AN ABSTRACT OF THE DISSERTATION OF

Jenny Cecilia Urbina Gonzalez for the degree of Doctor of Philosophy in Environmental Science presented on August 4, 2017.

Title: Ontogenetic Shifts in Disease Response in Native and Invasive Anuran Species

Abstract approved:

Tiffany Sacra Garcia Andrew R. Blaustein

This dissertation presents a different approach to understanding how amphibians are responding to disease through ontogeny. Although numerous efforts have been conducted to understand host responses to the fungus *Batrachochytrium dendrobatidis* (Bd), studies have been restricted to distinct developmental stages. This dissertation provides information on host response to Bd across life history transitions in native and invasive anuran species. My dissertation is an effort to understand several aspects of the host-pathogen dynamic in the amphibian- chytrid system from embryos to hatchlings, larvae, and juveniles to reproductive adults. I investigate how previous exposure at early life stages (embryos) carries over to impact host response in later life stages. Then, I explore how the virulence of the pathogen varies according to its origin and how this modifies host response. Finally I explore variation across geographic ranges in size at first reproduction, a life history trait that influences invasion potential, in the American bullfrog (*Lithobates catesbeianus*).

Biodiversity loss threatens ecosystems worldwide and several factors, such as habitat transformation, overexploitation, and pollution contribute to this unprecedented crisis. Additional threats include emerging infectious diseases and the introduction of invasive species, both included as central topics of my dissertation research.

I experimentally examined if embryonic exposure of anuran species to the fungus *Batrachochytrium dendrobatidis* (Bd) produces effects within the same stage. I exposed embryos of three anuran species found in the Willamette Valley, Oregon, to different strains of Bd at particular stages of embryonic development. I found that exposure to Bd resulted in direct effects on embryos; I found an increase in mortality after Bd exposure, and this response was conditioned by the host species, timing of exposure and Bd strain. I followed individuals through the hatching life history transition and into the larval stage. I detected both direct and latent effects of Bd exposure on the anuran larvae. Direct effects were observed in individuals exposed only as larvae, while latent effects were detected in individuals exposed only as embryos. Finally, repeated exposure to Bd as embryos and larvae resulted in species-specific mortality (Chapter 2).

Research on variation in host response to pathogens isolated from conspecifics in different distributional ranges is needed to understand how pathogen origin can mediate host response. Chapter 3 explores the susceptibility of wild-caught invasive American bullfrogs to different Bd strains isolated from conspecifics in different distributional ranges. I found larval bullfrogs were susceptible to a novel Bd strain despite it being isolated from conspecifics. The finding of lower infection loads over time suggests bullfrogs are potentially able to clear Bd infection, but this response seems to be strain-specific. In an era of emerging diseases and globalization, understanding the impacts of novel strains provides information about the importance of evolutionary relationships between hosts and pathogens.

In chapter 4, I studied the next anuran life history transition: reproductive adults. I quantified variation in a key life history trait, size at first reproduction, which contributes to reproductive, and thus invasion, success. I used field sampling and laboratory analysis to determine the minimum reproductive size in an invasive anuran species, the American bullfrog, in the Willamette Valley. I found the minimum reproductive size of bullfrogs is similar to the reported values for bullfrogs in other invaded ranges yet smaller than sizes reported from their native range at similar latitudes. The results obtained by this research may be applied to management actions towards controlling and minimizing the impacts of this invasive species over local species of conservation concern.

Chapter 5 summarizes the findings and implications of the studies presented in this dissertation.

Ontogenetic Shifts in Disease Response in Native and Invasive Anuran Species

by
Jenny Cecilia Urbina Gonzalez

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented August 4, 2017
Commencement June 2018

Doctor of Philosophy dissertation of Jenny Cecilia Urbina Gonzalez presented on August 4, 2017

APPROVED:

Co major Professor, representing Environmental Science

Co major Professor, representing Environmental Science

Director of the Environmental Sciences Graduate Program

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Jenny Cecilia Urbina Gonzalez, Author

ACKNOWLEDGEMENTS

First, I would like to thank my advisors, Dr. Tiffany Garcia Sacra and Dr. Andrew Blaustein for giving me the chance of working with them. I feel honored of have been part of their teams while receiving excellent training, I am now not only a better scientist but undoubtedly a better human being. I joined both laboratories as an international student coming with not the best English and they have always been super patient during all these years to understand my accent, weird sentences and impossible manuscripts to read. I will be eternally grateful for their time and all the support Andy and Tiff always and without hesitation provided me through all these years. Thanks for understanding and helped me during the hard times I experienced during my Ph. D, there are no words I can write to express how grateful I am for having you at that time.

Andy “Mi Querido Jefe”, always inspiring, always inviting me to accept challenges and be a better scientist. Andy taught me the value of a well design experiment and always encouraged me to publish and publish and publish without feeling down due to multiple rejections but keep working for a better paper or presentation. Thanks for your edits, you are a magician, it didn’t matter how bad written my versions were, after receiving your feedback every manuscript improved notoriously, sorry for making you suffer with my articles and prepositions, I was trying to make you lose your mind! But I couldn’t. Thanks Dr. Blaustein for all, including your dark comedy style, during this time your jokes were a breeze in stressful moments.

Tiff Thanks! During all this time you have been a great advisor, thanks for your constant guidance and insightfulness, thanks for your assistance, thanks for giving me the opportunity to share with you and your family, your constant encouragement and support bring me all the way to this moment. You are a great mentor, friend and I hope to be able to bring with me to everywhere at least a little bit of your enthusiasm. Thanks for your time, you were always there for me, for us.

I would also like to thank my graduate committee members for their assistance. Anna Jolles provided feedback that improved my methods and for her amazing ideas to be implemented in future experiments. Karen Warkentin, for her expertise in the initial planning of my dissertation. Jerri Bartholomew for accepting being part of my committee and for giving me the opportunity to work with her. Molly Kile, for accepting being my graduate council representative. To all of you, thanks for your support.

I am indebted to all my brilliant labmates, past and current members, in both Blaustein and Garcia lab. In several occasions I have told you, you are my family! Thanks for sharing all this time with me. In the Blaustein lab Lindsay Biga, Julia Buck, Steph Gervasi, Paul Bradley, Tara Chestnut, and Gisselle Xie, thanks for your critiques, assistance and mentorship. Steph Gervasi thanks for being a great mentor and friend. Thanks for giving me the opportunity to work with you and teaching me so many things. Tara “Maria” Chestnut thanks for your friendship, for your insightfulness and for your time. I am so lucky I can say you are my friend. Thanks for the amazing meals and for believe in me. You have been an inspirational lab mate. Paul Bradley thanks for your advice and critiques, Gisselle and Julia thanks for being great office mates. Thanks to the new generation of Blaustenians: Nathalie Hambalek “brujita”, Emily Reynolds, Cassie Nix, Paul Snyder, Carson Lillard, Farallon Broughton for bringing new ideas and research topics to the lab. Thanks to my cohort sister and passenger in the same trip, Trang Dang, for explaining me everything related to molecular techniques, thanks for your friendship and your constant cheering during interminable hours in several summers working in the cold room. Carmen “Carmentea” Harjoe, your patience with me is admirable, thanks for surviving my “disorganized” desk, thanks for being a great mentee, you learned fast. Thanks for answered every question about grammar every five minutes. Thanks for the good times in the office, for being a source of super healthy snacks and sharing those with me. I am grateful for your constant help as mentor especially when I began teaching and I was completely lost.

In the Garcia lab Jennifer Rowe, Stephen Selego, Emily Nebergall, Lindsey Thurman, Evan Bredeweg, Danielle Nelson. Thanks to all of you for helping me to be a better person, a better lab mate and a better researcher. Danielle, Lind and Evan, thanks for all your time, support and love. You are more than my academic sisters and brother. I am so happy we are friends and I am thankful for that. Thanks for your love, for your encouragement, thanks for being there in good and bad times. I have learned so much from you and I deeply admire you and I love you. Evan, thanks for being my friend, for always taking care of me and for helping me so much. There is no way I can return all that to you. Thanks for your support all these years, Thanks for your help and hard work for hours and hours in the field and in the lab, so many things I have to be thankful for. Thanks for your trust, for sharing your time, thanks for your wisdom, your ideas and for your enthusiasm, for understand me, thanks to your family Angie, Kai always helping me too, finally, thanks for your puns what a challenge for the language and the brain of a foreigner but I love being able to laugh when able to understand. Definitely, I could not ask for a better lab community.

I want to thank the collaborators to the Blaustein and Garcia lab. Working with other labs has taught me a lot about teamwork. Thanks to all the undergraduates who helped me during this process, special thanks to Catherine Lu, Justin Conner, Cassidy Medellin, Christopher Cousin working hard and learning, working even weekends and late hours.

I am grateful for the opportunity to have been sharing with people from three different departments, Fisheries and Wildlife, Integrative Biology and Environmental Sciences, amazing students and a great community. Thanks to the economic support provided through teaching assistantship position by the Integrative biology and the Fisheries and Wildlife department. Thanks to Lori Kayes coordinator of the BIO series for her help, energy and ideas to make the series a great opportunity to teach. Thanks for being a great mentor. All this work would not be possible without the help of Tara Bevandich, Traci Durrel-Khalife, Torri Givigliano, Trudy Powel, Jane van Order, and Tresa Bolling in the Integrative Biology Department. Thanks to Ann Leen, Gabrielle Fecteau, Leighann Auer, Amber Ahlgren and Nancy Allen in Fisheries and wildlife and the work of Renee Freeman in the office of Environmental Sciences Graduate program, your hard work is invaluable to keep all these department working. Thanks to all the students that were around during my time, past and present students. Thanks for your presence and for sharing with me. Beginning with my cohort thanks to Hannah Tavalire, Jessie Reimer, Cammie Crowder, Emily Bellis, and Reuben Biel. Just a few names of the wonderful community in Integrative Biology Allie Barner, Vanessa Constant, Allysa Rickborn, Caitlin Magel, Sarah Alto, Silke Bachhuber, Jenna Sullivan, Antonio Gomez, Emily Hartfield, Hailey Ohms, Holland Elder, Claire Couch, Alex Davis, Rhea

Hanselmann, Katherine Dziedzic, Will Fennie, Miram Gleiber, Carolin Gliden, Leah Segui, Jack Koch, Trevor, Lona Thompson, Lisa Wagner, Emily Uhrig, Heather Broughton, and many more. Thanks to great students from the Fisheries and Wildlife department working hard in outreach activities.

Thanks to my friends in other departments Aleja Sanchez, Jose Picado, Iria Gimenez, Dolores Vasquez, a mi ñañita Adriana Argoti. Thanks to the most nerd, brilliant and friendly Colombian gang Javier Tabima, Diego Zarrate, Andrea Jara, Paola Torres, Rafa Leano, Francisco Bolanos, Paulo Murillo Patricia Rincon, Ivania Ceron, Carlitos Moreno, I love you guys, thanks for being such an amazing representation of our country. To the Association of LatinAmerican students (ALAS), past and current members, thanks for being the island to escape from research, being able to dance and have fun. To the International Cultural Service Program (ICSP at OSU) for providing economic support and giving me the opportunity to teach and learn about my country. Thanks to the Fulbright – Colciencias program for all the funding and the logistical support all these years. This experience has been amazing and possible thanks to you. Nicolas F. Soria Zurita thanks for your amazing support and love, you are an incredible friend. Thanks for helping me so much. Thanks for all this great time! Your friendship and love are invaluable, thanks for all your patience during these last months of thesis work. Thanks to all my close friends in all around the world, Los Gatos (Sandrita Galeano, Juanita Correa, Diego Arcila), Victoria Estrada, Guillermo Penagos, Lina Monsalve, Magda Chinchilla, Alejo Colonia, Maya Socha, Sandrita Idarraga, I love you and I miss you and despite the distance I am blessed with having your support via email, WhatsApp or Instagram. Last but not least the most important thank goes to my family.

Thanks for your love, for your care and for understanding. Thanks for your companionship despite the distance. Thanks to my parents for providing me education, for that unconditional love every day everywhere.

CONTRIBUTION OF AUTHORS

Dr. Andrew R. Blaustein and Dr. Tiffany Garcia were my graduate co-advisors in the Environmental Sciences Graduate Program and contributed to all aspects of this research. My co-advisors contributed to the experimental design, implementation, interpretation and editing of all the chapter of this thesis. Dr. Anna Jolles, Karen Warkentin contributed to the experimental design of Chapter 2. Ph. D candidate Evan Bredeweg is a graduate student co-author who contributed to the implementation, analysis of data and interpretation of Chapters 2-4.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

DEDICATION

In memory of my daddy Manuel Francisco Urbina Sierra

To my mom for her unconditional love, friendship and constant support

Ontogenetic Shifts in Disease Response in Native and Invasive Anuran Species

CHAPTER 1 - INTRODUCTION

Diseases play a major role in determining the life history, population dynamics and community structure (Jones et al. 2008). Disease results in complex interactions among hosts, pathogens and the environment (Plowright et al. 2008, Skerratt et al. 2009, Haislip et al. 2011). Therefore, understanding disease dynamics requires study across multiple taxonomic and hierarchical scales ranging from molecular biology to ecosystem level. The integration of ecology, immunology, epidemiology, pathology, invasion biology and conservation biology will enrich our understanding and management of disease dynamics that can have significant impacts on wild organisms. To advance our knowledge of disease, we must integrate these diverse scientific disciplines to design comprehensive and holistic studies (Plowright et al. 2008).

In recent years, the emergence of infectious diseases has caused global concern due to the link with species extinctions, population declines and developmental anomalies (Jones et al. 2008). Biodiversity losses have been documented globally across multiple taxonomic groups (Mooney 2010) and amphibians are recognized as a group of serious conservation concern (Stuart et al. 2004, Wake & Vredenburg 2008, Kilpatrick et al. 2010) with extinction rates estimated to exceed 105 times the baseline for all species (McCallum 2007). Amphibian population declines are often the result of multiple factors and are strongly impacted by habitat loss, the introduction of non-native species, and emerging infectious diseases (Kats & Ferrer 2003, Cushman 2006, Blaustein et al. 2011). Different types of pathogens affect amphibians in complex ways and can cause mortality or sublethal damage (Blaustein & Kiesecker 2002, Blaustein et al. 2012). Bacterial and viral diseases such as red leg syndrome and ranaviruses affect both wildlife and captive amphibian populations (Cunningham et al. 2003, Densmore & Green 2007). Mycotic and mycotic-like organisms are also implicated with amphibian diseases, such as chytridiomycosis, zygomycoses, chromomycoses, saprolegniasis and ichtyophoniasis (Speare et al. 1994, Longcore et al. 1999, Taylor et al. 1999, Kiesecker et al. 2001, Juopperi et al. 2002, Densmore & Green 2007). Protozoan and metazoan parasites cause malformations, such as webbings, supernumerary digits and limbs, or missing limbs (Blaustein & Johnson 2003a, b, Johnson et al. 2003). However, only a fraction of these pathogens are implicated in the decline of multiple populations of amphibians (Daszak et al. 1999, Wake & Vredenburg 2008).

Emerging Infectious Diseases (EIDs) are those reported in new geographical ranges and host species, and whose incidence, pathogenicity and impact have increased recently (Daszak et al. 2003). *Batrachochytrium dendrobatidis* (Bd) is the pathogen that causes chytridiomycosis, an emerging infectious disease of amphibians ((Berger et al. 1998, Daszak et al. 1999, Longcore et al. 1999). The geographical distribution of Bd is extensive: Bd is present on every continent except Antarctica (Fisher, Garner, et al. 2009). Bd has been reported in 516 amphibian species (Olson et al. 2013), and is associated with the extinction and decline of at least 200 species (Skerratt et al. 2007). Considering future scenarios of climate change, model project Bd will spread to new regions threatening additional amphibians populations (Xie et al. 2016). Virulence of Bd is associated with its dynamic genome (Farrer et al. 2017); multiple lineages have been described revealing the complexity of Bd’s evolutionary history and significant diversity in lineage function (Rosenblum et al. 2013). So far, the global panzootic lineage emerging in the 20th century is recognized as producing the most aggressive strains (Farrer et al. 2011). Research on how hosts respond to different strains with different virulence must be done when considering the interaction among the host, Bd, and the environment.

The response of amphibians to Bd vary with species (Blaustein et al. 2005, Garner et al. 2006, Searle et al. 2011, Brannelly et al. 2012, Gervasi et al. 2017) and population (Briggs et al.

2005, Tobler & Schmidt 2010, Phillott et al. 2013, Bradley et al. 2015). Host response is also modified by factors such as environmental conditions (Schlaepfer et al. 2007), Bd strain (Berger et al. 2005, Fisher et al. 2009, Dang et al. 2017), host developmental stage (Garcia et al. 2006, Ortiz-Santaliestra et al. 2013, Bakar et al. 2016, Gervasi et al. 2017) and host immunity (Gervasi et al. 2014, McMahon et al. 2014, Bataille et al. 2015). Hosts can exhibit diverse responses to Bd infection which include reduced survivorship as well as indirect or sub-lethal impacts such as changes in host behavior (Han et al. 2011), activity (Kleinhenz et al. 2012), morphology (Venesky et al. 2010), and timing and size at metamorphosis (Parris & Beaudoin 2004, Parris & Cornelius 2004). These sub-lethal responses can have significant impacts on host fitness and disease dynamics within the ecosystem. As such, it is important to identify species- and stage-specific variables that influence the direct and indirect effects of Bd.

Host ontogeny and life stage are important factors when examining or predicting disease dynamics. Exposure to the pathogen during a particular developmental stage can drastically change host life history trajectories. Exposure of amphibian embryos to pathogens at early stages of development can trigger latent, or carry over development effects over ontogeny (Rohr et al. 2013). For example, exposure of amphibian egg masses to a pathogenic fungus (Order: Dothideales) and water molds (Order: Saprolegniales) resulted in earlier hatching in four different amphibian host species (Warkentin et al. 2001, Gomez- Mestre et al. 2006, Touchon et al. 2006). We currently lack information regarding latent effects of Bd exposure on development through ontogeny, as well as direct Bd impacts on embryos. Interestingly, amphibian embryos were not considered to be susceptible to the chytrid fungus. However, the Bd pathogen can produce enzymes that can destroy tissue, potentially impacting embryos by delaying or triggering key transitions such as hatching time (Rosenblum et al. 2010, McMahon et al. 2013, Fites et al. 2013). This may result in ontogenetic shifts or changes in life history strategies. Direct or latent effects may also vary with Bd strain and with host species, therefore evaluating different strains is critical to disentangle intrinsic aspects of the pathogen as virulence and how it changes among hosts.

The chapters included as part of this thesis are aimed to advance our understanding of the interaction host – pathogen in native and invasive amphibian species across life history transitions. My research is mainly empirically driven, but it also includes field sampling for sample collection and laboratory analysis. In this thesis, I examine the impact of exposure to different Bd strains on native and invasive amphibian species (Chapter 2). I exposed embryos and followed their development across the hatching life history transition (Gosner stage 26), describing patterns of susceptibility for *Pseudacris regilla*, *Anaxyrus boreas* and *Lithobates catesbeianus*. In my next chapter, using wild - caught metamorphs of *L. catesbeianus*, I evaluated susceptibility to different strains of Bd isolated from conspecifics (Chapter 3). This chapter examines different pathogenic strains and their differential effects on an invasive anuran species. In chapter 4, I focused on reproductive potential of *L. catesbeianus* in their invaded range. The results from this chapter may be useful for managers for understanding when an invasive species reaches its minimum reproductive size which can help management to make informed decisions for control. Finally, in the last chapter (5), I summarize my work in regards to future research efforts to better understand the dynamics of an emerging infectious disease (chytridiomycosis) in anuran hosts. This research represents a different approach to understanding how amphibians are responding to disease through ontogeny and provides novel information regarding disease ecology, particularly impacts of emerging infectious diseases in key life history stages.

Literature cited

Bakar AA, Bower DS, Stockwell MP, Clulow S, Clulow J, Mahony MJ (2016) Susceptibility to disease varies with ontogeny and immunocompetence in a threatened amphibian. Oecologia:1–13

Bataille A, Cashins SD, Grogan L, Skerratt LF, Hunter D, McFadden M, Scheele B, Brannelly LA, Macris A, Harlow PS, Bell S, Berger L, Waldman B (2015) Susceptibility of amphibians to chytridiomycosis is associated with MHC class II conformation. Proc R Soc B Biol Sci 282

Berger L, Marantelli G, Skerratt LF, Speare R (2005) Virulence of the amphibian chytrid fungus Batrachochytrium dendrobatidis varies with the strain. Dis Aquat Organ 68:47–50

Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, Hines HB, Lips KR, Marantelli G, Parkes H (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proc Natl Acad Sci 95:9031–9036

Blaustein AR, Gervasi SS, Johnson PTJ, Hoverman JT, Belden LK, Bradley PW, Xie GY (2012) Ecophysiology meets conservation: understanding the role of disease in amphibian population declines. Philos Trans R Soc B Biol Sci 367:1688

Blaustein AR, Han BA, Relyea RA, Johnson PTJ, Buck JC, Gervasi SS, Kats LB (2011) The complexity of amphibian population declines: understanding the role of cofactors in driving amphibian losses. Ann N Y Acad Sci 1223:108–119

Blaustein AR, Johnson P (2003a) Explaining frog deformities. Sci Am:60–65

Blaustein AR, Johnson PT (2003b) The complexity of deformed amphibians. Front Ecol Environ 1:87–94

Blaustein AR, Kiesecker JM (2002) Complexity in conservation: Lessons from the global decline of amphibian populations. Ecol Lett 5:597–608

Blaustein AR, Romansic JM, Scheessele EA, Han BA, Pessier AP, Longcore JE (2005) Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus Batrachochytrium dendrobatidis. Conserv Biol 19:1460–1468

Bradley PW, Gervasi SS, Hua J, Cothran RD, Relyea RA, Olson DH, Blaustein AR (2015) Differences in sensitivity to the fungal pathogen Batrachochytrium dendrobatidis among amphibian populations. Conserv Biol 29:1347–1356

Brannelly LA, Chatfield MWH, Richards-Zawacki CL (2012) Field and Laboratory Studies of the Susceptibility of the Green Treefrog (Hyla cinerea) to Batrachochytrium dendrobatidis Infection. PLOS ONE 7:e38473

Briggs CJ, Vredenburg VT, Knapp RA, Rachowicz LJ (2005) Investigating the population-level effects of chytridiomycosis: an emerging infectious disease of amphibians. Ecology 86:3149–3159

Cunningham A, Danzak P, Rodriguez J (2003) Pathogen Pollution: defining a parasitological threat to biodiversity conservation. J Parasitol 89:78-83

Cushman SA (2006) Effects of habitat loss and fragmentation on amphibians: a review and prospectus. Biol Conserv 128:231–240

Dang T, Searle CL, Blaustein AR (2017) Virulence variation among strains of the emerging infectious fungus Batrachochytrium dendrobatidis (Bd) in multiple amphibian host species. Dis Aquat Organ 124:233–239

Daszak P, Berger L, Cunningham AA, Hyatt AD, Green DE, Speare R (1999) Emerging infectious diseases and amphibian population declines. Emerg Infect Dis 5:735

Daszak P, Cunningham AA, Hyatt AD (2003) Infectious disease and amphibian population declines. Divers Distrib 9:141–150

Densmore CL, Green DE (2007) Diseases of amphibians. ILAR J 48:235–254

Farrer RA, Martel A, Verbrugghe E, Abouelleil A, Ducatelle R, Longcore JE, James TY, Pasmans F, Fisher MC, Cuomo CA (2017) Genomic innovations linked to infection strategies across emerging pathogenic chytrid fungi. 8:14742

Farrer RA, Weinert LA, Bielby J, Garner TWJ, Balloux F, Clare F, Bosch J, Cunningham AA, Weldon C, Preez LH du, Anderson L, Pond SLK, Shahar-Golan R, Henk DA, Fisher MC (2011) Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. Proc Natl Acad Sci 108:18732–18736

Fisher MC, Bosch J, Yin Z, Stead DA, Walker J, Selway L, Brown AJP, Walker LA, Gow NAR, Stajich JE, Garner TWJ (2009) Proteomic and phenotypic profiling of the amphibian pathogen Batrachochytrium dendrobatidis shows that genotype is linked to virulence. Mol Ecol 18:415–429

Fisher MC, Garner TW, Walker SF (2009) Global emergence of Batrachochytrium dendrobatidis and amphibian chytridiomycosis in space, time, and host. Annu Rev Microbiol 63:291–310

Fites JS, Ramsey JP, Holden WM, Collier SP, Sutherland DM, Reinert LK, Gayek AS, Dermody TS, Aune TM, Oswald-Richter K, Rollins-Smith LA (2013) The Invasive Chytrid Fungus of Amphibians Paralyzes Lymphocyte Responses. Science 342:366

Garcia TS, Romansic JM, Blaustein AR (2006) Survival of three species of anuran metamorphs exposed to UV-B radiation and the pathogenic fungus Batrachochytrium dendrobatidis. Dis Aquat Organ 72:163–169

Garner TW., Perkins MW, Govindarajulu P, Seglie D, Walker S, Cunningham AA, Fisher MC (2006) The emerging amphibian pathogen Batrachochytrium dendrobatidis globally infects introduced populations of the North American bullfrog, Rana catesbeiana. Biol Lett 2:455–459

Gervasi SS, Hunt EG, Lowry M, Blaustein AR (2014) Temporal patterns in immunity, infection load and disease susceptibility: understanding the drivers of host responses in the amphibian-chytrid fungus system. Funct Ecol 28:569–578

Gervasi SS, Stephens PR, Hua J, Searle CL, Xie GY, Urbina J, Olson DH, Bancroft BA, Weis V, Hammond JI, Relyea RA, Blaustein AR (2017) Linking Ecology and Epidemiology to Understand Predictors of Multi-Host Responses to an Emerging Pathogen, the Amphibian Chytrid Fungus. PLOS ONE 12:e0167882

Gomez- Mestre I, Touchon JC, Warkentin KM (2006) Amphibian embryo and parental defenses and a larval predator reduce egg mortality from water mold. Ecology:2570–2581

Haislip NA, Gray MJ, Hoverman JT, Miller DL (2011) Development and disease: How susceptibility to an emerging pathogen changes through anuran development. PLoS ONE 6:e22307

Han BA, Searle CL, Blaustein AR (2011) Effects of an Infectious Fungus, Batrachochytrium dendrobatidis, on Amphibian Predator-Prey Interactions. PLOS ONE 6:e16675

Johnson PTJ, Lunde KB, Zelmer DA, Werner JK (2003) Limb deformities as an emerging parasitic disease in amphibians: Evidence from museum specimens and resurvey data. Conserv Biol 17:1724–1737

Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P (2008) Global trends in emerging infectious diseases. Nature 451:990–993

Juopperi T, Karli K, De Voe R, Grindem CB (2002) Granulomatous dermatitis in a spadefoot toad (Scaphiopus holbrooki). Vet Clin Pathol 31:137–139

Kats LB, Ferrer RP (2003) Alien predators and amphibian declines: review of two decades of science and the transition to conservation. Divers Distrib 9:99–110

Kiesecker JM, Blaustein AR, Miller CL (2001) Transfer of a pathogen from fish to amphibians. Conserv Biol 15:1064–1070

Kilpatrick AM, Briggs CJ, Daszak P (2010) The ecology and impact of chytridiomycosis: an emerging disease of amphibians. Trends Ecol Evol 25:109–118

Kleinhenz P, Boone MD, Fellers G (2012) Effects of the Amphibian Chytrid Fungus and Four Insecticides on Pacific Treefrogs (Pseudacris regilla). J Herpetol 46:625–631

Longcore JE, Pessier AP, Nichols and DK (1999) Batrachochytrium dendrobatidis gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 91:219–227

McCallum ML (2007) Amphibian decline or extinction? Current declines dwarf background extinction rate. J Herpetol 41:483–491

McMahon TA, Brannelly LA, Chatfield MWH, Johnson PTJ, Joseph MB, McKenzie VJ, Richards-Zawacki CL, Venesky MD, Rohr JR (2013) Chytrid fungus Batrachochytrium dendrobatidis has nonamphibian hosts and releases chemicals that cause pathology in the absence of infection. Proc Natl Acad Sci 110:210–215

McMahon TA, Sears BF, Venesky MD, Bessler SM, Brown JM, Deutsch K, Halstead NT, Lentz G, Tenouri N, Young S, Civitello DJ, Ortega N, Fites JS, Reinert LK, Rollins-Smith LA, Raffel TR, Rohr JR (2014) Amphibians acquire resistance to live and dead fungus overcoming fungal immunosuppression. Nature 511:224–227

Mooney HA (2010) The ecosystem-service chain and the biological diversity crisis. Philos Trans R Soc B Biol Sci 365:31–39

Olson DH, Aanensen DM, Ronnenberg KL, Powell CI, Walker SF, Bielby J, Garner TWJ, Weaver G, Fisher MC, The Bd Mapping Group (2013) Mapping the Global Emergence of Batrachochytrium dendrobatidis, the Amphibian Chytrid Fungus. PLoS ONE 8:e56802

Ortiz-Santaliestra ME, Rittenhouse TAG, Cary TL, Karasov WH (2013) Interspecific and Postmetamorphic Variation in Susceptibility of Three North American Anurans to Batrachochytrium dendrobatidis. J Herpetol 47:286–292

Parris MJ, Beaudoin JG (2004) Chytridiomycosis impacts predator-prey interactions in larval amphibian communities. Oecologia 140:626–632

Parris MJ, Cornelius TO (2004) Fungal pathogen causes competitive and developmental stress in larval amphibian communities. Ecology 85:3385–3395

Phillott AD, Grogan LF, Cashins SD, McDonald KR, Berger LEE, Skerratt LF (2013) Chytridiomycosis and seasonal mortality of tropical stream-associated frogs 15 Years after introduction of Batrachochytrium dendrobatidis. Conserv Biol 27:1058–1068

Plowright RK, Sokolow SH, Gorman ME, Daszak P, Foley JE (2008) Causal inference in disease ecology: investigating ecological drivers of disease emergence. Front Ecol Environ 6:420–429

