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Original Contribution

How Temperature, Pond-Drying, and Nutrients Influence Parasite Infection and Pathology

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Abstract: The rapid pace of environmental change is driving multi-faceted shifts in abiotic factors that influence parasite transmission. However, cumulative effects of these factors on wildlife diseases remain poorly understood. Here we used an information-theoretic approach to compare the relative influence of abiotic factors (temperature, diurnal temperature range, nutrients and pond-drying), on infection of snail and amphibian hosts by two trematode parasites (*Ribeiroia ondatrae* and *Echinostoma* spp.). A temperature shift from 20 to 25 °C was associated with an increase in infected snail prevalence of 10–20%, while overall snail densities declined by a factor of 6. Trematode infection abundance in frogs was best predicted by infected snail density, while *Ribeiroia* infection specifically also declined by half for each 10% reduction in pond perimeter, despite no effect of perimeter on the per snail release rate of cercariae. Both nutrient concentrations and *Ribeiroia* infection positively predicted amphibian deformities, potentially owing to reduced host tolerance or increased parasite virulence in more productive environments. For both parasites, temperature, pond-drying, and nutrients were influential at different points in the transmission cycle, highlighting the importance of detailed seasonal field studies that capture the importance of multiple drivers of infection dynamics and the mechanisms through which they operate.

Keywords: Eutrophication, Global warming, Infectious disease, Malformations, Multiple stressors, Phenology

Introduction

Environmental changes involving temperature, drought, and nutrient-loading are altering aquatic environments in ways that influence pathogen transmission (Johnson et al. 2010; Altizer et al. 2013; Budria 2017), but the relative importance and cumulative effects of these abiotic factors can be challenging to assess. A wide variety of laboratory and mesocosm studies have examined the effects of isolated or paired drivers such as temperature or nutrient addition on disease systems, providing valuable mechanistic understanding (Paull et al. 2012; Decaestecker et al. 2015; Buck et al. 2016; Penttinen et al. 2016; Laverty et al. 2017). Fieldwork exploring these proposed mechanisms in a more natural context can offer additional insights into the relative importance of such drivers and their changes over time (Raffel et al. 2013; Marcogliese 2016).

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Field studies of disease dynamics over a full season can further improve our understanding of the key drivers of infection by clarifying mechanisms that are challenging to infer from single visits. For instance, temperature can have differential effects on the phenology of hosts and parasites, substantially altering the timing of host–parasite interactions with consequences for host infection intensity and pathology (Paull and Johnson 2014; Gethings et al. 2015). Additionally, for parasites with complex transmission cycles, the cumulative effects of abiotic drivers depend upon their influence on each stage of the life cycle. Collecting detailed data on each transmission stage allows for inference of the mechanisms through which abiotic drivers are influencing the system as a whole.

Multiple abiotic factors influence parasite transmission, oftentimes in complex or indirect ways, such that determining the net effects in a field setting can pose a significant challenge. Warming temperatures can have conflicting effects on net transmission dynamics, for instance by simultaneously increasing pathogen development rates and the mortality rates of intermediate hosts or vectors (Paull et al. 2012; Mordecai et al. 2013). Such direct effects occur alongside potential changes to the timing and consequences of host–parasite interactions due to differential changes in phenology (e.g., the timing of seasonal events such as breeding or flowering) among species (Paull and Johnson 2014; Gethings et al. 2015; Pastok et al. 2016; Thackeray et al. 2016). Temperature variability may further influence disease risk due to fluctuations above or below optimal performance temperatures of hosts or parasites, or acclimation rates that differ between hosts and parasites (Rohr and Raffel 2010; Lambrechts et al. 2011; Raffel et al. 2013; Paull et al. 2015; Altman et al. 2016). Wetland-drying and changes in nutrient-loading can similarly have multifaceted effects on the survival and infection success of parasites with consequences for disease risk (McKenzie and Townsend 2007; Gage et al. 2008; O'Connor et al. 2008; Smith and Schindler 2009; van Dijk et al. 2010; Johnson et al. 2010) For example, higher nutrient concentrations can enhance the densities of hosts or vectors while simultaneously altering host resistance, leading to net changes in infection (Johnson et al. 2010; Aalto et al. 2015). Examining the effects of these drivers across multiple stages of infection—for instance in host species utilized sequentially in the complex life cycle of a trematode—gives insight into likely mechanisms influencing patterns.

Here, we sampled trematode infections (*Ribeiroia ondatrae* and *Echinostoma* spp.) in snail and amphibian hosts at 18 wetlands at monthly intervals to obtain estimates of infection throughout the summer. Concurrently, we measured pond temperature (mean and variance), pond-drying (change in surface area), and nutrient concentrations at each pond. These two trematode parasite species are sequentially transmitted from ramshorn snails to amphibians or fishes and, finally, to birds or mammals. In amphibian hosts, *Ribeiroia* infection can cause limb deformities or mortality, while echinostomes can reduce survival or cause edema (Johnson and Mckenzie 2009). Our goals were to (1) assess the relative importance of abiotic variables at multiple stages of the infection process and (2) assess shifts in the seasonality of infections and their consequences for amphibian pathology. Recognizing the multidimensional nature of environmental change as well as the potential for such effects to manifest differentially across stages of infection, our approach captures the mechanisms through which environmental changes can drive cumulative seasonal changes to parasite transmission dynamics.

