Climate-driven shifts in host-parasite interactions: Consequences for parasite transmission and amphibian pathology

Sara Hellmuth Paull

Follow this and additional works at: https://scholar.colorado.edu/ebio_gradetds
Part of the Ecology and Evolutionary Biology Commons, Environmental Sciences Commons, and the Parasitology Commons

Recommended Citation
https://scholar.colorado.edu/ebio_gradetds/24
CLIMATE-DRIVEN SHIFTS IN HOST-PARASITE INTERACTIONS:
CONSEQUENCES FOR PARASITE TRANSMISSION AND HOST PATHOLOGY

by

Sara Hellmuth Paull

B.A. Dartmouth College, 2005

A thesis submitted to the
Faculty of the Graduate School of the
University of Colorado in partial fulfillment
of the requirement for the degree of
Doctor of Philosophy
Department of Ecology and Evolutionary Biology
2012
This thesis entitled: 
Climate-driven shifts in host-parasite interactions: Consequences for parasite transmission and amphibian pathology 
written by Sara Hellmuth Paull 
has been approved for the Department of Ecology and Evolutionary Biology 

______________________________________
Pieter Johnson 

______________________________________
Sharon Collinge 

______________________________________
Robert Guralnick 

______________________________________
Brett Melbourne 

______________________________________
Elisabeth Root 

______________________________________

Date

The final copy of this thesis has been examined by the signatories, and we Find that both the content and the form meet acceptable presentation standards Of scholarly work in the above mentioned discipline.
Interspecific differences in climate-driven changes to organismal physiology and phenology will alter the timing and consequence of many ecological interactions. The extent to which such changes may exacerbate or ameliorate disease risk is increasingly controversial. I used a combination of laboratory and field studies to simultaneously test both direct (i.e., physiological) and indirect (i.e., interspecific interactions) effects of climate change on disease using the parasite, *Ribeiroia ondatrae*, and its hosts as a model system. *Ribeiroia* is transmitted sequentially from snails to amphibians to birds, and when amphibians are infected early in development, they are more likely to die or become deformed. Because parasite development is enhanced by elevated temperatures while amphibian breeding is jointly constrained by temperature and rainfall, I hypothesized that climate change could lead to greater infection of tadpoles at earlier stages of development, causing elevated pathology. By infecting snails at different temperatures in the laboratory, I found that higher temperatures accelerated parasite
development and amplified snail pathology (via reduced egg output), but also decreased snail survival time. Similarly, in amphibians, parasite infectivity (host penetration) was enhanced, but establishment (encystment), persistence, and survival outside the host declined with rising temperature. There was a mid-temperature peak in deformities, likely resulting from increases in parasite infectivity with temperature coupled with reductions in host vulnerability owing to faster development. My field survey revealed a negative association between evaporation and the density of infected snails as well as amphibian infection intensity (independently of the snail effect), likely due to reduced host and parasite survival and production at sites subjected to drying. After accounting for *Ribeiroia* infection intensity, amphibian deformities were also positively correlated with nutrients, suggesting a potential effect of eutrophication on host tolerance or parasite virulence. Different aspects of climate change will thus act to enhance infection in some ways, but hinder it in others, suggesting that future research into climate-disease interactions should use a combination of field and laboratory studies that focus on potential nonlinearities between climate and disease risk, shifting host-parasite interactions, and abiotic changes beyond temperature.
DEDICATION

This thesis is dedicated to my parents for their limitless love and support, to my brother and sister for inspiring me in so many ways, and especially to my husband, for always making me smile.
ACKNOWLEDGEMENTS

There are numerous people without whom this thesis would not have been possible. I would first like to extend my sincerest thanks to my academic advisor, Dr. Pieter Johnson, who has been an extraordinary mentor. He taught me a tremendous amount about experimental design, analysis, and writing, and most importantly, how to think like a scientist. By providing unique insight and helpful feedback on each of my projects, he encouraged me to reach my full potential. He has always gone out of his way to provide fast and helpful input at short notice, to introduce his students to colleagues at conferences, and to build a fun and collaborative lab environment. I would also like to express gratitude to my committee members, Dr. Sharon Collinge, Dr. Robert Guralnick, Dr. Brett Melbourne, and Dr. Elisabeth Root. They were always willing to meet with me to provide extra support, advice and encouragement along the way. I am also very thankful to Dr. Chris Ray for taking the time to help get me started with the analyses of my field data. I am grateful to the other graduate students and post-docs that have spent time in our lab, including, Sarah Orlofske, Katie Dosch, Dan Preston, Joe Mihaljevic, Max Joseph, Dr. Esra Kellermanns, Dr. Brett Goodman, Dr. Yuri Springer, and Dr. Jason Hoverman for their insightful contributions to many of these chapters, and for the enjoyable times we had both in and out of the lab and field. Finally, a large number of volunteers and paid assistants helped to collect the data presented here, including Charissa Rujanavech, Liz Daly, Kayley Dorsa, Nik Mirhashemi, Ashley Camenson, Daniel Ross, Shelly Todd, Greg Walth, Kendra Gietzen, Clara Boland, Kelly King and Katherine Knezo. I would like to extend a particular thanks to Bryan
LaFonte and Patrick Hoffman, both of whom put in extremely long, frequently volunteer, hours to complete challenging projects.

The experiments described here were funded by a wide variety of funding agencies that I would also like to thank. I was supported for three years by the United States Environmental Protection Agency (EPA) under the Science to Achieve Results (STAR) Graduate Fellowship Program, which provided a stipend allowing me to focus on my research, as well as direct funding for research supplies. EPA has not officially endorsed this dissertation and the views expressed herein may not reflect the views of the EPA. I was also supported for one semester by the National Science Foundation GK-12 fellowship program, and for another semester by the University of Colorado Graduate School through a dissertation completion fellowship. Several other granting agencies provided funding for the research described herein including: Sigma Xi, The Scientific Research Society (Grant-In-Aid of Research) and the American Society of Ichthyologists and Herpetologists (Gaige award). I also received very generous support from the University of Colorado, through the Ecology and Evolutionary Biology Department, the Museum of Natural History, the Beverly Sears graduate student research program, the Graduate School travel grant program, and Undergraduate Research Opportunities Program.
CONTENTS

CHAPTER
I. Can we predict climate-driven changes to disease dynamics? Applications for theory and management in the face of uncertainty .................. 1

Complexities of predicting climate-driven changes in disease dynamics ..............................................................2

How does climate change affect patterns of disease? ......................... 3

Current methods for studying climate-driven changes in disease ........... 8

Where do we go from here? Predicting and mitigating the effects of climate change on disease ............................................. 10

Conclusion ......................................................................................17

II. Temperature enhances host pathology in a snail-trematode system:
Consequences of climate change for disease emergence? .................. 19

Abstract ........................................................................................19

Introduction ...................................................................................20

Methods ........................................................................................23

Results ..........................................................................................29

Discussion ......................................................................................32

III. Temperature-driven shifts in a host-parasite interaction drive nonlinear changes in disease risk ..................................................38

Abstract ........................................................................................38

Introduction ...................................................................................39

Methods ........................................................................................42

Results ..........................................................................................48

Discussion ......................................................................................51
IV. Beyond warming: comparing the roles of temperature, evaporation, and nutrients on amphibian disease risk in wetland ecosystems

Abstract

Introduction

Methods

Results

Discussion

V. Conclusion

Effects of climate change on the Ribeiroia system

Future directions

Implications of this work for studies of climate change ecology

REFERENCES
TABLES

Table

3.1 Model comparison showing that the deformity rate of infected tadpoles has a nonlinear relationship with temperature…………………………..50

4.1 List of models and the variables included for each AICc analysis of the effects of climate and nutrient variables on infection and deformities of snails and amphibians ……………………………………………69

4.2 Best supported models (ΔAICc<2) for densities of infected snails, residuals of amphibian infections (after accounting for infected snail densities), and residuals of deformities (after accounting for Ribeiroia infections) ……………………………………………………………..73

4.3 Model-averaged coefficients and standard errors calculated for the predictor variables in the best-supported models (ΔAICc<2) of densities of infected snails, residuals of amphibian infections (after accounting for infected snail densities), and residuals of deformities (after accounting for Ribeiroia infections)…………………………..74
Figures

1.1 Hypothetical trade-offs between temperature-dependent growth and mortality rates of a pathogen.................................................................4

1.2 Potential direct influence of climate change at different stages of pathogen transmission for hypothetical vectorborne, complex life-cycle, and directly transmitted diseases........................................12

1.3 Depiction of the thermal tolerance window of a hypothetical host and pathogen..........................................................................................13

2.1 Influence of temperature and infection by *Ribeiroia ondatrae* on mean growth per day (± 1 SE) of snail host (*Planorbella trivolvis*)............29

2.2 Total number of eggs produced per snail (*Planorbella trivolvis*) per day in different temperature treatments over time in the uninfected and infected treatments..................................................30

2.3 Influence of temperature and infection by *Ribeiroia ondatrae* on snail host survival..................................................................................31

2.4 Proportion of snails releasing cercariae in different temperature treatments..........................................................................................32

3.1 Percentage of cercariae surviving in each treatment over time...........48

3.2 Effect of temperature on the percentage of parasites penetrating tadpole skin and the percentage of the parasites that established once inside the host...........................................................................49

3.3 Mean ± SE number of metacercarial cysts recovered from *P. regilla* tadpoles necropsied 48 hours post-infection in the short-term trials, and from *P. regilla* necropsied upon metamorphosis in the long-term trials..................................................................................50

3.4 Percentage of *Ribeiroia*-exposed *Pseudacris regilla* that survived to metamorphosis and emerged deformed, or died before metamorphosis at each temperature ± 95% CI........................................51

3.5 Mean ± SE developmental stage (Gosner 1960) of tadpoles just prior to each of the four *Ribeiroia* exposure events at each temperature........52
4.1 The location of each of the twenty field sites monitored in our study is marked with a circled star. Note that some sites (particularly the two in the farthest northeast corner) are so close together that only one site marker is visible on the map.

4.2 Relationship between nutrients (first principle component of TDP and TDN) and deformity risk, where the y-axis is the residuals of the relationship between number of *Ribeiroia* metacercariae and percent of deformed metamorphs.

5.1 Photo of greenhouse structures placed over buried mesocosm tanks.
CHAPTER 1

CAN WE PREDICT CLIMATE-DRIVEN CHANGES TO DISEASE DYNAMICS? APPLICATIONS FOR THEORY AND MANAGEMENT IN THE FACE OF UNCERTAINTY


How climate change will affect diseases is rapidly becoming one of the most pressing and challenging questions for epidemiologists and conservationists. Advances in modeling techniques and climate science since publication of the first assessment of global climate change in 1990 have led to increasingly reliable predictions about temperature and precipitation changes (Solomon et al. 2007). Corresponding theoretical developments regarding ecological effects of climate on disease have also occurred, but consensus remains elusive (Wilson 2009). While there is a strong body of theoretical work exploring potential climate-disease interactions, there has been little consideration of the potential synergistic effects of climate change and disease on the resilience of wildlife populations and communities. This will be a key concept for identifying effective management strategies in the face of multiple interacting threats and uncertainty (Hoegh-Guldberg and Bruno 2010).

I begin this chapter by highlighting the debate over the role of climate in the spread of disease using human malaria and amphibian chytridiomycosis as case studies. Next, I discuss the mechanisms through which climate change can alter host-pathogen physiology, distribution, interactions, and evolution, emphasizing empirical examples that illustrate the predominant trends. Finally, I discuss current statistical and empirical methods used to evaluate climate-disease linkages before proposing novel methods
for studying, predicting, and managing the problems associated with climate-driven variations in disease.

**Complexities of predicting climate-driven changes in disease dynamics**

The effects of climate change on the distribution and severity of diseases is currently a source of vigorous debate (Lafferty 2009, Randolph 2009, Wilson 2009, Rohr et al. 2011). Multiple, interacting global change drivers, the complex interaction networks of many infectious diseases, and uncertainties in the specifics of temperature and precipitation predictions preclude simplistic generalizations about disease changes resulting from climate change (Tylianakis et al. 2008, Rohr et al. 2011). Given these complexities, it is not surprising that efforts to identify climate ‘signatures’ in disease patterns have yielded equivocal results. I illustrate this point with two case studies – one involving a human disease (malaria) and one a wildlife disease (amphibian chytridiomycosis).

Early attempts at projecting the consequences of climate change for malaria risk based on biological models of vectors predicted large range expansions for human disease risk, igniting over a decade of debate on the topic (e.g., Martens et al. 1995). Rogers and Randolph (2000) criticized initial models as overly simplistic and argued that they overestimated future malaria distributions. Using current malaria distributions to statistically model future ranges under climate change scenarios, they predicted little change in malaria risk. Ostfeld (2009), however, pointed out that such forecasts under-represented the climatic tolerances for malaria because they excluded regions of targeted malaria eradication.

Similar controversies have arisen over the role of climate change in the emergence and spread of *Batrachochytrium dendrobatidis* (*Bd*), a chytridiomycete pathogen that has caused
dramatic amphibian population declines and extinctions (Skerratt et al. 2007). To explain the
decline of tropical montane frogs, Pounds et al. (2006) proposed the “chytrid-thermal-optimum
hypothesis,” which suggests that climate-mediated increases in cloud cover shifted temperatures
towards the growth-optimum for *Bd*. Lips et al. (2008), on the other hand, found no support for
climate-driven *Bd* outbreaks in Central and South America, showing instead that disease patterns
were consistent with the epidemic spread of a recently introduced pathogen. An analysis by
Rohr et al. (2008) further argued that correlations between temperature and frog declines do not
necessarily imply that climate change has caused these species declines.

The case studies of malaria and *Bd* emphasize some of the fundamental problems that
plague the debate over climate-driven effects on diseases, particularly those of urgent human
health or conservation concern. Given the variety and complexity of disease systems and the
uncertainties inherent in making climate predictions, it is prudent to question at what scale can
we accurately forecast climate-mediated changes in disease? The answer is vitally important
because the success of mitigation strategies ultimately depends on the efficient allocation of
limited resources to regions most at risk of disease increases. To assess the feasibility of
predicting climate change effects on disease dynamics, I first discuss the mechanisms through
which climate change can influence host-pathogen physiology, distributions, interactions, and
evolution.

**How does climate change affect patterns of disease?**

*Physiological Changes*

Climate change can influence the physiology of hosts, vectors and pathogens in different
ways, introducing intriguing shifts in disease patterns. Nonlinear responses of pathogens to
rising temperature could have a major impact on their abundance. In one example, recent warming shifted the development time of Arctic nematode larvae from two years to a single season, thereby increasing the infection risk experienced by muskoxen (Kutz et al. 2005). If warming temperatures consistently accelerate parasite development more than that of their hosts, climate change could dramatically enhance parasite abundance and host pathology. Alongside changes in mean temperature, shifts in climate variability will also affect disease dynamics by changing pathogen development rates or host immune responses relative to constant temperatures (Paaijmans et al. 2009; Rohr and Raffel 2010).

Climate change will alter mortality rates as well as developmental rates, however, and the balance between these changes for hosts, vectors, and pathogens will influence disease severity in a system (Fig. 1.1). In temperate zones, warmer winters could enhance the overwintering survival of some pathogens and vectors (Harvell et al. 2002, Canto et al. 2009), but higher metabolic rates and temperatures that exceed thermal tolerance...
limits can also reduce vector and parasite survival (Lafferty 2009, Snall et al. 2009). Nor will the effects of climate change on disease necessarily be consistent across the distribution of a pathogen. For instance, elevated precipitation can reduce water salinity and therefore the survival of *Vibrio* bacteria in mesic areas, while increasing cholera risk in drier areas, possibly due to lower water availability and increased concentration of the pathogen in available water sources (Pascual et al. 2002).

A final complication of physiological influences on disease patterns is the influence of climate on host immunity, particularly for ectothermic species. Warming temperatures may either increase or decrease host immunity (e.g., Harvell et al. 2002, Canto et al. 2009). Other climatic changes, such as prolonged drought or increased atmospheric carbon dioxide concentrations, can also alter host resistance to pathogens (Garrett et al. 2006). These observations collectively suggest that the net effect of climate change on disease will depend on how the physiology of different hosts, vectors, and pathogens, respond to temperature and precipitation changes.

*Range Shifts*

Climate models predict a greater rise in minimum temperatures than maxima, such that temperatures may be more likely to approach the thermal optima of many organisms, leading to predictions that diseases will expand their ranges as temperate areas warm (Ostfeld 2009). Many plant and animal pathogens and vectors may shift poleward in latitude or upward in elevation with climate change (e.g., Pascual et al. 2006). For example, bluetongue virus has spread northward into Europe since 1998, likely as a result of northward expansion of its vector and increased overwinter virus persistence (Purse et al. 2005). However, many organisms face barriers to dispersal and physiological limitations that prevent range expansions (Root et al.)
2003, Lafferty 2009). For instance, Randolph and Rogers (2000) projected a net range reduction of tick-borne encephalitis (TBE) in Europe owing to the dependency of TBE on infected nymphal ticks feeding in close proximity to larval ticks – an event that only occurs in particular climatic conditions. Poleward movements of pathogens and vectors could have a strong effect on the immunologically naïve host populations they encounter, regardless of whether pathogens quantitatively expand their ranges. For instance, if malaria shifts its distribution upwards in elevation, it will move into the most populous regions of Africa and South America such that a net decrease in range could still translate into an increase in human impact (Pascual and Bouma 2009). An elevational increase in avian malaria in Hawai’i could have devastating effects on highly susceptible native bird populations (Atkinson et al. 2009). Proactive disease regulation measures (e.g., mosquito control) should be considered for areas where the latitudinal and elevational boundaries of pathogens seem to be determined by climatic factors rather than dispersal barriers.

