AN ABSTRACT OF THE DISSERTATION OF

Lindsey L. Thurman for the degree of Doctor of Philosophy in Wildlife Science presented on May 22, 2017.

Title: Amphibian Communities and Climate Change: Where Ecological Processes Meet Evolutionary Interactions.

Abstract approved:

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Forecasts of the impacts of climate change have traditionally focused on individual species and their phenotypes, phenology, or distribution. However, shifts in species distributions and the resulting reorganization of community composition represent an important violation to the assumption of species acting in isolation. Whereas species may respond individualistically to climate change, the manifestations of their responses will be largely influenced by interactions with other organisms. Tractably dealing with complex interaction networks in the face of climate change will require an understanding of community dynamics and the degree to which biotic interactions influence species' behavior, physiology, and survival – and ultimately their footprint on the landscape.

To improve our understanding of community-level responses to climate change, I explored amphibian response strategies from both a population- and community-level perspective and provided a critical evaluation of one of the primary methods for incorporating biotic interactions into predictive species distribution

models. In Chapter 2, I evaluated amphibian species' physiological constraints and the potential consequences of phenotypic plasticity as a first step to understanding their sensitivity, and ultimately, adaptive capacity to climate change. I experimentally quantified phenotypic plasticity in larval growth and development in three high elevation Anuran species (*Anaxyrus boreas, Pseudacris regilla,* and *Rana cascadae*) in response to projected climate warming scenarios for the Cascade Mountain Range. Warming initiated faster weight gain and accelerated larval growth rates in each of the species. However, any effort to achieve optimum body size (in both length and weight), while maintaining the necessary developmental trajectory under heat stress, was relatively unsuccessful.

In Chapter 3, I tested the response strategies of the same three Anuran species to a different climate stressors, hydroperiod reduction (i.e. drought), and included the additional stress of interspecific competition. I found that competition exacerbated the effects of drying on competitively inferior species (*Anaxyrus boreas* and *Pseudacris regilla*) and that, in general, species responses were largely context-dependent. My results emphasize the importance of biotic interactions in predictions of species response to climate change.

In Chapter 4, I provide a critical evaluation of standard methods for incorporating biotic interactions into predictive species distribution models. Most methods utilize observational data via species co-occurrences on the landscape to infer the role of biotic interactions in structuring species distributions. Results from a series of tests using two long-term amphibian co-occurrence datasets from Mt. Rainier National Park (Washington) and Mt. Hood (Oregon) show that the current best available methods are largely disconnected from community ecology theory and have yet to reconcile the complex dynamics within trophically-structured communities.

My research aims to fill a critical knowledge gap in the connection between community dynamics and biogeography, with significant implications for conservation and management of a severely threatened taxonomic group. I highlight the significant challenges of estimating species response to climate change across multiple levels of taxonomic organization and spatio-temporal scales.

Amphibian Communities and Climate change: Where Ecological Processes Meet Evolutionary Interactions

by
Lindsey L. Thurman

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APPROVED:

Major Professor, representing Wildlife Science

Head of the Department of Fisheries and Wildlife

Dean of the Graduate School

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

Lindsey L. Thurman, Author

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CONTRIBUTION OF AUTHORS

Chapter 2: Dr. Tiffany Garcia contributed to experimental design, interpretation of results, and edited the manuscript.

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Chapter 4: Dr. Allison Barner contributed to project development, implementation of statistical methods in R, analyses, interpretation of results, and manuscript preparation. Dr. Tiffany Garcia contributed input to the project’s conceptual development and edited the manuscript. Dr. Tara Chestnut provided data from Mt. Rainier National Park.

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DEDICATION

I dedicate this dissertation to my father, who started me on the path to knowledge.
Who gave me confidence and saw in me limitless potential.

Shine on you crazy diamond.

In memory
David Trent Thurman
11/14/1947 – 11/1/2012

AMPHIBIAN COMMUNITIES AND CLIMATE CHANGE: WHERE ECOLOGICAL PROCESSES MEET EVOLUTIONARY INTERACTIONS

1 – GENERAL INTRODUCTION

Reconciling the mechanisms, or processes, underlying the patterns we observe in nature is a unifying challenge across all ecological disciplines (Levin 1992). What drives the biogeographical patterns of ecological communities, for example, is one such question that we have been seeking to answer for more than 150 years. The challenge of discerning the mechanisms behind observable patterns in ecological communities is best explained by the evolution of community ecology theory. Theories on patterns in ecological communities are rooted in Charles Darwin's theories on species' struggle for existence. Darwin proposed that competition between organisms, most importantly organisms of the same species, was the chief mechanisms of evolutionary change and the resulting patterns of species on the landscape (Darwin 1859). Darwin's observations were crucial to the understanding of adaptation to the environment and biogeographical patterns, and stimulated many of the earliest natural historians to take a more rigorous approach to developing the science of ecology.

In the early 20th century, as the field of ecology was evolving, botanists emerged as some of the first scientists to experimentally test Darwin's ideas on adaptation. However, there was much debate over the processes by which species adapt to the environment, Clements' (1916; 1936) theories on plant communities and succession were based on the idea of communities as a superorganism (discrete entities with boundaries). He often used an organismal metaphor to demonstrate that communities changed over time much like the process of human development, resulting in a discrete endpoint or "climax" (Clements 1916; 1936). But Clements' work did not go unchallenged. One of the most vocal opponents of the organismal view of plant communities was Henry Allen Gleason. Gleason (1926) instead proposed an individualistic viewpoint that challenged Clements' ideas of fixed and bounded patterns. Gleason argued that plant associations were a product of fluctuating environmental conditions in space and time, and were based on individual environmental tolerances (a continuum view; Gleason 1926). This debate, and work by early animal ecologists like Charles Elton (Summerhayes & Elton 1923; Elton & Nicholson 1942; Elton 1949), spurred much of the development of ecological niche theory and the continued debate over the role of abiotic vs. biotic forces that structure ecological communities.

By the mid-20th Century, many ecologists, including Robert MacArthur, E. O. Wilson, and G. Evelyn Hutchinson (e.g., Hutchinson 1959; MacArthur & Pianka 1966; MacArthur & Wilson 1967), were interested in the mathematical and theoretical underpinnings of populations and communities. Much focus was directed towards topics like demographic processes, abundance and richness, spatial structuring, quantification of the ecological niche, and species interactions. For a more mechanistic understanding of community organization, MacArthur (1972) developed four themes of Geographical Ecology (and what is now referred to as Macroecology) that remain an incredibly influential part of modern biogeographical theory. These four themes include species abundance distributions, body size distributions, species area relationships, and the latitudinal diversity gradient (Brown

1999). The latitudinal diversity gradient stimulated much of MacArthur's work, and many others to this day, including investigations into predictable characteristics of ecological communities such as increases in turnover (beta diversity), geographic range size (Stevens 1989), and evenness in abundance distributions (Hubbell 1979).

As niche theory evolved, it catalyzed research into community succession and assembly, coexistence theory, and how species interactions regulate biodiversity patterns. Competition took the forefront of much ecological research and has become one of the most ubiquitous, and heavily studied, of species interactions. G. David Tilman has been a major player in developing the theory behind competition in spatially structured environments as a mechanism behind the structuring of ecological communities, particularly when resources are limited or nonsubstitutable (e.g., Tilman 1984; Tilman 1994). Competition is a mutually negative interaction because the acquisition of the resource by one individual simultaneously deprives others' access to it, which can negatively affect an individual's fitness and per capita population growth rates (Amarasekare 2003). Tilman's work on resource-ratio models of exploitative competition describes how coexistence can result when populations of several species that utilize the same limiting resources manage to persist within the same locality. This is based on species having differing abilities to compete for shared resources, such that coexistence is possible when species utilize resources that are more self-limiting (Tilman 1982). The idea of self-limitation has become a central tenet of community ecology; coexistence requires species to have stronger effects on regulating their own abundances than they have on the regulation of abundances of other species through interspecific interactions. Spatial-structuring of communities is another theme of Tilman's work that can be linked to numerous theories of community organization, such as competition-colonization trade-offs and the role of species interactions in a variable environment (Levins and Culver 1971, Hastings 1980, Tilman 1994).

Although trophic interactions have seen less attention, they are also considered a significant structuring component of ecological communities. Hairston et al. (1960) asked "why is the world green?" focusing attention on the role of top-down forces (e.g. predation) and indirect effects in shaping ecological communities. Menge and Sutherland (1976) presented their perspective on the roles of competition and predation in community structure and noted that the importance of predation as a regulating factor increased, as competition decreased, at lower trophic levels. By 1987 they had formulated the Environmental Stress Gradient Hypothesis and emphasized that competition, rather than predation, may be a primary structuring component in harsh environments because mobile predators are excluded or rendered ineffective by environmental severity (Menge and Sutherland 1987). The idea behind environmental gradients as they applied to disturbance was particularly novel and brought attention to short-term, temporal variation in community composition.

By the turn of the 21st Century, modern coexistence theory had emerged as a unifying framework for species coexistence that links the principles of environmental variability across space and time, species interactions, and spatial structuring, as previously described. Chesson (2000) described the mechanisms allowing species to stably coexist in a community through time. He described these mechanisms as either stabilizing, or equalizing. Stabilizing mechanisms (i.e. stabilizing niche differences) increase negative intraspecific interactions relative to interspecific interactions (the idea of self-limitation). Resource competition is a useful example of how stabilization occurs. If a species depends on a resource, and simultaneously reduces that resource, then a density-dependent feedback loop will result. Thus, when any one species increases in abundance, its per capita growth rate slows relative to other species (negative frequency dependence). On the other hand, equalizing mechanisms (i.e. relative fitness differences) are those differences between species that predict the outcome of competition in the absence of niche differentiation and have also been called fitness inequalities (Adler et al. 2007). If fitness inequalities are large, strong stabilization is required for long-term coexistence.

In this dissertation, I revisit some of the prevailing themes of community ecology theory. I explore amphibian species response to climate change as a means of understanding how species react to environmental stressors both individualistically, and collectively in a community context. I also expand my scale of inquiry to examine the connection between localized species interactions and their broader spatial distributions. I focused on the pond-breeding amphibian assemblage of the Cascade Mountain Range. This system contains a multi-trophic network of interacting amphibian species that overlap in occurrence and phenology in snowmelt dominated wetlands. Each chapter is linked by an effort to improve predictions of biological responses to climate change for amphibian species, a highly vulnerable taxonomic group.

In Chapter 2, I evaluated the physiological constraints imposed by climate warming and the consequences of phenotypic plasticity in individual species. It has been hypothesized that amphibians with phenotypically plastic traits may have the potential to combat environmental heterogeneity, allowing for persistence in regions expected to undergo variable climatic change. For example, plasticity in larval developmental time may be advantageous when larvae must undergo metamorphosis before ponds dry or freeze. However, significant trade-offs exist between accelerated larval development and optimal metamorphic body size as a size differential can have survival or reproductive fitness consequences. I examined the potential consequences of phenotypic plasticity in amphibian growth and development in response to climate warming to better understand species-specific vulnerability to climate change factors.

In Chapter 3, I tested these individualistic responses to environmental stress against antagonistic interactions to determine if species interactions, specifically competition, mediate amphibian response to climate change. With climate change, we may see shifts away from optimum environmental conditions for a given species and towards the optimum of another. This will in turn affect species' abundance and distribution, and the ecological context in which they interact. Therefore, exploring and quantifying biotic interactions in the context of changing environmental conditions is key to gaining more accurate predictions of their response to climate change. This is one of the first amphibian studies to explore, in a controlled experimental setting, how changing environmental conditions affect the strength and direction of interactions across multiple species and, conversely, how interactions can mediate the impact of environmental change. This study is a major step towards understanding context-dependencies in species interactions.

In Chapter 4, I provide a critical evaluation of the predominant method for incorporating species interactions into predictive distribution models: inferring interactions from statistical analyses of species co-occurrence patterns (or spatial associations). This practice is rooted in early community ecology theory where the primary assumption was that competition, specifically competitive exclusion, results in checkerboard distributions among competing species (Diamond 1975). While theory and methodologies have since advanced, we have yet to reconcile the connection between species associations estimated from observational data (pattern) and species interactions as quantified experimentally (process). I test the current best-available methods of co-occurrence analysis using two long-term datasets on amphibian species occurrence and show that this disconnect is particularly evident in trophically-structured communities (rich in species and interaction types) that vary over spatial and temporal gradients.

Overall, I utilize experimental, observational, and statistical approaches to quantify the role of amphibian species interactions in modulating their response to multiple climate change factors and evaluate new methods for improving the link between local (community) and landscape (biogeographic) drivers of biodiversity patterns. Amphibians are extremely vulnerable and responsive to environmental change, they exhibit diverse life history strategies, behavioral and physiological plasticity, and complex community dynamics. By leveraging their diverse ecological roles, I provide new insight into ecological community response to climate change and the potential conservation implications for amphibian species.

2 – DIFFERENTIAL PLASTICY IN RESPONSE TO SIMULATED CLIMATE WARMING IN A HIGH ELEVATION AMPHIBIAN ASSEMBLAGE

Lindsey L. Thurman and Tiffany S. Garcia

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Abstract

Climate change is expected to increase amphibian extinction rates, however little is known about the physiological responses of amphibian populations to climate change projections for their region. For the Cascade Mountain Range of the US Pacific Northwest, high-resolution climate models predict temperature increases during summer months. We evaluated phenotypic plasticity in larval growth and development in response to this projected increase in temperature in three co-occurring Anuran species: Cascades Frogs (*Rana cascadae*), Western Toads (*Anaxyrus boreas*), and Pacific Chorus Frogs (*Pseudacris regilla*). We exposed each species to two temperature regimes: the control treatment simulated the historical summer temperatures for the decade of 2001 to 2011; the warmed treatment mirrored this seasonal trend, but simulated a 4°C average increase in temperature. To quantify the magnitude of plasticity and any consequences to body size, we measured growth characteristics throughout larval development and metamorphosis. We found significant acceleration in larval developmental rates, and increased larval body mass under the warmed temperature treatment for all three species. However, when compared across Gosner (1960) developmental stages, the perceived weight gain in the warmed treatment was largely due to the advancement in development triggered by warming. As a consequence of rapid development, we observed differential shifts in body size features. We further identified an optimal temperature range (22-25°C) within which all three species showed maximum weight gain and development rates, and above which performance plateaued. We provide empirical evidence for species-

specific thermal tolerances and the potential for individualistic responses to climate change among co-occurring amphibian species.

Introduction

Modern climate has deviated from historical trends, resulting in measurable increases in global air and ocean temperatures and alterations to precipitation (Walther et al. 2005, IPCC 2013). Rapid changes in habitable climate space have increased species extinction rates and significantly altered the evolutionary trajectories of multiple taxonomic groups, including birds, butterflies, amphibians, and coral reef communities (Lawler et al. 2010, Thackeray et al. 2010, Foden et al. 2013). Various behavioral and physiological responses have been examined that may allow species to better cope with rapid environmental change (Bellard et al. 2013), including phenological shifts (Badeck et al. 2004, Buckley et al. 2015), poleward or elevational range shifts (Berg et al. 2010, Travis et al. 2013), broadened ecological breadth and niche requirements (Deutsch et al. 2008), and potential for contemporary evolution (Merilä and Hendry 2014). These responses reflect three strategies that can be utilized to withstand climate change: behavioral evasion, adaptive evolution, or phenotypic plasticity (Gienapp et al. 2008).

Phenotypic plasticity, or the expression of multiple phenotypes by a single genotype, is a means by which individuals can alter behavior, physiology, or morphology in response to environmental change and is assumed to be adaptive when individual fitness is maintained (Valladares et al. 2006, Canale and Henry 2010). The relationship between temperature and phenotypic plasticity in ectotherms, particularly amphibians, has been relatively well studied (Newman 1998, Álvarez and Nicieza 2002, Orizaola and Laurila 2009, Tejedo et al. 2010), with much effort directed at determining thermal optima, morphological variation, and growth performance. Recently, perspectives have shifted towards a call for applying these physiological and trait-based assessments of amphibian plasticity to determine the direct link between climate change factors and species' vulnerability (Li et al. 2013). Amphibians with phenotypically plastic traits are hypothesized to have the potential to combat environmental heterogeneity, allowing for persistence in regions expected to undergo significant climatic change (Urban et al. 2014). However, there has been little to no application of these temperature-plasticity studies to actual climate change scenarios and a species' perceived flexibility may not confer persistence (i.e. function) within their region under predicted climate futures.

Phenotypic plasticity in larval development rates may be advantageous when larvae must undergo metamorphosis before ponds dry or freeze (Perotti et al. 2011, Amburgey et al. 2012). Significant trade-offs, however, exist between larval development rates and adult body size as size can have significant survival and reproductive consequences (Werner 1986, Morin and Johnson 1988). Additionally, climatic shifts and consequent disruptions to community assembly may render plastic responses maladaptive (Reed et al. 2011). Phenotypic plasticity can also be costly when suboptimal phenotypes are expressed in a given environment (i.e. mismatch), or if simply possessing the ability to be plastic incurs lower fitness (Auld et al. 2010). Thus, the constraints and adaptive potential of plasticity are dependent upon species-environment interactions and may differ among species within the same environment.

High elevation amphibian breeding habitats, such as wetlands and ephemeral ponds, show marked variation in climate-controlled parameters, including temperature, precipitation patterns, and the length and depth of snow cover (Beniston et al. 1997, Diaz and Bradley 1997). High-resolution climate models for the Cascade Mountains in the U.S. Pacific Northwest have shown declining snowpack levels and significant summer temperature increases (Nolin and Daly 2006, Mote and Salathé 2010). The impacts of climate change in this mountain region will be particularly complex for amphibian species existing in snowmelt-dominated wetlands (Ryan et al. 2014). Due to the extreme weather patterns and high specialization among associated species, a comparative study of species-specific responses across a stress gradient can provide insight into the role of phenotypic plasticity in responding to climatic instability.

Our study compared phenotypic plasticity in larval growth and development rates among an assemblage of high elevation amphibian species in response to a warming climate scenario for their region (Cascade Mountain Range, U.S. Pacific Northwest). We examined temperature-induced phenotypic plasticity in multiple developmental characteristics in three species that overlap in distribution and phenology: Cascades Frogs (*Rana cascadae*), Western Toads (*Anaxyrus boreas*), and Pacific Chorus Frogs (*Pseudacris regilla*). Body size reductions incurred from phenotypic plasticity over ontogeny were expected to be dissimilar among the species, owing to their variable life histories and relative environmental sensitivities (e.g. degree of association with ephemeral wetlands). Species capable of optimizing trait responses while minimizing mortality risks in a warming environment may be relatively less vulnerable to changing climatic conditions.

Methods

Animal Collection and Husbandry

We collected egg masses of Cascades Frogs (*R. cascadae*), Western Toads (*A. boreas*), and Pacific Chorus Frogs (*P. regilla*) within 24-48 hours of being deposited from Little Three Creek Lake (Deschutes County, Oregon; 2046 m elevation) in July of 2012. Individuals were collected from at least six separate clutches per species to reduce clutch effects. Embryos were pooled by species and reared to hatching in a temperature-controlled environmental chamber (15 °C, 12L: 12D photoperiod) at Oregon State University. Embryos were held in 30-L HDPE plastic tubs with filtered, dechlorinated tap water. Upon hatching, individuals of each species were mixed and haphazardly assigned to an experimental treatment. Experimental densities were stocked at relatively low densities (30 individuals per tub [1 individual/Liter]) and fed *ad libitum* algal pellets and a rabbit chow/fish flake mix (3:1). Partial water changes were conducted every 3 days, with complete water changes every 7 days when necessary. Two temperature-controlled environmental chambers were used for this experiment, each set at a specific experimental temperature curve (Fig. 2.1). All experimental units were transferred between environmental chambers three times over the course of the experiment to control for sequence and room effects.

Experimental Design

We conducted the experiment from July 9 to September 26, 2012. We utilized a 3 x 2 fully factorial design with 12 replicates for each species and two temperature regime treatments (control and future warming). Temperature conditions for the control treatment were based on SNOTEL (Snowpack Telemetry; Natural Resource Conservation Service) data collected from the Three Creeks Meadow station (site 815), located approximately 5 km from the collection site. We averaged historical daily maximum air temperatures from July to September from 2001 to 2011 to estimate current maximum temperature conditions during the amphibian breeding and development season. The range of air temperatures selected for the control treatment were further supported by experimental and observational studies on these species collected from the same geographic area (Bancroft et al. 2008). Future temperature conditions were estimated based on high-resolution climate models for the latter half of the 21st Century (A1B emissions scenario) for the Cascade Mountain Range (Leung et al. 2004, Salathé et al. 2008, Mote and Salathé 2010). The A1B emissions scenario was selected because it represents an intermediate climate future. Using these temperature projections, we simulated a future warming scenario that increased daily temperatures relative to control conditions by 4ºC. Each time the temperature was adjusted in the environmental chambers, we monitored aquatic temperatures within a random subset of the experimental tubs (n=5 per treatment). Temperatures in the tubs remained within 1°C of ambient temperatures in the environmental chambers throughout the experiment.

During the experiment, we took a non-destructive random subsample of 5 individuals per tub every 10 days for a total of 7 sampling periods (70 days), or until completion of metamorphosis. We quantified snout-vent length (mm), total length (mm), wet weight (g), and Gosner (Gosner 1960) developmental stage at each sub-sampling interval and measured mortality daily. We checked experimental tubs twice daily for emergence of metamorphs starting at day 30 [or emergence of forelimbs; Gosner (1960) stage 42]. Larvae reaching metamorphosis were removed and measured for snout-vent length (mm) and wet weight (g). Time to metamorphosis was defined as the number of days (to nearest 12 hours) elapsed from hatching (day 0) until emergence. All individuals were humanely euthanized using MS-222 and preserved in 70% ethanol upon emergence, or at the termination of the experiment.

Statistical Analysis

To examine treatment effects on larval growth, development, and body size, we performed repeated measures analyses using linear mixed effects models. In all models, wherein the relationships appeared non-linear, we fit a polynomial function to the regression model by comparing a range of nth degree polynomials (quadratic to 4th degree polynomial) to the linear model using (Akaike 1974) information criterion (ΔAIC<2 equals equivalent support; fit with maximum likelihood). All variables were computed as the mean response per experimental unit (tub). In the first analysis, temperature treatment was included as a fixed factor and tub (subject) was treated as a random effect. We analyzed the effect of temperature treatment on body weight, length, and Gosner (1960) developmental stage by day 30, as well as larval duration and size (length and weight) at metamorphosis for each species independently. In the second analysis, we examined differences in larval growth rate (change in weight and length) with respect to Gosner (1960) developmental stage to determine if growth trajectories differed across larval development in each of the temperature treatments. For this comparison, we performed repeated measures analyses using linear mixed effects models, adjusted for non-linearity, wherein the interaction between temperature treatment (included as a factor) and Gosner (1960) developmental stage was included as a covariate and tub (subject) was treated as a random effect. We specified the appropriate covariance structure to stabilize heteroscedasticity between temperature treatments when necessary. In the third analysis, we evaluated growth and development over the continuous temperature gradient to determine treatment differences in the temperature at which maximum growth and development occurred. We calculated the instantaneous growth and development rates by deriving the tangent line to the fitted curve at each temperature and identifying the point (i.e. temperature) at which the slope of the tangent line was the highest. Lastly, to examine treatment effects on the proportion of individuals surviving to emergence, we fit a generalized linear mixed model with a binomial error distribution and logit link function for each species. Temperature treatment was included as a fixed effect and tub (subject) was treated as a random effect. To meet the assumption of circularity in all models, we evenly spaced sampling times with sufficient replicates based on *a priori* knowledge of species natural history and larval development rates. All statistical analyses were conducted using R Statistical software (version 3.2.4 © 2016) and the packages *nlme* (Pinheiro et al., 2016) and *lme4* (Bates et al., 2015).

Results

Larval Growth and Development

By day 30 of the experiment, larvae of all three species in the warmed treatment had reached significantly greater weight (Western Toad: $F_{1,22} = 30.86$, $P < 0.001$; Cascades Frog: $F_{1,22} = 21.16$, $P < 0.001$; Pacific Chorus Frog: $F_{1,22} = 8.03$, $P = 0.01$; Fig. 2.2) and length (Western Toad: $F_{1,22} = 54.11$, $P < 0.001$; Cascades Frog: $F_{1,22} = 22.34$, $P < 0.001$; Pacific Chorus Frog: $F_{1,22} = 7.05$, $P = 0.01$) relative to the control treatment. Warming significantly reduced average larval duration (i.e. time to emergence), such that all three species showed accelerated emergence times in the warmed temperature treatment (Western Toad: $F_{1,22} = 228.03$, $P < 0.001$; Cascades Frog: $F_{1,22} = 113.70$, $P < 0.001$; Pacific Chorus Frog: $F_{1,22} = 32.25$, $P < 0.001$; Fig. 2.3). Average larval duration between the two treatments was most disparate for Western Toads, with an average reduction in larval duration by 15.96 days (SE=0.99) in the warmed treatment. Pacific Chorus Frogs showed similar acceleration, with an average reduction in larval duration by 13.88 days (SE=1.28). For Cascades Frogs, average larval duration was also significantly shorter in the warmed treatment, but with the lowest relative reduction by 10.89 days (SE=1.00). Each of the species also emerged at significantly different experimental temperatures between treatments (Western Toad: $F_{1,22} = 855.56$, $P < 0.001$; Cascades Frog: $F_{1,22} = 651.97$, $P < 0.001$; Pacific Chorus Frog: $F_{1,22} = 349.94$, $P < 0.001$; Fig. 2.3). In the warmed temperature treatment, mean emergence dates for each of the species coincided with peak ambient temperatures (approximately 28-30ºC). In the control temperature treatment with a cooler temperature gradient, peak emergence was prolonged past peak ambient temperatures and occurred at approximately 22-23ºC for each species.