Materials and Methods

Study System

Our study sites were ponds in the oak-chaparral ecosystem east of the San Francisco Bay area of California on regional preserves used for recreation and livestock grazing (Fig. S1). Site elevation ranged from 135–1057 m, and the average amount of canopy cover within 5 m of each pond's shorelines was $< 10\%$. We monitored the intermediate snail (*Helisoma trivolvis*) and amphibian (*Pseudacris regilla*) hosts of two commonly occurring trematode parasites, *Ribeiroia ondatrae,* and parasites in the echinostome group (primarily *E. trivolvis*, but possibly including *Echinostoma revolutum* and *Echinoparyphium* spp.). Both taxa have a similar life cycle in which ramshorn snails become infected by free-swimming miracidia. These miracidia hatch from eggs that are released alongside the feces of definitive avian or mammalian hosts (Fried and Graczyk 2004; Johnson and Mckenzie 2009; Szuroczki and Richardson 2009). Free-swimming cercariae emerging from snails encyst within larval amphibians or fishes until they are consumed by the definitive host (Szuroczki and Richardson 2009). Within tadpoles, echinostome species infect the developing nephric system, sometimes causing pathology through edema, slowed growth rates, or early-stage mortality (Schotthoefer

et al. 2003). By contrast, *Ribeiroia* cercariae target the developing limb buds, causing mortality or severe limb deformities when tadpoles are infected during limb development (Johnson et al. 2004; Johnson et al. 2011).

Sampling Methods

We sampled ramshorn (*Helisoma trivolvis*) snails monthly from May through August 2010. To maximize parasite detectability, we selected 18 ponds at which one or both parasites had been detected in snails in 2009, that ranged broadly in size and hydroperiod. This technique resulted in ponds with a range of prevalence in snails for both parasites (*Ribeiroia*: 0–25% *Echinostoma*: 0–41%). Results from these fine-scale 2010 seasonal surveys have not been analyzed or published elsewhere. On each of four visits to the ponds, we quantified snail abundance using 10 dipnet sweeps every 15 m around the pond using a D-frame net (1.4 mm mesh, 2600 cm^2) to conduct a 1-m-long sweep just above the substrate and perpendicular to the shoreline. On each visit, we collected between 41 and 180 (mean per visit = 112, SD = 26) mature *H. trivolvis* snails ($>$ 8 mm in size). Snails were isolated into vials filled with 40 mL of aged and dechlorinated tap water, and we identified any released parasites over the following 24 h using morphological characteristics (Fried and Toledo 2004; Johnson et al. 2004; Szuroczki and Richardson 2009). For a subset of up to 15 snails per visit, we preserved all of the cercariae released over a 24 h period for subsequent quantification to estimate per-snail release rates of cercariae for each trematode.

During the May visit, we also recorded the developmental stage (Gosner 1960) of up to 35 (total mean per site-visit = 30.2, SD = 6.2) larval Pacific chorus frogs (*Pseudacris regilla*) collected using a D-frame dipnet. Later in the season, when *P. regilla* metamorphs were present, we also captured by hand and examined up to 100 metamorphs per visit (total mean per site from all visits = 169, SD = 113) for deformities along a transect 0–5 m from shore (Johnson et al. 2002). Deformities included missing, extra, and misshapen limbs, feet, or digits, as well as skin webbings and femoral projections(Johnson et al. 2001; Lunde and Johnson 2012). We collected, euthanized and necropsied $\sim$ 10 metamorphic amphibians per site (range = 6–16) in June (corresponding with peak metamorphosis), examining all skin, organs, limbs and tissues to quantify *Ribeiroia* and echinostome metacercariae (Sutherland 2005; Johnson and Hartson 2009; Johnson et al. 2013).

We measured June nutrient concentrations, seasonal water temperature, and proportional change in pond surface area (pond-drying) at each site. We measured temperature every 2 h at each pond by placing one Hobo underwater datalogger (Onset Computer Corp.) 50 cm below the water surface. From June 2 to August 11 (the date range when all sites had loggers present to avoid biasing calculations of the mean and range), we calculated both the mean temperature and the mean diurnal temperature range (average difference between the daily maximum and minimum recorded temperatures; DTR) at each site. We used a GPS unit to measure pond surface area (m^2) during the July and August visits, and calculated pond-drying as the proportional difference in pond area ((July − August)/July). In July, we collected and froze water samples in acid-washed 125 mL plastic Nalgene bottles for analysis of total dissolved phosphorus (TDP) and nitrogen (TDN) by the University of Colorado Arikaree laboratory. Samples from three sites were lost in transit, so we used data collected at these sites in July of 2009. For 15 sites sampled in both 2009 and 2010, the concentrations of nutrients in 2009 were highly correlated with 2010 concentrations, with a slope estimate close to 1 (TDP: slope = 1.09 $\pm$ 0.15, $R^2 = 0.83$, $P < 0.01$; TDN: slope = 0.91 $\pm$ 0.15, $R^2 = 0.78$, $P < 0.01$). Results of all analyses were the same regardless of whether these three sites were included or excluded. We therefore report results with all sites included. We used the first principal component axis of the combined TDP and TDN values, which explained 89% of the variance, for the nutrient value in the models (package 'stats' in R).

Analytical Approach, Hypotheses and Model Descriptions

We used an information-theoretic approach to compare among models with one of four abiotic variables (temperature, DTR, pond-drying, and the first principle component of the total dissolved nitrogen and phosphorous levels) and test targeted hypotheses about host and parasite dynamics (Burnham and Anderson 2002). For response variables measured multiple times over the season (e.g., snail density, infection prevalence in snails), models also included an effect of season (early vs. late summer) and its interaction with each abiotic variable (Table 1). Snail body size is known to influence trematode cercariae release primarily by dictating the amount of available space for rediae or sporocyst growth (Karvonen et al. 2006; Morley et al.