**Biotic Interactions**

Local differences in species’ physiological responses to climate change will scale up to influence biotic interactions that can have consequences for disease (Gilman et al. 2010; Yang and Rudolf 2010). For instance, in some disease systems, host immunity and/or pathology is dependent on the age or development stage of the host at infection (Woodland and Blackman 2006; Johnson et al. 2011; Patankar et al. 2011). If hosts and parasites in such disease systems have different developmental or phenological responses to climate change, this could shift the timing of their interaction, which could have consequences for pathogen virulence and host pathology (Yang and Rudolf 2011). Climate change can also shift host-parasite infection dynamics. For example, an analysis of a thirty-year dataset of chytrid-diatom dynamics revealed
that milder winters reduced chytrid fungal infections of the diatom host *Asterionella formosa* because the diatoms became infected before they bloomed, reducing population sizes of both species (Ibelings et al. 2011). Temperature-driven changes in predator-prey relationships can also affect disease transmission. Hall et al. (2006) showed that regulation of fungal epidemics in *Daphnia* by predatory fish may be stronger at warmer temperatures, because the fish respond more strongly to temperature. Climate-driven changes in the compositions of host and parasite communities will also lead to novel host-parasite interactions. For example, hosts may gain or lose parasites as a result of interspecific differences in movement rates (Garrett et al. 2006, Brooks and Hoberg 2007, Harvell et al. 2009). These novel parasite communities can influence host pathology via competition, facilitation and predation among different parasite species (Pederson and Fenton 2007). Novel communities could thus result in interactions between parasites that complicate efforts to predict disease patterns.

*Evolutionary Responses*

Changing climates will shift the selective pressures operating on pathogens and their hosts, providing a catalyst for evolutionary change. Pathogens tend to have short generation times and high mutation rates, which facilitates adaption to changing environmental conditions (Koelle et al. 2005). Range shifts may also enhance the evolution and spread of drug resistant pathogens by facilitating host movement and gene flow (Criscione and Blouin 2004, Bonizzoni et al. 2009). Strong selection for use of new hosts by pathogens may lead to disease emergence in previously unaffected populations (Marcogliese 2001, Brooks and Hoberg 2007). A greater understanding of pathogen evolutionary processes can be achieved using a phylodynamic approach to characterize the drivers of pathogen evolutionary dynamics across multiple scales (Holmes and Grenfell 2009). Pathogen evolution and changing climate will stimulate host and
vector adaptations as well, although evolutionary constraints may restrict the rate at which this occurs (Austin et al. in press). Climate is changing at a rate that is unprecedented for the last 50 million years (Solomon 2007). Adaptation will be constrained in most cases by the rate of microevolution, as well as antagonistic genetic correlations, which may not proceed at rates fast enough for the predicted pace of climate change (Etterson and Shaw 2001, Visser 2008). While mobile organisms may be able to escape regions of increasing parasitism, those with slow migration rates, such as plants, will be forced to rely more on adaptation to changing threats resulting from pathogens (Garrett et al. 2006). Reductions in plant genetic diversity resulting from local adaptation to rapid climate change could also reduce disease resistance in some populations (Jump and Peñuelas 2005).

**Current methods for studying climate-driven changes in disease**

Current techniques for forecasting the influence of climate change on disease risk include correlative studies along temporal and spatial climate gradients, synthetic meta-analyses, predictive models, and experimental investigations. I discuss the relative merits and weaknesses of each technique before suggesting novel strategies that can enhance their implementation. Because of the urgency of the problem, the complexity of host-parasite-climate systems, and the paucity of baseline data for most diseases, a combination of approaches is likely to generate the most reliable results for forecasting.

Statistical approaches attempt to link temporally or spatially variable climatic patterns with disease incidence. These approaches take two main forms: tests for connections between regional warming trends and disease, or correlations between the El Niño Southern Oscillation (ENSO) or the North Atlantic Oscillation (NAO) indices and disease. Studies linking disease
incidence to ENSO or NAO indices can provide a useful tool for forecasting disease severity up to a year in advance (Chaves and Pascual 2006). Interpreting the results requires some caution, however, as such models often lack mechanistic components and correlations involving large-scale, monotonic increases in both variables can be misleading (Rohr et al. 2008). Other intrinsic factors such as changes in the number of immune hosts could cause cyclical changes in disease, underscoring the need to consider the relative importance of extrinsic climatic forcing versus internal drivers (Dobson 2009, Harvell et al. 2009).

Meta-analyses and other synthetic approaches can distill large-scale patterns from the synthesis of numerous, small-scale experiments or surveys. By quantitatively summarizing current knowledge, meta-analyses have provided compelling evidence to support theoretical predictions about biotic responses to climate change (e.g., Parmesan and Yohe 2003, Root et al. 2003). Meta-analyses can be problematic, however, because working with published data can introduce bias (e.g., null results often go unpublished) or non-independent studies into the analysis (Lei et al. 2007). While these problems can be minimized with a careful literature selection process, the results typically do not mechanistically explain patterns and mask the variability among studies that is essential for fine-scale prediction (Lei et al. 2007).

There are two main types of bioclimatic models: mechanistic models that use physiological parameters to infer changes to disease distributions, and statistical models that use the climatic parameters associated with the current range of the disease to forecast future range shifts (Jeschke and Strayer 2008). The utility of bioclimatic models lies in their ability to generate quantitative hypotheses about disease shifts resulting from climate change that can be used to direct management efforts (Jeschke and Strayer 2008). Many bioclimatic models, however, depend on assumptions that are frequently violated, such as the assumptions that
species ranges are not limited by dispersal or biotic interactions, and a species’ genetic climate tolerance does not vary through space or time (Peterson and Shaw 2003, Jeschke and Strayer 2008, Gilman et al. 2010, Brodie et al., this volume). Incorporating other aspects known to influence disease risk, such as anticipated changes in host population size or limitations to dispersal will strengthen the predictive power of such models (Peterson and Shaw 2003).

Carefully designed experiments provide the most mechanistic evidence of how climate affects disease dynamics. While short-term laboratory studies are typically limited to one component of a complex disease system, they help clarify the mechanisms underlying climate-disease interactions and can be used to parameterize mechanistic predictive models. For example, Terblanche et al. (2008) experimentally measured the thermal tolerance of tsetse flies, vectors for human and animal trypanosomiases, and inferred that future warming would exceed their upper thermal limits and lead to a reduction in their geographic range. Field experiments are necessary to explore the effects of climate change on disease in a more realistic larger-scale context. For instance field studies that have elevated temperatures of plant-pathogen systems generally find that pathogens respond uniquely to the warming treatment, with some increasing and others decreasing (e.g., Wiedermann et al. 2007), suggesting that large-scale patterns may not be fully elucidated by smaller single-system experiments.

Where do we go from here? Predicting and mitigating the effects of climate change on disease

It is critical that we focus attention towards developing more quantitative predictions, greater mechanistic understanding, and explicit management advice regarding the effects of climate on disease. Here, I summarize novel strategies in the areas of modeling, empirical
research, and disease management, which can be used in conjunction with existing tools to provide an informative framework for mitigating the effects of climate change on disease.

Although forecasts about climate-driven changes in disease dynamics will always be plagued by the complexity of the issue, unpredictable stochastic forces, and variation in disease response across scales, a greater mechanistic understanding of the processes involved, including more cross-disciplinary research, and a focus on climate-sensitive aspects of disease transmission (Fig. 1.2) will enhance our ability to respond to changing disease risks.

Physiology meets ecology: using novel modeling strategies to enhance disease forecasting

Mechanistic models based on physiological parameters of specific host-pathogen systems and regional climate forecasts can facilitate management plans at the local level. Dynamic energy budget (DEB) models describe organismal physiologies in detail and can be adapted to model interactions between organisms at the population and community levels (e.g. Kooijman 2001, Vasseur and McCann 2005). Biophysical models have strong predictive power relative to correlative methods because they can detect effects across multiple scales and can distinguish between hypothesized drivers of disease change (Helmuth 2009, Kearney et al. 2009). For example, a biophysical model that incorporated the microclimatic effects of different water storage containers demonstrated that water storage had a larger impact than climate change on the distribution of dengue-carrying mosquitoes (Kearney et al. 2009). Such mechanistic models are rare, however, owing to the increase in parameters that occurs when extending bioenergetics models to the community level (Vasseur and McCann 2005). In the absence of such detailed physiological data, qualitative predictions about host-parasite interactions can still be made by considering easily measured physiological parameters such as thermal windows, which describe the range of temperatures across which an organism can maintain stable performance, and the
Climate effects on host-pathogen interactions

Fig. 1.2 Potential direct influence of climate change at different stages of pathogen transmission for hypothetical vectorborne (dashed line), complex life-cycle (dotted line), and directly transmitted (dot-dashed line) diseases. The changes that occur in the dynamics of any given disease will be affected by whether the organisms involved are ectothermic or endothermic, as well as by indirect interactions with the community and environment. Attempts to understand climate-driven changes in disease dynamics will be further complicated by evolutionary changes and distributional shifts of hosts, vectors, and pathogens as well.
Q_{10} coefficient, which describes a change in a given physiological rate for every 10°C change in temperature. These measures may be useful in predicting the direction of changes in host-pathogen interactions (Figure 1.3). Increased emphasis on physiological models for predicting climate-driven changes in biotic interactions within specific disease systems could complement the current use of bioclimatic models to provide a more complete overall picture.

![Graph showing metabolic rates of host and pathogen](image)

Fig. 1.3 Depiction of the thermal tolerance window of a hypothetical host (solid line) and pathogen (dotted line). The shaded region is the range of temperatures at which the host and parasite can co-exist. As temperatures warm they become more favorable for the parasite, shifting the character of the relationship.

**Experimental climate change: using novel empirical approaches to address disease effects**

Previous empirical research on the climate-disease linkage has been hindered by logistical complications of experimentally modifying environmental temperatures. I suggest two forms of empirical study to help advance research on climate change: (1) spatial gradients in temperature, and (2) experimental mesocosm and field studies. Because elevation gradients offer
a range of climatic conditions over relatively short distances, correlations between climate and
disease patterns across differing altitudes will have fewer covariates to influence the results
(Fukami and Wardle 2005). Relatively few studies have correlated biotic responses to climate
change using available spatial gradients, despite the ability of such geographical gradients in
temperature to provide powerful tools for understanding climate-driven changes in disease risk
(Hudson et al. 2006, Altizer et al. 2006). Semi-natural mesocosm experiments are also an
effective way to test theories about climate-driven changes in disease transmission that can
minimize the loss of realism. Designs for large outdoor mesocosms can range from expensive
computerized aquatic systems to simple plexiglass heat-trapping structures in terrestrial or
aquatic environments (Liboriussen et al. 2005, Netten et al. 2008). The use of simplified host-
parasite systems can further facilitate experimental testing of hypotheses about disease reactions
to climate change. The Arctic is a promising region for such studies due to its low anthropogenic
influence, low levels of biodiversity, and predictions for pronounced climatic shifts (Kutz et al.
2009).

Further study of the direct and indirect climatic drivers of disease dynamics other than
temperature is also necessary. Climate change involves alteration of a suite of climatic variables
beyond mean temperature, including precipitation, diurnal and seasonal temperature ranges, and
the frequency and severity of extreme weather events. For example, in aquatic systems, changes
in ice cover, acidification, eutrophication, lake mixing regimes, and UV exposure associated with
climate change will also affect hosts and parasites (Marcogliese 2001). Changes in terrestrial
ecosystems are expected in response to climate-driven alteration of snow cover, wildfire
disturbance regimes, the frequency of extreme weather events and carbon dioxide concentrations
(Garrett et al. 2006, Schumacher and Bugman 2006, Jentsch et al. 2009). Studies that test factors
other than changes in mean temperature and precipitation on disease systems are needed to assess whether climatic variability or indirect drivers may also play a role in disease dynamics.

Technological advances for research and collaboration

Interdisciplinary collaborations will be an important aspect of developing novel research methods to explore climate-driven changes in disease. For instance, molecular PCR-based techniques can improve disease surveillance methods (Polley and Thompson 2009). Recent breakthroughs in DNA sequencing techniques will also pave the way for phylodynamic approaches to epidemiology that could provide key insights into evolutionary responses of pathogens to climate change (Holmes and Grenfell 2009). Technological advances in sequencing and identification techniques have also made the direct analysis of paleoparasitological changes associated with changes in paleoclimate data an increasingly useful avenue of research (Dittmar 2009). Geographic information systems (GIS) offer another powerful epidemiological tool that can be used for mapping potential climate-driven changes to disease risk (Ostfeld et al. 2005). For example, remote sensing technology can be used to characterize regions with high disease risk (Glass et al. 2007). Collectively, these tools should be applied toward developing a large-scale, interactive and publicly accessible database of disease distribution and pathology that would provide an invaluable resource for global-scale analyses of climate-driven changes in disease (Semenza and Menne 2009). Because the study of climate change and disease spans disciplines ranging from atmospheric science to epidemiology, multidisciplinary efforts are key to developing the creative research and data acquisition strategies necessary for effective disease management.

Planning for the unpredictable: management and surveillance tools
Despite our best efforts to predict disease responses to climate change, the complexities and uncertainties within these systems ensure that ecological “surprises” will occur. Acting in the face of such uncertainties requires effective use of management strategies in combination with enhanced disease treatment and surveillance methods. Managing populations to increase their resilience will be critical given the uncertainties in predicting climate-driven changes to ecosystems (Hoegh-Guldberg and Bruno 2010). To increase the resilience of populations to climate-driven disease susceptibility, managers should focus efforts on maintaining high genetic and species diversity while reducing other environmental stressors (Evans and Perschel 2009, Hoegh-Guldberg and Bruno 2010). Simple changes in animal husbandry practices, including grazing, housing, and shearing can also reduce disease risk in domestic animals and the spill-over of infections into wildlife populations (Morgan and Wall 2009). In the case of diseases in humans or in threatened or economically valuable species, vaccination against infection may be necessary (Hampson et al. 2009). Flexibility will be a key component of plans for managing climate-driven changes in disease. Adaptive management is one such strategy that involves development of experimental management programs to test alternative hypotheses for effective resource management (Walters and Holling 1990). Another emerging strategy for dealing with stochastic events that cause unpredictable regime shifts is to develop alternative management plans for multiple future scenarios to cope with unexpected changes quickly and effectively (Bennett et al. 2003).

*Linking climate change, disease and conservation:*

Ultimately, the success of predictions about climate-disease interactions will be measured by their utility in mitigating the negative consequences of diseases for human and wildlife populations. Although low host population sizes can often reduce disease persistence, diseases
can still cause species extinctions by driving populations to unstably small numbers or residing in reservoir hosts (de Castro and Bolker 2005). Even if a disease does not cause extinction of a particular species, it can lead to genetic homogenization that reduces its ability to cope with other environmental stressors (Smith et al. 2006). An overall assessment of climate-driven changes to disease risk should use these characteristics to determine which diseases are most likely to pose a threat to wildlife populations.

Finally, the conservation of parasites and pathogens themselves may be an appropriate management consideration. Parasites play a vital role in many ecosystems, and their disappearance could have a cascading effect on other processes. Parasites serve as key links in food webs and as regulators of host populations (Dobson et al. 2008). Specialist parasites may have a higher extinction risk, which could allow generalist parasites (typically associated with higher pathological effects) to increase as a result of competitive release (Dunn et al 2009). Parasites and pathogens have a dramatic influence on the health of human and wildlife populations. Changes in their dynamics and abundance will be among the most important consequences of global climate change.

**Conclusions**

Given the complexities involved in disease dynamics and the uncertainties surrounding climate change predictions, the debate over whether climate change will increase or reduce global disease risk will continue. Diseases and species tend to respond idiosyncratically to climate change, with range shifts, physiological changes, phenological changes, and evolutionary rates differing among species. Nevertheless, the risk of changing diseases to human health and wildlife conservation is great enough that action is required. Our predictive abilities can be
enhanced through biophysical modeling, increased use of experimental manipulations incorporating the direct and indirect effects of climate change, and collaborative efforts that capitalize on recent technological innovations. Our forecasting capabilities may be limited to predicting the general behavior of specific, well-parameterized host-pathogen systems, or to identifying the factors that are important in driving the dynamics of specific classes of pathogens. The best approach for maximizing our predictive capabilities is to combine mechanistic empirical and modeling approaches. The uncertainty arising from the complexities of host-parasite-climate interactions, particularly in conjunction with other global change drivers, underscores the need for managing wildlife populations to increase resilience within a framework of flexible adaptive management practices.
CHAPTER 2

TEMPERATURE ENHANCES HOST PATHOLOGY IN A SNAIL-TREMATODE SYSTEM: CONSEQUENCES OF CLIMATE CHANGE FOR DISEASE EMERGENCE?


Abstract

Disease severity may be altered by the differential responses of hosts and parasites to rising temperatures leading to an increase or reduction of disease. The net effect of climate change on emerging diseases will reflect the effects of temperature on all life history stages of both hosts and parasites. To explore how climate change differentially influences hosts and parasites, I studied the effect of increasing temperatures on different life stages of the multi-host trematode parasite *Ribeiroia ondatrae*, which has been linked to the emerging phenomenon of amphibian limb malformations, and its snail intermediate host *Planorbeella trivolis*. I determined the effects of temperature on development of *R. ondatrae* eggs and redia larvae and the effects of parasite exposure (exposed and sham-exposed), temperature (13, 20, and 26°C), and their interaction on snail host vital rates, including growth, mortality and reproduction. *Ribeiroia eggs* developed four times faster at 26°C than at 17°C and did not develop at 12°C. Higher temperatures increased snail growth, egg production, and mortality. Infection interacted with temperature to enhance the growth of infected snails while reducing their fecundity at 26°C. These results suggest that pathology associated with infection is amplified at higher temperatures. The timing of interactions between *R. ondatrae* and *P. trivolis* may be influenced by their physiological responses to temperature. Temperature-driven increases in the growth of infected snails coupled with the cessation of parasite development at lower temperatures suggest that warming temperatures will change host-parasite dynamics. Taken together, these results
indicate that future climate change could alter parasite abundance and pathology by creating a ‘phenological mismatch’ between snail hosts and parasites, potentially leading to infection of both snail and amphibian hosts in earlier and, in the case of amphibians, more vulnerable stages of development.

