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Geophysiology of Wood Frogs: Landscape Patterns of Prevalence of Disease and Circulating Hormone Concentrations across the Eastern Range

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Synopsis One of the major challenges for conservation physiologists is to determine how current or future environmental conditions relate to the health of animals at the population level. In this study, we measured prevalence of disease, mean condition of the body, and mean resting levels of corticosterone and testosterone in a total of 28 populations across the years 2011 and 2012, and correlated these measures of health to climatic suitability of habitat, using estimates from a model of the ecological niche of the wood frog's geographic range. Using the core-periphery hypothesis as a theoretical framework, we predicted a higher prevalence and intensity of infection of *Batrachochytrium dendrobatidis* (*Bd*) and ranaviruses, two major amphibian pathogens causing disease, and higher resting levels of circulating corticosterone, an indicator of allostatic load incurred from living in marginal habitats. We found that *Bd* infections were rare (2% of individuals tested), while infections with ranavirus were much more common: ranavirus-infected individuals were found in 92% of ponds tested over the 2 years. Contrary to our predictions, rates of infection with ranaviruses were positively correlated with quality of the habitat with the highest prevalence at the core of the range, and plasma corticosterone concentrations measured when frogs were at rest were not correlated with quality of the habitat, the prevalence of ranavirus, or the intensity of infection. Prevalence and mean viral titers of ranavirus infection were higher in 2012 than in 2011, which coincided with lower levels of circulating corticosterone and testosterone and an extremely early time of breeding due to relatively higher temperatures during the winter. In addition, the odds of having a ranavirus infection increased with decreased body condition, and if animals had an infection, viral titers were positively correlated to levels of circulating testosterone concentration. By resolving these patterns, experiments can be designed to test hypotheses about the mechanisms that produce them, such as whether transmission of the ranavirus and tolerance of the host are greater or whether virulence is lower in populations within core habitats. While there is debate about which metrics serve as the best bioindicators of population health, the findings of this study demonstrate the importance of long-term monitoring of multiple physiological parameters to better understand the dynamic relationship between the environment and the health of wildlife populations over space and time.

Introduction

One of the major challenges for conservation physiologists is to assess how current or future environmental conditions affect animals' health across spatial scales (Wikelski and Cooke 2006; Cooke et al. 2013). While the overwhelming majority of studies measure the effects of natural or experimental variation in environmental conditions on physiology or disease at the individual level (Ostfeld et al. 2005;

Crespi et al. 2013), it is important to relate broad-scale environmental variation (e.g., patterns of climate and of land-use) to indicators of population-level health, such as incidence or prevalence of disease, reductions in physiological performance, or means of some measure of physiological stress (i.e., allostatic load) (McEwen and Wingfield 2003). Landscape epidemiological studies, for example, have revealed important mechanisms that underlie the risks for disease, and thus, drive interactions between the environment and host–pathogen dynamics (Ostfeld et al. 2005; Emmanuel et al. 2011; Meentemeyer et al. 2012). Similarly, studies of landscape physiology (i.e., macrophysiology) (Chown et al. 2004; Osovitz and Hofmann 2007) have also provided novel insights into organism–environment interactions or responses to climatic change that would otherwise not present themselves by focusing on individual-level responses (Gaston 2003; Somero 2005; Gaston et al. 2009; Huey et al. 2009).

Concepts of the health and physiological performance of animals have been an inherent component of biogeographic and macroecological ideas surrounding species' geographic distributions (Gaston 2003). The limits of species' ranges are set by species-specific physiological tolerances, ecological, and evolutionary dynamics (e.g., Kirkpatric and Barton 1997; Holt and Keitt 2005; Holt et al. 2005; Bridle and Vines 2007; Wiens 2011; Cahill et al. 2014; Kubisch et al. 2014). A common hypothesis explaining broad macroecological patterns in the dynamics of species' ranges is the core-periphery hypothesis (CPH), also called core-edge, center-abundance, or central-marginal hypothesis. The CPH, which has been called a "general rule" in biogeography (Hengeveld and Haeck 1982; Brown 1984; Brown et al. 1995) and a common feature shared by all species (Guo et al. 2005; reviewed by Sagarin et al. 2006), predicts that patterns of abundance across a species' range result from spatial variation in environmental quality that varies in predictable ways, from high-quality core regions to low-quality peripheral/edge regions. The CPH framework allows us to make specific predictions about the ways in which animals' health varies across a species' range. For example, several empirical studies have linked the spread of disease to low levels of genetic diversity in natural founder populations or in populations that have high incidences of inbreeding (reviewed by King and Lively 2012). Given that populations at the edge of species' ranges exhibit lower genetic diversity (Brown 1984; Eckert et al. 2008; Kawecki 2008; Micheletti and Storfer 2015) and might be at the limit of their physiological tolerance (and therefore at high allostatic loads), there also might be greater incidence of disease among populations at the edge of the range. Yet several other studies have failed to find a correlation between optimal performance and core habitats, suggesting that the relationships between environmental quality, genetic diversity, and health are much more complex than originally believed (Sagarin and Gaines 2002; Gaston 2003).

In this study, we took an integrative approach to assess the health of populations of wood frogs by measuring disease-related and physiological traits of adult males within the framework of the CPH. Wood frogs provide an ideal system in which to address these issues because these animals are common and widespread, their distribution is well studied, and populations exist in diverse climates and degrees of urbanization. We assessed spatial patterns of prevalence and intensity of infection (as measured by viral titers in the hosts) of two of the major pathogens of concern for amphibian populations, the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), and viruses in the genus *Ranavirus*, of adult male wood frogs from 28 populations. We also explicitly related patterns of environmental quality to physiological traits of individuals, such as body condition, circulating concentrations both of corticosterone as a measure of energetic state and allostatic load, and of testosterone as a measure of reproductive effort.

We had different predictions for the two pathogens. Frog Virus 3 (FV3) is the type virus for the genus *Ranavirus* (family *Iridoviridae*) which are emerging viruses of fish, reptiles, and amphibians (Granoff et al. 1965; Chinchar 2002). FV3-like virus isolates are often lethal to larval amphibians, but adults of many species are resistant or can be sublethal carriers, at least in the laboratory; little is known about infections in adults in natural populations (Wolf et al. 1968; Gray et al. 2009). Wood frogs, however, are easily infected and rapidly succumb to FV3 infection as both larvae and metamorphs (Haislip et al. 2011), so we predicted that ranavirus infections would be rare in adult frogs. By contrast, *Bd* is thought to be very common in North America (e.g., Richards-Hrdlicka et al. 2013). While *Bd* has caused massive declines and extinctions in amphibian communities in many parts of the world (Skerratt et al. 2007), many frog species can persist with *Bd* infections. The outcome of *Bd* infection depends on complex interactions between the strain of *Bd* and the host's thermal physiology, skin microbial community, and secreted antimicrobial peptides among other factors (Woodhams et al. 2007; Rollins-Smith et al. 2011; Rowley and Alford 2013).

Previous studies have documented sublethal *Bd* infections in many frog species within the geographical area surveyed in this study, including wood frogs (e.g., Longcore et al. 2007; Richards-Hrdlicka et al. 2013); therefore, we predicted *Bd* to be more widespread than ranavirus in adult wood frogs in our study.

Based on the CPH framework, we predicted that the prevalence and intensity of these pathogens would be relatively higher at the edge of the geographic range or in areas with poor environmental quality because those populations would be smaller, and thus have reduced genetic variation, and environments would be physiologically more challenging to inhabit (e.g., warmer climates at the southern edge of the range). Specifically, we expected that animals in environments of poor quality would show decreased body condition and increased corticosterone concentrations in their blood when measured at rest, which tends to be elevated when animals experience stressful environments, past or present (reviewed by Crespi et al. 2013), and tends to be negatively correlated to incidence and severity of disease (Sapolsky et al. 2000; Dhabhar 2002). Finally, although difficult to discern from a cross-sectional (rather than longitudinal) study, differences in prevalence of infection might reflect underlying differences in transmission dynamics resulting from differences in host density or habitat structure between the core and periphery. Alternatively, the frequency of *Bd* or ranavirus infection could vary with latitude or elevation rather than with environmental quality per se because viral replication rates and host susceptibility has been related to temperature and precipitation (*Bd*: Piovia-Scott et al. 2011; Raffel et al. 2015; ranavirus: Langdon 1989; Allender et al. 2013; reviewed by Brunner et al. 2015).

