (SVL) (Smith et al. 1998). Larvae are black and may reach 27 mm total length immediately prior to metamorphosis. The species was known to breed in shallow littoral water in ponds and lakes, floodplain ponds, and irrigated hay meadows within the Laramie Basin (Lewis et al. 1985). The toad was historically common throughout the Laramie Basin in the 1950s and 1960s. However, populations declined in the 1970s, and only few individuals were sporadically seen throughout the 1980s (Lewis et al. 1985; Baxter and Stone 1985; Odum and Corn 2005). The last known wild toads were collected and brought into captivity in the early 1990s, and it was declared extinct in the wild (Odum and Corn 2005). The species has since survived in captivity, and the Wyoming Toad is still listed as extinct in the wild by the IUCN Red List (Hammerson 2017). The Wyoming Toad conservation program has largely focused on reintroductions of captive-bred animals since 1992. However, the reestablishment of wild, self-sustaining populations has been unsuccessful even though thousands of animals have been released to the wild over the past 24 years (Hammerson 2004; Dodd 2013, Polasik et al. 2015). The main goals of our study are 1) to test if a delayed-release protocol (hereafter referred to as a soft release) improves release site fidelity and promotes a more natural spatial ecology of reintroduced captive-bred toads; 2) to study field-collected, overwintered toads and explore how their behavior and spatial ecology compares to released captive-bred toads; 3) to improve our understanding of the basic spatial ecology and behavior of the toad. Because of its historical rarity and subsequent declines, little information exists about the toad in the wild. We tested the hypothesis that there is no difference in the spatial ecology, burrowing behavior, substrate preference, and change in mass between soft- and hard-released toads. We tested the null hypotheses that there is that there are no difference between soft- and hard-released toads in the following parameters of movement: total distance moved, maximum displacement distance from starting point, and maximum displacement between any two relocations, mean distance per relocation, and tortuosity. Path tortuosity (i.e., the property of having many turns between the first and last relocation) was calculated as the ratio of the total path distance to the total displacement distance from the first to last relocation. We also tested the null hypothesis that there is no difference between the same movement parameters between soft-released toads and those that have been in the wild for at least one year (overwintered toads). The conservation and management implications of these findings are then discussed.

1.3. Tracking the Wyoming Toad

There are two methods typically used to attach telemetry devices to amphibians: internal implantation and external attachment. Surgically implanted intraperitoneal transmitters have been successfully used on many amphibians, including salamanders (e.g., Madison & Farrand 1998; Faccio 2003) and anurans (e.g., Parker & Anderson 2003; Long et al. 2010). However, surgical implantation is accompanied by inherent risks to the animal, such as visceral herniation (Heemeyer et al. 2012), expulsion of the transmitter and tearing of sutures (Weick et al. 2005). Furthermore, implanting transmitters into small amphibians may add further complications that result from the transmitter's size and weight relative to that of the animal (Kenward 1987). Tracking devices may also be attached to an amphibian externally, and while many of the risks involved with surgical implantation are absent, external tags may become snagged on vegetation, inhibit natural animal movement, make the animal more conspicuous to predators, or they can cause skin abrasions.

Two previous tracking studies focused on the Wyoming Toad (Table 4.1). Twenty toads were tracked in 1998 and 1999 by Parker and Anderson (2003), but the results have received criticism for erroneous habitat analyses for the toad (e.g., Dreitz 2006). In 2011, an additional 13 toads were tracked by Engbrecht et al. (unpublished). Both studies were limited by high mortality and small sample size resulting from the rarity of the toad. Furthermore, implanted intraperitoneal transmitters for radio telemetry typically weighed more than 10% the animal's total body weight (Parker & Anderson 2003). These limitations necessitated a more intensive tracking study testing multiple hypotheses and protocols to improve conservation planning and management for the toad.

In this study, we utilized a harmonic tracking system and externally attached tags. We performed three experimental reintroductions of adult, captive-bred toads to compare the effectiveness of soft- and hard-release methods for reintroduction. To make these comparisons we examined the spatial movement of toads after reintroduction. Ideally, these captive-bred toads could be compared to data on wild toads. However there are no known wild-born adult Wyoming Toads, because evidence of reproduction in the wild has remained elusive since the 1990s. The closest proxy to wild toads available were a small number of free ranging toads likely reintroduced as tadpoles or metamorphs prior to 2014. Overwintered toads had spent at least one winter *in situ*. Thus, overwintered toads were also studied to gain baseline data for subsequent comparisons to captive-bred toads.

MATERIALS AND METHODS

2.1. Study Sites

In the summer of 2014, an external harness for the toads was developed at the Red Buttes Environmental Biology Laboratory (RBEBL) Wyoming Toad breeding facility located in Laramie, Wyoming, USA. Field research was conducted at Mortenson Lake National Wildlife Refuge (MLNWR), Wyoming, USA (41.2098°N 105.8396°W). The MLNWR is 719 hectares in size and at approximately 2211 meters in elevation. The refuge encompasses four small, interconnected lakes. The refuge has a history of decades of cattle (*Bos taurus*) ranching and fishing. Recreational fishing and fish stocking were prohibited in the early 1990s at MLNWR. However, cattle grazing remains permitted on much of the refuge periodically. The intensity, timing, and number of cattle on the refuge has fluctuated over time. Most reintroductions of the toads have taken place at MLNWR, and releases have been ongoing since the early 1990s. Nevertheless, no self-sustaining wild population of the toads has become established.

2.2. Source of animals

We utilized a randomized block design. Each treatment (soft- and hard- release) consisted of equal numbers of males and female toads assigned to treatment groups using a random number table. For the 2014 release (Release A), 24 captive-bred *A. baxteri* were reared to adult size at Cheyenne Mountain Zoo and Como Zoo. In 2015, an additional 22 captive-bred, adult toads were supplied by Cheyenne Mountain Zoo and released as part of this study. The captive-bred adults were 20.0–43.4 g (mean = 27.3 g) in mass. Twelve overwintered, adult toads were field collected at MLNWR in 2015 and tracked concurrently with captive-bred toads (Table 4.2). The overwintered toads were likely from captive-bred stock that was released at the refuge as larva or reared in outdoor enclosures and released as metamorphs at least one year prior to our study (Polasik et al. 2015). We estimate that the largest overwintered adults had likely spent two to three years *in situ* prior to our study because of their large size. Only overwintered adults larger than 24 g were utilized, and an equal ratio of males and females were tracked.

2.3. Tracking protocol

Harmonic tracking is an effective system for tracking wildlife, particularly for very small animals (O'Neal et al. 2004). We used an R8 model harmonic detector (RECCO AB, Lindigo, Sweden). The system employs a harmonic transceiver that transmits a signal that is reflected, at the first harmonic of the original signal, off of a passive diode carried by the animal. The reflected signal is detected in the form of a highly directional audio signal (Pellet et al. 2006). The system we used had a detection range of approximately 2–10 m, depending upon field conditions (e.g., depth of toad underground, topography, or if the toad was submerged. We utilized two types of reflective diodes, including a Schottky diode SD101C (40 volt [VR] 30 milliamp [IF]; Allied Electronics, Fort Worth, Texas, USA) and a surface-mount diode from RECCO AB. The diodes are passive, do not contain a battery, and were attached to a flexible stainless steel, whip antenna (Figure 4.1). Colored glass beads or matte colored nail polish bands were placed on the tracking device to allow identification of the uniquely marked individuals from a distance. To attach the tag, we developed a small harness that fits around the pectoral region of the toad, with two small arm loops. The harmonic tracking device and harness cost approximately \$1.50 per animal tracked. The harmonic tracking device's total package weight (diode, antenna, and harness) had a mass 0.56–0.91 grams.