Rohr JR, Raffel TR, Halstead NT, McMahon TA, Johnson SA, Boughton RK, Martin LB (2013) Early-life exposure to a herbicide has enduring effects on pathogen-induced mortality. Proc R Soc B Biol Sci 280

Rosenblum EB, James TY, Zamudio KR, Poorten TJ, Ilut D, Rodriguez D, Eastman JM, Richards-Hrdlicka K, Joneson S, Jenkinson TS, Longcore JE, Parra Olea G, Toledo LF, Arellano ML, Medina EM, Restrepo S, Flechas SV, Berger L, Briggs CJ, Stajich JE (2013) Complex history of the amphibian-killing chytrid fungus revealed with genome resequencing data. Proc Natl Acad Sci 110:9385–9390

Rosenblum EB, Voyles J, Poorten TJ, Stajich JE (2010) The Deadly Chytrid Fungus: A Story of an Emerging Pathogen. PLoS Pathog 6:e1000550

Schlaepfer M, Sredl M, Rosen P, Ryan M (2007) High Prevalence of Batrachochytrium dendrobatidis in Wild Populations of Lowland Leopard Frogs Rana yavapaiensis in Arizona. EcoHealth 4:421–427

Searle CL, Gervasi SS, Hua J, Hammond JI, Relyea RA, Olson DH, Blaustein AR (2011) Differential Host Susceptibility to Batrachochytrium dendrobatidis, an Emerging Amphibian Pathogen. Conserv Biol 25:965–974

Skerratt L, Berger L, Speare R, Cashins S, McDonald K, Phillott A, Hines H, Kenyon N (2007) Spread of Chytridiomycosis Has Caused the Rapid Global Decline and Extinction of Frogs. EcoHealth 4:125–134

Skerratt LF, Garner TWJ, Hyatt AD (2009) Determining Causality and Controlling Disease is Based on Collaborative Research involving Multidisciplinary Approaches. EcoHealth 6:331–334

Speare R, Thomas AD, O’Shea P, Shipton WA (1994) Mucor amphibiorum in the toad, Bufo marinus, in Australia. J Wildl Dis 30:399–407

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller RW (2004) Status and Trends of Amphibian Declines and Extinctions Worldwide. Science 306:1783–1786

Taylor SK, Williams ES, Pier AC, Mills KW, Bock MD (1999) Mucormycotic dermatitis in captive adult Wyoming toads. J Wildl Dis 35:70–74

Tobler U, Schmidt BR (2010) Within- and Among-Population Variation in Chytridiomycosis-Induced Mortality in the Toad Alytes obstetricans. PLOS ONE 5:e10927

Touchon JTJ, Gomez-Mestre IG-MI, Warkentin KWK (2006) Hatching plasticity in two temperate anurans: responses to a pathogen and predation cues. Can J Zool 84:556–563

Venesky MD, Wassersug RJ, Parris MJ (2010) Fungal Pathogen Changes the Feeding Kinematics of Larval Anurans. J Parasitol 96:552–557

Wake DB, Vredenburg VT (2008) Are we in the midst of the sixth mass extinction? A view from the world of amphibians. Proc Natl Acad Sci 105:11466–11473

Warkentin KM, Currie CR, Rehner SA (2001) Egg-killing fungus induces early hatching of red-eyed treefrog eggs. Ecology 82:2860–2869

Xie GY, Olson DH, Blaustein AR (2016) Projecting the Global Distribution of the Emerging Amphibian Fungal Pathogen, Batrachochytrium dendrobatidis, Based on IPCC Climate Futures. PLOS ONE 11:e0160746

CHAPTER 2 - DIRECT AND LATENT EFFECTS OF PATHOGEN EXPOSURE ACROSS NATIVE AND INVASIVE AMPHIBIAN LIFE STAGES

Jenny Urbina[1], Evan Bredeweg[2], Andrew R. Blaustein[3], Tiffany S. Garcia[2]

[1] Environmental Sciences Graduate Program, Oregon State University, Corvallis Oregon 97331
[2] Department of Fisheries and Wildlife, Oregon State University, Corvallis Oregon 97331
[3] Department of Integrative Biology, Oregon State University, Corvallis Oregon 97331

Abstract

Emerging infectious diseases are one of the multiple factors contributing to the current biodiversity crisis. Amphibian populations are declining globally in part due to chytridiomycosis, an emerging infectious disease, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd). This fungus primarily affects keratinized structures in larval, juvenile, and adult amphibians. However, we know little about how Bd can impact early amphibian life history stages as well as potential latent effects of Bd exposure over ontogeny. We examined the effects of Bd exposure across Pacific chorus frog (*Pseudacris regilla*), Western toad (*Anaxyrus boreas*) and American bullfrog (*Lithobates catesbeianus*) life stages using two different Bd strains and multiple exposure times. Using a factorial experimental design, embryos of these three species were exposed to Bd at early or late embryonic stages, with some individuals re-exposed after hatching. Embryonic Bd exposure resulted in differential survival as a function of host species, Bd strain and timing of exposure. *P. regilla* experience embryonic mortality when exposed during later developmental stages to one of the Bd strains. There were no differences across the treatments in embryonic mortality of *A. boreas*. And embryonic mortality of *L. catesbeianus* occurred in all Bd exposure. We detected latent effects in *A. boreas* and *L. catesbeianus* larvae, as the odds of larval mortality increased when individuals had been exposed to any of the Bd strains during the embryonic stage. We also detected direct effects on larval mortality in all three-anuran species as a function of Bd strain. And when individuals were repeatedly exposed (late in the embryonic stage and again as larvae), we found an increase in the odds of mortality. Our results suggest that exposure to Bd can directly affect embryo survival and have direct and latent effects on larvae survival of both native and invasive species. However, these impacts were highly context dependent, with timing of exposure and Bd strain influencing the severity of the effects.

Introduction

In many organisms, exposure to stressors during embryonic or prenatal stages can result in both direct and latent effects on subsequent developmental stages. These effects can be on individual growth rates, behavior, locomotion, immunology, etc. (Pechenik 2006, Uller et al. 2009, Murillo-Rincón et al. 2017, Sniegula et al. 2017). For example, exposure to predator cues in the pinewoods tree frog *Hyla femoralis* slowed larval growth and development, resulting in metamorphs with relatively smaller body sizes (LaFiandra & Babbitt 2004). In amphibians, repeated exposure at early life stages to other environmental stressors, such as contaminants, predator cues, and pathogens can produce latent effects in juvenile and adult amphibians (Pechenik 2006, Richter-Boix et al. 2014, Garcia et al. 2017). As such, the timing of pathogen exposure might play a critical role on host susceptibility to infection (Rumschlag & Boone 2015).

Changes in individual susceptibility to pathogens occur throughout ontogeny in many organisms, including plants (Develey-Rivière & Galiana 2007), insects (Brutscher et al. 2015), birds (Mast & Goddeeris 1999), reptiles (Holgersson et al. 2016), mammals (Valkenburg et al. 2012) and amphibians (Rohr et al. 2010, Echaubard et al. 2016). The key, however, to understanding temporal association between pathogens and susceptibility is to empirically discern latent and direct effects within and across life history stages. We posit that amphibians can be model systems for testing these questions as they are a taxon of conservation concern, have complex life histories, and are susceptible to multiple emerging infectious diseases.

One of the most researched amphibian pathogens is the fungus *Batrachochytrium dendrobatidis* (Bd), which has been implicated in the decline of numerous amphibian species worldwide (Hatcher et al. 2012, Olson et al. 2013, Berger et al. 2016). Differential susceptibility to Bd has been documented across species (Blaustein et al. 2005, Searle et al. 2011, Gahl et al. 2012, Gervasi et al. 2013, Bielby et al. 2015, Gervasi et al. 2017), populations (Tobler & Schmidt 2010, Bradley et al. 2015), life stages (Briggs et al. 2005, Garner et al. 2009, Briggs et al. 2010, Piovia-Scott et al. 2011, Ortiz-Santaliestra et al. 2013) and *Bd* strains (Retallick & Miera 2007, Gervasi et al. 2013, Doddington et al. 2013, Piovia-Scott et al. 2015, Berger et al. 2016, Dang et al. 2017). However, how exposure to Bd in one developmental stage can produce latent effects in a later life stage is unclear. Information regarding direct Bd impacts on embryos is also lacking as Bd mainly affects keratinized structures, which are absent in embryos. Further, the importance of evolutionary relationships between Bd strain and the embryonic host may also have significant implications.

We explored the direct and latent effects of Bd exposure on both the embryonic and larval stages using three amphibian species with differential susceptibility to native and invasive Bd strains. We posit that amphibian embryos will be susceptible to the chytrid fungus as Bd can produce enzymes that can destroy tissue (Fites et al. 2013; McMahon et al. 2012; Rosenblum et al. 2010). Further, the release of fungal toxin (Blaustein et al. 2005, Voyles et al. 2009) could impact embryos by delaying or triggering key transitions resulting in ontogenetic shifts or latent effects on life history trajectories. Direct or latent effects may also vary with Bd strain and with host species, therefore evaluating different strains is critical to disentangle intrinsic aspects of the pathogen, such as virulence and how it changes among hosts. We also examined the influence of Bd exposure on larval survival predicting that repeated exposure to Bd across the embryonic/larval transition would result in decreased survival.

Methods

We studied three anuran species found in the US Pacific Northwest (PNW). The Pacific chorus frog (*Pseudacris regilla*) is a highly abundant species throughout its PNW range, the Western toad (*Anaxyrus boreas*) has experienced declines across much of its historic range and American bullfrogs (*Lithobates catesbeianus*) are an introduced species in the PNW (Blaustein et al. 1995, Muths et al. 2003, Jones et al. 2005). Twenty clutches of *P. regilla* were collected from Little Three creeks on 19 June 2014 (44°06'03.5" N, 121°38'34.7" WGS84 Deschutes County, OR, elevation = 2000 meters) and 600 eggs of *A. boreas* were collected from 20 different egg masses at Todd Lake (44°01'44.5" N, 121°41'07.6"W WGS84 Deschutes County, OR, elevation = 1870 meters) on 29 May 2015. We collected 600 newly laid eggs from six distinct *L. catesbeianus* egg masses from William L. Finley National Wildlife Refuge on 20 May 2014 (44°25'23.6" N, 123°18'41.8"W WGS84 Benton County, OR, elevation = 276 meters). After collection, eggs were immediately transported to a climate controlled environment at Oregon State University and held under constant temperature (14 – 15.5 C) and photoperiod (12L: 12D) conditions. Less than six hours after arrival, every clutch of *P. regilla* or group of eggs of *A. boreas* and *L. catesbeianus* were divided into three groups and each group for *P. regilla* and *A. boreas* contained ~10 eggs (±1.95 eggs), and 20 eggs for *L. catesbeianus*.

Pre-Hatch Exposure Regime-

Bd exposure treatments were administered in either the early embryonic developmental stages or closer to hatching. Early exposure (early) corresponded to the late gastrula stages, or Gosner Developmental Stage 12 (Gosner 1960) while closer to hatching exposure (late)

corresponded to embryos capable of muscular response, or Gosner Developmental Stage 18 (Gosner 1960). Bd strains (i.e. the isolate of the fungus used for the inoculation) included a novel Bd strain to Oregon freshwater habitats (JEL 627, hereafter ‘East’, isolated from *L. catesbeianus* in Maine USA), and an endemic Bd strain (JEL 630, hereafter ‘West’, isolated from *L. catesbeianus* in Oregon). Following Searle et al. (2011), Bd was cultured on a sterile tryptone – agar media plates with 0.5 ml of stock Bd broth coming from each particular strain of the fungi. Bd cultures were allowed to grow for 5-8 days at 20 °C before used in the experiment.
Using a hemocytometer, we quantified the zoospores from a pooled inoculation broth (8-12 plates per Bd strain). Five ml inoculations of the zoospore broth (30K zoospores/ml) were then administered to experimental units (18 cm H x 10 cm OD high - density polyethylene graduated beakers) containing 800 ml of dechlorinated water. A similar dose was previously tested in larvae of *P. regilla* (Gervasi et al. 2013), *A. boreas* (Marcum et al. 2010, Gervasi et al. 2013, Searle et al. 2014), and *L. catesbeianus* (Gahl et al. 2012, Eskew et al. 2015). Controls were inoculated with a sham inoculum created by rinsing the same number of sterile agar plates with 5 ml of dechlorinated water.

Using a factorial experimental design, each group of eggs was assigned to a time of exposure treatment (Early, Late) and a Bd strain treatment (West, East, Control) (Figure 2.1: pre-hatching). 60 experimental units (581 total eggs) were assigned for *P. regilla* (10 replicates per Early and Late treatment groups), 51 experimental units (506 total eggs) for *A. boreas* (8 replicates per Early exposure treatment, 9 replicates per Late exposure treatment), and 30 experimental units (600 total eggs) for *L. catesbeianus* (5 replicates per Early treatment groups, 6 replicates per East/Late treatment, and 4 replicates for West/Late exposure treatment) (Table

Table 2.1). The length of the pre-hatching phase varied by species, lasting 19 days for *P. regilla* and *A. boreas*, and 22 days for *L. catesbeianus*. Embryos that died were preserved individually in 2.0 ml Eppendorf tubes with 95 % ethanol. No water changes were performed during the pre-hatching phase as movement associated with water changes can induce hatching, thus influencing our results. Upon hatching, water changes were conducted weekly. We quantified the time of hatching by direct observation, and hatchling events and survival were recorded twice per day.

To analyze differences in hatching time, we compared proportions between treatments of eggs being exposed to Bd and control treatments (no exposure to the pathogen) using quasibinomial generalized linear models (GLM). All analyses were run in R (R Core Team 2016). To evaluate differences among strains and controls we calculated pairwise comparison using a Tukey HSD test.

Post-Hatch Exposure Regime

Upon hatching, survivors were pooled within pre-hatch treatment groups (Early or Late; East, West, Control) to standardize sample sizes for the post-hatch phase of the experiment. In this phase, larvae were either re-exposed to the same pre-hatch Bd strain or held as controls to estimate latent effects (Figure 2.1: Post-Hatch). For *P. regilla*, there were a total of 328 surviving hatchlings distributed across the larval exposure treatments for a total of 82 experimental units, resulting in 33 control replicates, 21 East strain replicates, and 28 West strain replicates. We reported complete mortality in the East/Late pre-hatch exposure treatment group; as such, there was no continuation of this treatment in the post-hatch phase. For *A. boreas*, we ran 42 control replicates, 28 East strain replicates, and 26 West strain that contained a total of 384 surviving hatchlings for a total of 96 experimental units. For *L. catesbeianus*, we ran 23 control replicates, 17 East strain replicates, and 16 West strain using a total of 228 surviving hatchlings with a total of 56 experimental units. Due to complete mortality in the East/Early and the West/Early pre-hatch phase, these treatments were not continued in the post-hatch phase (Table 2.2).

Larvae were held individually and those that were re-exposed to Bd were re-inoculated once a week (every 7 d) for the duration of the experiment. Individuals were held in rectangular plastic containers (31 x 18 x 8 cm) filled with 2000 ml dechlorinated water. Water changes occurred concurrently with re-inoculation using 5ml of 50K zoospores/ml. Animals that died during the experiment were preserved in 95% ethanol. At the end of the experiment, animals remaining alive were humanely euthanized in accordance with institutional animal care protocol in MS-222 (Tricaine methanesulfonate) and then preserved in 95% ethanol. The experimental trials for each species lasted until individuals reached Gosner stage 30-31 (distinctive foot paddle) or death. Total duration for the experiment was 65 days for *P. regilla*, 59 days for *A. boreas* and 19 days for *L. catesbeianus*.

We monitored survival twice per day and quantified developmental differences through time by staging all larvae (Gosner stage) every week during water changes. At the end of the post-hatch phase, we sampled a subset of all Bd-exposed animals of each species and also randomly sampled 5 control animals of each species to confirm no contamination happened across treatments. To assess infection load at the termination of the experiment, we dissected larvae mouthparts for *P. regilla* individuals, and we swabbed mouthparts using fine tipped sterile rayon swabs (Medical Wire and Equipment MW&E 113) for *A. boreas and L. catesbeianus*. Both protocols, swabbing and cutting mouthparts, are recommended as adequate protocols for assessing infection loads. Excised mouthparts and swabbing are similar in the likelihood of detecting Bd infection regardless of developmental stage and larval size (Retallick et al. 2006, Hyatt et al. 2007).

Each sample was analyzed using quantitative polymerase chain reaction (qPCR) following the methods of (Boyle et al. 2004). A small modification of the amount of Prepman Ultra (Applied Biosystems®, Life Technologies) was used to extract the DNA; we used 60 µL instead of 40 µL (Searle et al. 2013). Our extractions were diluted 1:10 and each sample was analyzed in triplicate to quantify the average number of genome equivalents per animal (7500 real-time PCR Applied Biosystems instrument). To analyze infection loads, we log transformed the qPCR results as log (genome equivalents per individual + 1) to normalize data.

Effect of exposure on survivorship was analyzed independently by species using odds ratios calculated with a generalized linear mixed model, family: binomial (logit). The values of the ratios represent the likelihood or the risk of mortality due to exposure to the pathogen in comparison to the controls. Therefore, odds ratios higher than 1 represent an increased risk after exposure, odds ratios equal to one represent no difference in the risk, and odds ratios lower than 1 represent a lower risk of the exposed group. All analyses were run in R (version 3.3.2).

Results

Pre-Hatching Phase:

Pseudacris regilla embryos exposed to both the East and West Bd strains in the Early exposure groups had a lower proportion of hatchlings relative to controls (East strain: $t = -4.40$, $p < 0.001$;

West strain: t=1.99, p=0.04). A post hoc Tukey test showed that this proportion was different in embryos exposed to the East strain in contrast to the control (z = -4.45, p<0.001) and the West strain (z = 6.14, p<0.001), with only a 50% hatching rate. Reduced hatching was also found in the Late/East treatment group (t=- 11.03, p<0.001) relative to the Late/West treatment (t=- 1.29, p= 0.19). In fact, less than 10% of embryos hatched after being exposed late in development to the non-native East strain (Figure 2.2, top). In *A. boreas*, the proportion of embryos that hatched was similar across both strains in comparison to controls across the Early (East strain: t= -0.49, p = 0.62; West strain: t=0.62, p=0.53) and Late exposure treatments (East strain: t= 1.31, p=0. 19; West strain: t=0.73, p=0. 46) (Figure 2.2, middle). The proportion of *L. catesbeianus* embryos that hatched was low when embryos were exposed early in development, with lower survival in the West Bd strain treatment relative to controls (West strain: t=3.58, p<0.001). There were no survivors in the East strain exposure treatment. The estimate of Bd strain as factor in our model was high (5329), potentially due to the 100% mortality, making the t and p- value not significant (t= 0.003, p = 0.99). The proportion of embryos that hatched in the Late exposure treatment was lower across both Bd strains in comparison to the controls (East strain: t= 2.89, p <0.01; West strain: t= 2.13, p=0.03) (Figure 2.2, bottom). A post hoc Tukey test showed that this proportion was different in embryos exposed to the East strain in contrast to the control (z = 2.89, p<0.01), but it was not different for embryos exposed to the West strain (z = 2.13, p= 0.08)

Post-Hatching Phase

Our generalized linear mixed model quantified as odds ratios (OR) the effects of exposure to a particular strain on larvae mortality in comparison to the controls given their history of exposure as embryos. As such, results are reported as an increase or decrease in odds of mortality.

Direct effects on larvae –only exposed to Bd as larvae (Control-Bd)

We found evidence for direct effects of Bd exposure on larval mortality for the three species. In *P. regilla*, post -hatching exposure to the East strain increased the odds of mortality ($OR_{\text{Early/ Control- East -}}$ 8.88, p = 0.01, CI: 1.43- 54.85) (Figure 2.3 Left panel). For *A. boreas,* we found that individuals exposed during the post-hatch phase to the West strain had lower odds of mortality relative to controls ($OR_{\text{Early/ Control- West}}$ 0.12, p = 0.03, CI: 0.018 – 0.84, Figure 2.4 Left panel). In contrast, larvae coming from the Late control group and exposed post-hatch to East or West had higher odds of mortality than controls ($OR_{\text{Late/ Control - East}}$ 14.38, p = 0.03, CI: 1.19 - 173.65; $OR_{\text{Late /Control -West}}$ 19.56, p=0.03, CI: 1.32- 288 (Figure 2.4 Right panel). Larvae of *L catesbeianus* increased their odds of mortality when exposed to either East or West strain (OR_{East} 9.9, p= 0.04, CI: 1.06 – 92; OR_{West} 539, p < 0.001, CI: 29.64 – 9801).

Latent Effects on larvae –only exposed to Bd as embryos (Bd-Control)

We did not find evidence for latent effects in *P. regilla*. In *A. boreas* odds of mortality changed according to the time of exposure and Bd strain. Odds of mortality for larvae decreased when embryos were exposed early in development to the West strain of Bd ($OR_{\text{Early/ West – control}}$ 0.14, p = 0.02, CI: 0.026 - 0.73, Figure 2.4, left panel). On the contrary, individuals exposed Late as embryos to the East strain had higher odds of mortality than controls ($OR_{\text{Late/ East – control}}$ 10.62, p = 0.04, CI: 1.07-105, Figure 2.4 right panel). In *L catesbeianus*, we found higher odds of mortality than controls for both Bd strains (OR $_{\text{Late/ East – control}}$ 31, p = 0.001, CI: 3.5- 272, OR $_{\text{Late/ West control}}$ 23.21, p = 0.006, CI: 2.42 – 222.14, Figure 2.5).

Repeatedly Exposed treatments- exposed to Bd as both embryos and larvae (Bd-Bd)

We found evidence that exposure to Bd in both the embryonic and larval stages affects the larval odds of mortality in all three species. We found in *P. regilla* that re- exposure to the West strain, (Late treatment group) increased the odds of mortality (OR $_{\text{Late/West–West}}$ 8.05, p = 0.04, CI 1.01-64.22, Figure 2.3). In *A. boreas* odds of mortality increased in re- exposed individuals to both the East and West strains (Late treatment groups) (OR $_{\text{Late/East - East}}$ 9.37, p = 0.05, CI: 0.92 – 95; OR $_{\text{Late/West -West}}$ 9.12, p= 0.05, CI: 0.91 – 91.32). In *L. catesbeianus*, odds of mortality were high for re-exposed animals to either Bd strain (OR $_{\text{East Late - East}}$ 58.3 p = 0.0003, CI: 6.35 – 534.6, OR $_{\text{West Late-West}}$ 101.29, p < 0.001, CI: 8.9 – 1145).

Infection loads

Real-time qPCR analyses of tadpole mouthparts for *P. regilla* and swabs for *A. boreas* and *L. catesbeianus* confirmed Bd infection in all species but not in all treatments. All tadpoles subsampled from control treatments were negative for Bd. Mean infection load did not differ by treatment and history of exposure across all species. We found infection loads from *P. regilla* when exposed to both strains at Early or Late treatment groups in the direct effect and the repeteadly exposure treatments (Table 2.3). In *A. boreas* infection loads were positive when individuals were exposed Early or Late to the East strain in the direct effect treatment and in individuals from the Early treatment group repeatedly exposed to the West strain (Table 2.3). Infection loads for *L. catesbeianus* were positive for individuals exposed in the Late treatment group. Positive results for this species were detected after exposure to the East strain in the direct effect treatment and for both strains in the latent effect treatment (Table 2.3).

Discussion

Life stage, time of exposure, and Bd strain influenced susceptibility to Bd in the embryo - larvae life history transition for three anuran species: *P. regilla*, *A. boreas*, and *L. catesbeianus*. We detected direct effects of Bd on embryonic and larval mortality, latent effects across the embryo/larval transition, and additive effects when repeatedly-exposed to Bd across both life stages. Exposure of embryos to Bd resulted in direct impacts on hatchling survivorship. We found direct, negative impacts of Bd strain and time of exposure on embryonic survival and proportion of hatching success for *P. regilla* and *L. catesbeianus*. Embryos of *P. regilla* were drastically affected by the non-native East Bd strain, resulting in 90% mortality when exposed later in embryonic development. Interestingly, embryos of invasive *L. catesbeianus* died when exposed to either Bd strain (East or West). When exposed early in embryonic development to the East strain, the number of viable hatchlings was zero and we detected a mortality of 90% in hatchlings after early exposure to the West Bd strain. When exposed later in development (East or West strains), only 50% of embryos hatched.

Post-hatching exposure resulted in both direct and latent impacts on larval survivorship. Direct effects on larvae are reported mainly as an increased in the odds of mortality for all three-anuran species. *P. regilla* was negatively affected by exposure to the non-native East Bd strain, while *A. boreas* and *L. catesbeianus* were affected by both strains (East and West). Odds of mortality in *A. boreas* were higher when exposed to the West strain (19.56) than when exposed to the East strain (14.38). On the contrary, the odds of mortality in *L. catesbeianus* were higher when exposed to the East strain (9.9) than when exposed to the West strain (5.39). This result was not wholly unexpected as larvae mortality has been reported in experimental studies exposing these same species to Bd. *A. boreas* has been particularly susceptible to Bd (Blaustein et al. 2005, Dang et al. 2017) while *P. regilla* and *L. catesbeianus* larvae have relatively high survivorship (Blaustein et al. 2005, Reeder et al. 2012, Gervasi et al. 2013, Dang et al. 2017). In this study, we found a direct effect of Bd on larval survivorship for all three species. The increase in the odds of mortality in *P. regilla* and *L. catesbeianus* larvae can be explained by the origin and characteristics of the East strain. Isolated from *L. catesbeianus* in Maine (USA), this strain has been identified as hypervirulent (Farrer et al. 2011, Schloegel et al. 2012, Rosenblum et al. 2013) and categorized as part of the North American clade in the Global Pandemic Lineage (GPL) (Schloegel et al. 2012). As such, we anticipated an increase in larval mortality due to a lack of evolutionary relationship with this strain. However, *L. catesbeianus* larvae were also susceptible to the East strain even though it was isolated from their conspecifics within their native range.

In terms of latent effects, we found an increase in the odds of larval mortality of both *A. boreas* and *L. catesbeianus*.as a function of Bd strain and timing of embryonic exposure. In *A. boreas*, embryos exposed early to the West strain showed a decrease in the odds of mortality. Conversely, when *A. boreas* were exposed to the East strain late in embryonic development, larvae were almost 10 times more likely to die than control individuals. There was a similar increase in the odds of larval mortality in *L. catesbeianus* when exposed as embryos to any of the Bd strains. The high mortality rates in *L. catesbeianus* when exposed early to Bd prevented us from understanding potential latent effects for this invasive species. Similarly, we could not evaluate potential latent effects after late exposure of *P. regilla* embryos to the East strain. Our results support the hypothesis that timing of pathogen exposure is a major factor that influences host survivorship.

We also found effects of repeatedly Bd exposure (exposed in both the embryonic and larval stages) in all three-anuran species. All species showed an increase in the odds of larval mortality when the first Bd exposure occurred at a later embryonic developmental stage (Gosner stage 18). In *P. regilla*, odds of mortality increased after repeated exposure to the West strain. *A. boreas* and *L. catesbeianus* increased the odds of mortality when exposed to both strains (East or West). Repeated exposure effects have been reported in experiments examining the larval/metamorph transition (Goater 1994, LaFiandra & Babbitt 2004, Kelehear et al. 2009, Saka et al. 2013), thus our experiment provides additional information concerning other life history transitions.

The differential response of *A. boreas* to Early/Late and East/West Bd treatments may be explained by the presence of a potential critical window of vulnerability for this species and by the virulence of Bd strain. Late exposure of *A. boreas* embryos to the East strain increased the odds of larval mortality of this species. Fernandez-Beneitez et al. (2011) found that embryos of natterjack toad (*Bufo calamita*) and Western spadefoot toad (*Pelobates cultripes*) exposed to *Saprolegnia* spp. at early developmental stage (Gosner stage 12) suffered no increase in mortality, while embryos challenged at later stages of embryonic development Gosner staged 15 and 19 were sensitive to the pathogen with mortality rates up to 90%. Understanding which species experience latent effects will help target management efforts by identifying how exposure in particular life history stages can change host response.

Our findings complement the information on susceptibility of *P. regilla* to Bd as larvae of this species had previously been reported to be tolerant to certain Bd strains (Blaustein et al.

2005, Dang et al. 2017). Interestingly, we found that this tolerance can change with an individual's previous exposure regime to non-native strains. Our experimental evaluation revealed that Bd strains isolated from an invasive species can have harmful consequences on native and even invasive conspecific hosts. Our findings for *A. boreas* support previous work showing species as being susceptible to both the East and West strains of Bd (Blaustein et al. 2005, Gervasi et al. 2013, Dang et al. 2017). In *L. catesbeianus*, larvae and adults have been reported as able to withstand infection loads of the chytrid in different regions (Hanselmann et al. 2004) and this species is suggested as an asymptomatic carrier or reservoir of Bd (Daszak et al. 2004, Garner et al. 2006). Our results indicate that larvae can also be susceptible to Bd but this response will be mediated by previous exposure in an early life stage. Individuals that received exposure as embryos and then were re- exposed as larvae to the chytrid were about 50 times more likely to die than individuals kept as controls. This contrasts with previous experimental studies reporting this species as a carrier of Bd (Blaustein et al. 2005, Gahl et al. 2012, Eskew et al. 2015). Generally, those studies directly exposed individuals in the larval stage (Gosner stage 26-30) without considering previous exposure regimes. In our study, *L. catesbeianus* were vulnerable to Bd exposure in response to direct exposure and across life history transitions.

We found species- specific embryonic mortality after exposure to Bd. Many pathogens impact anuran embryos, including ranavirus (Haislip et al. 2011), oomycetes (Kiesecker & Blaustein 1995, Fernández-Benéitez et al. 2008), filamentous ascomycetes (Warkentin et al. 2001) and microsporidia (Green & Converse 2005). But few studies have quantified direct effects of Bd on anuran embryos. Bd enzymatic action is one mechanism that could explain this result, as it can cause damage in skin tissue of hosts after exposure (Blaustein et al. 2005, Symonds et al. 2008, Moss et al. 2010, McMahon et al. 2013). A complex mix of proteolytic and hydrolyze enzymes (esterases) that degrade amphibian tissue have been described from different Bd isolates (Symonds et al. 2008, Moss et al. 2010, Brutyn et al. 2012). In addition, many hatching anurans release enzymes to assist with degradation of the egg capsule at the moment of hatching (Carroll & Hedrick 1974, Cohen et al. 2016); this could potentially facilitate the enzymatic action of Bd to degrade tissues. Recently, dose - dependent mortality and proliferation in zebrafish (*Danio rerio*) tissue was reported with toxins secreted after the establishment of Bd sporangia (Liew et al. 2017).