Table 1. A List of All Models Compared by AIC for Each of the Analyses (Described in Italics).

Temp[a]	TbS[b]	DTR[c]	DTRbS[d]	Dry[e]	DbS[f]	Nut[g]	NutbS[h]	S[i]	Pond	Inf[j]
Prevalence of snail Ribeiroia *or* Echinostoma										
1	0	0	0	0	0	0	0	0	1	0
0	1	0	0	0	0	0	0	0	1	0
0	0	1	0	0	0	0	0	0	1	0
0	0	0	1	0	0	0	0	0	1	0
0	0	0	0	1	0	0	0	0	1	0
0	0	0	0	0	1	0	0	0	1	0
0	0	0	0	0	0	0	0	1	1	0
0	0	0	0	0	0	0	0	0	1	0
Snail density										
1	0	0	0	0	0	0	0	0	1	0
0	1	0	0	0	0	0	0	0	1	0
0	0	1	0	0	0	0	0	0	1	0
0	0	0	1	0	0	0	0	0	1	0
0	0	0	0	1	0	0	0	0	1	0
0	0	0	0	0	1	0	0	0	1	0
0	0	0	0	0	0	1	0	0	1	0
0	0	0	0	0	0	0	1	0	1	0
0	0	0	0	0	0	0	0	1	1	0
0	0	0	0	0	0	0	0	0	1	0
Abundance of Ribeiroia *or* Echinostoma *cercariae*										
1	0	0	0	0	0	0	0	0	1	0
1	0	0	0	0	0	0	0	1	1	0
0	0	1	0	0	0	0	0	0	1	0
0	0	1	0	0	0	0	0	1	1	0
0	0	0	0	1	0	0	0	0	1	0
0	0	0	0	1	0	0	0	1	1	0
0	0	0	0	0	0	1	0	0	1	0
0	0	0	0	0	0	1	0	1	1	0
0	0	0	0	0	0	0	0	1	1	0
0	0	0	0	0	0	0	0	0	1	0
Tadpole stage										
1	0	0	0	0	0	0	0	0	1	0
0	0	1	0	0	0	0	0	0	1	0
0	0	0	0	1	0	0	0	0	1	0
0	0	0	0	0	0	1	0	0	1	0
0	0	0	0	0	0	0	0	0	1	0
Metacercariae of Ribeiroia *or* Echinostoma *and prevalence of deformities*										
1	0	0	0	0	0	0	0	0	1	1
0	0	1	0	0	0	0	0	0	1	1
0	0	0	0	1	0	0	0	0	1	1

Table 1. continued

Temp[a]	TbS[b]	DTR[c]	DTRbS[d]	Dry[e]	DbS[f]	Nut[g]	NutbS[h]	S[i]	Pond	Inf[j]
0	0	0	0	0	0	1	0	0	1	1
0	0	0	0	0	0	0	0	0	1	1
0	0	0	0	0	0	0	0	0	1	0

A 1 indicates that the predictor variable was included additively in the model, while a 0 indicates that it was not included in that model. Pond was included as a random effect even in the nonseasonal models because individuals were used as replicates.

[a]Temperature.

[b]Interaction with temperature and either season (prevalence, snail density) or snail size (cercariae abundance).

[c]Diurnal temperature range.

[d]Interaction with DTR and either season (prevalence, snail density) or snail size (cercariae abundance).

[e]Pond-drying.

[f]Interaction with drying and either season (prevalence, snail density) or snail size (cercariae abundance).

[g]Nutrients.

[h]Interaction with nutrients and either season (prevalence, snail density) or snail size (cercariae abundance).

[i]Season—either early or late (prevalence, snail density) or Size for cercariae abundance analysis.

[j]Measure of infection: for metacercariae, this value is the density of infected snails, while for deformities this value is the mean number of metacercariae in metamorphs collected from the site.

2010). We therefore included an additive effect of snail size into models of cercariae release rate. Past work has demonstrated no effect of tadpole density on per capita tadpole infection in the field (Johnson et al. 2013). Here, we also found no relationship between tadpole density and the per capita number of metacercariae in metamorphs from each site; therefore tadpole density was not used as a predictor in our models. Each set of compared models also included one null model with only an intercept, and another model with only the direct upstream driver of the response (e.g., density of infected snails for metacercariae analyses, *Ribeiroia* metacercariae abundance for deformity analyses Table 1). Analyses of infection prevalence and snail density included 18 sites, while those analyzing tadpole stage, metacercariae abundance and deformities included 16 sites due to a lack of amphibians at 2 sites. Analyses of the number of cercariae released per snail included 14 sites for *Ribeiroia,* and 9 sites for *Echinostoma*, since it was only possible to include sites in which snails with mature infections were collected.