2.4 Release protocol

On 19 July 2014, we performed a paired experimental reintroduction (Release A) of both hard-released (N = 12) and soft-released toads (N = 12) (Table 4.2). A second experimental reintroduction (Release B) of soft-released toads (N = 12) was performed on 20 June 2015 while simultaneously tracking overwintered toads (N = 12). Finally, a third group (Release C) of soft-released toads (N = 10) was tracked starting 28 August 2015. Release C was originally planned to take place while simultaneously tracking a second group of overwintered toads. However, few overwintered toads could be found at that time, and most of the overwintered toads located were exhibiting symptomatic signs of chytridiomycosis. It would have likely been an additional stressor to have external tracking harness and tags abrading their already irritated, infected skin. Thus, only the captive-bred individuals were tracked for a shortened period in Release C.

2.4.1. Soft release: Releases A, B, and C

The soft-released toads were moved into an outdoor enclosure at the release site 14 days prior to full release, to permit acclimatization to the ambient environment and habitat at the release site. The enclosure was the same design developed for use at MLNWR to head-start toadlets by Polasik et al. (2015). The outdoor enclosure measured approximately 2 x 2 x 1 m (Figure 2). The enclosures consisted of a PVC pipe frame, screen walls, and hinged lid. Inside the enclosure we placed a dog water-bowl and several broken, clay flower pots that could be used as refugia and provided a variety of microhabitats for the toads. The enclosure included a moisture gradient, with one end submerged in water and the other end relatively dry and sandy. The enclosure was located at the edge of a ditch on the southeast side of Mortenson Lake for all three soft releases. The toads in the enclosure were fed insects collected by field-sweeping the upland habitat at MLNWR every other day. Captive-reared toads, in both the soft- and hard-release groups, were fitted with the tracking devices at least 48 hours before full release allowing them to acclimate to the harness and limit the associated stress of handling.

2.4.2 *Hard release: Release A*

In Release A, the soft- and hard-release toads were released simultaneously at the same location. The hard-release treatment group were transported by vehicle from RBEBL to MLNWR in plastic containers in a cooler. Hard-released toads were placed inside the soft release enclosure (Figure 2), and the screen walls were immediately removed from the enclosure. At that point, both soft- and hard- release groups were fully released to the environment and tracking was initiated.

2.4.3. Overwintered toads: Release B

In 2015, overwintered toads were tracked at the same time as the second delayed release group (Table 4.2). Overwintered toads were found though visual encounter surveys around the East and South sides of Mortenson Lake. Toads were hand captured and fitted with a harness and tracking device. Each harness was custom sized and fitted to each toad. The toad's sex, SVL, and mass were recorded prior to release. Processing time from collection to release with the tracking package was approximately 15–20 minutes; toads were released the point of capture.

2.5. Field measurements and statistics

Data preparation and statistical analyses were performed using ARC 10.3 GIS software (Environmental Systems Research Institute). The ArcMET 10.3.1.v1 extension package (Wall 2014) was used for calculating distances moved, path statistics, and minimum convex polygons. Additionally, we utilized the R statistical package (R Core Team 2017) for statistical tests, including Mann-Whitney tests, Chi-square tests, and

Wilcoxan tests. For the one-way analysis of variance (ANOVA) test it was determined that the assumptions for homogeneity of variance were met (Sokal and Rohlf 1981). The Wyoming Toad is one of the world's rarest vertebrates so sample sizes were necessarily small, and results should be interpreted with that in mind.

The location of each toad was recorded using a Garmin eTrex 30x GPS unit (Model 010-01508-10) with a typical accuracy of one to two meters using the averaging location function. An attempt to relocate tagged toads was made every six hours for the first 48 hours after each release (days 1–2) in all releases. In Release A, we attempted to relocate toads twice each day approximately in the morning and near sunset from days 3–30, and approximately once per day from days 31–55. In Release B, we attempted to relocate toads twice daily from days 2–30. Systematic searching to find tracked animals took approximately one to three hours for each sampling bout. Not every animal was relocated in each sampling bout. Weather conditions (e.g., rain storms and lightening) forced some sampling bouts to be cancelled or end early. Detection range of the harmonic tracking device was reduced if the toad was burrowed underground or submerged in water. Some animals occasionally were not relocated for several days when burrowed deep below ground.

Time, ambient temperature and cloud cover was recorded at the start of each tracking session. Once a toad was located using the harmonic receiver, the toad was visually sighted and identified if possible. We recorded a subjective, categorical soil moisture metric at each location. The toad's location as recorded as (1) unsaturated soil, (2) saturated soil identified by water seeping upwards if a finger is pressed to the ground, or (3) the toad is in water or on floating/standing vegetation above water. As a metric to describe detectability during visual encounter surveys, we recorded if the toad could be visually spotted from a standing position without disrupting the toad or moving vegetation (yes or no) after the toad was located with the harmonic tracking device. We also recorded if a toad was burrowed underground (yes or no) upon relocation. Burrowed toads were also recorded as either self-burrowed or utilizing an existing mammal burrow. Toads were handled once a week to inspect animals for abrasions, visually assess physical status, and measure mass. We attempted to limit disturbance of the toads during the study.

Because repeated relocations were taken on the same animals, the relocation data are auto-correlated. Sub-sampling to achieve non-independence was not done because calculation of home range or habitat preference were not the goals of this study. Rather, to see if release method had any impact movement patterns of toads, performed comparisons between paired groups with equally auto-correlated data, as has been done in similar studies (e.g., Bright and Morris 1994). Furthermore, subsampling to remove auto-correlation within a dataset may reduce biological relevancy in some cases (Solla et al. 1999). Following data collection, we calculated the total distance moved, mean path length between each relocation, maximum displacement distance between any two relocations, and displacement from the starting point (i.e., the straight-line distance between the first and last relocation) for each toad. Our intention was to compare release strategies rather than to compare home range estimates. Home range size using the obtained tracking data, over a short period of time, is not biologically relevant to the Wyoming Toad. However, the area of activity was studied between groups using a minimum convex polygon (MCP) home range analysis to explore site fidelity. We calculated the 90% MVP area of relocations on tracking days 7–14 (week 2) to compare activity areas of toads (Worton 1987). Utilizing only relocations obtained from days 7–14 after tracking started was done to minimize the impact of handling and relocation stress, and to maximize the sample size of tracked toads available for comparison.

RESULTS

3.1. Movements

3.1.1 Soft- versus hard- release

We obtained tracking data from 48 captive-bred reintroduced toads and 12 overwintered toads (Table 4.2). A total of 1438 toad relocations were recorded. Toads in Release A moved a mean total path distance of 314.7 meters (Table 4.3). Within Release A, the soft- and hard-released toads had different patterns of movement. Hard-released toads moved more than double the total path distance of soft-released toads, traveling an average of 446.4 m and 183.2 m, respectively (Mann-Whitney Test, $P < 0.05$). The mean distance moved between each relocation of hard-released toads (23.1 m) was greater than soft-released toads (8.8 m) (Mann-Whitney Test, $P < 0.05$). Mean distance moved per each 24-hour period in the soft released group was lower than was found in the hard-released toads as well (Mann-Whitney Test, $P < 0.01$). The grouped mean path distances occurring between days 0–2, 2–4, and 46 also showed large differences between the soft- and hard-release treatments. Hard-released toads in the first two days post release moved 380% farther than did the soft-released toads in the same two-day period (Figure 4.3). Although there was a notable increase in movement immediately after release, throughout the study, hard-released toads generally moved further and more often, relative to soft-released toads. To analyze the toad's movement trends over a 36-day period, we grouped the movement data into four-day periods (Figure 4.4) to reduce the impacts of individual variation, short-term weather variations, and outlier long-distance dispersals on the overall trends of distance travelled between treatments. Over a 36-day period, greater movement by hard-released toads between days 12–16 occurred and was followed by a reduction in distances travelled between days 16–36. Soft-released toads showed greater site fidelity, with respect to the release site, than was observed in the hard-released toads. The mean straight-line, maximum displacement distance from the release site to their final relocation was 138.1 m for hard-released and 52.3 m for soft-released toads (Mann-Whitney Test, $P < 0.05$; Figure 4.5). The mean maximum distance between any two relocations was more than two-fold greater in the hard-released (194 m) than for the soft-released (73.4 m) toads (Mann-Whitney Test, $P < 0.01$). The tortuosity was higher for hard-released toads (11.1) than for the soft-released toads (15.0), but the values were not statistically different (Mann-Whitney Test, $P > 0.05$). Comparisons of the MCP sizes showed that hard-released toads in Release A utilized a larger MCP area over days 7–14 than did the over-wintered toads in Release B (34 m^2sq. m and 82 m^2, respectively; Mann-Whitney test, $P <0.05$).