The present study offers useful information about the complexity of host response to a pathogen, particularly with multiple exposures across life stages. Our study provides information about direct effects of Bd on anuran embryos, with significant impacts on mortality and the proportion of hatching success. Our results also quantified latent effects of Bd exposure over ontogeny (Hamdoun & Epel 2007). Despite being a relatively brief period, exposure to Bd in the egg led to increased mortality after hatching. Species-specific differences were due to the timing of embryonic exposure and re-exposure in the larval stage. Additional research exploring the mechanisms protecting the embryos is needed to better understand the susceptibility of this developmental stage to disease. Characteristics such as jelly thickness and composition, or size of the capsule, can be involved in resistance to chytrid. As eggs received material from their parents during oviposition, evaluating the role of parents in the immune response of their offspring can help us to understand more about embryonic immunity. Further studies are also required to better understand how variation in other environmental and biological parameters can affect the outcome of repeated Bd exposure in anuran species. Our results add information to the growing body of evidence concerning differential susceptibility to pathogens among amphibian species and across life stages.

Acknowledgments

We thank N. Soria, L. Londono, G. Mayorga for assistance measuring and staging tadpoles and assistance with animal care. D. Olson, C. Harjoe and L. Thurman for help during egg masses collection, Spatafora Lab., Tanguay Lab., and the Center for Genome Research and Biocomputing at Oregon State University for providing laboratory resources for analysis.

Table

Table 2.1 Number of replicates per treatment per species followed by total number of eggs per treatment between parentheses

Host species	**Pre-hatch exposure regime Bd strain x Time exposure treatments**					
	Control		**East**		**West**	
	Early	**Late**	**Early**	**Late**	**Early**	**Late**
Pseudacris regilla	10 (101)	10(96)	10(97)	10(94)	10(98)	10(95)
Anaxyrus boreas	8(80)	9(85)	8(84)	9(86)	8(84)	9(87)
Lithobates catesbeianus	5(100)	5(100)	5(100)	6(120)	5(100)	4(80)

Table 2.2 Number of replicated groups exposed in the different treatments per species as larvae. In parentheses, total number of individuals per treatment including all replicates; (-) no treatment

Pseudacris regilla		**Pre-hatching treatments**					
		Bd strain x Time					
		Control		East		West	
		Early	Late	Early	Late	Early	Late
Post -hatch Bd treatment	**Control**	8 (32)	7 (28)	5 (20)	0 (0)	7 (28)	6 (24)
	East	8 (32)	7 (28)	6 (24)	0 (0)	-	-
	West	8 (32)	7 (28)	-	-	7 (28)	6 (24)

Anaxyrus boreas		**Pre –hatching treatments**					
		Bd strain x Time					
		Control		East		West	
		Early	Late	Early	Late	Early	Late
Post -hatch Bd treatment	**Control**	5 (20)	3 (12)	8 (32)	8 (32)	9 (36)	9 (36)
	East	6 (24)	5 (20)	9 (36)	8 (32)	-	-
	West	5 (20)	4 (16)	-	-	9 (36)	8 (32)

Lithobates catesbeianus		**Pre-hatching treatments**					
		Bd strain x Time					
		Control		East		West	
		Early	Late	Early	Late	Early	Late
Post -hatch Bd treatment	**Control**	6 (24)	6 (24)	0 (0)	7 (28)	0 (0)	4 (16)
	East	5 (20)	5 (20)	0 (0)	7 (28)	-	-
	West	6 (24)	6 (24)	-	-	0 (0)	4 (16)

Table 2.3 Mean Bd loads (genome equivalents ± SD) at the end of experiment for *P. regilla*, *A. boreas* and *L. catesbeianus* exposed early during embryonic development (top panel) and Bd loads for the same species exposed late during embryonic development (bottom panel). Star * indicates treatments with significant effects in the odds of mortality of larvae. NA indicates treatments without samples due to mortality in the pre-hatching exposure part.

Species	EARLY	*Direct effects*		*Latent effects*		*Twofold effects*	
	CONTROL	CONTROL		EAST	WEST	EAST	WEST
	Control	East	West	Control		East	West
P. regilla	0	1.25*±1.009	1.86±1.24	0	0	0.005±0.009	1.45±1.77
A. boreas	0	0.008±0.008	0*	0	0*	0	0.012±0.02
L. catesbeianus	0	0	0*	NA	NA	NA	NA

Species	LATE	*Direct effects*		*Latent effects*		*Twofold effects*	
	CONTROL	CONTROL		EAST	WEST	EAST	WEST
	Control	East	West	Control		East	West
P. regilla	0	0.74±1.24	3.39±1.05	NA	0	NA	1.62*±1.17
A. boreas	0	0.019*± 0.01	0*	0.00016*± 0.0003	0	0.005*±0.006	0
L. catesbeianus	0	0.53*± 0.62	0	0.03**± 0.05	0.59**± 0.76	0	0

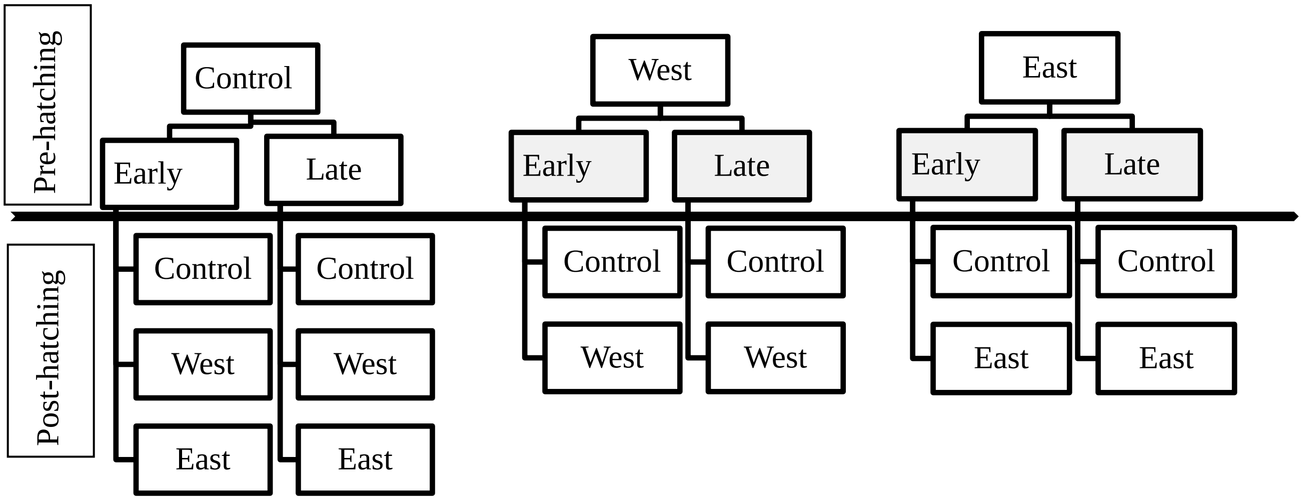
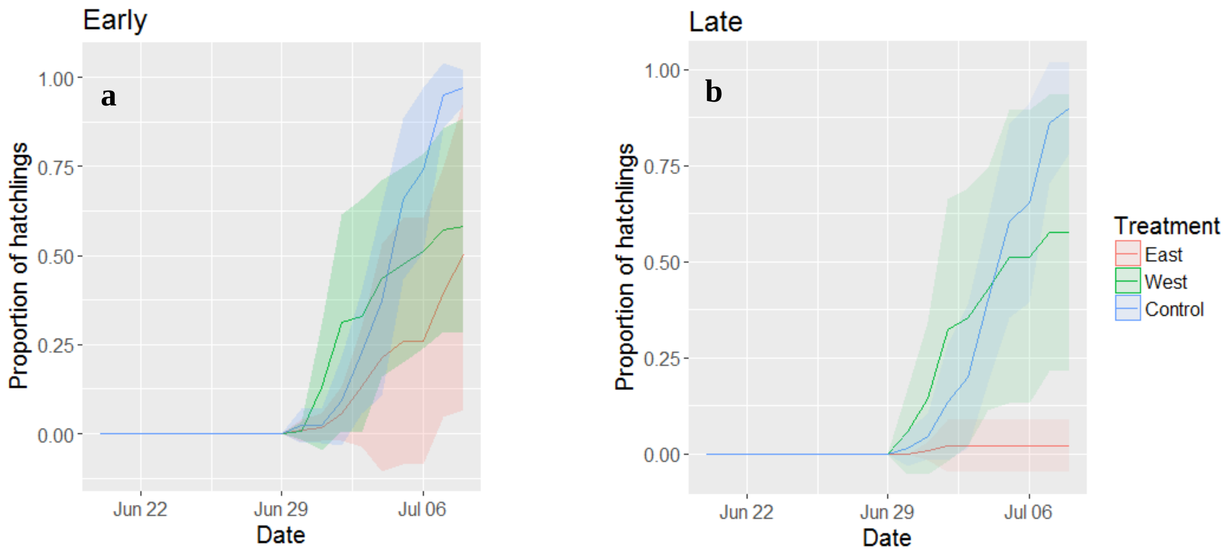
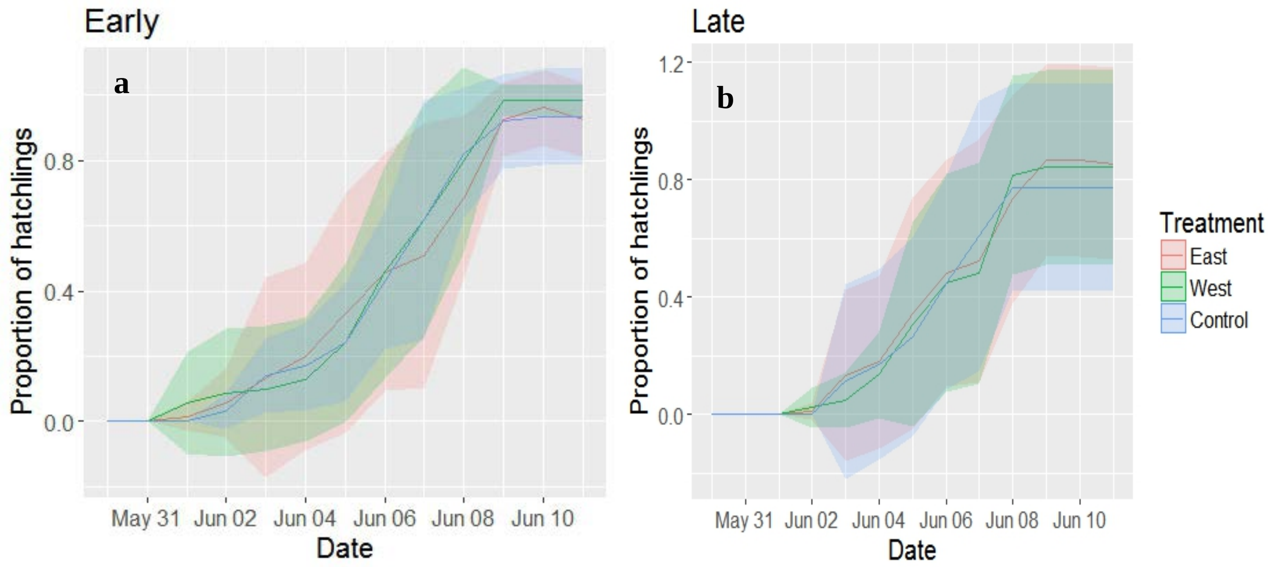


Figure 2.1 Exposure treatments of egg masses and tadpoles according to the time of exposure and chytrid strain. Pre-hatching treatments are indicated in gray and above the separation line. Treatments for tadpoles (Post-hatching) are listed below the separation line


Early
a
Proportion of hatchlings
1.00
0.75
0.50
0.25
0.00
Jun 22
Jun 29
Jul 06
Date
Late
b
Proportion of hatchlings
1.00
0.75
0.50
0.25
0.00
Jun 22
Jun 29
Jul 06
Date
Treatment
East
West
Control


Early
a
Proportion of hatchlings
0.8
0.4
0.0
May 31
Jun 02
Jun 04
Jun 06
Jun 08
Jun 10
Date
Late
b
Proportion of hatchlings
1.2
0.8
0.4
0.0
May 31
Jun 02
Jun 04
Jun 06
Jun 08
Jun 10
Date
Treatment
East
West
Control

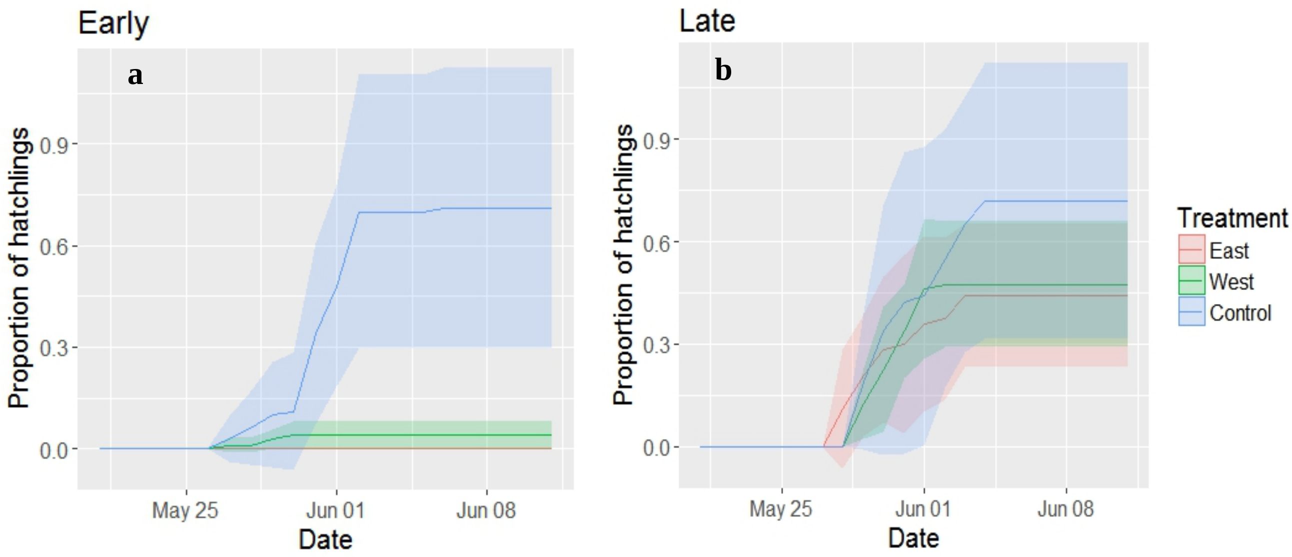

Early
a
Proportion of hatchlings
0.9
0.6
0.3
0.0
May 25
Jun 01
Jun 08
Date
Late
b
Proportion of hatchlings
0.9
0.6
0.3
0.0
May 25
Jun 01
Jun 08
Date
Treatment
East
West
Control

Figure 2.2 Proportion of hatchlings in *P. regilla* (top), *A. boreas* (middle), and *L. catesbeianus* (bottom) after exposure of eggs to different chytrid treatments. Red color represents exposure to the East strain, green color represents exposure to the West strain, and controls are indicated by the blue color. a) Treatments exposed early in development (Gosner stage 12) and b) Treatments exposed late in development (Gosner state 18)

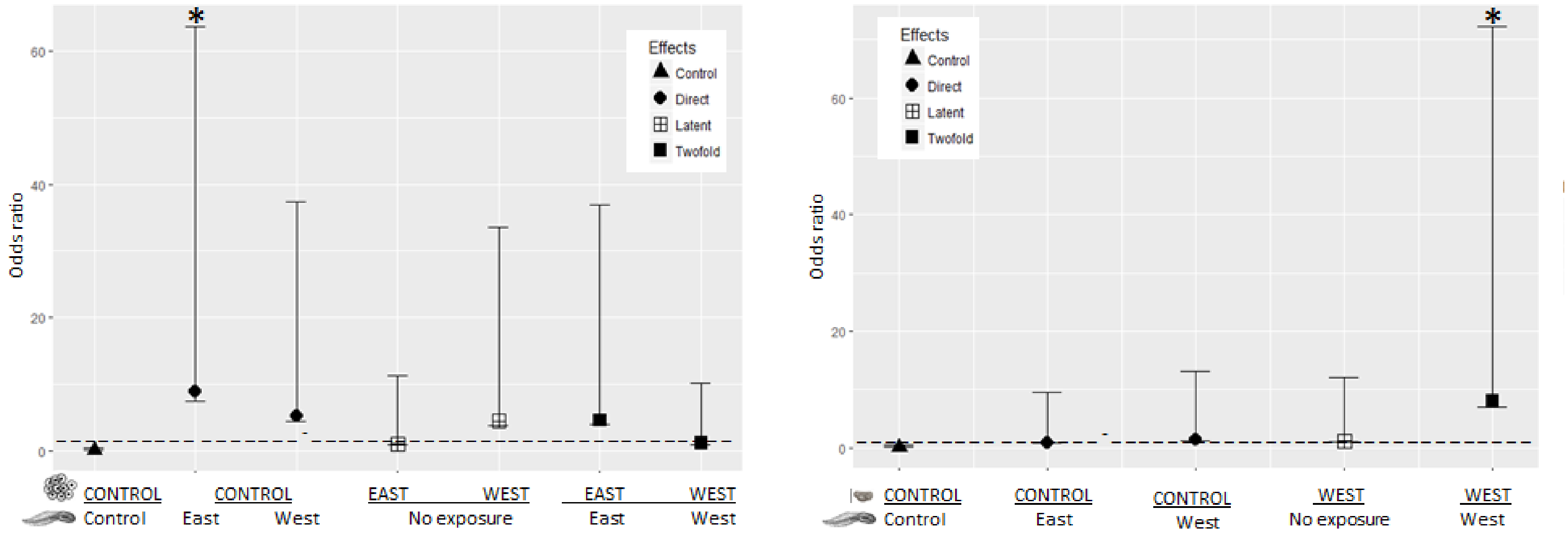


Figure 2.3 Odds ratio (OR) for *P. regilla* tadpoles according to their original exposure as embryos and their subsequent exposure as larvae. Left panel: early exposure, right panel: late exposure. Label of x- axis includes a fraction that indicates in the numerator the exposure regime as embryo and in the denominator exposure regime post-hatching. A dashed line indicates value 1. OR > 1 higher risk after exposure, OR = 1 no risk difference, OR< 1 lower risk after exposure. A star (*) indicates treatments with significant odds ratios

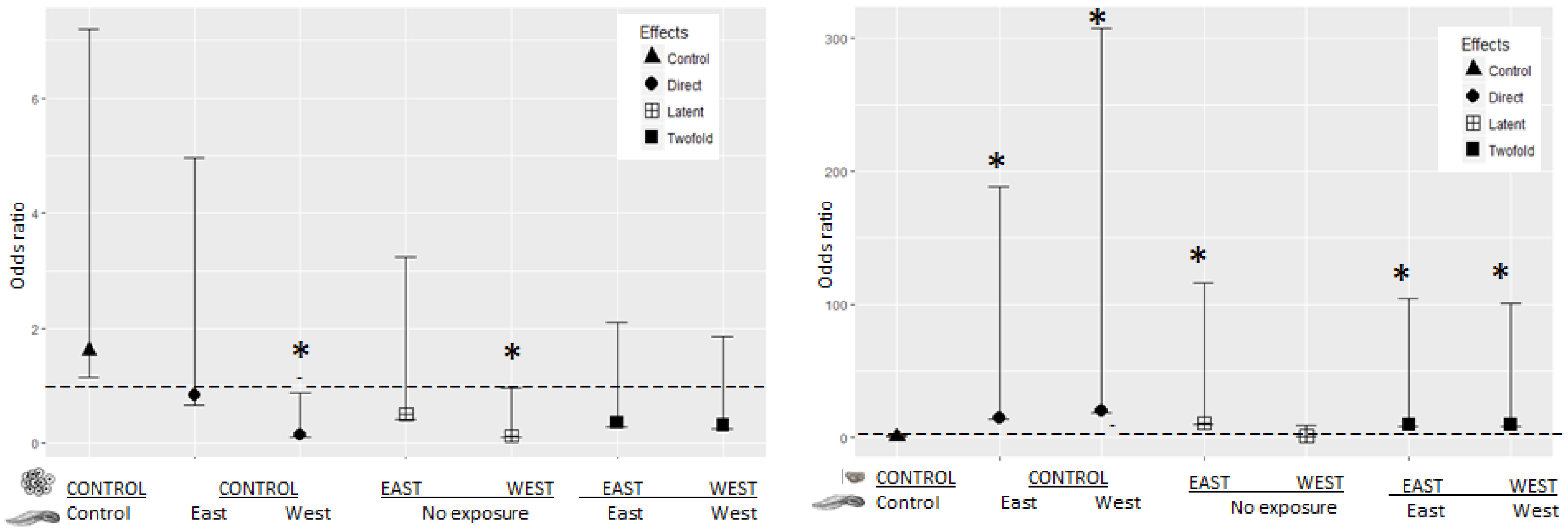


Figure 2.4 Odds ratio (OR) for *A. boreas* tadpoles according to their original exposure as embryos and their subsequent exposure as larvae. Left panel: early exposure, right panel: late exposure. Label of x- axis includes a fraction that indicates in the numerator the exposure regime as embryo and in the denominator exposure regime post-hatching. A dashed line indicates value 1. OR > 1 higher risk after exposure, OR = 1 no risk difference, OR< 1 lower risk after exposure. A star (*) indicates treatments with significant odds ratios

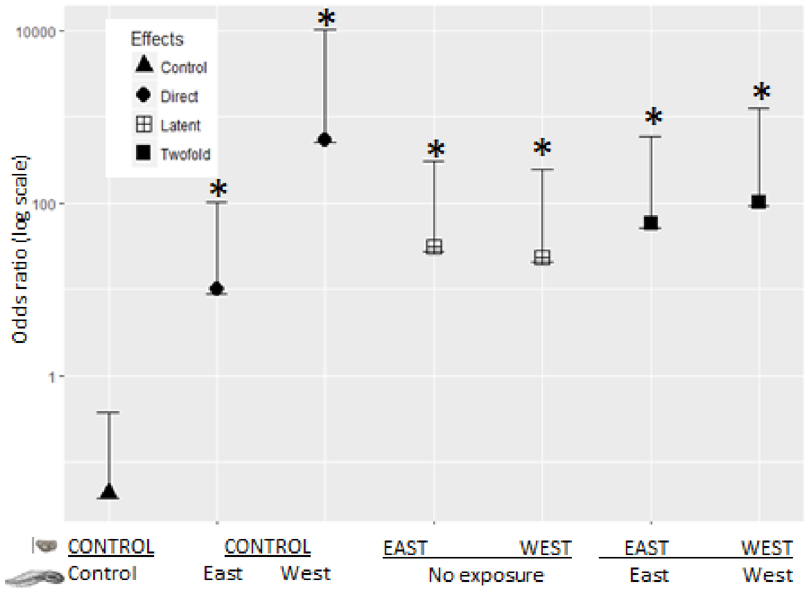


Figure 2.5 Odds ratio (OR) for *L. catesbeianus* tadpoles according to their original exposure as embryos and their subsequent exposure as larvae. Only one panel showing results of late exposure as embryos exposed early died. Label of x- axis includes a fraction that indicates in the numerator the exposure regime as embryo and in the denominator exposure regime post-hatching. A star (*) indicates treatments with significant odds ratios

Literature cited

Berger L, Roberts AA, Voyles J, Longcore JE, Murray KA, Skerratt LF (2016) History and recent progress on chytridiomycosis in amphibians. Aquat Fungi 19:89–99

Bielby J, Fisher MC, Clare FC, Rosa GM, Garner TWJ (2015) Host species vary in infection probability, sub-lethal effects, and costs of immune response when exposed to an amphibian parasite. Sci Rep 5:10828

Blaustein A, Beatty J, Olson D, Storm R (1995) The biology of amphibians and reptiles in old-growth forests in the Pacific Northwest.

Blaustein AR, Han BA, Relyea RA, Johnson PTJ, Buck JC, Gervasi SS, Kats LB (2011) The complexity of amphibian population declines: understanding the role of cofactors in driving amphibian losses. Ann N Y Acad Sci 1223:108–119

Blaustein AR, Romansic JM, Scheessele EA, Han BA, Pessier AP, Longcore JE (2005) Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus Batrachochytrium dendrobatidis. Conserv Biol 19:1460–1468

Bradley PW, Gervasi SS, Hua J, Cothran RD, Relyea RA, Olson DH, Blaustein AR (2015) Differences in sensitivity to the fungal pathogen Batrachochytrium dendrobatidis among amphibian populations. Conserv Biol 29:1347–1356

Briggs CJ, Knapp RA, Vredenburg VT (2010) Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. Proc Natl Acad Sci 107:9695–9700

Briggs CJ, Vredenburg VT, Knapp RA, Rachowicz LJ (2005) Investigating the population-level effects of chytridiomycosis: an emerging infectious disease of amphibians. Ecology 86:3149–3159

Brutscher LM, Daughenbaugh KF, Flenniken ML (2015) Antiviral defense mechanisms in honey bees. Soc Insects Vectors Med Vet Entomol 10:71–82

Brutyn M, D’Herde K, Dhaenens M, Rooij PV, Verbrugghe E, Hyatt AD, Croubels S, Deforce D, Ducatelle R, Haesebrouck F, Martel A, Pasmans F (2012) Batrachochytrium dendrobatidis zoospore secretions rapidly disturb intercellular junctions in frog skin. Fungal Genet Biol 49:830–837

Carroll EJ, Hedrick JL (1974) Hatching in the toad Xenopus laevis: Morphological events and evidence for a hatching enzyme. Dev Biol 38:1–13

Cohen KL, Seid MA, Warkentin KM (2016) How embryos escape from danger: the mechanism of rapid, plastic hatching in red-eyed treefrogs. J Exp Biol 219:1875

Dang T, Searle CL, Blaustein AR (2017) Virulence variation among strains of the emerging infectious fungus Batrachochytrium dendrobatidis (Bd) in multiple amphibian host species. Dis Aquat Organ 124:233–239

Daszak P, Strieby A, Cunningham AA, Longcore J, Brown C, Porter D (2004) Experimental evidence that the bullfrog (Rana catesbeiana) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. Herpetol J 14:201–207

Develey-Rivière M-P, Galiana E (2007) Resistance to pathogens and host developmental stage: a multifaceted relationship within the plant kingdom. New Phytol 175:405–416

Doddington BJ, Bosch J, Oliver JA, Grassly NC, Garcia G, Schmidt BR, Garner TWJ, Fisher MC (2013) Context-dependent amphibian host population response to an invading pathogen. Ecology 94:1795–1804

Echaubard P, Pauli BD, Trudeau VL, Lesbarrères D (2016) Ranavirus infection in northern leopard frogs: the timing and number of exposures matter. J Zool 298:30–36

Eskew EA, Worth SJ, Foley JE, Todd BD (2015) American Bullfrogs (Lithobates catesbeianus) Resist Infection by Multiple Isolates of Batrachochytrium dendrobatidis, Including One Implicated in Wild Mass Mortality. EcoHealth 12:513–518

Farrer RA, Weinert LA, Bielby J, Garner TWJ, Balloux F, Clare F, Bosch J, Cunningham AA, Weldon C, Preez LH du, Anderson L, Pond SLK, Shahar-Golan R, Henk DA, Fisher MC (2011) Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. Proc Natl Acad Sci 108:18732–18736

Fernández-Benéitez MJ, Ortiz-Santaliestra ME, Lizana M, Diéguez-Uribeondo J (2008) Saprolegnia diclina: another species responsible for the emergent disease “Saprolegnia infections” in amphibians. FEMS Microbiol Lett 279:23–29

Fernández-Benéitez M, Ortiz-Santaliestra M, Lizana M, Diéguez-Uribeondo J (2011) Differences in susceptibility to Saprolegnia infections among embryonic stages of two anuran species. Oecologia 165:819–826

Gahl MK, Longcore JE, Houlahan JE (2012) Varying Responses of Northeastern North American Amphibians to the Chytrid Pathogen Batrachochytrium dendrobatidis. Conserv Biol 26:135–141

Garcia TS, Urbina J, Bredeweg E, Ferrari MC. (2017) Embryonic learning and developmental carry-over effects in an invasive anuran. Oecologia

Garner TW., Perkins MW, Govindarajulu P, Seglie D, Walker S, Cunningham AA, Fisher MC (2006) The emerging amphibian pathogen Batrachochytrium dendrobatidis globally infects introduced populations of the North American bullfrog, Rana catesbeiana. Biol Lett 2:455–459

Garner TWJ, Walker S, Bosch J, Leech S, Marcus Rowcliffe J, Cunningham AA, Fisher MC (2009) Life history tradeoffs influence mortality associated with the amphibian pathogen Batrachochytrium dendrobatidis. Oikos 118:783–791

Gervasi S, Gondhalekar C, Olson DH, Blaustein AR (2013) Host Identity Matters in the Amphibian-Batrachochytrium dendrobatidis System: Fine-Scale Patterns of Variation in Responses to a Multi-Host Pathogen. PLoS ONE 8:e54490

Gervasi SS, Stephens PR, Hua J, Searle CL, Xie GY, Urbina J, Olson DH, Bancroft BA, Weis V, Hammond JI, Relyea RA, Blaustein AR (2017) Linking Ecology and Epidemiology to Understand Predictors of Multi-Host Responses to an Emerging Pathogen, the Amphibian Chytrid Fungus. PLOS ONE 12:e0167882