We further compared larval body length and weight between treatments with respect to Gosner (1960) developmental stage, since accelerated development may have contributed to the larger body size in the warmed treatment by day 30. For example, Figure 2.4 illustrates the mechanism behind increased weight gain in the warmed treatment by day 30 (Sampling Period 4). Western Toad larvae in the warmed treatment were heavier than those in the control treatment, but they were also nearly 10 stages further along in development. Therefore, if we remove the effect of time and compare across larval developmental stages, two of the three species actually weighed less in the warmed treatment over the course of larval development (Pacific Chorus Frog: $F_{1,101} = 20.21$, $P < 0.001$; Cascades Frog: $F_{1,122} = 20.99$, $P < 0.001$; Fig. 2.5) compared to the control treatment. The difference in Western Toad weight at day 30 was no longer evident when the effect of time was removed, individuals were able to maintain a similar body weight over development between treatments ($F_{1,86} = 0.02$, $P = 0.88$). Pacific Chorus Frog and Western Toad larvae exhibited reduced body length through development in the warmed temperature treatment (Pacific Chorus Frog: $F_{1,86} = 5.25$, $P = 0.02$; Western Toad: $F_{1,86} = 143.21$, $P < 0.001$), but Cascades Frogs were able to maintain a similar larval body length through development between treatments ($F_{1,122} = -0.97$, $P = 0.33$). Thus, Cascades Frogs and Western Toads exhibit a tradeoff in their ability to maintain similar body weight vs. length over larval development in the warmed treatment.

We also compared growth trends across the temperature gradient (another proxy for time). For all three species, individuals in the warmed treatment invested earlier in larval development and weight gain than those in the control treatment, but the temperatures at which maximum growth occurred were relatively similar across temperature treatments within and among species (Western Toad: 22-24 °C, Cascades Frog: 23-24 °C, Pacific Chorus Frog: 23-25 °C; Fig. 2.6). However, the investment in growth in body length over the temperature gradient was relatively consistent within each species and showed no similar pattern in temperature optima across treatments; individuals invested early in length gain.

Size at emergence

For Pacific Chorus Frogs and Cascades Frogs, the gain in larval weight by day 30 in the warmed treatment was reversed at metamorphosis; these individuals exhibited smaller body weight (Pacific Chorus Frog: $F_{1,22} = 10.44$, $P = 0.004$; Cascades Frog: $F_{1,22} = 29.76$, $P < 0.001$; Fig. 2.7) and length relative to controls at emergence (Pacific Chorus Frog: $F_{1,22} = 5.38$, $P = 0.03$; Cascades Frog: $F_{1,22} = 16.63$, $P < 0.001$). Conversely, emerging Western Toads in the warmed temperature treatment were able to maintain a similar metamorph body weight as individuals in the control temperature treatment ($F_{1,22} = 2.35$, $P = 0.14$), however metamorph body length was reduced ($F_{1,22} = 11.10$, $P = 0.003$).

Survivorship

The likelihood of Cascades Frog larvae surviving to emergence in the warmed temperature treatment (odds ratio: 1.26; 95% C.I.: 0.94 - 1.69) was not significantly different from the likelihood of surviving to emergence in the control treatment (odds ratio: 0.82; 95% C.I.: 0.66 - 1.01). Similarly, the likelihood of Pacific Chorus Frog larvae surviving to emergence in the warmed temperature treatment (odds ratio: 0.866; 95% C.I.: 0.62 - 1.20) was not significantly different from the control temperature treatment (odds ratio: 2.43; 95% C.I.: 1.92 - 3.08). There was a higher likelihood of Western Toad larvae surviving to emergence in the warmed temperature treatment (odds ratio: 3.88; 95% C.I.: 2.40 - 6.26) compared to the control treatment (odds ratio: 0.40; 95% C.I.: 0.28 - 0.56).

Discussion

This study offers an analysis of the possible range of responses to projected climate warming within an amphibian assemblage and quantifies the trade-offs associated with phenotypic plasticity over larval development. By combining historical and future predicted temperature data for this region, we were able to show that amphibian species respond individualistically to temperature shifts regardless of distributional or phenological overlap. Further, temperatures departing from the historical trends, such as those utilized in this study, were predicted to stress all species to the point of either plastic response or mortality. While warming initiated faster weight gain and accelerated larval growth rates in each of the species, there was a significant tradeoff in larval and juvenile body size. Any effort to achieve optimum body size (in both length and weight), while maintaining the necessary developmental trajectory under heat stress, was relatively unsuccessful. Due to the differences in developmental rates between treatments, mismatches in body size arose over time.

Early in the experiment, individuals in the warmed temperature treatment were expectedly larger given their advanced developmental stage, but smaller in either weight or length in relation to control treatment individuals when compared at each developmental stage.

While temperatures used in the warmed treatment do not supersede the hypothesized critical thermal maxima for these species, they do approach the limits of tolerable temperatures (e.g. Bancroft et al., 2008). Across species, there appears to be a thermal optimum in the range of 22-25°C wherein larval development rates and weight gain are maximized and above which growth rates plateau (Fig. 2.6). In the warmed treatment, individuals reached this temperature optimum earlier in development, thus the energetic demands of accelerated development and stress of continued warming beyond this thermal range likely prevented these individuals from matching the body size of their control treatment counterparts (Dewitt et al. 1998).

The difference between body size over time versus body size over developmental stage may have interesting implications for natural populations. Discussions on the consequences of phenotypic plasticity in amphibians often revolve around the fitness costs of rapid larval development post-emergence. Reduced juvenile body size can negatively affect fitness as adults given that size at metamorphosis is a key indicator of future reproductive success (Semlitsch et al. 1988). However, when competing as larvae in the aquatic environment, developmental stage may be arguably less important than body size (Morin and Johnson 1988, Werner 1992, Vogel and Pechmann 2010), thus reductions in larval body size can also have immediate costs. Smaller body size can reduce survival via increased predation risk or loss of competitive edge (Werner 1986), but it also has consequences within the aquatic food web as amphibian larvae contribute a substantial portion of the prey biomass in aquatic environments (Gibbons et al. 2006). Combined with the truncated availability of amphibian prey as a result of rapid development, reductions in biomass may significantly disrupt food web dynamics, depending on the response of other species in the network.

For all three species, size differentials between treatments persisted post-metamorphosis (Fig. 2.7). Pacific Chorus Frogs showed the greatest overall size disparity between temperate treatments as individuals in the warmed treatment had reduced body weight and length through larval development and post-emergence. The pattern of weight loss over development in the warmed temperature treatment relative to controls was also maintained at the time of emergence for Cascades Frogs. Cascades Frogs further experienced a reduction in body length post-emergence that was not evident through larval development, an interesting ontogenetic shift in susceptibility to the consequences of warming. Western Toads maintained similar body weight between treatments, but exhibited reduced body length through development and post-emergence. Thus, multi-species' response to climate warming may not be predictable by traits in isolation as differential responses were observed among species, across multiple growth characteristics, and through ontogeny. The observed variability among these species gives support to the hypothesis that community dynamics in the natural environment may be disrupted as species respond individualistically to climate stress throughout development and into metamorphosis. It also highlights the complex nature of metamorphosis itself, and the need for more information on this metamorphic window in terms of physiological requirements and ecological consequences.

The survivorship analyses revealed another interesting trend for Western Toads, which experienced an unexpectedly higher likelihood of survival to emergence under warming. There did not appear to be any disease- or density-related mortality within the replicate tubs. It may be an acute mortality event in the Western Toad's control group that drove this result. When animals were transferred between the two temperature-controlled environmental chambers, toads experienced an abrupt mortality event possibly due to an unforeseen sensitivity to the physical stress of movement. There were no differences in the likelihood of surviving to emergence between temperature treatments for both Pacific Chorus Frogs and Cascades Frogs. This suggests that the costs of phenotypic plasticity in response to warming may be restricted to body size during these early life stages and potentially buffered by juvenile survival, in the absence of other stressors. However, for amphibian species at high elevations, continued survival post-emergence is directly impacted by a suite of environmental and biological factors. Maintaining an optimal body size is necessary to withstand these pressures and any stress-associated shifts in body size may leave individuals more vulnerable to mortality (Werner 1986).

Although plasticity is often viewed as a potential buffering mechanism for climate change, this study supports recent evidence that suggests the opposite may be true, particularly for ectotherms (Gunderson and Stillman 2015). The long-term benefits of plasticity are entirely dependent upon organisms' ability to predict the strength and direction (or variability) of stressful environmental conditions, or rely on flexible maternal effects that offer a fitness advantage through mechanisms like transgenerational plasticity (Galloway and Etterson 2007). Regardless, phenotypic plasticity is a bet-hedging strategy whereby phenotypic variation within a population improves the potential for an appropriate response to changing conditions (Chown et al. 2010). However, plasticity can also constrain the potential for evolutionary adaptation (van Buskirk and Steiner 2009) and in the case of increasing climatic unpredictability, a broadening of performance curves and a reduction in phenotypic plasticity may reduce the likelihood of fitness costs due to inappropriate responses (Chown et al. 2010). Our understanding of the costs versus benefits of plasticity as an amphibian response strategy will thus require utilizing projections of climate shifts to determine the degree of variability in environmental conditions and concordant tradeoffs in species' performance.

Phenotypic plasticity is also not the only mechanism for responding to climate change and alternative responses may be employed in cooperation, or as an alternative to, physiological changes (e.g. phenological shifts and behavioral thermoregulation). Further, the effects of climate change transcend warming temperatures to include changes in a suite of environmental parameters. Thus, the potential for species to have individualistic responses to multiple climate change factors may have cascading impacts on community structure and function and further alter the interactions between organisms and their environment (Miner et al. 2005). This problem of rapid climate change altering ecological patterns and processes, amplified by variation in species' response strategies, will require a better understanding of multi-species dynamics at a broader landscape scale. This study contributes to this research need and provides an important context in which to view disparity in phenotypic plasticity within an amphibian assemblage.

Acknowledgments

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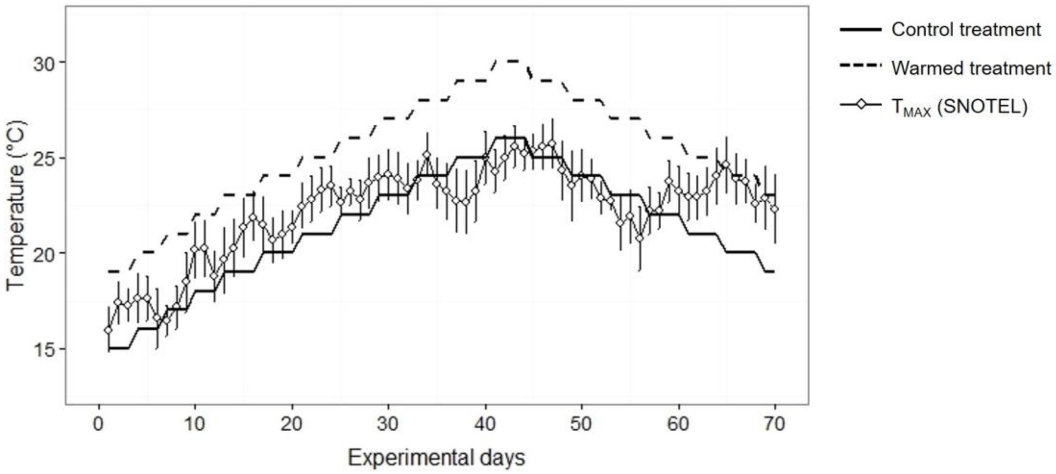

Figure 2.1 Experimental temperature regimes and historical temperature conditions. Control temperature treatment indicated by solid black line and warmed temperature treatment indicated by dashed red line. Average daily maximum temperatures (solid black line with open circles) for the summer months averaged for the decade 2001-2011 taken from SNOTEL site 815 (http://www.wcc.nrcs.usda.gov/snow/); represents historical temperature conditions upon which the temperature treatments were based. Warmed temperature treatment was 4ºC warmer than the control temperature treatment.

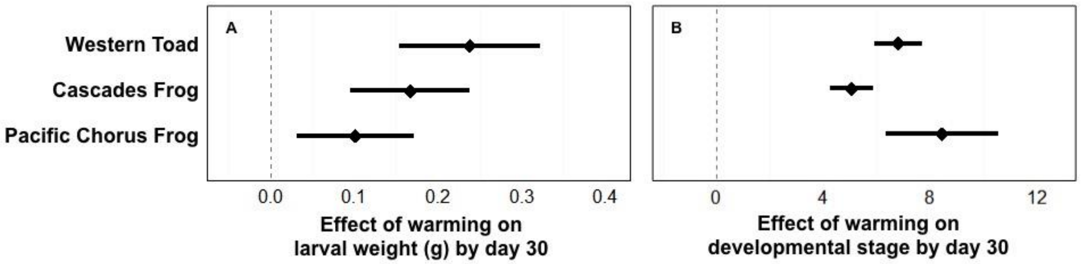

Figure 2.2 Species-level effects of warming on larval characteristics. Coefficient values and 95% confidence intervals for (A) increases in larval body weight and (B) increases in developmental stage by day 30 (n = 12 for each species). Species include the Western Toad (*Anaxyrus boreas*), Cascades Frog (*Rana cascadae*), and Pacific Chorus Frog (*Pseudacris regilla*).

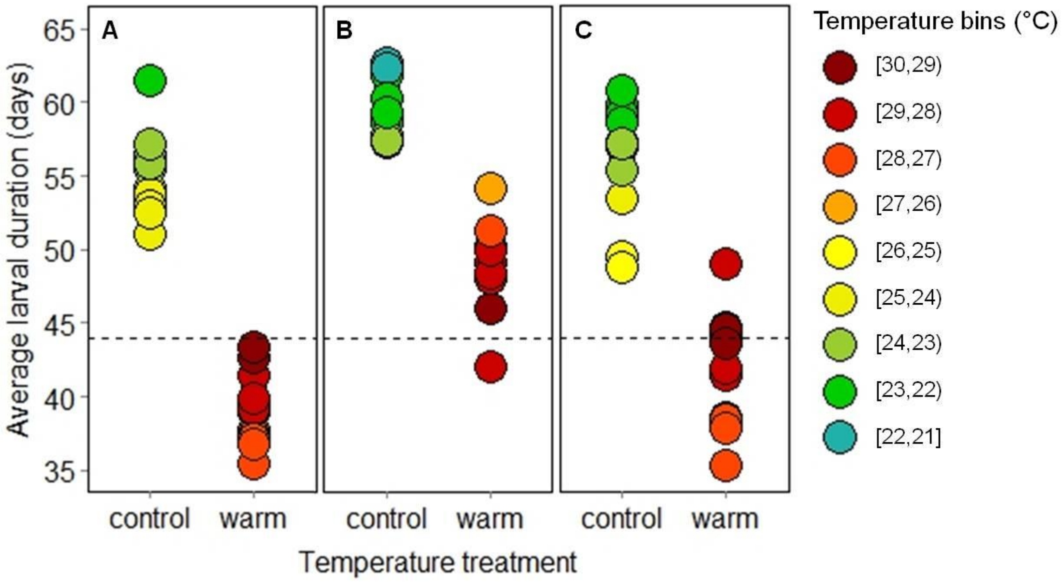

Figure 2.3 Average larval duration (days) in the control and warmed temperature treatments. (A) Western Toads (n = 12 per treatment), (B) Cascades Frogs (n=12 per treatment), and (C) Pacific Chorus Frogs (n = 12 per treatment). The color gradient represents 1ºC temperature ranges for the average temperature at emergence for each treatment tub. The horizontal dashed line indicates the day of peak temperature (day 44) during the experiment. All individuals in the control treatment emerged after the peak temperature (26ºC) and individuals in the warmed treatment emerged around the time of peak temperature (30ºC).

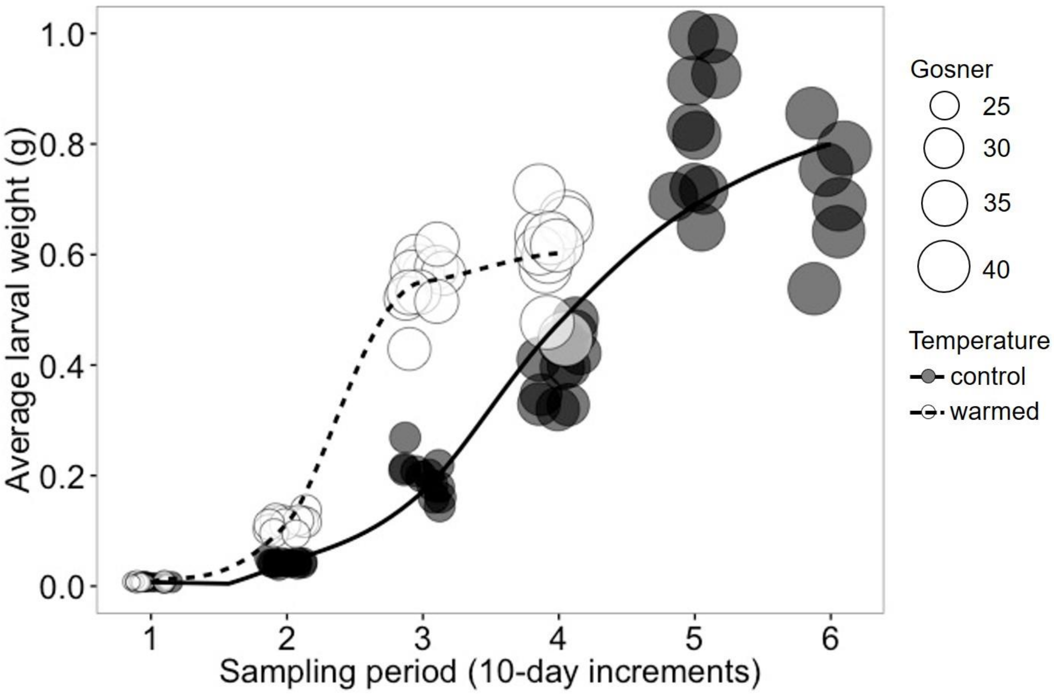

Figure 2.4 Difference in average weight gain (g) between the control and warmed temperature treatments over the course of the experiment for Western Toad larvae. The control temperature treatment is represented by shaded circles with a solid line, the warmed temperature treatment is represented by unshaded circles with a dashed line (n = 12 per treatment). The size of the circle indicates Gosner (1960) developmental stage.

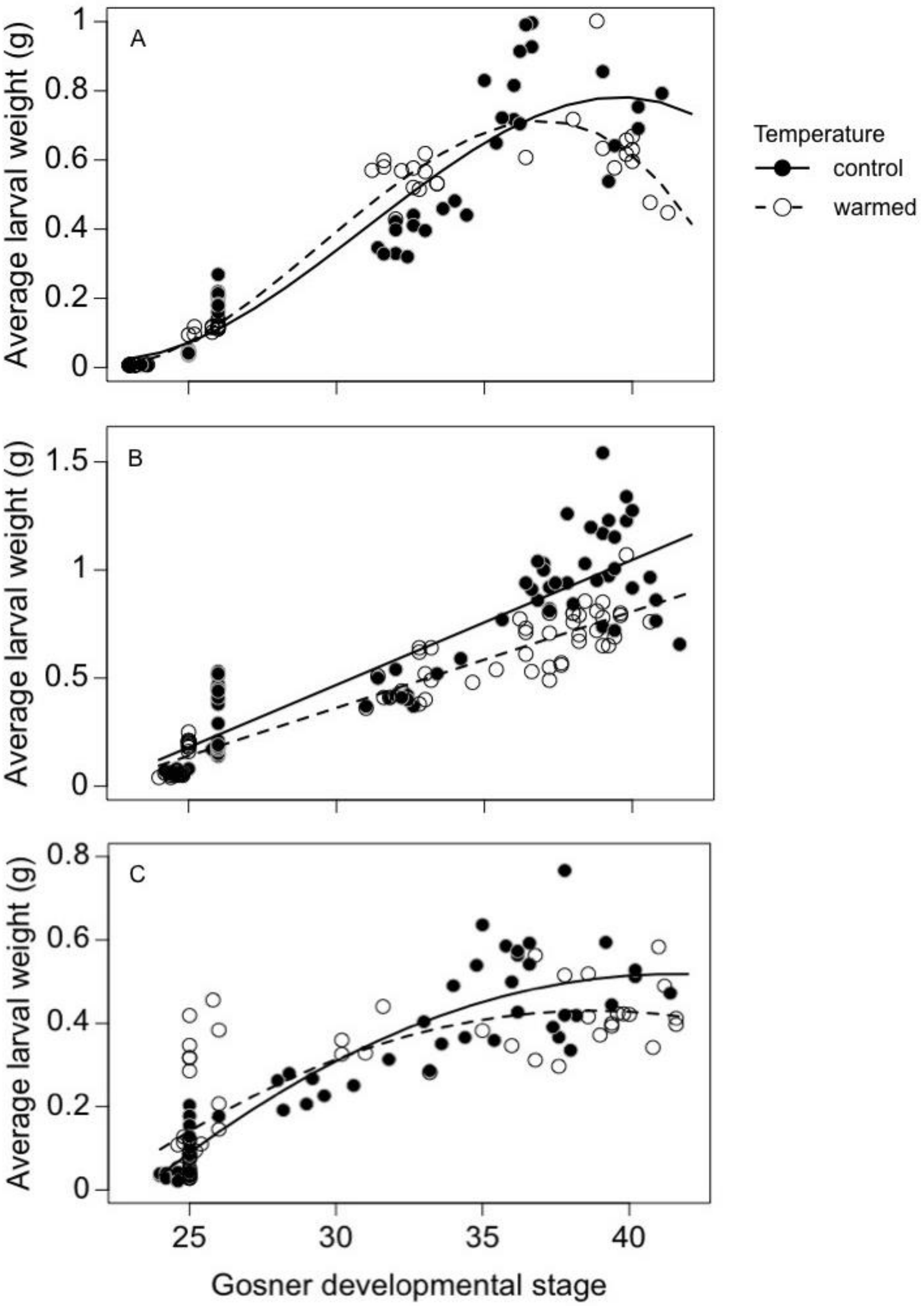

Figure 2.5 Difference in average weight gain (g) between the control and warmed temperature treatments over larval development. (A) Western Toads (n = 12 per treatment), (B) Cascades Frogs (n = 12 per treatment), and (C) Pacific Chorus Frogs (n = 12 per treatment). The control temperature treatment is represented by the black circles with a solid line and the warmed temperature treatment is represented by the white circles with a dashed line.

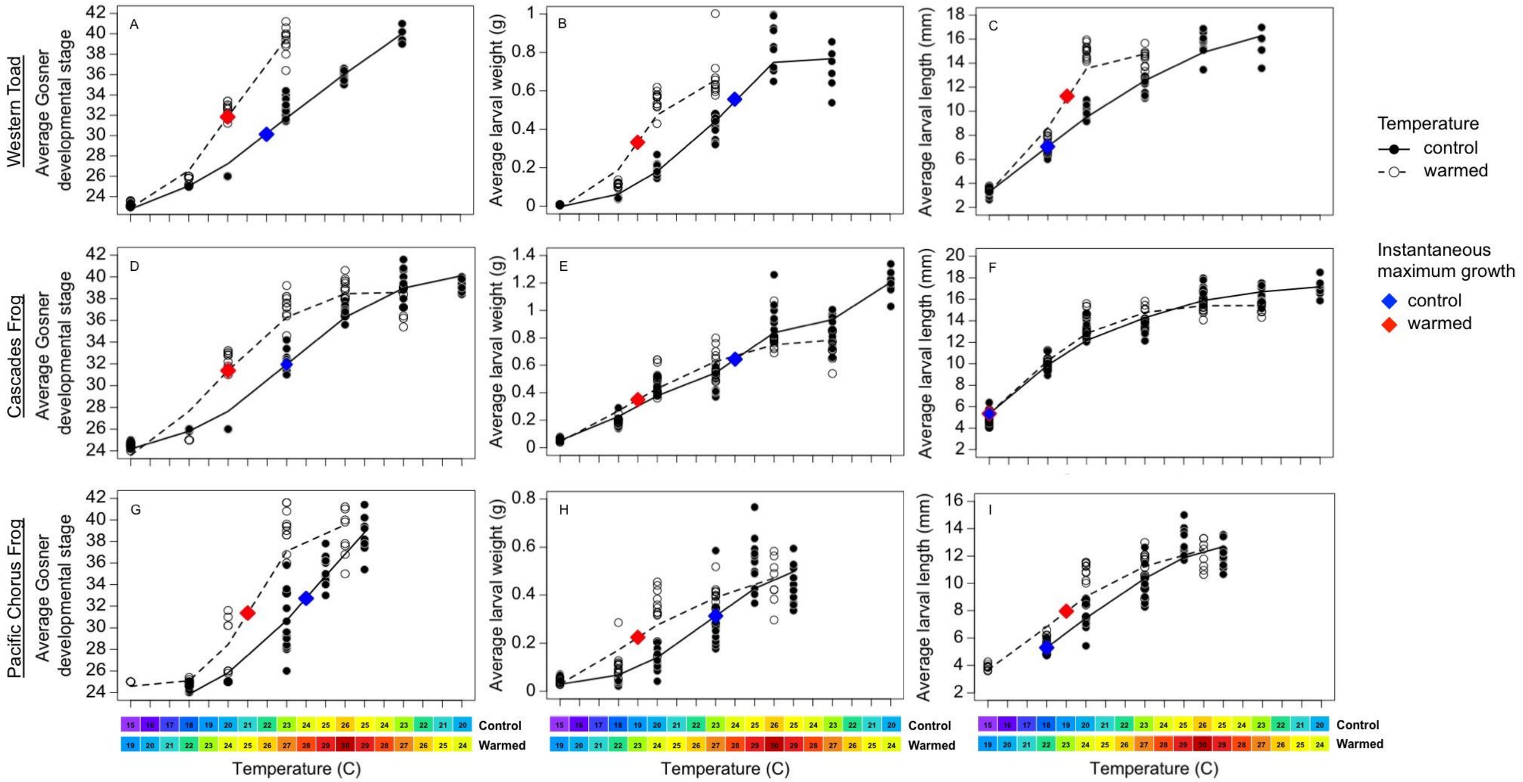

Figure 2.6 Differences in larval development rates and growth characteristics over the control and warmed experimental temperature gradients for Western Toads (A-C), Cascades Frogs (D-F), and Pacific Chorus Frogs (G-I). The control temperature treatment is represented by black circles with a solid line and the warmed temperature treatment is represented by white circles with a dashed line. The point of instantaneous maximum growth or development rate (calculated from the tangent line to the curve at each point along the curve) is indicated by a blue diamond for the control treatment and a red diamond for the warmed treatment.