Methods

Study species

The range of the wood frog (*Lithobates sylvaticus*) spans nearly 40° in latitude (33°–70° N), from Alabama to New Brunswick and Nova Scotia in the east and north to the Arctic Circle and Alaska in the west (Conant and Collins 1998) (Fig. 1). There are two evolutionary clades within the species (Lee-Yaw et al. 2008): the eastern clade, which is the focus of this study, is distributed across the eastern United States and north to the maritime provinces of Canada; the range of the western clade includes the northern parts of the American Midwest, the Great Lakes region, and western Canada. Wood frogs are explosive breeders that emerge in late winter/early spring to breed in wetlands and vernal pools, laying eggs in masses (~300–1500 eggs per female) just underneath the surface of the water around the edges of vernal pools and in fishless wetlands (Conant and Collins 1998). Males typically aggregate at ponds before females arrive, and breeding occurs for 1–7 days, after which frogs disperse into forested habitats for the remainder of the year (Berven and Grudzien 1990).

Collections

In the early spring of 2011 and 2012, from January to April depending on breeding phenology across latitudes, we collected wood frogs returning to ponds or just having arrived at ponds to breed (Table 1). We collected only males because of their reduced demographic importance compared with egg-laying females, and because we wanted to reduce variation in our samples (i.e., avoid differences in circulating hormones between the sexes) while maximizing the sample size of frogs we could collect. Frogs were collected by hand or with dip nets during peak breeding activity (17:00 to 1:00) from 22 active breeding ponds in 2011 and 18 in 2012 that spanned the entire eastern clade (resolved by Lee-Yaw et al. 2008), from Alabama through Maine, USA and into Nova Scotia, Canada (Fig. 1, Table 1; see Supplementary Table S1 for information about collection permits). Breeding ponds were selected at random within a targeted region such that we had variation in environmental suitability from core to edge habitats throughout the range, based on ecological projections of the niche model (see below).

Upon collection, dermal swabs for the detection of *Bd* were taken following standard protocols (Hyatt et al. 2007) and stored in 70% ethanol until extractions of DNA could be conducted for *Bd* analysis. We also measured snout-to-vent length (SVL), femur length, and mass of the body, which we used to calculate body condition as the residuals of mass against SVL. Frogs were individually housed in opaque plastic shoeboxes overnight for approximately 8 h to allow the frogs to return to a resting state (evidenced by the fact that frogs were typically not moving, heart rates were low, and they did not resist handling the next morning) and to standardize the handling time needed to collect blood samples from frogs, as the time it took to catch frogs upon arriving at a breeding pond varied within and across sites. After this time, 20% benzocaine solution (Extra-strength Oragel) was applied to the ventral throat and abdomen and the frogs left in their box

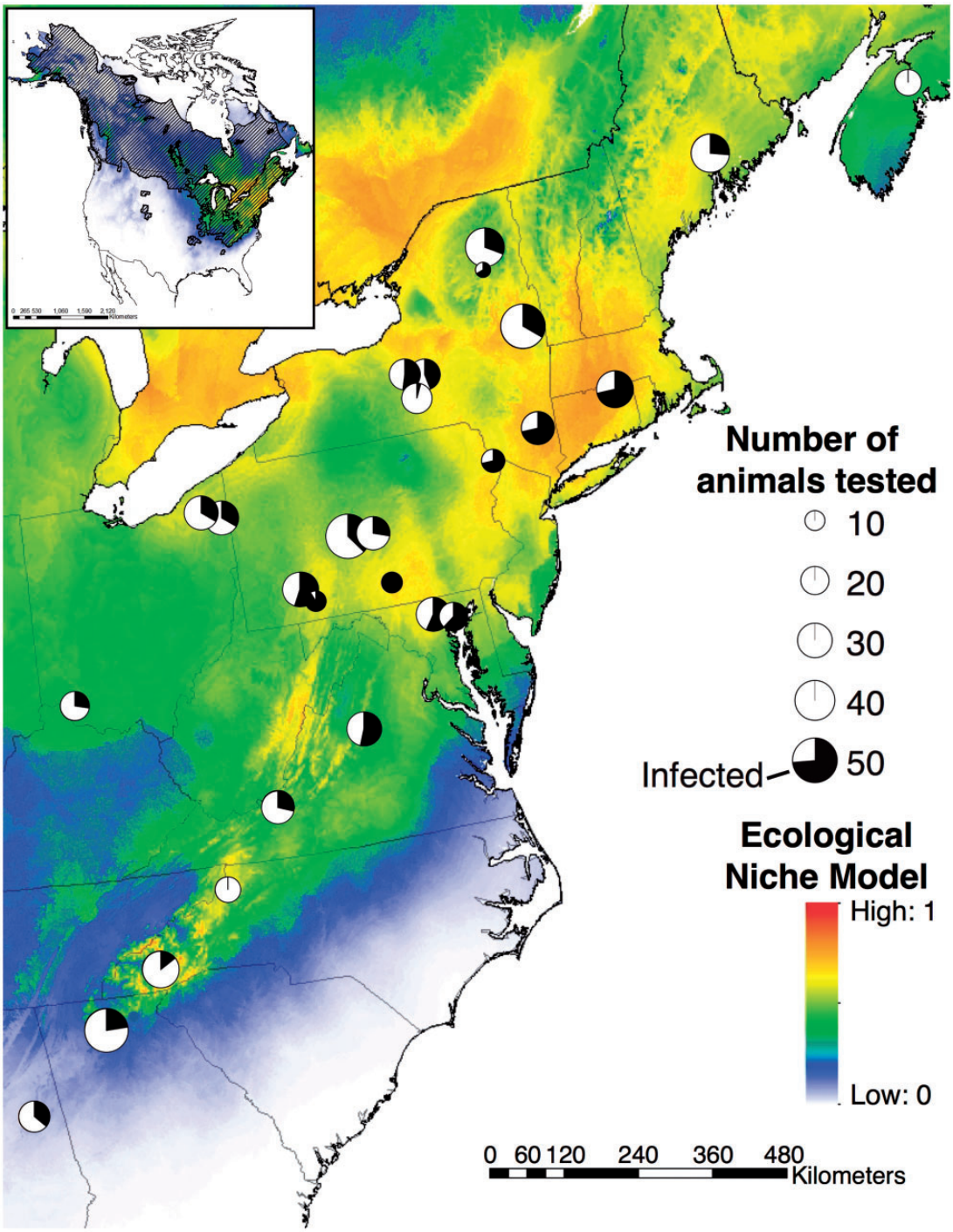

Fig. 1 Proportion of adult male wood frogs detected to have ranaviral infections at each site where we collected frogs as they approached breeding ponds in 2011 and 2012 combined, plotted against estimates of the suitability of habitat derived from our ENM, with red shading, reflecting high suitability, to blue shading, reflecting poor suitability. The size of the pie is proportional to the sample size for that location and black shading represents the proportion of individuals who tested positive for ranavirus (FV3) out of 100% (full circle; see Table 1 for specific locations of sites, and Supplementary Table S2 for numerical percent of frogs testing positive for infections by population and year). Inset: Entire extent of the wood frog's geographic range (International Union for Conservation of Nature) overlapped on a range-wide map of habitat suitability.

until they were non-responsive (~2 min), and blood was collected via cardiac puncture within the next 2–3 min. Animals were euthanized via exsanguination, and tissues were harvested and stored for gene expression and population genetic analyses (not reported herein). Blood samples were centrifuged and the resulting plasma frozen until hormone analysis. Liver tissue was dissected using flame-sterilized

Table 1 Geographic location and elevation of collection sites, dates sampled, and sample sizes for disease and hormone assays for each site