Gervasi SS, Urbina J, Hua J, Chestnut T, Relyea RA, Blaustein AR (2013) Experimental evidence for American bullfrog (Lithobates catesbeianus) susceptibility to chytrid fungus (Batrachochytrium dendrobatidis). EcoHealth 10:166–171

Goater CP (1994) Growth and survival of postmetamorphic toads: Interactions among larval history, density, and parasitism. Ecology 75:2264–2274

Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190

Green DE, Converse KA (2005) Diseases of amphibian eggs and embryos. In: S.K. Majumdar, J.E. Huffman, F.J. Brenner, A.I. Panah (eds) Wildlife Diseases: Landscape Epidemiology, Spatial Distribution and Utilization of Remote Sensing Technology. The Pennsylvania Academy of Science, Easton, PA, p 62–71

Haislip NA, Gray MJ, Hoverman JT, Miller DL (2011) Development and disease: How susceptibility to an emerging pathogen changes through anuran development. PLoS ONE 6:e22307

Hamdoun A, Epel D (2007) Embryo stability and vulnerability in an always changing world. Proc Natl Acad Sci 104:1745–1750

Hanselmann R, Rodríguez A, Lampo M, Fajardo-Ramos L, Alonso Aguirre A, Marm Kilpatrick A, Paul Rodríguez J, Daszak P (2004) Presence of an emerging pathogen of amphibians in introduced bullfrogs Rana catesbeiana in Venezuela. Biol Conserv 120:115–119

Hatcher MJ, Dick JTA, Dunn AM (2012) Disease emergence and invasions. Funct Ecol 26:1275–1287

Holgersson MCN, Nichols WA, Paitz RT, Bowden RM (2016) How important is the eggshell as a source for initial acquisition of Salmonella in hatchling turtles? J Exp Zool Part Ecol Genet Physiol 325:142–148

Hyatt AD, Boyle DG, Olsen V, Boyle DB, Berger L, Obendorf D, Dalton A, Kriger K, Hero M, Hines H, Phillott R, Campbell R, Marantelli G, Gleason F, Colling A (2007) Diagnostic assays and sampling protocols for the detection of Batrachochytrium dendrobatidis. Dis Aquat Organ 73:175–192

Jones LLC, Leonard WP, Olson DH (2005) Amphibians of the Pacific Northwest. Seattle Audubon Society, Seattle

Kelehear C, Webb JK, Shine R (2009) Rhabdias pseudosphaerocephala infection in Bufo marinus: Lung nematodes reduce viability of metamorph cane toads. Parasitology 136:919–927

Kiesecker JM, Blaustein AR (1995) Synergism between UV-B radiation and a pathogen magnifies amphibian embryo mortality in nature. Proc Natl Acad Sci U S A 92:11049–11052

LaFiandra EM, Babbitt KJ (2004) Predator induced phenotypic plasticity in the pinewoods tree frog, Hyla femoralis: Necessary cues and the cost of development. Oecologia 138:350–359

Liew N, Mazon Moya MJ, Wierzbicki CJ, Hollinshead M, Dillon MJ, Thornton CR, Ellison A, Cable J, Fisher MC, Mostowy S (2017) Chytrid fungus infection in zebrafish demonstrates that the pathogen can parasitize non-amphibian vertebrate hosts. Nat Commun 8:15048

Marcum R, St-Hilaire S, Murphy P, Rodnick K (2010) Effects of Batrachochytrium dendrobatidis infection on ion concentrations in the boreal toad Anaxyrus (Bufo) boreas boreas. Dis Aquat Organ 91:17–21

Mast J, Goddeeris BM (1999) Development of immunocompetence of broiler chickens. Vet Immunol Immunopathol 70:245–256

McMahon TA, Brannelly LA, Chatfield MWH, Johnson PTJ, Joseph MB, McKenzie VJ, Richards-Zawacki CL, Venesky MD, Rohr JR (2013) Chytrid fungus Batrachochytrium dendrobatidis has nonamphibian hosts and releases chemicals that cause pathology in the absence of infection. Proc Natl Acad Sci 110:210–215

Moss A, Carty N, San Francisco M (2010) Identification and partial characterization of an elastolytic protease in the amphibian pathogen Batrachochytrium dendrobatidis. Dis Aquat Organ 92:149–158

Murillo-Rincón AP, Laurila A, Orizaola G (2017) Compensating for delayed hatching reduces offspring immune response and increases life-history costs. Oikos 126:565–571

Muths E, Corn PS, Pessier AP, Green DE (2003) Evidence for disease-related amphibian decline in Colorado. Biol Conserv 110:357–365

Olson DH, Aanensen DM, Ronnenberg KL, Powell CI, Walker SF, Bielby J, Garner TWJ, Weaver G, Fisher MC, The Bd Mapping Group (2013) Mapping the Global Emergence of Batrachochytrium dendrobatidis, the Amphibian Chytrid Fungus. PLoS ONE 8:e56802

Ortiz-Santaliestra ME, Rittenhouse TAG, Cary TL, Karasov WH (2013) Interspecific and Postmetamorphic Variation in Susceptibility of Three North American Anurans to Batrachochytrium dendrobatidis. J Herpetol 47:286–292

Pechenik JA (2006) Larval experience and latent effects—metamorphosis is not a new beginning. Integr Comp Biol 46:323–333

Piovia-Scott J, Pope K, Joy Worth S, Rosenblum EB, Poorten T, Refsnider J, Rollins-Smith LA, Reinert LK, Wells HL, Rejmanek D, Lawler S, Foley J (2015) Correlates of virulence in a frog-killing fungal pathogen: evidence from a California amphibian decline. ISME J 9:1570–1578

Piovia-Scott J, Pope KL, Lawler SP, Cole EM, Foley JE (2011) Factors related to the distribution and prevalence of the fungal pathogen Batrachochytrium dendrobatidis in Rana cascadae and other amphibians in the Klamath Mountains. Biol Conserv 144:2913–2921

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

Reeder NM, Pessier AP, Vredenburg VT (2012) A reservoir species for the emerging amphibian pathogen Batrachochytrium dendrobatidis thrives in a landscape decimated by disease. PLoS One 7:e33567

Retallick RWR, Miera V (2007) Strain differences in the amphibian chytrid Batrachochytrium dendrobatidis and non-permanent, sub-lethal effects of infection. Dis Aquat Organ 75:201–207

Retallick RWR, Miera V, Richards KL, Field KJ, Collins JP (2006) A non-lethal technique for detecting the chytrid fungus Batrachochytrium dendrobatidis on tadpoles. Dis Aquat Organ 72:77–85

Richter-Boix A, Orizaola G, Laurila A (2014) Transgenerational phenotypic plasticity links breeding phenology with offspring life-history. Ecology 95:2715–2722

Rohr JR, Raffel TR, Hall CA (2010) Developmental variation in resistance and tolerance in a multi-host–parasite system. Funct Ecol 24:1110–1121

Rosenblum EB, James TY, Zamudio KR, Poorten TJ, Ilut D, Rodriguez D, Eastman JM, Richards-Hrdlicka K, Joneson S, Jenkinson TS, Longcore JE, Parra Olea G, Toledo LF, Arellano ML, Medina EM, Restrepo S, Flechas SV, Berger L, Briggs CJ, Stajich JE (2013) Complex history of the amphibian-killing chytrid fungus revealed with genome resequencing data. Proc Natl Acad Sci 110:9385–9390

Rumschlag SL, Boone MD (2015) How Time of Exposure to the Amphibian Chytrid Fungus Affects Hyla chrysoscelis in the Presence of an Insecticide. Herpetologica 71:169–176

Saka M, Tada N, Kamata Y (2013) Application of an amphibian (Silurana tropicalis) metamorphosis assay to the testing of the chronic toxicity of three rice paddy herbicides: Simetryn, mefenacet, and thiobencarb. Ecotoxicol Environ Saf 92:135–143

Schloegel LM, Toledo LF, Longcore JE, Greenspan SE, Vieira C., Lee M, Zhao S, Wangen C, Ferreira CM, Hipolito M, Davies AJ, Cuomo CA, Daszak, P P, James TY (2012) Novel, panzootic and hybrid genotypes of amphibian chytridiomycosis associated with the bullfrog trade. Mol Ecol 21:5162–5177

Searle CL, Belden LK, Du P, Blaustein AR (2014) Stress and chytridiomycosis: Exogenous exposure to corticosterone does not alter amphibian susceptibility to a fungal pathogen. J Exp Zool Part Ecol Genet Physiol 321:243–253

Searle CL, Gervasi SS, Hua J, Hammond JI, Relyea RA, Olson DH, Blaustein AR (2011) Differential Host Susceptibility to Batrachochytrium dendrobatidis, an Emerging Amphibian Pathogen. Conserv Biol 25:965–974

Sniegula S, Janssens L, Stoks R (2017) Integrating multiple stressors across life stages and latitudes: Combined and delayed effects of an egg heat wave and larval pesticide exposure in a damselfly. Aquat Toxicol 186:113–122

Symonds EP, Trott DJ, Bird PS, Mills P (2008) Growth Characteristics and Enzyme Activity in Batrachochytrium dendrobatidis Isolates. Mycopathologia 166:143–147

Tobler U, Schmidt BR (2010) Within- and Among-Population Variation in Chytridiomycosis-Induced Mortality in the Toad Alytes obstetricans. PLOS ONE 5:e10927

Uller T, Sagvik J, Olsson M (2009) Pre-hatching exposure to water mold reduces size at metamorphosis in the moor frog. Oecologia 160:9–14

Valkenburg SA, Venturi V, Dang THY, Bird NL, Doherty PC, Turner SJ, Davenport MP, Kedzierska K (2012) Early Priming Minimizes the Age-Related Immune Compromise of CD8+ T Cell Diversity and Function. PLOS Pathog 8:e1002544

Voyles J, Young S, Berger L, Campbell C, Voyles WF, Dinudom A, Cook D, Webb R, Alford RA, Skerratt LF, Speare R (2009) Pathogenesis of Chytridiomycosis, a Cause of Catastrophic Amphibian Declines. Science 326:582–585

Warkentin KM, Currie CR, Rehner SA (2001) Egg-killing fungus induces early hatching of red-eyed treefrog eggs. Ecology 82:2860–2869

CHAPTER 3 - HOST-PATHOGEN DYNAMICS AMONG THE INVASIVE AMERICAN BULLFROG (*LITHOBATES CATESBEIANUS*) AND CHYTRID FUNGUS (*BATRACHOCHYTRIUM DENDROBATIDIS*)

Jenny Urbina[1], Evan Bredeweg[2], Tiffany S. Garcia[2], Andrew R. Blaustein[3]

[1] Environmental Sciences Graduate Program, Oregon State University, Corvallis Oregon 97331
[2] Department of Fisheries and Wildlife, Oregon State University, Corvallis Oregon 97331
[3] Department of Integrative Biology, Oregon State University, Corvallis Oregon 97331

Abstract

The combination of introduced host species and emerging pathogens can result in unanticipated disease dynamics and novel host-pathogen interactions. The American bullfrog (*Lithobates catesbeianus*) is a successful invasive amphibian in the western U.S. that can act as a host to the emerging fungal pathogen, *Batrachochytrium dendrobatidis* (Bd) that has been implicated in the decline of amphibian populations worldwide. However, how host-pathogen interactions vary across populations and strains is relatively unknown. We experimentally examined if wild caught invasive bullfrogs were differentially susceptible to two regionally distinct isolates of Bd. Newly metamorphosed bullfrog individuals were exposed to either a Bd strain originally isolated from bullfrogs in their endemic range or a strain from the invaded range in the western USA. Juvenile bullfrogs were collected from a breeding site in the western U.S. where the western US strain of Bd is also found. We quantified initial infection load of bullfrogs and compared mortality rates and changes in infection load after 30 days to determine strain-specific susceptibility. We found that wild caught bullfrogs from the western U.S. were particularly susceptible to an eastern Bd strain (JEL 627). In contrast, infection loads and survival were higher in individuals exposed to the western strain (JEL 630); suggesting individuals were more susceptible to a novel strain. Individuals infected upon collection were able to clear their infection over time. We found lower infection loads over time in individuals exposed to the western strain, indicating the ability to clear this infection. This suggests rapid evolution of resistance to sympatric pathogens and indicates a cost to maintaining resistance to historic strains.

Introduction

Biodiversity loss threatens ecosystem function and ecosystem services worldwide (Naeem et al., 1999; Balvanera et al., 2006; Oliver et al., 2015). Habitat transformation, introduction of invasive species, pollution, overpopulation, and overexploitation are human activities explaining the unprecedented biodiversity loss (Brook et al., 2008; Butchart et al., 2010; Barnosky et al., 2012; Dirzo et al., 2014). Another global threat for biodiversity conservation and human health are emerging infectious diseases (EID's) (Fisher et al., 2012; Tompkins et al., 2015). EID's may have substantial ecological and economic costs (Hatcher et al., 2012). Population declines and extinctions across multiple taxa have been associated with EID's (Daszak et al., 2000; Frick et al., 2010; Rogers & Miller, 2013; Lorch et al., 2015). For example, amphibians represent one of the most threatened vertebrate group whose numbers have been decimated worldwide, in part, by disease (Skerratt et al., 2007; Crawford et al., 2010; Olson et al., 2013).

The fungal pathogen *Batrachochytrium dendrobatidis* (Bd) is especially prominent with regard to amphibians population declines, range reductions and extinctions (Hatcher et al., 2012; Berger et al., 2016). Bd infects more than 600 amphibian species globally (Olson et al. 2013) and recent distribution models suggest shifts and potential expansion in Bd ranges under projected scenarios of climate change (Xie et al., 2016). Bd causes chytridiomycosis, which can cause excessive skin shedding, loss of reflex, lethargy and mortality in susceptible juveniles and adults. In tadpoles, chytridiomycosis affects mainly mouthpart structures (Voyles et al., 2009; Brutyn et al., 2012), although mortality can occur when larvae are exposed to Bd (Blaustein et al., 2005; Garner et al., 2009; Searle et al., 2013). Susceptibility to Bd varies across host species (Blaustein et al., 2005; Searle et al., 2011; Gahl et al., 2012; Bielby et al., 2015; Gervasi et al., 2017), population (Tobler & Schmidt, 2010; Bradley et al., 2015), life stage (Blaustein et al., 2005;

Briggs et al., 2010; Ortiz-Santaliestra et al., 2013; Searle et al., 2013) and, pathogen strain (Berger et al. 1998, Retallick & Miera 2007, Gervasi et al. 2013, Doddington et al. 2013, Eskew et al. 2015).

Despite extensive research effort since the discovery of Bd, differential impacts of Bd strain on amphibian hosts are poorly understood (Morehouse et al., 2003; Retallick & Miera, 2007; Farrer et al., 2011; Gahl et al., 2012). The response of a host to a particular strain gives us insight about virulence of the pathogen as well as tolerance and resistance of the host. Multiple lineages of Bd have been identified using genetic and genomic information from multiple geographic locations (Farrer et al., 2011; Rosenblum et al., 2013). Pathogenicity of these lineages can differ according to amphibian host and/or location (Schloegel et al., 2012). Some Bd Global pandemic lineage (Bd-GPL) strains, however, have shown different virulence levels when tested in common hosts (Berger et al., 1998), highlighting the need for additional research on strain-specific interactions.

Several amphibian hosts are considered to be more resistant to Bd than other. Among anuran amphibians, it has been suggested that the American bullfrog (*Lithobates catesbeianus*) is a relatively tolerant carrier of Bd, harboring the pathogen without signs of morbidity or mortality (Daszak et al., 2004; Garner et al., 2006). However, reports of mass mortality events in farmed American bullfrog populations suggested that exposure to novel Bd strains may cause chytridiomycosis outbreaks (Mazzoni et al., 2003). Moreover, Gervasi et al. (2013) found differential susceptibility in juvenile bullfrogs experimentally exposed to different Bd strains. As such, American bullfrogs offer a unique opportunity to study the ecological and evolutionary relationship between an EID and its host.

Bullfrogs are endemically distributed in the east and central regions of the United States and have established wild invasive populations after introductions in the western US (Moyle, 1973) and other continents (Ficetola et al., 2007; Nori et al., 2011) and island chains around the world (Lever, 2003). In their invaded range, direct and indirect effects of bullfrogs on native amphibian species have been documented by field surveys and experimental studies (Kats & Ferrer, 2003; Bucciarelli et al., 2014). Direct effects occurred by predation (D’Amore et al., 2009) and competition (Both & Grant, 2012; Preston et al., 2012; Medeiros et al., 2017) while indirect effects involved altering the use of habitat (D’Amore et al., 2009), changes in behavior (Kiesecker et al., 2001), and changes in activity and refuge use (Kiesecker & Blaustein, 1997, 1998). Other indirect effect involved the role play by bullfrogs as disease vector (Garner et al., 2006; Greenspan et al., 2012), especially of chytridiomycosis.

Bd strains have been isolated from bullfrogs in their native and invasive ranges (Schloegel et al., 2012) with the earliest detection of Bd in invasive California populations reported from specimens in 1928 (Huss et al., 2013). We investigated if invasive bullfrogs in Oregon USA had differential susceptibility to Bd strains isolated from their endemic and invasive ranges. Therefore, we experimentally exposed wild caught juveniles to Bd isolated from bullfrogs in Maine, USA (eastern strain, JEL 627) and Bd isolated from bullfrogs in Oregon, USA (western strain, JEL 630). We used wild caught animals to estimate infection loads at the time of capture and to estimate the impact of previous exposure on strain specific susceptibility. While virulence typically depends on the interaction among host, pathogen and environment (Poulin & Combes, 1999), some Bd traits such as zoosporangium size (Fisher et al., 2009), inhibition of growth in immune cells (Fites et al., 2013) and zoospores production (Langhammer et al., 2013) have been linked to virulence. We quantified the mean number of zoospores produced by Bd while in culture in agar media to identify activity differences in both Bd strains outside the host. While a high zoospore number can be linked mechanistically to a high infection rate (Briggs et al., 2010); some strains with low zoospore production can still have major impacts on their host. We hypothesized that bullfrogs from Oregon would show greater mortality when exposed to a novel Bd strain (eastern strain). We also hypothesized that individuals infected at the time of capture would be more susceptible to a novel strain.

Materials and methods

We collected 90 recently juvenile bullfrogs at Gosner stage 45 when a tail stub was still detected in the individuals (Gosner, 1960), ($\bar{x}$ = 3.3–8.9 g, SD = 5.6 g ± 1.15) from a seasonal pond with no resident fish populations (44°24'47.0"N 123°19'38.0"W) in William L. Finley National wildlife refuge, OR (USA). Our survey followed a Visual Encounter Survey method (VES); we used head-lamps and manual flashlights to spotlight individual frogs on the vegetation along the margin of the pond. Once an animal was detected, we hand-collected the individual wearing new gloves per individual to avoid cross contamination.

Initial Infection Load Assessment

We handled each individual with fresh gloves and swabbed fifteen strokes along the ventral side and along each thigh and rear foot using one sterile swab per individual (MW113, Medical Wire & Equipment). Swabs were placed in sterile 1.5 ml microcentrifuge tubes and kept on a cooler with ice. We immediately transported the frogs and swabs to Oregon State University in individual containers and randomly assigned individuals to one of three experimental treatments: Eastern Bd treatment (strain JEL 627), Western Bd treatment (strain JEL 630) or control (No Bd). Although the use of terbinafine hydrochlorine in ethanol has been found as effective at curing infection in *L. catesbeianus* (Bowerman et al., 2010), we did not use this substance or a different fungicide as their action could change the response of the individuals when exposed to the pathogen. We use wild caught animals to obtain information about how previous exposure in nature affect the response of individuals when repeatedly exposed to Bd.

Bd Culture Methodology

Bd strain JEL 627 is an isolate from the native range of American bullfrogs in Maine, USA; JEL 630 was isolated from the local invasive range of American bullfrogs in Oregon, USA. Both strains were obtained from cryogenically preserved material by J. Longcore in May 2013. Colonies from the plates were sterilely moved into tryptone broth 1% before being plated in sterile agar for at least 7 days before inoculation. For control animals (n=30), inoculum without Bd but from sterile agar plates was used (Searle et al. 2011, Gervasi et al. 2013).

Bd virulence Methodology

To quantify the virulence of Bd, we counted the number of zoospores produced in culture over time for both strains JEL 627 and JEL 630 (i.e., strain growth rate). We cultured on the same day: 50 agar plates per strain using 1 ml of tryptone broth 1% per plate. After 6 days of culture, we harvested zoospores from 5 plates per strain using 10 ml of water and counted the number of active zoospores using a hemocytometer. We repeated the harvesting and counting of zoospores on days 8 through 15. We stopped our observations when the number of zoospores and their activity began to decline (Day 15).

Bd Exposure Methodology

We housed frogs individually in petri dishes (140 x 30 mm) with holes in the lid and 10 ml of water covering the bottom. All units were held at 18 ° C and on a 12hr light: 12hr dark photoperiod. Animals were acclimated for three days and then exposed to one of three treatment groups: JEL 627 (n=30), JEL 630 (n=30), or a control. Individuals were given to a concentration of 1.7 X 10^4 zoospores/ml in 15 ml of total inoculate weekly.

Survival of individuals was monitored for 30 days post initial treatment exposure. Individuals found dead during the experiment were immediately preserved in 95% ethanol. After 30 days, all surviving animals were euthanized (MS-222) and preserved in 95% ethanol. All animals were swabbed after preservation following the same protocol used to assess initial infection loads (15 strokes along the ventral side and along each thigh and rear foot using one sterile swab per individual). We quantified infection load of all animals before and after the experiment using quantitative-PCR (qPCR) (Boyle et al., 2004). All samples were analyzed in triplicate and reported as positive when replicates showed Bd DNA in at least two wells. Average number of genome equivalents per individual (infection loads) were log transformed to normalize data distribution during statistical analysis.

Statistical Analysis

Using a *post hoc* analysis of variance (ANOVA), we evaluated if initial infection load differed among individuals randomly assigned to the treatments. We hypothesized that some individuals would be infected with the western Bd strain upon capture, thus we used a linear regression model to determine if body size (snout-vent length) or body condition (Băncilă et al., 2010) impacted initial Bd infection loads. We used an analysis of covariance (ANCOVA) to determine treatment effects on infection loads upon death or at the termination of the experiment controlling for initial infection load.

Using Kaplan-Meier analyses, we compared survival of animals in control versus Bd-exposed treatments (JEL 627 and JEL 630). We used a Cox's proportional hazards model to statistically compare survival of each treatment group and its associated "hazard ratio" as well as the effect of initial infection load. A hazard ratio including 1 indicates there is no difference in the probability of mortality associated with a factor, in a comparative way (a hazard ratio > 1 indicates an increase in the probability of mortality). To analyze differences in growth rate between strains, we calculated a log-level multiple linear regression to predict mean number of zoospores based on strains (treatments) and time. Statistical analyses were performed in R (version 3.3.1, 2016).

Results

The overall prevalence of Bd in wild-caught juveniles of *L. catesbeianus* was 43 %, with 39 out of 90 frogs testing positive for Bd at the time of capture (Table 3.1). Individuals were randomly assigned to treatment groups without a priori information on infection status. Treatment groups were significantly different in proportion of initially infected individuals and infections loads ($F_{2, 87} = 4.52$, $p < 0.001$), with the control group having a high number of individuals with higher infection loads than either exposure group (Table 3.1). However, initial infection load was not a significant predictor of infection load after the experiment ($F_{1,86} = 0.29$, $p = 0.59$).

Infection loads of juveniles collected in the field were in average 11.2 genome equivalents and this initial infection load was not related with snout-vent length ($t_{87} = 1.71$, $p = 0.09$) or body condition ($t_{87} = 0.17$, $p = 0.86$) of the animals. At the termination of the experiment, we found reduced infection loads in the control treatment. As expected, animals exposed to Bd strains during the experiment had significantly higher infection loads than controls, with the JEL 630 (t_{86} = 3.47, $p < 0.001$) group showing higher loads than JEL 627 (t_{86} = 2.63, $p = 0.01$). Infection loads after 30 days of treatment exposure were largely explained by treatment level ($F_{2,86}$ = 6.34, $p = 0.0027$, Table 3.2 and Figure 3.1).

After experimental exposure to Bd treatments, the rate of mortality in animals exposed to JEL 627 was significantly greater than the rate of mortality in control animals (Figure 3.2, Cox proportional hazards model $p < 0.008$; hazard ratio = 3.7). The rate of mortality of individuals exposed to JEL 630 was not significantly greater than the rate of mortality in control animals (Figure 3.3; Cox proportional hazards model $p = 0.63$; hazard ratio = 0.72). During the first 15 days of the experiment, 15 of 30 animals died when exposed to JEL 627. In comparison, only 4 animals died when exposed to JEL 630 (Figure 3.3) in the first half of the experiment. These treatment effects were independent of initial infection load which was included in the Cox model, but did not significantly influence mortality (Figure 3.3; Cox proportional hazards model $p=0.74$; hazard ratio = 1.11). The following log-level multiple linear regression model predicted the number of zoospores for strains in culture as -2.15 + 0.28 (days) + 0.07 (strain) ($F_{2,13}$ = 70.42, $p < 0.001$, R- square = 0.9155). There was a significant effect of strain on growth rate ($F_{1,13}$ = 22.73, $p = 0.00036$); JEL 627 had a 7.1% higher growth rate per day after culture than JEL 630 (CI 3.98 to 10.95%) (Figure 3.4).

Discussion

Wild-caught American bullfrog (*L. catesbeianus*) juveniles from a population within their western USA invasion range were susceptible to a novel Bd strain. In a factorial experiment, we found that bullfrogs exposed to a Bd strain isolated from the bullfrog's endemic range (eastern strain, JEL 627) suffered higher mortality rates compared to controls (no Bd exposure), bullfrogs exposed to a western Bd strain (JEL 630) isolated from Oregon. During the first half of the experiment, almost 50% of the individuals exposed to the eastern Bd strain died. In contrast, 86 % of the individuals exposed to the western Bd strain survived and harbored a higher pathogen burden. This suggests strain-specific resistance in this invasive anuran population.

We found that 43% of the bullfrog juveniles were infected with Bd prior to experimental exposure. Interestingly, 19 out of 30 infected individuals randomly placed within the control group were able to clear the infection over time. The initial infection loads may not have reached a threshold for the onset of disease (McConnell, 2007). The levels of initial Bd infection were low in terms of prevalence and intensity (mean 11.1 raw genome equivalents), and were similar to other wild-caught bullfrogs swab samples from the USA (Garner et al., 2006; Schloegel et al., 2009; Walke et al., 2015).

Lower infection loads were detected in individuals exposed to the eastern strain upon termination of the study. This observed reduction in infection load was not associated with a lower incidence of the disease as concluded in several chytridiomycosis studies (Stockwell et al. 2010, Gervasi et al. 2013). These lower infections loads were related to an increased probability of mortality in animals exposed to the eastern strain. Conversely, individuals exposed to the western strain had higher infection loads and a lower mortality risk. We posit that this is a statistical relationship and argue for closer examination of the relationships between infection load, morbidity and strain pathogenicity.

Infection loads can vary considerably among individuals due to host susceptibility but also to pathogen virulence (Beldomenico & Begon, 2010). Although we did not characterize individual host immunity via immune response (see Gervasi et al. 2013), we characterized the eastern and western strain growth rate while in culture to infer virulence (Fisher et al 2009, Langhammer et al 2013). Our results indicate that the eastern strain had a higher growth rate relative to the western JEL 630 while in culture. A greater number of active zoospores through time could lead to an increase in the risk of mortality in animals exposed to JEL 627. However, *in-vitro* growth rates of the pathogen are not always consistent with pathogen growth in susceptible hosts. Strains with lower *in-vitro* growth rates can represent higher Bd loads in their hosts (Piovia-Scott et al., 2015). In this study, bullfrogs exposed to the novel eastern strain with an higher *in vitro* growth rate were more susceptible and died faster, even with a lower infection load relative to animals exposed to the western strain.

Individuals exposed to a novel Bd strain can experience a higher mortality risk than individuals exposed to strains isolated from conspecifics (Gervasi et al., 2013b; Eskew et al., 2015). When exposed to the native strain, individuals have high infection loads and survive which could be indicative of co-evolutionary dynamics. The host-pathogen interaction in a particular geographic distribution is expected to lead toward coexistence, with reduced susceptibility in the host and reduced pathogenicity in the pathogen (Doddington et al., 2013).

Bd is an emerging pathogen globally (Olson et al., 2013; Balaz et al., 2014; Van Rooij et al., 2015) and it is projected to spread with changes in climate (Liu et al., 2012; Xie et al., 2016). In the absence of a shared evolutionary history, the impact of a new Bd strain on the host is a higher probability of host mortality. Invasive species capable of transporting novel strains to new geographic ranges can potentially cause chytridiomycosis outbreaks with unusual severity and magnitude (Farrer et al., 2011; Van Rooij et al., 2015). This disruption of evolved trade- offs between the host and the pathogen can be devastating to local amphibian assemblages. At least 17 different Bd strains have been isolated from *L. catesbeianus* from different geographic distributions (Schloegel et al., 2012) and archived collections from the California Academy of Sciences (CAS) reported the presence of Bd in specimens dated back to 1928 (Huss et al., 2013). While it is unknown which Bd strain was detected at 1928, we hypothesize that invasive bullfrogs in the western USA have coexisted with Bd, and after reaching stable pathogen-host equilibrium this strain is not virulent to their host. A similar result was found in *Taudactylus eungellensis*, a stream dwelling frog in Australia where populations were able to persist with endemic infections of Bd (Retallick et al., 2004).

Understanding the variation in host response to pathogens isolated from conspecifics in different distributional ranges is needed to understand how pathogen origin can mediate host response. The strains used in this experiment are part of the North American clade - Global Panzootic Lineage (Bd- GPL1). However, they are grouped within distinct clusters and thus vary in distributional range and heterozygosity (James et al., 2009; Rosenblum et al., 2013). Although the GPL contains many of the deadliest Bd isolates, our findings support there are differences in virulence properties inside this lineage that deserve more research.