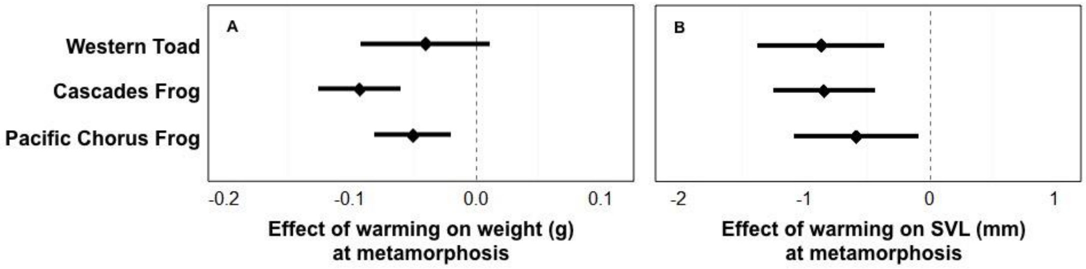

Figure 2.7 Species-level effects of warming on juvenile (metamorph) characteristics. Coefficient values and 95% confidence intervals for (A) body weight and (B) length (n = 12 for each species). Species include the Western Toad (*Anaxyrus boreas*), Cascades Frog (*Rana cascadae*), and Pacific Chorus Frog (*Pseudacris regilla*). The Western Toad did not exhibit a significant reduction in body weight at emergence in the warmed temperature treatment.

3 – ASYMMETRIC COMPETITION SHAPES AMPHIBIAN RESPONSE TO RAPID ENVIRONMENTAL CHANGE

Lindsey L. Thurman and Tiffany S. Garcia

Abstract

Pond-breeding amphibians experience climatic variability primarily in the form of seasonal temperature fluctuations and water availability, two factors that are expected to significantly depart from the norm as climate change progresses. Larval amphibians are also concurrently exposed to biological stressors as they often co-occur in diverse, multi-species assemblages. Thus, to accurately predict species' response to climate change, we must acknowledge not only the abiotic drivers, but the biological dynamics that influence response strategies. We focus on three amphibian species that overlap in distribution and phenology across high elevations of the Cascade Mountain Range of the U.S. Pacific Northwest; the Pacific chorus frog (*Pseudacris regilla*), Cascades frog (*Rana cascadae*), and Western toad (*Anaxyrus boreas*). Amphibians in this region are threatened by climate-induced habitat loss resulting from the shortening, and in some areas elimination, of seasonal wetlands. We conducted a fully-factorial experiment to determine the role of competition in shaping amphibian response to hydroperiod reduction (i.e. rapid drying). We found competitive interactions were largely non-additive and condition-specific with regard to drying, and that competition exacerbated the effects of drying on competitively inferior species. Our results suggest that ignoring biotic interactions in predictions of species response to climate change may be misleading.

Introduction

Climate change is the major global threat to biodiversity (Pimm 2009). As novel environmental conditions continue to emerge, ecological communities are being altered in ways that have no modern equivalent (Hobbs et al. 2009). Much progress has been made in predicting species' population-level responses to climate change through effects on physiology (Chown et al. 2010, Seebacher et al. 2014), range shifts (Foden et al. 2013), and phenology (Badeck et al. 2004, Richardson et al. 2013). However, species do not exist in isolation and are connected by a network of interactions with other members of their community (Walther 2010). Most predictive models ignore important underlying mechanisms like biotic interactions, because their integrative role with climate change is difficult to quantify (Angert et al. 2013). It is, therefore, important to further our understanding of the role of biotic interactions in modulating or compounding the effects of climate change (Gilman et al. 2010, Alexander et al. 2015, Urban et al. 2016). Accurate predictions of species' response to climate change should account for not only abiotic drivers, but the biological dynamics that directly (or indirectly) influence their response strategies.

The complex interplay between biotic interactions and the abiotic constraints associated with climate change suggests continued evaluation, such as how changing environmental conditions affect the strength and direction of multi-species interactions (e.g. (Hoover et al. 2012, Milazzo et al. 2013, Rysavy et al. 2016)) and, conversely, how interactions can mediate the impact of environmental stress (e.g. (McGuire and Agrawal 2005, Suttle et al. 2007, Alexander et al. 2015). A more detailed understanding of these relationships should significantly improve the predictive accuracy of ecological forecasts used in conservation planning.

We tested the interplay between biotic and abiotic constraints on amphibian responses, as these animals are sensitive to environmental stress and have long been used to investigate antagonistic interactions, particularly competition, predation, and parasite/pathogen dynamics (e.g., Peacor and Werner 1997, Preston et al. 2014, Youngquist et al. 2015). Despite the breadth of literature on amphibian community dynamics, few studies have investigated the interaction between drying and multi-species interactions beyond pairwise assessments (for a review of amphibian experiments see Tables A1 and A2). Of the studies investigating the combined effect of multi-species interactions and drying, most are limited in their inference to net interaction strengths and net effects of abiotic stressors due to their limited experimental design (Table A2).

A stronger understanding of the effects of environmental change on amphibian communities requires scaling from pairwise to multi-species interactions wherein direct and indirect effects may emerge (Tylianakis et al. 2008, Buck et al. 2012, Harvey et al. 2016). Amphibian species have been shown to adjust their behavior and morphology in the presence of different predators and competitors (Relyea 2000), as well as under novel environmental conditions (O'Regan et al. 2014b). Thus, species response strategies catered to either biotic interactions or abiotic stress (or a combination of both) may be context-specific (Chesson 1986, Dunson and Travis 1991, Taniguchi and Nakano 2000) and result in markedly different outcomes.

In this study, we use three amphibian species that overlap in distribution and phenology in snowmelt-dominated wetlands across high elevations of the Cascade Mountain Range of the U.S. Pacific Northwest: Pacific chorus frog (*Pseudacris regilla*), Cascades frog (*Rana cascadae*), and Western toad (*Anaxyrus boreas*).

Amphibians in this region are threatened by climate-induced habitat loss resulting from shortened wetland hydroperiods, and in some areas, complete elimination of seasonal wetlands (Ryan et al. 2014). Hydrological models for ephemeral and intermediate wetlands at high elevations in the Pacific Northwest predict earlier drawdown, more rapid summer recession rates, and reduced minimum water levels, resulting in a longer dry season in summer (Lee et al. 2015). While some species are adapted to withstand a degree of seasonal dry-down, increased evaporation rates and the rapid drying of wetlands may leave some species more susceptible to drought conditions. The potentially shortened breeding window can result in significant spatial and temporal overlap in phenologies among larval amphibian competitors, with multiple species developing and competing for food resources simultaneously. Thus, differences in sensitivity to rapid drying and drought conditions may result in unequal fitness among competing species.

To determine the role of combined biotic and abiotic stress in structuring amphibian species response, we conducted a fully-factorial competition experiment that tested three-species competition under permanent and rapidly drying (i.e. ephemeral) hydroperiods. To our knowledge, this is the first fully-factorial experiment to investigate the combined effects of drying and interspecific interactions involving at least three amphibian species (Table A1 and A2). We included all combinations of interspecific competition to determine if species interactions changed with respect to the assemblage of competitors. We hypothesized that competition intensities would be dependent upon abiotic conditions (hydroperiod). More specifically, we hypothesized that the need to accelerate development in a drying environment would impose enough physiological stress on individuals to exacerbate competitive effects and result in reduced survival and body weight through ontogeny.

Methods

Collection

Egg masses of Pacific chorus frog (*Pseudacris regilla*), Cascades frog (*Rana cascadae*), and Western toad (*Anaxyrus boreas*) were collected from five pond breeding sites in the central Oregon Cascades between 1800 - 2050 m elevation during the summer breeding season of 2014 (Deschutes County, Oregon). Breeding sites were semi-permanent ponds (dry in late Fall or in summer during drought years), which are projected to experience earlier and faster rates of drawdown, reduced overall water availability, and increased frequency of complete drying under climate change (Lee et al. 2015). Individuals (embryos) were collected from a minimum of six separate clutches per species per population to reduce clutch effects. Embryos were pooled by species and reared to hatching in a temperature-controlled environmental chamber (15°C, 12L: 12D) at Oregon State University. Within 8 hours of hatching, individuals of each species were mixed and haphazardly assigned to their competition and hydroperiod treatments.

Experimental Design

We utilized a 7x2 (seven species combinations x two hydroperiod regimes) fully factorial, blocked experimental design with five replicate tubs for each treatment combination. The seven species combinations included three intraspecific competition treatments, three pairwise interspecific competition treatments, and one three-species competition treatment (Fig. 3.1). We held the total number of tadpoles constant at 30 across treatments (single species: 30 individuals per mesocosm, pairwise combinations: 15 individuals per species per mesocosm, three-species combination: 10 individuals per species per mesocosm). The experiment was conducted in outdoor mesocosms (120-L HDPE plastic tubs filled with well water) at Oregon State University's Lewis-Brown Horticulture Farm. All treatment combinations were randomly assigned to their respective mesocosms and arrayed beneath canopy cover. A ceramic tile was used in each tub to monitor algal growth and verify that consumptive competition was occurring across all treatments. We conducted partial water changes as needed to avoid algal overgrowth.

For the permanent hydroperiod treatment, water volume was maintained at 100 L throughout the experiment, resulting in a density of 0.3 individuals/L. For the drying hydroperiod treatment, water volume was reduced at a rate of 8 L every 5 days, reducing the original 100 L water volume (0.3 individuals/L) to 12 L water volume (2.5 individuals/L without mortality) over the course of the 60-day experiment. Temperature sensors (Maxim iButton® Thermochron Temperature Data Loggers) were deployed for the duration of the experiment in the bottom of mesocosms within Block 1. We predicted higher variability in daily temperatures over the course of the experiment in the drying treatment relative to the mesocosms with permanent hydroperiods given the reduction in water volume over time (Fig. B1).

We measured incremental growth via changes in average larval weight and Gosner developmental stage (Gosner 1960). We measured juvenile performance by quantifying time to emergence, proportion emerging, and weight at emergence throughout the experiment. The experiment was blocked so that individuals in each of the five treatment combination replicates were sampled sequentially over the course of five days (e.g. first day of sampling = 1 replicate of all 7 x 2 treatment combinations). The experiment lasted for 60 days, with 10-day intervals between each sampling period (7 sampling periods total, including sampling on day 0). Given that metamorphosis is not a discrete event (Werner 1986), measures of juvenile performance were taken post-emergence when individuals were considered fully terrestrial (complete tail resorption). Mesocosm tubs were checked daily for emergence of metamorphosed individuals onto floating platforms starting after the development of forelimbs. All individuals were euthanized using MS-222 and preserved in 70% ethanol at the termination of the experiment.

Statistical Analyses

To examine treatment effects on growth and development, we performed repeated measures analyses using linear mixed effects models with a random effect of tub nested within block. For each species, the response variables of interest included average larval weight (g) at day 30 and Gosner developmental stage at day 30, time to emergence, and weight at emergence. All variables were computed as the mean response per mesocosm (experimental unit). To examine treatment effects on the proportion of individuals surviving to emergence, we fit a generalized linear mixed model with a binomial distribution and logit link function. For each species, we tested the response variables as a function of the combination of hydroperiod and competition treatments. We evaluated whether our data supported incorporating a two-way interaction between the treatments by comparing support for the models using Akaike's information criterion (ΔAIC<2 equals equivalent support; fit with maximum likelihood). We also tested for differences in mean and standard deviation of water temperature throughout the course of the experiment between the permanent and drying hydroperiod treatments using a linear mixed effects model with the random effect of tub. We found significantly greater variability in temperature in the drying treatment, but no difference in mean temperature throughout the experiment (Table B1). Therefore, drying also increased temperature variability. All linear mixed model analyses were conducted using R Studio Statistical software (version 0.99.903 © 2016) and the packages *nlme* (Pinheiro et al. 2016) and *lme4* (Bates et al. 2015) with restricted maximum likelihood (REML).

For each species, we used the interaction between rapid drying and the interspecific competition treatments (two pairwise competition treatments and one three-species competition treatment) to determine if the multi-stressor effects on the response variables of interest were antagonistic, synergistic, or additive when significant (Piggott et al. 2015, Côté et al. 2016). We compared the observed responses to an expected additive value; additive responses were calculated by adding the responses to each stressor in isolation. For example, we compared the observed response of Pacific chorus frogs in the three-species competition and drying treatment to the expected response calculated by adding the effect of the three-species competition treatment (with no drying) to the drying treatment (with no competition). We used the classification methods as described in Piggott et al. (2015) to elucidate whether the effect of an interaction between factors was positively synergistic (more positive than predicted additively), or negatively synergistic (more negative than predicted additively), when appropriate.

Results

Effect of drying

There was no significant main effect of drying on larval or post-metamorphic traits in Pacific chorus frogs (larval development: t_{31}=0.63, P=0.53; larval weight: t_{31}=0.96, P=0.35; metamorph weight: t_{30}=0.96, P=0.35; time to emergence: t_{30}=1.30, P=0.20). For Western toads and Cascades frogs, the main effect of drying manifested during metamorphosis; both species showed no main effect of drying on larval body weight (Western toads: t_{31}=-0.17, P=0.86; Cascades frog: t_{31}=0.52, P=0.61) or early larval development rates (Western toads: t_{31}=1.44, P=0.16; Cascades frog: t_{31}=-0.25, P=0.81). Western toad metamorphs weighed 18% less (t_{29}=2.64, P=0.01) in drying compared to permanent hydroperiod treatments (Fig. 3.2d). For Cascades frogs, drying accelerated average time to emergence by 2.37 days (t_{30}=2.43, P=0.02; Fig. 3.2c), but metamorphs weighed 16% less (t_{29}=1.98, P=0.05; Fig. 3.2d).

Effect of competition

Western toads showed no main effect of interspecific competition on body weight through development (competition with Cascades frogs: t_{31}=-1.92, P=0.06;

with Pacific chorus frogs: t_{30}=-1.26, P=0.22; with both: t_{30}=-1.33, P=0.19) and post-emergence (competition with Cascades frogs: t_{29}=-0.54, P=0.59; with Pacific chorus frogs: t_{29}=-0.14, P=0.89; with both: t_{29}=1.49, P=0.15). However, interspecific competition resulted in persistent developmental delays. Western toad competition with Cascades frogs resulted in a delay in early larval development by 0.67 Gosner stages (t_{31}=-2.23, P=0.03; Fig. 3.2a) and a delay of 7.71 days in the time to emergence (t_{30}=3.66, P=0.001; Fig. 3.2c) relative to no interspecific competition. Three-species competition with Cascades frogs and Pacific chorus frogs also resulted in a delay in Western toad early larval development by 0.73 Gosner stages (t_{31}=-2.41, P=0.02; Fig. 3.2a) and a delay of 6.21 days in the time to emergence (t_{30}=2.95, P=0.006; Fig. 3.2c). Pacific chorus frogs showed an opposite pattern in their response as they were the only species to exhibit reductions in body size through ontogeny from competition. When directly competing with Cascades frogs, larvae weighed 17% less by day 30 (t_{31}=-2.99, P=0.005; Fig. 3.2b) and emerged with 15% reduction in metamorph body weight (t_{30}=-2.31, P=0.03; Fig. 3.2d). Pacific chorus frog metamorphs also weighed 17% less in the three-species competition treatment with Cascades frogs and Western toads (t_{30}=-2.77, P=0.01; Fig. 3.2d). Cascades tadpoles were significantly heavier by day 30 in all interspecific competition treatments, including 17%, 20%, and 27% increases when competing with Pacific chorus frogs (t_{31}=2.17, P=0.04), Western toads (t_{31}=2.49, P=0.02), and both species (t_{31}=3.38, P=0.002), respectively (Fig. 3.2b). Additionally, competition with Western toads significantly accelerated time to emergence by 4.37 days (t_{30}=-3.12, P=0.004; Fig. 3.2c).

Interactive effect of drying and competition

The effect of each experimental treatment was compared to the control treatment (a permanent hydroperiod with no interspecific competition). Western toads exhibited a synergistically-negative response to drying and three-species competition with persistent developmental delays; larvae were 1.30 Gosner stages behind in development by day 30 (t_{28}=-2.65, P=0.013; Fig. 3.3a) and time to emergence was delayed by 9.73 days (t_{27}=3.27, P=0.003; Fig. 3.3c). As an example of non-additivity in responses, if this delay in time to emergence had been additive for Western toads, they would have delayed emergence by approximately 3.76 days (drying + three-species competition = -2.45 + 6.21 days). Therefore, the interactive effect of drying and three-species competition on Western toad time to emergence was synergistic as the response was more than additive. Western toads also showed a synergistically-negative response to the interactive effect of drying and competition with Cascades frogs, which resulted in a delay in time to emergence by 7.81 (t_{27}=2.62, P=0.01; Fig. 3.3c). There were no consequences of these interactive effects on body weight through ontogeny for Western toads. The interactive effect of three-species competition and drying on Pacific chorus frog body weight was synergistically-negative throughout development. Pacific chorus frog larvae weighed 16% less (t_{28}=-1.96, P=0.05; Fig. 3.3b) and metamorphs weighed 28% less at emergence (t_{27}=-3.24, P=0.003; Fig. 3.3d) than individuals in the control treatment. Pacific chorus frog body weight was also negatively impacted by the interactive effect of drying and competition with Cascades frogs, however this affect was only synergistically-negative as larvae and remained additive as metamorphs. In this treatment, Pacific chorus frog larvae weighed 19% less by day 30 (t_{28}=-2.34, P=0.03; Fig. 3.3b) and metamorphs weighed 19% less at emergence (t_{27}=-2.08, P=0.05; Fig. 3.3d). Cascades frogs benefited from the interactive effects of drying and competition and the effects were synergistically-positive when significant. By day 30, Cascade frog larvae weighed 24% more when competing with Western toads in the drying treatment (t_{28}=2.09, P=0.05; Fig. 3.3b), but this relative weight gain did not persist through metamorphosis (t_{26}=-1.35, P=0.19;). Cascades frogs were also able to accelerate time to emergence by 4.56 days when competing with Western toads in the drying treatment (t_{27}=2.54, P=0.02; Fig. 3.3c) and by 3.9 days in the combined three-species competition and drying treatment (t_{27}=2.17, P=0.04; Fig. 3.3c).

Proportion emerging (*survivorship*)

Across all treatments, Cascades frogs exhibited a significantly lower likelihood of surviving to emergence than Western toads and a marginally significant likelihood of lower survivorship relative to Pacific chorus frogs (odds ratio: 0.57, 95% CI: 0.42-0.76; Appendix C). However, Cascades frogs exhibited no significant difference in the likelihood of surviving to emergence across their respective competition and hydroperiod treatments (Fig. 3.4c). For Western toads, the interaction between drying and competition with Cascades frogs resulted in a reduced likelihood of surviving to emergence compared to the control treatment (permanent hydroperiod with no interspecific competitors; odds ratio = 0.52, 95% CI: 0.35-1.12; Fig. 3.4a). The likelihood of Pacific chorus frogs surviving to emergence was also significantly reduced when competing with Cascades frogs (under permanent hydroperiod: odds ratio = 0.30, 95% CI: 0.12–0.74; under drying hydroperiod: odds ratio = 0.35, 95% CI: 0.14–0.87). The interaction between drying and three-species competition with Cascades frogs and Western toads also reduced their likelihood of surviving to emergence (odds ratio: 0.34, 95% CI: 0.13-0.90; Fig. 3.6b: Dry, C+W).

Discussion

We quantified the interactive effects of rapid drying, a climate change factor predicted to increase in frequency and extent in the Cascade Mountain Range, and interspecific competition on amphibian growth, development, and survival. We showed that community dynamics play a large role in structuring amphibian response to drying in this system. In line with our expectations, we found that competition was a significant driver of larval amphibian growth and development rates and that species responded individualistically to different stressor (i.e. treatment) combinations. Further, the combination of biotic and abiotic stressors resulted in primarily non-additive effects on species growth and development through ontogeny. Most often, the interactive effects were synergistically-negative, which suggests that the effects of rapid drying on amphibian species may be intensified in natural communities where antagonistic interactions impose additional energetic constraints.

It is also evident that a competitive hierarchy exists among these species as larvae and that competitive rank remains relatively unchanged, only exacerbated, when stressed with a rapidly drying hydroperiod. Thus, the impact of drying on species' performance was largely dependent upon interspecific competition dynamics; no main effect of drying manifested until emergence or post-emergence as terrestrial juveniles. For instance, Pacific chorus frogs did not plastically respond to drying alone and only showed a response when drying was combined with competition. The importance of biotic interactions in this context may help to explain why other studies of the experimental effects of drying on amphibian species have shown little or no negative impacts in the absence of other stressors (e.g. Amburgey et al. 2012, Cook et al. 2013). These results also suggest that drying alone may not be a significant stressor on larvae, but instead may result in latent effects as juveniles.

The main effect of larval competition was negative for the two weaker competitors (Pacific chorus frogs and Western toads) and positive for the competitively dominant Cascades frogs. Cascades frogs increased larval body weight across all competition treatments, whereas competition with Cascades frogs resulted in persistent developmental delays in Western toads and reduced body size through ontogeny in Pacific chorus frogs. The differences in response to competition among the three species may be reflective of inherent body size differences. A size advantage, as was seen in larval Cascades frogs, can result in asymmetric competition for limited algal resources (Wilbur 1972, Morin and Johnson 1988, Werner 1992, Richter-Boix et al. 2004). Ranids are typically the largest-bodied Anuran larvae and Bufonids are often the smallest-bodied amphibian species in lentic aquatic systems, resulting in inherent size differences regardless of treatment effects on growth (Wells 2007), as was seen in this experiment. Thus, the inherent size advantage of Cascades frogs may have contributed to its competitive advantage as larvae, particularly under stressful environmental conditions. For Western toads, the smallest of the three species, body size may be constrained to a smaller size class regardless of experimental conditions. This body size constraint has been shown in other experiments of their environmental stress response and is hypothesized to be due to energetic trade-offs, wherein energy devoted to maintaining a minimum body size in stressful conditions causes developmental delays (Thurman and Garcia 2017). Interestingly, although Cascades frogs showed the greatest resilience to both stressors as larvae, juveniles exhibited a marginally reduced size at emergence in the drying treatment and had lower survival to emergence relative to the other two species. Thus, trade-offs in growth versus survival may be one consequence of maintaining a competitive advantage in stressful environmental conditions.

The competitive dominance relationship among these species was maintained under drying conditions. We expect that this was because rapid drying did not minimize the body size disparity between Cascades frogs and the two weaker competitors, allowing them to persist as a larger, more dominant competitor in all treatments. More experimental tests are needed to determine if, and when, other conditions may cause a reversal in competitive dominance, or level the playing field so as to avoid competitive exclusion and maintain species coexistence (*sensu* mechanisms of multi-species coexistence; Chesson 1994). An important component to these tests of competition in a variable environment is population density. A species at high density is likely to dominate the system when conditions are favorable and have a strong competitive effect, whereas a species at low density in that same environment will have a weaker competitive effect (Chesson 2000). Given female Cascades frogs produce relatively fewer eggs and have relatively lower survival rates overall (as seen in this experiment), it is possible that this species exists at lower densities in shared environments with Pacific chorus frogs and Western toads, which may mitigate their competitive advantage. Additionally, species-specific responses to environmental change suggests that favorable conditions should be different for each species and allow for buffering against population decline and competitive exclusion. However, it has yet to be determined which species will find favorable environments in the face of a rapidly changing climate, or how species' interactions will evolve. Thus, testing multi-species interactions across different or fluctuating environmental conditions (i.e. climate change factors) at varying densities, may elucidate more nuanced interactions and outcomes.

The interspecific variability in response to combined abiotic and biotic stressors further supports our main hypothesis that investigating species response to environmental stressors in isolation is not indicative of their response in natural communities. While this three-species approach does not necessarily paint a complete picture of the community web for this region, it provides much needed evidence for the significance of direct and indirect interactions beyond pairwise assessments. Not only do species show non-additive responses to multiple stressors (drying and competition), but their response to drying most often depended upon the combination of species (i.e. pairwise vs. three-species) and their life stage.