						Sample sizes			
Site	**Latitude**	**Longitude**	**Elev (m)**	**State**	**Date sampled**	**Rv**	***Bd***	**C**	**T**
1	33.4811	−85.8142	635	AL	February 1, 2011	0	19	0	0
				AL	January 8, 2012	25	0	12	12
2	34.5558	−84.2507	541	GA	February 18, 2011	25	25	11	14
				GA	January 17, 2012	24	0	11	11
3	34.5606	−84.3008	535	GA	February 17, 2011	0	11	5	2
4	34.6871	−83.7582	464	GA	February 21, 2011	0	6	2	0
5	35.2912	−83.1801	651	NC	February 26, 2011	0	7	3	0
6	35.3003	−83.1880	661	NC	February 28, 2011	0	26	10	9
7	35.3118	−83.0886	733	NC	March 1, 2011	10	10	5	0
				NC	January 21, 2012	25	0	12	12
8	36.2719	−83.0886	363	NC	March 5, 2011	17	17	7	1
9	37.2874	−80.4591	675	VA	February 4, 2012	28	0	14	14
10	38.1280	−78.7107	248	VA	March 1, 2012	30	0	15	15
11	39.0459	−84.1181	265	OH	March 7, 2012	22	0	11	11
12	39.4514	−76.6268	133	MD	March 2, 2012	21	0	11	11
13	39.4781	−76.6849	122	MD	February 29, 2012	30	0	15	15
14	40.0295	−77.4493	289	PA	March 18, 2011	0	5	3	0
				PA	March 2, 2012	11	0	6	6
15	40.1950	−79.2729	462	PA	March 15, 2011	31	31	14	17
15	40.1592	−79.2732	463	PA	March 8, 2012	11	0	4	4
16	40.7789	−78.0074	416	PA	April 4, 2011	25	25	12	16
				PA	March 12, 2012	26	0	14	14
17	40.7958	−77.9522	402	PA	April 3, 2011	29	28	14	16
18	41.3389	−80.7726	280	OH	April 7, 2011	30	0	13	15
19	41.3752	−74.9526	395	PA	March 16, 2012	14	0	7	7
20	41.4255	−80.8889	251	OH	March 21, 2011	30	16	14	14
21	41.6768	−73.8916	38	NY	March 31, 2011	14	21	6	8
21				NY	March 15, 2012	14	0	7	7
22	41.9677	−72.1582	255	CT	April 5, 2011	35	49	15	6
23	42.4780	−76.0375	389	NY	April 8, 2011	24	24	0	0
23	42.7966	−76.0626	387	NY	April 10, 2011	25	25	12	15
23	42.7857	−76.0537	365	NY	March 19, 2012	25	0	13	13
24	43.1048	−73.7028	95	NY	April 10, 2011	27	23	12	16
24				NY	March 21, 2012	24	0	13	13
25	43.9745	−74.2211	498	NY	April 26, 2011	0	14	5	0
25	43.9789	−74.2374	510	NY	April 14, 2012	6	0	3	3
26	44.3055	−74.0956	513	NY	April 25, 2011	19	30	9	12
				NY	April 4, 2012	20	0	9	9
27	44.7262	−68.8377	34	ME	April 19, 2011	30	30	13	14
				ME	April 9, 2012	8	0	3	3
28	44.8163	−64.3079	195	NS, CA	April 15, 2011	18	20	8	10

Notes: When the exact pond was collected in 2 years, no latitude/longitude information is given for 2012; but for instances where we collected individuals in the same area but not exactly the same pond, we include the specific latitude and longitude for each pond but consider them to be from the same site (Rv = ranavirus, *Bd* = *Batrachochytrium dendrobatidis*, C = corticosterone, T = testosterone).

forceps and scalpel and stored in RNAlater (QIAGEN, Valencia, CA) until DNA extractions could be conducted for analysis of ranaviruses. All procedures were approved by the Washington State Institutional Animal Care and Use Committee (Protocol No. 04167-006).

Detection of pathogens

We extracted DNA from the swabs using PrepMan Ultra (Life Technologies, Carlsbad, CA) and screened it for *Bd* DNA using a Taqman real-time quantitative polymerase chain reaction (qPCR) assay with a StepOne Plus Thermocycler (Life Technologies) following a standard protocol, including the use of an exogenous internal positive control (Applied Biosystems) in the third well of each sample to detect PCR inhibition (Boyle et al. 2004; Hyatt et al. 2007). We used a serial dilution from 10^{-1} to 10^{4} zoospores per 5 μL of DNA, extracted from zoospores of Bd isolate JEL 197 grown on 1% tryptone agar, harvested with 1× PBS, and counted three times with a hemocytomer as a standard for quantifying *Bd* titers (provided by A.P. Pessier, Amphibian Disease Laboratory, Institute for Conservation Research, San Diego Zoo Global). Samples with amplification in two or three wells after 40 cycles were scored as positive. Those without amplification in any of the wells were scored as negative. Ambiguous samples were re-run and if at least one well showed amplification the sample was scored as positive. Titers of *Bd* are reported as the $\log_{10}$ (average zoospore equivalents) of all replicate reactions of the sample (i.e., including any zeros).

To assay for infection by ranavirus, we extracted DNA from liver samples using the Qiagen DNEasy Blood and Tissue kit following the manufacturer's instructions (QIAGEN Inc, Valencia, CA). The concentration of extracted DNA was measured using a NanoDrop-2000 (Thermo-Scientific) and, if necessary, diluted to approximately 20 ng DNA/μL with elution buffer to meet the manufacturer's guidelines of approximately 100 ng of template in the reaction. Extracted DNA from each sample was screened for ranavirus in triplicate 20 μL reactions on 96-well plates with 5 μL of DNA template (~100 ng) using a qPCR reaction with primers and probe that amplify a 70-bp region within the major capsid protein (MCP) of all known amphibian ranaviruses in North America (Brunner and Collins 2009). A 10-fold serial dilution of DNA extracted from a FV3-like ranavirus grown in *Epithilium papilloma cyprinia* cells from 10^{2} to 10^{7} plaque-forming units (pfus) was used as a standard against which unknown samples were quantified. We used the same criteria for PCR amplification used for *Bd* analysis to detect individuals that were positive for infection. Titers of ranavirus are reported as $\log_{10}$ (average pfu) of all replicates of the sample (i.e., including any zeros).

Because of the low titers in many samples, we verified the presence of ranavirus DNA in a subset of the original samples from 2011 by re-extracting DNA from them and then using conventional PCR with a separate set of primers flanking those used in the qPCR reaction (MCP 4 and 5, following Mao et al. 1997) and visualized with ethidium bromide on a 1% agarose gel. The presence/absence of ranavirus DNA was also confirmed in a separate subset of samples with qPCR in an independent laboratory by researchers who were blind to the nature of the samples. To verify whether the ranavirus DNA we detected represented active or quiescent infections, we extracted RNA from the liver tissue of a subsample of infection and uninfected individuals (confirmed with methods above) and conducted quantitative RT-PCR for expression of the ranavirus immediate-early (IE) and MCP following the methods of Mazzoni et al. (2009).

Landscape environmental variables

To relate our measures of disease prevalence across wood frog populations to spatially explicit measure of habitat suitability or quality based on climate and geography, we developed an ecological niche model (ENM, see details below, Franklin 2009). We also extracted various landscape parameters from ecoinformatic databases to quantify relevant geographic and landscape factors. Range position (i.e., location relative to the core or edge of the range) was determined as the distance from the geographic core, which was calculated as a non-weighted centroid in ArcGIS 10.0 using zonal geometry in the spatial analyst extension toolbox. The boundary of the range for the eastern clade was based on 454 high-quality precision points accessed from GBIF.org and HerpNet.org (April 27, 2012). We estimated wetland density, as a measure of the number of neighboring breeding ponds within the vicinity of the collection site (i.e., a measure of the size of the wood frog metapopulation), by counting the number of wetlands within a 5-km radius of the sampled pond using maps from the National Wetlands Inventory (U.S. Fish and Wildlife Service 2015). Every type of wetland (e.g., marsh, bog) that was not a contiguous water body of water to the collection site or to each other was counted as an independent pond; if wetlands shared a border, they were counted as one.

Finally, we recorded distance from the nearest road for each collection site as an indicator of the potential for anthropogenic introductions of pathogens into ponds and of the general impact of human disturbance.

To define habitat suitability for wood frogs across the landscape, an ENM was generated with MaxEnt version 3.3.3k (Phillips et al. 2006; Phillips and Dudík 2008). Maxent uses environmental data from sites where the species has been detected and predicts the probability of occurrence of the species at all sites across a designated geographic region (e.g., see Elith et al. 2011; Gurutzeta et al. 2015)—in our case the entire species' range. We used 1360 high-quality locality points spanning the species' range downloaded from the public portals HerpNet.org and GBIF.org (April 27, 2012), which was well above the minimum 30 locations often recommended for such models (Wisz et al. 2008). Nineteen high-resolution (30 arc s) climate files were downloaded from WorldClim (Hijmans et al. 2005) plus elevation, and ArcMap version 10.2.2 (ESRI Redlands, CA) was used for all management of environmental layers and for editing. The geographic ranges of the layers were clipped to North America and reprojected to Albers equal-area conic to ensure all cells of the environmental layers encapsulated the same physical area. Settings were 20 replicates with cross validation, random seed, 80% training points, and maximum iterations at 10,000. Only linear, product, and quadratic features were used for the model to prevent over-fitting and to make the response curves more biologically realistic. All other settings were left at default values.

Analyses of hormones

Steroid hormones were extracted from plasma using a solid-phase extraction process with SepPack C18 extraction columns (Waters Inc.). Concentrations of corticosterone and testosterone were measured using an enzyme immunoassay kit (EIA, Cayman Chemicals) according to manufacturers' protocol. Prior to running the sample assays, the extraction columns plus the corticosterone and testosterone EIA kits were validated using a plasma pool of combined samples from wood frogs across the species' range. Serial dilutions and column cold spikes were performed to optimize recovery and ensure accuracy for the species. Parallelism of the standard curve and serial dilution was achieved for corticosterone ($r^2 = 0.9991$, $t = 0.2787$, $P = 0.7857$) and testosterone ($r^2 = 0.9999$, $t = -0.0158$, $P = 0.9876$); column recovery was high across all standards for corticosterone (min.%: 84.0, slope = 1.167, $r^2 = 0.9778$), as well as testosterone (min%: 74.9, slope = 0.4744, $r^2 = 0.9493$).