**Introduction**

Anthropogenic environmental changes are frequently cited as primary drivers of disease emergence in human and wildlife populations (Daszak et al. 2000; Jones et al. 2008). Disturbances of aquatic habitats by eutrophication, urbanization and invasive species are shifting the dynamics of freshwater diseases (Daszak et al. 2000; Gajadhar and Allen 2004; Johnson et al. 2007; Okamura et al. 2011; Poulin et al. 2011). Climate change will likely interact with other forms of global environmental change to further alter pathogen transmission in freshwater habitats (Hakalahti et al. 2006; Marcogliese 2008). Evidence already suggests that climate change is influencing the distribution and abundance of freshwater diseases of both medical and conservation significance (Pascual et al. 2006; Atkinson et al. 2009; Johnson and Paull 2011). However, the multifaceted interactions between climate change and freshwater environments make predicting the response of freshwater diseases to climate change particularly challenging (Ibelings et al. 2011; Paull and Johnson in press). Climate change will likely lead to both direct (i.e., physiological) and indirect (i.e., interspecific interactions) effects on parasite transmission, some of which may increase disease while others will reduce infection or pathology.

Temperature can act directly on disease by altering the susceptibility of hosts, the virulence of pathogens, and the growth rates of both hosts and pathogens, which can in turn influence host pathology and disease emergence (Cairns et al. 2005; Raffel et al. 2006). For instance, Bally and Garrabou (2007) demonstrated that infection with four bacterial pathogens
induced complete mortality of corals at temperatures above 24°C, whereas no disease symptoms were observed at 16°C. They attributed this result to elevated pathogen virulence and greater host susceptibility at higher temperatures. Increased pathogen growth rates can also lead to population-level changes in host disease incidence. For example, warming temperatures in the cool waters of Finland could cause a crustacean ectoparasite to switch from completing one generation per season to two, potentially leading to higher disease incidence in farmed fish (Hakalahti et al. 2006). These direct effects of temperature on host pathology can be compounded by indirect changes acting on host-parasite interactions.

Growing evidence suggests that climate-driven changes in interspecific interactions may lead to important consequences for host-pathogen relationships and disease emergence (Gilman et al. 2010). Because temperature patterns control growth and reproduction in a variety of organisms (Stenseth and Mysterud 2002), changes in temperature are likely to influence the rate and timing of development of some species more strongly than others. This asymmetrical response to temperature has led to mismatched interactions between plants and pollinators as well as predators and prey (Both and Visser 2001; Memmott et al. 2007). For example, over the last four decades in Lake Washington, warming temperatures have caused the peak algal bloom to shift 20 days earlier in the season; however, there has been no corresponding shift in peak densities of Daphnia, which depend on the algae for food, likely because Daphnia use photoperiod more than temperature as a cue for egg hatching (Winder and Schindler 2004). Such ‘phenological mismatch’ in a host-parasite system could affect the pathology associated with infection. Small organisms tend to have faster generation times, stronger growth responses to temperature, and wider thermal windows (Pörtner 2002), suggesting that climate change could cause pathogens to become abundant more quickly than their hosts during warm seasons. This
could lead to more severe infections in immature or immunologically naïve hosts, or alternatively to an absence of susceptible hosts during peak parasite abundance, thereby reducing infections.

Parasites with complex life cycles require multiple host species to reach maturity, making them particularly susceptible to the direct and indirect effects of climate change (Harvell et al. 2002; Marcogliese 2008; Mas-Coma et al. 2009). The trematode parasite, *Ribeiroia ondatrae* (Price), has recently been linked to widespread observations of limb deformities in amphibians, including missing or extra limbs as well as skin webbings (Johnson et al. 1999). *Ribeiroia*, which is transmitted sequentially from birds to snails to amphibians via free-living infectious stages, causes amphibian mortality and limb deformities in experimental studies (Johnson et al. 1999; Johnson et al. 2004). Analysis of historical accounts of amphibian populations suggests that the frequency, severity and distribution of amphibian deformities have likely increased in recent years (Hoppe 2000; Johnson et al. 2003; Johnson and Chase 2004). Although not all malformations are due to *Ribeiroia* infection, a diverse suite of anthropogenic influences including eutrophication, pesticide exposure, and introduced fish can exacerbate infection and may have contributed to increases in infection (Johnson et al. 2007, 2010; Rohr et al. 2008). As of yet, however, no studies have examined the potential influence of climate change – historical or forecasted – on interactions between *Ribeiroia* and its hosts, despite laboratory findings indicating that the life span of free-living trematode stages and development within ectothermic hosts is highly temperature-dependent (Fried and Ponder 2003; Poulin 2006; Yang et al. 2007). The life cycle of *Ribeiroia* is similar to that of other trematodes causing diseases in humans or wildlife, including schistosomiasis, fascioliasis and cercarial dermatitis. In view of this
similarity, the effects of temperature on R. ondatrae may illustrate general consequences of climate change for the epidemiology of trematode diseases and the conservation of hosts.

To explore the potential effects of climate change on host pathology and host-parasite interactions, I experimentally evaluated the effects of temperature on the development of R. ondatrae eggs and the pathology associated with infection of snail hosts, Planorbella trivolvis (Say). In particular, I sought to mechanistically determine (i) the direct effects of temperature on infection pathology of the snail host, and (ii) the potential for indirect effects of temperature on host-parasite interactions resulting from differences in the responses of R. ondatrae and P. trivolvis to changing temperatures. To this end, I assessed the effects of temperature on host growth, fecundity and mortality, as well as parasite development at multiple stages of the life cycle. Extending studies of the direct effects of temperature on disease to explore their ecological implications for host-parasite interactions is important for understanding the complex responses of hosts and parasites to climate change. This approach allowed me to compare changes in all aspects of snail pathology, including changes in growth, fecundity and mortality between infected and uninfected hosts. Given the role of R. ondatrae in causing deformities and mortality in natural amphibian populations (Johnson et al. 1999), understanding the influence of climate change on the physiology of this pathogen and its interactions with intermediate snail hosts is relevant to its overall effects on amphibian populations.

**Methods**

**Study System**

The complex life cycle of Ribeiroia ondatrae involves transmission from bird or mammal definitive hosts to snail intermediate hosts and then to amphibian or fish second intermediate
hosts (Johnson et al. 2004). Definitive hosts release parasite eggs into aquatic systems where they hatch into miracidia that live for 12-24 hours. Miracidia locate and infect snails in the family Planorbidae, ultimately developing into rediae within the snail over the course of several weeks. At night, mature rediae release mobile cercariae, which have about one to two days to locate and encyst within a larval amphibian host, often around the developing limb buds. When larval amphibians are infected early in development, this process can cause developmental abnormalities in amphibians, including extra, missing, or deformed limbs (Johnson et al. 1999).

*Planorbella trivolvis* (previously *Helisoma trivolvis*) is a pulmonate snail found in fresh waters throughout North America (Russell-Hunter et al. 1984). Pulmonate snails are hermaphroditic although self-fertilization is rare (Norton and Bronson 2006). Pulmonate reproductive cycles are typically semelparous; however, there are some cases of iteroparous *P. trivolvis* in mesotrophic habitats (Eversole 1978; Dillon 2000). The life span of *P. trivolvis* in wild populations is typically one to two years (Dillon 2000). Growth in pulmonate snails is indeterminate, with no pre-determined size at maturation, and continues throughout life (Russell-Hunter et al. 1984). These snails serve as one of the main hosts for *Ribeiroia ondatrae* in the United States (Johnson et al. 2004). Infection of *P. trivolvis* by trematodes leads to reproductive castration and accelerated growth known as gigantism (Lagru et al. 2007).

Effects of temperature on parasite free-living stages

To obtain eggs of *R. ondatrae*, I exposed five rats to 50 metacercariae removed from laboratory-infected *Lithobates catesbeianus* (Shaw) tadpoles. After two weeks, I collected feces on wet paper towels below wire rat cages. After filtering the feces through a series of five sieves (smallest = 47 μm pore size), I placed 12 mL of material (approximately 11,000 eggs) into 0.5 L glass jars filled with spring water. I covered jars to prevent light exposure, which is a known
stimulus for parasite hatching (Johnson et al., 2004). Water baths within temperature-control chambers were used to maintain specific water temperatures. I placed three jars in each temperature bath (12°C, 17°C, and 26°C) and used temperature-control chambers to maintain water baths at the 12°C and 17°C temperature treatments, and 250 Watt heaters (Jager brand) to warm the 26°C temperature treatment for 30 weeks. Power heads (Rio Mini 50) were placed in each water bath to keep water circulating and at a uniform temperature, and Hobo underwater dataloggers (Onset Computer Corporation; Bourne, MA) were placed in one water bath at each temperature to record temperatures every hour during the course of the experiment. I aerated jars continuously to reduce bacterial growth, changed the water weekly, and rotated jar locations in the water baths regularly to avoid positional effects.

I sampled 25-30 eggs from each jar twice a week at 200x magnification and characterized the fraction of eggs in each of the following categories: ostensibly viable but without signs of development (stage zero), initial concentration of material towards the centre of the egg (stage one), outline of developing miracidium visible (stage two), developed miracidium with a visible eyespot (stage three), hatched with exit pore visible (stage four), or dead.

Effects of temperature on intra-host development and host pathology

I studied the effects of temperature on R. ondatrae intra-snail development and host pathology using a two-by-three factorial experiment with two levels of snail infection (exposed and sham-exposed to R. ondatrae eggs) and three temperatures (13°C, 20°C and 26°C). Snails ranged in shell length from 7.2 to 12.3 mm, and came from a laboratory stock of snails originally collected in Minnesota and bred in the laboratory for multiple generations. I randomly assigned 360 snails to each of the six treatments (60 snails per treatment), taking care to divide different size classes (grouped in 1 mm increments) evenly among treatments. To infect the snails, I
obtained eggs from the faeces of *R. ondatrae*-infected rats and incubated them at 29°C. After 17 days of incubation, I quantified the number of *R. ondatrae* mature eggs (with a visible eyespot) per 20 μL subsample. I placed snails from the infected treatment into an 80 L container, exposing them as a group to 100 eggs per snail. Snails from the uninfected treatment were sham-exposed to filtered rat faeces collected from uninfected rats. All snails were held in their exposure tanks for 1 week prior to the start of the experiment.

At the start of the experiment I marked each snail shell with “queen bee” tags (Beeworks, Ontario, Canada) for individual identification and placed snails in groups of five within 2.5 L containers (12 containers in each of the six temperature x infection treatments for a total of 72 containers) to ensure reproductive activity since isolated *P. trivolvis* produce few to no eggs (Paull, pers. obs). Each 40 L temperature bath held six of these containers (three from each infection treatment), for a total of four replicate temperature baths for each treatment. Temperatures were controlled as described in the egg experiment. I rotated the position of containers and water baths weekly and moved snails among containers to avoid persistent container effects. In addition to the 360 experimental snails described above, I kept a supplementary 15 ‘replacement’ snails in identical conditions to experimental snails at each temperature and infection treatment to replace any snails that died in order to maintain equivalent snail densities in each experimental container. Data from replacement snails were only included in the egg analyses because their growth and mortality were not recorded unless they entered the experiment. When all replacement snails had been used, I shifted snails between containers of the same treatment to maintain equal densities. Containers were filled with dechlorinated tapwater, which was changed every five days. I fed snails a mixture of lettuce and TetraMin Spirulina flake food ad libitum and changed the water every five days.
I recorded snail mortality daily, and snail growth weekly, using digital calipers to measure shell length. At five day intervals I counted the number of eggs in each container. Beginning three weeks post-infection, dead snails were dissected to look for evidence of developing infections (i.e. rediae). One month post-exposure, I checked for mature infections in all surviving snails weekly by placing snails individually into 50 mL centrifuge tubes overnight at their respective temperature treatments. The following morning I removed the snails from their vials and quantified released cercariae.

Temperature Range

I used a total temperature range spanning 12 to 26°C to reflect both within-season variation in pond temperatures as well as projected changes to lake water temperatures over the next century. The mean spring-summer temperature measured every four hours by a datalogger in 2006 in the Minnesota pond from which these snails were collected was 19°C ± 7°C (SD). Over the course of a full year, the temperature in the pond ranged from 0°C to 30°C. Most projections for changes in the temperature of small lakes across the United States and Canada (the known range of this parasite) predict a temperature change of between 5-10°C by the year 2100 with lakes at higher latitudes expected to exhibit the greatest degree of change (Sharma et al. 2007; Fang and Stefan 2009). Thus I chose temperature intervals that would encompass expected changes due to climate change between each temperature category while still representing the wide range of seasonal temperatures in ponds where the parasite occurs.

Analyses

I used a repeated-measures ANOVA to test the effect of temperature on *R. ondatraceae* egg development over time (mean developmental stage per sampling date). Because I rotated snails randomly among containers and water baths during the course of the experiment, I used fixed
effects models for the analyses of snail growth, mortality, fecundity and parasite development.

In addition, I ran the analyses with models that included each snail’s initial water bath and container identity as random nested factors and the findings were the same (results not shown). The growth (mm/snail/day) and fecundity (eggs/container/day) data were also square-root transformed to reduce positive skew and improve homogeneity of variance. The effects of temperature and infection on snail growth were analyzed using a factorial two-way ANOVA with infection status and temperature treatment as fixed factors. Because I expected infection to reduce fecundity over time, I used a two-way repeated measures ANOVA to analyze the influence of infection and temperature on snail egg production. The first four intervals (20 days) of egg data were used in this analysis since mortality in the warmest treatment precluded the use of longer-term data. To analyze the effects of treatment on snail mortality, I used a proportional hazards test with infection status and temperature as fixed factors, and snails that were sacrificed or that did not die during the course of the experiment labeled as “censored”. I tested the effect of temperature on the time required for infected snails to begin releasing cercariae (patency) using a proportional hazards test with individuals that never shed labeled as “censored”. I used JMP 8.0 (SAS Institute, Cary, NC) for all statistical analyses.

To compare the relative influence of temperature on infected and uninfected snail growth rates, I calculated physiological Q_{10} rates using the formula: 

$$\log Q_{10} = \frac{10\log(R_2 - R_1)}{T_2 - T_1}$$

(Poulin, 2006). In this formula, R_1 and R_2 are growth rates (mm/day), at the 20°C (T_1) and 26°C (T_2) temperature treatments respectively. Conceptually a Q_{10} rate is the relative change in a physiological rate over a ten degree change in temperature, and describes the temperature sensitivity of physiological rates. The 13°C treatment was excluded from the Q_{10} analyses because growth was negligible.
Results

Parasite Egg Development

Temperature significantly enhanced the rate of *R. ondatrae* egg development (RM-ANOVA, $F_{2,6} = 300.66, P < 0.001$). Eggs in the $26^\circ C$ treatment began to hatch within 15 days, while eggs in the $17^\circ C$ treatment did not begin hatching until 58 days after the experiment began. Eggs incubated at $12^\circ C$ never developed beyond stage 0 after 52 days of observation, even after being warmed to the $26^\circ C$ treatment for the final 7 days of observation.

Host Growth

I found a significant, positive main effect for temperature on host growth (2-way ANOVA, $F_{2,303} = 115.13, P < 0.001$). Snails grew over five times faster at $26^\circ C$ than at $13^\circ C$ (Fig. 2.1). While there was no main effect for infection ($F_{1,303} = 0.72, P = 0.397$), I observed a significant infection-by-temperature interaction, such that warmer temperatures enhanced growth of infected snails more than uninfected snails ($F_{2,303} = 9.27, P < 0.001$). The $Q_{10}$ value for infected snail growth was 4.21 relative to 1.77 for uninfected snails. Among snails maintained at $26^\circ C$, infected snails grew nearly twice as much per day relative to uninfected snails.

Host Fecundity

Fig. 2.1 Influence of temperature and infection by *Ribeiroia ondatrae* on mean growth per day ($\pm 1$ SE) of snail host (*Planorbiella trivolvis*). Snail growth was calculated as the total shell growth for each snail divided by the number of days the snail survived.
Host egg production within each container increased with time and was significantly enhanced by temperature (RM-ANOVA, time: $F_{3,64} = 9.30, P < 0.001$; temperature: $F_{2,66} = 50.113, P < 0.001$). Snails in the 26°C treatment produced, on average, 7.5 times more eggs per day than snails in the 13°C treatment. This is reflected in the greater total numbers of eggs per snail per day at the different temperatures (Fig. 2.2). I found a significant temperature-by-infection interaction ($F_{2,66} = 10.40, P < 0.001$), such that the fecundity of infected snails was significantly reduced only in the 26°C treatment ($F_{1,22} = 76.72, P < 0.001$), but not at 20°C ($F_{1,22} = 1.24, P = 0.277$) or 13°C ($F_{1,22} = 1.69, P = 0.207$). On average, snails in the uninfected 26°C treatment produced nearly 3 times more eggs than snails in the infected 26°C treatment.

![Graph](image.png)

Fig. 2.2 Total number of eggs produced per snail (*Planorbella trivolvis*) per day in different temperature treatments over time in the (a) uninfected and (b) infected treatments. Eggs are shown for the first 80 days of recording.
**Host Mortality**

Higher temperatures significantly reduced snail survival, whereas infection had no effect on time to death (Proportional Hazards, temperature: $\chi^2 = 272.56, P < 0.001$; infection: $\chi^2 < 0.01, P = 0.986$). The median survival time of snails at 13°C was 130 days, which was five times longer than for snails at 26°C (Fig. 2.3). There was no significant temperature-by-infection interaction on snail survival (temperature-by-infection: $\chi^2 = 5.41, P = 0.067$).