While it is increasingly apparent that climate change is negatively impacting ecological communities (Walther 2010, Norberg et al. 2012), predictive models show minimal regard for the role of multi-species interactions as an additional constraint to their flexibility in response strategies (Urban et al. 2016). We incorporated naturally relevant biotic interactions into an experimental framework and determined that amphibian species exhibit qualitatively different responses to environmental stress in a community context. Justification for the inclusion of biotic interactions stems from mounting evidence that even sympatric amphibian species show markedly different responses to a suite of environmental stressors (O'Regan et al. 2014b). We emphasize that interpretations of the effects of climate change stressors on amphibian species could be misleading without this community context. Biotic interactions and more specifically, competition, are significant structuring components of larval amphibian communities and we have shown that they may additionally shape species' response to abiotic climate change factors. Thus, predictions of species response to climate change should no longer ignore the biotic multipliers (Zarnetske et al. 2012b) and much effort should be directed at quantifying the interconnectedness of species interactions and their combined resiliency at relevant spatial and temporal scales.

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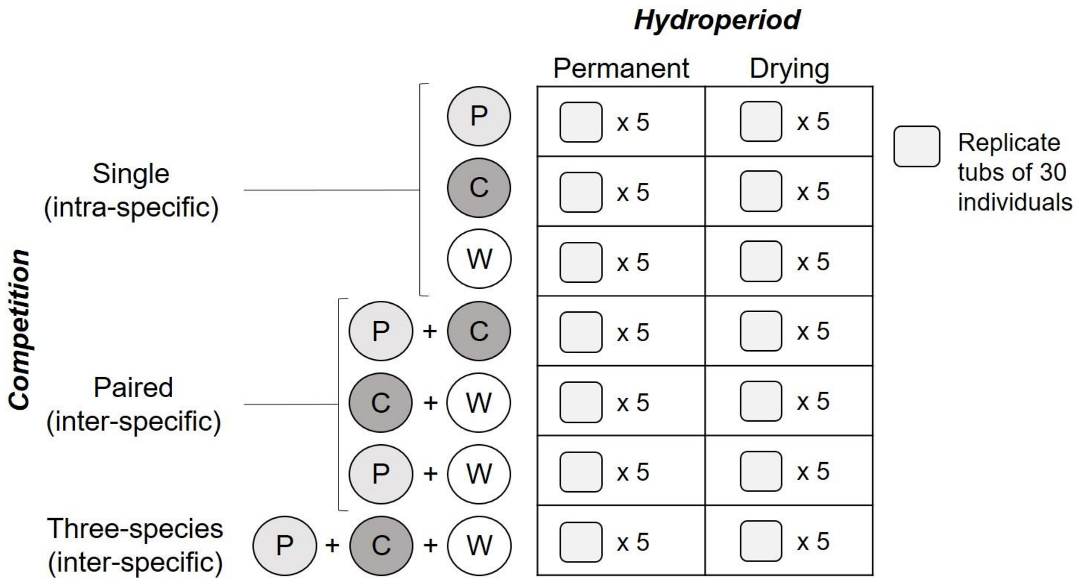

Figure 3.1. Diagram of the experimental design indicating the competition and hydroperiod treatment combinations. Each of the species is represented by the first letter of their common name (P: Pacific chorus frog; C: Cascades frog; W: Western toad). All treatment combinations were replicated in five mesocosm tubs.

Figure 3.2. Fitted values and 95% confidence intervals from linear mixed effects models of the main effects of drying and competition on (A) developmental stage by day 30, (B) weight by day 30, (C) time to emergence, and (D) metamorph weight. The response variables are centered on the control (scaled to zero; indicated by dashed, vertical line). Symbols outlined in red indicate a significantly negative response to the main effects of either competition or drying (P<0.05); symbols outlined in green indicate a significantly positive response (P<0.05).

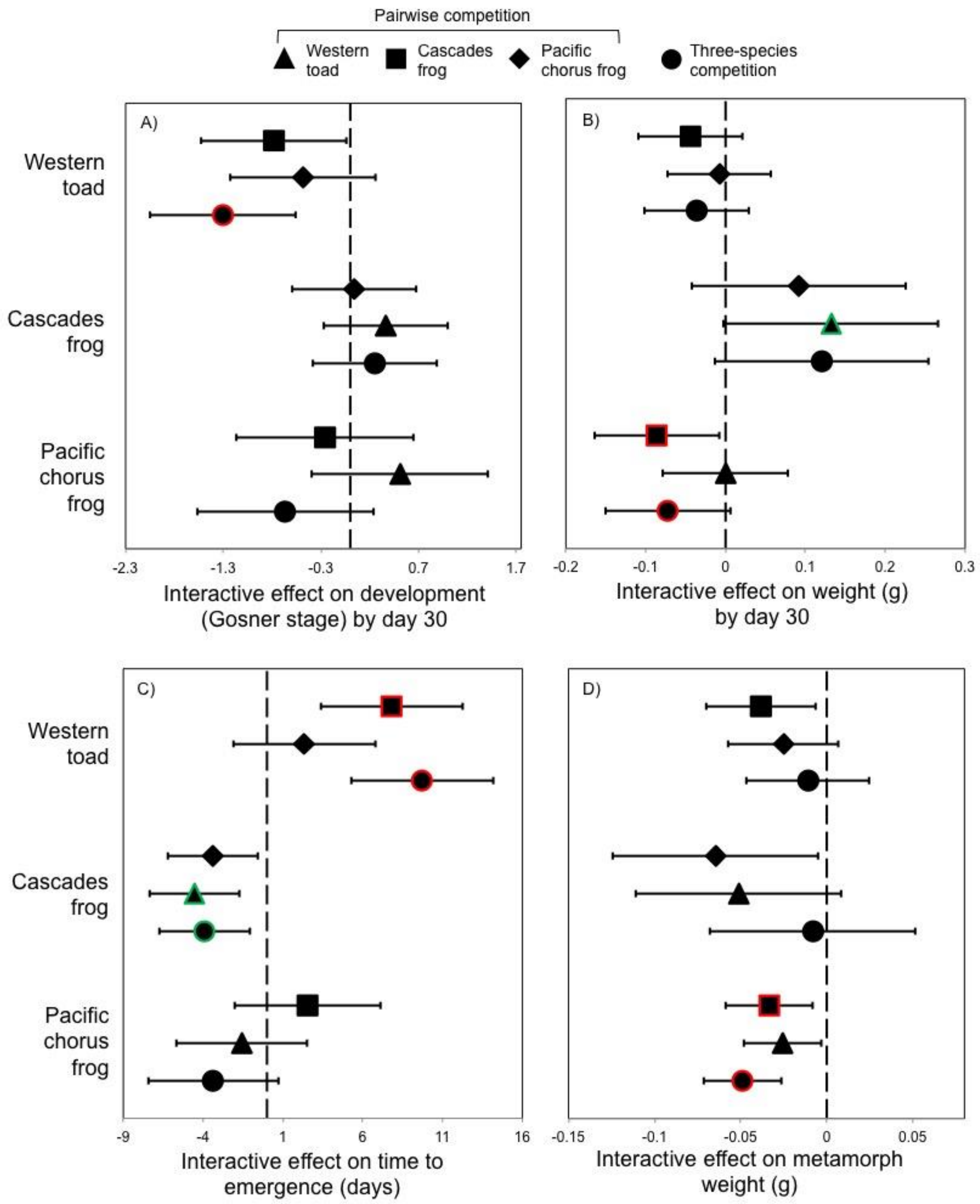

Figure 3.3. Fitted values and 95% confidence intervals from linear mixed effects models of the interactive effects of drying and competition treatment combinations on (A) developmental stage by day 30, (B) weight by day 30, (C) time to emergence, and (D) metamorph weight. The response variables are centered on the control (scaled to zero; indicated by dashed, horizontal line). Symbols outlined in red indicate a significant negative response to the interactive effects of competition and drying (*P*<0.05); symbols outlined in green indicate a significant positive response (*P*<0.05).

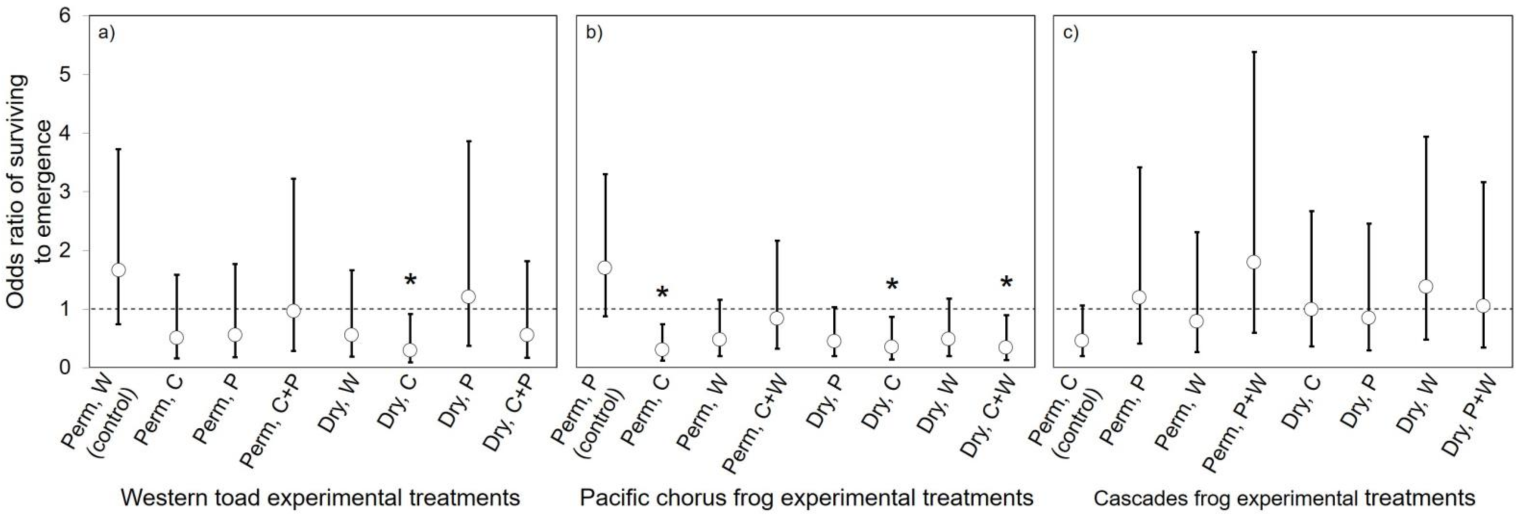

Figure 3.4. Odds ratios and 95% confidence intervals of the single and interactive effects of hydroperiod and species competition treatment combinations on the likelihood of surviving to emergence from generalized linear mixed models for (A) Western toads, (B) Pacific chorus frogs, and (C) Cascades frogs. When statistically significant, an odds ratio value > 1 indicates a higher probability of survival due to treatment effect; an odds ratio value < 1 indicates a lower probability of survival due to treatment effect. Odds-ratios were back-transformed from log scale for ease of interpretation. The hydroperiod treatment is labeled as Perm (permanent) or Dry (drying). The competition treatment is labeled with the first initial of the competing species (P: Pacific chorus frog; C: Cascades frog; W: Western toad). An asterisk indicates statistically significant difference from the control treatment in the probability of survival.

4 – TESTING THE LINK BETWEEN SPECIES INTERACTIONS AND SPECIES ASSOCIATIONS IN A TROPHIC NETWORK

Lindsey L. Thurman, Allison K. Barner, Tara Chestnut, and Tiffany S. Garcia

Abstract

Species interactions are dynamic processes that vary across environmental and ecological contexts, and operate across scale boundaries, making them difficult to quantify. Nevertheless, ecologists are increasingly interested in inferring species interactions from observational data using statistical analyses of their spatial co-occurrence patterns. To test the accuracy of various co-occurrence methods in inferring trophic interactions, we utilized two long-term datasets of pond-breeding amphibian species co-occurrences from Mt. Rainier National Park (Washington) and Mt. Hood (Oregon). We compared four statistical methods for inferring species interactions from their spatial associations, and implemented one novel approach using a time-series analysis of temporal co-occurrence. We used the best performing method, the Markov network approach, to further investigate the sensitivity of interaction inferences to spatial scale-dependencies and trophic-structuring. We found significant turnover, or dissimilarity, among the resulting spatial interaction networks; none correctly identified the known interaction network among amphibian species. We also show that negative interactions, specifically among predators and prey, are scale-dependent and that predator presence in a network can permeate indirect effects and dilute the detectability of competitive interactions among prey species. Overall, our study highlights a limiting frontier in co-occurrence theory and the disconnect between widely implemented methodologies and their ability to accurately infer interactions in trophically-structured communities.

Introduction

What drives community assembly across space and time is a central question in ecology, as species distributions and their associational patterns are shaped by a diverse set of processes (Vellend 2010). The contribution of species interactions to this search for ubiquitous patterns in species associations remains a frontier of ecological research. Species interactions in natural communities (e.g. competition, mutualism, and predation) give rise to non-random patterns and complex networks of direct and indirect effects that link species across environmental contexts, space, and time. It is widely accepted that species interactions directly underlie these non-random patterns in species spatiotemporal associations (Gotelli and Ulrich 2010). However, we have no unifying theory for how different types of interactions acting across multiple scales result in emergent community structure, from which significant "associations" are detected.

Inquiries into the connection between species co-occurrences and their underlying interactions find their origin in Diamond's (1975) general assembly rules, in which he argued for the role of interspecific competition as a primary driver of species co-occurrence patterns. Through the implementation of a null-model approach to test these assembly rules (Connor and Simberloff 1979) and the subsequent decades-long debate about these null models (Connor et al. 2013), considerable effort has been directed at inferring species pairwise interactions from their co-occurrence patterns (e.g. using combinatorics: Veech 2013; checkerboard pairs: Gotelli and Ulrich 2010; residuals: Ovaskainen et al. 2010; partial correlations: Morueta-Holme et al. 2016). In these methods, species found co-occurring in a survey more or less often than expected by chance are inferred to have a significant association, often leading to untested assumptions about species interactions. A similar inference can be made using correlations in temporal patterns in species abundances (Fig. 4.1), with roots in time series regression methods to estimate the interaction coefficients of a Lotka-Volterra competition model (see Pfister 1995 for review and evaluation). Modern implementations have crossed over from genomic applications (Faust et al. 2015). While extensions of these methods attempt to integrate potentially important environmental drivers (e.g. Araújo et al. 2011, Wisz et al. 2013, Blois et al. 2014, Tikhonov et al. 2017, Ovaskainen et al. 2017), co-occurrence analyses tend to evaluate model outcomes relative to unrealistic null expectations of spatial independence among species and untested assumptions about species interactions. As new co-occurrence methodologies are continuously being developed, there has yet to be a consensus on best available method or practices.

The conceptual and theoretical challenges of inferring biotic interactions from co-occurrence patterns stem from observational biases, context dependencies, and complex interactions in multi-species assemblages. The mechanisms underlying ecological patterns (e.g. species interactions) are often different from the scale of observation (Levin 1992). For instance, co-occurrence data provide snapshots of extant community structure, whereas non-stationarity in species occurrences and their interactions through space and time are widely documented (Poisot et al. 2015). Further, species interactions can be context-dependent, resulting in shifts in interaction dynamics across community contexts or environmental gradients (e.g. Bar-Massada and Belmaker 2016). The relationship between species associations and their interactions is also circular; interactions can drive co-occurrence patterns, but co-occurrences are also themselves the foundation for interactions. This variation in species interactions through space, time, and across contexts is compounded by the fact that most interactions are probabilistic in nature. That is, co-occurrences among species do not always result from, or lead to, interactions (Poisot et al. 2015). Further, the foundations of co-occurrence theory were derived specifically for the effects of competition on spatial patterns. Nevertheless, these methods have been applied to predator-prey communities without additional theoretical development (Bell et al. 2010, Faisal et al. 2010, Aderhold et al. 2012, Morales-Castilla et al. 2015).

Metacommunity theory (Leibold et al. 2004, Pillai et al. 2011), spatial food web theory (Massol et al. 2011), and the trophic theory of island biogeography (Gravel et al. 2011), provide insight into various processes leading to spatial associations among species in natural communities. These bodies of theory provide a solid foundation for a more robust co-occurrence theory, as species are continuously responding to a suite of processes (abiotic and biotic) acting on their distribution, abundance, and persistence. However, our ability to accurately infer the type (e.g. competition, predation, etc.) and effect (e.g. direct, indirect, or net) of interactions is highly constrained when all we have is a snapshot of species spatial associations. Despite these limitations, co-occurrence methods are increasingly being used to infer species interactions due to readily available data. This can have significant implications for conservation decision-making if these models are incorrectly estimating, for example, the role of biotic interactions in mediating species range shifts under climate change.

Significant advancements in co-occurrence theory could be made with improved integration of empirically-derived hypotheses for *indirect effects* and *trophic species interactions*. For species pairs, spatial association is likely a consequence of both the direct interactions between them, their direct interactions with other species in the community, and the indirect interactions that such direct effects produce (Barner 2016). Thus, interaction inferences resulting from co-occurrence analyses may represent the *net effects* of direct and indirect interactions, rather than direct effects *per se* (Novak et al. 2011, Barner 2016). Complex interactions across trophic levels are also a barrier to accurately inferring predator-prey interactions from their co-occurrence patterns. Methods to detect trophic interactions offer vague predictions about whether predators and prey should have positive or negative spatial associations and at what scale those patterns emerge (Schluter 1984, Bell et al. 2010, Faisal et al. 2010, Aderhold et al. 2012, Morales-Castilla et al. 2015). The prevailing hypothesis is that positive interactions result in positive associations and that negative interactions result in negative associations.

We argue that co-occurrence analyses are currently insufficient to accurately describe the complex interactions between predators and their prey and that predator-prey species pairs will exhibit a mixture of positive and negative associations. Complexity in predator-prey associations may arise due to: (1) Asymmetries in interaction outcomes for trophic interactions, wherein predators benefit from the interactions with their prey and the effect of that interaction on the prey species is costly (+/- interaction). (2) Complex indirect effects that arise in predator-prey systems through multi-species interactions, such as apparent competition and keystone predation, which complicate interpretations of pairwise associations. For example, competing prey may exhibit a positive association on the landscape because of shared predator avoidance and, therefore, be misinterpreted as having a positive interaction. (3) The game-like interactions among predators and their prey can result in dynamic co-occurrence patterns as they strategically distribute themselves across space to optimize foraging and survival (i.e. the “space race”; Sih 1984, Hammond et al. 2012). Generally, predators prefer locations with high prey densities, whereas prey prefer areas with lower predation risk. Depending on the outcome of this space race, their joint space use can indicate either a positive association (prey tracking), or a negative association (predator avoidance).

Improvements in co-occurrence theory would also benefit from tests of *spatial and temporal scale-dependencies* in species associations. At fine spatial scales, networks ideally describe the interactions among co-occurring species in a habitat, but it is not necessarily obvious what the relevant spatial boundaries are for most systems (i.e. the scale at which species interact). Further, spatial analyses of species co-occurrence networks largely assume that species’ patterns are static through time. However, changes in network structure and dynamics due to biotic interactions, stochastic processes, shifts in local abundances, or higher-order environmental effects, can cause temporal variability in co-occurrence patterns (Poisot et al. 2015). Therefore, species spatial co-occurrence patterns are also reflective of their covariances in time.

In this study, we examine the link between empirically-determined species interactions and their inferred interactions (as “associations”) from co-occurrence analyses in a multi-trophic amphibian community. Species co-occurrence data was obtained from two long-term amphibian monitoring datasets in Oregon and Washington. We first tested for potential methodological biases by implementing four statistical methods for inferring species interactions from spatial associations. We also implemented a temporal co-occurrence method known as a Boolean Dynamic Model, commonly used in molecular and cellular biology (Stein et al. 2013, Steinway et al. 2015), as a method to estimate species associations from time series data and identify the potential biases in temporally-blind models. We then incrementally expanded the spatial grain of observation of species co-occurrences from individual sites to local site networks to help elucidate the more nuanced spatial dynamics in species associations, particularly among predators and prey. Finally, we evaluated community context-dependencies via a test of the “dilution effect” (Diamond and Gilpin 1982) to evaluate the potential role of predator presence in diluting the effect of competition among prey species. Analyzing the entire species pool, rather than the guild of competitors, is hypothesized to bury the effect of competition on species associations. Evidence for a dilution effect would be a plausible explanation for why co-occurrence networks may reflect net effects rather than direct interactions *per se* in trophically-structured communities.

Amphibian species association networks were compared to empirically-determined interactions in two regions within the Pacific Northwest: Mt. Rainier National Park (Washington) and Mt. Hood (Oregon). Species interaction data was synthesized from the literature through a review of experiments and field observations examining predation (including intraguild predation) and interspecific competition (Fig. 4.2; Appendix D). Among the four spatial co-occurrence methods and one temporal co-occurrence method we test, we expect they should detect similar patterns in species associations, given the same underlying data. Under the assumption that associations should reflect the interactions among species, we hypothesize:

i. Accounting for the effect of various environmental covariates in statistical models will decrease the number of predicted associations and resulting associations will be more similar to known interactions (Blois et al. 2014).
ii. Non-random associations among amphibian species will be dependent upon scale; at finer spatial grains of observation, inferred species interactions will be more negative (Araújo and Rozenfeld 2014, Belmaker et al. 2015) and exhibit increasingly positive interactions when local, small-scale pond networks are more connected.
iii. Inferred interactions will also be community-context dependent; competitive species will have non-random, negative associations when predators are removed from the dataset (Diamond 1975). As predators are reintroduced, inferred interactions will become increasingly positive (i.e. diluted).

Methods

Study system

Pond-breeding amphibians at mid to high elevations in the Cascade Mountain Range of the U.S. Pacific Northwest exhibit within-taxa trophic structuring. At these altitudes, interactions among amphibian species occur during the summer breeding season in aquatic habitats. In ephemeral wetlands, larval salamanders and newts (Order Caudata) often comprise the highest trophic level and prey upon larval frogs and toads (Order Anura), which themselves are competitors for algal food resources and refugia. Between Oregon and Washington, 9 species of pond-breeding amphibians were detected in both the Mt. Rainier (hereafter “MORA”) and Mt. Hood (hereafter “HOOD”) co-occurrence datasets (Table 4.1).

Species interaction data

We conducted a literature review in Web of Science to gather manipulative experimental studies and field-based observations (i.e. mensurative experiments) of interspecific interactions between the nine pond-breeding amphibian species included in this study (Fig. 4.2; Appendix D). Since interaction strengths were not quantified across all of the amphibian experiments, we binarized pairwise interactions in a directed graph with negative effects (-1) for prey and competing species, or positive effects (+1) for a predator species. We included studies on cannibalism for the purposes of the review, but removed cannibalistic interactions from the analyses. We also removed interactions for the two species unique to HOOD (*Lithobates catesbeianus* and *Rana pretiosa*) from the known interaction network used in the spatial statistical methods comparison, since all other methods were constructed from the MORA dataset.

Co-occurrence data acquisition

Data on MORA amphibian co-occurrences were obtained from the National Park Service for the years 1984-2015. Data on HOOD amphibian co-occurrences were obtained from surveys conducted by Char Corkran of the Northwest Ecological Research Institute for the years 1986-2014. Prior to analyses, datasets were cleaned to remove observations with unknown species (e.g. identified only to *Genus*), or missing locale information. We limited our analyses to pond-breeding amphibian species (Table 4.1) and removed all data on terrestrial and stream-dwelling amphibian species. For the purposes of this study, "ponds" refer to all lentic waterbodies used for breeding by these species. The HOOD dataset contained two unique species, the invasive American bullfrog (*Lithobates catesbeianus*) and the Oregon spotted frog (*Rana pretiosa*).

To visualize species co-occurrences in the two regions, we calculated the joint proportions of co-occurrence (p_j) between each species pair, or the proportion of shared sites occupied out of the total possible sites occupied for each species (Eq.1; Fig. 4.3).

$$p_J = n_S/((n_A - n_S) + (n_B - n_S) + n_S) \qquad \text{[Eq. 1]}$$

Where n_S is the shared number of sites in which both species co-occur, n_A is the number of sites in which species A occurs in the absence of species B, and n_B is the number of sites in which species B occurs in the absence of species A.

Spatial data preparation in GIS

The HOOD dataset contained consistent, unique site identifiers for attributing species observations to sites. To attribute species observations at MORA to sites, we drew polygons around observations by site at the finest scale (i.e. individual ponds), which were indicated by some combination of site name and description, GPS location, survey/habitat type, and species observed. When species observations could not be accurately attributed to a particular site, they were removed. Each polygon was given a unique site identification code. We calculated the polygon centroids and performed a spatial join so that each observation within a polygon (site) could be linked in space and time for analyses of species associations. This process of data cleaning and spatial joining resulted in 4,738 species observations (reduced from 12,935) across 235 sites in MORA. The HOOD dataset originally contained observations in the area surrounding Mt. Hood, as well as other sites distributed throughout Oregon. We clipped the observations to just those near Mt. Hood, which resulted in 1,611 pond-breeding amphibian species observations across 117 sites. All GIS analyses were conducted in QGIS (version 2.14.3-Essen).