3.1.1 Overwintered versus soft release

In Release B, the average total distance moved during the 30-day tracking period was greater in the captive-bred individuals (256.3 m) than in the overwintered toads (182.6 m), however the results were not statistically different (Mann-Whitney Test, $P = 0.25$, NS) (Table 4.4). However, some differences between captive-bred and overwintered toads were still apparent. Soft-released toads in release B dispersed farther from their first tracking location than did the overwintered toads, moving 72.0 m and 38.2 m, respectively (Mann-Whitney Test, $P < 0.05$). Additionally, tortuosity was higher for soft-released toads (18.2) than for overwintered toads (5.5).

The data obtained from Release C can be described, but not compared to other releases, because of high mortality, lower tracking time (14 days), and the addition of cattle to the release site. The cattle changed the release site (e.g., grazing of vegetation, compaction of soil) making robust comparisons difficult. Toads in Release C moved a mean total distance of 111.4 m, the mean maximum displacement between any two relocations was 38.2 m, and mean displacement from the starting point was 42.9 m.

3.2. Immediate post-release survival

The toads tracked in our study had lower known mortality rates than previous tracking studies (Table 4.1). During Release A, one toad was found dead from a suspected predation even. Another toad was found alive, but with severe injuries to the dorsum, also attributable to a predation attempt; the toad was euthanized. Mustelids, and large wading birds were frequently seen actively foraging in the canal areas around Mortenson Lake frequented by toads. Four individuals (16.6%) in Release A were lost, and their tracking devices were not recovered.

During tracking of toads in Release B one animal (4% of the trial group) was found dead and desiccated after it dispersed approximately 300 m south of the release point into a dry cattle pasture. Four toads went missing in Release B, and their tracking devices were not recovered. Toads in Release C experienced higher mortality than any other release group, with 50% known mortality during the 14- day tracking period. Four toads died of suspected predation, one was crushed by a cow, and one was lost and its tracking device was not recovered.

3.3. Detectability, behavior and mass

While we could relocate toads with the harmonic tracking, most toads could not be sighted visually by an observer. We found that 64.7% of relocated toads in releases A and B were not observable because they were either burrowed underground or hidden in foliage. Toads were underground in 26.1% of relocations in releases A and B. Toads either utilized existing mammal burrows or self-buried. In Release B, the overwintered toads spent more time underground than soft-released toads (Chi-square = 14.08, $P <$ 0.01), and overwintered toads were less likely to be visually sighted than soft-released toads (Chi-square = 8.96, $P < 0.05$). After relocation with the harmonic tracking system, toads in Release C were visually sighted 18% more often than captive-bred toads in releases A and B (Chi-square = 13.59, $P < 0.01$).

Microsite moisture varied, and 25.5% relocations were on unsaturated soil, 56.3% on saturated soil, and 18% in standing water wherein the toad was typically on emergent vegetation above the water. Typically, these animals were found in the drainage canals running along the sides of the two lakes at MLNWR. No toads were found in the water of Mortenson Lake. However, some toads were relocated in the shallow marsh area in the smaller lake to the East of Mortenson Lake. Soft-released toads in releases A, B, and C did not change in mass while in the soft-release enclosure (Wilcoxon test, $P > 0.05$).

After release, there was no difference in mass between toad's starting weight and when re-measured at 14 days and 21 days (Wilcoxon test, $P > 0.05$).

DISCUSSION

4.1. Release methods

The results of our study support the hypothesis that utilizing a soft-release strategy in amphibian reintroductions increase site fidelity and may result in animals displaying behaviors considered to be typical of wild individuals. Although the rarity of the toad necessitated small sample sizes, our paired-release design controlled for environmental variation and terrain experienced by released toads, which allowed us to make a controlled comparison of movement patterns of different release strategies. We found differences in nearly every spatial metric used to compare the soft- and hard-released toads in Release A. The differences between the treatments add further evidence that the soft-release enclosures affected the post-release spatial ecology of the toads throughout the tracking period. However, it is unclear why soft releasing toads increased site fidelity and reduced movement. Hard-released animals have been suggested to be disoriented and stressed when placed into an entirely new environment (Biggins et al. 1999; Tweed et al. 2003, Sullivan et al. 2004; Letty et al. 2007). The stress associated with translocation has contributed to failures of many reintroductions according to several authors (reviewed by Teixeira et al. 2007). For example, handling and transportation to the release site may induce an acute stress response resulting in altered short-term behavior and increased movement or dispersal tendencies. Narayan et al.

(2012) found that cane toads (*Rhinella marina*) experienced elevated urinary corticosterone levels associated with handling stress for several hours. Although we identified 380% greater movement distances in hard-released toads over the first 48 hours after release, an acute stress response from handling and transportation would not explain why the spatial ecology differed for several weeks between soft and hard released toads (Figure 4.5). However, chronic stress associated with adaptation to a novel environment (such as being released to the wild) likely resulted in the observed long-term altered behavior. Released animals must cope with numerous new stimuli and learn its nearby environment (Baker et al 1998; Sullivan et al. 2004; Teixeira et al. 2013). Acclimation likely takes many days. In a study of *R. marina,* Narayan et al. (2013) found that elevated corticosterone metabolites levels associated with capture and acclimation to various temperatures took 14 days to return to baseline levels. Large, rapid dispersal behaviors undertaken by only hard-released toads in our study highlights the disparity between soft- and hard-released toads (Figure 4.5). Although, amphibian dispersal tendencies can vary by individual and population (e.g., Lindstrom et al. 2013), this is unlikely to explain the observed differences. The aggregate mean paths distance in each four-day period (Figure 4.5) appeared to decrease after Day 16 for hard-released individuals, which is a similar in time to the soft-release acclimation period. We hypothesize that hard-released toad's long-distance movements resulted from the stress of moving across a novel environment, similar to results found in other studies of translocated, hard-released herpetofaunal. Butler et al. (2005) found that translocated tiger snakes (*Notechis scutatus*) had home ranges six times larger than resident snakes. In a study of translocated Gila Monsters (*Heloderma suspectum*), Sullivan et al. (2014) found that translocated individuals had significantly higher mean daily speed than non-translocated individuals. Our results are another example of increased levels of movement and dispersal immediately following hard releases.