**Host and Parasite Developmental Rates**

I estimate that at least 95% of exposed snails and 0% of sham-exposed snails became infected. Among snails that died during the experiment, rediae were visible upon dissection between 1 and 3 months after exposure, depending on the temperature treatment. Of the exposed snails that survived to the point that rediae could reliably be detected in each temperature, 95% had visible rediae upon dissection. Exposed snails maintained at 26°C released cercariae significantly earlier than those at 20°C (Proportional Hazards: $\chi^2 = 81.76, P < 0.001$). Snails at 26°C first began releasing cercariae 28 days after the experiment began, compared with 50 days in the 20°C treatment (Fig. 2.4). Snails maintained at the 13°C treatment did not produce cercariae during the experiment.

![Fig. 2.3 Influence of temperature and infection by *Ribeiroia ondarrae* on snail host survival. Depicted is the mean number of days (± 1 SE) that snails survived as a function of experimental treatment.](image-url)
entire duration of the seven month experiment, despite the fact that dissection of these snails revealed pre-patent infections after three months (Fig. 2.4). When snails in the 13°C treatment failed to produced cercariae after four months, I randomly selected 15 infected snails from this treatment and warmed them to 20°C. Two of these snails survived long enough at this temperature to produce cercariae 22 and 29 days after warming, suggesting that, while R. ondatrae infection can develop below 13°C, snails do not release cercariae until temperatures are warmed past this threshold.

**Discussion**

The net effect of climate change on infectious disease dynamics depends on the full spectrum of direct and indirect effects of climate on host and pathogen life histories. Importantly, these effects will extend beyond simple changes in host or parasite geographic distributions to include significant shifts in the physiology and temporal interactions between hosts and parasites that could alter disease dynamics in natural populations. By experimentally manipulating temperature and multiple stages in the life cycle of the pathogenic trematode, Ribeiroia ondatrae, I demonstrate that increases in temperature enhance the growth, mortality
and development of both hosts and parasites in different ways, leading to elevated pathology in snail hosts. Given the role of *R. ondatrae* in causing amphibian limb deformities, these results have additional implications for amphibian conservation and disease emergence.

**Climate change, host pathology and disease emergence**

Parasite-induced pathology in snail (*Planorbarbella trivolvis*) hosts, including castration and accelerated growth, known as “gigantism” (Sorensen and Minchella 2001; Johnson et al. 2004), were exacerbated at warmer temperatures. Infected snails in the 26°C treatment ceased reproduction within 1.5 months, whereas those in the 13°C treatment continued to reproduce for the duration of the seven month experiment, regardless of whether they were exposed to parasites. Such temperature-mediated pathology likely occurs through one of two main mechanisms: higher temperatures facilitate parasite production and virulence (Mouritsen and Jensen 1997; Kocan et al. 2009), or temperature suppresses host immune responses (Cairns et al. 2005). My results are consistent with faster development of trematode rediae within snails at higher temperatures, which lead to castration of infected snails before they reached peak egg output (Fig. 2.2). The energy saved by this reduction in host reproduction can be allocated to snail growth and parasite biomass (Mouritsen and Jensen 1994), which likely contributed to the larger influence of temperature on the growth rates of infected snails. While the role of snail immunity in these patterns cannot be discounted, immune defenses such as phagocytosis in other mollusks occur efficiently at temperatures ranging from 5-37°C (Prieur et al. 1990).

The influence of warmer temperatures on host-parasite interactions between *Planorbarbella* and *Ribeiroia* populations in natural systems will also depend on the net difference in reproduction and mortality of both the host and the parasite (Harvell et al. 2002; Lafferty 2009). I found that despite temperature-driven increases in snail mortality, total egg production by
uninfected snails over the duration of the experiment was still highest in the 26°C treatment, suggesting that, over the temperature range measured in this study, warmer temperatures could lead to a net increase in uninfected snail populations. Trematode infection could, however, reduce this positive influence of temperature on snail fecundity by castrating snail hosts, consistent with observations that trematode infections frequently have negative effects on snail population densities (Lafferty 1993; Fredensborg et al. 2005).

Whether climate change is contributing to the current emergence of amphibian limb deformities remains an open question. Environmental stressors such as pesticides, elevated UV-B radiation and eutrophication occurring alone or in combination with *R. ondatrae* have all been proposed as possible explanations for increased observations of malformed amphibians (Blaustein and Johnson 2003; Johnson et al. 2007; Rohr et al 2008). No studies, however, have yet comprehensively examined the role of climate change in the emergence of amphibian limb deformities. This experiment demonstrates that warmer temperatures drive faster parasite development, increased growth of infected snails, and greater net production of susceptible snail hosts. Further mesocosm and field studies that include the full host community for this complex life cycle parasite will help to determine whether these effects in snail hosts translate into greater pathological effects on amphibians.

**Phenological mismatch in host-parasite interactions**

These results indicate that temperature change will differentially affect the development rates of *P. trivolvis* and *R. ondatrae*. For example, the Q₁₀ value for the growth rate of infected snails was more than twice that of uninfected snails. This comparatively high growth rate in infected snails at elevated temperatures is likely driven by accumulation of both snail and parasitic tissue (Sousa 1983; Probst and Kube 1999), which allows for greater parasite
production. In a review of the experimental literature, Poulin (2006) noted a dramatic effect of temperature on the \( Q_{10} \) rate for the release of several species of trematode cercariae from their snail hosts. Similarly, Yang et al. (2007) described an increase in the development rate of schistosome rediae up to the experiment’s limit at 30°C. These studies indicate that trematode parasites are highly sensitive to temperature changes and benefit from increasing temperatures up to relatively high thermal limits. The small size of trematodes may allow them to capitalize on increasing temperatures more efficiently than their hosts, although a thorough comparison of the temperature dependence of host and parasite vital rates requires direct measurement of actual metabolic energy use (i.e. oxygen consumption rates).

This study also demonstrated differences in threshold temperatures for reproduction and development between *P. trivolvis* and *R. ondatrae* that will likely have important consequences for the timing of host-parasite interactions under scenarios of future climate change. In this study, parasite eggs failed to develop below a threshold temperature of <12°C. Similarly, cercarial production did not occur in snails raised at 13°C until the temperature was elevated to 20°C. By contrast, snails continued to grow and produce eggs at all temperatures experienced within the study. Threshold temperatures for the development of trematode infections within snails have also been observed at 6°C for rediae of *Philophthalmus rhionica* (Ataev 1991), and calculated at 15°C for schistosome sporocysts (Zhou et al. 2008). These results suggest that localized warming trends could cause trematodes to become active earlier in the season as temperatures rise above the threshold for parasite development. This could lead to interactions with hosts in earlier more vulnerable stages of development, particularly if the hosts experience slower, more consistent (non-threshold driven) increases in developmental rates with temperature.
Mismatched timing of host-parasite interactions could alter pathology or infection prevalence depending on the abundance and developmental stage of hosts during parasite activity. *Planorbella trivolvis* live for between one to two years and exhibit seasonal egg production in spring (Morris and Boag 1982), suggesting that developing juvenile snails are the dominant age-class when trematode eggs begin to hatch. In this study, snails would have grown more prior to *Ribeiroia* egg hatching in the medium temperature treatment compared to snails at 26°C because of the increased time for growth before egg hatching at the cooler temperature. These results indicate that earlier spring warming due to climate change may disproportionately increase the hatching rate of *R. ondatrae* eggs relative to snail development, such that snails may be infected at a smaller size. This could alter the dynamics of *R. ondatrae*, since many aspects of trematode infections in snails are influenced by the size of the snail at infection, including both infection success and snail mortality (Kuris 1980; Theron et al. 1998).

Such phenological mismatches could persist or even amplify across different stages in the parasite life cycle. The release of trematode cercariae by infected snails is highly sensitive to temperature in some species (Poulin 2006; Koprivnikar and Poulin 2009). For *Ribeiroia*, earlier and more pronounced release of cercariae by infected snails could lead to infection of larval amphibians at earlier stages of development when they are most vulnerable to deformities and mortality (Schotthoefer et al. 2003), thereby enhancing pathology. However, if peak infection precedes amphibian breeding, which is jointly controlled by temperature and rainfall (Duellman and Trueb 1987), temperature shifts could reduce parasite transmission. Thus, temperature-driven mismatches in the phenology of hosts and parasites in this system may have consequences at multiple stages of the life cycle.

*Conclusions*
Physiological data can provide a useful framework for understanding not only the effects of climate change on individual species ranges (Jeschke and Strayer 2008), but also for forecasting likely changes in species interactions, including those of hosts and parasites. While the direct influences of climate change on the geographic range and growth rates of pathogens are the subject of increasing study (Kutz et al. 2005; Pascual et al. 2006), less effort has focused on identifying the indirect changes in pathogen transmission that are likely to result from temperature-mediated shifts in host-parasite interactions. Threshold temperatures, Q_{10} rates and other measures of temperature sensitivity as well as physiological responses such as thermal limits are a promising way forward in the development of a mechanistic understanding of how climate change could indirectly shift disease dynamics. Climate change will almost certainly cause net increases in some diseases and net decreases in others (Lafferty 2009). Identifying patterns in the temperature sensitivity of groups of pathogens and hosts may offer insight into both the direct and indirect mechanisms for climate-driven changes in disease. Understanding the potential responses of multiple parasite life stages and host physiological responses to temperature will allow more robust inferences about the influence of temperature on parasite dynamics and may identify which portions of their life cycles will be most susceptible to change.
CHAPTER 3

TEMPERATURE-DRIVEN SHIFTS IN A HOST-PARASITE INTERACTION DRIVE NONLINEAR CHANGES IN DISEASE RISK


Abstract:

Climate change may shift the timing and consequences of interspecific interactions, including those important to disease spread. Because hosts and pathogens may respond differentially to climate shifts, however, predicting the net effects on disease patterns remains challenging. Here, I used field data to guide a series of laboratory experiments that systematically evaluated the effects of temperature shifts on the full infection process, including the survival, infectivity, establishment, persistence, and virulence of a highly pathogenic trematode (*Ribeiroia ondatrae*), and the development and survival of its amphibian host. My results revealed nonlinearities in disease patterns as a function of temperature, which resulted from changes in both host and parasite processes. Both hosts and parasites responded strongly to temperature; hosts accelerated development while parasites showed enhanced infectivity (host penetration) but reduced establishment (encystment), persistence, and survival outside the host. While there were no differences in host survival among treatments, I observed a mid-temperature peak in parasite-induced deformities (63% at 20°C), with the lowest frequency of deformities (12%) occurring at the highest temperature (26°C). This nonlinear effect likely resulted from increases in parasite infectivity with temperature coupled with reductions in host vulnerability owing to faster development. My results suggest that, for this and likely many other systems, climate-driven changes to disease will depend critically on underlying shifts in host and parasite development rates and the timing of their interactions. Furthermore, despite strong temperature-
driven changes in parasite infectivity, survival, and establishment, the opposing nature of these effects lead to no difference in tadpole parasite burdens shortly after infection. These findings reveal that temperature-driven changes to the infection process may not be easily observable from comparison of parasite burdens alone, but multi-tiered experiments quantifying the responses of hosts, parasites and their interactions can enhance our ability to predict temperature-driven changes to disease risk.

**Introduction:**

A fundamental challenge in climate research is to understand how current and forecasted shifts in temperature will affect species interactions. Climate change has been linked to poleward range-shifts, earlier phenologies, and declining body sizes of individual species (Parmesan and Yohe 2003; Menzel et al. 2006; Gilman et al. 2010; Walther 2010; Gardner et al. 2011). Not all species are responding equivalently, however, leading to growing interest in the implications of such changes for interspecific interactions. Differences among species in their individual responses to climate change can scale up to alter interspecific interactions by creating novel communities, shifting the timing of interactions, and altering competitive dominance (Gilman et al. 2010; Traill et al. 2010; Walther 2010; Woodward et al. 2010). Such changes will have broad-reaching implications for the resilience of communities and the ecosystem services that they provide (Folke et al. 2004; Hoegh-Guldberg and Bruno 2010; Thackeray et al. 2010).

Despite our growing understanding of these ecological responses to climate change, their effects on disease risk remain a pressing and controversial topic (Wilson 2009; Rohr et al. 2011; Hoverman et al. under review). Some of this controversy stems from the complex effects of temperature on hosts and parasites, leading to difficulties predicting how such changes will alter
their interactions and the disease outcome (Paull and Johnson in press). Temperature can directly alter both parasite virulence and host immunity, each of which affect parasite burdens and host pathology (Braid et al. 2005; Studer et al. 2010; Møller 2010). For example, warmer temperatures enhanced both the growth rate of the fungal pathogen, *Aspergillus sydowii*, as well as the anti-fungal activity of its coral host, although the pathology associated with infection (measured as change in zooxanthellae abundance), was still greatest at high temperatures (Ward et al. 2007). Similarly, temperature often elevates both the development and mortality rates of individual hosts or parasites and changes the seasonal windows of transmission such that the balance between these factors becomes important for determining the net effect of elevated temperatures on parasite encounter rates by hosts (Kutz et al. 2005; Lafferty 2009; Paull and Johnson in press). For instance, development rates of the dengue vector, *Aedes aegypti*, increased over the temperature range of 10-35°C, while survival rates declined at temperatures greater than 30°C (Tun-Lin et al. 2000). Increased temperatures could thus decrease transmission, even in the face of elevated pathogen replication, if parasite or vector survival rates decline simultaneously. A further complication to predicting the response of host-parasite interactions to climate change is that host and parasite responses to temperature are not generally linear, with the performance of organisms increasing up to a particular temperature, beyond which it declines (Thieltges and Rick 2006; Angilletta 2009; Lafferty 2009). This suggests that the range of temperatures experienced under future climate change will be an important determinant of the net response of particular diseases to climatic shifts.

Understanding the effects of temperature on parasite and host responses is further complicated by the potential for indirect climate-effects on the timing of host-parasite interactions. Increasingly, disruptions of interspecific interactions such as predator-prey and
plant-herbivore dynamics have been observed because seasonal events such as breeding, migration, and flowering, are advancing earlier in the season in some species but not in others (Memmott et al. 2007; Post et al. 2008; Primack et al. 2009). Yang and Rudolf (2010) recently noted that the types and consequences of many interspecific interactions are mediated by the developmental stages of the interactors, suggesting that the inclusion of ontogeny in phenological models could enhance our predictions of climate-driven changes to these interactions. This could be particularly important for host-parasite dynamics, given that host developmental stage can be a critical determinant of both host resistance and tolerance to disease (Kelly et al. 2010; Johnson et al. 2011). For instance, if hosts and parasites differ in the sensitivities of their growth rates to temperature, or in the phenological cues (e.g., temperature, precipitation, or daylength) used for reproduction, hosts could be exposed to parasites earlier or later in their development with climate change, with potential consequences for host infection rates and pathology (Paull and Johnson 2011).

The complexities inherent to understanding climate-driven changes to host-parasite interactions highlight the need for mechanistic approaches to studying temperature-driven changes in disease dynamics. Here, I used an interaction between an amphibian host and a highly virulent trematode parasite to systematically evaluate the effects of temperature on multiple stages of the infection process. I assessed how responses of the host and parasite to temperature combine to influence this host-parasite interaction and host pathology. The goals of the experiment were to (1) determine how temperature influences both lethal and non-lethal forms of host pathology (e.g., mortality, developmental malformations, and growth) and (2) assess whether differences in pathology were the result of changes to parasite or host responses to temperature, or a combination of factors at each step of the infection process. My study offers
a detailed look at this issue by considering both short- and long-term effects of temperature on host infection and pathology and by differentiating between effects on parasite establishment and persistence within hosts.

**Materials and Methods:**

*Study System:*

*Ribeiroia ondatrae* (Trematoda: Digenea) is a complex life cycle parasite that must be transmitted sequentially from a first intermediate host (snail) to a second intermediate host (amphibian or fish) to a definitive host (bird or mammal) to complete its life cycle (Johnson et al. 2004). Parasites are released from snails as mobile cercariae that burrow into the skin of developing larval amphibians. Once the cercariae have penetrated amphibian skin, they become subcutaneously encysted as metacercariae, usually in a region localized near the larval amphibian’s developing hind limbs (Sessions and Ruth 1990; Johnson et al. 1999). The pathology associated with infection (mortality and severe limb deformities) depends on when larval amphibians become infected (earlier infection leads to more severe pathology) and the number of parasites with which they are infected (Schotthoefer et al. 2003; Johnson et al. 2011). The malformations resulting from infection may benefit the parasite by increasing the likelihood that the amphibian host is consumed by the definitive host (Johnson et al. 2004; Goodman and Johnson 2011). Throughout the manuscript, I refer to parasite survival as survival in the absence of the host, parasite infectivity as the success of host skin penetration, parasite establishment as the success of cyst formation, parasite persistence as the maintenance of cysts until host metamorphosis, and virulence as the level of pathology caused by the parasite.

*Overview of approach*
I recorded the temperature at 19 Ribeiroia-positive wetlands in the East Bay area of California and used these measurements to define a realistic temperature range for experiments that would incorporate temperatures currently observed during the amphibian-growing season, as well as temperatures that are expected to occur with future climate change. I conducted two short-term experiments: one to study the effects of temperature on parasite survival in the absence of hosts, and another to study parasite infectivity and establishment in hosts. To test how temperature influences parasite persistence and virulence, and host pathology, I performed a long-term experiment, exposing amphibians at different temperatures and raising them to metamorphosis. Both tadpole exposure experiments were conducted at the same time, in the same location, with tadpoles and parasites from the same population pool to ensure that conditions were as similar as possible, and to strengthen inferences made between experiments. While Ribeiroia infects a wide variety of amphibian host species, I chose to focus on Pseudacris regilla because of its sensitivity to developing pathology as a result of infections, and its ubiquity in California wetlands where I focused my field survey efforts (Johnson et al. 2002).