Co-occurrence analyses: methods overview

We implemented five statistical methods for analyzing species co-occurrences: odds ratio (Lane et al. 2014), Markov network (Harris 2016), partial correlations (Harris 2016), residuals from a Bayesian Joint Species Distribution Model ("Bayesian JSDM"; Golding et al. 2015), and a Boolean dynamic network model (Steinway et al. 2015). The first four methods are representative of two of three categories of spatial co-occurrence analyses, as described in Barner (2016), which include constraint-based methods, correlation methods, and partial/residual correlation and covariance methods. We did not include a correlation method as this method performs poorly (Weiss et al. 2016). For these four spatial co-occurrence methods, each sample (observation for a particular site and year) was considered independent and the potential effect of time on species co-occurrences was ignored and considered to be a replicate observation. The fifth approach is a novel implementation of a Boolean dynamic network model that generates an interaction network and dynamic model based on time series data (Steinway et al. 2015). We applied each of the four spatial co-occurrence methods to the amphibian community data from MORA described previously. The time series method was applied to the amphibian community data from HOOD, as it had more continuous, long-term occurrence data.

Two of the spatial co-occurrence methods, odds ratio and Bayesian JSDM residuals methods, allowed for the inclusion of environmental covariates. We focused on climatic conditions to account for potential abiotic influences on species associations and obtained spatial climate data from the PRISM Climate Group (http://prism.oregonstate.edu) for the breeding season (May-September). Temperature and precipitation patterns are considered significant climatic drivers of amphibian occupancy, as they are determinants of the hydroperiod regimes of wetlands, which can affect the vital rates, abundance, and distribution of wetland-dependent species (Ray et al. 2016). Variables included: mean seasonal temperature, seasonal maximum temperature, seasonal minimum temperature, and total seasonal precipitation for each site, across all years of the study.

Co-occurrence analyses: implementation

We followed the recommended implementation of each method and used the default parameter values given in the R package for each method unless otherwise stated (R Core Team 2016). The *odds ratio* method of Lane et al (2014) is representative of the more traditional, constraint-based methods typified by null-modeling approaches (e.g. Gotelli 2000). This is the only spatial co-occurrence method we implemented that claims to determine the direction of species associations (i.e. which species tend to predict the presence of another). This method quantifies the sign and strength of associations between species in terms of two odds ratios: the odds of the first species being present when the second one is also present, divided by the odds of the first species occurring regardless of the second (and vice versa). As recommended in Lane et al (2014), we removed rare species occurring in less than 10% of samples, which included *Anaxyrus boreas* and *Rana aurora.* No species met the removal criteria for “over-abundance” (those occurring in more than 95% of samples). The authors state that this method is a hypothesis-generation tool that “enables assessments of mechanisms giving rise to observed patterns of co-occurrence”. However, positive and negative associations are often described interchangeably as positive and negative interactions, respectively. This method was implemented in R using the ‘sppairs’ package (Westgate and Lane 2015), with and without environmental correction. Environmental correction was incorporated as a categorical random effect in the model. To generate habitat categories for this random effect, we ran a hierarchical cluster analysis on the climate data, converted the clusters to factors and reduced the number of clusters until no singletons remained.

Harris (2016) provides a method for disentangling marginal (net) and conditional (direct) associations through the application of *Markov networks* (undirected graphical models) from statistical physics. The Markov model predicts pairwise associations by conditioning the relationship between species pairs based on their global associations with all other species in the network. Harris (2016) describes the β estimates derived from the Markov probability model as estimates of direct interactions. This method was implemented in R using the ‘rosalia’ package (Harris 2015).

Another method that may be used to account for indirect effects is the *partial correlations* method (Harris 2016), which summarizes species’ conditional relationships. In our implementation, we utilized the off-diagonal elements of the inverse correlation matrix, which in graphical Gaussian models, represent the direct associations between two species, conditioned on all remaining species. This method was implemented in R using the ‘corpcor’ package (Schäfer and Strimmer 2005).

JSDMs are emerging as a new method for inferring the effect of species interactions on multi-species distributions based on their spatial co-occurrences. Partial covariances, calculated from the *residuals of Bayesian JSDMs*, are used to estimate the degree of association among species pairs. We used the ‘BayesComm’ package (Golding and Harris 2015) in R to implement the JSDM of Golding et al (2015), a non-hierarchical version of the Bayesian multivariate probit regression model in Pollock et al. (2014) and conceptually similar to the model of Ovaskainen et al. (2010). This method was implemented with and without environmental correction. To incorporate environmental correction, we ran forward stepwise selection across all climate covariates for each species. From this, a list of covariates to assign to each species was included as a conditioning variable in the model.

Co-occurrence analyses: spatial methods comparison

We first assessed the relative complexity of the networks, including the species interaction network constructed from experimental and observational field data reviewed in Appendix D. Network complexity was measured via connectance (C), the fraction of all possible links between species that are realized in a network. Connectance is calculated from the number of significant nodes in each network (S) and the number of significant links inferred (L). For undirected networks, connectance is calculated as $C = L/(S \times (S - 1))$; for directed networks, connectance is calculated as $C = L/S^2$.

To assess potential methodological differences in network structure and estimated interactions, we applied the concept of network turnover (Poisot et al. 2012, Barner 2016). Network turnover, or the dissimilarity of networks, is comprised of multiple components that account for both species and interaction turnover. These components include: the dissimilarity in species composition communities (β_S), or in this case, the dissimilarity in species determined to be significantly associated; the dissimilarity of interactions (i.e. links) established between species present in both networks being compared (β_{OS}); the dissimilarity in interaction structure introduced by dissimilarity in species composition (β_{ST}); and the dissimilarity of interactions overall (β_{WN}). By definition, β_{WN} is the additive combination of β_{OS} and β_{ST}. Network turnover ranges from 0 (complete similarity) to 1 (complete dissimilarity).

Network statistics were calculated using the ‘betalink’ (Poisot et al 2012) and ‘NetIndices’ (Kones et al. 2009, Soetaert et al. 2014) packages.

Spatial-scale dependencies

Even pond-breeding amphibians, which co-occur within relatively delineated habitats for significant portions of their life cycle, are an example of how species can interact across a gradient of spatial (and temporal) scales. These scale-dependencies can arise due to the interconnectedness of pond networks, wherein local communities are linked by the movement and dispersal of individuals between nearby ponds, resulting in joint local and regional dynamics (*sensu* metacommunity dynamics; Urban 2004). To test the influence of increasing the spatial grain of observation of species co-occurrences on resulting spatial association networks, we incrementally linked sites by nearest neighbor distance thresholds ranging from 250m, 500m, 1km, to 2km. The distance thresholds for linking sites into local site-networks were based on meager empirical data of dispersal distances for amphibian species. Generally, pond-breeding amphibians are considered highly philopatric and are typically found within 500m of their natal sites, with few individuals documented dispersing up to 5km (Wells 2007). Dispersal distances are also likely taxon-specific considering the diversity of life histories and morphologies among pond-breeding amphibian species. Thus, the shortest distance thresholds (up to 500m) represent local pond networks that arguably exchange the majority of species, even those with limited dispersal abilities. To link the sites in QGIS, we created fixed width buffers of half the required distance between pond breeding sites, ranging from 125m to 1km buffers. Pond breeding sites connected by these distance thresholds were joined and considered one sampling unit in co-occurrence analyses (Fig. 4.4). We used the Markov network approach because it is the only spatial co-occurrence method designed to detect direct species interactions, as opposed to net interactions. We evaluated changes in the sign of species interactions across spatial scales.

Test of dilution effect

To determine if co-occurrence with Caudate predators dilutes the inference of competitive interactions among Anurans in the amphibian network, we assembled occurrence data starting with the Anuran guild (four species) and adding the occurrence data of each of three Caudate predators to the community matrix incrementally from single to pairwise combinations to the full assemblage. If removing a predator species observation, or some combination of predator species, resulted in samples (unique site/year combinations) with no amphibian species present, then the samples were removed from analyses. Through this pseudo-assembly process, we estimated network turnover (β_{WN}; as described previously) across community contexts and evaluated shifts in the sign and strength of interactions between Anurans across community contexts. We also compared the interaction inferences from the Anuran guild to those from the known interaction network. For this test, we again estimated interactions using the Markov network method.

Temporal co-occurrence

Modern temporal co-occurrence analysis is a rapidly emerging field, with few methods having openly available software. We implemented one of the few available methods, based on a probabilistic Boolean model of the community (Campbell et al. 2011, Steinway et al. 2015), rather than on the more common ordinary differential equation approach (e.g., the approaches in Pfister 1995). In the Boolean framework, each species (as a “node”) is either present (“ON”) or absent (“OFF”). Whether a target species is present or absent can depend on the inhibitory (negative) or facilitative (positive) effects of other species (nodes) in the system. The effect of those species on the target species occurrence (called “rules” in this framework) is inferred using an algorithm that finds the set of interactions that explains the variation in the target species time-series of occurrence with the least amount of error (Steinway et al. 2015). We used the ‘BoolNet’ R package (Mussel et al. 2010) to implement this Boolean framework, which returns a set of interaction rules with an associated probability. We analyzed only the time series of HOOD sites that were annually sampled for more than 15 years (n = 8 sites; Fig. 4.5).

Results

Spatial methods comparison

Network structure, and the interactions estimated therein, differed substantially across each of the spatial co-occurrence methods relative to the network of known species interactions (β_{WN} ranged from 0.167 to 0.667; Fig. 4.6). The Markov network and partial correlation network were equivalent in structure (i.e.

complexity measured via connectance; Table 4.2) and they performed best at predicting the known direct interactions among amphibian species (similar results found in Harris 2016; Table 4.3). Among the spatial co-occurrence methods, there was 21% dissimilarity in species predicted to have significant associations across methods (mean β_S = 0.209, 95% CI: 0.140, 0.278). Mean interaction turnover (β_{WN}) was 0.535 (95% CI: 0.387, 0.683), meaning there was approximately 53.5% dissimilarity in estimated interactions. This interaction turnover is the additive effect of 24.9% dissimilarity in estimated interactions between shared species pairs across methods (mean β_{OS} = 0.249; 95% CI: 0.180, 0.318) and 28.6% dissimilarity in interaction structure due to turnover in species predicted to have significant associations (mean β_{ST} = 0.286; 95% CI: 0.202, 0.370).

Out of 21 possible undirected species interactions in a metaweb of seven amphibian species, only four species pairs were consistently identified across all six spatial co-occurrence methods, regardless of the type of interaction inferred. Of these four consistently identified pairs, only two pairs were estimated to have the same sign of interaction (positive or negative) across all six methods. This means the probability of these six methods inferring the same species pairs with the same interaction type was only 9.5%. The first of the two species pairs that were consistently identified with the same sign of interaction across all spatial co-occurrence methods was *Taricha granulosa* and *Pseudacris regilla*, a predator and prey predicted to have a consistently positive interaction. The second species pair was *Taricha granulosa* and *Ambystoma gracile,* competing Caudate species that exhibit intraguild predation, and they were also consistently identified as having a positive interaction. Overall, the number of significantly associated species pairs and the sign of their interactions were highly sensitive to co-occurrence method.

Spatial-scale dependencies

To test the effect of increasing the spatial grain of observation of species co-occurrences, we implemented the Markov network approach using the MORA dataset to evaluate scale-dependencies in shared interactions between species pairs and their inferred interaction type. We connected local pond sites by incrementally increasing the distance thresholds for linkage (from 0 to 2 km) to determine if changes in small-scale connectivity would confer changes in estimated interactions, particularly for predator-prey pairs that may shift across the landscape in a "space race" for survival (*sensu* Sih 1984; Hammond et al. 2012). Of the 21 possible undirected species interactions, 12 species pairs were consistently identified as having the same interaction type across all spatial grains of observation. These species pairs and their interactions were, therefore, spatial scale-independent at this extent. Of these 12 significantly interacting pairs, nine pairs were identified as having consistently positive interactions and three pairs were identified as having consistently negative interactions. However, for this test we were more interested in the inconsistent interactions. For the nine species pairs whose interactions shifted across scales, six pairs shifted from negative interactions at 0 km connectivity to positive at a maximum connectivity distance of 2 km. Of the six species pairs that transition from negative to positive interactions as connectivity increases, five were interactions between predator and prey (including intraguild predation between *Ambystoma* *macrodactylum – Taricha granulosa*). Therefore, as the spatial grain of observation of species co-occurrences increases (i.e. increased local-site connectivity), the number of positive interactions is estimated to increase, particularly for predator-prey species pairs (Fig. 4.7).

Dilution effect

A pseudo-community assembly process using the Markov network method and the MORA dataset uncovered a dilution effect of predators on the interpretation of competition among prey species (*sensu* Diamond & Gilpin 1982). The Markov network of four Anuran species most closely resembled their known set of competitive interactions, taken from empirical data, when predators were excluded entirely from the dataset (β_{OS} = 0.200; Fig. 4.8). Four out of six possible undirected interactions among the Anurans species were identified as negative in the absence of predators. The two species pairs identified as having positive interactions included *Anaxyrus boreas – Pseudacris regilla* and *Pseudacris regilla – Rana aurora* and these interactions remained positive, and increased in strength, across community contexts. As predators were introduced singly, pairwise, and in the full assemblage, the negative interactions for two of the four remaining pairs shifted to positive interactions. These two pairs of competing Anurans included *Anaxyrus boreas – Rana aurora* and *Anaxyrus boreas –Rana cascadae.* For the two species pairs that remained negative, the strength of the negative interaction was diluted with the introduction of predators. For example, the estimated interaction strength between *Rana cascadae –*

Pseudacris regilla went from -2.62 in the absence of predators to -0.51 in the full assemblage.

Temporal co-occurrence

An evaluation of the time-series of co-occurrence data from HOOD revealed that most species exhibit patchy presence/absence through time, with only a few species present at a given site during all years sampled (Fig. 4.5). To determine if fluctuations in species co-occurrence patterns through time can reveal more detail about their interspecific interactions, we analyzed the HOOD time-series data from eight sites using a probabilistic Boolean network model. In this model, interspecific effects can be described as a species either having inhibitory (negative) effects, or facilitative (positive) effects on the presence of another species through time. Half of the sites (n=4) used in the time-series analysis of co-occurrence predicted species to have no interspecific effects on their presence. Appendix E provides a summary of the output of the Boolean network analysis for all eight sites (Table E1), including alternative transition functions (i.e. interaction rules) for each species and their probabilities and error rates. Many of the species showed self-regulatory behavior, meaning only intraspecific effects were predicted. Some species were even “knocked out”, with transition functions equal to zero, meaning their occurrence patterns were not estimated to be affected by interactions. The four sites with evidence of interspecific effects had divergent interaction pathways (Fig. 4.9), with no consistent positive or negative regulatory behavior between species pairs across all sites (Table E1). Very few transition functions predicted pairwise, direct effects between species, rather they indicate more nuanced, multi-species pathways. For example, *Rana aurora* at site LCM had 64 equiprobable transition functions with substantial trade-offs in inhibitory vs. facilitative effects of *Anaxryus boreas, Pseudacris regilla, Rana cascadae,* and *Taricha granulosa.* These complex pathways are also evident at site WEENS wherein the presence of *T. granulosa* can have inhibitory or facilitative effects on *Rana cascadae* depending on the effect of *A. boreas* on *T. granulosa.* Essentially, *R. cascadae* presence is predicted when the inhibitory effect of competing *A. boreas* is mitigated by the predatory *T. granulosa,* and vice versa. A testable hypothesis of this three-species interaction pathway could be that it is indicative of either apparent competition between *R. cascadae* and *A. boreas*, or long-term, prey-switching behavior by *T. granulosa*.

Discussion

The complexity of multi-trophic interactions complicates interpretations of interaction networks inferred from species associations. A test of sensitivity to methodologies for inferring species interactions from their spatial associations revealed substantial turnover (i.e. dissimilarity) in network structure and inferred interactions across different method types. Consistency among methods in inferring the same interaction between two species was only 9.5%. The best performing methods were the Markov network and partial correlation approaches, which were similar in their approximations and have been shown to perform comparably in other studies (Harris 2016). Both methods attempt to tease apart the indirect, or net effects, from direct effects, by conditioning each pairwise interaction on their global associations with all other species in the network. Accounting for this “background noise” may be the reason why these networks more closely resemble the known species interaction network. However, even these two methods cannot differentiate reciprocal (bi-directional) predator-prey interactions. Across all spatial statistical methods compared, their inability to assign two interaction estimates (i.e. asymmetric interactions) for each species pair is a primary reason for their dissimilarity to the known interaction network.

Accounting for the effect of the environment in model formulas decreased the number of significant associations in the Bayesian JSDM residuals method as predicted, but not the odds ratio method. Neither improved the accuracy of resulting associations relative to the known interaction network. In fact, accounting for the environment in the Bayesian JSDM residuals approach weakened the accuracy of inferred interactions. This could simply be due to our choice of environmental covariates; climate conditions may be less explanatory of species associations than other environmental factors. These statistical methods could be tested with additional (or alternative) environmental covariates, such as the type of lentic waterbody (e.g. emergent wetland, tarn, lake, etc.), elevation, slope/aspect, or adjacent terrestrial land cover type. Given that this study was conducted within the west-slope Cascade Mountain ecoregion, dominated by moist coniferous forest, it may be that environmental covariates are not divergent enough to explain differences in species associations at this scale. Instead, species may be responding to more fine-scaled, microclimatic or environmental conditions hidden within broader regional patterns

(Frey et al. 2016), such as wetland hydroperiod, aquatic temperatures, or within-season variability in ambient temperature and precipitation.

The estimated interactions among amphibian species were also dependent upon spatial scale. We used the best performing method, the Markov network, to determine if the spatial grain of observation of species co-occurrences affected interaction network structure. We tested the hypothesis proposed in Belmaker et al. (2015) and Araújo and Rozenfeld (2014) that spatial grain size influences the detectability of biotic interactions. More specifically, we tested if negative interactions are only discernible and important in species distribution model predictions at fine spatial grains. We linked sites into local site-networks across a gradient of connectivity distance thresholds. These site-distance thresholds fell within the expected dispersal distance of amphibian species. As hypothesized, at smaller spatial grains of observation (minimal connectivity among ponds), species were estimated to have more negative interactions. The negative interactions became increasingly positive when local pond networks became more connected (i.e. at broader resolutions). However, one question remained: *why* were negative interactions lost at increasing scales of resolution? The answer to this question may be found in the spatial dynamics of the game-like interaction, or space race, between predators and prey (Sih 1984). The predator-prey space race is the preference for predators to prefer areas with high prey density and, alternatively, for prey to seek areas with lower predation risk. However, the space race can also be scale-dependent (*sensu* the life-dinner principle; Dawkins and Krebs 1979, Hammond et al. 2012). At fine scales, if predators do not track prey effectively, the costs are minimal; a matter of a meal sooner vs. later. Whereas for prey, the interaction is a matter of life vs. death. At broader spatial scales, the costs become higher for the predator if there are too many missed foraging opportunities. Therefore, at finer spatial scales, negative associations may reflect a degree of successful predator avoidance, but at broader spatial scales predators and prey exhibit positive associations as predators must successfully track prey to avoid starvation. This tendency for interaction networks to infer increasingly positive interactions between predator and prey across broader spatial resolutions may, therefore, be capturing this space race dynamic.

We also used the Markov network to further investigate the spatial associations between trophically vs. non-trophically interacting species from the perspective of Diamond's (1975) dilution effect theory. Our results supported his hypothesis that including multiple trophic levels in co-occurrence analyses would dilute competitive effects. Anuran competitors were identified as having more non-random, negative interactions when predators were excluded from the dataset. As predators were reintroduced via a pseudo-assembly process, estimated interactions among competitors became increasingly positive. All competitor interactions tended towards positive, even those that remained negative. This assembly process provides evidence that multi-species interactions can introduce complex indirect effects and that methods for inferring species interactions from their spatial associations is currently insufficient for trophically-structured communities.

An evaluation of long-term dynamics in species co-occurrence revealed another interesting limitation to traditional methods for inferring species interactions from their spatial association patterns. In general, presence and absence of species fluctuated through time, and only a few species were ubiquitously present at a given site across the 28-year study (Fig. 4.5). Results from the probabilistic Boolean network model revealed that complex multi-species interaction pathways dominated over pairwise, direct effects when interspecific interactions were predicted in the model. Temporal analyses, such as the Boolean network model, do not appear to be limited to quantifying pairwise (direct) interactions like spatial co-occurrence methods. This approach could go a long way to improving our understanding of the link between species associations and their interactions by accounting for indirect effects through multi-species pathways. However, we were limited to using inter-annual presence/absence data, which is informative but less so than abundance data for time-series analysis of species interactions. Larval abundance data could reveal more nuanced interspecific interaction pathways via per capita effects. Overall, this time-series analysis supports our hypothesis that species' spatial associations may be reflective of the net outcome of multi-species interactions rather than direct effects *per se*. Further, spatial analyses ignore important temporal structuring of interaction dynamics that may be informative when interpreting spatial associations.

We have offered one of the first tests of the link between empirically-derived trophic interactions and their spatio-temporal associations. The well-studied network of interspecific interactions in this amphibian food web offers valuable insight into the complexities of inferring species interactions from their associations. Even by generating a known interaction network for these species, we are over-simplifying their complex dynamics. These amphibian species can have multiple interaction types for a given species pair, which may not be accurately characterized in traditional association network frameworks. For instance, when conditions allow for larval salamanders to over-winter, they grow substantially in body size and some individuals plastically alter their head morphology to cannibalize or predate upon other competing Caudates (Walls et al. 1993). This over-wintering behavior creates an additional trophic level of intraguild predators. However, intraguild predation is not unique to Caudates. For example, Western toad (*Anaxryus boreas*) larvae have been observed consuming competing Pacific chorus frog (*Pseudacris regilla*) larvae and Cascades frog (*Rana cascadae*) larvae (Jordan et al. 2004). Further, competitive interactions among larvae can be asymmetric, often depending on body size, and shift in outcome through ontogeny (see Chapter 3 and, e.g., Bar-Massada and Belmaker 2016). Thus, a single species can fill divergent ecological roles throughout their lifetime, and all life stages of a species can be present in a pond simultaneously. This asymmetry and stage-structuring of interaction dynamics are just two examples of potential higher-order effects that can further limit inferences of species interactions using available co-occurrence methods.

Conclusion

We provide substantial evidence that inferring trophic interactions, and the complex network of indirect effects they create, is the limiting frontier in modern co-occurrence theory and methodology. Each of the methods tested generated different species interaction networks, most of which were significantly dissimilar from the known interaction network for pond-breeding amphibian species of the Pacific Northwest. Further, we revealed spatial-, temporal-, and community context-dependencies in species interactions. Therefore, tacit assumptions about the link between patterns and processes, if not thoroughly examined, can cause incorrect inferences.

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Table 4.1. Species observations in the Mt. Rainier National Park (MORA) and Mt. Hood (HOOD) datasets. The frequency of occurrence refers to the raw (and proportional) occurrence of each species across sites included in this study. It therefore represents the spatial prevalence of a species over the survey period for each region.

	Species	**Species code**	**Frequency of occurrence (spatially)**	
			MORA	**HOOD**
Caudates	*Ambystoma gracile* (Northwestern salamander)	AMGR	169 (71.9% of sites)	49 (41.9% of sites)
	Ambystoma macrodactylum (Long-toed salamander)	AMMA	112 (47.7% of sites)	23 (19.7% of sites)
	Taricha granulosa (Rough-skinned newt)	TAGR	45 (19.1% of sites)	63 (53.8% of sites)
Anurans	*Anaxyrus boreas* (Western toad)	ANBO	19 (8.1% of sites)	21 (17.9% of sites)
	Lithobates catesbeianus (American bullfrog; invasive)	LICA	0	3 (2.6% of sites)
	Pseudacris regilla (Pacific chorus frog)	PSRE	33 (14.0% of sites)	55 (47.0% of sites)
	Rana aurora (Northern red-legged frog)	RAAU	13 (5.5% of sites)	25 (21.4% of sites)
	Rana cascadae (Cascades frog)	RACA	212 (90.2% of sites)	61 (52.1% of sites)
	Rana pretiosa (Oregon spotted frog)	RAPR	0	1 (0.9% of sites)

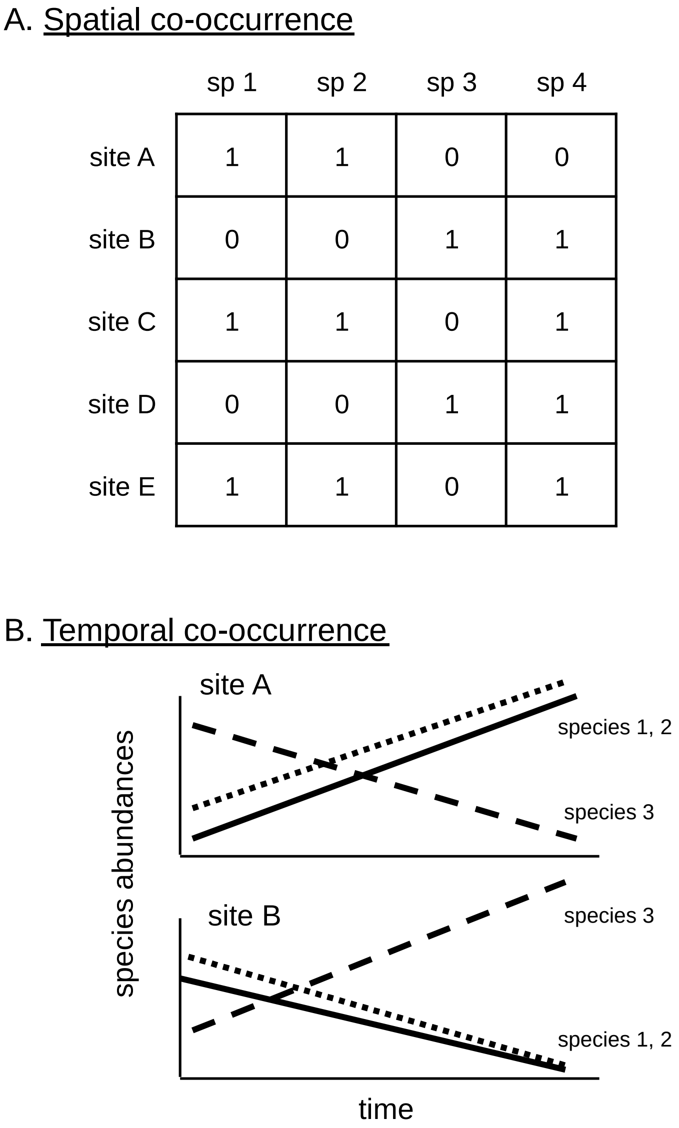
Table 4.2. Network complexity measured as connectance ($C = L/(S \times (S-1))$) for undirected graphs and ($C = L/S^2$) for directed graphs, compared across the spatial co-occurrence methods. Connectance of the amphibian species interaction network (top row), derived from experimental and observational data, has a connectance of 0.612 (as a directed graph). To compare connectance across methods, directed networks were converted to undirected networks.