The soft-release method clearly influences reintroduced toads, but is the altered spatial ecology induced by the soft release beneficial? Although, the overwintered toads tracked in this study were not truly wild, they offer a useful baseline for the behavior and spatial ecology of Wyoming Toads currently surviving *in situ.* The overwintered toads in Release B showed reduced movement compared to the combined movement data of captive-reared toads. However, distances traveled by overwintered toads was not different from those of soft-released toads in Release B. The primary conclusion of Release B suggests that the spatial ecology of soft-released toads is more like that of overwintered toads than of hard-released toads. Similarity to overwintered toads is likely preferable, in the context of conservation programs, given that the overwintered individuals have survived in a natural setting. The overwinter survival of reintroduced Wyoming Toads has been historically very low. The behavior and activity patterns of the few surviving overwintered toads display their ability to avoid predators, locate suitable habitat, and find food in a difficult environment.

4.2. Survival

Although, we found evidence of predation upon several tracked animals, the sample size was not large enough to do a robust comparison of predation rates. Our results indicate that captive-bred toads may benefit from a soft-release strategy if increased site fidelity and natural behaviors are preferred, however our experiments were not designed to compare long-term survival and fitness of the toads. It might be hypothesized that hard-released animals would have an enhanced risk of predation with increased movement as has been as described for other taxa (e.g., Skelly 1994; Yoder et al. 2004). Site fidelity may be hypothesized to be very important for survival within a patchy habitat. Translocations to suitable habitat closely surrounded by a matrix of poor habitat, such as at MLNWR, may carry increased risk of animals rapidly dispersing into unsuitable habitat immediately following reintroduction. Deleterious post-release dispersal into poor habitat may be a substantial source of mortality (Le Gouar et al. 2012). For example, one hard-released animal in Release B did disperse south of Mortenson Lake into the xeric, upland habitat. The toad was found dead several hundred meters south of appropriate habitat.

Mortality occurring in toads in this study was greatly reduced compared to previous tracking studies of the Wyoming Toad (Table 4.1). The use of the light-weight external tags likely contributed to this result. Our tracking devices were 2.5 5 times lighter than those used in previous studies. We could not attribute any mortalities in our study directly to the tracking devices during tracking. However, two toads experienced skin abrasions resulting from the harness and were subsequently released after removing the tracking harness, their fate is unknown. The reduced detection distance of the harmonic tracking system used in this study may have resulted in toads not being detected. Efforts to detect “missing” toads were extensive, and relocation sweeps of areas far beyond the furthest relocation of a toad from the release site were performed. Some toads would disappear from tracking detection near a large mammal burrow for several days, and then reappear at the surface.

Our results indicate the importance of further research on the long-term impacts of soft-release strategies for amphibians. The potential benefits of such techniques to increasing survival is supported by other studies in a variety of vertebrate groups (e.g., Wanless et al. 2002; Mitchell et al. 2011). However, long-term monitoring is necessary to fully assess a soft releases impact on survival. For example, a robust long-term comparison of soft and hard-released animals might be obtained by marking (e.g., toe-clipping or PIT tagging) many toads split into two release treatments and subsequently recording overwinter survival.

4.3 Behavior and activity

Captive-reared toads displayed a wide range of natural behaviors immediately upon release to the wild (e.g., foraging for insects, burrowing, and utilizing mammal burrows). It is interesting that the captive-bred adults released in this study were reared in sparse enclosures, and they had never experienced a dirt substrate or natural vegetation prior to release. Additionally, captive-bred adults were reared with a limited variety of prey items (e.g., crickets, mealworms, and earthworms). The prey items available to toads after reintroduction were of much greater variety than was their diet in captivity. We observed released toads foraging on insects gathered around cow feces (Linhoff and Donnelly 2016). Following release, we anecdotally surmise that captive-bred toads appeared to have no problem identifying and consuming a naturally wide variety of prey species of various size. It appears that an acclimation or learning time is not necessary for foraging skills to develop in Wyoming Toads as supported by the observed lack of body mass changes after release in captive-bred toads. Thus, captive bred toads were able to maintain themselves in a natural environment. However, it appears there are still differences the behavior and habitat use between captive-reared and overwintered toads

Among the three treatments groups, the amount of time toads spent underground was the lowest in hard-released toads and highest in overwintered toads. At MLNWR, mammal burrows were important microhabitat sites and refugia for all groups of tracked toads. Toads used mammal burrows more often than self-burrowing. The presence of burrowing mammals might be critical for the long-term persistence of Wyoming Toads in some habitats. Overwintered toads were found in areas with thick vegetation, and they utilized a smaller activity area compared to captive-bred toads and often returned to the same mammal burrow or thick patch of vegetation during the day, while moving in more open areas in the evening and night. Overwintered toads were also more cryptic to people, being approximately half as likely to be visually sighted by a standing observer. These results suggest that overwintered toads may be more behaviorally adapted to avoid predators than captive-reared animals.

The high incidence of burrowing behavior and utilization of mammal burrows by Wyoming Toads also has implications for captive husbandry of the species. Toads of many species are often reared in sparse enclosures lacking a dirt substrate or vegetation (e.g., Scherff-Norris et al. 2002). Utilizing dirt and premade burrows may be useful environmental enrichment for conditioning Wyoming Toads prior to release. Rearing amphibians in more natural enclosures, or transitioning animals to more naturalistic enclosures for a period immediately prior to their release, may increase wild-type behaviors post reintroduction. However, these hypotheses remain untested in amphibians. The impact of husbandry and transitioning amphibians to the wild appears to be an area fertile for further study.

4.4 Toad cattle interactions

Prior to Release C the addition of many cattle at MLNWR greatly changed the local environment. The cattle altered the vegetation structure and eliminated many microhabitat sites through grazing activity, thus making data collected during Release C difficult to compare to previous releases. The toads in Release C experienced higher mortality than any other release, including one toad found crushed while burrowed in soft soil in the imprint of a hoof (Linhoff and Donnelly 2016). While the small sample size of Release C precludes a statistical analysis, the reduction in vegetation and microhabitat sites utilized by toads from cattle grazing and trampling appeared to have contributed to higher rates of mortality compared to the other release groups. Toads in Release C were less likely to be found underground than were soft-released toads in releases A and B. We suggest that cattle contribute to soil compaction and closing the entrances of mammal burrows, thus making it more difficult for toads to get underground. The impact of cattle on amphibians has been found to be species dependent and likely correlates with grazing intensity. For example, Burton et al. (2009) found that American toads (*Anaxyrus americanus*) increased with cattle presence, but green frogs (*Lithobates clamitans*) declined. Interestingly, Wyoming Toads were frequently recaptured on water saturated soils similar to some ranid frogs negatively impacted by cattle.

During releases A and B, toads were typically were found in the thickest vegetation, and rarely found in the open. Toads in Release C were visually sighted by researchers more often than were captive-bred soft- or hard-released toads, and we hypothesis that their increased visibility may have resulted from reduced vegetation from grazing and trampling that occurred prior to and during Release C. Predation may be a factor limiting toad survival once vegetation is removed by cattle grazing. Because overwintered toads spent more time underground than did captive-bred toads, overwintered toads may be much better at coping with cattle-induced stressors than are newly released toads, which are possibly undergoing a stressful acclimation period. Cattle preferred the habitat immediately around the lake with the thickest vegetation for grazing, resulting in high spatial and temporal overlap with toads. The increased mortality in Release C may suggest that releasing toads prior to cattle on the refuge or earlier in the season may be preferable, however further study on how cattle impact toad survival is critical for future conservation efforts at MLNWR. The impact of cattle on the Wyoming Toad remains untested.

4.5 Conclusions and implications for future amphibian reintroductions

Our findings show that acclimating non-larval captive-bred toads through a soft-release approach is likely preferable to a hard release. However, the results presented in our study may not be applicable to adults of all amphibian species. The optimum amphibian life history stage (e.g., eggs, larvae, juveniles, or adults) for translocation is dependent on both the goals of the translocation, and the survival rates of each life stage utilized for reintroduction. Amphibians are an incredibly diverse group, and attention to the translocated species' natural history, behavior, and reproductive mode should be considered early in the planning stages of planning an appropriate release strategy.