We developed hypotheses for each abiotic variable based on previous laboratory experiments and the literature, with the goal of determining the relative importance of each variable on the two parasites (*Ribeiroia ondatrae* and *Echinostoma* spp.), and their respective states (snail infection prevalence, cercariae release from snails, amphibian infection abundance and deformities). We also consider the factors influencing snail host population density and amphibian development since these can also influence parasite infection. First, we hypothesized that increasing temperature would increase the developmental rates of parasites, snails and tadpoles, but also increase mortality and potentially reduce snail density (Paull and Johnson 2011; Paull et al. 2012). This would translate into an increase in the observed parasite prevalence in snails. However, given that warmer temperatures can also reduce snail densities and increase tadpole development, if the increases in prevalence do not out-pace these mitigating factors, we would expect a reduction in amphibian infections or deformities with rising temperatures (Studer et al. 2010). Second, we hypothesized that higher DTR would accelerate parasite and tadpole development rates if oscillating around the optimal performance temperature for those species, or slow development if that increase in range tended to exceed such optima (Paaijmans et al. 2009; Rohr et al. 2013). Based on previous experimental studies, the optimal temperature for *Ribeiroia* cercariae release is $\sim$ 22 °C (Paull et al. 2015). Third, we recognized the potential for high rates of pond-drying to have conflicting effects on infection. If reduced water levels concentrated hosts and parasites closer together, transmission would likely increase and lead to greater infection and pathology (Kiesecker and Skelly 2001); however, if reduced water levels caused greater mortality of parasites (O'Connor et al. 2008; Martinaud et al. 2009) or snails (Thomas and McClintock 1996; Sandland and Minchella 2004) and/or

accelerated amphibian larval development (Doughty and Roberts 2003; Koprivnikar et al. 2014), infection loads in emerging frogs could decrease. Fourth and finally, we expected nutrients to alter the virulence of parasites (Aalto et al. 2015), or to affect infection indirectly by increasing algal growth, which can promote snail densities or their production of cercariae (Johnson et al. 2007), thereby increasing amphibian infections.

We conducted repeated-measures analyses in R (R Core Team 2015) for each of the response variables that was measured more than once over the season. Pond identity was included as a random intercept term, thereby accounting for the nonindependence of samples collected from the same site over time. We used a generalized linear mixed effects model (GLMM) with a binomial distribution (lme4 package) to model the proportion of infected snails (using the R function 'cbind' to analyze individual-level 'successes' and 'failures') and a GLMM with a negative binomial distribution (glmmADMB package) to model snail density and the number of parasites released per snail (Zuur et al. 2009). Because of low overall infection prevalence values, we combined May and June values of snail infection and snail density, and compared them to the combined values of July and August values, thereby focusing on the contrast between early and late summer. For analyses of amphibian responses, we used a linear mixed effects model (nlme package) to model changes in tadpole development stage, a GLMM with a negative binomial distribution (glmmADMB package) to model amphibian infection intensities, and a GLMM with a binomial distribution (lme4 package) to model deformity prevalence (using cbind to analyze individual-level successes and failures). Amphibian responses (tadpole stage, infection abundance and deformities) were analyzed at the individual host level with site as a random intercept term to account for the nonindependence of animals collected from the same pond.

We checked for multicollinearity (R package HH) and spatial autocorrelation in the residuals (R package spdep) using Moran's I with distance to nearest neighbor set at 10 km to reflect the average foraging flight distance of waterbirds such as egrets and herons (Kelly et al. 2008). We selected the best models (R package MuMIn) following the methods of Burnham and Anderson (2002). Thus, rather than considering all possible models, we developed a subset of models based on previous research and theory to predict changes to transmission (Table 1). We used Type II sums of squares to determine the individual significance of all terms in the final models (R package car).

Results

Site Characteristics and Model Evaluations

Mean summer water temperatures across sites (May-August) averaged 21.9 °C, SD = 1.8 °C (average site temperatures ranged from 18–25 °C), while diurnal temperature range

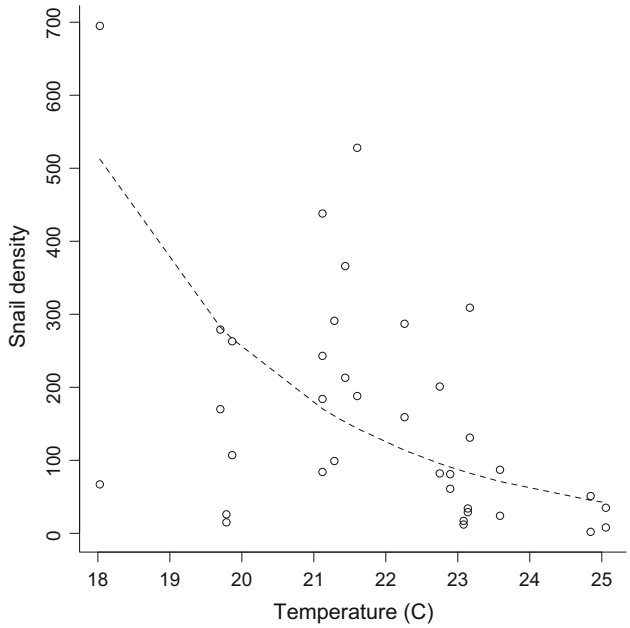

Figure 1. Snail density (number of snails per 1 m sweep) declines with mean water temperature at a site. The dashed line shows the predicted values from the best fit model (R^2 = 0.15). Points indicate the mean values for both early and late-season visits to each site.