Method	**Node richness (S)**	**Number of links (L)** (directed/undirected)	**Connectance** (directed/undirected)
Known interactions (from empirical data)	7	30 / 15	0.612 / 0.357
Odds ratio	5	10 / 10	0.400 / 0.500
Odds ratio (environmental correction)	5	10 / 10	0.400 / 0.500
Markov network	7	21	0.500
Bayesian JSDM residuals	6	10	0.333
Bayesian JSDM residuals (environmental correction)	5	7	0.350
Partial correlation	7	21	0.500

Table 4.3. A comparison of network turnover statistics across spatial co-occurrence methods. β_S is the dissimilarity in species composition (i.e. species with significant associations) of communities, β_{OS} is the dissimilarity of interactions between species common to both realizations, β_{ST} is the dissimilarity of interactions due to species turnover, and β_{WN} is the dissimilarity of interactions. The Markov network and partial correlation approaches performed best with respect to the network of known amphibian species interactions.

Method A	**Method B**	$\boldsymbol{\beta_S}$	$\boldsymbol{\beta_{OS}}$	$\boldsymbol{\beta_{ST}}$	$\boldsymbol{\beta_{WN}}$
Known interactions	Markov network	0.000	0.167	0.000	0.167
Known interactions	Partial correlation	0.000	0.167	0.000	0.167
Known interactions	Bayes JSDM residuals	0.077	0.300	0.140	0.440
Known interactions	Bayes JSDM residuals + environ	0.167	0.286	0.260	0.545
Known interactions	Odds ratio	0.273	0.333	0.333	0.667
Known interactions	Odds ratio + environ	0.273	0.333	0.333	0.667
Odds ratio + environ	Odds ratio	0.000	0.000	0.000	0.000
Partial correlation	Markov network	0.000	0.000	0.000	0.000
Bayes JSDM residuals + environ	Bayes JSDM residuals	0.091	0.125	0.051	0.176
Bayes JSDM residuals	Markov network	0.077	0.200	0.155	0.355
Partial correlation	Bayes JSDM residuals	0.077	0.200	0.155	0.355
Bayes JSDM residuals + environ	Markov network	0.167	0.176	0.324	0.500
Partial correlation	Bayes JSDM residuals + environ	0.167	0.176	0.324	0.500
Markov network	Odds ratio	0.273	0.333	0.417	0.750
Markov network	Odds ratio + environ	0.273	0.333	0.417	0.750
Partial correlation	Odds ratio	0.273	0.333	0.417	0.750
Partial correlation	Odds ratio + environ	0.273	0.333	0.417	0.750
Bayes JSDM residuals	Odds ratio	0.400	0.333	0.436	0.769
Bayes JSDM residuals	Odds ratio + environ	0.400	0.333	0.436	0.769
Bayes JSDM residuals + environ	Odds ratio	0.333	0.429	0.371	0.800
Bayes JSDM residuals + environ	Odds ratio + environ	0.333	0.429	0.371	0.800

Figure 4.1. A conceptual figure of (A) the spatial co-occurrences among species indicated in a site-by-species community matrix where 1 represents presence and 0 indicates absence; and (B) temporal co-occurrence patterns, determined by changes in species abundances through time theoretically due to interactions between the three species at two sites.



A. Spatial co-occurrence

	sp 1	sp 2	sp 3	sp 4
site A	1	1	0	0
site B	0	0	1	1
site C	1	1	0	1
site D	0	0	1	1
site E	1	1	0	1

B. Temporal co-occurrence

Figure 4.2. Known species interactions among pond-breeding amphibians in this study from experimental tests and field observations of predation (including intraguild predation) and interspecific competition. The combined network, including both predation and competition, was used in analyses when comparing statistical co-occurrence methods to known interactions. Methodological comparisons were conducted on the MORA dataset, so LICA and RAPR were removed from the combined network since they were unique to the HOOD dataset. All networks are directed, allowing for bi-directional interactions between species pairs. For predation, this allows for a positive effect on the predator (+1; blue arrows) and a negative effect on the prey (-1; red arrows). Competition is assumed to be negative for both species. Strength of interactions (i.e. per capita effect) is not typically quantified and was therefore not indicated in this figure or in analyses.

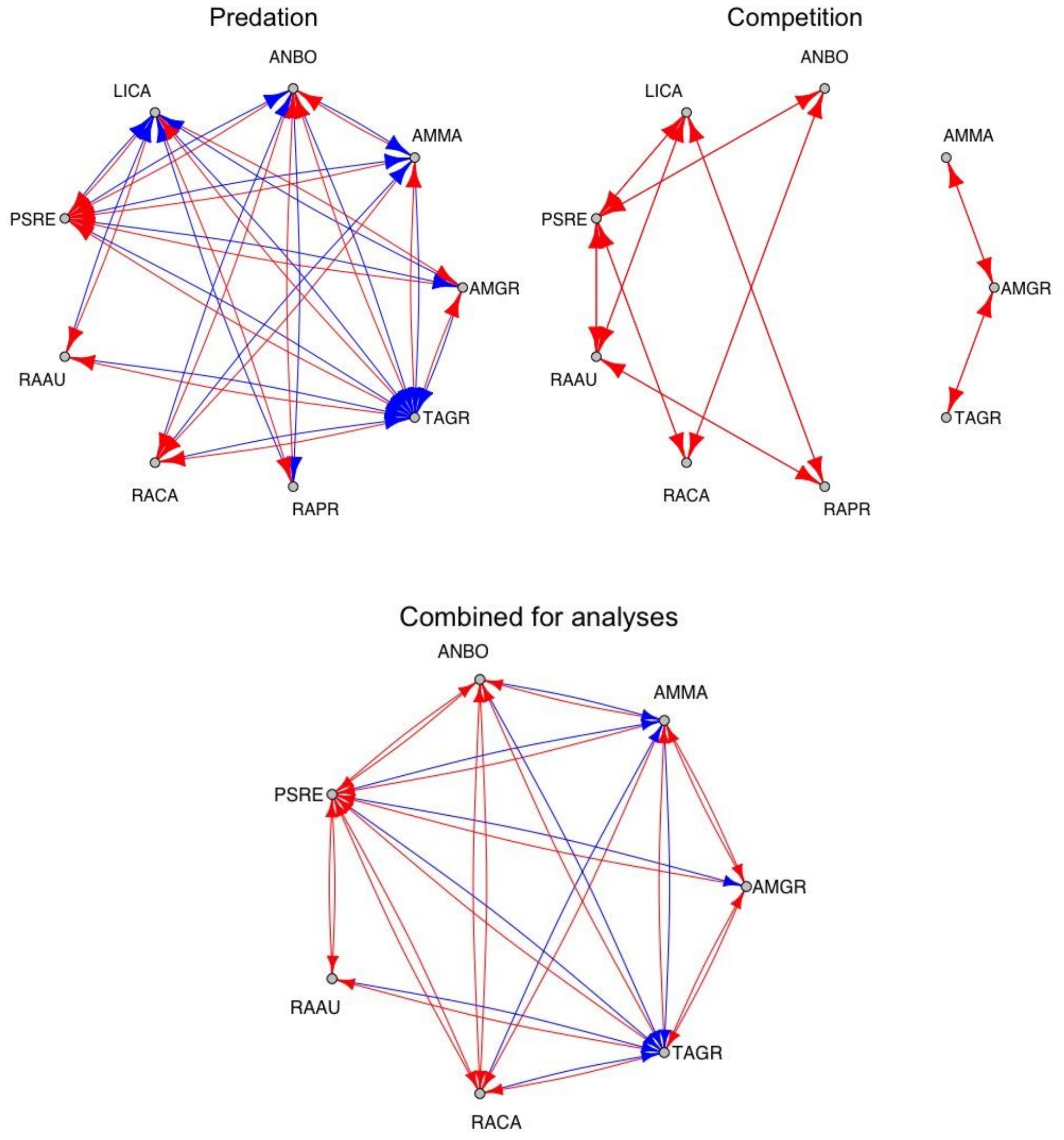


Figure 4.3. A network of the raw co-occurrences among species found in each dataset (MORA=Mt. Rainier; HOOD=Mt. Hood). HOOD contains two additional, rare species: the invasive American bullfrog (*Lithobates catesbeianus*) and Oregon spotted frog (*Rana pretiosa*). Line strength indicates the joint proportions of co-occurrence between each species pair; bolder lines indicate a higher relative frequency of co-occurrence for a given species pair. The difference in species' joint proportions of co-occurrence between the two regions suggests that in MORA there are either more single-species ponds, or many ponds dominated by one species (e.g. RACA) and other species are relatively rare. Comparatively, HOOD appears to have species rich ponds.

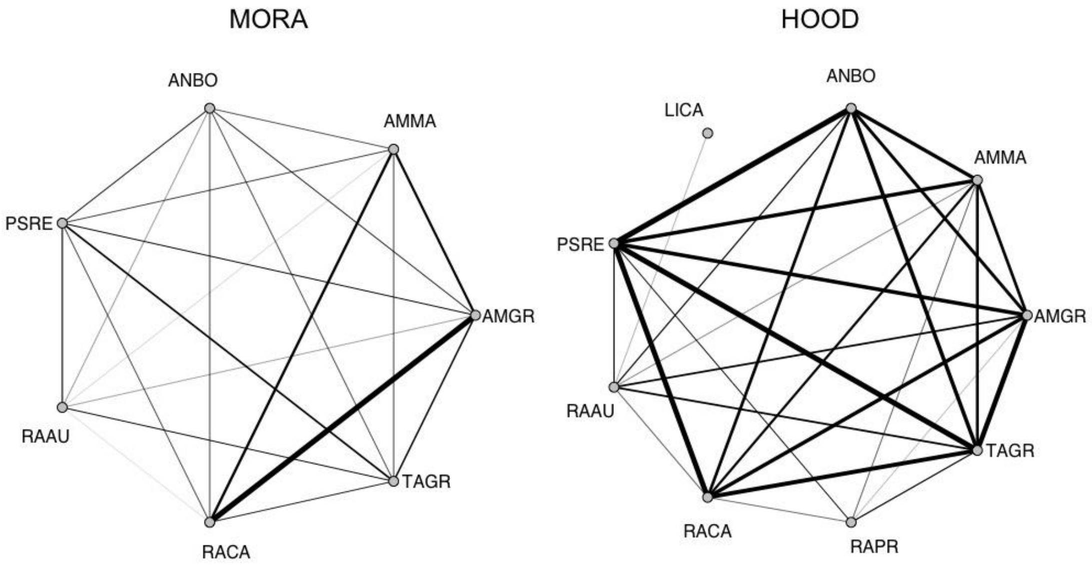
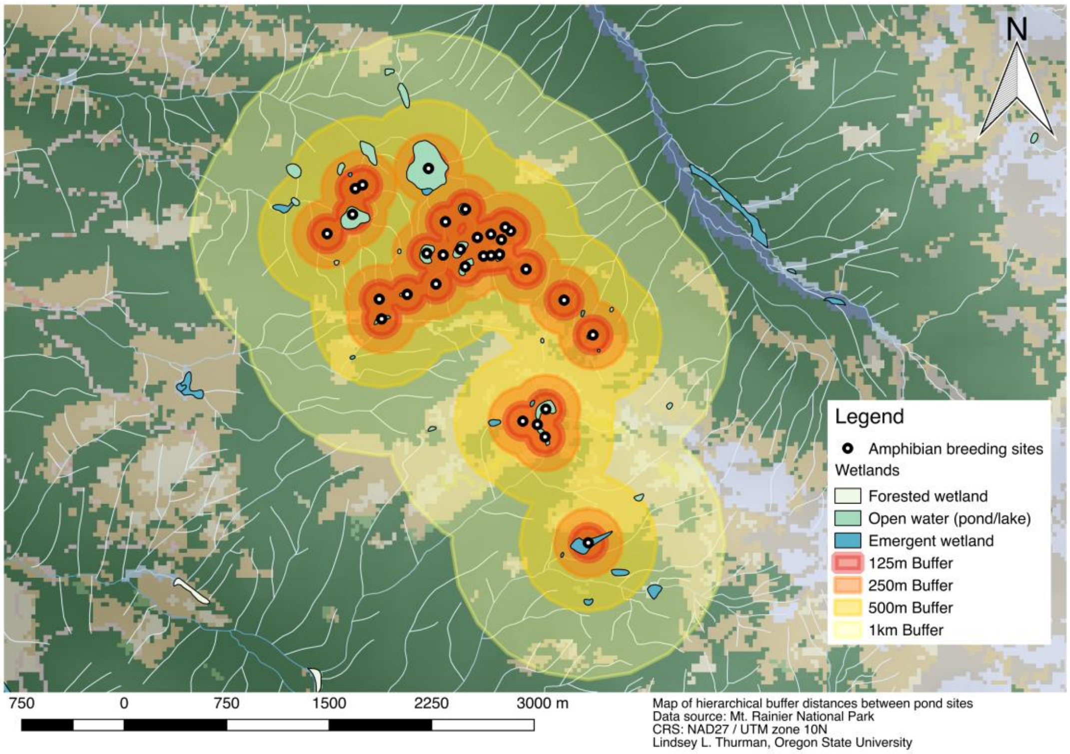


Figure 4.4. Example from MORA of fixed width buffers used to link amphibian pond-breeding sites into local networks, taken at increments from 125m to 1km (i.e. nearest neighbor distances of 250m to 2km).



Map of hierarchical buffer distances between pond sites
Data source: Mt. Rainier National Park
CRS: NAD27 / UTM zone 10N
Lindsey L. Thurman, Oregon State University

Figure 4.5. Eight sites in the Mt. Hood region (HOOD) were sampled at least 15 years throughout the 30-year dataset (1984-2014). Long-term monitoring began at these sites in 1987. Black boxes indicate species presences for a given year; white boxes indicated species absences. Columns with no outlined boxes for presence or absence reflect years where a site was not sampled.

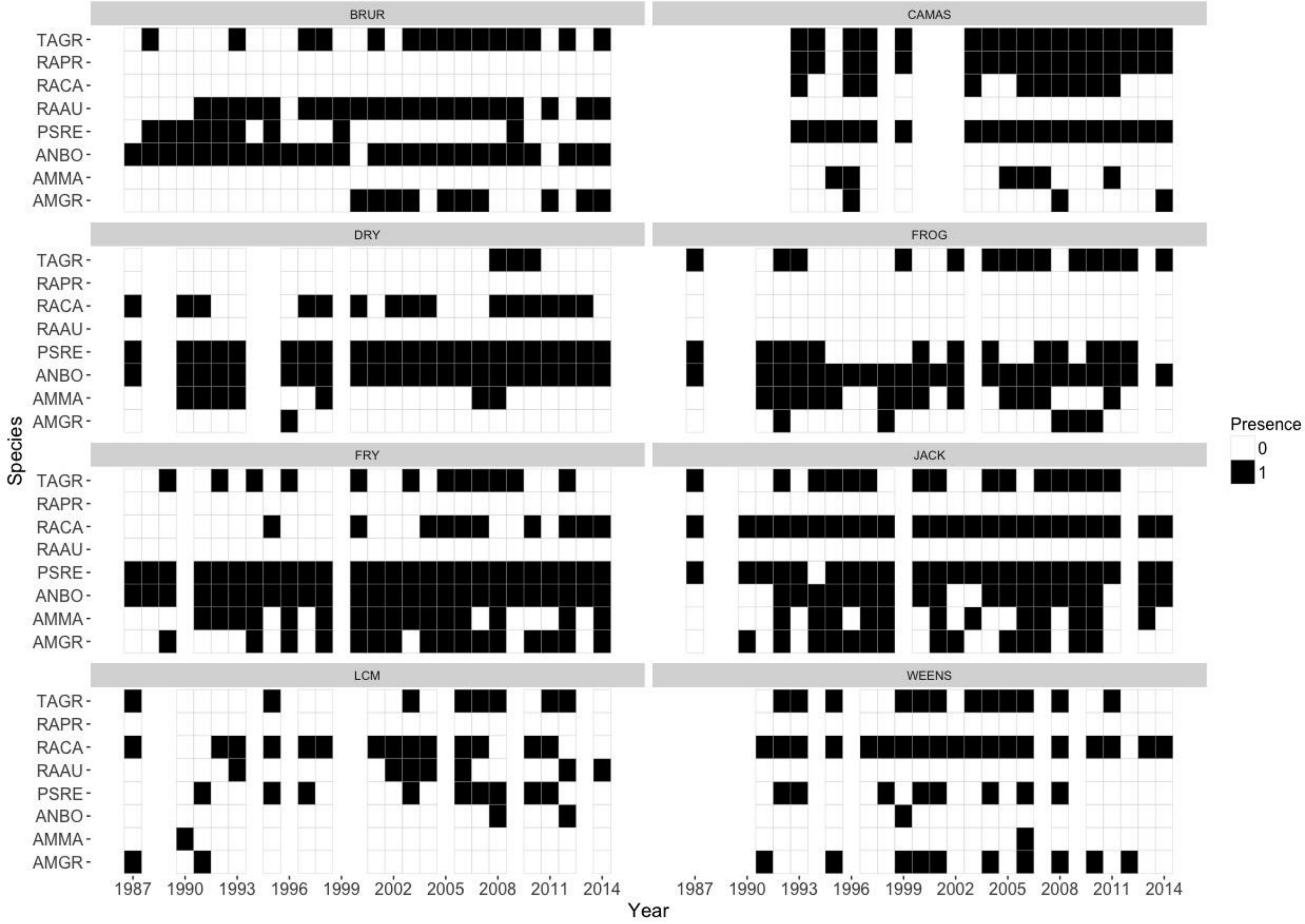


Figure 4.6. Comparing results from four statistical methods for analyzing species' spatial co-occurrences. Two of the methods, odds ratio and Bayes JSDM residuals, allow for environmental influence. Each network diagram shows the same set of 7 amphibian species from MORA. Links among taxa (interactions, associations) are blue for positive associations and red for negative associations. Arrows, when present, indicate the direction of effect between species pairs. Boldness of the links indicates relative strength of association; processes for determining association strengths are unique to each method.

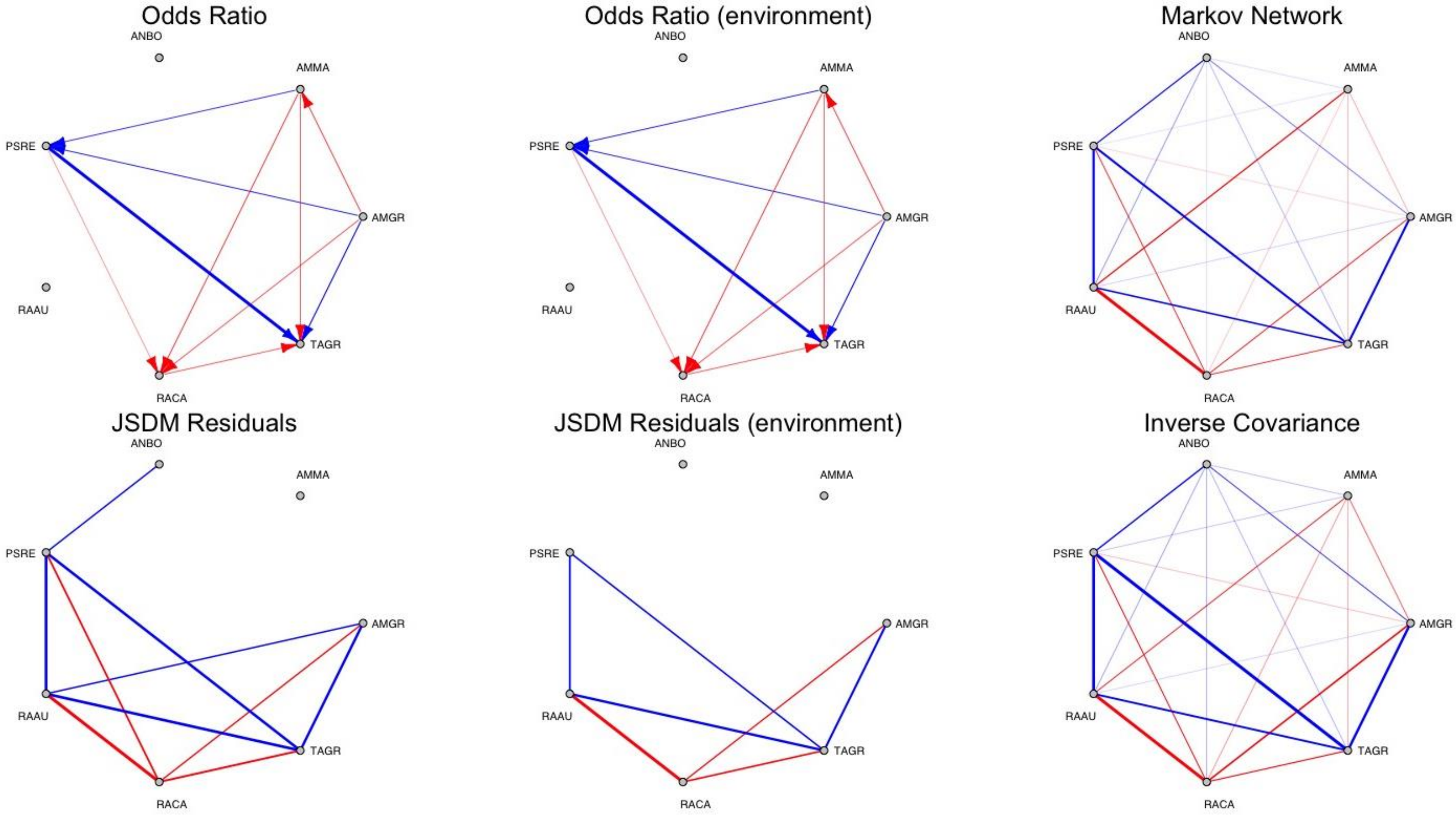


Figure 4.7. Comparing network structure and turnover across different spatial grains of observation of amphibian species co-occurrences in MORA using the Markov network approach. Distance thresholds for linking ponds into local networks incrementally increased from 250m to 2km. Red lines indicate negative interactions between species pairs; blue lines indicate positive interactions. Boldness of the links indicates relative strength of interaction.

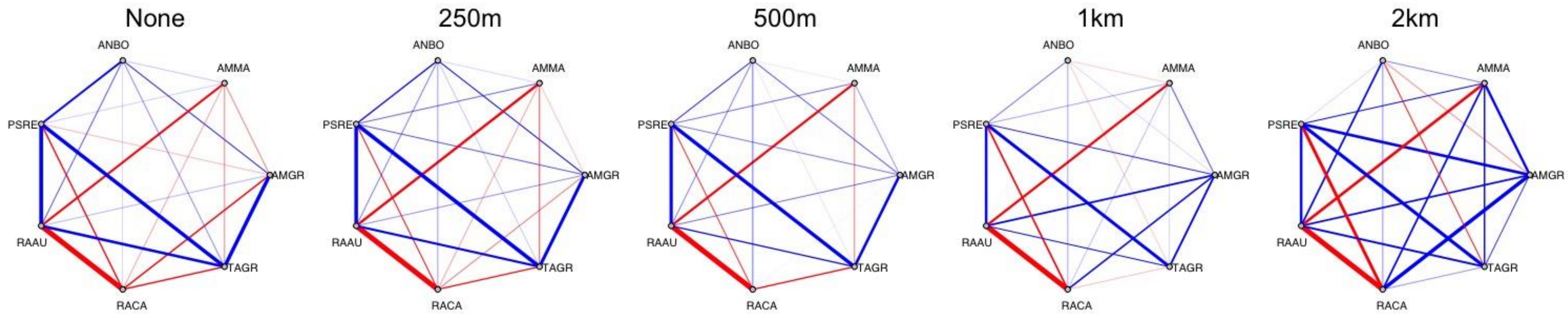


Figure 4.8. A test of the dilution effect using a pseudo-assembly process of incrementally incorporating predator species into a four-species Anuran (competitor) network. In the top row, each predator species is assembled into the network individually. In the second row, predators are assembled pairwise factorially and, lastly, all species are included together in the network. Red lines indicate negative interactions between species pairs; blue lines indicate positive interactions. Boldness of the links indicates relative strength of interaction.