While our study is the first direct comparison of soft- and hard-release methods for an adult amphibian, it introduces an important area of study in amphibian reintroductions. Releasing larvae may circumvent some behavioral problems associated with reintroductions of captive-bred adults. Implementing soft releases for larvae may be useful as well (e.g., Polasik et al. 2015). However, reintroductions of larvae may also be impossible for some amphibian species, necessitating rearing captive animals to later life stages. For example, numerous groups of amphibian taxa demonstrate parental care making reintroduction of larva problematic (Crump 1996).

Because of the rapid decline of the Wyoming Toad and its extinction in the wild, robust studies on their spatial ecology and behavior are lacking. Our study has provided new insights on one of the world's rarest vertebrates. However, further study of the long-term effectiveness of soft release methods needs urgent assessment for both the Wyoming Toad and other amphibian species.

ACKNOWLEDGMENTS

Funding and support for this project was provided by a National Science Foundation Graduate Research Fellowship to LL (grant #2013172527), and the study was conducted under the IACUC protocol 14-004 approved by the FIU Animal Care and Use Committee. All toads were handled and kept in captivity in compliance with the Wyoming Department of Fish and Wildlife with project approval from the Wyoming Toad Research Committee. Additional funding and facilities were provided by the Wyoming Department of Fish and Wildlife. We thank Jason Palmer, Dawn Moon and Kurt Linnell at RBEBL for providing logistic support, workspace, and daily husbandry needs to all captive toads in 2014. The Como Zoo and Cheyenne Mountain Zoo helped rear the captive-bred adults used for the study. We thank Melanie Murphy, Julie Polasik, Doug Keinath, Elizabeth Mac, Jay Vance, and Jessica Sellers who were all instrumental in providing the necessary logistical support, advice, and assistance with fieldwork to complete this project.

LITERATURE CITED

Attum, O., Otoum, M., Amr, Z., & Tietjen, B. (2011). Movement patterns and habitat use of soft-released translocated spur-thighed tortoises, *Testudo graeca*. *European Journal of Wildlife Research*, *57*, 251-258.

Baker, M. L., Gemmell, E. & Gemmell, R. T. (1998). Physiological changes in brushtail possums, *Trichosurus vulpecula*, transferred from the wild to captivity. *Journal of Experimental Zoology*, 280, 203-212.

Baxter, G. T., & Stone, M. D. (1985). *Amphibians and reptiles of Wyoming*. Wyoming Game and Fish Department, Cheyenne, Wyoming.

Biggins, D.E., Vargas, A., Godbey, J.L., Anderson, S.A., (1999). Influence of prerelease experience on reintroduced black-footed ferrets (*Mustela nigripes*). *Biological Conservation* 89, 121–129.

Bosch, J., Sanchez-Tomé, E., Fernández-Loras, A., Oliver, J. A., Fisher, M. C., & Garner, T. W. (2015). Successful elimination of a lethal wildlife infectious disease in nature. *Biology Letters*, *11*, 20150874.

Bright, P. W. & Morris, P. A. (1994). Animal translocation for conservation: performance of dormice in relation to release methods, origin and season. *Journal of Applied Ecology*, *31, 699–708.*

Burton, E. C., Gray, M. J., Schmutzer, A. C., & Miller, D. L. (2009). Differential responses of postmetamorphic amphibians to cattle grazing in wetlands. *Journal of Wildlife Management*, 73(2), 269-277.

Butler, H., Malone, B., & Clemann, N. (2005). The effects of translocation on the spatial ecology of tiger snakes (*Notechis scutatus*) in a suburban landscape. *Wildlife Research*, *32*(2), 165-171.

Clark, Joseph D., Djuro Huber, and Christopher Servheen. (2002): Bear reintroductions: lessons and challenges: invited paper. *Ursus* 335-345.

Crump, M. L. (1996). Parental care among the amphibia. in *Advances in the Study of Behaviour* 25 pg 109-144. ed.. J. Rosenblatt, C. Snowdon. Academic Press, San Diego, CA

Dawson, J., Patel, F., Griffiths, R. A., & Young, R. P. (2016). Assessing the global zoo response to the amphibian crisis through 20-year trends in captive collections. *Conservation Biology*, *30*, 82-91.

Dodd, C. K. (2013). *Frogs of the United States and Canada, 2-vol. set* (Vol. 1). JHU Press. Baltamore, Maryland, pg. 43-46.

Dreitz, V. J. (2006). Issues in species recovery: an example based on the Wyoming Toad. *BioScience*, *56*, 765-771.2.

Eastridge R. and Clark J. (2001). Evaluation of 2 soft-release techniques to reintroduce black bears. *Wildlife Society Bulletin* Vol 29, No. 4 Winter.

Faccio, S. D. (2003) Postbreeding emigration and habitat use by Jefferson and spotted salamanders in Vermont. *Journal of Herpetology*, 37, 479-489.

Germano, J. M., and P. J. Bishop. (2009). Suitability of amphibians and reptiles for translocation*.* *Conservation Biology* 23,7-15.

Hall, J. A., & Fleishman, E. (2010). Demonstration as a means to translate conservation science into practice. *Conservation Biology*, *24*, 120-127.

Hammerson. G (2004). *Anaxyrus baxteri*. *The IUCN Red List of Threatened Species 2004*: e.T54583A11155140. Available at: http://dx.doi.org/10.2305/IUCN.UK.2004.RLTS.T54583A11155140.en. Downloaded on 02 January 2018.

Harding, G., Griffiths, R. A., & Pavajeau, L. (2016). Developments in amphibian captive breeding and reintroduction programs. *Conservation Biology*, *30*(2), 340-349.

Hardy, B. M., Pope, K. L., Piovia-Scott, J., Brown, R. N., & Foley, J. E. (2015). Itraconazole treatment reduces *Batrachochytrium dendrobatidis* prevalence and increases overwinter field survival in juvenile Cascades frogs. *Diseases of Aquatic Organisms*, *112*(3), 243-250.

Heemeyer, J. L., P. J. Williams, and M. J. Lannoo. (2012). Obligate crayfish burrow use and core habitat requirements of crawfish frogs. *Journal of Wildlife Management,* 76, 1081-1091.

Kenward, R. (1987). *Wildlife Radio Tagging*. Academic Press, Inc., London, UK.

Le Gouar, P., Mihoub, J.-B. & Sarrazin, F. (2011) Dispersal and habitat selection: behavioural and spatial constraints for animal translocations. In *Reintroduction Biology: Integrating Science and Management*, eds J.G. Ewen, D.P. Armstrong, K.A. Parker & P.J. Seddon. Wiley-Blackwell, Oxford, UK.

Letty, J., Marchandeau, S., Aubaneau, J., (2007). Problems encountered by individuals in animal translocations: Lessons from field studies. *EcoScience* 14, 420–431.

Letty, J., Marchandeau, S., Clobert, J. and Aubineau, J. (2000), Improving translocation success: an experimental study of anti-stress treatment and release method for wild rabbits. *Animal Conservation*, 3: 211–219.

Lewis, D. L., Baxter, G. T., Johnson, K. M., & Stone, M. D. (1985). Possible extinction of the Wyoming Toad, *Bufo hemiophrys baxteri*. *Journal of Herpetology*, *19*, 166-168.

Lindström, T., Brown, G. P., Sisson, S. A., Phillips, B. L., & Shine, R. (2013). Rapid shifts in dispersal behavior on an expanding range edge. *Proceedings of the National Academy of Sciences*, *110*(33), 13452-13456.