(DTR) (mean difference between the daily maximum and minimum recorded temperatures across the season) averaged 8.8 °C, SD = 3.1 °C (range from 3.3 to 16.6 °C). Factors influencing this between-site variability in diurnal temperature range could include variation in cover by pond surface vegetation or pond depth. On average, ponds decreased in surface area between July and August by 16.4% (range = 0–55%) as drying progressed throughout the summer. Total dissolved phosphorus concentrations averaged 0.07 mg/L, SD = 0.07 mg/L (range from 0.01 to 0.25 mg/L), giving a range in Trophic State Index from 37 to 83 (Carlson 1977), suggesting the wetlands range from oligotrophic to hypereutrophic. TDN concentrations averaged 1.1 mg/L, SD = 0.66 mg/L (range from 0.44 to 3.2 mg/L). We found no evidence for multicollinearity among predictor variables, for which variance inflation factors ranged from 1.1 to 1.6. We also found no evidence for spatial autocorrelation in residuals of the best-supported models (Moran's I $P > 0.33$ for all models), suggesting that nonspatial models were adequate.

Snail infection patterns

Snail density declined strongly with rising temperature (Coef = -0.65, $\chi^2 = 10.6$, $df = 1$, $P < 0.01$), such that an increase in temperature from 20 to 25 °C was associated with a nearly 6-fold reduction in snail density (Fig. 1). The best-supported model for *Ribeiroia* infection prevalence in snails included the temperature-by-season interaction (Temp: Coef = -0.8, $\chi^2 = 3.5$, $df = 1$, $P = 0.06$; Season: Coef = 0.2, $\chi^2 = 1.1$, $df = 1$, $P = 0.30$; Temp-by-Season: Coef = 1.6, $\chi^2 = 37.9$, $df = 1$, $P < 0.01$), such that prevalence increased faster in warmer ponds over the season (Table 2; Fig. 2). The best-supported model for *Echinostoma* infection prevalence had a DTR-by-season interaction (DTR: Coef =2.2, $\chi^2 = 7.1$, $df = 1$, $P = 0.01$; Season: Coef = 2.4, $\chi^2 = 6.5$, $df = 1$, $P = 0.01$; DTR-by-Season: Coef =-1.5, $\chi^2 = 65.6$, $df = 1$, $P < 0.01$) such that echinostome prevalence declined more strongly at sites with high DTR (Fig. 3). Results are similar when the outlier is excluded. The number of *Ribeiroia* and *Echinostoma* cercariae released per snail increased as a function of snail size (*Rib.:* Coef = 0.2, $\chi^2 = 12.2$, $df = 1$, $P < 0.01$; *Echino.:* Coef = 0.2, $\chi^2 = 12.6$, $df = 1$, $P < 0.01$; Fig. 4), with the top model for *Ribeiroia* also including a nonsignificant positive trend with DTR (Coef = 0.35, $\chi^2 = 3.1$, $df = 1$, $P = 0.08$). Note that when the 3 snails from the outlier site with the highest *Echinostoma* cercariae release rate are excluded, the top model shifts to include both snail size and a nonsignificant positive trend for an effect of DTR.

Table 2. Best-supported Models (ΔAICc < 2) for Glmms Describing the Binomially Distributed Prevalence of Infected Snails Using Individual Successes and Failures as Replicates.

Response	Model[a]	Log(*L*)[b]	K[c]	AICc[d]	ΔAICc[e]	*w*[f]
Ribeiroia prevalence in snails	Temp.-by-Season	− 69.5	5	151.4	0	1
Echinostome prevalence in snails	DTR-by-Season	− 82.2	5	176.3	0	0.75
	Temp.-by-Season	− 83.3	5	178.5	2.2	0.25
Ribeiroia released per snail	DTR & Size	− 775.6	5	1561.6	0	0.30
	Size	− 776.8	4	1561.9	0.2	0.27
	Temp. & Size	− 776.0	5	1562.5	0.9	0.19
	Nut. & Size	− 776.4	5	1563.3	1.6	0.13
Echinostomes released per snail	Size	− 523.0	4	1054.6	0	0.34
	Nut. & Size	− 522.4	5	1055.5	0.9	0.22
	Dry & Size	− 522.6	5	1055.9	1.3	0.18
	Temp. & Size	− 622.7	5	1056.2	1.7	0.15
Snail density	Temp.	− 213.8	4	437	0	0.77

Results are also shown for glmms with negative binomial distributions describing the number of parasites released per infected snail (analyzed at the individual level), and changes to snail density over the season (analyzed at the site level). All models included a random effect of site.

[a]List of predictor variables included in the best models.

[b]Log likelihood.

[c]Number of parameters.

[d]Akaike information criterion corrected for small sample size.

[e]Difference in AICc value between the best ranked model and the current model.

[f]Akaike weight for the model.

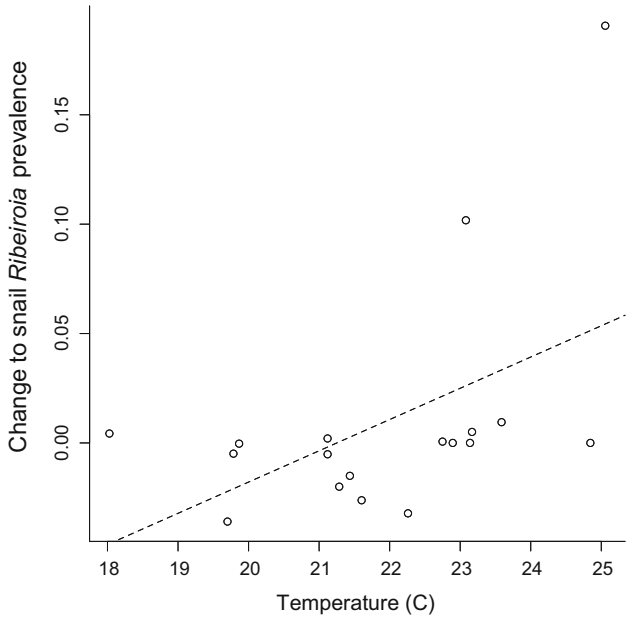

Figure 2. Change in the prevalence of *Ribeiroia*-infected snails (late–early season) as a function of mean water temperature over the season. Because temperature affected the rate of change in prevalence across the season, we represent the seasonal change as the difference between early and late-season measures. Each point represents the average at a single site, and the fitted lines represent the linear relationship between change in *Ribeiroia* prevalence and temperature with (dashed, relationship nonsignificant) and without the outlier ($R^2 = 0.24$).