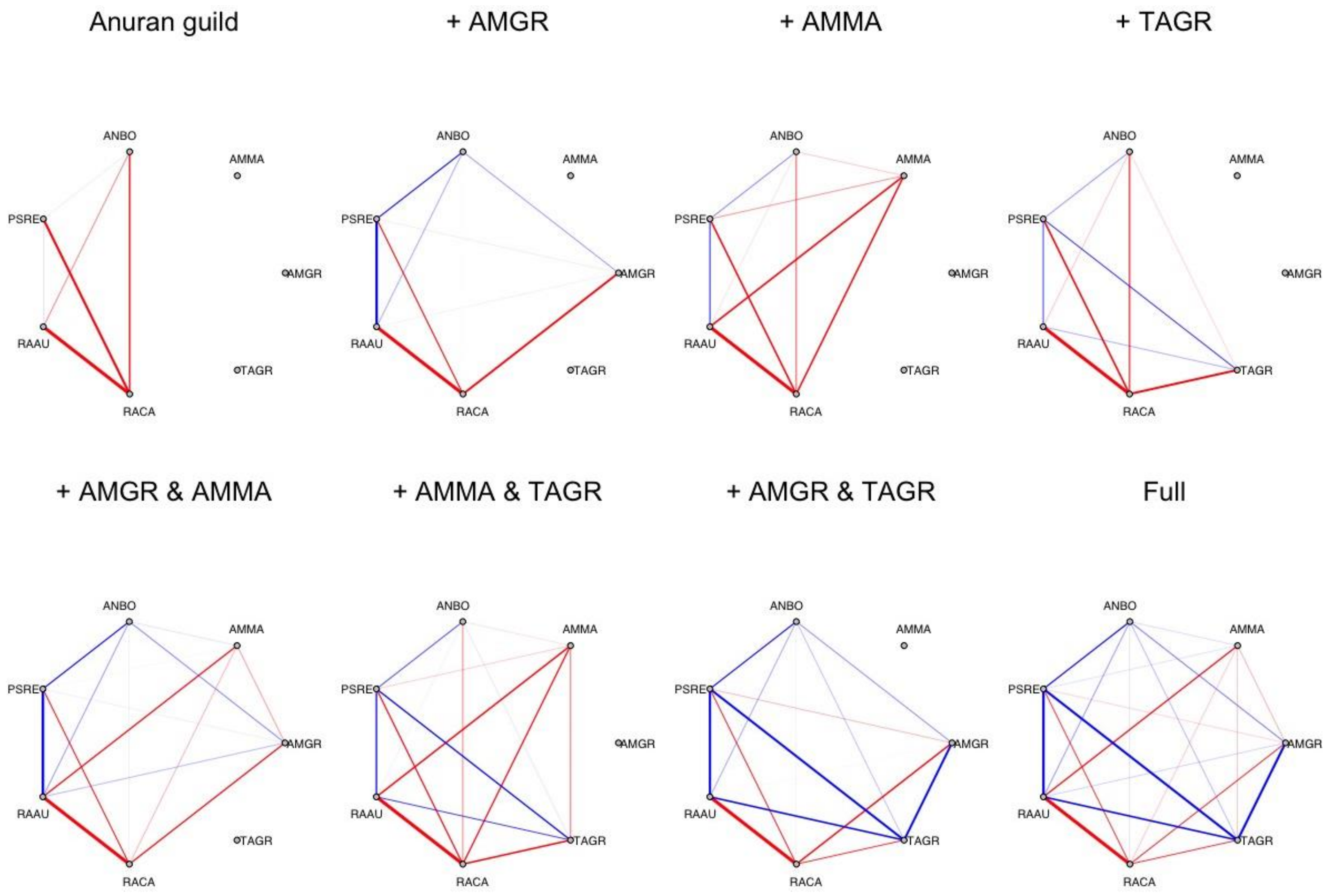
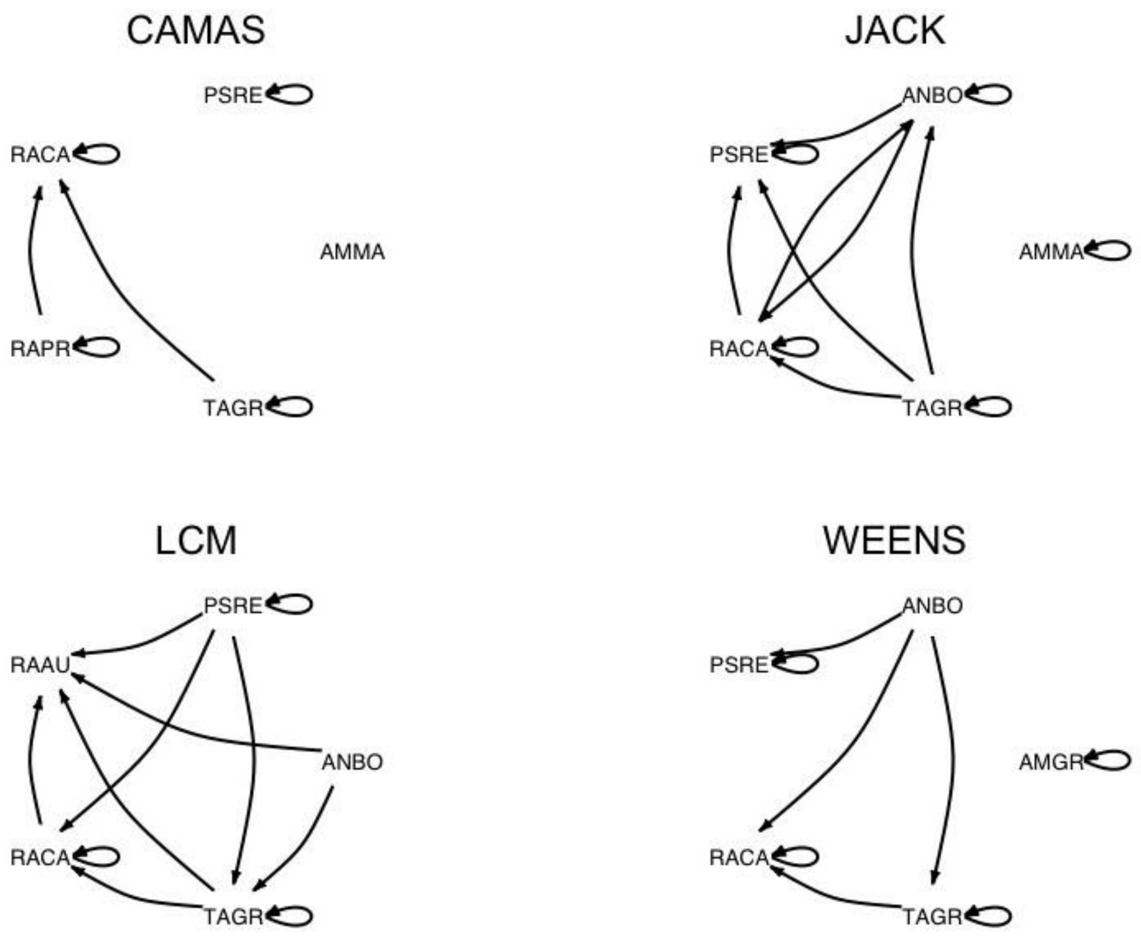


Figure 4.9. Boolean dynamic networks resulting from time series analysis of co-occurrence for four sites in HOOD. Edges with arrows indicate probabilistic transition functions, or the regulation of one species by another. Some species are entirely self-regulated (loops), meaning no other species is estimated to impact its occurrence.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5 – CONCLUSION

Over the last century, ecologists have engaged in the challenge of understanding the dynamic processes that structure species distributional patterns. Multiple sub-fields of ecology have contributed substantially to this task, working from the scale of local communities to entire biogeographic regions. Despite studying relevant and interrelated processes, ecologists struggle to integrate this knowledge across disparate spatio-temporal scales and ecological sub-disciplines (Araújo and Rozenfeld 2014). This synthesis is becoming more urgent given the need to forecast the effects of global climate change on ecological systems. Currently, attempts to tackle this issue rely on a suite of niche models that correlate species' environmental constraints with their large-scale geographic distributions (e.g. species distribution models, bioclimatic envelope models, etc.). These models exclude important ecological knowledge about species interactions gained over decades of experimental and observational studies because of a nearly axiomatic claim that these interactions do not matter at broader spatial extents (Sandel 2015). This problematic discrepancy has been widely debated and challenged for the past decade (e.g. Pearson and Dawson 2003, Zarnetske et al. 2012a, Wisz et al. 2013, Blois et al. 2013). However, there is a growing consensus that biotic interactions contribute to ecological community structure, and therefore species' broader distributional patters, and contribute to species persistence.

In this dissertation, I show that amphibian species exhibit differential sensitivities to climate change stressors and that biotic interactions can significantly structure species response strategies. The variability in sensitivity to climate warming among each of the amphibian species examined in Chapter 2, was an indication that community dynamics in the natural environment may be disrupted as species respond individualistically to climate stress throughout ontogeny. In Chapter 3, I tested this hypothesis and exposed the same three amphibian species to the combined stress of competition and rapid pond drying (associated with increasing drought conditions throughout the Cascade Mountain Range). I found competitive interactions were largely context-dependent with regard to drying and that competition exacerbated the effects of drying on competitively inferior species. These results provide substantial evidence for the importance of biotic interactions in modulating species response to climate change stressors.

Although there is increasing evidence that these biotic signals may be important at broader spatial scales (e.g. Belmaker et al. 2015), we lack a broadly applicable platform upon which we can include spatial constraints based on the strength of interactions and the dynamics of the biological community. This will require extensive data on ecological networks (indirect and direct interactions, trophic and non-trophic interactions) across broad spatio-temporal extents, which is not readily available. Instead, we have an abundance of local-scale, short-term, experimentally-derived datasets on pairwise species interactions and broad-scale occurrence (presence/absence) patterns. This data gap has forced ecologists to exclude biotic interactions and model species distributions by correlating occurrence patterns with current and future trends in the climate or environment; an environmental niche-based approach. When biotic interactions are included, it is typically through the misuse of co-occurrence data. This is problematic because co-occurrence patterns do not necessarily result from, or lead to, interactions. Species interactions, inferred by co-occurrence patterns, can become obscured by the complex web of direct and indirect interactions in real networks, as I have shown in Chapter 4.

In Chapter 4, I evaluated the accuracy of a suite of methods used to infer species interactions from their spatial associations. I tested these methods using two long-term amphibian co-occurrence datasets with the prediction that they would be insufficient at characterizing trophic interactions and unable to reconcile spatial- and temporal-scale dependencies in species interactions. While no method perfectly characterized known species interactions, all detected significant, non-random associations. Based on the results from all three chapters, predictions about species response to climate change, in the absence of important biological processes like species interactions, may be misleading. I argue that the discussion should now shift from the *relevance* of biotic interactions in structuring species distributions, to the *scale* at which these patterns manifest and how we can more accurately integrate the network of *direct, indirect,* and *net* effects of species interactions that arise in complex communities. Ultimately, the fundamental challenge to accurately inferring biotic interactions is that it requires bridging theoretical, process-based models of species interaction networks with spatially explicit, correlative models of species geographic distributions. Bridging the gap between these two disciplines will require the integration and application (or refinement) of new methods to better inform predictions of species' response to climate change.

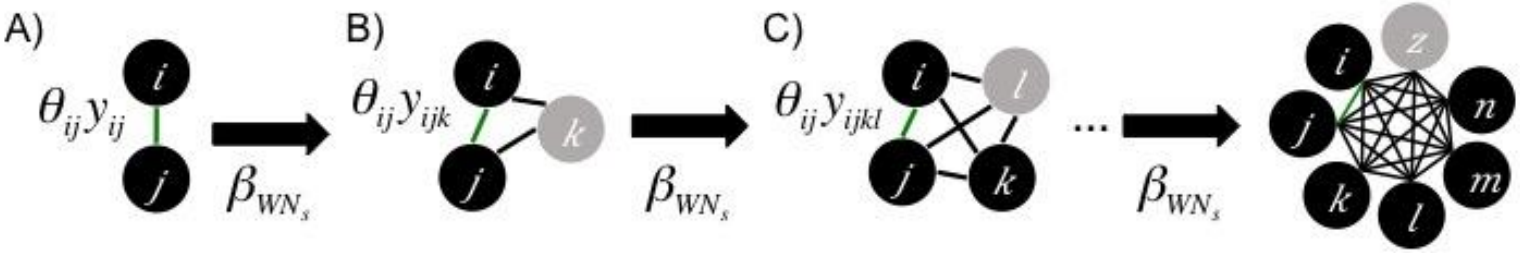
Future directions

For co-occurrence methods to be informative, particularly in trophically-structured communities, they must also disentangle direct, indirect, and net effects. Indirect and net effects can reveal important information about the strength of direct species interactions in complex communities. In many communities, only a few species will have strong direct effects, whereas most will have weak or diffuse effects (Berlow 1999). Collectively, these connections can be important in regulating community structure, particularly when no single direct interaction dominates. For instance, Aschehoug and Callaway (2015) found that the strength of pairwise competition among perennial plants was weakened in complex assemblages due to the increase in potential indirect interactions acting on the species pairs. Further, the increase in species and interaction complexity had an opposing net effect on the strength of pairwise competition. Indirect interactions can therefore create large variation in the net effects on species in the community. Given that indirect and net effects can play such a large role in community structure, it is surprising that there has been little effort to improve estimates of their effects.

Two recent attempts have been made to isolate the observed net effects in co-occurrence data from the direct associations among species. Cazelles et al. (2016) present a probabilistic framework to differentiate observed co-occurrences (net effects) from conditional co-occurrences (direct associations). However, the authors provide a limited framework for testing hypotheses regarding the emergence of indirect and net effects, restricted to linear food chains and exploitative or apparent competition. Harris (2016) provides a more promising avenue for disentangling marginal (net) and conditional (direct) associations using Markov networks (undirected graphical models) as applied in this study. Similar to Cazelles et al. (2016), the collective net effect of all other species extraneous to the pair of interest is considered background noise to their direct interaction, and indirect and net effects cannot be disentangled. Improvements in the quantification of net and indirect effects through the use of graphical models could improve our understanding of the link between species associations and species interactions.

Until now, the estimation of species interactions from a graphical model of co-occurrences has yet to be applied to multi-trophic communities. Changing from an undirected graphical model, as implemented in Harris (2016), to directed graphical model may improve our interpretation of asymmetric relationships (e.g. predator-prey). To disentangle direct, indirect, and net effects, we could incrementally “assemble” communities from single to pairwise to more complex assemblages and estimate the change, or turnover, in interactions between species pairs across community contexts (see process outlined in Box 5.1). Each step in the spatial assembly process would represent all permutations of a community set taken from the regional pool of species and their spatial associations. Unlike previous attempts to disentangle direct, indirect, and net effects (Cazelles et al. 2016), the assembly of communities would not be constrained based on assumed interactions (e.g. linear food chains, apparent competition, etc.). As communities become increasingly complex, each new species assembled into the community would have a quantifiable direct, indirect, and net effect on the interaction estimate (θ) between each species pair. Determining the relative magnitude of the indirect and net effects across community contexts has been largely discounted in co-occurrence analyses. This process could be a first step towards evaluating how the direct interactions between two species are either amplified, diluted, or maintained as new species are assembled into the community.

Box 5.1



A theoretical assembly process in space. (A) A pairwise network of species i and j, wherein $\theta_{ij}y_{ij}$ is the direct interaction estimate for species i and j estimated from locations, y, containing *only* species i and j. (B) A three-species network of species i, j, and k, wherein $\theta_{ij}y_{ijk}$ is the conditional interaction estimate for species i and j, after accounting for the indirect effects of species k at locations, y, containing *only* species i, j, and k. (C) A four-species network of species i, j, k, and l wherein $\theta_{ij}y_{ijkl}$ is the net interaction estimate for species i and j, after accounting for the net effects of species k and l at locations, y, containing species i, j, k and l. β_{WN_s} is the turnover in interaction estimates between species across each sub-network, s, from 1 to n sub-networks.

The turnover in interaction estimates between each network, or β_{WN} (adapted from Poisot et al. 2012), for all permutations of species pairs across the assembly process is a function of: 1) turnover in community structure (i.e. changes in species composition across a community complexity gradient; β_S), and 2) turnover in the sign of interactions (i.e. different estimated interactions between the same species pairs; β_{OS}). β_{WN} would therefore reflect the complexity of incrementally increasing species *and* potential interaction richness (i.e. the propagation of indirect and net effects through the assembly process) acting on species direct interaction estimates.

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APPENDICES

APPENDIX A - Review of experimental studies examining the effects of hydroperiod and biotic interactions on amphibian species

Table A1. Experimental studies investigating the effect of rapid drying on a single amphibian species (or a group of species) in the absence of interspecific interactions.

Study	**Species**	**Comments**
	Pseudacris maculata	
Brady and Griffiths 2000	*Bufo bufo* *Bufo calamita* *Rana temporaria*	
Charbonnier and Vonesh 2015	*Physalaemus pustulosus*	
Cook et al. 2013	*Lithobates catesbeianus*	
Denver et al. 1998	*Scaphiopus hammondii*	
Gervasi and Foufopoulos 2008	*Rana sylvatica*	
Kehr et al. 2014	*Argenteohyla siemersi pedersen*	Light intensity and intraspecific density included as factors
Leips et al. 2000	*Hyla gratiosa* *Hyla cinerea*	
Loman and Claesson 2003	*Rana temporaria*	
Maciel and Juncá 2009	*Pleurodema diplolister* *Rhinella granulosa*	Temperature included as a factor
Merilä et al. 2000	*Rana temporaria*	
Newman 1989	*Scaphiopus couchii*	Food limitation and intraspecific density included as factors
O'Regan et al. 2014	*Spea intermontana* *Pseudacris regilla* *Rana aurora*	Temperature included as a factor
Perotti et al. 2011	*Pleurodema thaul* *Pleurodema bufoninum* *Rhinella spinulosa*	
Rohr et al. 2004	*Ambystoma barbouri*	Food limitation and Atrazine included as factors
Ryan and Winne 2001	*Rana sphenocephala*	
Semlitsch and Wilbur 1988	*Ambystoma talpoideum*	
Székely et al. 2010	*Pelobates syriacus*	
Tejedo and Reques 1994	*Bufo calamita*	Intraspecific density included as a factor
Thumm and Mahony 2006	*Pseudophryne australis*	

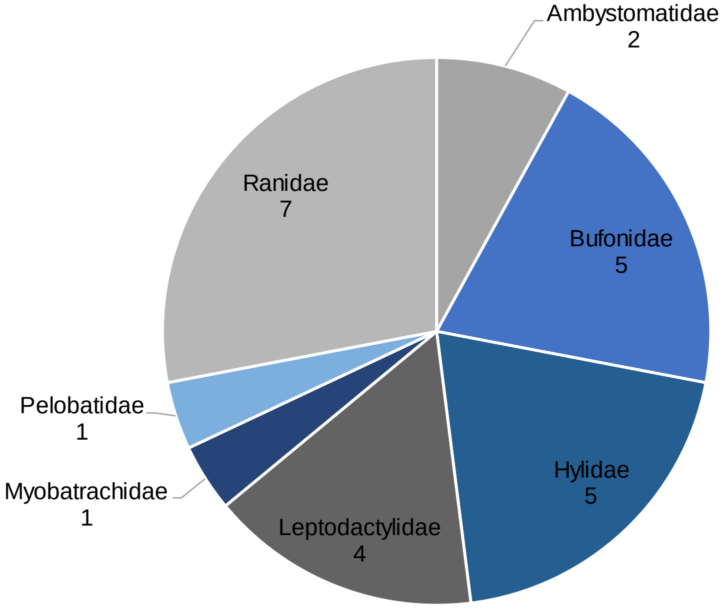

Figure A1. Experimental studies from Table A1 categorized by the number of studies per Family.

Table A2. Experimental studies investigating amphibian response to multiple stressors (biotic, abiotic, or a combination of both). This list does not include pairwise amphibian interaction experiments, unless in combination with abiotic stressors. It also does not include experiments investigating the effects of multiple abiotic stressors on a single amphibian species, unless drying is included as a factor.

Authors & Date	**Abiotic Factor**	**Inter-specific interaction type**	**# of interacting Species**	**Effect type**	**Comments**
Studies investigating multiple abiotic stressors, including drying					
Kehr et al. 2014	Drying + Light	-	-	Direct and Indirect	Tadpole density was also included as a factor.
O'Regan et al. 2014	Drying + Temperature	-	-	Direct and Indirect	See Maciel and Juncá (2009) for a similar experimental design.
Rohr et al. 2004	Drying + Contaminant + Food	-	-	Direct and Indirect	
Studies investigating multi-species interactions					
Kiesecker and Blaustein 1999	-	Pathogen + Competition	3	Direct and Indirect	
Raffel et al. 2010	-	Predation + Parasitism	3	Direct and Indirect	
Walls 1995	-	Predation + Competition	3	Direct and Indirect	
Werner and Anholt 1996	-	Predation + Competition	3	Net	In each of the experimental units, both *R. catesbeiana* and *R. clamitans* were constantly present, but at varying densities. Therefore, the effect of predation on either species could not be differentiated from the possible underlying effect

Authors & Date	Abiotic Factor	Inter-specific interaction type	# of interacting Species	Effect type	Comments
					of competition between them and was therefore a net predation effect.
Youngquist et al. 2015	-	Competition	2 and 3	Direct and Indirect	Multiple experiments conducted, including one fully-factorial, three-species competition experiment.
Relyea and Yurewicz 2002	-	Predation	2 and 3	Direct and Indirect	Multiple experiments conducted. In the mesocosm experiment with 4 species, each experimental unit received bullfrog and green frog tadpoles. Therefore, the effect of predation on, for example, green frogs was actually a net effect given the constant presence of bullfrogs.
			4	Net	
Werner and McPeek 1994	-	Predation	3 and 4	Net	Multiple experiments conducted with 3 and 4 species, although total # of species used was 5. The 2 prey species (*R. catesbeiana* and *R. clamitans*) were always together, therefore the effect of predation on either prey species was a net effect.
Peacor and Werner 1997	-	Predation + Competition	2 and 3	Direct and Indirect	Multiple experiments conducted. In the mesocosm experiment with 4 species, each experimental unit received bullfrog and green frog tadpoles. Therefore, the effect of the treatments on, for example, green frog tadpoles was actually a net effect given the constant presence of bullfrog tadpoles.
			4	Net	
Relyea 2000	-	Predation + Competition	3 and 4	Direct and Indirect	Multiple experiments conducted.

Authors & Date	Abiotic Factor	Inter-specific interaction type	# of interacting Species	Effect type	Comments
Wojdak et al. 2014	-	Predation	4	Direct and Net	Treatments included each pairwise predator-prey combination (2 species) and all three predators together with the prey (4 species), but no combinations of two predators with the prey (3 species).
Preston et al. 2014	-	Parasitism + Predation	4	Direct and Indirect	Multiple experiments conducted. In the 5-species mesocosm experiment, *A. boreas* was added as another amphibian prey item and both *A. boreas* and *P. regilla* were maintained as an assemblage across all treatments. Johnson et al. 2013 conducted a similarly complex host-parasite experiment.
			5	Net	
Rohr et al. 2015	-	Predation + Parasitism	5	Direct and Indirect	
Studies combining abiotic stressors with biotic interactions					
Kiesecker and Skelly 2001	Drying	Pathogen	2	Direct	
Laurila and Kujasalo 1999	Drying	Predation	2	Direct	For similar experimental designs, see Lane and Mahony 2002, Mogali et al. 2016, Shanbhag et al. 2016
Christenson et al. 2014	Contaminant	Predation	2	Direct	
Zhao et al. 2014	Temperature	Predation	2	Direct	
Buck et al. 2012	Contaminant	Pathogen + Competition	3	Direct and Indirect	Two amphibian species and a pathogen.
Rowe and Dunson 1995	Drying	Predation + Competition	3	Net	Overall tadpole density manipulated, but assemblage constant across treatments.
Boone and James	Drying +	Competition	4	Net	Overall tadpole density manipulated, but

Authors & Date	Abiotic Factor	Inter-specific interaction type	# of interacting Species	Effect type	Comments
2003	Contaminants				assemblage constant across treatments and only compared to controls (each species in isolation).
Boone et al. 2004	Drying	Competition	4	Net	Overall tadpole density manipulated, but assemblage constant across treatments.
Purrenhage and Boone 2009	Habitat	Competition	4	Net	Tadpole assemblage constant across treatments.
Boone and Semlitsch 2002	Drying + Contaminant	Predation + Competition	5	Net	Tadpole prey assemblage constant across treatments and all ponds contained same "background community" of predators.
Wilbur 1987	Drying	Predation + Competition	5	Net	Overall tadpole density manipulated, but assemblage constant across treatments.

APPENDIX B – Ch.3 supplemental results for water temperature profiles (in °C) in Block 1 of the hydroperiod treatments.