Optimized dilutions of samples were selected for corticosterone (1:40) and testosterone (1:160). Samples were run in duplicate and were randomized across plates. The absorbance of each well was read at 405 nm using a BIOTEK spectrophotometer, and the plasma's concentration of the hormone was interpolated from a standard curve run in duplicate on each plate. Some hormone samples were too concentrated at initial dilutions and were further diluted with enzyme immunoassay buffer. Resulting dilutions varied for corticosterone from 1:40 to 1:400 and testosterone from 1:160 to 1:400. All EIA preparation and plates were performed according to the protocol provided by the manufacturer. In 2011, the average (±standard deviation) intra-assay coefficient of variation (CV) was 7.7 ± 7.5% and inter-assay CV was 13.47% across 22 plates for corticosterone; the intra-assay CV was 7.5 ± 5.3% and inter-assay CV was 9.48% across 15 plates for testosterone. In 2012, the average intra-assay CV was 8.15 ± 2.48% and inter-assay CV was 17.75% for corticosterone; the average intra-assay CV was 7.73 ± 1.86% and inter-assay CV was 12.58% (across 17 plates for each hormone). Comparison to plasma testosterone and corticosterone concentrations we obtained after overnight housing were comparable to those of other frogs whose blood was collected within 2–3 min upon collection via cardiac puncture during the breeding season (e.g., Hopkins et al. 1997; Eikenaar et al. 2012); and on average were less than male wood frogs blood samples collected upon capture the morning after being placed in experimental breeding arenas (Swierk et al. 2014). Therefore, we considered that the corticosterone and testosterone concentrations we obtained as those reflecting a "resting" state.

Statistical analysis

We analyzed patterns of prevalence with landscape variables using logistic regressions with the glmer() function in the lme4 package (Bates et al. in press) in R (R Development Core Team 2013). We used linear mixed models (with the lmer() function) to analyze differences in the intensity of infection ($\log_{10}$-transformed viral titers) among populations within and between years. We included a random intercept for "pond" in all models because some ponds were sampled in both years of the study, and also to account for the extra-binomial variation in prevalence from pond-to-pond (i.e., a greater

amount of variation in our data than expected by simple binomial, "coin-flip" variation). Significance of individual parameters was assessed by generating parametric bootstrap 95% confidence intervals on the parameters (1000 simulations with the bootMer() function using default parameters).

To determine relationships among individual traits (body condition and $\log_{10}$-transformed plasma corticosterone and testosterone concentrations) and the probability of infection and intensity of infection ($\log_{10}$ viral titer), we again used mixed models (with the lmer() function) including pond as a random intercept and an interaction term between the environment/host variable and year to determine whether relationships varied between 2011 and 2012. We used linear mixed models with pond as a random intercept as described above to determine whether individual traits varied across years. Finally, we calculated Spearman's rank correlations among our environmental predictor variables and among individual predictor variables to better understand the relationships among variables and guard against collinearity.

Results

Geographic distribution of prevalences of *Bd* and ranavirus

We tested a total of 878 adult male wood frogs for ranavirus (in 2011 and 2012) or *Bd* (in 2011 only), spanning 28 sites over 2 years (514 frogs from 22 sites in 2011 and 364 frogs from 18 sites in 2012; 7 sites were assayed for ranavirus in both years). Only 10 of 462 frogs (2.16%, 95% CI: 1.2–3.9%) tested for *Bd* in 2011 had detectable infections (no frogs were tested for *Bd* in 2012 for this reason), and all of these were very low-level infections ($\leq$0.03 zoospore equivalents). We had no indication of PCR inhibition in these samples based on the clear amplification of the exogenous internal positive controls, so this low prevalence is likely not due to false negatives. These infections were found in 6 out of the 21 sites visited in 2011: two in Georgia, two in North Carolina, one in Maine, and one in Nova Scotia (see Fig. 1, Supplementary Table S2). Of the 337 frogs that were tested for both *Bd* and ranavirus none was co-infected, but this was not a significant negative association (Fisher's exact test $P = 0.190$).

Infection by ranavirus was much more common than *Bd* in adult male wood frogs: positive individuals were found in 25 out of 27 ponds tested for ranavirus over the 2 years (Fig. 1, Supplementary Table S2), and prevalence of infection across all populations was 38.9% (95% CI: 35.5–42.4%; 293/753 frogs tested). We detected ranavirus in more populations in 2012 than in 2011: 100% of ponds had infected frogs in 2012 versus 81% of ponds in 2011. Prevalence of infection was higher as well: 50.3% (95% CI: 45.2–55.4%; 183/364 frogs) in 2012 versus 28.3% (95% CI: 24–32.9%; 110/389 frogs) in 2011. Of the nine ponds we sampled in both years, prevalence of infection increased in eight of them; however, prevalence was not significantly correlated between years (Spearman's rank correlation $\rho = 0.357$, $P = 0.444$), largely because several ponds with very low prevalence in 2011 had high prevalence in 2012 (Supplementary Table S2).

Of those frogs that tested positively for ranavirus, the average viral titer on a $\log_{10}$ scale was generally low (0.881 ± 0.805, mean $\pm$ SD pfu-equivalents in 2011, and 1.514 ± 0.892 in 2012), but varied from a low of -2.021 to a maximum of 6.313 (Supplementary Table S2). We did not note any external signs of infection (e.g., red legs, lesions) at collection, but we did detect mRNA expression of genes (IE, MCP) associated with viral replication in a subset of animals tested, suggesting that these frogs were experiencing asymptomatic, low-level, but active infections.

Environmental correlates of infection

In both years, we found that the odds of ranavirus infection increased by 22-fold across the full range of suitability scores projected from the ENM for these ponds, and decreased with distance from the geographic centroid ($\log_{10}$-km) of the range of the eastern clade by 26.5-fold from the center to edge (Fig. 2, Table 2). Note that suitability scores are negatively correlated with distance from the centroid, with highest climatic suitability in the center and decreasing suitability as the distance away from the centroid increases (Pearson $r = -0.82$, $P < 0.001$, Supplementary Fig. S1). The relationships between the prevalence of the disease and the ENM score or distance from the geographic centroid were rather consistent between years. There was no relationship between prevalence of ranaviral infection in wood frogs returning to ponds and the distance to the nearest road (Fig. 3, Table 2). The relationship between prevalence of infection and wetland density varied, depending on the year: there was no clear relationship in 2011, but in 2012 there was a 12.6-fold increase in the odds of infection from the lowest to the highest density of wetlands (Fig. 3, Table 2). Wetland density tended to be positively correlated with ENM score, although statistically significant

only in 2011 (Pearson $r = 0.67$) and tended to be negatively correlated with distance from the centroid of the range (Supplementary Fig. S1).

Hosts' correlates of infection

At the level of the population, we did not detect significant relationships between the prevalence of ranavirus and the mean of frogs' body condition or mean concentration of plasma testosterone (Table 2). The relationship between prevalence and concentration of resting plasma corticosterone depended on the year of collection (Table 2); however, the relationship was not significant in either year (Table 2). This result likely reflects the significantly different means and distributions of corticosterone we measured among males in each year (Supplementary Fig. S1). At the individual level, there was a negative relationship between individual body condition and the odds of infection (7-fold across the full range of body conditions; $\beta_{\text{Body Condition}} = -0.079$; 95% CI: -0.153 to -0.001, Fig. 4), but the odds of infection did not change significantly with either resting corticosterone or testosterone ($\beta_{\text{CORT}} = 0.164$; 95% CI: -0.516 to 0.896; $\beta_{\text{T}} = 0.289$; 95% CI: -0.232 to 0.808).