In conclusion, this study underscores the importance of experimental studies to shed light on infection dynamics in invasive species and its implication in the movement of individuals to different geographic locations. Translocation of an invasive species will mean the arrival of not only of a potential predator and competitor of native species but also a host species harboring pathogens that affect natives. Arrival of non-native bullfrogs and their associated pathogens can represent an ‘invasional meltdown’ increasing their likelihood of survival and the magnitude of their ecological impacts (Simberloff & Von Holle, 1999). We need to evaluate the interactions among Bd strains from different geographic locations and susceptible hosts to understand if the interaction is facilitating or precluding the onset of a disease. In an era of emerging diseases and globalization, understanding the impacts of a novel strain can help managers better mitigate these dangers, potentially through stronger regulation of importation of live animals, reducing the trade of species and applying informed legislation in conservation actions.

Table 3.1 *Batrachochytrium dendrobatidis* (Bd) mean initial infection load values (raw genome equivalents GE) for all individuals upon field capture. Bd occurrences CI = 95% Clopper-Pearson binomial confidence interval for prevalence (%)

Assigned Exposure treatment (n=30 per treatment)	**Infection loads mean Bd raw GE (low-high)**	**Prevalence Bd No. Bd-positive / Total no. samples (%)**	**Prevalence Bd CI**
Control	21.8 (0-343)	26/30 (86)	69-96
JEL 627 (East strain)	10.7 (0-303)	8/30 (26)	12-45
JEL 630 (West strain)	0.8 (0- 12)	5/30 (16)	5-34
Total	11.1 (0-343)	39/90 (43)	32-54

Table 3.2 *Batrachochytrium dendrobatidis* (Bd) mean infection load values (raw genome equivalents GE) for all individuals after being Bd-exposed to JEL 627 and JEL 630. Bd occurrences CI = 95% Clopper-Pearson binomial confidence interval for prevalence (%)

Exposure treatment	**Infection loads mean Bd raw GE (low-high)**	**Prevalence Bd No. Bd-positive / Total no. samples (%)**	**Prevalence Bd CI**
Control	7.3 (0-145)	11/30 (36)	19-56
JEL 627 (East strain)	30.7 (0-613)	28/30 (93)	77-99
JEL 630 (West strain)	76.6 (0- 1721)	29/30 (96)	83-99

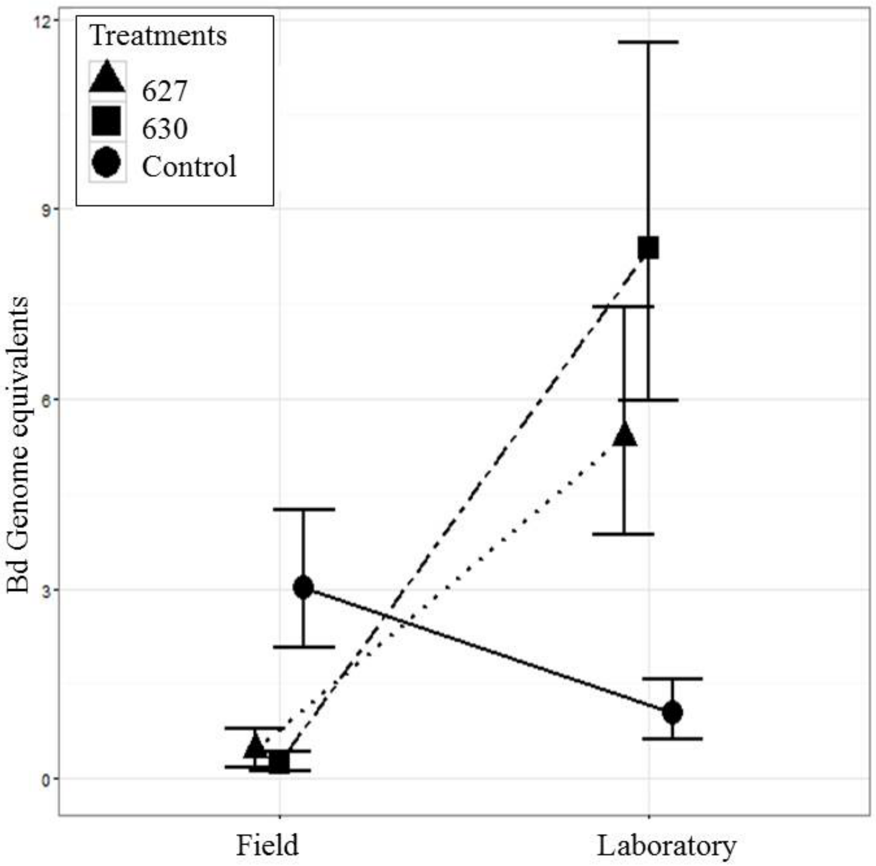


Figure 3.1 Genome equivalents of Bd before and after exposure to the pathogen. Bars represent standard error range. Field values represent infection loads of animals collected in the field. Laboratory infection loads represent animals after being exposed to a particular treatment. Individuals selected as controls decreased their infection loads through the experiment. Individuals exposed to JEL 630 were able to sustain higher infection loads than individuals exposed to JEL 627.

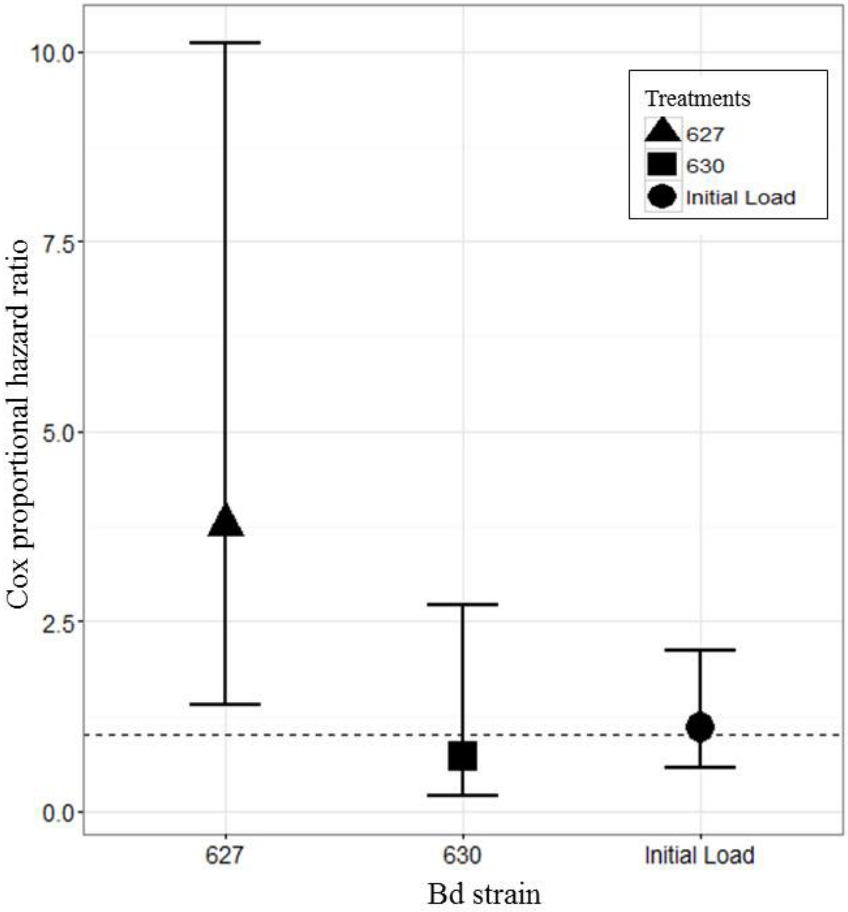


Figure 3.2: Cox proportional hazard ratios for factors of exposure treatment and initial infection load compared with a base level of one. Bars represent the 95% confidence interval for the hazard ratios. A hazard ratio of 1 indicates there is no difference in the probability of mortality associated with a factor, in a comparative way (a hazard ratio > 1 indicates an increase in the probability of mortality).

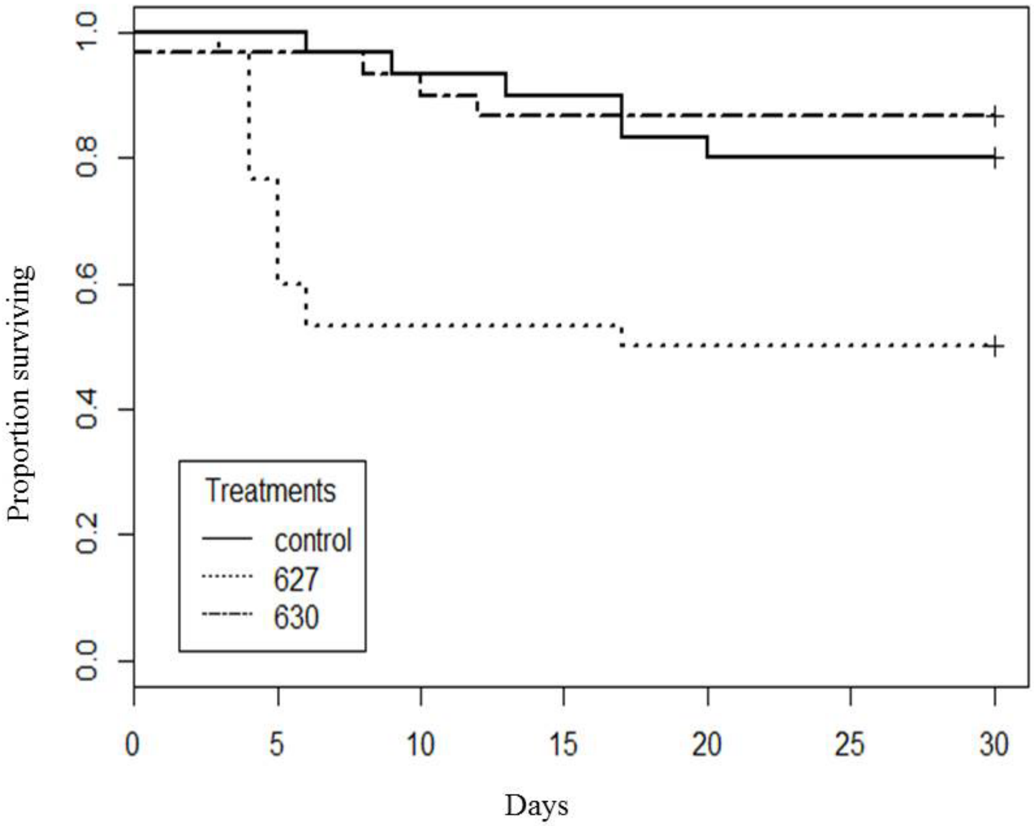


Figure 3.3: Survival curves of invasive bullfrogs after exposure to amphibian chytrid fungus strains JEL 627 (dotted lines) and JEL 630 (dashed line). Survival was significantly reduced in the pathogen treatment for JEL 627. No differences in survival occurred between Control (solid line) and pathogen-exposed animals in the JEL 630 treatment (dashed line)

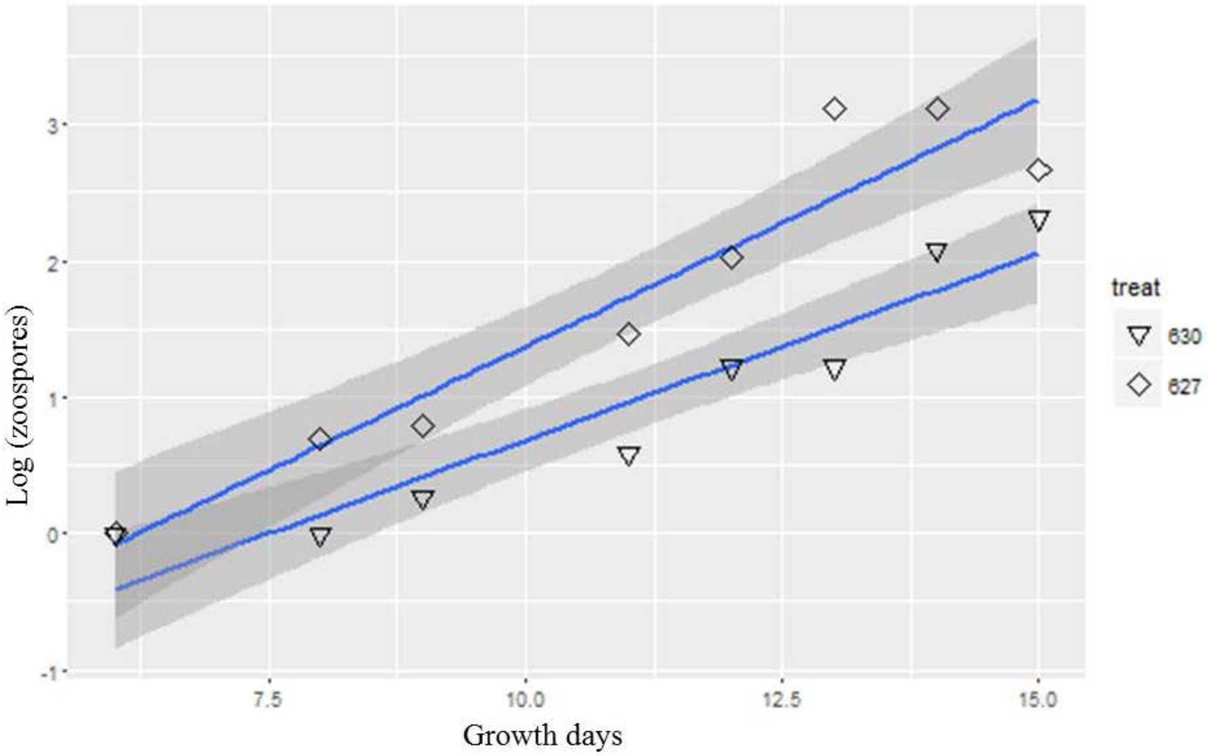


Figure 3.4 Growth curves in days after culture for two Bd strains. JEL 627 represented by diamonds (top line) and JEL 630 represented by triangle (bottom line). The shadow represents the estimated standard errors per strain.

Acknowledgments

Angie Soken, Trang Dang, Julia Buck, Cassidy Medellin for collection of samples. Funding Fulbright- Colciencias- Colombia and Society Northwestern Vertebrate Biology (SNVB)

Literature cited

Balaz, V., J. Voros, P. Civis, J. Vojar, A. Hettyey, E. Sos, R. Dankovics, R. Jehle, D. G. Christiansen, F. Clare, M. Fisher, T. Garner, & J. Bielby, 2014. Assessing Risk and Guidance on Monitoring of Batrachochytrium dendrobatidis in Europe through Identification of Taxonomic Selectivity of Infection. Conservation Biology 28: 213–223.

Balvanera, P., A. B. Pfisterer, N. Buchmann, J.-S. He, T. Nakashizuka, D. Raffaelli, & B. Schmid, 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecology Letters 9: 1146–1156.

Băncilă, R., T. Hartel, Plăiaşu Rodica, J. Smets, & D. Cogălniceanu, 2010. Comparing three body condition indices in amphibians: a case study of yellow-bellied toad Bombina variegata. Amphibia Reptilia 31: 558–562.

Barnosky, A. D., E. A. Hadly, J. Bascompte, E. L. Berlow, J. H. Brown, M. Fortelius, W. M. Getz, J. Harte, A. Hastings, P. A. Marquet, N. D. Martinez, A. Mooers, P. Roopnarine, G. Vermeij, J. W. Williams, R. Gillespie, J. Kitzes, C. Marshall, N. Matzke, D. P. Mindell, E. Revilla, & A. B. Smith, 2012. Approaching a state shift in Earth/'s biosphere. Nature 486: 52–58.

Beldomenico, P. M., & M. Begon, 2010. Disease spread, susceptibility and infection intensity: vicious circles?. Trends in Ecology & Evolution 25: 21–27.

Berger, L., A. A. Roberts, J. Voyles, J. E. Longcore, K. A. Murray, & L. F. Skerratt, 2016. History and recent progress on chytridiomycosis in amphibians. Aquatic Fungi 19: 89–99.

Berger, L., R. Speare, P. Daszak, D. E. Green, A. A. Cunningham, C. L. Goggin, R. Slocombe, M. A. Ragan, A. D. Hyatt, K. R. McDonald, H. B. Hines, K. R. Lips, G. Marantelli, & H. Parkes, 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Sciences 95: 9031–9036.

Bielby, J., M. C. Fisher, F. C. Clare, G. M. Rosa, & T. W. J. Garner, 2015. Host species vary in infection probability, sub-lethal effects, and costs of immune response when exposed to an amphibian parasite. Scientific Reports 5: 10828.

Blaustein, A. R., J. M. Romansic, E. A. Scheessele, B. A. Han, A. P. Pessier, & J. E. Longcore, 2005. Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus Batrachochytrium dendrobatidis. Conservation Biology 19: 1460–1468.

Both, C., & T. Grant, 2012. Biological invasions and the acoustic niche: the effect of bullfrog calls on the acoustic signals of white-banded tree frogs. Biology Letters 8: 714.

Bowerman, J., C. Rombough, S. R. Weinstock, & G. E. Padgett-Flohr, 2010. Terbinafine Hydrochloride in Ethanol Effectively Clears Batrachochytrium dendrobatidis in Amphibians. Journal of Herpetological Medicine and Surgery 20: 24–28.

Boyle, D., D. Boyle, V. Olsen, J. Morgan, & A. Hyatt, 2004. Rapid quantitative detection of chytridiomycosis (Batrachochytrium dendrobatidis) in amphibian samples using real-time Taqman PCR assay. Diseases of aquatic organisms 60: 141–148.

Bradley, P. W., S. S. Gervasi, J. Hua, R. D. Cothran, R. A. Relyea, D. H. Olson, & A. R. Blaustein, 2015. Differences in sensitivity to the fungal pathogen Batrachochytrium dendrobatidis among amphibian populations. Conservation Biology 29: 1347–1356.

Briggs, C. J., R. A. Knapp, & V. T. Vredenburg, 2010. Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. Proceedings of the National Academy of Sciences 107: 9695–9700.

Brook, B. W., N. S. Sodhi, & C. J. A. Bradshaw, 2008. Synergies among extinction drivers under global change. Trends in Ecology & Evolution 23: 453–460.

Brutyn, M., K. D’Herde, M. Dhaenens, P. V. Rooij, E. Verbrugghe, A. D. Hyatt, S. Croubels, D. Deforce, R. Ducatelle, F. Haesebrouck, A. Martel, & F. Pasmans, 2012. Batrachochytrium dendrobatidis zoospore secretions rapidly disturb intercellular junctions in frog skin. Fungal Genetics and Biology 49: 830–837.

Bucciarelli, G. M., A. R. Blaustein, T. S. Garcia, & L. B. Kats, 2014. Invasion Complexities: The Diverse Impacts of Nonnative Species on Amphibians. Copeia 2014: 611–632.

Butchart, S. H. M., M. Walpole, B. Collen, A. van Strien, J. P. W. Scharlemann, R. E. A. Almond, J. E. M. Baillie, B. Bomhard, C. Brown, J. Bruno, K. E. Carpenter, G. M. Carr, J. Chanson, A. M. Chenery, J. Csirke, N. C. Davidson, F. Dentener, M. Foster, A. Galli, J. N. Galloway, P. Genovesi, R. D. Gregory, M. Hockings, V. Kapos, J.-F. Lamarque, F. Leverington, J. Loh, M. A. McGeoch, L. McRae, A. Minasyan, M. H. Morcillo, T. E. E. Oldfield, D. Pauly, S. Quader, C. Revenga, J. R. Sauer, B. Skolnik, D. Spear, D. Stanwell-Smith, S. N. Stuart, A. Symes, M. Tierney, T. D. Tyrrell, J.-C. Vié, & R. Watson, 2010. Global Biodiversity: Indicators of Recent Declines. Science 328: 1164.

Crawford, A. J., K. R. Lips, & E. Bermingham, 2010. Epidemic disease decimates amphibian abundance, species diversity, and evolutionary history in the highlands of central Panama. Proceedings of the National Academy of Sciences 107: 13777–13782.

D’Amore, A., E. Kirby, & M. McNicholas, 2009. Invasive species shifts ontogenetic resource partitioning and microhabitat use of a threatened native amphibian. Aquatic Conservation: Marine and Freshwater Ecosystems 19: 534–541.

Daszak, P., A. A. Cunningham, & A. D. Hyatt, 2000. Emerging Infectious Diseases of Wildlife-- Threats to Biodiversity and Human Health. Science 287: 443–449.

Daszak, P., A. Strieby, A. A. Cunningham, J. Longcore, C. Brown, & D. Porter, 2004. Experimental evidence that the bullfrog (Rana catesbeiana) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. Herpetological Journal 14: 201–207.

Dirzo, R., H. S. Young, M. Galetti, G. Ceballos, N. J. B. Isaac, & B. Collen, 2014. Defaunation in the Anthropocene. Science 345: 401.

Doddington, B. J., J. Bosch, J. A. Oliver, N. C. Grassly, G. Garcia, B. R. Schmidt, T. W. J. Garner, & M. C. Fisher, 2013. Context-dependent amphibian host population response to an invading pathogen. Ecology 94: 1795–1804.

Eskew, E. A., S. J. Worth, J. E. Foley, & B. D. Todd, 2015. American Bullfrogs (Lithobates catesbeianus) Resist Infection by Multiple Isolates of Batrachochytrium dendrobatidis, Including One Implicated in Wild Mass Mortality. EcoHealth 12: 513–518.

Farrer, R. A., L. A. Weinert, J. Bielby, T. W. J. Garner, F. Balloux, F. Clare, J. Bosch, A. A. Cunningham, C. Weldon, L. H. du Preez, L. Anderson, S. L. K. Pond, R. Shahar-Golan, D. A. Henk, & M. C. Fisher, 2011. Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. Proceedings of the National Academy of Sciences 108: 18732–18736.

Ficetola, G. F., C. Coïc, M. Detaint, M. Berroneau, O. Lorvelec, & C. Miaud, 2007. Pattern of distribution of the American bullfrog Rana catesbeiana in Europe. Biological Invasions 9: 767–772.

Fisher, M. C., J. Bosch, Z. Yin, D. A. Stead, J. Walker, L. Selway, A. J. P. Brown, L. A. Walker, N. A. R. Gow, J. E. Stajich, & T. W. J. Garner, 2009. Proteomic and phenotypic profiling of the amphibian pathogen Batrachochytrium dendrobatidis shows that genotype is linked to virulence. Molecular Ecology 18: 415–429.

Fisher, M. C., D. A. Henk, C. J. Briggs, J. S. Brownstein, L. C. Madoff, S. L. McCraw, & S. J. Gurr, 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature 484: 186–194.

Fites, J. S., J. P. Ramsey, W. M. Holden, S. P. Collier, D. M. Sutherland, L. K. Reinert, A. S. Gayek, T. S. Dermody, T. M. Aune, K. Oswald-Richter, & L. A. Rollins-Smith, 2013. The Invasive Chytrid Fungus of Amphibians Paralyzes Lymphocyte Responses. Science 342: 366.

Frick, W. F., J. F. Pollock, A. C. Hicks, K. E. Langwig, D. S. Reynolds, G. G. Turner, C. M. Butchkoski, & T. H. Kunz, 2010. An Emerging Disease Causes Regional Population Collapse of a Common North American Bat Species. Science 329: 679.

Gahl, M. K., J. E. Longcore, & J. E. Houlahan, 2012. Varying Responses of Northeastern North American Amphibians to the Chytrid Pathogen Batrachochytrium dendrobatidis. Conservation Biology 26: 135–141.

Garner, T. W. ., M. W. Perkins, P. Govindarajulu, D. Seglie, S. Walker, A. A. Cunningham, & M. C. Fisher, 2006. The emerging amphibian pathogen Batrachochytrium dendrobatidis globally infects introduced populations of the North American bullfrog, Rana catesbeiana. Biology Letters 2: 455–459.

Garner, T. W. J., S. Walker, J. Bosch, S. Leech, J. Marcus Rowcliffe, A. A. Cunningham, & M. C. Fisher, 2009. Life history tradeoffs influence mortality associated with the amphibian pathogen Batrachochytrium dendrobatidis. Oikos 118: 783–791.

Gervasi, S., C. Gondhalekar, D. H. Olson, & A. R. Blaustein, 2013a. Host Identity Matters in the Amphibian-Batrachochytrium dendrobatidis System: Fine-Scale Patterns of Variation in Responses to a Multi-Host Pathogen. PLoS ONE 8: e54490.

Gervasi, S. S., P. R. Stephens, J. Hua, C. L. Searle, G. Y. Xie, J. Urbina, D. H. Olson, B. A. Bancroft, V. Weis, J. I. Hammond, R. A. Relyea, & A. R. Blaustein, 2017. Linking Ecology and Epidemiology to Understand Predictors of Multi-Host Responses to an Emerging Pathogen, the Amphibian Chytrid Fungus. PLOS ONE 12: e0167882.

Gervasi, S. S., J. Urbina, J. Hua, T. Chestnut, R. A. Relyea, & A. R. Blaustein, 2013b. Experimental evidence for American bullfrog (Lithobates catesbeianus) susceptibility to chytrid fungus (Batrachochytrium dendrobatidis). EcoHealth 10: 166–171.

Gosner, K. L., 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16: 183–190.

Greenspan, S. E., A. J. K. Calhoun, J. E. Longcore, & M. G. Levy, 2012. Transmission of Batrachochytrium dendrobatidis to wood frogs (Lithobates sylvaticus) via a bullfrog (L. catesbeianus) vector. Journal of Wildlife Diseases 48: 575–582.

Hatcher, M. J., J. T. A. Dick, & A. M. Dunn, 2012. Disease emergence and invasions. Functional Ecology 26: 1275–1287.

Huss, M., L. Huntley, V. Vredenburg, J. Johns, & S. Green, 2013. Prevalence of Batrachochytrium dendrobatidis in 120 Archived Specimens of Lithobates catesbeianus (American Bullfrog) Collected in California, 1924–2007. EcoHealth 10: 339–343.

James, T. Y., A. P. Litvintseva, R. Vilgalys, J. A. T. Morgan, J. W. Taylor, M. C. Fisher, L. Berger, C. Weldon, L. du Preez, & J. E. Longcore, 2009. Rapid Global Expansion of the Fungal Disease Chytridiomycosis into Declining and Healthy Amphibian Populations. PLOS Pathogens 5: e1000458.

Kats, L. B., & R. P. Ferrer, 2003. Alien predators and amphibian declines: review of two decades of science and the transition to conservation. Diversity and Distributions 9: 99–110.

Kiesecker, J. M., & A. R. Blaustein, 1997. Population differences in responses of Red legged frogs (Rana aurora) to introduced bullfrogs. Ecology 78: 1752–1760.

Kiesecker, J. M., & A. R. Blaustein, 1998. Effects of Introduced Bullfrogs and Smallmouth Bass on Microhabitat Use, Growth, and Survival of Native Red-Legged Frogs (Rana aurora). Conservation Biology 12: 776–787.

Kiesecker, J. M., A. R. Blaustein, & C. Miller, 2001. Potential mechanisms underlying the displacement of native Red-legged frogs by introduced bullfrogs. Ecology 82: 1964–1970.

Langhammer, P. F., K. R. Lips, P. A. Burrowes, T. Tunstall, C. M. Palmer, & J. P. Collins, 2013. A Fungal Pathogen of Amphibians, Batrachochytrium dendrobatidis, Attenuates in Pathogenicity with In Vitro Passages. PLOS ONE 8: e77630.

Lever, C., 2003. Naturalized Reptiles and Amphibians of the World. http://dx.doi.org/10.1086/421639.

Liu, X., J. R. Rohr, & Y. Li, 2012. Climate, vegetation, introduced hosts and trade shape a global wildlife pandemic. Proceedings of the Royal Society B: Biological Sciences 280: http://rspb.royalsocietypublishing.org/content/280/1753/20122506.abstract.

Lorch, J. M., J. Lankton, K. Werner, E. A. Falendysz, K. McCurley, & D. S. Blehert, 2015. Experimental Infection of Snakes with Ophidiomyces ophiodiicola Causes Pathological Changes That Typify Snake Fungal Disease. mBio 6: http://mbio.asm.org/content/6/6/e01534-15.abstract.

Mazzoni, R., A. A. Cunningham, P. Daszak, A. Apolo, E. Perdomo, & G. Speranza, 2003. Emerging Pathogen in Wild Amphibians and Frogs (Rana catesbeiana) Farmed for International Trade. Emerging Infectious Disease journal 9: 995.

McConnell, T. H., 2007. The nature of disease: Pathology for the health professions. Lippincott Williams & Wilkins, Baltimore.

Medeiros, C. I., C. Both, T. Grant, & S. M. Hartz, 2017. Invasion of the acoustic niche: variable responses by native species to invasive American bullfrog calls. Biological Invasions 19: 675–690.

Morehouse, E. A., T. Y. James, A. R. D. Ganley, R. Vilgalys, L. Berger, P. J. Murphy, & J. E. Longcore, 2003. Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. Molecular Ecology 12: 395–403.

Moyle, P. B., 1973. Effects of Introduced Bullfrogs, Rana catesbeiana, on the Native Frogs of the San Joaquin Valley, California. Copeia 1973: 18–22.

Naeem, S., F. Chapin III, R. Costanza, P. R. Ehrlich, F. B. Golley, D. U. Hooper, J. H. Lawton, R. V. O'Neill, H. A. Mooney, & O. E. Sala, 1999. Biodiversity and ecosystem functioning: maintaining natural life support processes. Issues in ecology 4.

Nori, J., J. N. Urbina-Cardona, R. D. Loyola, J. N. Lescano, & G. C. Leynaud, 2011. Climate Change and American Bullfrog Invasion: What Could We Expect in South America?. PLOS ONE 6: e25718.

Oliver, T. H., N. J. B. Isaac, T. A. August, B. A. Woodcock, D. B. Roy, & J. M. Bullock, 2015. Declining resilience of ecosystem functions under biodiversity loss. Nature Communications 6: 10122.

Olson, D. H., D. M. Aanensen, K. L. Ronnenberg, C. I. Powell, S. F. Walker, J. Bielby, T. W. J. Garner, G. Weaver, M. C. Fisher, & The Bd Mapping Group, 2013. Mapping the Global

Emergence of Batrachochytrium dendrobatidis, the Amphibian Chytrid Fungus. PLoS ONE 8: e56802.