Selection of temperature range for experiments:

To determine a realistic temperature range for my experiments, I used field data from the same region where I collected P. regilla eggs and Ribeiroia-infected Helisoma trivolvis. I placed dataloggers 50 cm below the water surface from May-August 2010 at 19 California ponds, and a subset of two sites had loggers year-round. I selected a temperature range of 12 to 23°C for the cercarial study to span the range of current mean early spring (March-April) temperatures (12.6°C, SD=3.3°C), and 2-3°C above current mean summer (May-August) temperatures (20.8°C, SD=2.3°C). However, because of very low cercarial activity levels at 12°C, I increased the temperatures to a range of 17-26°C for the tadpole exposure experiments (mean May-August
temperature at the coolest site was 17.7°C, SD=3.3°C) to enhance the biological realism and response surface over a realistic temperature range (i.e., no parasite activity would occur at 12°C). Given that the temperature of North American small lakes is forecasted to change between 5-10°C by 2100 (Sharma et al. 2007; Fang and Stefan 2009), the use of an upper temperature limit (26°C) that was 5 degrees above mean May-August pond temperatures is likely a conservative test of climate-driven changes to pond temperatures in the region.

**Temperature effects on parasite activity and survival:**

I tested the effect of temperature (12, 19, and 23°C, SD = 1°C for all) on cercarial activity and survival in the laboratory. I collected *Ribeiroia*-infected rams-horn snails (*H. trivolvis*) from wetlands in the east bay area of California. *Ribeiroia ondatrae* cercariae pooled from five of these snails were isolated into 96-well-plates containing 5 mL of water and one parasite per well (n=21 parasites at 12 and 19°C, and n=20 parasites at 23°C). I submerged 1.4 L waterproof containers holding the well plates and a Hobo datalogger (Onset Computer Corp.) in three water baths (33 L). I used water baths within temperature-control chambers to experimentally manipulate temperatures. I controlled temperature in heated baths (19 and 23°C) with 250 Watt heaters (Jager brand), while temperature in the remaining bath was maintained at the level of the temperature-control chamber (12°C). It should be noted that constraints prevented replicate water baths from being used at each temperature in this experiment, and that pooling cercariae from five snails may have limited the genetic variation of cercariae in the study.

The trays were submerged at 23:00 hrs, at which point cercariae were a maximum of three hours old. I first recorded cercarial survival five hours later (and at 2 hr intervals thereafter) by briefly examining each well under a microscope. I rotated trays 90° when they
were returned to their temperature treatments to limit any positional effects with respect to the heaters. The trials were concluded when all cercariae were either dead or no longer moving.

**Temperature effects on parasite infectivity and establishment:**

I tested the effects of temperature (17, 20 and 26°C, SD = 1°C for all) on parasite infectivity (e.g., skin penetration) and establishment (e.g., metacercarial cyst formation) in tadpoles. For tadpole experiments, clutches of Pacific chorus frogs (*P. regilla*) eggs were collected from wetlands in California, hatched in the laboratory, and raised communally until tadpoles reached Gosner (1960) stages 28-29. I fed tadpoles *ad libitum* a water-based slurry containing equal parts ground TetraMin and *Spirulina* fish flakes on a daily basis, and changed the water every three days using aged and treated tapwater. I acclimated cercariae (15 minutes) and tadpoles (36 hours) to the appropriate temperature treatment prior to exposing tadpoles in 800 mL mason jars held in a total of nine water baths of the appropriate temperature. I exposed each tadpole (n = 21 per temperature) to 25 cercariae (< four hours old at time of exposure) for 45 minutes before transferring tadpoles into 2.5 L containers maintained for 48 hours in the nine water baths. To quantify the number of cercariae that failed to infect the tadpole, I filtered the 800 mL of exposure water using a vacuum pump with fiberglass 0.7 micron filters. Filters were stained with a solution composed of 88% water, 11.7% acetic acid, and 0.3% light green to dye the cercariae, which were subsequently quantified on a stereodissecting microscope (Daly and Johnson 2011). To test filtration reliability, I counted cercariae on filters from control jars containing no tadpoles (n = 5 per temperature). After metacercarial cysts had a chance to form within the tadpoles (48 hours), all tadpoles were preserved in formalin after being euthanized in a solution of tricaine methanesulfonate (MS-222) buffered with sodium bicarbonate. Tadpoles were subsequently necropsied to quantify the number of metacercarial cysts.
Long-term effects of temperature on parasite persistence and host pathology:

I conducted a longer-term experiment with multiple tadpole exposures over time to assess the influence of temperature (17, 20 and 26°C, SD = 1°C for all) on host pathology (growth, survival and limb malformations). I maintained tadpoles individually in 2.5 L containers, with eight containers positioned in each water bath, and a total of 18 water baths (six per temperature). Each temperature treatment consisted of 16 uninfected control tadpoles, and 32 Ribeiroia-infected tadpoles. I randomly assigned tadpoles between stages 27-28 to each treatment and acclimated them for 24 hours prior to the first exposure. Tadpoles were exposed four times to a dose of seven cercariae every three days (28 parasites per tadpole over nine days). Ribeiroia cercariae were temperature-acclimated for 15 minutes prior to each exposure. Because developmental stage at infection can influence host pathology, I randomly staged five tadpoles from each treatment several hours prior to infection to determine how temperature was influencing tadpole development rates. To limit positional effects, I rotated container positions within baths every three days, and water baths every two weeks. When tadpoles metamorphosed, they were euthanized, weighed, measured (snout-vent-length), checked for deformities, preserved, and necropsied to quantify Ribeiroia infection.

Analyses:

For the cercarial survival experiment, I used a parametric survival analysis to test the effects of temperature on parasite mortality, “censoring” the five parasites that were not dead after 50 hrs. For the short-term tadpole infection experiment, I used generalized linear mixed effects models (glmmms) for split-plot designs to analyze the effect of temperature (fixed effect) and bath (random effect) on the percentage (coded as number of “successes” and “failures”) of parasites penetrating and forming metacercarial cysts within hosts using the binomial
distribution. I statistically controlled for pseudoreplication within my studies by nesting individuals within water baths, including this as a random effect in all of the mixed-effect models (Crawley 2007, Zuur et al. 2009, Rohr et al. 2011). This analysis recognizes and accounts for the fact that tadpoles from the same water bath, even though maintained in separate individual containers, could nonetheless have correlated responses if there is a bath-level effect. In the long-term tadpole pathology experiment, I tested the effects of parasite exposure, temperature, and their interaction (fixed effects) and bath (random effect) on host mortality using a glmm with the binomial distribution, and on time-to- and size-at metamorphosis using linear mixed effects models. Among animals that survived to metamorphosis, I further tested the effects of temperature (fixed effect) and bath (random effect) on malformations (0 vs. 1) and the percentage of cercariae recovered as metacercarial cysts using a binomial distribution. I used Akaike Information Criterion (AICc), corrected for small sample size, to determine the relative support for either a linear or nonlinear relationship between temperature and deformities (including bath as a random effect in both models). To determine whether a detectable temperature-driven acceleration in tadpole development occurred during the infection process, I used a linear mixed effects model to test the effects of date of exposure, temperature, and their interaction (fixed effects) and bath (random effect) on tadpole developmental stage (Gosner 1960). Tadpoles that died during infection or had incomplete information were excluded from analyses. Following Bolker et al. (2009), glmms were fit using the Laplace approximation method using the lme4 package (lmer function) while the linear mixed effects analyses were performed using the nlme package (lme function) in R (R Development Core Team 2008). The survival analysis was performed using JMP 8.0.2.
**Results:**

*Cercarial activity:*

Increases in temperature reduced cercarial survival. Cercarial survival time correlated negatively with temperature, such that cercariae survived for a mean ± standard error (SE) of 47.9 ± 0.5 hours at 12°C, 25.7 ± 0.5 hours at 19°C, and 21.3 ± 0.4 hours at 23°C ($\chi^2 = 176.7, P < 0.01$, Fig. 3.1).

*Temperature effects on parasite infectivity and establishment:*

I found contrasting effects of temperature on host skin penetration (infectivity) and parasite cyst formation (establishment), suggesting that parasite processes were changing in response to temperature. Higher temperature significantly increased the percentage of parasites that successfully penetrated tadpole skin ($Z = 2.5, P = 0.01$), which was estimated as the number of cercariae not recovered through filtering (Fig. 3.2a). The filtration process was highly reliable, with a mean percentage recovery of parasites from control filters of 98.4%, 99.2%, and 96.8% at 17, 20 and 26°C respectively out of a total of 25 parasites. By contrast, the percentage of cercariae that successfully encysted following skin penetration declined with temperature ($Z = -2.1, P = 0.04$, Fig. 3.2b). These conflicting

![Graph showing cercarial survival over time at different temperatures.](image-url)
effects of temperature on parasite infectivity and establishment lead to no significant difference in the number of metacercarial parasites detected among tadpoles examined 48 hrs after parasite exposure ($Z = -0.3, P = 0.79$, Fig. 3.3a).

![Fig. 3.2 Effect of temperature on the percentage of parasites penetrating tadpole skin, measured as the percentage of parasites not recovered by filtration after the exposure period (a) and the percentage of the parasites that established once inside the host (calculated as the number of metacercarial cysts divided by the number of parasites that penetrated the hosts) (b). All bars show mean ± SE.](image)

**Long-term effects of temperature on parasite persistence and host pathology:**

Parasite persistence: In the long-term exposure study, fewer metacercarial cysts were recovered from metamorphosing hosts raised at 26°C compared to the other temperature treatments ($Z = -2.0, P = 0.05$). While metamorphs in the 17°C and the 20°C had a mean ± SE of 11.29 ± 0.77 and 12.79 ± 0.73 metacercariae, respectively, only 8.93 ± 0.83 metacercariae were recovered in metamorphs from the 26°C treatment (Fig. 3.3b).
Fig. 3.3 Mean ± SE number of metacercarial cysts recovered from *P. regilla* tadpoles necropsied 48 hours post-infection in the short-term trials (a), and from *P. regilla* necropsied upon metamorphosis in the long-term trials (b). Please note that these results are from the two separate short- and long-term experiments.

Mortality and malformations: The prevalence of severe limb deformities varied significantly with temperature (Z = -2.0, *P* = 0.04), such that deformities peaked in the 20°C treatment at 63% of metamorphosing frogs compared to 38% in the 17°C treatment and 12% in the 26°C treatment (Fig. 3.4). The relationship between deformities and temperature was nonlinear, with the second-order polynomial model having an AICc of 90.6, compared to an AICc of 94.4 for the linear model (Table 3.1).

Table 3.1: Model comparison showing that the deformity rate of infected tadpoles has a nonlinear relationship with temperature.

<table>
<thead>
<tr>
<th>Model</th>
<th>K^b</th>
<th>AICc^c</th>
<th>ΔAICc^d</th>
<th>w^e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quadratic</td>
<td>4</td>
<td>90.6</td>
<td>0</td>
<td>0.87</td>
</tr>
<tr>
<td>Linear</td>
<td>3</td>
<td>94.4</td>
<td>3.8</td>
<td>0.13</td>
</tr>
</tbody>
</table>

^aThese describe the modeled relationship with temperature  
^bNumber of parameters (bath was included as a random effect)  
^cCorrected Akaike’s Information Criteria for small sample sizes  
^dDifference in AIC values from top model  
^eModel weight
Observed deformities included polymelia (extra limbs), taumelia (folds in the leg bones forming a bony triangle), cutaneous fusion (skin webbing connecting the upper and lower leg bones) and femoral projections (small extension of bone or skin from the hind limb). Neither exposure to *Ribeiroia*, nor temperature had an effect on tadpole mortality (exposure: $Z = -0.2$, $P = 0.82$, temperature: $Z = -0.6$, $P = 0.58$, Fig. 3.4). Survival to metamorphosis was 77%, 85%, and 81% in the 17°C, 20°C, and 26°C treatments, respectively, and none of the control tadpoles exhibited malformations at metamorphosis.

![Diagram showing percentage of Ribeiroia-exposed Pseudacris regilla that survived to metamorphosis and emerged deformed (shaded bars), or died before metamorphosis (white bars) at each temperature ± 95% CI.](image)

Growth: Warming temperatures caused a strong increase in tadpole development rate (time x temperature: $t = 7.8$, df = 3, $P < 0.01$). As a result, by the end of the exposures (day 10), tadpoles raised at 17°C were at an average of stage 31, compared to an average stage of 37 among tadpoles raised at 26°C (Fig. 3.5). Temperature accelerated time to metamorphosis and
reduced length and mass of metamorphs (time: \( t = -6.7, df = 14, P < 0.01 \), length: \( t = -3.7, df = 14, P < 0.001 \); mass: \( t = -4.15, df = 14, P < 0.001 \)).

Fig. 3.5 Mean ± SE developmental stage (Gosner 1960) of tadpoles just prior to each of the four *Ribeiroia* exposure events at each temperature. Pictures along the y-axis show development of the limb buds for each corresponding stage.
Discussion:

Mechanistically assessing the effects of climate change on disease requires an understanding of the influence of temperature on the parasite, the host, and the product of their interactions. For hosts, tadpole development was accelerated in the warmest treatment, which reduced the time period in which hosts were highly sensitive to infection and pathology (Johnson et al. 2011). Parasites also showed strong responses to temperature both inside and outside of the host. Although parasite infectivity (penetration of hosts) was enhanced at warmer temperatures, the survival of parasites outside of the host (as free-swimming cercariae) as well as their ability to establish (encyst) and persist within hosts until metamorphosis all had negative relationships with temperature. These contrasting effects of temperature on host and parasite processes lead to an interesting, nonlinear relationship between temperature and host pathology. I observed a mid-temperature peak in the frequency of severe limb malformations, which are expected to be lethal in nature (Goodman and Johnson 2011), likely because higher temperatures enhanced parasite infectivity but reduced host vulnerability to deformities via faster growth rates. Furthermore, despite strong effects of temperature on parasite infectivity, survival, and establishment, the opposing nature of these effects lead to no net change in the initial parasite burden found in tadpoles 48 hours after infection. Because each component of the infection process (infectivity, establishment, and persistence) may respond differently to temperature, simple measures of parasite burden alone may not be adequate to predict or understand host pathology in response to climate shifts, underscoring the value of considering temperature-driven changes to each component of the infection process.

Nonlinearities in temperature-driven changes to host pathology are likely to result when rising temperatures influence both parasite and host processes. Here, the prevalence of limb
deformities peaked in the mid-temperature treatment, with deformities among metamorphs raised at 20°C being over five times more prevalent than at 26°C, and almost twice as prevalent than at 17°C. This pattern likely resulted from enhanced host tolerance as well as parasite infectivity as temperatures increased. Malformation frequency declines steadily in tadpoles exposed to *Ribeiroia* at Gosner stages 30 and beyond. (Schotthoefer et al. 2003; Johnson et al. 2011). Thus, the observed reduction in the prevalence of deformities at 26°C is consistent with the hypothesis that the sharp increase in host development in the 26°C treatment effectively moved hosts outside of the ‘critical window’ of sensitivity. At 26°C, 75% of the parasites were administered after tadpoles had surpassed a mean developmental stage of 30 (Gosner 1960), whereas mean developmental stage of tadpoles in the cooler treatments, did not surpass this point until the final exposure (Fig. 3.5). By contrast, the reduction in tadpole deformity prevalence from the 20 to 17°C treatments is consistent with the hypothesis that reductions in cercarial infectivity (skin penetration) at lower temperatures lead to less disruption of developing cells in the limb buds of larval amphibians. At 17°C an average of only 13 parasites successfully penetrated the tadpole skin, compared to 17 parasites at 26°C. Although there were no differences in parasite burdens among tadpoles exposed at different temperatures (Fig 3.2a), higher penetration of hosts at warmer temperatures may have caused damage to the developing limb tissue, even though not all of the parasites encysted successfully as metacercariae (see Stopper et al. 2002). Given that stage-dependent parasite virulence and host tolerance are common to a variety of disease systems (Kelly et al. 2010; Patankar et al. 2011), incorporating ontogeny into studies of climate-driven changes to host-parasite interactions will enhance our understanding of how host pathology may respond to climate change (Ryce et al. 2004; Yang and Rudolf 2010), including the potential for nonlinear effects of temperature on host pathology.
Trade-offs in the response of parasites to increased temperature are likely common in natural systems and have the potential to cause unanticipated effects of climate-change on disease. I observed an apparent trade-off between parasite infectivity (ability to penetrate the skin) and both its survival outside the host and ability to encyst within the host. The trade-off with survival is consistent with the “energy limitation hypothesis”, which states that the finite energy stores of this free-living stage of trematodes are used up more quickly at high temperatures as a result of higher activity levels (Pechenik and Fried 1995; Thietges and Rick 2006; Studer et al. 2010). Thus, the enhanced cercarial infectivity observed here likely resulted from greater parasite activity levels at warmer temperatures, which increased the likelihood that parasites encountered and penetrated hosts. However, increases in activity may come at the cost of establishment: parasites in the warmest treatment successfully established metacercarial cysts only 71% of the time after penetration, compared to 87% in the coldest treatment. Similar tradeoffs have been reported in other disease systems; for instance, Woodhams et al. (2008) found that lower growth rates and establishment success of the pathogen *Batracochytrium dendrobatidis* at low temperatures were counteracted by longer survival and greater release of infectious stages, allowing the pathogen to maintain high performance over a wide range of temperatures. Such contrasting effects of temperature on development and mortality rates of parasites, vectors and hosts are common in many disease systems, highlighting the important potential for nonlinearities in disease responses to temperature that will likely over-ride more simplistic predictions of increased disease due to faster parasite development rates (Lafferty 2009; Paull and Johnson 2011).