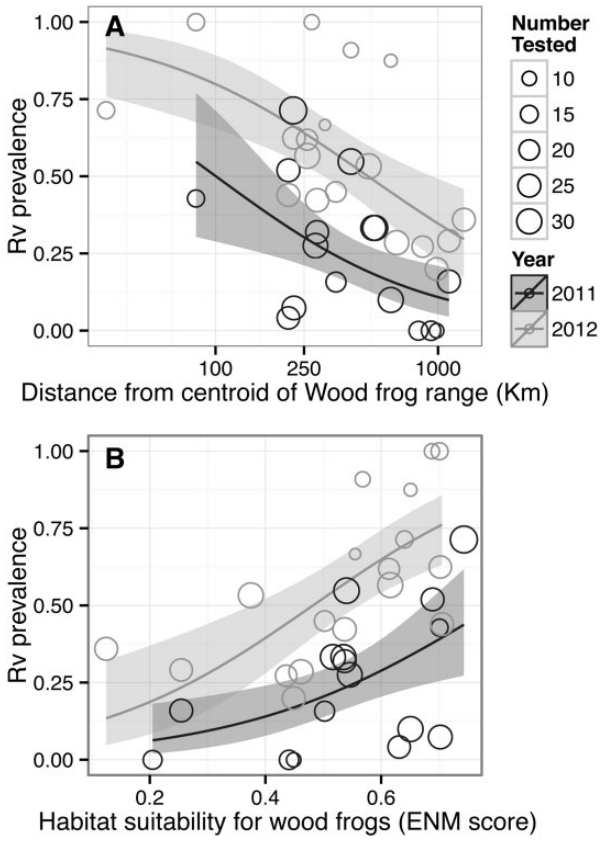

Fig. 2 Relationships between the prevalence of ranavirus (Rv) and the distance from the center of the eastern wood frog's geographic range (**A**) and the habitat suitability for wood frogs (i.e., predicted probability of occurrence of the wood frog estimated by our ENM) (**B**). Both factors are significantly correlated in 2011 (black) and 2012 (gray), and prevalence was significantly greater in 2012. The size of the circle is proportional to the number of individuals collected and shading represents 95% confidence intervals about the best-fit regression line (see Table 2 for associated statistical results).

In terms of intensity of infections, viral titer was not correlated with body condition or with resting levels of corticosterone, but increases with plasma *T* concentrations, by 1.18 orders of magnitude across the range of testosterone concentrations (Fig. 4). Like the prevalence of ranavirus, intensity of infection (i.e., viral titer) was significantly higher in 2012 compared with 2011, as were resting plasma corticosterone ($\beta_{\text{CORT}} = -0.471$, 95% CI: -0.564 to -0.379) and testosterone ($\beta_{\text{T}} = -0.508$; 95% CI: -0.602 to 0.417), but not body condition. Neither level of corticosterone nor of testosterone was correlated with body condition at collection, but resting levels of corticosterone and testosterone were positively correlated (Supplementary Fig. S1).

Discussion

The focus of this study was to determine whether the prevalence of infection by pathogens, along with other physiological measures of population health in adult male wood frogs, followed patterns predicted by the CPH. Although we found significant geographic patterns in the prevalence and intensity of infections, they were counter to our initial predictions. First, we predicted that *Bd* would be common and ranavirus rare, but instead *Bd* infections were rare in our samples, and ranavirus was found in almost all populations, with an average prevalence of 39% among individuals over both years. Second, contrary to our prediction that populations within less climatically suitable habitats would have higher prevalence of infection due to relatively greater physiological stress and an inability to resist or recover from disease, we found that wood frogs living in the climatically suitable habitats at the center of the eastern range had the highest prevalence of ranavirus.

Why *Bd* infections were so rare in adult wood frogs remains an open question. Wood frog metamorphs can be experimentally infected with *Bd* (Gahl et al. 2004; Searle et al. 2011), and *Bd*-infected adults have been identified in field surveys, although not at high prevalence relative to other species (Longcore et al. 2007; Richards-Hrdlicka et al. 2013; Lenker et al. 2014). In a survey of the prevalence of *Bd* across species and types of habitat in Australia,

Table 2 Statistical relationships between population-level ranavirus prevalence of infection and environmental or host variables of interest in this study.

Factor	β	95% Confidence interval
Environmental variables		
ENM	**4.993**	**2.806 to 7.742***
Distance from centroid of range	**−2.039**	**−3.136 to −1.079***
Distance from road	−0.023	−0.079 to 0.025
Wetland density × year	**0.011**	**−0.001 to 0.023***
Wetland density in 2011	0.010	−0.007 to 0.028
Wetland density in 2012	**0.015**	**0.005 to 0.028***
Host variables		
Average body condition	−0.030	−0.20 to 0.128
Average resting plasma CORT × Year	**−3.846**	**−7.345 to −0.987***
Average resting CORT in 2011	1.132	−1.807 to 4.221
Average resting CORT in 2012	0.779	−1.776 to 3.447
Average resting plasma *T*	−0.188	−1.383 to 0.978

Note: asterisk (*) indicates significant factors affecting ranavirus prevalence, type also in boldface.

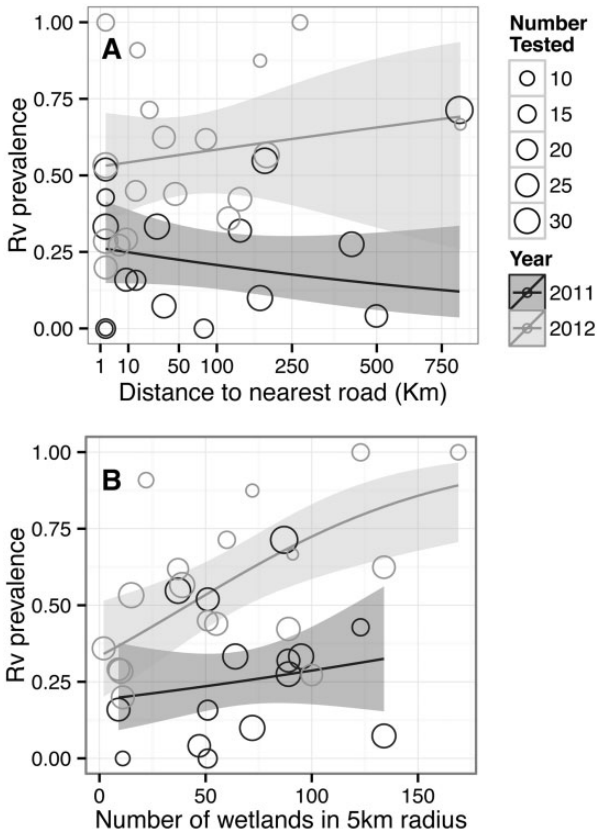

Fig. 3 Relationships between the prevalence of ranavirus (Rv) and the distance of the collection site from the nearest road (**A**) and the number of wetlands in a 5-km radius from collection site (**B**) in 2011 (black) and 2012 (gray). Size of the circle is proportional to the number of individuals collected and shading represents 95% confidence intervals about the best-fit regression line (see Table 2 for associated statistical results).

Kriger and Hero (2007) did not find *Bd* in any ephemeral pond species, which the authors attributed to the inability of *Bd* to persist after ponds dry. In the case of wood frogs, it is possible that the low prevalence is due to adults leaving ponds before *Bd* has had the opportunity to colonize and spread widely, as wood frogs are typically one of the first species to breed upon spring thaw. Alternatively, adult wood frogs could be relatively more resistant to *Bd* infection, or have more effective mechanisms of clearing infections, than other species. The *Bd*-positive frogs we identified were from populations at both southern and northern extremes of the range, but with so few individuals we could not assess this hypothesis.

By contrast, adult wood frogs are apparently an excellent reservoir for ranavirus, as we found infections in adult males throughout the range. Ranavirus epidemics in amphibians primarily occur in ponds and thus infect, and often kill, larvae (Green et al. 2002; Gray et al. 2009; Brunner et al. 2015); however, some individuals are able to survive with ranaviral infection and presumably retain these infections as adults (Brunner et al. 2004; Reeve et al. 2013). Ranavirus-related die-offs of larvae have been reported in populations of wood frogs across the eastern range and up to the most northern extent of the range in northern Canada and Alaska (Schock et al. 2010; Duffus et al. 2015), but these reports are sporadic and published distributions of ranaviruses