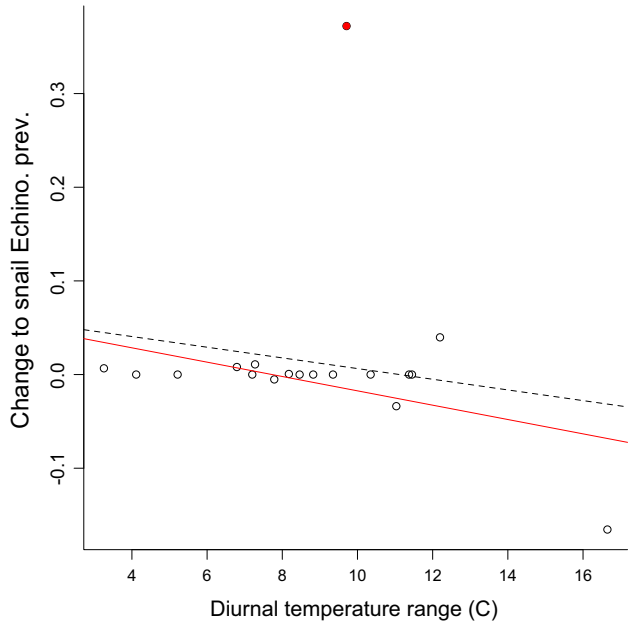

Figure 3. Effects of mean DTR on the change in the prevalence of echinostome-infected snails (late – early season) at each wetland (filled red circle shows outlier). Because temperature affected the rate of change in prevalence across the season, we represent the seasonal change as the difference between early and late-season measures. Each point represents the average at a single site, and the fitted line is the linear relationship between change in *Echinostoma spp.* prevalence and DTR (dashed black line is fitted with outlier: $R^2 = 0.03$, solid red line is fitted without outlier, $R^2 = 0.30$)

Amphibian infection, pathology, and development

The top model explaining developmental Gosner stage of tadpoles collected at sites in May included a positive effect of pond-drying, but this was not a significant relationship (Coef = *1.1*, $\chi^2 = 1.9$, $df = 1$, $P = 0.16$; Fig. S2). Mean infection abundance in *P. regilla* at a site ranged from 0.1 to 56.1 metacercariae per host (*Ribeiroia*) and 0.6–606.5 metacercariae per host (echinostomes). The best-supported model for predicting *Ribeiroia* infection included a negative effect of accelerated drying and a nonsignificant positive relationship with the density of *Ribeiroia*-infected snails (Table 3; Fig. 5; Drying: Coef = − 1.0, $\chi^2 = 6.9$, $df = 1$, $P < 0.01$; Density *Ribeiroia* snails: Coef = 0.5, $\chi^2 = 1.7$, $df = 1$, $P = 0.20$). The model including only the density of echinostome-infected snails was the best model explaining echinostome infection load in amphibians (Table 3; Coef = 0.9, $\chi^2 = 8.7$, $df = 1$, $P < 0.01$).

We examined an average of 186 metamorphic *Pseudacris regilla* per site at *Ribeiroia*-positive sites, for which the overall deformity prevalence ranged from 0 to 41% of examined individuals (mean: 13 ± 4% (SE), grand total of frogs examined = 2,983). At sites where *Ribeiroia* was not detected in metamorphs ($N = 2$), deformity prevalence averaged 3.6 ± 1% (SE). The best-supported model to predict *P. regilla* deformities included a positive effect of both *Ribeiroia* infection intensity and nutrient concentrations (Table 3; Fig. 6; Nut: Coef = − 0.7, $\chi^2 = 23.5$, $df = 1$, $P < 0.01$; Rib: Coef = 0.9, $\chi^2 = 45.9$, $df = 1$, $P < 0.01$). Note that because both TDP and TDN loaded negatively onto this axis, negative coefficients represent a positive effect of nutrients.

Discussion

Our study explored the relative importance of abiotic variables at multiple points in the infection processes of two complex life cycle trematode parasites (e.g., snail infection, amphibian infection, and amphibian pathology). Results were broadly similar between the two species,

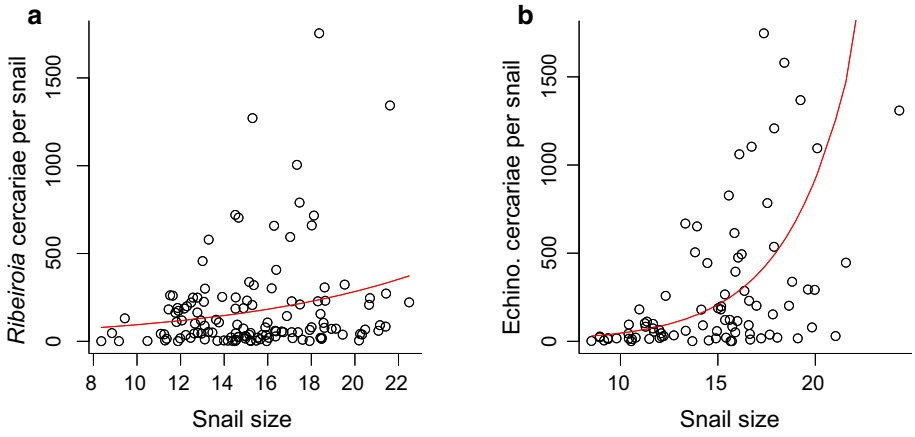

Figure 4. Snail size (mm) positively predicted the number of *Ribeiroia* (**a** $R^2 = 0.04$) and *Echinostoma spp.* (**b** $R^2 = 0.19$) released per snail. Fitted line shows results of negative binomial generalized linear model.