Mechanistically identifying the factors driving differences in (or similarities between) parasite burdens across a range of temperatures has important implications for predicting how
climate change will influence disease risk. Here, I found that tadpoles raised at 26°C had a mean of only 9 parasites at metamorphosis, compared to a mean of 13 parasites found in metamorphs at 20°C (Fig. 3.3). This decline is surprising because these animals reached metamorphosis nearly 3 times faster than hosts at lower temperatures, allowing less time for parasite clearance to occur (e.g., Johnson et al. 2011). Two potential hypotheses could explain the reduced parasite burden in metamorphs at 26°C: (1) differences in parasite infection or establishment success in tadpoles exposed at different stages or temperatures, or (2) temperature-driven differences in parasite persistence. The first hypothesis is unlikely to be correct, because previous work has demonstrated that tadpole stage has limited effects on parasite burdens (despite having large effects on pathology) within the range of stages used in this study (Johnson et al. 2011). Similarly, results from the short-term experiment showed no effect of temperature on parasite burden 48 hours after infection (Fig. 3.2a). The second hypothesis, that parasite persistence, or the ability of cysts to maintain themselves after establishment, was decreased by warmer temperatures, is more consistent with the literature. Because tadpoles lose parasite infections over time (Johnson et al. 2011), high temperatures could reduce parasite persistence via improvements to the tadpole immune response and the clearance rate of parasites. Field and laboratory studies in other systems have demonstrated that lymphocytes and eosinophils are higher in amphibians living in warmer conditions (Maniero and Carey 1997; Raffel et al. 2006), and eosinophils are considered to be an important component of the tadpole response to R. ondatrae metacercariae (Kiesecker 2002). Experiments directly exploring the question of how climate change may influence the amphibian immune response will provide further insight into how climate change may influence disease risk.
While many studies have focused on temperature-driven changes to host resistance, or the ability of hosts to prevent or reduce infection, these results suggest that analyses of how temperature alters host tolerance may provide another important measure of the potential effects of climate change on disease risk. Conceptually, the ‘resistance-tolerance’ framework recognizes that hosts not only have the capacity to limit initial parasite infection (resistance), but they can also reduce the damage caused by each parasite (tolerance) (Råberg et al. 2009). A traditional analysis of tolerance, which compares the slope of the regression between parasite-burden and pathology (Råberg et al. 2009), was precluded in this study because both temperature and time-to-metamorphosis affect the number of metacercariae observed, making it impossible to reliably compare estimates of parasite burden between treatments. Furthermore, these two variables are strongly collinear (i.e., temperature accelerates host development, which correspondingly reduces the amount of time available for hosts to clear infections). Nevertheless, temperature-driven changes in host tolerance may help explain the reduction in pathology observed in the 26°C treatment. Johnson et al. (2011) recently showed that the tolerance of P. regilla to R. ondatrae increases with developmental stage, which is consistent with my observations that warmer temperatures accelerated host development and may have lead to a reduction in host pathology during exposure (i.e., an increase in host tolerance). Similarly, a recent study of bacterial parasites of Daphnia showed that rising temperatures enhanced host tolerance, possibly due to changes in levels of resource conflict between the host and parasite (Vale et al. 2011). Further exploration of potential temperature-driven changes to tolerance will be important because of its influence on host-pathogen co-evolution (Miller et al. 2006; Råberg et al. 2009).
Ecologically relevant laboratory experiments testing temperature-driven changes to the full infection process can provide insight into how future climate change will influence disease in host populations. Because I used temperature and infection levels guided by field data, I can combine this mechanistic understanding of how temperature influences host pathology with inferences about potential changes to the density and seasonal abundance of hosts and parasites in this system to better understand how climate change may influence pathology risk. Here, I found that temperature hastened tadpole development and shortened the window during which hosts were susceptible to deformities. Under field conditions, however, temperature will also accelerate parasite development within upstream hosts such as snails, leading to earlier cercarial release and often greater cercarial production (Poulin 2006; Paull and Johnson 2011). Additionally, because amphibian breeding is jointly constrained by precipitation and temperature (Duellman and Trueb 1987), climate change may not always lead to earlier amphibian breeding (Blaustein et al. 2001; Gibbs and Breisch 2001; Todd et al. 2011), particularly in systems where precipitation is projected to decline. Thus, the timing of precipitation events and the magnitude of temperature-driven increases in parasite and host development rates will be central in governing the timing of host-parasite interactions and host pathology. Importantly, however, elevated mortality of snail hosts with increasing temperature or evaporation could counteract this temperature-driven increase in cercarial production, emphasizing the value of extending the approach used here to simultaneously evaluate the effects of climate-driven changes to the population dynamics of the snail, amphibian and avian hosts to predict climate-driven changes in this system.

Conclusion:
Predicting how diseases will respond to climate change requires a mechanistic understanding of how temperature will influence hosts, parasites, and their interactions. Because hosts and parasites experience trade-offs between life history characteristics such as development rates and survival in response to temperature increases, nonlinear relationships between temperature and infection or pathology are likely to be common. Taken together, these results suggest that predictions of climate-driven changes to disease risk should incorporate an understanding of how climate affects the ontogeny and phenology of hosts and parasites, and the predicted range of temperatures most likely to be experienced at the time of infection. My results also indicate that contrasting effects of temperature on the infection process may not be apparent from simple comparisons of parasite burdens across temperatures, requiring instead a more thorough investigation of the temperature-dependence of different stages of the infection process. Temperature-driven changes to host immunity and parasite infectivity may become particularly important when considering the impact of other environmental changes on hosts and parasites.
CHAPTER 4

BEYOND WARMING: COMPARING THE ROLES OF TEMPERATURE, EVAPORATION, AND NUTRIENTS ON AMPHIBIAN DISEASE RISK IN WETLAND ECOSYSTEMS

Abstract

Hosts and parasites are often exposed simultaneously to multiple environmental changes, each of which can influence host-parasite interactions. Because these changes tend to be multi-faceted, involving shifts in numerous abiotic conditions, there is additional potential for such factors to interactively affect disease risk at different stages of the infection process. The goal of this study was to compare the relative influence of climate variables (temperature, diurnal temperature range, and evaporation) and nutrients (total dissolved phosphorus and nitrogen) on seasonal changes in the interactions of two trematode parasites (*Ribeiroia ondatrae* and *Echinostoma* spp.) with their intermediate hosts (aquatic snails and larval amphibians). Both taxa are transmitted from snail hosts to larval amphibians, and infection of early-stage tadpoles can lead to mortality or severe limb deformities. Between May and August 2010, I sampled 20 wetlands in the East Bay region of California four times (80 total site visits) and used an information theoretic approach to compare models relating climatic and nutrient variables to host density, infection and pathology. Evaporation negatively predicted the density of infected snails for both *Ribeiroia* and *Echinostoma*, which may have resulted from greater mortality of parasite eggs or snail estivation at these sites. Evaporation was also negatively correlated with amphibian *Ribeiroia* infection intensity, even after factoring out the density of infected snails. This could be due to either reduced parasite output by infected snails subjected to desiccation, or faster amphibian development (and consequently reduced exposure) at sites with higher evaporation. Nutrients were the best predictor of amphibian deformities after taking *Ribeiroia*-infection intensity into account- an effect potentially mediated by reduced host tolerance or increased
parasite virulence in eutrophic environments. This study highlights the importance of considering the effects of a wide spectrum of environmental changes at different points of the infection process to determine their net influence on host-parasite interactions.

Introduction:

Climate change is predicted to alter a range of abiotic factors beyond simple changes in mean temperature, including diurnal temperature variability, precipitation, and evaporation (Räisänen 2002; Karl and Trenberth 2003; Solomon et al. 2007). These shifts in turn could lead to indirect changes in ecosystems, with cascading effects on community members. For example, eutrophication of wetlands could occur if greater evaporation concentrates the nutrients that are already present, or if higher precipitation increases nutrient runoff into these systems (Schindler 1997; Jeppesen et al. 2011). Furthermore, eutrophication and elevated temperatures can interact to exacerbate hypoxic conditions in freshwater systems because of lower oxygen solubility in warm water and longer periods of stratification (Foley et al. 2012). Greater recognition of these multidimensional abiotic components of climate change and their potential interactive effects on organisms will be key to assessing the ecological challenges posed by climate change (Clusella-Trullas et al. 2011).

The multifaceted nature of climate change makes it particularly challenging to forecast how climate shifts will affect species interactions, including those between hosts and parasites (Rohr et al. 2011; Hoverman et al. under review; Paull and Johnson in press). The potential for changes in temperature variability, precipitation, or evaporation to counter-act or reinforce temperature-driven changes to disease risk remains uncertain. Climate change is expected to cause reduced temperature variability in the winter in the Northern hemisphere, but increased
variability in the summer (Räisänen 2002), and several studies have indicated that temperature variability may be an important factor influencing disease risk (Paaijmans et al. 2009; Rohr and Raffel 2010; Lambrechts et al. 2011). For instance, Lambrechts et al. (2011) found that mosquitoes that experienced a diurnal temperature range of 20°C had a lower prevalence of infection and died more quickly when exposed to Dengue virus than mosquitoes that experienced less temperature variability, but the same mean temperature. The warming temperatures associated with climate change are also theoretically expected to enhance evaporation globally, although regional changes are also dependent on other factors such as cloud cover, solar irradiance and water vapor concentration (Karl and Trenberth 2003; Huntington 2006). The drier conditions that may result in some regions could negatively influence the survival and development of parasites and vectors, with potential consequences for disease risk (Gage et al. 2008; van Dijk et al. 2010). For example, the percentage of eggs of the trichostrongyloid parasite, *Haemonchus contortus*, that developed to the stage that is transmissible to ruminants was negatively related to evaporation and positively related to amount of precipitation, likely because desiccation limited egg survivorship (O’Connor et al. 2008).

Changes in the seasonality and phenology of organisms also have the potential to alter the timing and consequence of host-parasite interactions, further complicating predictions of climate-driven changes to disease. Some species are already displaying phenological shifts, for example, in the timing of breeding or flowering (Parmesan and Yohe 2003; Menzel et al. 2006). Other species, however, are more resistant to phenology changes, leading to mismatches in predator-prey and plant-pollinator interactions (Memmott et al. 2007; Post et al. 2008; Primack et al. 2009). Similar mismatches could also have consequences for host-parasite interactions. For example, Saino et al. (2009) showed that over the past 40 years parasitic cuckoo (*Cuculus*
canorus) populations have advanced their arrival date by a mean of only 5.6 days, compared to 14.6 days for their host species with shorter migration routes- a mismatch that could lead to host-switching or cuckoo declines. In many parasite systems, host ontogeny can influence the development of infection and pathology (Johnson et al. 2011; Kelly et al. 2010; Yang and Rudolf 2010; Patankar et al. 2011), and thus climate-driven changes to the seasonality of host or parasite abundance could be important to disease risk.

Nutrient enrichment is another form of environmental change that has complicated effects on disease risk. The increased resources associated with nitrogen and phosphorus inputs can elevate the abundance of a wide variety of vectors, hosts, and parasites (McKenzie and Townsend 2007; Smith and Schindler 2009; Johnson et al. 2010). Nutrients can also influence host resistance as well as parasite virulence, which can have implications for parasite transmission and host pathology (Johnson et al. 2010). For example, when Daphnia magna hosts were maintained on phosphorus-poor diets (which would be less likely in eutrophic wetlands), Daphnia reproduction was reduced. This effect was more pronounced in hosts infected with the parasite Pasteuria ramosa, suggesting that virulence could decline in eutrophic conditions (Frost et al. 2008).

It is important to consider how the multiple forms of environmental change occurring simultaneously in many ecosystems may exacerbate or counter-act effects on disease risk. Here, I sampled trematode infections in snail and amphibian hosts at 20 wetlands at monthly intervals to obtain estimates of changes in infection throughout the summer season. Concurrently, I measured pond temperature (mean and variability), evaporation (using area comparisons), and nutrient concentrations (nitrogen and phosphorus) at each pond. The goals of this study were to (1) assess the relative importance of various climatic (mean temperature, diurnal temperature
range, or evaporation) and nutrient (total dissolved phosphorus and nitrogen) variables at multiple stages of the infection process and (2) determine how climate variables affected the seasonality of infections and their consequences for amphibian pathology. Thus, I used an information theoretic approach to compare the relative importance of various climatic and nutrient variables in explaining patterns of infection of two parasite taxa in two distinctive stages of the life cycle (snail and amphibian hosts). My approach recognizes the multidimensional components of environmental change, as well as the potential for these factors to act at different stages of a complex infection process over time.

**Materials and Methods:**

**Study System:**

The study sites were ponds located in the oak-chaparral ecosystem east of the San Francisco Bay area of California on regional preserves used for recreation and livestock grazing (Fig 4.1). I monitored two commonly-occurring trematode parasites, *Ribeiroia ondatrae*, and *Echinostoma* spp. because they are known to cause pathology in larval amphibians, particularly among early development stages (Schotthoefer et al. 2003; Johnson et al. 2011), suggesting that factors altering the seasonal abundance of these two parasites could have important consequences for amphibian disease risk. Both taxa have a similar life cycle; parasite eggs are released into the wetland by a definitive avian or mammalian host, which hatch and infect planorbid snails (Szuroczki and Richardson 2009). After several weeks of development within snails, the parasites are released as free-swimming cercariae, which encyst within larval amphibians or fish until they are trophically transmitted to the definitive host (Szuroczki and Richardson 2009). Within tadpoles, *Echinostoma* spp. infect the developing nephric system,
Fig. 4.1 The location of each of the twenty field sites monitored in our study is marked with a circled star. Note that two of the sites in the northeast corner are so close together that only one site marker is visible on the map.
sometimes causing pathology through edema, slowed growth rates, and early-stage mortality (Schotthoefer et al. 2003). By contrast, *R. ondatrae* target the developing limb bud tissue, causing mortality or severe limb deformities when tadpoles are infected in earlier stages of development (Johnson et al. 2004; Johnson et al. 2011). I also sampled *Helisoma trivolvis* snails, because they are the only intermediate hosts for *Ribeiroia* that occur in this system, and *Pseudacris regilla* tadpoles, because they are widespread, abundant, and susceptible to deformities caused by *Ribeiroia*.

**Sampling methods:**

I designed a monthly sampling scheme to detect how environmental factors influenced seasonal fluctuations in host and parasite abundance and overall host pathology. To maximize parasite detectability and temperature variation among ponds, I selected 20 sites along an elevation gradient (135-1,057 m) that had high *Ribeiroia* abundance in 2009. I sampled planorbid snails and other invertebrates at these sites once each month from May – August 2010. I visited sites roughly in order from north to south, and preserved the order of site visits at each sampling round. I quantified the abundance of snails and tadpoles using dipnet sweeps, which were conducted every 15 m around the pond by rapidly pulling a D-frame net (1.4 mm mesh, 2600 cm²) 1 m through the water in the area just above the substrate. At each visit, I used dipnets to collect between 41-180 (mean-per-visit = 112, SD = 26) *H. trivolvis* snails, collecting all snails > 8 mm in size. To check for parasitic infections, I “shed” snails in the laboratory by isolating them into vials filled with 40 mL of aged and dechlorinated tap water between 17-21:00. I visually inspected vials for parasites and identified them under the microscope the following morning and evening.
At each visit, I also recorded the developmental stage (Gosner 1960) of up to 30 larval amphibians, and the abundance and malformation status of *Pseudacris regilla* metamorphs by recording the number of metamorphs observed while walking a transect between 0-5 m from the shoreline. I visually examined up to 100 metamorphs collected along the transects for deformities, including missing, extra, or misshapen limbs, feet, or digits, as well as skin webbings and femoral projections (Johnson et al. 2002). I examined a mean of 169 (SD = 113) individuals from each site over the course of the season. A mean of 10 metamorphic amphibians per site (range = 6-16) were collected at the June visit and necropsied to quantify infection with both *Ribeiroia* and *Echinostoma* metacercariae (Sutherland 2005; Johnson and Hartson 2009).

I recorded nutrient levels, temperature, and proportional change in area as a proxy for evaporation at each site to evaluate the relative importance of these abiotic variables in explaining infection abundance in snails and amphibians and how these patterns changed throughout the season (see section on hypothesis generation). I collected water samples in acid-washed 125 mL plastic Nalgene bottles during the July visit, immediately froze and sent them to the University of Colorado Kiowa laboratory for analysis of total dissolved phosphorus (TDP) and nitrogen (TDN). Three sites were missing nutrient samples during my visits, so I used data collected at these sites in July of 2009. This should have little effect on the results, since there was a significant linear relationship with a slope estimate range including 1 between the 2009 and 2010 values at the fifteen sites for which I have data in both years (TDP: slope = 1.09 ± 0.15, $R^2 = 0.83$, $P < 0.01$; TDN: slope = 0.91 ± 0.15, $R^2 = 0.78$, $P < 0.01$). To limit the number of parameters in the models to appropriate numbers for the sample size, I chose to combine the measures for TDP and TDN into a single variable with a principle components analysis using the package stats in the program R (R Core Development Team 2008). The first principle
component explained 89% of the variance, and I used this single value for nutrients in all of the models. Because both TDP and TDN loaded negatively onto the axis, negative coefficients actually represent a positive effect of nutrients. I measured temperature at the sites by placing Hobo underwater dataloggers (Onset Computer Corp.) 50 cm below the water surface (where amphibians and snails were observed to spend a majority of time) during the first visit in May. I adjusted logger location slightly (< 3 m) at each site visit to maintain the same depth (50 cm) even if water levels changed. Loggers recorded temperature and light levels at two-hour intervals. I calculated both the mean temperature and mean diurnal temperature range (average difference between the daily maximum and minimum recorded temperatures; DTR) at each site over the range of dates on which I had data for all sites. I used a GPS unit to measure pond area (m$^2$) during the July and August visits, and used this information to approximate evaporation by calculating the difference in pond area (July minus August) divided by July pond area. The majority of the sites were permanent, with only three being classified as semi-permanent or temporary. As such, using proportional change in area as a continuous predictor, rather than the binary variable of hydroperiod offers a more resolved picture of the ecological effects of evaporation.