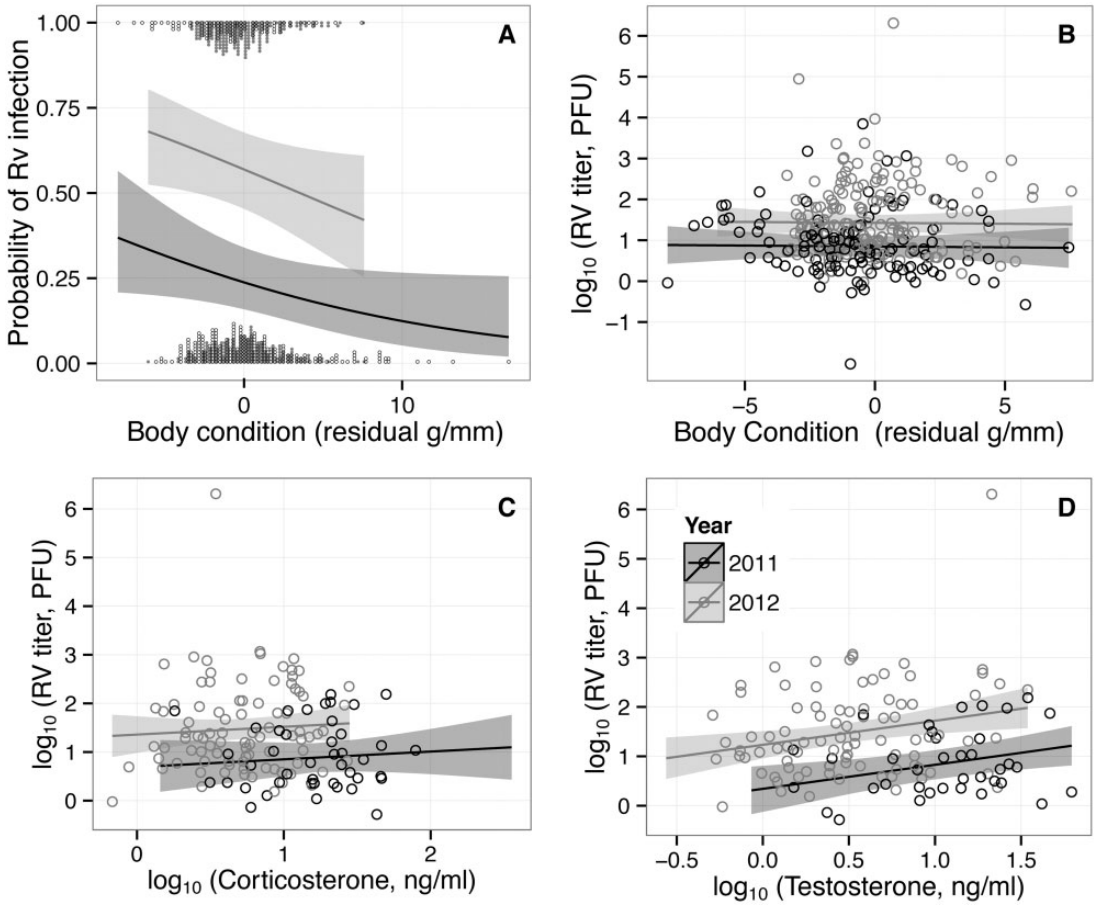

Fig. 4 Individual-level analysis of hosts' traits and the status of ranavirus in 2011 (black line) and 2012 (gray line). (**A**) Relationship between the probability of infection by ranavirus (Rv) and body condition (residual of weight on SVL linear regression) plotted with histograms of body conditions for those testing positive along the top, and those testing negative along the bottom. Relationships between viral titers (log-transformed, in plaque-forming units, PFU) and (**B**) body condition (residual of regression of mass on SVL), (**C**) concentration of plasma corticosterone and (**D**) testosterone (both log transformed, ng/mL) measured when the frog was at rest. Circles represent individuals and shading represents 95% confidence intervals about the best-fit regression line (see Table 2 for associated statistical results).

likely reflect sampling effort and awareness of the disease more than the true incidence of die-offs. While several studies have documented relationships between environmental conditions and ranaviral die-offs or prevalence in tadpoles (Gahl and Calhoun 2010), particularly with anthropogenic factors (e.g., pesticides or agriculture) (Gray et al. 2007; St-Amour et al. 2008), our measurement of ranaviral infections among adults across populations is unique in its spatial extent and focus on adults, and revealed significant patterns of broad-scale environmental variation of the prevalence of ranavirus over space and time.

If we consider the epidemiology of ranaviruses, there are several possible reasons why greater prevalence occurs in the high-quality habitat at the center of the range. First, the higher prevalence of infection among adults could reflect differences among populations in the ability to tolerate infections. Under better conditions larvae and metamorphs might be better able to suppress viral loads below lethal levels (Brunner et al. 2005) or minimize the pathology associated with ranaviral infections such that many, or most, infections persist at sublethal levels after metamorphosis. Conversely, if wood frog tadpoles or metamorphs at the edges of the range are less able to tolerate infections, and thus have a higher mortality rate, prevalence of ranavirus among adults would be low in these habitats. The environmental conditions in the core of the species' range also could be optimal for ranavirus growth or persistence, while the northern and southern edges are too cold or too warm, respectively, for the virus. The outcomes of ranaviral infections are often temperature sensitive, although often in complex ways (reviewed in Brunner et al. 2015). Finally, there could be geographic variation in the virulence of ranavirus strains

across the eastern range, although we cannot address this hypothesis at this time because researchers are just beginning to document the phylogeography of ranaviruses and empirical studies characterizing geographic patterns of viral traits (e.g., virulence, persistence, and transmissibility) are virtually nonexistent.

The geographic pattern of prevalence might instead result from differences in the transmission of ranaviruses. Wood frog populations are more abundant in the core of their distribution, which could lead to more movement between ponds and introduction of ranavirus-infected individuals, thereby maintaining infections at the metapopulation level. Indeed, the prevalence of ranavirus increased with increases in the abundance of wetlands within a 5-km radius (a measure of metapopulation density) in 2012, although this relationship did not reach statistical significance in the 2011 sample. Interestingly, the climatically most suitable habitat for wood frogs also coincides with high densities of the human population, and human-mediated movement or introductions of ranavirus cannot be ruled out as a contributing factor. We did not see a correlation between the distance from roads and the probability of ranaviral prevalence, but we did not design our sampling scheme to test the impact of human disturbance or access to sites in this study. Correlating the prevalence of infection in a broader sampling of ponds of different habitat types (urban, suburban, agricultural) with GIS layers that capture anthropogenic impacts on the environment (i.e., impervious surface, canopy cover, or natural landscape) could address this question. Future studies focused on resolving the geographic differences in the likelihood and outcome of ranaviral epidemics and the fate of infected metamorphs within and between years will be helpful in testing these hypotheses.

Our findings also bolster a hypothesis explaining how ranaviruses might persist from year to year in highly seasonal, often ephemeral, populations in ponds. Brunner et al. (2004) proposed that some fraction of amphibian larvae escapes their natal pond with chronic, sublethal infections and then (re)introduce the virus into the ponds when they return to breed. Their support for this hypothesis, however, rested on the observation of just two infected adult tiger salamanders (*Ambystoma mavortium* [formerly *tigrinum*] *nebulosum*) out of 30 returning to a single pond (Brunner et al. 2004), although sublethal infections have also been noted in other adult amphibians, often from biological suppliers (Wolf et al. 1968; Clark et al. 1969; Robert et al. 2007). Our observation of ranavirus-infected male wood frogs returning to 25 out of 27 ponds suggests that this (re)introduction pathway might be at work more broadly. While most individuals had very low viral titers, and would thus be unlikely to succumb to infection or shed virions into the environment (Fig. 4), a few had titers well above approximately 1000 virus particles in the range observed in experimental infections (Brunner and Collins 2009; Haislip et al. 2012). We suspect that these individuals would have shed viral particles in their breeding ponds.

Moreover, we found a moderate, but consistent, positive relationship between testosterone and ranaviral titers. In explosively breeding frogs, testosterone increases in males as they migrate to ponds and during mating choruses (Houck and Woodley 1995; Harvey et al. 1997; Hopkins et al. 1997), and testosterone can be immunosuppressive (i.e., the immunocompetence handicap hypothesis; Folstad and Karter 1992; also see Zuk 1996; Casto et al. 2001; Fuxjager et al. 2011). We captured frogs as they were just arriving at breeding ponds, and it is likely that competitive interactions with males as well as prolonged calling and amplexus would have increased concentrations of testosterone above the levels we measured and could have led to more virus shed into pond water or even some individuals dying of the infection (thus, inoculating the pond with virus). It would be interesting to measure testosterone and ranaviral titers of frogs during amplexus or after breeding to test this testosterone-mediated recrudescence hypothesis, and potentially add to other examples of male-driven transmission of parasites (Hawley and Altizer 2011).

Our initial predictions of higher prevalence and intensity of ranaviral infection in low-quality habitats on the edge of the range were partially based on the assumption that the individuals living there are experiencing greater physiological stress that is manifest in chronically higher levels of corticosterone, which can be immunosuppressive (Sapolsky et al. 2000; Dhabhar 2002). Our data are generally not consistent with this hypothesis. Although we detected a weak interaction between year and circulating corticosterone concentration on the prevalence of ranavirus, within years there was no clear relationship at the population or individual levels and viral titers of the infected frogs were not related to corticosterone levels. We also did not see a strong correlation between corticosterone and either habitat suitability (ENM score) or the distance from the centroid of the range. Circulating levels of corticosterone are at their highest in male frogs during breeding (Hopkins et al. 1997), and variance in hormonal levels due to

environmental conditions could be diminished at this time of year, especially if only the most healthy frogs are able to breed. Measurement of other aspects of the regulation of hypothalamo–pituitary–interrenal axis (e.g., stress-responsiveness) might be better indicators of environmental stress in this system (see Romero 2004), or our results may simply suggest that wood frogs in lower-quality habitats are not experiencing greater physiological stress.