Linhoff, L. J., and Donnelly, M. A. (2016) *Anaxyrus baxteri* (Wyoming Toad) Interactions with cattle. *Herpetological Review* 47, 103-104.

Long, Z. L., Prepas, E., and Lannoo., M. J. (2010). Assessment of intracoelomic implantation and waistband harness radiotransmitter attachment on wood frogs (*Lithobates sylvaticus*) and boreal toads (*Anaxyrus boreas boreas*). *Herpetol. Rev*., 41(3), 315.

Madison, D. M., and L. Farrand III. (1998). Habitat use during breeding and emigration in radio-implanted tiger salamanders, *Ambystoma tigrinum*. *Copeia,* 402-410.

Maxwell, J. M., & Jamieson, I. G. (1997). Survival and recruitment of captive-reared and wild-reared Takahe in Fiordland, New Zealand. *Conservation Biology*, *11*, 683-691.

Mitchell, A. M., Wellicome, T. I., Brodie, D., & Cheng, K. M. (2011). Captive-reared burrowing owls show higher site-affinity, survival, and reproductive performance when reintroduced using a soft-release. *Biological Conservation*, *144*, 1382-1391.

Narayan, E. J., Cockrem, J. F., & Hero, J. M. (2013). Sight of a predator induces a corticosterone stress response and generates fear in an amphibian. *PLoS One*, *8*, e73564.

Narayan, E. J., Hero, J. M., & Cockrem, J. F. (2012). Inverse urinary corticosterone and testosterone metabolite responses to different durations of restraint in the cane toad (*Rhinella marina*). *General and Comparative Endocrinology*, *179*, 345-349.

Odum R. A., and Corn P. S. (2005). *Bufo baxteri* Porter, 1968, Wyoming Toad. In M. J. Lannoo, ed., *Amphibian Declines: The Conservation Status of United States Species*. pp. 390–392. University of California Press, Berkeley, California.

O'Neal, M. E., Landis, D. A., Rothwell, E., Kempel, L., & Reinhard, D. (2004). Tracking insects with harmonic radar: a case study. *American Entomologist*, *50*, 212-218.

Parker, J. M., & Anderson, S. H. (2003). Habitat use and movements of Wyoming Toads. *The Journal of Wildlife Management*, 67, 439-446.

Parker, K. A., Dickens, M. J., Clarke, R. H. and Lovegrove, T. G. (2012) The Theory and Practice of Catching, Holding, Moving and Releasing Animals, in *Reintroduction Biology: Integrating Science and Management*. eds J. G. Ewen, D. P. Armstrong, K. A. Parker and P. J. Seddon), John Wiley & Sons, Ltd, Chichester, UK.

Pellet, J., L. Rechsteiner, A. K. Skrivervik, J. F. Zürcher, and N. Perrin. (2006). Use of the harmonic direction finder to study the terrestrial habitats of the European tree frog (*Hyla arborea*). *Amphibia Reptilia,* 27(1), 138-142.

Polasik, J. S., Murphy, M. A., Abbott, T., & Vincent, K. (2015). Factors limiting early life stage survival and growth during endangered Wyoming Toad reintroductions. *The Journal of Wildlife Management*, *80*, 540-552.

R Core Team (2017). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Scheele, B. C., Hunter, D. A., Grogan, L. F., Berger, L. E. E., Kolby, J. E., McFadden, M. S., ... & Driscoll, D. A. (2014). Interventions for reducing extinction risk in chytridiomycosis-threatened amphibians. *Conservation Biology*, *28*(5), 1195-1205.

Scherff-Norris, K. L., Livo, L. J., Pessier, A., Fetkavich, C., Jones, M., Kombert, M., ... & Spencer, B. (2002). Boreal toad husbandry manual. *Colorado Division of Wildlife*.

Seddon, P. J., Armstrong, D. P., & Maloney, R. F. (2007). Developing the science of reintroduction biology. *Conservation Biology*, *21*(2), 303-312.

Skelly, D. K. (1994). Activity level and the susceptibility of anuran larvae to predation. *Animal Behaviour*, *47*(2), 465-468.

Smith, H. M., Chiszar, D., Collins, J. T., & van Breukelen, F. (1998). The taxonomic status of the Wyoming Toad, Bufo baxteri Porter. *Contemporary Herpetology*, *22*.

Sokal, R. R., & Rohlf, F. J. (1981). *Biometry* (second edition).

Sullivan, B. K., Kwiatkowski, M. A., & Schuett, G. W. (2004). Translocation of urban Gila monsters: a problematic conservation tool. *Biological Conservation*, *117*(3), 235-242.

Stiles, J. A. (2013). *Evaluating the Use of Enclosures to Reintroduce Eastern Indigo Snakes* (Doctoral dissertation, Auburn University).

Teixeira, C. P., De Azevedo, C. S., Mendl, M., Cipreste, C. F., & Young, R. J. (2007). Revisiting translocation and reintroduction programmes: the importance of considering stress. *Animal Behaviour*, *73*(1), 1-13.

Ting, R. T. H., (2015) Spatial ecology and habitat enrichment of the Cinnamon Bush Frog (*Nyctixalus pictus*). (Undergraduate thesis, National University of Singapore).

Tweed, E. J., Foster, J. T., Woodworth, B. L., Oesterle, P., Kuehler, C., Lieberman, A. A., Powers, A. T., Whitaker, K., Monahan, W. B., Kellerman, J., Telfer, T., (2003). Survival, dispersal, and home-range establishment of reintroduced captive-bred puaiohi, *Myadestes palmeri*. *Biological Conservation,* 111, 1–9.

Wall, J. (2014). Movement Ecology Tools for ArcGIS (ArcMET) v.10.2.2 vX. Available at: www.movementecology.net. Accessed: 6, 15 2016.

Wanless, R. M., Cunningham, J., Hockey, P. A., Wanless, J., White, R. W., & Wiseman, R. (2002). The success of a soft-release reintroduction of the flightless Aldabra rail (*Dryolimnas* [*cuvieri*] *aldabranus*) on Aldabra Atoll, Seychelles. *Biological Conservation*, *107*, 203-210.

Weick, S. E., M. G. Knutson, B. C. Knights, and B. C. Pember. (2005). A comparison of internal and external radio transmitters with northern leopard frogs (*Rana pipiens*). *Herpetological Rev*iew, 36:415-421.

Worton, B. J. (1987) A review of models of home range for animal movement. *Ecological Modelling,* 38:277–298.

Yoder, J. M., Marschall, E. A., & Swanson, D. A. (2004). The cost of dispersal: predation as a function of movement and site familiarity in ruffed grouse. *Behavioral Ecology*, *15*(3), 469-476.

Figure 4.1. An adult, captive-bred *Anaxyrus baxteri* wearing the harness and harmonic tracking device. At the time of the photo, the toad had been tracked for 38 days post release. Three glass beads (white, green, and black) can be seen on the tag that were used to uniquely identify the toad.

Figure 4.2. The delayed release enclosure used at Mortenson Lake National Wildlife Refuge. The same enclosure was used for all delayed releases, and it was kept in the same location for Release A, B, and C.