Ortiz-Santaliestra, M. E., T. A. G. Rittenhouse, T. L. Cary, & W. H. Karasov, 2013. Interspecific and Postmetamorphic Variation in Susceptibility of Three North American Anurans to Batrachochytrium dendrobatidis. Journal of Herpetology 47: 286–292.

Piovia-Scott, J., K. Pope, S. Joy Worth, E. B. Rosenblum, T. Poorten, J. Refsnider, L. A. Rollins-Smith, L. K. Reinert, H. L. Wells, D. Rejmanek, S. Lawler, & J. Foley, 2015. Correlates of virulence in a frog-killing fungal pathogen: evidence from a California amphibian decline. ISME J 9: 1570–1578.

Poulin, R., & C. Combes, 1999. The Concept of Virulence: Interpretations and Implications. Parasitology Today 15: 474–475.

Preston, D. L., J. S. Henderson, & P. T. J. Johnson, 2012. Community ecology of invasions: direct and indirect effects of multiple invasive species on aquatic communities. Ecology 93: 1254–1261.

Retallick, R. W. R., H. McCallum, & R. Speare, 2004. Endemic Infection of the Amphibian Chytrid Fungus in a Frog Community Post-Decline. PLOS Biology 2: e351.

Retallick, R. W. R., & V. Miera, 2007. Strain differences in the amphibian chytrid Batrachochytrium dendrobatidis and non-permanent, sub-lethal effects of infection. Diseases of Aquatic Organisms 75: 201–207.

Rogers, C. S., & J. Miller, 2013. Coral Diseases Cause Reef Decline. Science 340: 1522.

Rosenblum, E. B., T. Y. James, K. R. Zamudio, T. J. Poorten, D. Ilut, D. Rodriguez, J. M. Eastman, K. Richards-Hrdlicka, S. Joneson, T. S. Jenkinson, J. E. Longcore, G. Parra Olea, L. F. Toledo, M. L. Arellano, E. M. Medina, S. Restrepo, S. V. Flechas, L. Berger, C. J. Briggs, & J. E. Stajich, 2013. Complex history of the amphibian-killing chytrid fungus revealed with genome resequencing data. Proceedings of the National Academy of Sciences 110: 9385–9390.

Schloegel, L. M., A. M. Picco, A. M. Kilpatrick, A. J. Davies, A. D. Hyatt, & P. Daszak, 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: 1420–1426.

Schloegel, L. M., L. F. Toledo, J. E. Longcore, S. E. Greenspan, C. Vieira, M. Lee, S. Zhao, C. Wangen, C. M. Ferreira, M. Hipolito, A. J. Davies, C. A. Cuomo, P. Daszak, P, & T. Y. James, 2012. Novel, panzootic and hybrid genotypes of amphibian chytridiomycosis associated with the bullfrog trade. Molecular Ecology 21: 5162–5177.

Searle, C. L., S. S. Gervasi, J. Hua, J. I. Hammond, R. A. Relyea, D. H. Olson, & A. R. Blaustein, 2011. Differential Host Susceptibility to Batrachochytrium dendrobatidis, an Emerging Amphibian Pathogen. Conservation Biology 25: 965–974.

Searle, C. L., G. Y. Xie, & A. R. Blaustein, 2013. Development and Infectious Disease in Hosts with Complex Life Cycles. PLoS ONE 8: e60920.

Simberloff, D., & B. Von Holle, 1999. Positive Interactions of Nonindigenous Species: Invasional Meltdown?. Biological Invasions 1: 21–32.

Skerratt, L., L. Berger, R. Speare, S. Cashins, K. McDonald, A. Phillott, H. Hines, & N. Kenyon, 2007. Spread of Chytridiomycosis Has Caused the Rapid Global Decline and Extinction of Frogs. EcoHealth 4: 125–134.

Stockwell, M. P., J. Clulow, & M. J. Mahony, 2010. Host species determines whether infection load increases beyond disease-causing thresholds following exposure to the amphibian chytrid fungus. Animal Conservation 13: 62–71.

Tobler, U., & B. R. Schmidt, 2010. Within- and Among-Population Variation in Chytridiomycosis-Induced Mortality in the Toad Alytes obstetricans. PLOS ONE 5: e10927.

Tompkins, D. M., S. Carver, M. E. Jones, M. Krkošek, & L. F. Skerratt, 2015. Emerging infectious diseases of wildlife: a critical perspective. Trends in Parasitology 31: 149–159.

Van Rooij, P., A. Martel, F. Haesebrouck, & F. Pasmans, 2015. Amphibian chytridiomycosis: a review with focus on fungus-host interactions. Veterinary Research 46: 137.

Voyles, J., S. Young, L. Berger, C. Campbell, W. F. Voyles, A. Dinudom, D. Cook, R. Webb, R. A. Alford, L. F. Skerratt, & R. Speare, 2009. Pathogenesis of Chytridiomycosis, a Cause of Catastrophic Amphibian Declines. Science 326: 582–585.

Walke, J. B., M. H. Becker, S. C. Loftus, L. L. House, T. L. Teotonio, K. P. C. Minbiole, & L. K. Belden, 2015. Community Structure and Function of Amphibian Skin Microbes: An Experiment with Bullfrogs Exposed to a Chytrid Fungus. PLoS ONE 10: 1–18.

Xie, G. Y., D. H. Olson, & A. R. Blaustein, 2016. Projecting the Global Distribution of the Emerging Amphibian Fungal Pathogen, Batrachochytrium dendrobatidis, Based on IPCC Climate Futures. PLOS ONE 11: e0160746.

CHAPTER 4 - REPRODUCTIVE CHARACTERISTICS OF INVASIVE AMERICAN BULLFROGS (*LITHOBATES CATESBEIANUS*) IN THE PACIFIC NORTHWEST INVADED RANGE

Jenny Urbina[1], Evan Bredeweg[2], Andrew R. Blaustein[3], Tiffany S. Garcia[2]

[1] Environmental Sciences Graduate Program, Oregon State University, Corvallis Oregon 97331
[2] Department of Fisheries and Wildlife, Oregon State University, Corvallis Oregon 97331
[3] Department of Integrative Biology, Oregon State University, Corvallis Oregon 97331

Abstract

Invasive species pose a major threat to global biodiversity. Invasion potential of exotic populations can be strongly influenced and potentially mediated, by reproductive characteristics such as fecundity, egg production and duration and number of reproductive events. The minimum body size at first reproduction can also play a role in the establishment of an invasive species, with breeding at smaller sizes facilitating establishment and spread. The American bullfrog (*Lithobates catesbeianus*) is an invasive anuran that has successfully invaded more than 40 countries across 4 continents. In the Willamette Valley, Oregon, USA, this nonnative species is having profound effects on native ecosystems. We characterized reproductive characteristics with emphasis on the minimum size at which males and females reach sexual maturity. We collected and dissected 105 individuals between 2013 and 2017, quantifying characteristics of sexual maturity including snout-vent length, total length, sex, tympanum diameter, presence of distended oviducts or eggs for females, and testes length and sperm activity in males. The minimum reproductive size we measured is smaller than predicted, with both males and females exhibiting smaller minimum reproductive sizes relative to bullfrogs within their native range. Our results are similar to other studies on minimum reproductive size on invasive bullfrog populations within the South American invasion ranges. Reduction in size at reproductive maturity is likely impacting the invasive success of American bullfrogs within these regions and this study gives us insight into potential control mechanisms.

Introduction

Biological invasions are a significant driver of global change in biodiversity (Elton 1958, Simberloff 2013). Intentional and unintentional species introduction can result in degraded ecosystem function (Ehrenfeld 2010, Simberloff 2011), changes in interspecific interactions (Simberloff 2006, Fukami et al. 2006) and contributes to native population declines (Gibbons et al. 2000, Blackburn et al. 2004). In addition to environmental impacts, changes to economic growth (i.e agriculture, Paini et al. 2016) and human health (Juliano & Lounibos 2005) make invasive species one of the more costly anthropogenic disturbances at a global scale (Pimentel et al. 2000, Paini et al. 2016, Rogers 2017). However, a reasonably small proportion of exotic species succeed in establishing populations within novel regions (Booth et al. 2003) or, after becoming established, do not directly impact the invaded ecosystems (White et al. 2006). Therefore, it is of utmost importance to understand and evaluate the potential of introduced species to invade within a novel range. Predicting invasiveness can be difficult as biotic and abiotic factors both play a role determining the establishment of exotic populations (Hui et al. 2016).

Trait-based inquiry can be useful when characterizing biological invaders (Ricciardi et al. 2013), including life history characteristics such as growth and reproduction rates, home range size, and diet breadth (Kolar & Lodge 2001). Although the strategies by which invasive species establish and spread vary significantly, reproductive traits, such as average clutch size and size at first reproduction, can disproportionally affect population dynamics (Lodge 1993). In particular, invasion potential can be strongly impacted by body size at first reproduction. For example, lionfish (*Pterois* spp) and the brown tree snake (*Boiga irregularis*) have larger body sizes in their invaded ranges, resulting in increased propagule pressure (Savidge et al. 2007, Gardner et al. 2015). Species-specific information on the relationship between body size and reproductive capacity can be useful for management actions that target invasive species with significant conservation impacts (Van Kleunen et al. 2010).

The unprecedented loss of amphibian biodiversity at a global scale (Barnosky et al. 2011, Ceballos et al. 2015) contrasts to several anuran species that are successful invaders (Beard et al. 2003, Lobos & Jaksic 2005, Ortega et al. 2005, Vargas Salinas 2006, Urban et al. 2007, Rebelo et al. 2010, Bucciarelli et al. 2014). American bullfrogs (*Lithobates catesbeianus*) have established in over 40 countries across 4 continents and been implicated in the decline of native species across multiple taxonomic groups (Adams & Pearl 2007). Trait-based research has largely attributed successful bullfrog invasions to initial propagule pressure and biotic tolerance to varying climate regimes (Pearl et al. 2005, Govindarajulu et al. 2006, Adams & Pearl 2007, Rago et al. 2012, Bai et al. 2012). The ability to reproduce at a smaller size improves invasion and range expansion potential of a newly established bullfrog population. In Brazil, established populations of American bullfrogs are able to reproduce when males reach 7.6 cm and females reach 6.5 cm (Lima et al. 1998, Leivas et al. 2012), which is smaller than what is reported in the bullfrog's native range. In the northern extent of the bullfrog's Pacific Northwest range, Govindarajulu et al. (2006) reported reproductive sizes similar to those found in the native populations at similar latitudes (Bruneau & Magnin 1980; Shirose et al. 1993).

We evaluated the size at first reproduction in bullfrogs in the southern extent of their Pacific Northwest invaded range and compared minimum sizes with other invaded and native populations for this species. Bullfrogs were introduced to the Pacific Northwest during the early 1900's to establish frog farms with the intention of exporting to international markets and are now densely populated throughout the region’s low-elevation freshwater habitats (Jennings & Hayes 1985). We evaluated the minimum reproductive size for males and females of American bullfrogs in the Willamette Valley (OR, USA) using macroscopic and microscopic characteristics of their reproductive system. We hypothesized the minimum reproductive size in the Willamette Valley would be smaller than the size reported in bullfrog populations at similar latitudes within their native range, and invasive populations from the northern extent of the Pacific Northwest invaded range. Further, we predicted minimum reproductive sizes would be similar to invasive populations in Brazil.

Methods

We collected American bullfrogs (*Lithobates catesbeianus*) samples from 6 locations in Lane (43° 57' 39.5994" N, 122° 39' 42.4794" W) and Benton County (44° 37' 41.5194" N, 123° 23' 14.6394" W) in the Willamette Valley (Oregon). We sampled 4 ponds with no resident fish populations and 2 permanent ponds with fish populations (Figure 4.1 and Table 4.1). Using Visual Encounter Surveys (Crump & Scott Jr. 1994) we sampled and collected bullfrogs during spring and summer breeding seasons of 2013 and 2017 for a total sampling effort of 150 hours. Individuals were transported to Oregon State University where they were euthanized using MS-222 and preserved in 90% ethanol. We followed all institutional and national guidelines for the care and use of animals.

Determination of sexual maturity is more rigorously done by examination of the gonads (Govindarajulu et al. 2006), as relying only on secondary sexual characteristics can be problematic. Yellow throat coloration and swollen nuptial pads in males are indicators of sexual maturity, but are only present in males. Further, gender differences in tympanum size are not obvious in young individuals. As such, we determined the stage of gonad development for both males and females in addition to measured snout-vent length (SVL), total body length, eye and tympanum diameter, and determined body mass for each individual. For males, we excised, measured and weighed testes with a precision of 0.001 g for mass and 0.01 mm for length. The right gonad was macerated to test for the presence of active sperm while the left gonad was preserved in 90% ethanol for microscopic analysis (Costa et al. 1998a, b). For females, ovarian maturation stages were described following the protocol developed in Costa et al. (1998a). Ovarian maturation in female American bullfrogs can be categorized into five distinct stages: 1) juvenile with thin ovaries, hyaline to whitish and no oocytes distinguishable; 2) beginning of maturation with yellowish ovaries and deeper invaginations, oocytes present; 3) intermediate maturation grayish ovaries with pigmented post-vitellogenic oocytes; 4) advanced maturation high proportion of post-vitellogenic oocytes; and 5) spent ovaries: flaccid, with reduced volume and atresic oocytes (Costa et al. 1998, Kaefer et al. 2007).

The minimum reproductive size was determined as the minimum SVL when females presented convoluted oviducts or ovaries with eggs inside their thoracic cavity (stages ≥ 2). In the case of males, their minimum reproductive size was the minimum SVL when they exhibited active sperm. We macerated the right testis in 0.5 ml of Holtfreter’s solution 10% to count actively motile sperm using a hemocytometer (Browne & Zippel 2007). We evaluated if body size of adults were different by analyzing the SVL measurement and body mass using a Student’s t- test with a Welsh correction. To evaluate the logistic regression accuracy to predict sexual maturity in males and females, we used a ROC (Receiving Operator Characteristic Curve). This graphic representation shows the ability of the logistic regression to correctly classify cases meeting certain condition (sexually maturity) and cases not meeting the condition of interest. The estimated threshold indicates the point at which the prediction for values meeting the condition is optimal; this is the point at which the sum of the false positives and false negatives is the least.

Results

We collected a total of 105 *L. catesbeianus*: 51 females, 50 males, and 4 individuals that were undetermined. Of this total, 21 were adult females and 38 were adult males. In females, SVL varied between 3.8 cm to 17.6 cm, total length between 8.46 cm and 34 cm, and body mass ranged from 4.63 g to 500 g. In males, SVL varied between 4.01 mm to 16.5 mm, total length between8.77 cm and 36 cm, and body mass ranged from 5.37 g to 357 g. We found that the minimum reproductive size for females was 6.7 cm and for the males 6.6 cm. For mature individuals we did not observe gender differences in size (SVL Welsh t-test, t = 1.244, df= 41.02, and p =0.22; mean ± SD: Males SVL = 10.98 ± 2.75 cm, and females SVL = 11.93 ± 2.80 cm) or body mass (Welsh t-test, t = 0.90, df= 34.67, and p = 0.37; mean ± SD: Males BM =144 ± 105 g, and females BM = 174.11 ± 129 g). The threshold at which ROC curves estimated the accuracy of the logistic regression to predict sexual maturity in males and females as optimal was 0.69 and 0.48 respectively. The SVL value for these thresholds is 7.8 cm in males and 10 cm for females (Figure 4.2). At these thresholds, males and females were predicted to be sexually mature, with the minimum number of false positives and false negatives.

Discussion

We found that the minimum reproductive size for male and female American bullfrogs in the Willamette Valley was 6.6 cm and 6.7 cm respectively, which is smaller relative to populations within the native range at similar latitudes with male and females being mature at 9.5 cm and 10.8 cm respectively (Howard 1981) (Table 4.2). Further, our minimum reproductive size is similar to those found in invasive bullfrog populations in Brazil, South America, where the minimum reproductive size at sexual maturity was found to be 7.6 cm for males and 6.5 cm for females (Leivas et al. 2012). Minimum reproductive sizes in our study were smaller relative to other invaded ranges in the United States and Canada, including populations from the northern extent of the Pacific Northwest invaded range (Washington and British Columbia; Table 4.2). This reduction in minimum reproductive size is likely increasing the number of reproductive events for breeding individuals, thus increasing the propagule pressure of invasive populations in Oregon (Leivas et al. 2012). Reaching sexual maturity at a smaller body size is thus likely enhancing invasion potential for populations within the Willamette Valley, Oregon, with individuals reaching breeding age before secondary sexual characteristics are present.

The allocation of energy towards reproduction provides advantages to invading species. The reproductive cycle of American bullfrogs in Oregon is mainly restricted to the summer season when individuals congregate in lentic freshwater systems. Critical factors for breeding include calm water and air temperature above 20° C (Jones et al. 2005). In the Willamette Valley, females can lay egg masses with 6,000 to 20,000 eggs with body size positively correlated with egg number (Nussbaum et al. 1983). In warm water, hatching occurs in two to five days and tadpoles can take up to two years to reach metamorphosis. However, tadpoles from some populations in the Pacific Northwest invaded range have been documented as being able to metamorphose less than four months after hatching (Govindarajulu et al. 2005, Cook et al. 2013). Males and females in the Willamette Valley may therefore be reaching their minimum reproductive size less than two years after metamorphosis. This size shift may be explained by reaching metamorphosis faster, resulting in smaller juvenile body sizes. The estimated thresholds for males and females to be sexually mature were similar to measurements reported in an invaded range from Brazil (Lima et al. 1998). The estimated threshold was greater for females (10 cm) that could indicate females reach maturity delayed relative than males. Females of the western clawed frog (*Xenopus tropicalis*), another successful anuran invader, are able to mature 6 months after metamorphosis at only 6.5 cm in length, providing an advantage in the widespread of the invaded range of this species (McCoid & Fritts 1989).

Life history characteristics that value adult survivorship over reproductive potential can also increase invasion success (Sol et al. 2012). This highlights the tradeoff between earlier sexual maturity and smaller juvenile body size in Oregon bullfrog populations. We posit that bullfrog females in the Willamette Valley are allocating energy to early maturation with a correlated reduction in egg mass size. Individuals may also be capable of double clutching, or spawning multiple times during a breeding season. Multiple clutching increases propagule pressure as increases in the number of offspring within each generation. Additionally, multiple clutching can lead to improving the genetic diversity of the invading populations as one female’s eggs can be fertilized by multiple males (Howard 1983). However, this aspect of reproductive biology has not been studied or reported in the Willamette Valley.

Understanding key traits that predict or enhanced invasion success is critical for the implementation of management and control actions (Adams & Pearl 2007). Characterizing the reproductive activity of breeding bullfrog populations in the Willamette Valley in connection with abiotic factors can be critical in managing the establishment of new populations of this species. Our study identified a decrease in the minimum reproductive size of males and females in invasive American bullfrogs in the Willamette Valley relative to native populations. This finding indicates that we need to modify our view of what constitutes a mature bullfrog in the Willamette Valley, and potentially in other invasion ranges. Although smaller females can have limited reproductive output, the potential for longer reproductive longevity both over ontogeny and within a breeding season could significantly increase the invasion potential of this critical invasive species.

Acknowledgments

We thank A. Soken, S. Gervasi, J. Giarrizo, N. Salinas, D. Paoletti, T. Chestnut, C. Medellin, G. Xie, L. Thurman, S. Selegos, D. Jones, T. Dang, S. Gregory, J. Doyle, J. Rowe for their invaluable help and company during sampling events. We acknowledge R. Hanselmann and JM. Urbina for their help disentangle equations. A special thanks to C. Cousins for his hard work in 2017. A special thanks to the Blaustein lab, Garcia Lab, and Saavedra Lab for space and resources.

“… They are present in hundreds, or even thousands. The transformed individuals present a spirited sight as one approach… Wright 1920 “…

Table 4.1 Sampled locations for American bullfrogs (*Lithobates catesbeianus*) in the Willamette Valley.

Location	**Coordinates**	**Fish presence (Yes=Y/No=N)**	**Hydroperiod**
William L. Finley National Wildlife refuge -Lower 22	44°24'47.0"N 123°19'38.0"W	N	Mostly permanent, dry by management
LCC wetlands	44°00'49.5"N 123°02'22.1"W	N	Permanent
Timberline	44° 01' 13.07 "N 123° 08' 52.07"W	N	Permanent
Barger	44° 04' 35.8"N 123° 12' 14.7"W	N	Permanent
William L. Finley National wildlife refuge –Cattail pond	44° 24' 05.0"N 123° 19' 27.8"W	Y	Mostly permanent, dry by management
Green Island	44°08'23.6"N 123°06'14.4"W	Y	Permanent

Table 4.2 Minimum reproductive size for American bullfrogs (*Lithobates catesbeianus*) in native and invaded ranges of distribution (ND =no data)

Location	**Country**	**Invasive Population (Y-N)**	**Minimum reproductive size males (cm)**	**Minimum reproductive size females (cm)**	**Reference**
Oregon	USA	Y	6.61	6.77	This study
State of Parana	Brazil	Y	7.6	6.5	(Leivas et al. 2012)
Washington	USA	Y	10.5	11.5	(Jones et al. 2005)
State of Rio Grande do sul	Brazil	Y	9.025	12.083	(Kaefer et al. 2007)
British Columbia	Canada	Y	mean 14.8	mean 14.7	(Govindarajulu et al. 2006)
State of Minas Gerais	Brazil	Y	8.09	10.33	(Lima et al. 1998)
Quebec	Canada	N	9.5-11	9.5-11	(Bruneau & Magnin 1980)
Ontario	Canada	N	9.1	11.3	(Shirose et al. 1993)
Michigan	USA	N	9.5	10.8	(Howard 1981)
New Jersey	USA	N	Mean 15.12	Mean 14.03	(Ryan 1980)
Missouri	USA	N	ND	12.3	(Willis et al. 1956)
ND	USA	N	8.5	8.9	(Wright & Wright 1949)
New York	USA	N	8.5	11	(Raney & Ingram 1941)
Louisiana	USA	N	ND	12.7	George 1940 (thesis unpublished) **
ND	USA	N	ND	10.16	(Wright 1920)

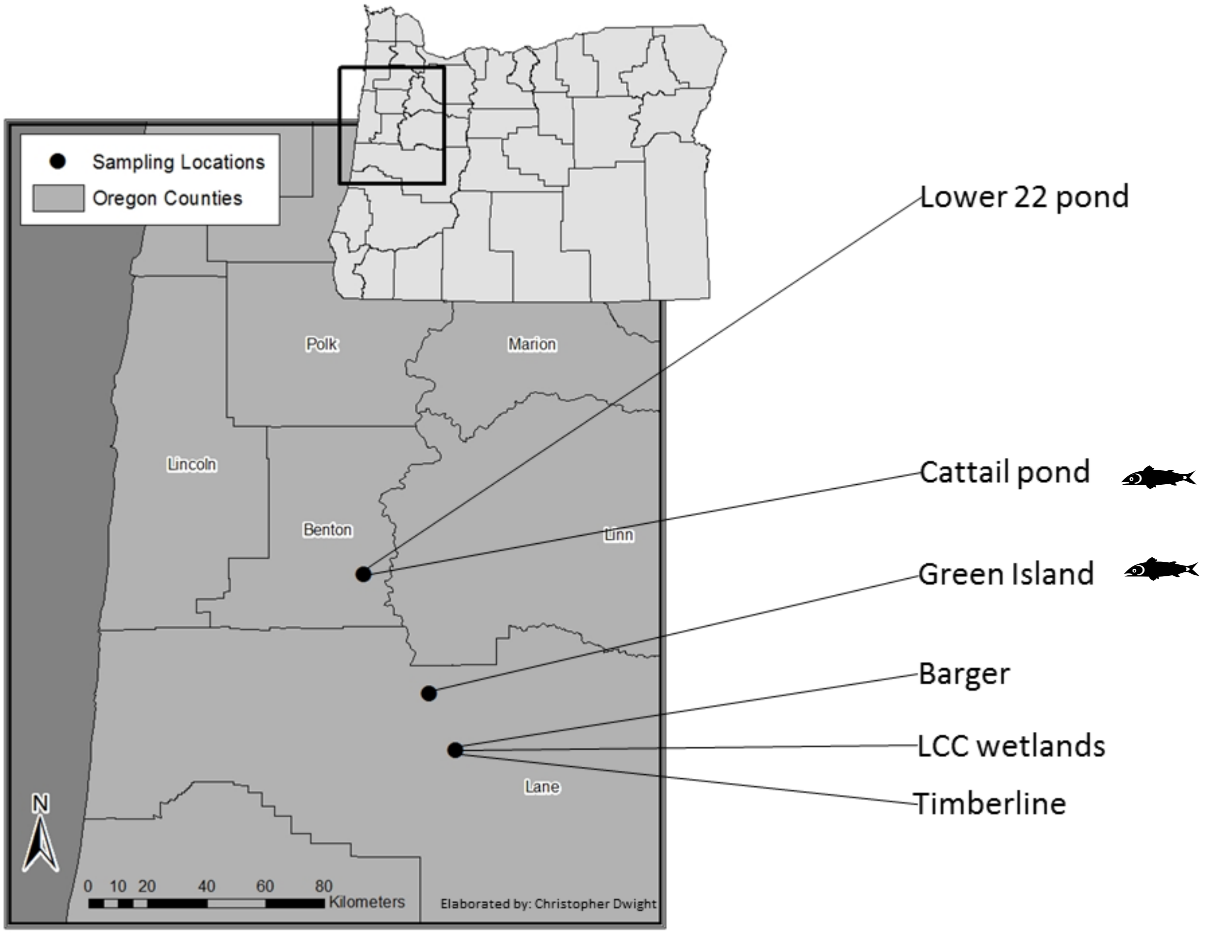


Figure 4.1 Sampling sites in the Willamette Valley from where American bullfrogs (*L. catesbeianus*) were collected

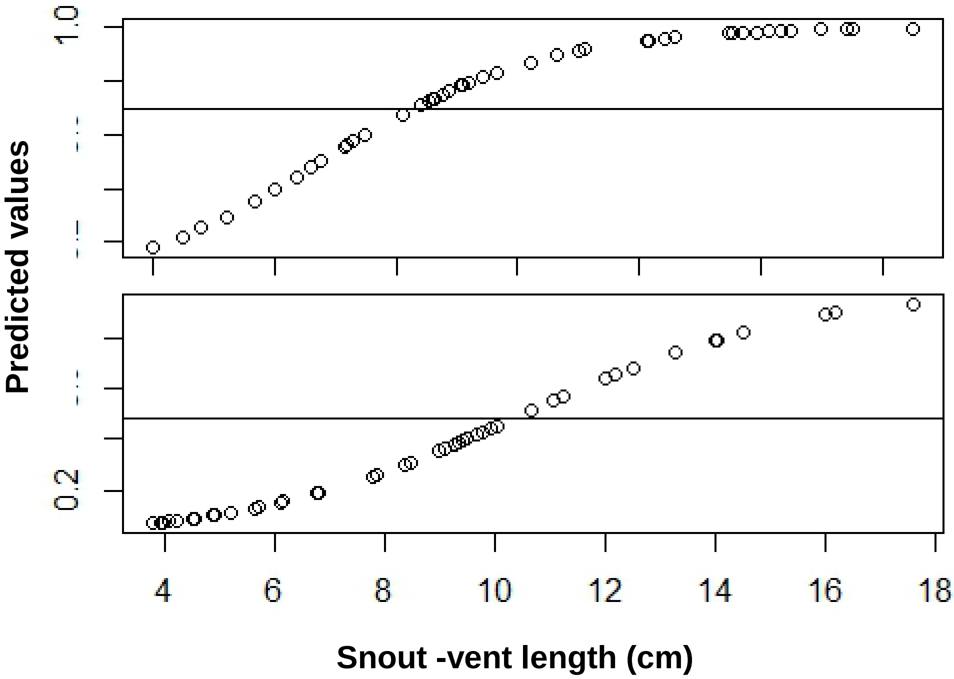


Figure 4.2 Estimated values for the first maturation of males (top) and females (bottom) of America bullfrog (*L. catesbeianus*) in an invaded range (Willamette Valley, OR). The horizontal line represents the threshold at which males and females are sexually mature

Literature cited

Adams MJ, Pearl CA (2007) Problems and opportunities managing invasive Bullfrogs: is there any hope? In: Gherardi F (ed) Biological invaders in inland waters: Profiles, distribution, and threats. Springer Netherlands, Dordrecht, p 679–693

Bai C, Ke Z, Consuegra S, Liu X, Li Y (2012) The role of founder effects on the genetic structure of the invasive bullfrog (Lithobates catesbeianaus) in China. Biol Invasions 14:1785–1796

Barnosky AD, Matzke N, Tomiya S, Wogan GOU, Swartz B, Quental TB, Marshall C, McGuire JL, Lindsey EL, Maguire KC, Mersey B, Ferrer EA (2011) Has the Earth/'s sixth mass extinction already arrived? Nature 471:51–57

Beard KH, Eschtruth AK, Vogt KA, Vogt DJ, Scatena FN (2003) The effects of the frog Eleutherodactylus coqui on invertebrates and ecosystem processes at two scales in the Luquillo Experimental Forest, Puerto Rico. J Trop Ecol 19:607–617

Blackburn TM, Cassey P, Duncan RP, Evans KL, Gaston KJ (2004) Avian Extinction and Mammalian Introductions on Oceanic Islands. Science 305:1955

Booth B., Murphy S., Swanton C. (2003) Weed Ecology in Natural and Agricultural Systems. CABI publishing, Cambridge, MA

Browne RK, Zippel K (2007) Reproduction and Larval Rearing of Amphibians. ILAR J 48:214–234

Bruneau M, Magnin E (1980) Croissance, nutrition et reproduction des ouaouarons Rana catesbeiana Shaw (Amphibia Anura) des Laurentides au nord de Montreal. Can J Zool 58:175–183

Bucciarelli GM, Blaustein AR, Garcia TS, Kats LB (2014) Invasion Complexities: The Diverse Impacts of Nonnative Species on Amphibians. Copeia 2014:611–632