While we did not see a relationship between corticosterone and prevalence of ranavirus infection over space, we did see changes in both of these measures over time. Both prevalence and intensity of ranavirus infections were higher in 2012, while corticosterone was significantly lower. While we cannot determine whether the changes in hormone and viral titers are functionally related or what caused this inter-annual variation, we do know that environmental conditions prior to breeding were conspicuously different between years. Because of warmer overwintering temperatures between 2011 and 2012, breeding occurred 10–30 days earlier in 2012 than in 2011 (Table 1). When compared with a broader range of years, populations in the vicinity of Syracuse, NY, bred 10 days earlier than the average date recorded for the area between 1990 and 1999 (Gibbs and Briesch 2001), and breeding dates for other sites within New York were among the earliest dates on record (North American Amphibian Monitoring Program, http://www.pwrc.usgs.gov/naamp/; Weir and Mossman 2005). Warmer overwintering temperatures and low snowfall have been associated with reduced body condition, survival, and fecundity in female frogs (*Bufo bufo*: Reading 2007; *L. sylvaticus*: Benard 2015), and there is concern that warmer overwintering temperatures could also affect host–pathogen interactions in amphibians (Garner et al. 2011). Monitoring ranavirus prevalence and viral titers across populations over multiple years, as well as conducting controlled experiments, is needed to determine the effects of warmer climates on immune responses to ranaviruses in wood frogs.

While there is debate about which metrics to use as bioindicators of population health (Stephen 2014; Bonier et al. 2009; Dickens and Romero 2013), our study demonstrates the importance of measuring multiple dimensions of health across space and time. Our study of 28 populations revealed for the first time a geographic pattern to the incidence of ranavirus infection across the eastern range, and yielded a greater understanding of the epidemiology of the ranaviruses and endocrine–pathogen interactions. The animals in our study did not show signs of disease, but frogs with high viral titers are at risk of becoming sick, moribund, or dying, particularly after expending energy involved with mating (Howard 1980). At the population level, an increase in prevalence also could relate to an increase in the probability of epidemics among tadpoles in the next generation (Brunner et al. 2015). Our study also showed that the relationships among environmental stressors, glucocorticoids, and susceptibility to disease at the population-level are not always direct or clear (Haislip et al. 2012; Reeve et al. 2013), and can change from year to year. This highlights the need for long-term monitoring to understand the dynamic relationships between the environment, physiology, and disease status, particularly in the face of climate change and habitat alteration (Crespi et al. 2013). While we are left with more questions than answers, the patterns generated by this study contribute to several testable hypotheses to advance our overall understanding of how the environment affects animals' health.

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Supplementary data

Supplementary data available at *ICB* online.

References

Allender MC, Mitchell MA, Torres T, Sekowska J, Driskell EA. 2013. Pathogenicity of frog virus 3-like virus in red-eared slider turtles (*Trachemys scripta elegans*) at two environmental temperatures. J Comp Pathol 149:356–67.

Bates D, Maechler M, Bolker BM, Walker S. In press. Fitting linear mixed-effects models using {lme4}. J Stat Software.

Benard MF. 2015. Warmer winters reduce frog fecundity and shift breeding phenology, which consequently alters larval development and metamorphic timing. Glob Change Biol 21:1058–65.

Berven KA, Grudzien TA. 1990. Dispersal in the wood frog, *Rana sylvatica*: Implications for genetic population structure. Evolution 44:2054–6.

Bonier F, Martin PR, Moore IT, Wingfield JC. 2009. Do baseline glucocorticoids predict fitness? Trend Ecol Evol 24:634–42.

Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Dis Aquat Organ 60:141–8.

Bridle JR, Vines TH. 2007. Limits to evolution at range margins: When and why does adaptation fail? Trend Ecol Evol 22:140–7.

Brown JH. 1984. On the relationship between abundance and distribution of species. Am Nat 124:255–79.

Brown JH, Mehlman DW, Stevens GC. 1995. Spatial variation in abundance. Ecology 76:2028–43.

Brunner JL, Collins JP. 2009. Testing assumptions of the trade-off theory of the evolution of parasite virulence. Evol Ecol Res 11:1169–88.

Brunner JL, Richards K, Collins JP. 2005. Dose and host characteristics influence virulence of ranavirus infections. Oecologia 144:399–406.

Brunner JL, Schock DM, Collins JP, Davidson EW. 2004. The role of an intraspecific reservoir in the persistence of a lethal ranavirus. Ecology 85:560–6.

Brunner JL, Storfer A, Gray MJ, Hoverman JT. 2015. Ranavirus ecology and evolution: From epidemiology to extinction. In: Gray MJ, Chinchar VG, editors. Ranaviruses: Lethal pathogens of ectothermic vertebrates. Secaucus, NJ: Springer. p. 71–104.

Cahill AE, Aiello-Lammens ME, Fisher-Reid MC, Hua X, Karanewsky CJ, Ryu HY, Sbeglia GC, Spagnolo F, Waldron JB, Wiens JJ. 2014. Causes of warm-edge range limits: Systematic review, proximate factors and implications for climate change. J Biogeogr 41:429–42.

Casto JM, Nolan V Jr, Ketterson ED. 2001. Steroid hormones and immune function: Experimental studies in wild and captive dark-eyed juncos (*Junco hyemalis*). Am Nat 157:408–20.

Chinchar VG. 2002. Ranaviruses (family Iridoviridae): Emerging cold-blooded killers. Archiv Virol 147:447–70.

Chown SL, Gaston KJ, Robinson D. 2004. Macrophysiology: large scale patterns in physiological traits and their ecological implications. Funct Ecol 18:159–67.

Clark HF, Gray C, Fabian F, Zeigel R, Karzon DT. 1969. Comparative studies of amphibian cytoplasmic virus strains isolated from the leopard frog, bullfrog, and newt. In: Mizell M, editor. Biology of amphibian tumors. New York, NY: Springer-Verlag. p. 310–26.

Conant R, Collins JT. 1998. A field guide to reptiles and amphibians. New York: Houghton Mifflin Harcourt.

Cooke SJ, Sack L, Franklin CE, Farrell AP, Beardall J, Wikelski M, Chown SL. 2013. What is conservation physiology? Perspectives on an increasingly integrated and essential science. Conserv Physiol 1:1–23.

Crespi EJ, Williams TD, Jessop TS, Delehanty B. 2013. Life history and the ecology of stress: How do glucocorticoid hormones influence life-history variation in animals? Funct Ecol 27:93–106.

Dhabhar FS. 2002. Stress-induced augmentation of immune function: The role of stress hormones, leukocyte trafficking, and cytokines. Brain Behav Immun 16:785–98.

Dickens MJ, Romero LM. 2013. A consensus endocrine profile for chronically stressed wild animals does not exist. Gen Comp Endocrinol 191:177–89.

Duffus ALJ, Marschang RE, Waltzek TB, Stöhr A, Allender MC, Gotesman M, Whittington R, Hick P, Hines M. 2015. Distribution and host range of ranaviruses. In: Gray MJ, Chinchar VG, editors. Ranaviruses: Lethal pathogens of ectothermic vertebrates. Secaucus, NJ: Springer. p. 9–57.

Eckert CG, Samis KE, Lougheed SC. 2008. Genetic variation across species' geographical ranges: The central-marginal hypothesis and beyond. Mol Ecol 17:1170–88.

Eikenaar C, Husak J, Escallón C, Moore IT. 2012. Variation in testosterone and corticosterone in amphibians and reptiles: Relationships with latitude, elevation, and breeding season length. Am Nat 180:642–54.

Elith J, Phillips SJ, Hastie T, Dudik M, En Chee Y, Yates CJ. 2011. A statistical explanation of MaxEnt for ecologists. Divers Distrib 17:43–57.

Emmanuel NN, Loha N, Okolo MO, Ikenna OK. 2011. Landscape epidemiology: An emerging perspective in the mapping and modelling of disease and disease risk factors. Asian Pac J Trop Dis 1:247–50.

Folstad I, Karter AJ. 1992. Parasites, bright males, and the immunocompetence handicap. Am Nat 139: 603–22.

Franklin J. 2009. Mapping species distributions: Spatial inference and prediction. Cambridge, UK: Cambridge University Press.

Fuxjager MJ, Foufopoulos J, Diaz-Uriarte R, Marler CA. 2011. Functionally opposing effects of testosterone on two different types of parasite: Implications for the immunocompetence handicap hypothesis. Funct Ecol 25:132–8.

Gahl MK, Calhoun AJK. 2010. The role of multiple stressors in ranavirus-caused amphibian mortalities in Acadia national park wetlands. Can J Zool 88:108–21.