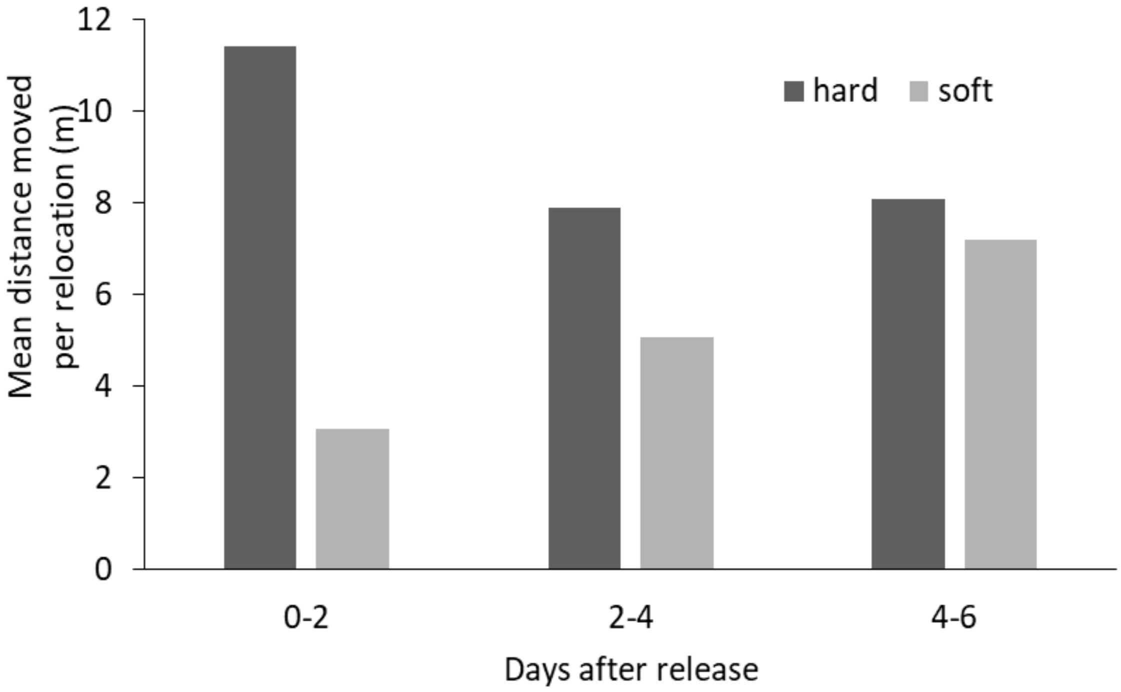

Figure 4.3. The Release A mean path distances during the first six days after reintroduction of soft and hard-released animals. All path distances between relocations were binned together within each two-day sample period.

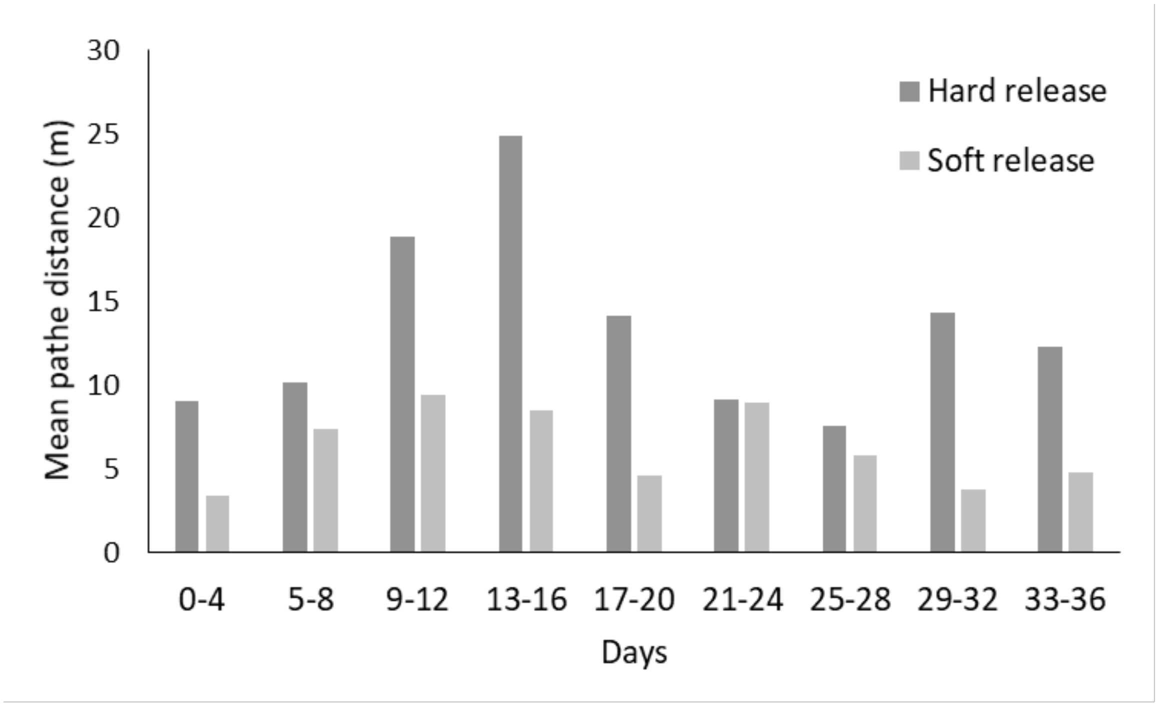

Figure 4.4. The mean path distances within each four-day period for hard and soft-released animals in Release A. All relocation path distances in each period were binned together and averaged.


Hard release


Soft release

Figure 4.5. (Preceding Page) The displaced distances of toads in release A is the straight-line measurement from their first location (i.e., from the soft release enclosure) to their final relocation point. The hard-released toads were placed in the soft release enclosure, and then the walls immediately remove

Release year	Release	Number of toads tracked	Known toad mortalities during tracking	Percentage of known survival	Percentage of tracking device mass to toad's body mass
1998	*Parker & Anderson 2003*	10	9 (90%)	0%	10-15%
1999	*Parker & Anderson 2003*	10	2 (20%)	0%	10-15%
2011	*Engbrecht (unpublished)*	13	7 (54%)	15%	n/a
2014	Release A	24	2 (8%)	66%	2-4%
2015	Release B	24	1 (4%)	79%	2-4%
2015	Release C	10	5 (50%)	40%	2-4%

Table 4.1. A summary of previous tracking efforts of the Wyoming Toad (*Anaxyrus baxteri*). The three releases in 2014 and 2015 are detailed in this study. Known mortalities indicate a toad carcass was found during tracking. Known survival indicates animals that were known to be alive when the animal was no longer tracked. If a toad went missing and the tracking device was not recovered it was not included in this number as the animal's fate was unknown.

Release	Source	Release Type	Number tracked	tracking period (days)	Known Mortality
A	Captive-bred	soft	12	55	1 (8%)
A	Captive-bred	hard	12	55	1 (8%)
B	Captive-bred	soft	12	30	1 (8%)
B	Overwintered	tagged *in situ*	12	30	0 (0%)
C	Captive-bred	soft	10	14	5 (50%)
C	Overwintered	tagged *in situ*	0	0	n/a
Total			58		8

Table 4.2. The source, release type and number of *Anaxyrus baxteri* tracked in each release group for this study. Max tracking period is when remaining animals still being tracked had their harness removed and tracking concluded. Mortality was confirmed only if a whole or partial toad carcass was recovered during tracking. No individuals in the planned Release C group were tracked because few healthy overwintered toads could be found.

Release A Type	Mean total distance (m)	Mean distance moved per relocation (m)	Max displacement (m)	Displacement from starting point (m)	Tortuosity
Soft n = 12	183.1	8.8	73.4	52.3	6.9
Hard n = 12	446.4	23.1	194.6	138.0	15.0
Combined	314.79	16.0	136.6	97.0	11.1

Table 4.3. Distanced moved by all animals in the Release A. Mean total distance is the total path length taken by the toad while being tracked. Max displacement is the maximum distance between any two relocations in the trajectory. Tortuosity is the ratio of the number of movement segments in the trajectory to total displacement in any two points.