Ceballos G, Ehrlich PR, Barnosky AD, García A, Pringle RM, Palmer TM (2015) Accelerated modern human–induced species losses: Entering the sixth mass extinction. Sci Adv 1

Cook MT, Heppell SS, Garcia TS (2013) Invasive bullfrog larvae lack developmental plasticity to changing hydroperiod. J Wildl Manag:n/a-n/a

Costa CLS, Lima SL, Andrade DR, Agostinho CA (1998) Morphological characterization of the development stages of female reproduction apparel of bullfrog, Rana catesbeiana, in the intensive Anfigranja systems. Rev Bras Zootec-Braz J Anim Sci 27:642–650

Costa CL, Lopes Lima S, Andrade DR, Agostinho CA (1998a) Caracterização Morfológica dos Estádios de Desenvolvimento do Aparelho Reprodutor Feminino da Rã-Touro, Rana catesbeiana, no Sistema Anfigranja de Criação Intensiva. Rev Bras Zootec 27:642–650

Costa CL, Lopes Lima S, Andrade DR, Agostinho CA (1998b) Caracterização Morfológica dos Estádios de Desenvolvimento do Aparelho Reprodutor Masculino da Rã-Touro, Rana catesbeiana, no Sistema Anfigranja de Criação Intensiva. Rev Bras Zootec 27:651–657

Crump ML, Scott Jr. NJ (1994) Chapter 2. Visual encounter surveys. In: W.R. Heyer, M.A. Donnelly, R.W. McDiarmid, L.C. Hayek, M.S. Foster (eds) Measuring and monitoring biological diversity: Standard methods for amphibians. Smithsonian Institution Press, Washington, DC, p 84–92

Ehrenfeld JG (2010) Ecosystem Consequences of Biological Invasions. Annu Rev Ecol Evol Syst 41:59–80

Elton C (1958) The Ecology of Invasions by Animals and Plants. Methuen, London

Fukami T, Wardle DA, Bellingham PJ, Mulder CPH, Towns DR, Yeates GW, Bonner KI, Durrett MS, Grant-Hoffman MN, Williamson WM (2006) Above- and below-ground impacts of introduced predators in seabird-dominated island ecosystems. Ecol Lett 9:1299–1307

Gardner PG, Frazer TK, Jacoby CA, Yanong RPE (2015) Reproductive biology of invasive lionfish (Pterois spp.). Front Mar Sci 2:7

Gibbons JW, Scott DE, Ryan TJ, Buhlmann KA, Tuberville TD, Metts BS, Greene JL, Mills T, Leiden Y, Poppy S, Winne CT (2000) The Global Decline of Reptiles, Deja Vu Amphibians. Bioscience 50:653–666

Govindarajulu P, Altwegg R, Anholt BR (2005) Matrix Model Investigation of Invasive Species Control: Bullfrogs on Vancouver Island. Ecol Appl 15:2161–2170

Govindarajulu P, Price WMS, Anholt BR (2006) Introduced Bullfrogs (Rana catesbeiana) in Western Canada: Has Their Ecology Diverged? J Herpetol 40:249–260

Howard RD (1981) Sexual Dimorphism in Bullfrogs. Ecology 62:303–310

Howard RD (1983) Sexual Selection and Variation in Reproductive Success in a Long-Lived Organism. Am Nat 122:301–325

Hui C, Richardson DM, Landi P, Minoarivelo HO, Garnas J, Roy HE (2016) Defining invasiveness and invasibility in ecological networks. Biol Invasions 18:971–983

Jennings MR, Hayes MP (1985) Pre-1900 Overharvest of California Red-Legged Frogs (Rana aurora draytonii): The Inducement for Bullfrog (Rana catesbeiana) Introduction. Herpetologica 41:94–103

Jones LLC, Leonard WP, Olson DH (2005) Amphibians of the Pacific Northwest. Seattle Audubon Society, Seattle

Juliano SA, Lounibos LP (2005) Ecology of invasive mosquitoes: effects on resident species and on human health. Ecol Lett 8:558–574

Kaefer IL, Boelter RA, Cechin SZ (2007) Reproductive biology of the invasive bullfrog Lithobates catesbeianus in southern Brazil. Ann Zool Fenn 44:435–444

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol Evol 16:199–204

Leivas P., Moura M., Favaro L. (2012) The reproductive biology of the invasive Lithobates catesbeianus (Amphibia:Anura). J Herpetol 46:153–161

Lima SL, Costa CLS, Agostinho CA, Andrade DR, Pereira HP (1998) Estimate of bullfrog size at first sexual maturation, Rana catesbeiana, in the intensive growing Anfigranja system. Rev Bras Zootec-Braz J Anim Sci 27:416–420

Lobos G, Jaksic FM (2005) The ongoing invasion of African clawed frogs (*Xenopus laevis*) in Chile: causes of concern. Biodivers Conserv 14:429–439

Lodge DM (1993) Biological invasions: Lessons for ecology. Trends Ecol Evol 8:133–137

McCoid MJ, Fritts TH (1989) Growth and Fatbody Cycles in Feral Populations of the African Clawed Frog, Xenopus laevis (Pipidae), in California with Comments on Reproduction. Southwest Nat 34:499–505

Nussbaum RA, Brodie ED, Storm RM (1983) Amphibians and reptiles of the Pacific northwest. University Press of Idaho, Moscow, Idaho

Ortega JE, Serrano VH, Ramírez-Pinilla MP, Lannoo MJ (2005) Reproduction of an Introduced Population of Eleutherodactylus johnstonei at Bucaramanga, Colombia. Copeia 2005:642–648

Paini DR, Sheppard AW, Cook DC, De Barro PJ, Worner SP, Thomas MB (2016) Global threat to agriculture from invasive species. Proc Natl Acad Sci 113:7575–7579

Pearl C, Adams M, Leuthold N, Bury R (2005) Amphibian occurrence and aquatic invaders in a changing landscape: Implications for wetland mitigation in the willamette valley, Oregon, USA. Wetlands 25:76–88

Pimentel D, Lach L, Zuniga R, Morrison D (2000) Environmental and economic costs of nonindigenous species in the United States. BioScience 50:53–65

Rago A, While GM, Uller T (2012) Introduction pathway and climate trump ecology and life history as predictors of establishment success in alien frogs and toads. Ecol Evol 2:1437–1445

Raney EC, Ingram WM (1941) Growth of tagged frogs (Rana catesbeiana Shaw and Rana clamitans Daudin) under natural conditions. Am Midl Nat 26:201–206

Rebelo R, Amaral P, Bernardes M, Oliveira J, Pinheiro P, Leitão D (2010) Xenopus laevis (Daudin, 1802), a new exotic amphibian in Portugal. Biol Invasions 12:3383–3387

Ricciardi A, Hoopes MF, Marchetti MP, Lockwood JL (2013) Progress toward understanding the ecological impacts of nonnative species. Ecol Monogr 83:263–282

Rogers WE (2017) Invasive Species. In: Reference Module in Earth Systems and Environmental Sciences. Elsevier

Ryan MJ (1980) The reproductive behavior of the Bullfrog (Rana catesbeiana). Copeia 1980:108–114

Savidge JA, Qualls FJ, Rodda GH (2007) Reproductive Biology of the Brown Tree Snake, Boiga irregularis (Reptilia: Colubridae), during Colonization of Guam and Comparison with That in Their Native Range. Pac Sci 61:191–199

Shirose LJ, Brooks RJ, Barta JR, Desser SS (1993) Intersexual differences in growth, mortality, and size at maturity in bullfrogs in central Ontario. Can J Zool 71:2363–2369

Simberloff D (2006) Invasional meltdown 6 years later: important phenomenon, unfortunate metaphor, or both? Ecol Lett 9:912–919

Simberloff D (2011) How common are invasion-induced ecosystem impacts? Biol Invasions 13:1255–1268

Simberloff D (2013) Invasive species: What everyone needs to know. Oxford University Press, New York

Sol D, Maspons J, Vall-llosera M, Bartomeus I, García-Peña GE, Piñol J, Freckleton RP (2012) Unraveling the Life History of Successful Invaders. Science 337:580

Urban MC, Phillips BL, Skelly DK, Shine R (2007) The cane toad’s (Chaunus [Bufo] marinus) increasing ability to invade Australia is revealed by a dynamically updated range model. Proc R Soc B Biol Sci 274:1413–1419

Van Kleunen M, Dawson W, Schlaepfer D, Jeschke JM, Fischer M (2010) Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. Ecol Lett 13:947–958

Vargas Salinas F (2006) Breeding behavior and colonization success of the Cuban treefrog Osteopilus septentrionalis. Herpetologica 62:398–408

White EM, Wilson JC, Clarke AR (2006) Biotic indirect effects: a neglected concept in invasion biology. Divers Distrib 12:443–455

Willis YL, Moyle DL, Baskett TS (1956) Emergence, Breeding, Hibernation, Movements and Transformation of the Bullfrog, Rana catesbeiana, in Missouri. Copeia 1956:30–41

Wright A (1920) Frogs: Their Natural History and Utilization. Washington, DC

Wright A, Wright A (1949) Handbook of frogs and toads of the United States and Canada. Comstock, New York

CHAPTER 5 CONCLUSIONS

Disease is a product of interactions among hosts and pathogens within in a particular environment. The amphibian - chytrid interaction is an ideal system to study the impact of an emerging pathogen and host response, as there is significant variation in species and population response, in addition to differential impacts across strain and life stage. My thesis examines amphibian host response to *Batrachochytrium dendrobatidis* (Bd) across life history transitions: embryos to larvae, larvae to metamorphs and juveniles to adults in different host species.

To investigate how host exposure to Bd during early life stages (embryos) impacts later life history stages, I used a factorial experimental design. Embryos of three anuran species (*Pseudacris regilla*, *Anaxyrus boreas* and *Lithobates catesbeianus*) were exposed to Bd at early or late embryonic stages, with some individuals re-exposed after hatching. I evaluated how time of exposure and Bd strain influenced host response (Chapter 2). Embryonic Bd exposure resulted in differential survival as a function of host species, Bd strain and timing of exposure. *P. regilla* experienced embryonic mortality when exposed during later developmental stages to one of the Bd strains. There were no differences across the treatments in embryonic mortality in *A. boreas*. Embryonic mortality of *L. catesbeianus* occurred in all Bd exposure treatments. We detected latent effects in *A. boreas* and *L. catesbeianus* larvae, as mortality increased when individuals had been exposed to any of the Bd strains during the embryonic stage. We also detected direct effects on larval mortality in all three-anuran species and it varied with Bd strain. When individuals were repeatedly exposed (late in the embryonic stage and again as larvae), we found an increase in mortality. Our results suggest that exposure to Bd can directly affect embryo survival and have direct and latent effects on larval survival of both native and invasive species. However, these impacts were highly context dependent, with timing of exposure and Bd strain influencing the severity of the effects. This chapter emphasizes how important is to consider that, despite being a relatively brief period, exposure to Bd in the embryonic stage can lead to increased mortality after hatching. Additional research exploring the mechanisms protecting the embryos is needed to better understand the susceptibility of this developmental stage to disease. As eggs receive material from their parents during oviposition, evaluating the role of parental contributions to the immune response of their offspring can help us better understand embryonic immunity. Further studies are also required to understand how variation in other environmental and biological parameters can affect the outcome of repeated Bd exposure in anuran species. Our results add information to the growing body of evidence concerning differential susceptibility to pathogens among amphibian species and across life stages.

I explored the next life transition, metamorphosis, by evaluating how wild - caught invasive *Lithobates catesbeianus,* respond to Bd strains isolated from conspecifics. My results showed that wild caught bullfrog metamorphs from the Western, US were susceptible to a Bd strain isolated from conspecifics from the Eastern US. Bullfrogs exposed to this novel strain suffered higher mortality rates compared to controls and to bullfrogs exposed to a Bd strain isolated from conspecifics in the Western US. Individuals exposed to the Western Bd strain survived and were able to harbor a higher pathogen burden. I found 43% of the wild - caught animals were infected prior to experimental exposure. Interestingly, they were able to clear the infection. Understanding the variation in host response to pathogens isolated from conspecifics across different distributional ranges can help us to understand how pathogen origin mediates host responses. Future work about virulence of Bd in isolates from conspecifics deserves more research. Upcoming research should include characterization of aspects such as individual immunity and pathogen virulence with and without the interaction with the host. This chapter illustrates how the movement of individuals to different geographic locations contributes to the arrival of pathogens that can affect local organisms.

Reproductive strategies and propagule pressure are key determinants of the invasive potential of a species. We studied reproductively mature adult bullfrogs. We quantified reproductive characteristics with an emphasis on the minimum size at which males and females reach sexual maturity in bullfrog population within the Willamette Valley Oregon USA (Chapter 4). After sampling six locations in the Willamette Valley during 2013 and 2017, results suggest the minimum reproductive size for males and females is smaller relative to sizes of adults in the native bullfrog range and similar to minimum reproductive sizes observed in bullfrog invasion ranges in South America. Reduction in size at reproductive maturity could impact the invasive success of American bullfrogs and give us insight into the history of their founder populations.

The research presented in this thesis sheds light on the complexity of host-pathogen interactions. Our experimental and observational studies indicate that the incorporation of a neglected life stage (embryos) is needed to understand the direct and latent impacts of Bd on later life stages. Future research can benefit from the inclusion of life history transitions in conjunction with changes in environmental conditions to better understand the host response and the pathogen role in an integral way. Results from my dissertation related to the minimum size of an invasive species can be taken into account in planning management actions to control this species. This dissertation provides new information on the impact of Bd on embryonic life stages, as well as information about susceptibility of American bullfrogs to Bd, a species that has generally been considered tolerant to Bd. Our results provide necessary information for conducting future research projects on the role of life history transition, repeated exposure, and strain virulence in different hosts.

Wildlife and human health are threatened by emergent infectious diseases and information about factors that influence the response of hosts and how that response changes is critical to establish actions to prevent or control disease spread. Ultimately, it is in our hands to conduct research and plan conservation actions that include a holistic view of disease. I posit there is a need to involve more sub disciplines in this research, and a demand to work together to understand disease dynamics. Understanding the amphibian –chytrid system is a first step to obtain information to disentangle disease dynamics and applied our knowledge in different systems affected by diseases.

BIBLIOGRAPHY

Adams MJ, Pearl CA (2007) Problems and opportunities managing invasive Bullfrogs: is there any hope? In: Gherardi F (ed) Biological invaders in inland waters: Profiles, distribution, and threats. Springer Netherlands, Dordrecht, p 679–693

Bai C, Ke Z, Consuegra S, Liu X, Li Y (2012) The role of founder effects on the genetic structure of the invasive bullfrog (Lithobates catesbeianaus) in China. Biol Invasions 14:1785–1796

Bakar AA, Bower DS, Stockwell MP, Clulow S, Clulow J, Mahony MJ (2016) Susceptibility to disease varies with ontogeny and immunocompetence in a threatened amphibian. Oecologia:1–13

Balaz V, Voros J, Civis P, Vojar J, Hettyey A, Sos E, Dankovics R, Jehle R, Christiansen DG, Clare F, Fisher M, Garner T, Bielby J (2014) Assessing Risk and Guidance on Monitoring of Batrachochytrium dendrobatidis in Europe through Identification of Taxonomic Selectivity of Infection. Conserv Biol 28:213–223

Balvanera P, Pfisterer AB, Buchmann N, He J-S, Nakashizuka T, Raffaelli D, Schmid B (2006) Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecol Lett 9:1146–1156

Băncilă R, Hartel T, Plăiaşu Rodica, Smets J, Cogălniceanu D (2010) Comparing three body condition indices in amphibians: a case study of yellow-bellied toad Bombina variegata. Amphib Reptil 31:558–562

Barnosky AD, Hadly EA, Bascompte J, Berlow EL, Brown JH, Fortelius M, Getz WM, Harte J, Hastings A, Marquet PA, Martinez ND, Mooers A, Roopnarine P, Vermeij G, Williams JW, Gillespie R, Kitzes J, Marshall C, Matzke N, Mindell DP, Revilla E, Smith AB (2012) Approaching a state shift in Earth/'s biosphere. Nature 486:52–58

Barnosky AD, Matzke N, Tomiya S, Wogan GOU, Swartz B, Quental TB, Marshall C, McGuire JL, Lindsey EL, Maguire KC, Mersey B, Ferrer EA (2011) Has the Earth/'s sixth mass extinction already arrived? Nature 471:51–57

Bataille A, Cashins SD, Grogan L, Skerratt LF, Hunter D, McFadden M, Scheele B, Brannelly LA, Macris A, Harlow PS, Bell S, Berger L, Waldman B (2015) Susceptibility of amphibians to chytridiomycosis is associated with MHC class II conformation. Proc R Soc B Biol Sci 282

Beard KH, Eschtruth AK, Vogt KA, Vogt DJ, Scatena FN (2003) The effects of the frog Eleutherodactylus coqui on invertebrates and ecosystem processes at two scales in the Luquillo Experimental Forest, Puerto Rico. J Trop Ecol 19:607–617

Beldomenico PM, Begon M (2010) Disease spread, susceptibility and infection intensity: vicious circles? Trends Ecol Evol 25:21–27

Berger L, Marantelli G, Skerratt LF, Speare R (2005) Virulence of the amphibian chytrid fungus Batrachochytrium dendrobatidis varies with the strain. Dis Aquat Organ 68:47–50

Berger L, Roberts AA, Voyles J, Longcore JE, Murray KA, Skerratt LF (2016) History and recent progress on chytridiomycosis in amphibians. Aquat Fungi 19:89–99

Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, Hines HB, Lips KR, Marantelli G, Parkes H (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proc Natl Acad Sci 95:9031–9036

Bielby J, Fisher MC, Clare FC, Rosa GM, Garner TWJ (2015) Host species vary in infection probability, sub-lethal effects, and costs of immune response when exposed to an amphibian parasite. Sci Rep 5:10828

Blackburn TM, Cassey P, Duncan RP, Evans KL, Gaston KJ (2004) Avian Extinction and Mammalian Introductions on Oceanic Islands. Science 305:1955

Blaustein A, Beatty J, Olson D, Storm R (1995) The biology of amphibians and reptiles in old-growth forests in the Pacific Northwest.

Blaustein AR, Gervasi SS, Johnson PTJ, Hoverman JT, Belden LK, Bradley PW, Xie GY (2012) Ecophysiology meets conservation: understanding the role of disease in amphibian population declines. Philos Trans R Soc B Biol Sci 367:1688

Blaustein AR, Han BA, Relyea RA, Johnson PTJ, Buck JC, Gervasi SS, Kats LB (2011) The complexity of amphibian population declines: understanding the role of cofactors in driving amphibian losses. Ann N Y Acad Sci 1223:108–119

Blaustein AR, Johnson P (2003a) Explaining frog deformities. Sci Am:60–65

Blaustein AR, Johnson PT (2003b) The complexity of deformed amphibians. Front Ecol Environ 1:87–94

Blaustein AR, Kiesecker JM (2002) Complexity in conservation: Lessons from the global decline of amphibian populations. Ecol Lett 5:597–608

Blaustein AR, Romansic JM, Scheessele EA, Han BA, Pessier AP, Longcore JE (2005) Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus Batrachochytrium dendrobatidis. Conserv Biol 19:1460–1468

Booth B., Murphy S., Swanton C. (2003) Weed Ecology in Natural and Agricultural Systems. CABI publishing, Cambridge, MA

Both C, Grant T (2012) Biological invasions and the acoustic niche: the effect of bullfrog calls on the acoustic signals of white-banded tree frogs. Biol Lett 8:714

Bowerman J, Rombough C, Weinstock SR, Padgett-Flohr GE (2010) Terbinafine Hydrochloride in Ethanol Effectively Clears Batrachochytrium dendrobatidis in Amphibians. J Herpetol Med Surg 20:24–28

Boyle D, Boyle D, Olsen V, Morgan J, Hyatt A (2004) Rapid quantitative detection of chytridiomycosis (Batrachochytrium dendrobatidis) in amphibian samples using real-time Taqman PCR assay. Dis Aquat Organ 60:141–148

Bradley PW, Gervasi SS, Hua J, Cothran RD, Relyea RA, Olson DH, Blaustein AR (2015) Differences in sensitivity to the fungal pathogen Batrachochytrium dendrobatidis among amphibian populations. Conserv Biol 29:1347–1356

Brannelly LA, Chatfield MWH, Richards-Zawacki CL (2012) Field and Laboratory Studies of the Susceptibility of the Green Treefrog (Hyla cinerea) to Batrachochytrium dendrobatidis Infection. PLOS ONE 7:e38473

Briggs CJ, Knapp RA, Vredenburg VT (2010) Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. Proc Natl Acad Sci 107:9695–9700

Briggs CJ, Vredenburg VT, Knapp RA, Rachowicz LJ (2005) Investigating the population-level effects of chytridiomycosis: an emerging infectious disease of amphibians. Ecology 86:3149–3159

Brook BW, Sodhi NS, Bradshaw CJA (2008) Synergies among extinction drivers under global change. Trends Ecol Evol 23:453–460

Browne RK, Zippel K (2007) Reproduction and Larval Rearing of Amphibians. ILAR J 48:214–234

Bruneau M, Magnin E (1980) Croissance, nutrition et reproduction des ouaouarons Rana catesbeiana Shaw (Amphibia Anura) des Laurentides au nord de Montreal. Can J Zool 58:175–183

Brutscher LM, Daughenbaugh KF, Flenniken ML (2015) Antiviral defense mechanisms in honey bees. Soc Insects Vectors Med Vet Entomol 10:71–82

Brutyn M, D’Herde K, Dhaenens M, Rooij PV, Verbrugghe E, Hyatt AD, Croubels S, Deforce D, Ducatelle R, Haesebrouck F, Martel A, Pasmans F (2012a) Batrachochytrium dendrobatidis zoospore secretions rapidly disturb intercellular junctions in frog skin. Fungal Genet Biol 49:830–837

Brutyn M, D’Herde K, Dhaenens M, Rooij PV, Verbrugghe E, Hyatt AD, Croubels S, Deforce D, Ducatelle R, Haesebrouck F, Martel A, Pasmans F (2012b) Batrachochytrium dendrobatidis zoospore secretions rapidly disturb intercellular junctions in frog skin. Fungal Genet Biol 49:830–837

Bucciarelli GM, Blaustein AR, Garcia TS, Kats LB (2014) Invasion Complexities: The Diverse Impacts of Nonnative Species on Amphibians. Copeia 2014:611–632

Butchart SHM, Walpole M, Collen B, Strien A van, Scharlemann JPW, Almond REA, Baillie JEM, Bomhard B, Brown C, Bruno J, Carpenter KE, Carr GM, Chanson J, Chenery AM, Csirke J, Davidson NC, Dentener F, Foster M, Galli A, Galloway JN, Genovesi P, Gregory RD, Hockings M, Kapos V, Lamarque J-F, Leverington F, Loh J, McGeoch MA, McRae L, Minasyan A, Morcillo MH, Oldfield TEE, Pauly D, Quader S, Revenga C, Sauer JR, Skolnik B, Spear D, Stanwell-Smith D, Stuart SN, Symes A, Tierney M, Tyrrell TD, Vié J-C, Watson R (2010) Global Biodiversity: Indicators of Recent Declines. Science 328:1164

Carroll EJ, Hedrick JL (1974) Hatching in the toad Xenopus laevis: Morphological events and evidence for a hatching enzyme. Dev Biol 38:1–13

Ceballos G, Ehrlich PR, Barnosky AD, García A, Pringle RM, Palmer TM (2015) Accelerated modern human–induced species losses: Entering the sixth mass extinction. Sci Adv 1

Cohen KL, Seid MA, Warkentin KM (2016) How embryos escape from danger: the mechanism of rapid, plastic hatching in red-eyed treefrogs. J Exp Biol 219:1875

Cook MT, Heppell SS, Garcia TS (2013) Invasive bullfrog larvae lack developmental plasticity to changing hydroperiod. J Wildl Manag:n/a-n/a

Costa CLS, Lima SL, Andrade DR, Agostinho CA (1998) Morphological characterization of the development stages of female reproduction apparel of bullfrog, Rana catesbeiana, in the intensive Anfigranja systems. Rev Bras Zootec-Braz J Anim Sci 27:642–650

Costa CL, Lopes Lima S, Andrade DR, Agostinho CA (1998a) Caracterização Morfológica dos Estádios de Desenvolvimento do Aparelho Reprodutor Feminino da Rã-Touro, Rana catesbeiana, no Sistema Anfigranja de Criação Intensiva. Rev Bras Zootec 27:642–650

Costa CL, Lopes Lima S, Andrade DR, Agostinho CA (1998b) Caracterização Morfológica dos Estádios de Desenvolvimento do Aparelho Reprodutor Masculino da Rã-Touro, Rana catesbeiana, no Sistema Anfigranja de Criação Intensiva. Rev Bras Zootec 27:651–657

Crawford AJ, Lips KR, Bermingham E (2010) Epidemic disease decimates amphibian abundance, species diversity, and evolutionary history in the highlands of central Panama. Proc Natl Acad Sci 107:13777–13782

Crump ML, Scott Jr. NJ (1994) Chapter 2. Visual encounter surveys. In: W.R. Heyer, M.A. Donnelly, R.W. McDiarmid, L.C. Hayek, M.S. Foster (eds) Measuring and monitoring biological diversity: Standard methods for amphibians. Smithsonian Institution Press, Washington, DC, p 84–92

Cunningham A, Danzak P, Rodriguez J (2003) Pathogen Pollution: defining a parasitological threat to biodiversity conservation. J Parasitol 89 (suppl.):S78-83

Cushman SA (2006) Effects of habitat loss and fragmentation on amphibians: a review and prospectus. Biol Conserv 128:231–240

D'Amore A, Kirby E, McNicholas M (2009) Invasive species shifts ontogenetic resource partitioning and microhabitat use of a threatened native amphibian. Aquat Conserv Mar Freshw Ecosyst 19:534–541

Dang T, Searle CL, Blaustein AR (2017) Virulence variation among strains of the emerging infectious fungus Batrachochytrium dendrobatidis (Bd) in multiple amphibian host species. Dis Aquat Organ 124:233–239

Daszak P, Berger L, Cunningham AA, Hyatt AD, Green DE, Speare R (1999) Emerging infectious diseases and amphibian population declines. Emerg Infect Dis 5:735

Daszak P, Cunningham AA, Hyatt AD (2000) Emerging Infectious Diseases of Wildlife--Threats to Biodiversity and Human Health. Science 287:443–449

Daszak P, Cunningham AA, Hyatt AD (2003) Infectious disease and amphibian population declines. Divers Distrib 9:141–150

Daszak P, Strieby A, Cunningham AA, Longcore J, Brown C, Porter D (2004) Experimental evidence that the bullfrog (Rana catesbeiana) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. Herpetol J 14:201–207

Densmore CL, Green DE (2007) Diseases of amphibians. ILAR J 48:235–254

Develey-Rivière M-P, Galiana E (2007) Resistance to pathogens and host developmental stage: a multifaceted relationship within the plant kingdom. New Phytol 175:405–416

Dirzo R, Young HS, Galetti M, Ceballos G, Isaac NJB, Collen B (2014) Defaunation in the Anthropocene. Science 345:401

Doddington BJ, Bosch J, Oliver JA, Grassly NC, Garcia G, Schmidt BR, Garner TWJ, Fisher MC (2013) Context-dependent amphibian host population response to an invading pathogen. Ecology 94:1795–1804

Echaubard P, Pauli BD, Trudeau VL, Lesbarrères D (2016) Ranavirus infection in northern leopard frogs: the timing and number of exposures matter. J Zool 298:30–36

Ehrenfeld JG (2010) Ecosystem Consequences of Biological Invasions. Annu Rev Ecol Evol Syst 41:59–80

Elton C (1958) The Ecology of Invasions by Animals and Plants. Methuen, London

Eskew EA, Worth SJ, Foley JE, Todd BD (2015) American Bullfrogs (Lithobates catesbeianus) Resist Infection by Multiple Isolates of Batrachochytrium dendrobatidis, Including One Implicated in Wild Mass Mortality. EcoHealth 12:513–518

Farrer RA, Martel A, Verbrugghe E, Abouelleil A, Ducatelle R, Longcore JE, James TY, Pasmans F, Fisher MC, Cuomo CA (2017) Genomic innovations linked to infection strategies across emerging pathogenic chytrid fungi. 8:14742

Farrer RA, Weinert LA, Bielby J, Garner TWJ, Balloux F, Clare F, Bosch J, Cunningham AA, Weldon C, Preez LH du, Anderson L, Pond SLK, Shahar-Golan R, Henk DA, Fisher MC (2011) Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. Proc Natl Acad Sci 108:18732–18736

Fernández-Benéitez MJ, Ortiz-Santaliestra ME, Lizana M, Diéguez-Uribeondo J (2008) Saprolegnia diclina: another species responsible for the emergent disease “Saprolegnia infections” in amphibians. FEMS Microbiol Lett 279:23–29

Fernández-Benéitez M, Ortiz-Santaliestra M, Lizana M, Diéguez-Uribeondo J (2011) Differences in susceptibility to Saprolegnia infections among embryonic stages of two anuran species. Oecologia 165:819–826

Ficetola GF, Coïc C, Detaint M, Berroneau M, Lorvelec O, Miaud C (2007) Pattern of distribution of the American bullfrog Rana catesbeiana in Europe. Biol Invasions 9:767–772

Fisher MC, Bosch J, Yin Z, Stead DA, Walker J, Selway L, Brown AJP, Walker LA, Gow NAR, Stajich JE, Garner TWJ (2009) Proteomic and phenotypic profiling of the amphibian pathogen Batrachochytrium dendrobatidis shows that genotype is linked to virulence. Mol Ecol 18:415–429

Fisher MC, Garner TW, Walker SF (2009) Global emergence of Batrachochytrium dendrobatidis and amphibian chytridiomycosis in space, time, and host. Annu Rev Microbiol 63:291–310

Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ (2012) Emerging fungal threats to animal, plant and ecosystem health. Nature 484:186–194

Fites JS, Ramsey JP, Holden WM, Collier SP, Sutherland DM, Reinert LK, Gayek AS, Dermody TS, Aune TM, Oswald-Richter K, Rollins-Smith LA (2013) The Invasive Chytrid Fungus of Amphibians Paralyzes Lymphocyte Responses. Science 342:366