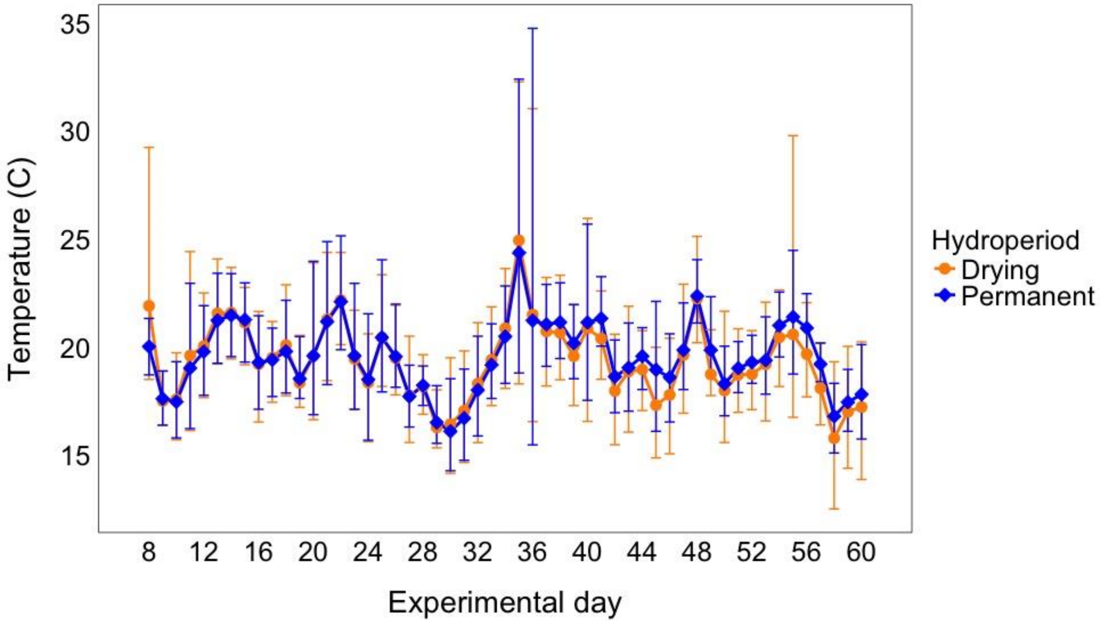

Figure B1. Daily water temperature profiles for the permanent (blue) and drying (orange) hydroperiod treatments. Maxim iButton® Thermochron Temperature Data Loggers were set to record at 1-hour intervals from days 8 through 60 of the experiment (July 2 to August 23, 2014; temperature sensors were not deployed until day 7 of the experiment). Points represent mean water temperatures and vertical bars represent average maximum and minimum temperatures for a given day.

Table B1. Linear mixed model results for the response variables of mean temperature and standard deviation of temperature expressed as a function of hydroperiod treatment (permanent or drying) and the random effect of tub. Values presented are predicted model estimates and standard errors, numerator degrees of freedom (*df*N), denominator degrees of freedom (*df*D), *F* statistics, and *P*-values.

Response variable: Mean temperature					
Predictor	**Estimate ± SE**	***df*N**	***df*D**	**F**	***P*-value**
Hydroperiod	0.266 ± 0.14	1	12	3.48	0.086
Response variable: Standard deviation of temperature					
Predictor	**Estimate ± SE**	***df*N**	***df*D**	**F**	***P*-value**
Hydroperiod	-0.42 ± 0.069	1	12	37.15	<0.001

APPENDIX C – Ch. 3 Supplemental results for survivorship

Table C1. Generalized linear mixed model results comparing the likelihood of survival between species across all experimental treatments (odds ratios > 1 indicates significantly higher probability of survival due to treatment effect; odds ratios < 1 indicates significantly lower probability of survival due to treatment effect). Odds-ratios were back-transformed from log scale for ease of interpretation.

Species	**Estimate (odds ratio)**	**95% CI**
Western toad	1.766	(1.313, 2.374)
Pacific chorus frog	1.326	(0.984, 1.788)
Cascades frog	0.583	(0.402, 0.846)

Table C2. Average proportion surviving to emergence, average number of larvae remaining unmetamorphosed by the completion of the experiment (after 60 days), and average mortalities (not due to completion of experiment) across species and treatments. These three variables sum to approximately one. In the drying hydroperiod treatment, larvae remaining at the completion of the experiment represent mortalities that would likely occur due to those individual’s inability to metamorphose before pond drying. For the interspecific competition treatments, a letter indicating the first initial of the competing species (P: Pacific chorus frog; C: Cascades frog; W: Western toad) is provided.

Species	**Treatment**	**Proportion surviving (mean ± SE)**	**Proportion larvae remaining (mean ± SE)**	**Proportion mortalities (mean ± SE)**
Western toad	Control: Permanent hydroperiod, Intraspecific competition (W)	0.61 ± 0.09	0.28 ± 0.04	0.11 ± 0.01
	Permanent hydroperiod, Pairwise competition (C)	0.47 ± 0.13	0.37 ± 0.04	0.16 ± 0.03
	Permanent hydroperiod, Pairwise competition (P)	0.49 ± 0.13	0.36 ± 0.06	0.15 ± 0.02
	Permanent hydroperiod, Three-species competition (C+P)	0.60 ± 0.16	0.30 ± 0.09	0.10 ± 0.02
	Drying hydroperiod, Intraspecific competition (W)	0.48 ± 0.09	0.37 ± 0.03	0.15 ± 0.02
	Drying hydroperiod, Pairwise competition (C)	0.33 ± 0.12	0.59 ± 0.03	0.08 ± 0.02
	Drying hydroperiod, Pairwise competition (P)	0.64 ± 0.12	0.29 ± 0.07	0.07 ± 0.02
	Drying hydroperiod, Three-species competition (C+P)	0.48 ± 0.16	0.42 ± 0.04	0.10 ± 0.03

Species	**Treatment**	**Proportion surviving (mean ± SE)**	**Proportion larvae remaining (mean ± SE)**	**Proportion mortalities (mean ± SE)**
Pacific chorus frog	Control: Permanent hydroperiod, Intraspecific competition (P)	0.62 ± 0.09	0.24 ± 0.03	0.14 ± 0.02
	Permanent hydroperiod, Pairwise competition (C)	0.35 ± 0.12	0.32 ± 0.05	0.33 ± 0.03
	Permanent hydroperiod, Pairwise competition (W)	0.45 ± 0.13	0.27 ± 0.03	0.28 ± 0.05
	Permanent hydroperiod, Three-species competition (C+W)	0.58 ± 0.16	0.24 ± 0.05	0.18 ± 0.06
	Drying hydroperiod, Intraspecific competition (P)	0.44 ± 0.09	0.43 ± 0.04	0.13 ± 0.02
	Drying hydroperiod, Pairwise competition (C)	0.39 ± 0.13	0.40 ± 0.04	0.21 ± 0.03
	Drying hydroperiod, Pairwise competition (W)	0.45 ± 0.13	0.25 ± 0.02	0.29 ± 0.04
	Drying hydroperiod, Three-species competition (C+W)	0.38 ± 0.16	0.36 ± 0.06	0.26 ± 0.08
Cascades frog	Control: Permanent hydroperiod, Intraspecific competition (C)	0.33 ± 0.09	0.43 ± 0.02	0.23 ± 0.01
	Permanent hydroperiod, Pairwise competition (P)	0.37 ± 0.13	0.39 ± 0.04	0.24 ± 0.06
	Permanent hydroperiod, Pairwise competition (W)	0.31 ± 0.12	0.40 ± 0.03	0.29 ± 0.07
	Permanent hydroperiod, Three-species competition (P+W)	0.46 ± 0.16	0.42 ± 0.06	0.12 ± 0.03
	Drying hydroperiod, Intraspecific competition (C)	0.33 ± 0.09	0.35 ± 0.02	0.32 ± 0.03
	Drying hydroperiod, Pairwise competition (P)	0.31 ± 0.12	0.40 ± 0.05	0.29 ± 0.06
	Drying hydroperiod, Pairwise competition (W)	0.41 ± 0.13	0.41 ± 0.17	0.42 ± 0.07
	Drying hydroperiod, Three-species competition (P+W)	0.34 ± 0.15	0.36 ± 0.06	0.30 ± 0.07

APPENDIX D – Review of experimental and field-based studies examining Pacific Northwest pond-breeding amphibian species interactions.

Table D1. A review of manipulative experiments and field observations (i.e. mensurative experiments) examining predation, including intraguild predation and cannibalism, and interspecific competition among Pacific Northwest amphibian species.

Predation			
Authors & Date	**Predator species**	**Prey species**	**Comments**
Adams 2000	*Lithobates catesbeianus* (=*Rana catesbeiana*)	*Pseudacris* (=*Hyla*) *regilla* *Rana aurora*	LICA → RAAU also in Pearl et al. 2005
Garcia et al. 2009	*Taricha granulosa*	*Ambystoma macrodactylum* *Pseudacris* (=*Hyla*) *regilla*	Intraguild predation (TAGR → AMMA)
Jordan et al. 2004	*Anaxyrus* (=*Bufo*) *boreas*	*Anaxyrus* (=*Bufo*) *boreas* *Pseudacris* (=*Hyla*) *regilla* *Rana cascadae*	Cannibalism and intraguild predation
Kiesecker and Blaustein 2003	*Taricha granulosa*	*Rana aurora*	Also observed in Wilson and Lefcort 1993
Lefcort and Eiger 1993	*Taricha granulosa*	*Lithobates catesbeianus* (=*Rana catesbeiana*)	
MacCracken 2007	*Taricha granulosa*	*Ambystoma gracile*	Intraguild predation
Pearl and Hayes 2002	*Rana pretiosa*	*Anaxyrus* (=*Bufo*) *boreas*	Intraguild predation
Pearl et al. 2004	*Lithobates catesbeianus* (=*Rana catesbeiana*)	*Rana aurora* *Rana pretiosa*	LICA → RAAU also in Blaustein et al. 1998 and Adams 2000
Peterson and Blaustein 1991	*Taricha granulosa*	*Anaxyrus* (=*Bufo*) *boreas* *Pseudacris* (=*Hyla*) *regilla* *Rana cascadae*	
Puttlitz et al. 1999	*Ambystoma gracile*	*Pseudacris* (=*Hyla*) *regilla*	
Romansic et al. 2009	*Taricha granulosa*	*Rana cascadae*	
Rowe 2013 (MS Thesis)	*Lithobates catesbeianus*	*Ambystoma gracile* *Lithobates catesbeianus*	Cannibalism
Thurman & Garcia *data unpubl.*	*Ambystoma macrodactylum*	*Anaxyrus boreas* *Pseudacris regilla* *Rana cascadae*	
Walls et al.	*Ambystoma*	*Ambystoma macrodactylum*	Cannibalism; also

1993	*macrodactylum*		observed in Wildy et al. 2001

Interspecific competition

Authors & Date	**Competing species**	**Comments**
Barnett and Richardson 2002	*Rana aurora* *Rana pretiosa*	
Hamilton et al. 2012	*Pseudacris regilla* *Rana aurora*	
Kiesecker and Blaustein 1999	*Pseudacris* (=*Hyla*) *regilla* *Rana cascadae*	
Kiesecker et al. 2001	*Lithobates catesbeianus* (=*Rana catesbeiana*) *Rana aurora*	
Monello et al. 2006	*Lithobates catesbeianus* (=*Rana catesbeiana*) *Pseudacris regilla*	*Rana luteiventris* also included in study
Pearl et al. 2005	*Lithobates catesbeianus* (=*Rana catesbeiana*) *Rana pretiosa* *Rana aurora*	Reproductive interference by LICA
Pearman 2002	*Ambystoma gracile* *Ambystoma macrodactylum*	
Taylor 1984	*Ambystoma gracile* *Taricha granulosa*	
Thurman & Garcia (Ch. 2 Dissertation)	*Anaxyrus boreas* *Pseudacris regilla* *Rana cascadae*	also in Han et al. 2015

APPENDIX E – Supplementary results from time series analysis of species co-occurrence using Boolean dynamic model

Background

Probabilistic Boolean networks (PBN) allow for specifying more than one transition function per species. Transition functions model the dependencies among species throughout the time series of the network and represent a probabilistic set of interactions. For example, species A is negatively affected by species B and C. However, when species D is present, the negative effects of species B are inhibited. Expressed as a transition function:

A = (!B & ! C) | (D & B & !C)

As a rule, inhibitions (i.e. negative interactions) are represented by a Boolean negation, expressed as “!”. The second statement “(D & B & !C)” is an exception (or alternative) to the first statement and is expressed as a logical OR, for which the “|” character is used. When multiple transition functions for a species are estimated with minimum error, all functions are included as alternative functions with equal probability. The probabilities of all transition functions for one species sum to one (Mussel et al. 2010).

Table E1. Transition functions for Boolean networks for each of the eight HOOD sites included in the time series analysis of species co-occurrences. Some species are self-regulating [e.g. ANBO = (ANBO)], meaning no interspecific effects, or "knocked-out" (i.e. equal to zero, no intraspecific or interspecific effects).

Site	**Species**	**Transition functions**
BRUR	AMGR	= 0 (probability: 1, error: 2)
	ANBO	= (ANBO) (probability: 1, error: 41)
	PSRE	= (PSRE) (probability: 1, error: 14)
	RAAU	= 0 (probability: 1, error: 8)
	TAGR	= (TAGR) (probability: 1, error: 34)
CAMAS	AMMA	= 0 (probability: 1, error: 2)
	PSRE	= (PSRE) (probability: 1, error: 26)
	RACA	= (RACA & TAGR) = (RACA & RAPR) } (probability: 0.5, error: 5)
	RAPR	= (RAPR) (probability: 1, error: 19)
	TAGR	= (TAGR) (probability: 1, error: 28)
DRY	ANBO	= (ANBO) (probability: 1, error: 8)
	PSRE	= (PSRE) (probability: 1, error: 18)
	RACA	= (RACA) (probability: 1, error: 1)
	TAGR	= (TAGR) (probability: 1, error: 6)
FROG	AMGR	= (AMGR) (probability: 1, error: 2)
	AMMA	= (AMMA) (probability: 1, error: 16)
	ANBO	= (ANBO) (probability: 1, error: 24)
	PSRE	= (PSRE) (probability: 1, error: 12)
	TAGR	= (TAGR) (probability: 1, error: 22)
FRY	AMMA	= (AMMA) (probability: 1, error: 2)
	ANBO	= (ANBO) (probability: 1, error: 14)
	PSRE	= (PSRE) (probability: 1, error: 16)
	RACA	= (RACA) (probability: 1, error: 1)
	TAGR	= (TAGR) (probability: 1, error: 16)
JACK	AMMA	= (AMMA) (probability: 1, error: 2)
	ANBO	= (ANBO & TAGR) | (ANBO & RACA) (probability: 1, error: 11)

Site	Species	Transition functions
JACK Cont’d	PSRE	= (!ANBO & PSRE) | (ANBO & !PSRE & !RACA & !TAGR) | (PSRE & RACA & TAGR) = (!ANBO & PSRE) | (ANBO & !PSRE & !RACA & !TAGR) | (PSRE & RACA) = (!ANBO & PSRE) | (ANBO & !PSRE & !RACA & !TAGR) | (PSRE & TAGR) = (!ANBO & PSRE) | (ANBO & !PSRE & !RACA & !TAGR) | (PSRE & TAGR) | (PSRE & RACA) = (PSRE & !RACA & !TAGR) | (!ANBO & PSRE) | (ANBO & !RACA & !TAGR) | (PSRE & RACA & TAGR) = (PSRE & !TAGR) | (!ANBO & PSRE) | (ANBO & !RACA & !TAGR) | (PSRE & RACA) = (PSRE & !RACA) | (!ANBO & PSRE) | (ANBO & !RACA & !TAGR) | (PSRE & TAGR) = (PSRE) | (ANBO & !RACA & !TAGR) } (probability: 0.125, error: 28)
	RACA	= (RACA) | (ANBO & !TAGR) (probability: 1, error: 28)
	TAGR	= (TAGR) (probability: 1, error: 23)
LCM	ANBO	= 0 (probability: 1, error: 3)
	PSRE	= (PSRE) (probability: 1, error: 10)
	RAAU	= (!ANBO & !PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA & !TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA & !TAGR) | (ANBO & PSRE & RACA & TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA & !TAGR) | (ANBO & PSRE & RACA & !TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA & !TAGR) | (ANBO & PSRE & RACA) = (!ANBO & !PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA & !TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA & !TAGR) | (ANBO & PSRE & TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | } (probability: 0.015625, error: 7)

Site	Species	Transition functions
LCM Cont'd	RAAU Cont'd	(ANBO & !PSRE & !RACA & !TAGR) | (ANBO & PSRE & RACA & !TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA & !TAGR) | (ANBO & PSRE & RACA) = (!ANBO & !PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA & !TAGR) | (ANBO & !PSRE & RACA & TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA & !TAGR) | (ANBO & RACA & TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA & !TAGR) | (ANBO & !PSRE & RACA & TAGR) | (ANBO & PSRE & RACA & !TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA & !TAGR) | (ANBO & RACA & TAGR) | (ANBO & PSRE & RACA) = (!ANBO & !PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA & !TAGR) | (ANBO & !PSRE & RACA & TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA & !TAGR) | (ANBO & RACA & TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA & !TAGR) | (ANBO & !PSRE & RACA & TAGR) | (ANBO & PSRE & RACA & !TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA & !TAGR) | (ANBO & RACA & TAGR) | (ANBO & PSRE & RACA) = (!PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) = (!PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & PSRE & RACA & TAGR) = (!PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & RACA & !TAGR) = (!PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & RACA & !TAGR) | (ANBO & } (probability: 0.015625, error: 7)

Site	Species	Transition functions
LCM Cont'd	RAAU Cont'd	PSRE & RACA) = (!PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) = (!PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & PSRE & TAGR) = (!PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & RACA & !TAGR) = (!PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & RACA & !TAGR) | (ANBO & PSRE & TAGR) = (!PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & !PSRE & RACA) = (!PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & RACA & TAGR) = (!PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & !PSRE & RACA) | (ANBO & RACA & !TAGR) = (!PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & RACA) = (!PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & !PSRE & RACA) = (!PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & RACA & TAGR) = (!PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & !PSRE & RACA) | (ANBO & RACA & !TAGR) = (!PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & RACA) = (!ANBO & !PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA) = (!ANBO & !PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA) | (ANBO & PSRE & RACA & TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA) | (ANBO & PSRE & RACA } (probability: 0.015625, error: 7)

Site	Species	Transition functions
LCM Cont’d	RAAU Cont’d	& !TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA) | (ANBO & PSRE & RACA) = (!ANBO & !PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA) = (!ANBO & !PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA) | (ANBO & PSRE & TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA) | (ANBO & PSRE & RACA & !TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA) | (ANBO & PSRE & RACA) = (!ANBO & !PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA) | (ANBO & !PSRE & TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA) | (ANBO & RACA & TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA) | (ANBO & !PSRE & TAGR) | (ANBO & PSRE & RACA & !TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA) | (ANBO & RACA & TAGR) | (ANBO & PSRE & RACA) = (!ANBO & !PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA) | (ANBO & !PSRE & TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA) | (ANBO & TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA) | (ANBO & !PSRE & TAGR) | (ANBO & PSRE & RACA & !TAGR) = (!ANBO & !PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !RACA) | (ANBO & TAGR) | (ANBO & PSRE & RACA) = (!PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & !PSRE & !RACA) = (!PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & !PSRE & !RACA) | (ANBO & } (probability: 0.015625, error: 7)

Site	**Species**	**Transition functions**
LCM Cont’d	RAAU Cont’d	PSRE & RACA & TAGR) = (!PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & !PSRE & !RACA) | (ANBO & RACA & !TAGR) = (!PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & !PSRE & !RACA) | (ANBO & RACA & !TAGR) | (ANBO & PSRE & RACA) = (!PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & !RACA & TAGR) = (!PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & !RACA & TAGR) | (ANBO & PSRE & TAGR) = (!PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & !RACA & TAGR) | (ANBO & RACA & !TAGR) = (!PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE & !TAGR) | (ANBO & !RACA & TAGR) | (ANBO & RACA & !TAGR) | (ANBO & PSRE & TAGR) = (!PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE) = (!PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE) | (ANBO & RACA & TAGR) = (!PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE) | (ANBO & RACA & !TAGR) = (!PSRE & RACA & !TAGR) | (!ANBO & PSRE & !RACA & TAGR) | (ANBO & !PSRE) | (ANBO & RACA) = (!PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE) = (!PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE) | (ANBO & TAGR) = (!PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE) | (ANBO & RACA & !TAGR) = (!PSRE & RACA & !TAGR) | (PSRE & !RACA & TAGR) | (ANBO & !PSRE) | (ANBO & RACA) } (probability: 0.015625, error: 7)

Site	**Species**	**Transition functions**
LCM Cont’d	RAAU Cont’d	} (probability: 0.015625, error: 7)
	RACA	= (RACA & TAGR) | (PSRE & RACA) (probability: 1, error: 10)
	TAGR	= (!ANBO & TAGR) | (ANBO & !PSRE & !TAGR) = (!ANBO & TAGR) | (ANBO & !PSRE & !TAGR) | (PSRE & TAGR) = (!PSRE & TAGR) | (!ANBO & TAGR) | (ANBO & !PSRE) = (TAGR) | (ANBO & !PSRE) } (probability: 0.25, error: 14)
WEENS	AMGR	= (AMGR) (probability: 1, error: 2)
	ANBO	= 0 (probability: 1, error: 1)
	PSRE	= (!ANBO & PSRE) | (ANBO & !PSRE) = (PSRE) | (ANBO) } (probability: 0.5, error: 7)
	RACA	= (!ANBO & RACA & TAGR) | (ANBO & !RACA & !TAGR) = (RACA & TAGR) | (ANBO & !RACA & !TAGR) = (!ANBO & RACA & TAGR) | (ANBO & !TAGR) = (RACA & TAGR) | (ANBO & !TAGR) = (!ANBO & RACA & TAGR) | (ANBO & !RACA) = (RACA & TAGR) | (ANBO & !RACA) = (!ANBO & RACA & TAGR) | (ANBO & !TAGR) | (ANBO & !RACA) = (RACA & TAGR) | (ANBO) } (probability: 0.125, error: 8)
	TAGR	= (!ANBO & TAGR) | (ANBO & !TAGR) = (TAGR) | (ANBO) } (probability: 0.5, error: 11)

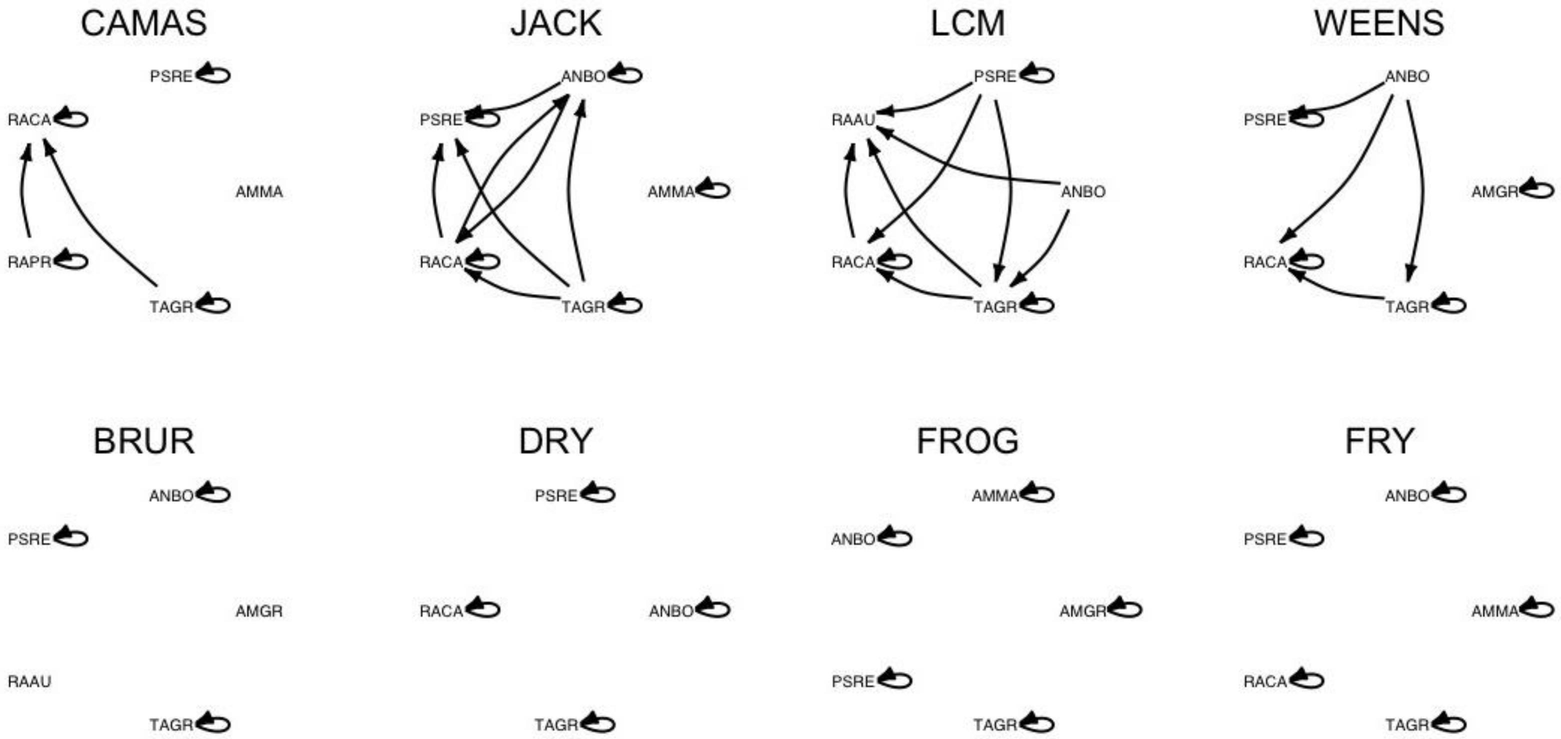

Figure E1. Boolean dynamic networks resulting from time series analysis of co-occurrence for all eight HOOD sites used in analysis. Edges with arrows indicate probabilistic transition functions, or the regulation of one species by another. For four of the sites (bottom row), species are either entirely self-regulated (i.e. no interspecific effects), or their functions equal zero (i.e. no intraspecific or interspecific effects).