Analytical approach and hypothesis generation:

I used an information theoretic approach to compare models that use climate and nutrient variables to explain host and parasite dynamics (Burnham and Anderson 2002). I developed these models based on previous literature and my own hypotheses. Specifically, I hypothesized that the density of infected snails and tadpole developmental stage (Gosner 1960) would peak earlier in the summer at warmer ponds, because laboratory experiments have indicated that, *Ribeiroia*, snails, and amphibians all develop faster at higher temperatures (Paull and Johnson
Because diurnal temperature range has also been shown to influence parasite development rates both negatively and positively, depending on the mean temperature around which they oscillate (e.g., Paaijmans et al. 2009), I included DTR in all models. I anticipated that evaporation could have either a positive or a negative influence on hosts and parasites. By reducing the volume of a pond, high evaporation could concentrate individuals closer together, leading to higher densities and facilitating transmission (e.g., Kiesecker and Skelly 2001); however, because evaporation can also lead to mortality of snail hosts (Thomas and McClintock 1996; Sandland and Minchella 2004), and faster amphibian development (e.g., Doughty and Roberts 2003), it could also reduce snail density and resultant infections and deformities. I expected nutrients to positively influence snail densities and host infection because a previous experimental study of *R. ondatrae* showed that nutrients increased the density of snail hosts as well as the number of parasites they released (Johnson et al. 2007).

*Models of the density of infected snails:*

Using these hypotheses, I developed nine candidate models (Table 4.1) to explain seasonal changes in the density of infected snails (*Ribeiroia* n=15 sites, *Echinostoma* n=9 sites). The number of sites included in each analysis was determined by whether the parasite was detected at that site within the host of interest and whether I had complete data for all predictor variables. For each parasite separately, I calculated infected snail density as the percentage of infected snails multiplied by the density of infected snails (snails per netsweep). To help meet assumptions of normality, I used log-transformed values for infected snail density. Because I expected these responses to change over the course of a summer in accordance with shifts in the predictor variables, I analyzed the data using a repeated-measures approach with pond identity included as a random effect (Zuur et al. 2009). The value for the first time point was an average
Table 4.1: List of models and the variables included for each AICc analysis

<table>
<thead>
<tr>
<th>Model Category&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Climate&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Nutrients&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Seasonal&lt;sup&gt;d&lt;/sup&gt;</th>
<th>A&lt;sup&gt;e&lt;/sup&gt; priori&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature</td>
<td>DTR</td>
<td>Evaporation</td>
<td>PC of TDP and TDN</td>
</tr>
<tr>
<td>Global</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>NI</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SV</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SV</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SV</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>SV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S + N</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Seasonal Analyses<sup>f</sup>:

<table>
<thead>
<tr>
<th></th>
<th>Temperature</th>
<th>DTR</th>
<th>Evaporation</th>
<th>PC of TDP and TDN</th>
<th>Time</th>
<th>Time x Temperature</th>
<th>Pond Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>NI</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>SV</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>SV</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SV</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S + N</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Overall<sup>g</sup>:

<table>
<thead>
<tr>
<th></th>
<th>Temperature</th>
<th>DTR</th>
<th>Evaporation</th>
<th>PC of TDP and TDN</th>
<th>Time</th>
<th>Time x Temperature</th>
<th>Pond Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Global</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SV</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SV</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SV</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C + N</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C + N</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C + N</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>Model category describes the types of explanatory variables included in models. Models in which only the <i>a priori</i> variable or the intercept was fit to the data are described as null. Global models include all variables in the set, the NI model includes all variables except the time-by-temperature interaction, SV models include only single variables, S (seasonal) models include a time-by-temperature interaction, and models labeled C + N or S + N pair a nutrient variable with either a single climate (C) variable or the seasonal (S) time-by-temperature interaction.

<sup>b</sup>Climate variables are factors expected to shift with changing climate, including: Temperature, DTR, and evaporation.

<sup>c</sup>The nutrient variable is the first principle component of TDP and TDN.

<sup>d</sup>Seasonal variables include time as a factor.

<sup>e</sup>Pond identity was included in all seasonal models as a random effect since these analyses involved response variables collected at the same pond over two different time periods.

<sup>f</sup>Seasonal analyses were completed for response variables expected to change over the season including the density of infected snails and tadpole developmental stage.

<sup>g</sup>Overall mean analyses were completed for response variables for which I only had data from one time point, or that I did not expect to change over the season, including: the residuals of amphibian infection intensity after accounting for the density of infected snails, and the residuals of amphibian deformities after accounting for infection intensity.
of May and June values, and the second an average of July and August values. I chose to use averaged values across two visits because infection prevalence on an individual date can be low (1%). I used the nlme package in the program R (R Core Development Team 2008) for the repeated-measures linear mixed effects models.

Amphibian infection, development and pathology models:

To explain changes in tadpole developmental stage (n = 17 sites), I used the same set of candidate models developed to explain seasonal changes in snail patterns (Table 4.1). Tadpole developmental stages were compared only between the May and June visits (omitting July and August data), because tadpole densities declined as individuals emerged. To evaluate the factors that influenced *Ribeiroia* and *Echinostoma* infections within metamorphosing amphibians (*Ribeiroia* n=16 sites, *Echinostoma* n=15 sites) and the prevalence of parasite-induced deformities (n=16 sites), I developed a set of nine candidate models (Table 4.1). Detecting parasites in amphibians is easier than in snails, which explains the larger sample sizes for these models. To better detect environmental factors acting at each stage of the infection process separately (rather than carry-over effects from factors influencing parasite abundance or transmission in the upstream hosts), I analyzed the residuals of amphibian infection intensity after accounting for the average density of infected snails in May and June (e.g., before the majority of tadpoles had metamorphosed). I calculated deformity prevalence over the entire season at a site as the total number of deformed individuals divided by the total number caught. I analyzed the residuals of deformity prevalence after accounting for *Ribeiroia* infections in amphibians. These models were not analyzed using a repeated-measures approach because amphibian infections were only measured in June, and I did not expect deformity prevalence to vary seasonally.
Model evaluation and selection

For each response variable, I checked for multicollinearity among the predictor variables using variance inflation factors. To test for spatial autocorrelation in the residuals (after fitting the global models), I used a Moran’s I with distance to nearest neighbor set at 10 km to reflect the average foraging flight distance of waterbirds such as egrets and herons (Kelly et al. 2008). I also checked the overall model fit by determining the correlation between observed values and those predicted by the global models. Following the methods of Burnham and Anderson (2002), I considered the best models to be those with \( \Delta AIC_c < 2 \), while those with \( 4 < \Delta AIC_c < 7 \) had considerably weaker support, and \( \Delta AIC_c > 7 \) had essentially no support (Burnham and Anderson 2002). I also evaluated the relative importance of individual variables by comparing the sum of the Akaike weights across all models that included that variable (Burnham and Anderson 2002). To calculate parameter estimates for the predictor variables in the strongest models (\( \Delta AIC_c < 2 \)), I calculated the model averaged coefficients and weighted unconditional standard errors based on the subset of models in which that predictor was present. I used the package HH to calculate the variance inflation factors, spdep for the Moran’s I, MuMIn to calculate the AICc values and Akaike weights, and AICcmodavg for model-averaged parameter and standard error estimates in the program R (R Core Development Team 2008).

Results:

Site characteristics:

Values for mean temperature, diurnal temperature range (DTR), proportional area loss, and nutrient concentrations were variable across sites. Mean May-August temperatures at the sites was 21.9°C, SD = 1.8°C (range from 18-25°C), while mean DTR was 5.0°C, SD = 1.7°C
(range from 1.8-9.3°C). Although I chose wetlands along a 922 m elevation gradient to maximize temperature differences, there was no significant effect of elevation on temperature ($R^2 < 0.01, P = 0.82$). Evaporation ranged from a loss of 0-55% of the pond area between July and August. Sites ranged from mesotrophic to hypertrophic (Smith et al. 1999), with a mean TDP concentration of 0.08 mg/L, SD = 0.07 mg/L (range from 0.01-0.36 mg/L), and mean TDN concentration of 1.2 mg/L, SD = 0.68 mg/L (range from 0.44-3.2 mg/L).

Model evaluation:

I found no evidence for multicollinearity among the predictor variables used in the models, with the variance inflation factors ranging from 1.1-1.6. I also found no evidence for autocorrelation in the residuals ($P > 0.19$ for all models), suggesting that nonspatial models were adequate.

Density of infected snails:

The observed and predicted values for the global models of infected snail density were correlated (Ribeiroia snail density: $R^2 = 0.52$; Echinostoma snail density: $R^2 = 0.79$). A negative coefficient for evaporation was present in the best-supported models to explain changes in the density of both Ribeiroia-infected and Echinostoma-infected H. trivolvis snails among sites and over time (Tables 4.2 and 4.3). Evaporation was the most important variable explaining the density of Ribeiroia infected snails, with a summed Akaike weight of 0.82 (Table 4.3). By contrast, nutrient concentration was the best predictor of the density of Echinostoma-infected snails with a summed Akaike weight of 0.58, while evaporation had the next highest summed Akaike weight of 0.29 (Table 4.3).
Table 4.2. Best supported models (ΔAICc<2) for densities of infected snails, residuals of amphibian infections (after accounting for infected snail densities), and residuals of deformities (after accounting for *Ribeiroia* infections)

<table>
<thead>
<tr>
<th>Response</th>
<th>Modela</th>
<th>Log(L)b</th>
<th>Kc</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density <em>Ribeiroia</em> snails</td>
<td>Evaporation</td>
<td>-18.2</td>
<td>4</td>
<td>46.1</td>
<td>0</td>
<td>0.82</td>
</tr>
<tr>
<td>Density <em>Echinostoma</em> snails</td>
<td>Nutrients</td>
<td>-12.1</td>
<td>4</td>
<td>35.8</td>
<td>0</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>Evaporation</td>
<td>-12.8</td>
<td>4</td>
<td>37.2</td>
<td>1.4</td>
<td>0.29</td>
</tr>
<tr>
<td><em>Ribeiroia</em> metacecariae residuals</td>
<td>Evaporation</td>
<td>-29.3</td>
<td>3</td>
<td>66.7</td>
<td>0</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Null</td>
<td>-31.6</td>
<td>2</td>
<td>68.1</td>
<td>1.4</td>
<td>0.23</td>
</tr>
<tr>
<td><em>Echinostoma</em> metacecariae residuals</td>
<td>Null</td>
<td>-26.0</td>
<td>2</td>
<td>57.0</td>
<td>0</td>
<td>0.50</td>
</tr>
<tr>
<td>Tadpole developmental stage</td>
<td>Time</td>
<td>-78.9</td>
<td>4</td>
<td>167.3</td>
<td>0</td>
<td>0.80</td>
</tr>
<tr>
<td>Deformity residuals</td>
<td>Nutrients</td>
<td>14.0</td>
<td>3</td>
<td>-19.9</td>
<td>0</td>
<td>0.64</td>
</tr>
</tbody>
</table>

aList of predictor variables included in the best models  
bLog likelihood  
cNumber of parameters  
dAkaike information criterion corrected for small sample size  
eDifference in AICc value between the best ranked model and the current model  
fAkaike weight for the model

Amphibian infection, development and pathology:

Correlation between the observed and predicted values based on the global models was weak for the residuals (after accounting for infected snail density) of amphibian *Ribeiroia* infections ($R^2 = 0.23$). Mean infection intensity in *P. regilla* at infected sites was highly variable (*Ribeiroia*: 14.7, SD=17.6; *Echinostoma*: 62.4, SD=157.9). The best-supported model for amphibian *Ribeiroia* infections included a negative coefficient for evaporation, even after taking into account the negative influence of evaporation on the density of infected snails (Tables 4.2 and 4.3). The null model was also among the top models explaining *Ribeiroia* infection residuals (Table 4.2). The global model explained very little of the residual amphibian *Echinostoma* infection ($R^2 = 0.02$) intensity, and the null model was the best supported (Table 4.2).

The predicted value of tadpole developmental stage (Gosner 1960) from the global model was strongly correlated with the observed values ($R^2 = 0.71$). Time was the only variable in the model that best explained the average developmental stage of tadpoles (Table 4.2). The model
containing a positive effect of evaporation on tadpole development was also weakly supported ($\Delta$AICc = 3.2). An effect of temperature on tadpole development was not supported by the data ($\Delta$AICc = 17.7).

Table 4.3. Model-averaged coefficients and standard errors calculated for the predictor variables in the best-supported models ($\Delta$AICc$<$2) of densities of infected snails, residuals of amphibian infections (after accounting for infected snail densities), and residuals of deformities (after accounting for *Ribeiroia* infections).

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Predictor Variable</th>
<th>Model-Averaged Coefficient$^a$</th>
<th>Weighted unconditional SE</th>
<th>Cumulative Akaike weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density <em>Ribeiroia</em> snails</td>
<td>Evaporation</td>
<td>-0.88</td>
<td>0.57</td>
<td>0.82</td>
</tr>
<tr>
<td>Density <em>Echinostoma</em> snails</td>
<td>Nutrients</td>
<td>-0.52$^b$</td>
<td>0.17</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>Evaporation</td>
<td>-1.38</td>
<td>1.24</td>
<td>0.29</td>
</tr>
<tr>
<td><em>Ribeiroia</em> metacercariae residuals</td>
<td>Evaporation</td>
<td>-5.26</td>
<td>2.52</td>
<td>0.53</td>
</tr>
<tr>
<td><em>Echinostoma</em> metacercariae residuals</td>
<td>Nutrients</td>
<td>0.09$^b$</td>
<td>0.30</td>
<td>0.17</td>
</tr>
<tr>
<td>Tadpole developmental stage</td>
<td>Time$^c$</td>
<td>2.09</td>
<td>0.73</td>
<td>0.80</td>
</tr>
<tr>
<td>Deformity residuals</td>
<td>Nutrients</td>
<td>-0.13$^b$</td>
<td>0.04</td>
<td>0.99</td>
</tr>
</tbody>
</table>

$^a$Model averaging across models in which the predictor is present

$^b$Negative coefficients represent a positive effect of nutrients because TDP and TDN loaded negatively onto the first principle component axis

$^c$Estimated after excluding models with interaction terms from the set of candidate models

The global model predicting the residuals of the percentage of deformed *P. regilla* (after accounting for *Ribeiroia* infection intensity) was correlated with the observed values ($R^2 = 0.53$). At *Ribeiroia*-positive sites, overall deformity prevalence ranged from 0-41% of all *P. regilla* metamorphs. Nutrients were the strongest predictor of the residuals of the linear relationship between *Ribeiroia* infection and deformities (Table 4.2; Fig. 4.2). The summed Akaike weight for nutrients was 0.99 compared to 0.13 for DTR and 0.11 for temperature and evaporation.
Discussion:

This study explored the relative importance of climate and nutrient variables at multiple points (e.g., snail infection, amphibian infection, and amphibian pathology) in the infection processes of two complex life-cycle trematode parasites. I found that higher evaporation negatively influenced the density of infected snails for both parasites. Even after accounting for the density of infected snails, similar effects of evaporation were found for the residuals of
Ribeiroia infection intensity in frogs, although overall model fit was weaker for this response variable. Evaporation could negatively influence infected snail densities by causing parasite egg or snail mortality, or by inducing estivation of these hosts. Similarly, evaporation could affect amphibian infection independently of its effect on the density of infected snails by either reducing parasite output in snails exposed to desiccation, or hastening amphibian development and thus reducing the time for exposure. Neither climate nor nutrients were strong predictors of the residuals of amphibian Echinostoma infections. After factoring out Ribeiroia infection intensities and modeling the residuals, nutrients were the best predictor of amphibian deformities, pointing to possible effects of eutrophication on host tolerance or parasite virulence. Contrary to my predictions, temperature was not among the top models explaining infections and deformities, which may reflect the inherent challenge associated with obtaining data from a large enough sample size of sites at a small enough time interval to detect changes in prevalence or seasonality in a highly complex and variable field settings. Our data highlight the importance of evaporation and eutrophication for Ribeiroia infection and pathology in the arid and eutrophic systems in which this study was conducted.

The higher desiccation risk, changing water chemistry, and loss of vegetation and habitat area associated with high evaporation in aquatic systems have the potential to reduce host or parasite survival, or alter host behavior in a way that compromises the observed density of infected hosts. Snails can suffer high mortality rates or initiate estivation during drying events (Thomas and McClintock 1996; Sandland and Minchella 2004). These data are consistent with the hypothesis that evaporation leads to either mortality or estivation of snail hosts, and subsequent reduction in the observed density of infected snails. A similar loss of snail hosts and their associated parasites was seen in a French lake after a drought (Gérard 2001). Desiccation
can also directly reduce the survival of parasite eggs and free-living stages (O’Connor et al. 2008; Martinaud et al. 2009; van Dijk et al. 2010). If infected shorebirds tend to spend time along wetland edges, large reductions in pond surface area could have a negative effect on the number of eggs that are able to successfully hatch and infect snail hosts. Given that soil drying can limit vector and parasite survival in terrestrial landscapes as well (Gage et al. 2008; O’Connor et al. 2008; van Dijk et al. 2010), I suggest that greater consideration be given to moisture and hydroperiod in future studies exploring the potential effects of climate change on disease.