Frick WF, Pollock JF, Hicks AC, Langwig KE, Reynolds DS, Turner GG, Butchkoski CM, Kunz TH (2010) An Emerging Disease Causes Regional Population Collapse of a Common North American Bat Species. Science 329:679

Fukami T, Wardle DA, Bellingham PJ, Mulder CPH, Towns DR, Yeates GW, Bonner KI, Durrett MS, Grant-Hoffman MN, Williamson WM (2006) Above- and below-ground impacts of introduced predators in seabird-dominated island ecosystems. Ecol Lett 9:1299–1307

Gahl MK, Longcore JE, Houlahan JE (2012) Varying Responses of Northeastern North American Amphibians to the Chytrid Pathogen Batrachochytrium dendrobatidis. Conserv Biol 26:135–141

Garcia TS, Romansic JM, Blaustein AR (2006) Survival of three species of anuran metamorphs exposed to UV-B radiation and the pathogenic fungus Batrachochytrium dendrobatidis. Dis Aquat Organ 72:163–169

Garcia TS, Urbina J, Bredeweg E, Ferrari MC. (2017) Embryonic learning and developmental carry-over effects in an invasive anuran. Oecologia

Gardner PG, Frazer TK, Jacoby CA, Yanong RPE (2015) Reproductive biology of invasive lionfish (Pterois spp.). Front Mar Sci 2:7

Garner TW., Perkins MW, Govindarajulu P, Seglie D, Walker S, Cunningham AA, Fisher MC (2006) The emerging amphibian pathogen Batrachochytrium dendrobatidis globally infects introduced populations of the North American bullfrog, Rana catesbeiana. Biol Lett 2:455–459

Garner TWJ, Walker S, Bosch J, Leech S, Marcus Rowcliffe J, Cunningham AA, Fisher MC (2009) Life history tradeoffs influence mortality associated with the amphibian pathogen Batrachochytrium dendrobatidis. Oikos 118:783–791

Gervasi S, Gondhalekar C, Olson DH, Blaustein AR (2013) Host Identity Matters in the Amphibian-Batrachochytrium dendrobatidis System: Fine-Scale Patterns of Variation in Responses to a Multi-Host Pathogen. PLoS ONE 8:e54490

Gervasi SS, Hunt EG, Lowry M, Blaustein AR (2014) Temporal patterns in immunity, infection load and disease susceptibility: understanding the drivers of host responses in the amphibian-chytrid fungus system. Funct Ecol 28:569–578

Gervasi SS, Stephens PR, Hua J, Searle CL, Xie GY, Urbina J, Olson DH, Bancroft BA, Weis V, Hammond JI, Relyea RA, Blaustein AR (2017) Linking Ecology and Epidemiology to Understand Predictors of Multi-Host Responses to an Emerging Pathogen, the Amphibian Chytrid Fungus. PLOS ONE 12:e0167882

Gervasi SS, Urbina J, Hua J, Chestnut T, Relyea RA, Blaustein AR (2013) Experimental evidence for American bullfrog (Lithobates catesbeianus) susceptibility to chytrid fungus (Batrachochytrium dendrobatidis). EcoHealth 10:166–171

Gibbons JW, Scott DE, Ryan TJ, Buhlmann KA, Tuberville TD, Metts BS, Greene JL, Mills T, Leiden Y, Poppy S, Winne CT (2000) The Global Decline of Reptiles, Deja Vu Amphibians. Bioscience 50:653–666

Goater CP (1994) Growth and survival of postmetamorphic toads: Interactions among larval history, density, and parasitism. Ecology 75:2264–2274

Gomez- Mestre I, Touchon JC, Warkentin KM (2006) Amphibian embryo and parental defenses and a larval predator reduce egg mortality from water mold. Ecology:2570–2581

Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190

Govindarajulu P, Altwegg R, Anholt BR (2005) Matrix Model Investigation of Invasive Species Control: Bullfrogs on Vancouver Island. Ecol Appl 15:2161–2170

Govindarajulu P, Price WMS, Anholt BR (2006) Introduced Bullfrogs (Rana catesbeiana) in Western Canada: Has Their Ecology Diverged? J Herpetol 40:249–260

Green DE, Converse KA (2005) Diseases of amphibian eggs and embryos. In: S.K. Majumdar, J.E. Huffman, F.J. Brenner, A.I. Panah (eds) Wildlife Diseases: Landscape Epidemiology, Spatial Distribution and Utilization of Remote Sensing Technology. The Pennsylvania Academy of Science, Easton, PA, p 62–71

Greenspan SE, Calhoun AJK, Longcore JE, Levy MG (2012) Transmission of Batrachochytrium dendrobatidis to wood frogs (Lithobates sylvaticus) via a bullfrog (L. catesbeianus) vector. J Wildl Dis 48:575–582

Haislip NA, Gray MJ, Hoverman JT, Miller DL (2011) Development and disease: How susceptibility to an emerging pathogen changes through anuran development. PLoS ONE 6:e22307

Hamdoun A, Epel D (2007) Embryo stability and vulnerability in an always changing world. Proc Natl Acad Sci 104:1745–1750

Han BA, Searle CL, Blaustein AR (2011) Effects of an Infectious Fungus, Batrachochytrium dendrobatidis, on Amphibian Predator-Prey Interactions. PLOS ONE 6:e16675

Hanselmann R, Rodríguez A, Lampo M, Fajardo-Ramos L, Alonso Aguirre A, Marm Kilpatrick A, Paul Rodríguez J, Daszak P (2004) Presence of an emerging pathogen of amphibians in introduced bullfrogs Rana catesbeiana in Venezuela. Biol Conserv 120:115–119

Hatcher MJ, Dick JTA, Dunn AM (2012) Disease emergence and invasions. Funct Ecol 26:1275–1287

Holgersson MCN, Nichols WA, Paitz RT, Bowden RM (2016) How important is the eggshell as a source for initial acquisition of Salmonella in hatchling turtles? J Exp Zool Part Ecol Genet Physiol 325:142–148

Howard RD (1981) Sexual Dimorphism in Bullfrogs. Ecology 62:303–310

Howard RD (1983) Sexual Selection and Variation in Reproductive Success in a Long-Lived Organism. Am Nat 122:301–325

Hui C, Richardson DM, Landi P, Minoarivelo HO, Garnas J, Roy HE (2016) Defining invasiveness and invasibility in ecological networks. Biol Invasions 18:971–983

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 10:339–343

Hyatt AD, Boyle DG, Olsen V, Boyle DB, Berger L, Obendorf D, Dalton A, Kriger K, Hero M, Hines H, Phillott R, Campbell R, Marantelli G, Gleason F, Colling A (2007) Diagnostic assays and sampling protocols for the detection of Batrachochytrium dendrobatidis. Dis Aquat Organ 73:175–192

James TY, Litvintseva AP, Vilgalys R, Morgan JAT, Taylor JW, Fisher MC, Berger L, Weldon C, Preez L du, Longcore JE (2009) Rapid Global Expansion of the Fungal Disease Chytridiomycosis into Declining and Healthy Amphibian Populations. PLOS Pathog 5:e1000458

Jennings MR, Hayes MP (1985) Pre-1900 Overharvest of California Red-Legged Frogs (Rana aurora draytonii): The Inducement for Bullfrog (Rana catesbeiana) Introduction. Herpetologica 41:94–103

Johnson PTJ, Lunde KB, Zelmer DA, Werner JK (2003) Limb deformities as an emerging parasitic disease in amphibians: Evidence from museum specimens and resurvey data. Conserv Biol 17:1724–1737

Jones LLC, Leonard WP, Olson DH (2005) Amphibians of the Pacific Northwest. Seattle Audubon Society, Seattle

Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P (2008) Global trends in emerging infectious diseases. Nature 451:990–993

Juliano SA, Lounibos LP (2005) Ecology of invasive mosquitoes: effects on resident species and on human health. Ecol Lett 8:558–574

Juopperi T, Karli K, De Voe R, Grindem CB (2002) Granulomatous dermatitis in a spadefoot toad (Scaphiopus holbrooki). Vet Clin Pathol 31:137–139

Kaefer IL, Boelter RA, Cechin SZ (2007) Reproductive biology of the invasive bullfrog Lithobates catesbeianus in southern Brazil. Ann Zool Fenn 44:435–444

Kats LB, Ferrer RP (2003) Alien predators and amphibian declines: review of two decades of science and the transition to conservation. Divers Distrib 9:99–110

Kelehear C, Webb JK, Shine R (2009) Rhabdias pseudosphaerocephala infection in Bufo marinus: Lung nematodes reduce viability of metamorph cane toads. Parasitology 136:919–927

Kiesecker JM, Blaustein AR (1995) Synergism between UV-B radiation and a pathogen magnifies amphibian embryo mortality in nature. Proc Natl Acad Sci U S A 92:11049–11052

Kiesecker JM, Blaustein AR (1997) Population differences in responses of Red legged frogs (Rana aurora) to introduced bullfrogs. Ecology 78:1752–1760

Kiesecker JM, Blaustein AR (1998) Effects of Introduced Bullfrogs and Smallmouth Bass on Microhabitat Use, Growth, and Survival of Native Red-Legged Frogs (Rana aurora). Conserv Biol 12:776–787

Kiesecker JM, Blaustein AR, Miller CL (2001a) Transfer of a pathogen from fish to amphibians. Conserv Biol 15:1064–1070

Kiesecker JM, Blaustein AR, Miller C (2001b) Potential mechanisms underlying the displacement of native Red-legged frogs by introduced bullfrogs. Ecology 82:1964–1970

Kilpatrick AM, Briggs CJ, Daszak P (2010) The ecology and impact of chytridiomycosis: an emerging disease of amphibians. Trends Ecol Evol 25:109–118

Kleinhenz P, Boone MD, Fellers G (2012) Effects of the Amphibian Chytrid Fungus and Four Insecticides on Pacific Treefrogs (Pseudacris regilla). J Herpetol 46:625–631

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol Evol 16:199–204

LaFiandra EM, Babbitt KJ (2004) Predator induced phenotypic plasticity in the pinewoods tree frog, Hyla femoralis: Necessary cues and the cost of development. Oecologia 138:350–359

Langhammer PF, Lips KR, Burrowes PA, Tunstall T, Palmer CM, Collins JP (2013) A Fungal Pathogen of Amphibians, Batrachochytrium dendrobatidis, Attenuates in Pathogenicity with In Vitro Passages. PLOS ONE 8:e77630

Leivas P., Moura M., Favaro L. (2012) The reproductive biology of the invasive Lithobates catesbeianus (Amphibia: Anura). J Herpetol 46:153–161

Lever C (2003) Naturalized Reptiles and Amphibians of the World.

Liew N, Mazon Moya MJ, Wierzbicki CJ, Hollinshead M, Dillon MJ, Thornton CR, Ellison A, Cable J, Fisher MC, Mostowy S (2017) Chytrid fungus infection in zebrafish demonstrates that the pathogen can parasitize non-amphibian vertebrate hosts. Nat Commun 8:15048

Lima SL, Costa CLS, Agostinho CA, Andrade DR, Pereira HP (1998) Estimate of bullfrog size at first sexual maturation, Rana catesbeiana, in the intensive growing Anfigranja system. Rev Bras Zootec-Braz J Anim Sci 27:416–420

Liu X, Rohr JR, Li Y (2012) Climate, vegetation, introduced hosts and trade shape a global wildlife pandemic. Proc R Soc B Biol Sci 280

Lobos G, Jaksic FM (2005) The ongoing invasion of African clawed frogs (*Xenopus laevis*) in Chile: causes of concern. Biodivers Conserv 14:429–439

Lodge DM (1993) Biological invasions: Lessons for ecology. Trends Ecol Evol 8:133–137

Longcore JE, Pessier AP, Nichols and DK (1999) Batrachochytrium dendrobatidis gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 91:219–227

Lorch JM, Lankton J, Werner K, Falendysz EA, McCurley K, Blehert DS (2015) Experimental Infection of Snakes with Ophidiomyces ophiodiicola Causes Pathological Changes That Typify Snake Fungal Disease. mBio 6

Marcum R, St-Hilaire S, Murphy P, Rodnick K (2010) Effects of Batrachochytrium dendrobatidis infection on ion concentrations in the boreal toad Anaxyrus (Bufo) boreas boreas. Dis Aquat Organ 91:17–21

Mast J, Goddeeris BM (1999) Development of immunocompetence of broiler chickens. Vet Immunol Immunopathol 70:245–256

Mazzoni R, Cunningham AA, Daszak P, Apolo A, Perdomo E, Speranza G (2003) Emerging Pathogen in Wild Amphibians and Frogs (Rana catesbeiana) Farmed for International Trade. Emerg Infect Dis J 9:995

McCallum ML (2007) Amphibian decline or extinction? Current declines dwarf background extinction rate. J Herpetol 41:483–491

McCoid MJ, Fritts TH (1989) Growth and Fatbody Cycles in Feral Populations of the African Clawed Frog, Xenopus laevis (Pipidae), in California with Comments on Reproduction. Southwest Nat 34:499–505

McConnell TH (2007) The nature of disease: Pathology for the health professions. Lippincott Williams & Wilkins, Baltimore

McMahon TA, Brannelly LA, Chatfield MWH, Johnson PTJ, Joseph MB, McKenzie VJ, Richards-Zawacki CL, Venesky MD, Rohr JR (2013) Chytrid fungus Batrachochytrium dendrobatidis has nonamphibian hosts and releases chemicals that cause pathology in the absence of infection. Proc Natl Acad Sci 110:210–215

McMahon TA, Sears BF, Venesky MD, Bessler SM, Brown JM, Deutsch K, Halstead NT, Lentz G, Tenouri N, Young S, Civitello DJ, Ortega N, Fites JS, Reinert LK, Rollins-Smith LA, Raffel TR, Rohr JR (2014) Amphibians acquire resistance to live and dead fungus overcoming fungal immunosuppression. Nature 511:224–227

Medeiros CI, Both C, Grant T, Hartz SM (2017) Invasion of the acoustic niche: variable responses by native species to invasive American bullfrog calls. Biol Invasions 19:675–690

Mooney HA (2010) The ecosystem-service chain and the biological diversity crisis. Philos Trans R Soc B Biol Sci 365:31–39

Morehouse EA, James TY, Ganley ARD, Vilgalys R, Berger L, Murphy PJ, Longcore JE (2003) Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. Mol Ecol 12:395–403

Moss A, Carty N, San Francisco M (2010) Identification and partial characterization of an elastolytic protease in the amphibian pathogen Batrachochytrium dendrobatidis. Dis Aquat Organ 92:149–158

Moyle PB (1973) Effects of Introduced Bullfrogs, Rana catesbeiana, on the Native Frogs of the San Joaquin Valley, California. Copeia 1973:18–22

Murillo-Rincón AP, Laurila A, Orizaola G (2017) Compensating for delayed hatching reduces offspring immune response and increases life-history costs. Oikos 126:565–571

Muths E, Corn PS, Pessier AP, Green DE (2003) Evidence for disease-related amphibian decline in Colorado. Biol Conserv 110:357–365

Naeem S, Chapin III F, Costanza R, Ehrlich PR, Golley FB, Hooper DU, Lawton JH, O'Neill RV, Mooney HA, Sala OE (1999) Biodiversity and ecosystem functioning: maintaining natural life support processes. Issues Ecol 4

Nori J, Urbina-Cardona JN, Loyola RD, Lescano JN, Leynaud GC (2011) Climate Change and American Bullfrog Invasion: What Could We Expect in South America? PLOS ONE 6:e25718

Nussbaum RA, Brodie ED, Storm RM (1983) Amphibians and reptiles of the Pacific northwest. University Press of Idaho, Moscow, Idaho

Oliver TH, Isaac NJB, August TA, Woodcock BA, Roy DB, Bullock JM (2015) Declining resilience of ecosystem functions under biodiversity loss. Nat Commun 6:10122

Olson DH, Aanensen DM, Ronnenberg KL, Powell CI, Walker SF, Bielby J, Garner TWJ, Weaver G, Fisher MC, The Bd Mapping Group (2013) Mapping the Global Emergence of Batrachochytrium dendrobatidis, the Amphibian Chytrid Fungus. PLoS ONE 8:e56802

Ortega JE, Serrano VH, Ramírez-Pinilla MP, Lannoo MJ (2005) Reproduction of an Introduced Population of Eleutherodactylus johnstonei at Bucaramanga, Colombia. Copeia 2005:642–648

Ortiz-Santaliestra ME, Rittenhouse TAG, Cary TL, Karasov WH (2013) Interspecific and Postmetamorphic Variation in Susceptibility of Three North American Anurans to Batrachochytrium dendrobatidis. J Herpetol 47:286–292

Paini DR, Sheppard AW, Cook DC, De Barro PJ, Worner SP, Thomas MB (2016) Global threat to agriculture from invasive species. Proc Natl Acad Sci 113:7575–7579

Parris MJ, Beaudoin JG (2004) Chytridiomycosis impacts predator-prey interactions in larval amphibian communities. Oecologia 140:626–632

Parris MJ, Cornelius TO (2004) Fungal pathogen causes competitive and developmental stress in larval amphibian communities. Ecology 85:3385–3395

Pearl C, Adams M, Leuthold N, Bury R (2005) Amphibian occurrence and aquatic invaders in a changing landscape: Implications for wetland mitigation in the willamette valley, Oregon, USA. Wetlands 25:76–88

Pechenik JA (2006) Larval experience and latent effects—metamorphosis is not a new beginning. Integr Comp Biol 46:323–333

Phillott AD, Grogan LF, Cashins SD, McDonald KR, Berger LEE, Skerratt LF (2013) Chytridiomycosis and seasonal mortality of tropical stream-associated frogs 15 Years after introduction of Batrachochytrium dendrobatidis. Conserv Biol 27:1058–1068

Pimentel D, Lach L, Zuniga R, Morrison D (2000) Environmental and economic costs of nonindigenous species in the United States. BioScience 50:53–65

Piovia-Scott J, Pope K, Joy Worth S, Rosenblum EB, Poorten T, Refsnider J, Rollins-Smith LA, Reinert LK, Wells HL, Rejmanek D, Lawler S, Foley J (2015) Correlates of virulence in a frog-killing fungal pathogen: evidence from a California amphibian decline. ISME J 9:1570–1578

Piovia-Scott J, Pope KL, Lawler SP, Cole EM, Foley JE (2011) Factors related to the distribution and prevalence of the fungal pathogen Batrachochytrium dendrobatidis in Rana cascadae and other amphibians in the Klamath Mountains. Biol Conserv 144:2913–2921

Plowright RK, Sokolow SH, Gorman ME, Daszak P, Foley JE (2008) Causal inference in disease ecology: investigating ecological drivers of disease emergence. Front Ecol Environ 6:420–429

Poulin R, Combes C (1999) The Concept of Virulence: Interpretations and Implications. Parasitol Today 15:474–475

Preston DL, Henderson JS, Johnson PTJ (2012) Community ecology of invasions: direct and indirect effects of multiple invasive species on aquatic communities. Ecology 93:1254–1261

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

Rago A, While GM, Uller T (2012) Introduction pathway and climate trump ecology and life history as predictors of establishment success in alien frogs and toads. Ecol Evol 2:1437–1445

Raney EC, Ingram WM (1941) Growth of tagged frogs (Rana catesbeiana Shaw and Rana clamitans Daudin) under natural conditions. Am Midl Nat 26:201–206

Rebelo R, Amaral P, Bernardes M, Oliveira J, Pinheiro P, Leitão D (2010) Xenopus laevis (Daudin, 1802), a new exotic amphibian in Portugal. Biol Invasions 12:3383–3387

Reeder NM, Pessier AP, Vredenburg VT (2012) A reservoir species for the emerging amphibian pathogen Batrachochytrium dendrobatidis thrives in a landscape decimated by disease. PLoS One 7:e33567

Retallick RWR, McCallum H, Speare R (2004) Endemic Infection of the Amphibian Chytrid Fungus in a Frog Community Post-Decline. PLOS Biol 2:e351

Retallick RWR, Miera V (2007) Strain differences in the amphibian chytrid Batrachochytrium dendrobatidis and non-permanent, sub-lethal effects of infection. Dis Aquat Organ 75:201–207

Retallick RWR, Miera V, Richards KL, Field KJ, Collins JP (2006) A non-lethal technique for detecting the chytrid fungus Batrachochytrium dendrobatidis on tadpoles. Dis Aquat Organ 72:77–85

Ricciardi A, Hoopes MF, Marchetti MP, Lockwood JL (2013) Progress toward understanding the ecological impacts of nonnative species. Ecol Monogr 83:263–282

Richter-Boix A, Orizaola G, Laurila A (2014) Transgenerational phenotypic plasticity links breeding phenology with offspring life-history. Ecology 95:2715–2722

Rogers WE (2017) Invasive Species. In: Reference Module in Earth Systems and Environmental Sciences. Elsevier

Rogers CS, Miller J (2013) Coral Diseases Cause Reef Decline. Science 340:1522

Rohr JR, Raffel TR, Hall CA (2010) Developmental variation in resistance and tolerance in a multi-host–parasite system. Funct Ecol 24:1110–1121

Rohr JR, Raffel TR, Halstead NT, McMahon TA, Johnson SA, Boughton RK, Martin LB (2013) Early-life exposure to a herbicide has enduring effects on pathogen-induced mortality. Proc R Soc B Biol Sci 280

Rosenblum EB, James TY, Zamudio KR, Poorten TJ, Ilut D, Rodriguez D, Eastman JM, Richards-Hrdlicka K, Joneson S, Jenkinson TS, Longcore JE, Parra Olea G, Toledo LF, Arellano ML, Medina EM, Restrepo S, Flechas SV, Berger L, Briggs CJ, Stajich JE (2013) Complex history of the amphibian-killing chytrid fungus revealed with genome resequencing data. Proc Natl Acad Sci 110:9385–9390

Rosenblum EB, Voyles J, Poorten TJ, Stajich JE (2010) The Deadly Chytrid Fungus: A Story of an Emerging Pathogen. PLoS Pathog 6:e1000550

Rumschlag SL, Boone MD (2015) How Time of Exposure to the Amphibian Chytrid Fungus Affects Hyla chrysoscelis in the Presence of an Insecticide. Herpetologica 71:169–176

Ryan MJ (1980) The reproductive behavior of the Bullfrog (Rana catesbeiana). Copeia 1980:108–114

Saka M, Tada N, Kamata Y (2013) Application of an amphibian (Silurana tropicalis) metamorphosis assay to the testing of the chronic toxicity of three rice paddy herbicides: Simetryn, mefenacet, and thiobencarb. Ecotoxicol Environ Saf 92:135–143

Savidge JA, Qualls FJ, Rodda GH (2007) Reproductive Biology of the Brown Tree Snake, Boiga irregularis (Reptilia: Colubridae), during Colonization of Guam and Comparison with That in Their Native Range. Pac Sci 61:191–199

Schlaepfer M, Sredl M, Rosen P, Ryan M (2007) High Prevalence of Batrachochytrium dendrobatidis in Wild Populations of Lowland Leopard Frogs Rana yavapaiensis in Arizona. EcoHealth 4:421–427

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). Biol Conserv 142:1420–1426

Schloegel LM, Toledo LF, Longcore JE, Greenspan SE, Vieira C., Lee M, Zhao S, Wangen C, Ferreira CM, Hipolito M, Davies AJ, Cuomo CA, Daszak, P P, James TY (2012) Novel, panzootic and hybrid genotypes of amphibian chytridiomycosis associated with the bullfrog trade. Mol Ecol 21:5162–5177

Searle CL, Belden LK, Du P, Blaustein AR (2014) Stress and chytridiomycosis: Exogenous exposure to corticosterone does not alter amphibian susceptibility to a fungal pathogen. J Exp Zool Part Ecol Genet Physiol 321:243–253

Searle CL, Gervasi SS, Hua J, Hammond JI, Relyea RA, Olson DH, Blaustein AR (2011) Differential Host Susceptibility to Batrachochytrium dendrobatidis, an Emerging Amphibian Pathogen. Conserv Biol 25:965–974

Searle CL, Xie GY, Blaustein AR (2013) Development and Infectious Disease in Hosts with Complex Life Cycles. PLoS ONE 8:e60920

Shirose LJ, Brooks RJ, Barta JR, Desser SS (1993) Intersexual differences in growth, mortality, and size at maturity in bullfrogs in central Ontario. Can J Zool 71:2363–2369

Simberloff D (2006) Invasional meltdown 6 years later: important phenomenon, unfortunate metaphor, or both? Ecol Lett 9:912–919

Simberloff D (2011) How common are invasion-induced ecosystem impacts? Biol Invasions 13:1255–1268

Simberloff D (2013) Invasive species: What everyone needs to know. Oxford University Press, New York

Simberloff D, Von Holle B (1999) Positive Interactions of Nonindigenous Species: Invasional Meltdown? Biol Invasions 1:21–32

Skerratt L, Berger L, Speare R, Cashins S, McDonald K, Phillott A, Hines H, Kenyon N (2007) Spread of Chytridiomycosis Has Caused the Rapid Global Decline and Extinction of Frogs. EcoHealth 4:125–134

Skerratt LF, Garner TWJ, Hyatt AD (2009) Determining Causality and Controlling Disease is Based on Collaborative Research involving Multidisciplinary Approaches. EcoHealth 6:331–334

Sniegula S, Janssens L, Stoks R (2017) Integrating multiple stressors across life stages and latitudes: Combined and delayed effects of an egg heat wave and larval pesticide exposure in a damselfly. Aquat Toxicol 186:113–122

Sol D, Maspons J, Vall-llosera M, Bartomeus I, García-Peña GE, Piñol J, Freckleton RP (2012) Unraveling the Life History of Successful Invaders. Science 337:580

Speare R, Thomas AD, O’Shea P, Shipton WA (1994) Mucor amphibiorum in the toad, Bufo marinus, in Australia. J Wildl Dis 30:399–407

Stockwell MP, Clulow J, Mahony MJ (2010) Host species determines whether infection load increases beyond disease-causing thresholds following exposure to the amphibian chytrid fungus. Anim Conserv 13:62–71

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller RW (2004) Status and Trends of Amphibian Declines and Extinctions Worldwide. Science 306:1783–1786

Symonds EP, Trott DJ, Bird PS, Mills P (2008) Growth Characteristics and Enzyme Activity in Batrachochytrium dendrobatidis Isolates. Mycopathologia 166:143–147

Taylor SK, Williams ES, Pier AC, Mills KW, Bock MD (1999) Mucormycotic dermatitis in captive adult Wyoming toads. J Wildl Dis 35:70–74

Tobler U, Schmidt BR (2010) Within- and Among-Population Variation in Chytridiomycosis-Induced Mortality in the Toad Alytes obstetricans. PLOS ONE 5:e10927

Tompkins DM, Carver S, Jones ME, Krkošek M, Skerratt LF (2015) Emerging infectious diseases of wildlife: a critical perspective. Trends Parasitol 31:149–159

Touchon JTJ, Gomez-Mestre IG-MI, Warkentin KWK (2006) Hatching plasticity in two temperate anurans: responses to a pathogen and predation cues. Can J Zool 84:556–563

Uller T, Sagvik J, Olsson M (2009) Pre-hatching exposure to water mold reduces size at metamorphosis in the moor frog. Oecologia 160:9–14

Urban MC, Phillips BL, Skelly DK, Shine R (2007) The cane toad's (Chaunus [Bufo] marinus) increasing ability to invade Australia is revealed by a dynamically updated range model. Proc R Soc B Biol Sci 274:1413–1419

Valkenburg SA, Venturi V, Dang THY, Bird NL, Doherty PC, Turner SJ, Davenport MP, Kedzierska K (2012) Early Priming Minimizes the Age-Related Immune Compromise of CD8+ T Cell Diversity and Function. PLOS Pathog 8:e1002544

Van Kleunen M, Dawson W, Schlaepfer D, Jeschke JM, Fischer M (2010) Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. Ecol Lett 13:947–958

Van Rooij P, Martel A, Haesebrouck F, Pasmans F (2015) Amphibian chytridiomycosis: a review with focus on fungus-host interactions. Vet Res 46:137

Vargas Salinas F (2006) Breeding behavior and colonization success of the Cuban treefrog Osteopilus septentrionalis. Herpetologica 62:398–408

Venesky MD, Wassersug RJ, Parris MJ (2010) Fungal Pathogen Changes the Feeding Kinematics of Larval Anurans. J Parasitol 96:552–557

Voyles J, Young S, Berger L, Campbell C, Voyles WF, Dinudom A, Cook D, Webb R, Alford RA, Skerratt LF, Speare R (2009) Pathogenesis of Chytridiomycosis, a Cause of Catastrophic Amphibian Declines. Science 326:582–585

Wake DB, Vredenburg VT (2008) Are we in the midst of the sixth mass extinction? A view from the world of amphibians. Proc Natl Acad Sci 105:11466–11473

Walke JB, Becker MH, Loftus SC, House LL, Teotonio TL, Minbiole KPC, Belden LK (2015) Community Structure and Function of Amphibian Skin Microbes: An Experiment with Bullfrogs Exposed to a Chytrid Fungus. PLoS ONE 10:1–18

Warkentin KM, Currie CR, Rehner SA (2001) Egg-killing fungus induces early hatching of red-eyed treefrog eggs. Ecology 82:2860–2869

White EM, Wilson JC, Clarke AR (2006) Biotic indirect effects: a neglected concept in invasion biology. Divers Distrib 12:443–455

Willis YL, Moyle DL, Baskett TS (1956) Emergence, Breeding, Hibernation, Movements and Transformation of the Bullfrog, Rana catesbeiana, in Missouri. Copeia 1956:30–41

Wright A (1920) Frogs: Their Natural History and Utilization. Washington, DC

Wright A, Wright A (1949) Handbook of frogs and toads of the United States and Canada. Comstock, New York

Xie GY, Olson DH, Blaustein AR (2016) Projecting the Global Distribution of the Emerging Amphibian Fungal Pathogen, Batrachochytrium dendrobatidis, Based on IPCC Climate Futures. PLOS ONE 11:e0160746