Release B Type	Mean total distance	Mean distance moved per relocation	Mean max displacement	Mean displacement from starting point	Tortuosity
Soft n = 12	256.3	13.7	109.4	72.0	18.2
Overwintered n = 12	182.62	5.9	54.3	38.2	5.5
Combined	209.	10.1	83.5	56.13	12.2

Table 4.4. Distanced moved by all animals in Release B. Mean total distance is the total path length taken by the toad while being tracked. Max displacement is the maximum distance between any two relocations in the trajectory. Tortuosity is the ratio of the number of movement segments in the trajectory to total displacement in any two points.

CONCLUSIONS

My dissertation research examined four interconnected aspects of managing wildlife in captivity for conservation. The results I have presented have challenged several fundamental assumptions in amphibian conservation relating to amphibian trade, husbandry, domestication processes, and how captive-bred amphibians are introduced back to the wild. I utilized a combination of economic modeling, systematic literature review, and field experiments to better understand how amphibians in captivity can benefit global conservation goals.

Chapter 1 of my dissertation highlights the importance of monitoring wildlife trade market dynamics. Collection of wild animals for the pet trade or human consumption is a global problem that can rapidly result in overexploitation of wildlife. I compared the traded prices of a 52 species of amphibian over a 28-year period. The resulting average yearly price increase of 29.3% indicates a meteoric increase in demand for live amphibians in the last decades. The dataset is the longest period of time for any analysis of amphibian trade dynamics globally, and it will serve as a useful baseline for continued monitoring of US amphibians sold in the pet trade. My results are particularly concerning given the continued decline of amphibian populations throughout the United States. As some species increase in rarity, their demand is likely to increase resulting from an economic Allee effect, further contributing to their decline. However, amphibians kept as pets may foster greater awareness and interest in preserving local amphibian populations by pet owners. To foster a sustainable pet trade, I urge greater monitoring of market pressures and price dynamics of traded amphibians.

In Chapter 2, I describe how our knowledge of husbandry for captive amphibians is critical for global amphibian conservation. However, my results indicate major knowledge gaps in our ability to care for amphibians in captivity, including numerous species at risk of extinction. Using a combination of taxonomic gap analysis and conservation needs assessments, I evaluated all 289 native amphibian species in the United States for husbandry research prioritizations. My review found that the majority of native amphibians in the United States have no taxon-specific husbandry information of any kind. Chapter 2 demonstrates how a systematic literature review can providing clear guidance for future conservation efforts. Amphibian husbandry is an area largely forgotten in amphibian research, and the resulting dataset will allow conservation practitioners to easily locate and assess husbandry requirements for any species in the dataset.

Chapter 3 describes the first description of unintentional domestication processes occurring in adult captive amphibian populations. The results challenge a paradigm in amphibian *ex situ* conservation programs that domestication processes do not occur for amphibians in captivity. My results show reduced defensive responses to a simulated predator indicating that captive-reared amphibians likely have reduced ability to avoid predators if returned to the wild. I hypothesize that frogs in captivity became habituated to looming stimuli, such as a person performing tank maintenance. Furthermore, captive-bred frog's tonic immobility reaction had increased frequency and length compared to wild frogs. My Chapter 3 results are supported by husbandry practices currently utilized for animals in other taxonomic groups (e.g., mammals, non-reptilian birds, and fish) intended for reintroduction that are designed to reduce habitation to people and retain wild-type behaviors. Implementing novel amphibian husbandry protocols, such as predator avoidance training, may be necessary to increase reintroduction success. Utilizing naturalistic enclosures may also facilitate the development of wild-type behaviors. The results of my study indicate an urgent need for further study of how current amphibian husbandry practices may reduce their ability to survive once returned to the wild.

In chapter 4, I report on reintroductions of the critically-endangered Wyoming Toad (*Anaxyrus baxteri*) at Mortenson Lake National Wildlife Refuge, Wyoming, USA. By performing three reintroductions of captive-bred toads comparing release strategies, I discovered that acclimating toads in an outdoor enclosure (a soft-release treatment) at the release site increased site fidelity and significantly reduced movement compared to toads simply released to the wild (a hard-release treatment). The soft-released toads were also more cryptic than hard-released toads. I compared these data to data for reintroduced, overwintered toads that were either introduced as adults or larvae. Acclimating toads to the release site resulted in behavior and movement patterns more similar to overwintered toads indicating it is likely the best release method. While my study is not a long-term assessment of survival and fitness of reintroduced toads, I expect that soft releasing toads will improve reintroduction success. These results are particularly important because there are dozens of amphibian species currently kept in captivity for planned reintroductions. By showing that amphibians are behaviorally dynamic and care must be taken in choosing a release methods, my study has challenged standard thinking that the behaviors of captive-bred amphibians in reintroductions are hardwired and do not require acclimation procedures that have been found helpful in other taxa groups.

My dissertation highlights the importance of understanding the nexus between animals in the wild and captivity. The increasing market pressures for amphibians in the pet trade described in Chapter 1 highlights the need to increase monitoring of the amphibian trade. Pet amphibians may facilitate greater understanding and appreciation of amphibians, but the pet trade must be sustainable. Improving amphibian husbandry will increase the number of captive-bred animals available in the pet trade and thus reduce collection of wild amphibians and the threat of overexploitation. However, the lack of amphibian husbandry described in Chapter 2, coupled with continuing global amphibian declines indicates urgent husbandry research is needed. In Chapter 3, I describe how amphibian behavior can be altered by their experiences in captivity, which demonstrations how amphibian behavior is malleable and directly related to their husbandry and experiences in captivity. These principles were applied in Chapter 4, wherein I demonstrated how providing a transitionary period to captive animals moving to a novel, wild environment can improve reintroduction success. My dissertation has integrated topics across the spectrum of animals in captivity and the wild, and it is my hope that the results will provide useful recommendations for conservation action.

VITA

LUKE J. LINHOFF

Born, Minneapolis, Minnesota

2004-2008	B.S., Biology University of Hawaii, Hilo Hilo, Hawaii
2009-2011	Henry Doorly Zoo and Aquarium
2016	M.S., Biology Florida International University Miami, Florida
2011-2018	Doctoral Candidate Florida International University Miami, Florida

PUBLICATIONS AND PRESENTATIONS

Harding, G, Linhoff, L.J., Griffiths, R. (2017) Amphibian reintroduction guidelines are coming! *FrogLog*. 25 (1) 118.

Linhoff, L.J. & Donnelly, M.A. (2016) *Anaxyrus baxteri* (Wyoming Toad) Interactions with cattle. *Herp. Rev.* 47 (1), 103-104.

Aaltonen J.T., Bohlender T., Snyder W.,Krebs, J., Linhoff L.; Snoza M.; Plesuk S., Kouba A., Germano J., Reichling S., Loskutoff N.M. (2011) The developmental competency of tadpoles produced in vitro from the endangered Dusky Gopher frog (*Rana capito ervosa*) using exogenous hormone treatment. *Reprod. Fertil. Dev.* 24, 172.

Kouba A.; Germano J., Hasenstab S., Reichling S., Krebs J., Linhoff L., Snoza M., Langhorne C., Willis E., Vance C. (2011) Development of assisted reproduction technologies for the endangered Mississippi Gopher Frog (*Rana sevosa*) and sperm transfer for in vitro fertilization. *Reprod. Fertil. Dev.* 24, 170.

GRANTS AND AWARDS

Frederick H. Stoye Conservation Award. American Society of Ichthyologists and Herpetologists. 2017

Dissertation Year Fellowship. Florida International University. 2017-2018

Graduate Research Fellowship Program. National Science Foundation. 2014-2017

East Asia Pacific Summer Institute Fellow. National Science Foundation. 2014