reflecting their similar transmission modes and life cycles. We found that the prevalence of infected snails increased more quickly over the season in warmer ponds for both parasites, but that snail density was negatively associated with rising temperature. Higher DTR was also associated with reductions in echinostome prevalence in snails, possibly due to time spent at temperatures that are suboptimal for parasite development. The risk of *Ribeiroia*-induced deformities associated positively with the average number of parasites per frog and the concentrations of nutrients, while accelerated pond-drying tended to negatively influence malformation risk. This latter effect could stem from the reduced duration of tadpole exposure with accelerated drying, which was consistent with a trend for faster tadpole development at drying sites. The alternative hypothesis—that drying reduced the per-snail release of cercariae—was not supported by our data for either trematode species. Although parasite release per snail is rarely measured, its inclusion here allowed us to explicitly test multiple causal pathways with respect to host exposure. Collectively, our results suggest that drying and eutrophication may overwhelm any temperature effects in the arid and eutrophic systems in which we conducted our study.

Temperature had strong but contrasting effects on snail infection prevalence and total snail density, such that temperature-driven increases in prevalence were counteracted by declines in snail density over the season. Thus, even while snail infection prevalence increased over the summer, these effects were likely offset by the overall decrease in snail density. Increases in *Ribeiroia* prevalence at warmer sites is consistent with laboratory work in this system showing temperature-driven increases to *Ribeiroia* development within snails, and declines in snail density at warm ponds may be due to reduced snail survival (Paull and Johnson 2011). This faster increase in prevalence at warm sites did not result in a direct effect of temperature on the prevalence of deformities, despite the fact that exposure of tadpoles at earlier developmental stages is associated with greater deformity risk (Johnson et al. 2011; Paull et al. 2012). However, if snail mortality due to warm temperatures caused parasite production to peak prior to this vulnerability window for amphibians, we would not expect to see temperature-driven increases to deformities (Paull and Johnson 2014).

Our results demonstrate the potential for drying to moderate the effects of infected snail density on amphibian infection abundance. Amphibian infections were best predicted by the density of infected snails, consistent with the life cycle of these trematodes, but drying also negatively influenced the average abundance of *Ribeiroia* metacercariae per frog after accounting for snail-mediated effects. Our data suggest that the release of parasites was not affected by pond-drying, indicating that the reductions in amphibian infection were not driven by corresponding reductions in the per-snail release of *Ribeiroia* cercariae. Rather, the data on tadpole developmental stage suggested a weak trend for *P. regilla* to be at later stages in ponds with greater drying (Fig. S2), suggesting that faster development may have helped them 'escape' exposure to *Ribeiroia* cercariae during particularly vulnerable periods of limb growth (Stopper et al. 2002; Schotthoefer et al. 2003; Rohr et al. 2010; Johnson et al. 2011). This trend for larval amphibians to be more developed in ponds with greater drying may have been weak in our data because of the tendency for multiple cohorts of tadpoles to be present in the pond at the same time. Larvae of many anuran species accelerate development rates in response to pond-drying (Newman 1992; Denver 1998; Doughty and Roberts 2003).

Table 3. Best-supported Models (ΔAICc < 2, or $AIC_c \geq$ Null) for Glmms Describing Amphibian Infections with a Negative Binomial Distribution and Amphibian Deformity Prevalence with a Binomial Distribution.

Response	Model[a]	Log(*L*)[b]	K[c]	AICc[d]	ΔAICc[e]	*w*[f]
Ribeiroia metacercariae	Dry + DRS[g]	− 474.1	5	958.5	0	0.71
Echinostome metacercariae	DES[h]	− 521.4	4	1051	0	0.40
Tadpole developmental stage	Dry	− 1557.5	4	3123	0	0.32
	Temp.	− 1557.9	4	3123.9	0.86	0.20
	Null	− 1559.1	3	3124.2	1.18	0.17
Deformities	Nut. + *Rib.* metacerc.	− 49.6	4	110.7	0	0.99

Results are also shown for a linear mixed effects model of tadpole developmental stage. All analyses used individual amphibians as the replicates, and included site as a random effect.

[a]List of predictor variables included in the best models.

[b]Log likelihood.

[c]Number of parameters.

[d]Akaike information criterion corrected for small sample size.

[e]Difference in AICc value between the best ranked model and the current model.

[f]Akaike weight for the model.

[g]Density of *Ribeiroia*-infected snails.

[h]Density of echinostome-infected snails.