Even after accounting for differences in the density of infected snails, evaporation could alter amphibian infections by reducing the number of parasites released per infected snail, limiting parasite survival, or enhancing amphibian development rates and thus reducing the duration of exposure. Desiccation can limit the number of cercariae produced per snail (Badger and Oyerinde 1996; Zekhnini et al. 2002). For instance, Zekhnini et al. (2002) showed that 5-10 day periods of desiccation reduced the duration of shedding as well as the total number of Schistosoma haematobium parasites that were released by snails. Such a per capita reduction in parasite output could reduce amphibian infection in ponds subjected to greater drying regimes, even in the absence of effects on infected snail densities. Additionally, many tadpole species accelerate development rates in response to pond drying (e.g., Newman 1992; Denver 1998; Doughty and Roberts 2003), and my data provide moderate support for the hypothesis that P. regilla develop more quickly at ponds with greater evaporation. Accelerated development would be expected to reduce amphibian infection intensity by limiting the amount of time in which tadpoles are exposed to parasites within the aquatic environment. The overall fits for the
amphibian infection intensity models were weaker than for other response variables, suggesting that unmeasured variables or stochasticity may also be important.

While infection intensity is one important component of pathology risk, host tolerance, or the ability to limit damage caused by a given parasite burden, and parasite virulence are also important factors to consider (Råberg et al. 2009). Here, I found that after factoring out mean *Ribeiroia* parasite burdens at a site, nutrients had a positive effect on the percentage of amphibians emerging deformed at a wetland. One hypothesis to explain this pattern could be a negative association between eutrophication and host tolerance of this parasite. The mechanisms involved in host tolerance may include tissue repair, or reducing the negative effects of immunopathology and/or toxins released by parasites (Råberg et al. 2009). The hypoxic conditions that often accompany eutrophication and direct toxic effects of some nitrogenous compounds can impair the growth and development of some amphibian larvae (e.g., Jofre and Karasov 1999; Marco and Blaustein 1999; Peltzer et al. 2008), which could also indicate a limited capacity for tissue repair mechanisms in response to infection. Another hypothesis to explain these results is that nutrients may have elevated the virulence of *Ribeiroia*. In plant disease systems, the “nutrient-disease” hypothesis states that elevated nitrogen can enhance fungal disease severity by increasing the nitrogen resources available to the pathogen (Mitchell et al. 2003). If cercariae were able to grow larger or become more active (e.g., through greater stored energy reserves) within snails in eutrophic environments, it is plausible that they may cause more host damage during infection, increasing deformity risk. Although my results show that nutrients enhanced the density of *Echinostoma* snails at these sites, it is unlikely that the higher deformity risk at eutrophic sites is the result of greater cumulative parasite load in amphibians (e.g., more *Ribeiroia-Echinostoma* coinfections), because a recent laboratory study
showed no interactive effects of co-infection with these two parasites on amphibian pathology (Johnson and Buller 2011). One caveat to this interpretation of the results is that predators can also cause some of the deformities observed in the field (Johnson and Bowerman 2010), and if associations exist between nutrients and sublethal predation risk, these could also explain the patterns observed. Further investigation of potential mechanisms behind associations between eutrophication and host pathology are warranted given the vast anthropogenic alteration of nutrient cycling that has occurred in recent decades (Vitousek et al. 1997; Carpenter et al. 1998).

In contrast to the large effects of evaporation and nutrients, temperature was not a strong predictor of host and parasite infection dynamics, and did not vary predictably with elevation. Because elevated temperatures enhance parasite development rates within snails while amphibian breeding is jointly constrained by temperature and rainfall (Duellman and Trueb 1987; Paull and Johnson 2011), I hypothesized that higher temperature could cause tadpoles to be infected earlier in the season and suffer greater pathology. However, the time-by-temperature interaction term in the models of infected snail density was not supported by the data. This may be due to the fact that estimates were only possible at the coarse scale of ‘early’ and ‘late’ season, which may have masked an effect of temperature on the seasonality of infected snail densities. It is also possible that the effects of temperature could be muted if hosts or parasites mitigate the effects of warming temperatures through behavioral thermoregulation (Kearney et al. 2009; Sears et al. 2011). I also found no relationship between temperature and elevation, suggesting that other factors such as pond volume, surface vegetation, or aspect may be playing a role in governing temperature differences among sites. This result highlights the importance of confirming that proxy variables (e.g., latitude or elevation) used in studies that substitute space for time to infer the potential ecological effects of climate change are indeed related to
temperature. Finally, the study was conducted based on current ambient temperatures at the sites. Climate change is forecasted to elevate pond temperatures by anywhere from 5-10°C in the next 100 years (Sharma et al. 2007; Fang and Stefan 2009), and it is possible that elevated temperatures beyond the ranges observed in the current study would have had a stronger effect on host-parasite interactions and disease risk. Similarly, in regions where wetland hydroperiod is a less dominant structuring force, effects of temperature could become more important.

Because climate change is multidimensional and affects organisms that are concurrently being influenced by other forms of environmental change, such as nutrient enrichment and disease, developing a framework to explore interactions among these factors within complex systems will be key for understanding their net influence on ecosystems and species. Several studies speculate that climate change and eutrophication may interact to enhance a variety of diseases (Horák and Kolárová 2011; Okamura et al. 2011). For instance, warmer temperatures can enhance the prevalence and severity of proliferative kidney disease in fish, while eutrophication promotes the growth of its alternate host, bryozoans (Okamura et al. 2011). Field studies comparing the relative influence of the various components of each factor at different stages of the infection process can further clarify when environmental factors are likely to interact positively, negatively, or not at all. Further insight can also be gathered from multi-year studies that consider how infection dynamics in previous years interact with abiotic variables to explain patterns in host-parasite dynamics.
CONCLUSION

Taken together, my results can be used to make preliminary predictions about climate-driven changes to the *Ribeiroia* system, and can also inform future research into climate-disease interactions in this and other systems. The overarching theme, common to each study described, is that climate-driven changes to parasite transmission are multi-dimensional, leading to changes that will both benefit and hinder transmission at different stages of the infection process. A clearer picture of cumulative changes to disease risk in this system can be obtained from modeling temperature-driven changes to the parasite and all hosts, conducting an over-winter mesocosm experiment of the effects of elevated temperature on all of the hosts simultaneously, and long-term field studies of climate-driven changes in bird behavior and parasite seasonality in different regions across the range of the parasite. Furthermore, climate change studies would benefit from a better understanding of the ecological consequences of behavioral thermoregulation as a potential response to climate change. My results suggest that future research into climate-disease interactions should use a combination of field and laboratory studies that focus on potential nonlinearities between climate and disease risk, shifting host-parasite interactions, and abiotic changes in addition to mean temperature. Below, I consider each of these themes in more detail.

Effects of climate change on the *Ribeiroia* system

Results from laboratory infections of *Helisoma trivolvis* snails and *Pseudacris regilla* tadpoles separately with *Ribeiroia* indicated that temperature has strong effects on parasite survival, host growth, survival and pathology, and host-parasite interactions. Generally, warmer
temperatures tended to increase snail fecundity and the growth rates of parasite eggs, snails, and tadpoles, but reduced survival of snails and parasitic cercariae. In my experimental infections of tadpoles, I observed a nonlinear relationship between temperature and the frequency of deformities, likely because temperature increased the initial tadpole infectivity of *Ribeiroia*, but also enhanced tadpole growth rates, making them vulnerable to deformities for a shorter period of time. Because higher temperatures in the field would also accelerate parasite development in snails (Paull and Johnson 2011), while amphibian breeding is constrained by temperature and rainfall (Duellman and Trueb 1987), I hypothesized that warmer temperature could lead to infection of earlier stages of amphibians and greater consequences for host pathology. Although temperature was not a strong predictor of *Ribeiroia* infections and amphibian deformities in the field, this may be due to the greater complexity associated with field studies, rather than a lack of influence of temperature. Trade-offs between the number of sites sampled and the frequency of sampling may have reduced my ability to detect effects of temperature. Similarly, the relatively small range of temperatures observed (7°C) could have also limited my ability to detect temperature effects. Finally, the field data represent a single year ‘snapshot’ of hosts and parasites, and if host-parasite dynamics experience complex or multi-year cycles, legacy effects of temperature could be obscured. Attempts to model the temperature-driven changes to the overlap between parasites and vulnerable amphibian stages, as well as mesocosm experiments that explore the effects of warming on infected snails and amphibians maintained within a communal tank will clarify the potential net role of temperature for this host-parasite interaction.

If multiple years of data were to confirm the patterns that I observed in the field study, it is possible that increased evaporation rates stemming from climate change could reduce the risk of *R. ondatrae* infection and pathology in *P. regilla* in the Bay Area of California. I found that
high evaporation rates in the field correlated negatively with densities of infected snails and amphibian infection intensity. Regional projections for future precipitation changes in California tend to predict slight increases in winter precipitation, but drier summers (Pan et al. 2011). These regional predictions suggest that evaporation rates during the transmission season will increase with climate change, having negative effects on host snail abundance and amphibian infections. However, multiple years of data incorporating a greater number of sites combined with studies of how bird infections and behavior may shift with climate change would be needed to confirm such a prediction.

Furthermore, reduced *Ribeiroia* disease risk with climate change may not be a trend that is generalizable across amphibian species and regions where the parasite occurs. *Ribeiroia* is a generalist parasite that infects numerous amphibian species and is abundant in several regions across North America (Johnson et al. 2002). Physiological responses of different species to climate change will be variable, leading to interspecific differences in climate-driven changes to disease risk (Garrett et al. 2006). For instance, temperature was recently shown to alter the immune response associated with exposure to a simulated pathogen differently across three coral species (Palmer et al. 2011). Furthermore, differing immune responses to *Ribeiroia* may be responsible for some of the interspecific variation in the frequency of deformities observed in the field (Johnson and Hartson 2009). Interspecific differences in the temperature-sensitivity of the immune system could mean that some amphibian species will be more susceptible to climate-driven changes in disease risk than others. Similarly, the reduction in snail host density with increased evaporation that likely drove declines in *P. regilla* infection and pathology during this study year may not occur in wetter years or less arid regions of the country where the parasite occurs. Longer-term, larger-scale monitoring of host and parasite demographics in the field will
be necessary to clarify how wetland hydroperiod and the weather context in a given year
influences associations between *Ribeiroia* and climate variables. Furthermore, the field study
only explored temporal overlap between amphibians and parasites under current climatic
conditions with a limited range of temperatures. It is possible that future increases in
temperature could lead to more dramatic shifts in the timing of peak parasite abundance that
could have consequences for amphibians.

**Future directions**

Coupling system models with additional experiments will offer greater mechanistic
understanding of the effects of temperature on this disease system, helping to make the results
and predictions more generalizable across a wider range of study sites, particularly for those
areas of North America where hydroperiod plays a less dominant role than it did at my field
sites. I plan to model the effects of temperature on this system using a series of differential
equations for each host and parasite stage (e.g., Mouritsen et al. 2005). This model will allow
comparison of the projected temporal overlap between amphibians and *Ribeiroia* under a wider
range of potential future temperature scenarios. I have collected much of the data needed to
parameterize such a model; however, additional data will be needed to determine how
temperature alters snail infection success and the abundance of parasites produced by snails. I
am also currently conducting a mesocosm study that experimentally manipulates the temperature
of simulated aquatic ecosystems, to add to the mechanistic understanding of the likely net
influence of temperature on this system. I am using a 2 x 3 factorial design manipulating
temperature (elevated or ambient) and infection status (none, low, high) within 379 L water
tanks, with six replicates per treatment. All tanks were buried for insulation, and temperature is
being elevated using greenhouse structures placed over half of the tanks (Fig 5.1; Netten et al. 2008). I added snails and parasite eggs on August 13, 2011, and I am allowing the tanks to overwinter at different temperatures. I will add eggs of the amphibian species, *Pseudacris triseriata* on a date in late March that corresponds to observations of the start of breeding by local populations. By incorporating the effects of earlier ice melt and spring warming on all hosts simultaneously, this experiment will allow me to directly test hypotheses about temperature-driven changes to the timing and consequence of host-parasite interactions.

Differences are already being observed between heated and unheated tanks. Heated tanks are between 4-6°C warmer than unheated, and infected snails stopped laying eggs in heated tanks (due to parasitic castration) six weeks earlier than in unheated tanks. Furthermore, I checked for parasite infections on September 25, 2011 and found that in the heated tanks, 44% of snails in the high infection treatment and 38% of snails in the low infection treatment shed cercariae, while none of the snails in the cooler tanks shed parasites. Using an experimental design that manipulates temperature of the full aquatic host-parasite community offers greater realism than laboratory experiments on infection of each host separately, while still maintaining the mechanistic understanding characteristic of controlled experimental designs (Paull and Johnson in press).

![Fig. 5.1 Photo of greenhouse structures placed over buried mesocosm tanks.](image-url)
Additional field studies are also needed to assess interannual fluctuations in the seasonality of host and parasite abundance at both ephemeral and permanent sites, and to determine how the population dynamics and behavior of bird hosts are related to climate. A multi-year field study similar to the one already completed that explicitly compares host and parasite population fluctuations throughout the season at ephemeral and permanent sites would offer tremendous insight into the dual influences of hydroperiod and weather on host-parasite interactions in this system. Field studies of birds are also important, because although birds are endothermic, they can still experience thermal stress, and the energetic costs of maintaining a set body temperatures are dependent on environmental temperatures (Angilletta 2009). As such, increased or decreased consumption rates could alter the likelihood of contracting *Ribeiroia* infections (because it is a trophically transmitted parasite), and behavioral thermoregulation by birds could change the input of *Ribeiroia* eggs into the system if they spend more or less time in the aquatic habitat as a result. Additionally, climate-driven changes to freshwater bird populations could include reduced abundance, if lower precipitation or higher evaporation leads to habitat loss, or increased abundance if milder winters aid juvenile survival, and will likely be species-specific (Kingsford and Norman 2002; Fasola et al. 2010). Further research into the relationship between climate and aspects of the life history and behavior of freshwater birds could offer insight into potential climate-driven changes to *Ribeiroia* dynamics within these hosts.

Finally, more studies of the ecological effects of climate change need to consider the potential for organisms to behaviorally thermoregulate by explicitly choosing to spend time in cooler microclimates (Kearney et al. 2009; Sears et al. 2011). While such changes in behavior could mitigate the effects of thermal stress, it may also have unanticipated indirect consequences.
Aquatic environments are particularly important habitats in which to study this phenomenon since in the summer, water temperature tends to decrease with depth (Brönmark and Hansson 2005). A laboratory study found that snails parasitized by a variety of trematode species chose cooler microhabitats than unparasitized snails, likely in an attempt to reduce parasite development rates (Żbikowska 2004). Such information is not only crucial for understanding temperature-driven changes to disease risk, but also for determining how other ecological interactions may change as well. In addition to being cooler, deeper water also has lower light availability and differences in the abundance of both food and predators (Burks et al. 2002; Brönmark and Hansson 2005). Using a series of factorial laboratory experiments to manipulate these multiple constraints on animal behavior would aid understanding of future effects of climate change on interspecific interactions.

Implications of this work for studies of climate change and disease ecology

Several patterns emerge from my data that are important to consider in future studies of climate-disease interactions, including threshold and nonlinear responses of hosts and parasites to temperature. I found that *Ribeiroia* eggs and rediae (the parasite stage within snails) did not develop below a threshold temperature of 12 and 13°C respectively. Future studies should consider the potential for such threshold temperatures and what role they could play in climate-disease dynamics (Codeço et al. 2008; Rohr et al. 2011). Similarly, I found that amphibian pathology had a nonlinear relationship with temperature, peaking at the mid-temperature treatment, because temperature enhanced parasite infectivity, but also reduced host vulnerability. Because the performance of organisms is not linearly related to temperature, and infection is the
combination of both host and parasite processes, temperature-driven changes in disease may be more likely to be nonlinear than linear (Angilletta 2009; Lafferty 2009).

My results also suggest that greater emphasis should be placed on understanding climate-driven changes to the timing and consequence of interspecific interactions (Gilman et al. 2010; Paull and Johnson in press). I found that tadpoles developed faster at the warmest temperature in my laboratory study and were consequently less vulnerable to deformities than tadpoles at cooler temperatures after the first exposure event. Climate change could have differential effects on the phenology or development rates of hosts and parasites (Møller 2010), with potentially strong consequences for disease in systems where host pathology is governed by the developmental stage of the host at infection (e.g., Johnson et al. 2011; Patankar et al. 2011). Given that other ecological interactions, such as predation and competition can also be governed by the relative size or abundance of the interacting species, it will be key to develop greater understanding of interspecific differences in the response to temperature that could alter the timing and consequence of their interactions (Gilman et al. 2010; Yang and Rudolf 2010).

Finally, understanding ecological responses to climate change requires a combination of field and laboratory experiments considering variables besides changes in mean temperature (Marcogliese 2001; Clusella-Trullas et al. 2011). Controlled laboratory experiments offer excellent mechanistic understanding of climate-disease interactions that could be challenging to interpret based on simpler comparisons between, for example, parasite burdens under different conditions in the field (Paull and Johnson under revision). Field studies, however, offer more realism, and can often uncover important factors that are difficult to effectively study in the laboratory. For instance, my field study revealed that negative effects of evaporation rate on snail populations may have reduced amphibian infection and pathology- a relationship that
would have been challenging to detect in a laboratory setting; however results from my laboratory studies with temperature allow for predictions about temperature-driven changes to infection that are more challenging to document in a complex field setting. Thus, both laboratory and field studies offer valuable information about potential future climate-driven changes in disease risk in this system.
REFERENCES


Helmuth B. 2009. From cells to coastlines: how can we use physiology to forecast the impacts of climate change? Journal of Experimental Biology 212:753-760.


Miller M., A. White, and M. Boots. 2006. The evolution of parasites in response to tolerance in their hosts: The good, the bad, and apparent commensalism. Evolution 60:945-956.