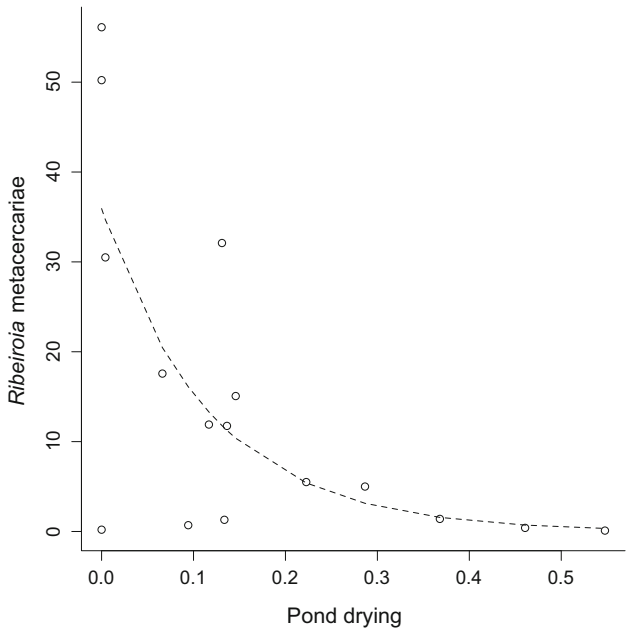

Figure 5. Relationship between the number of *Ribeiroia* metacercariae recovered from individual *P. regilla* metamorphs, and drying, measured as the proportional change in area from July to August. The dashed line shows the predicted values from a negative binomial generalized linear model of the mean site values ($R^2 = 0.46$).

The current findings also provide insights into the relationship between host infection and the resultant level of pathology (i.e., host tolerance) (Råberg et al. 2009). Both field and experimental work have shown that amphibian limb deformities are positively related to *Ribeiroia* infection loads (Johnson et al. 1999; Johnson et al. 2002), because cercariae induce intensity-dependent pathology, such that heavily infected hosts are more likely to become malformed or die prior to metamorphosis. Our data show that both amphibian infection loads and nutrient concentrations were associated with higher deformity prevalence at wetlands. While infection load is often the most important component of pathology risk, high nutrient levels may have further increased pathology due to changes in host tolerance—or the ability to limit damage caused by a given parasite burden—and/or parasite virulence (Råberg et al. 2009). When nutrient concentrations reach above-optimal conditions, further increases can lead to a decline in host condition due to the hypoxic water or directly toxic effects of nitrogenous compounds on the growth and development of amphibian larvae (Jofre and Karasov 1999; Marco and Blaustein 1999; Peltzer et al. 2008). Under such conditions, hosts may have limited capacity to support the tissue repair mechanisms associated with host tolerance of parasites. Nutrients could also contribute to elevated parasite virulence by increasing nitrogen resources available to the pathogen (Mitchell et al. 2003; Johnson et al. 2010; Aalto et al. 2015). Importantly, however, the link between deformity prevalence and nutrient concentrations was not due to increased host vulnerability, given that there was no relationship between nutrients and tadpole developmental stage at our sites.

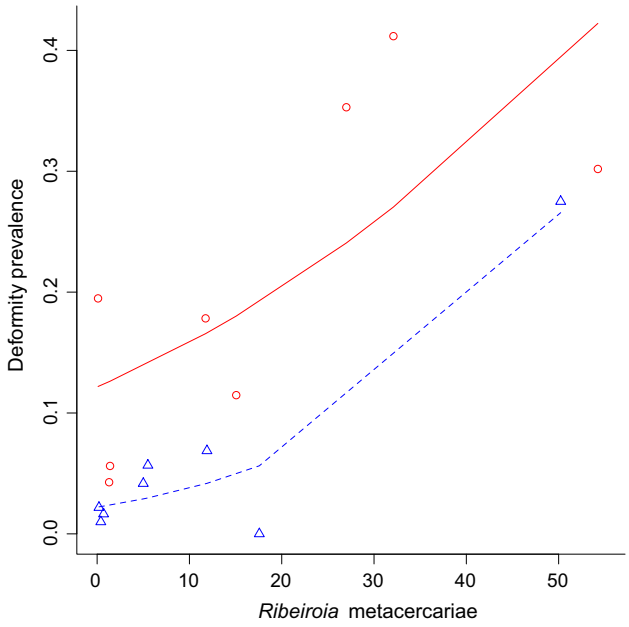

Figure 6. The prevalence of *P. regilla* with deformities at a wetland increased with the abundance of *Ribeiroia* metacercariae. The prevalence of deformities at a given infection abundance was greater at sites with nutrient concentrations above the median (red points) than those with below-median nutrients (blue triangles). The lines show the predicted values for a binomial generalized linear model fit separately to the mean values at sites with high (red solid line, $R^2 = 0.48$) and low (blue dashed line, $R^2 = 0.91$) nutrient concentrations.

Environmental change is multidimensional and studies that explore interactions among these factors within complex systems will be key for understanding their net influence on ecosystems and species. Several studies speculate that shifts in temperature, extreme climate events (e.g., drought), and nutrient-loading may interact to enhance a variety of diseases (Horák and Kolářová 2011; Okamura et al. 2011). For instance, warmer temperatures can enhance the prevalence and severity of proliferative kidney disease in fish, while eutrophication promotes the growth of its alternate host, bryozoans (Okamura et al. 2011). Field studies incorporating each stage of the infection process can further clarify when environmental factors are likely to interact positively, negatively, or not at all. Our results suggest that increased drying rates may actually have a mitigating effect on trematode parasite infections in pond systems subjected to high rates of drying. Eutrophication, however, exacerbated the pathology experienced by amphibians as a result of *Ribeiroia* infection, indicating that this factor may be more important to consider for management strategies in the study region.

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