Gahl MK, Longcore JE, Houlahan JE. 2004. Varying responses of northeastern North American amphibians to the chytrid pathogen *Batrachochytrium dendrobatidis*. Conserv Biol 26:135–41.

Garner TWJ, Rowcliffe M, Fisher MC. 2011. Climate change, chytridiomycosis or condition: An experimental test of amphibian survival. Glob Change Biol 17:667–75.

Gaston KJ, Chown SL, Calosi P, Bernardo J, Bilton DT, Clarke A, Clusella-Trullas S, Ghalambor CK, Konarzewski M, Peck LS, et al.. 2009. Macrophysiology: A conceptual reunification. Am Nat 174:595–612.

Gaston KJ. 2003. The structure and dynamics of geographic ranges. New York: Oxford University Press.

Gibbs JP, Breisch AR. 2001. Climate warming and calling phenology of frogs near Ithaca, New York, 1900–1999. Conserv Biol 15:1175–8.

Granoff AP, Came E, Rafferty KA. 1965. The isolation and properties of viruses from *Rana pipiens*: Their possible relationship to the renal adenocarcinoma of the leopard frog. Ann N Y Acad Sci 126:237–55.

Gray MJ, Miller DL, Hoverman JT. 2009. Ecology and pathology of amphibian ranaviruses. Dis Aquat Organ 87:243–66.

Gray MJ, Miller DL, Schmutzer AC, Baldwin CA. 2007. Frog virus 3 prevalence in tadpole populations inhabiting cattle-access and non-access wetlands in Tennessee, USA. Dis Aquat Organ 77:97–103.

Green DE, Converse KA, Schrader AK. 2002. Epizootiology of sixty-four amphibian morbidity and mortality events in the USA, 1996–2001. Ann N Y Acad Sci 969:323–39.

Guo Q, Taper M, Schoenberger M, Brandle J. 2005. Spatial–temporal population dynamics across species range: From centre to margin. Oikos 108:47–57.

Gurutzeta G-A, Lahoz-Monfort JJ, Elith J, Gordon A, Kujala H, Lentini PE, McCarthy MA, Tingley R, Wintle BA. 2015. Is my species distribution model fit for purpose? Matching data and models to applications. Glob Ecol Biogeogr 24:276–92.

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

Haislip NA, Hoverman JT, Miller DL, Gray MJ. 2012. Natural stressors and disease risk: Does the threat of predation increase amphibian susceptibility to ranavirus? Can J Zool 90:893–902.

Harvey LA, Propper CR, Woodley SK, Moore MC. 1997. Reproductive endocrinology of the explosively breeding desert spadefoot toad, *Scaphiopus couchi*. Gen Comp Endocrinol 105:102–13.

Hawley DM, Altizer SM. 2011. Disease ecology meets ecological immunology: Understanding the links between organismal immunity and infection dynamics in natural populations. Funct Ecol 25:48–60.

Hengeveld R, Haeck J. 1982. The distribution of abundance. 1. Measurements. J Biogeogr 9:303–16.

Hijmans R, Cameron S, Parra J. 2005. Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25:1965–98.

Holt RD, Keitt TH. 2005. Species' borders: A unifying theme in ecology. Oikos 108:3–6.

Holt RD, Keitt TH, Lewis MA, Maurer BA, Taper ML. 2005. Theoretical models of species' borders: Single species approaches. Oikos 108:18–27.

Hopkins WA, Mendonca MT, Congdon JD. 1997. Increased circulating levels of testosterone and corticosterone in southern toads, *Bufo terrestris*, exposed to coal combustion waste. Gen Comp Endocrinol 108:237–46.

Houck LD, Woodley SK. 1995. Field studies of steroid hormones and male reproductive behaviour in amphibians. In: Heatwole H, Sullivan BK, editors. Amphibian biology, Vol. 2. Social behavior. New SouthWales, Australia: Surrey Beatty. p. 677–703.

Howard RD. 1980. Mating behaviour and mating success in woodfrogs *Rana sylvatica*. Anim Behav 28:705–16.

Huey RB, Deutsch CA, Tewksbury JJ, Vitt LJ, Hertz PE, Alvarez Perez HJ, Garland T Jr. 2009. Why tropical forest lizards are vulnerable to climate warming. Proc R Soc B 276:1939–48.

Hyatt AD, Boyle DG, Olsen V, Boyle DB, Berger L, Obendorf D, Dalton A, Kriger K, Hero M, Hines H, et al.. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Dis Aquat Organ 73:175–92.

Kawecki TJ. 2008. Adaptation to marginal habitats. Ann Rev Ecol Evol Syst 39:321–42.

King KC, Lively CM. 2012. Does genetic diversity limit disease spread in natural host populations. Heredity 109:199–203.

Kirkpatrick M, Barton NH. 1997. Evolution of a species' range. Am Nat 150:1–23.

Kriger KM, Hero J-M. 2007. The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. Divers Distrib 13:781–8.

Kubisch A, Holt RD, Poethke H-J, Fronhofer EA. 2014. Where am I and why? Synthesizing range biology and the eco-evolutionary dynamics of dispersal. Oikos 123:5–22.

Langdon JS. 1989. Experimental transmission and pathogenicity of epizootic haematopoietic necrosis virus (EHNV) in redfin perch, *Perca fluviatilis* L., and 11 other teleosts. J Fish Dis 12:295–310.

Lee-Yaw JA, Irwin JT, Green DM. 2008. Postglacial range expansion from northern refugia by the wood frog, *Rana sylvatica*. Mol Ecol 17:867–84.

Lenker MA, Savage AE, Becker CG, Rodriguez D, Zamudio KR. 2014. *Batrachochytrium dendrobatidis* infection dynamics vary seasonally in upstate New York, USA. Dis Aquat Organ 21:51–60.

Longcore JR, Longcore JE, Pessier AP, Halteman WA. 2007. Chytridiomycosis is widespread in anurans of northeastern United States. J Wildl Manage 71:435–44.

Mao J, Hedrick RP, Chinchar VG. 1997. Molecular characterization, sequence analysis, and taxonomic position of newly isolated fish iridoviruses. Virology 229:212–20.

Mazzoni R, de Mesquita AJ, Fleury LF, de Brito WM, Nunes IA, Robert J, Morales H, Coelho AS, Barthasson DL, Galli L, et al.. 2009. Mass mortality associated with a frog virus 3-like ranavirus infection in farmed tadpoles *Rana catesbeiana* from Brazil. Dis Aquat Organ 86:181–91.

McEwen BS, Wingfield JC. 2003. The concept of allostasis in biology and biomedicine. Horm Behav 43:2–15.

Meentemeyer RK, Haas SE, Vaclavik T. 2012. Landscape epidemiology of emerging infectious diseases in natural and human-altered ecosystems. Annu Rev Phytopathol 50:379–402.

Micheletti SJ, Storfer A. 2015. A test of the central-marginal hypothesis using population genetics and ecological niche modelling in an endemic salamander (*Ambystoma barbouri*). Mol Ecol 24:967–79.

Osovitz CJ, Hofmann GE. 2007. Marine macrophysiology: Studying physiological variation across large spatial scales in marine systems. Comp Biochem Physiol A Mol Integr Physiol 147:821–7.

Ostfeld RS, Glass GE, Keesing F. 2005. Spatial epidemiology: An emerging (or re-emerging) discipline. Trend Ecol Evol 20:328–36.

Piovia-Scott J, Pope KL, Lawler SP, Cole EM, Foley JE. 2011. Factors related to the distribution and prevalence of the fungal pathogen *Batrachochytrium dendrobatidis* in *Rana cascadae* and other amphibians in the Klamath Mountains. Biol Conserv 144:2913–21.

Phillips SJ, Anderson RP, Schapire RE. 2006. Maximum entropy modeling of species geographic distributions. Ecol Model 190:231–59.

Phillips SJ, Dudík M. 2008. Modeling of species distributions with Maxent: New extensions and a comprehensive evaluation. Ecography 31:161–75.

R Development Core Team. 2013. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Raffel TR, Halstead NT, McMahon TA, Davis AK, Rohr JR. 2015. Temperature variability and moisture synergistically interact to exacerbate an epizootic disease. Proc Biol Sci 282:20142039.

Reading CJ. 2007. Linking global warming to amphibian declines through its effects on female body condition and survivorship. Oecologia 151:125–31.

Reeve BC, Crespi EJ, Whipps CM, Brunner JL. 2013. Natural stressors and ranavirus susceptibility in larval wood frogs (*Rana sylvatica*). EcoHealth 10:190–200.

Richards-Hrdlicka KL, Richardson JL, Mohabir L. 2013. First survey for the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in Connecticut (USA) finds widespread prevalence. Dis Aquat Organ 102:169–80.

Robert J, Abramowitz L, Gantress J, Morales HD. 2007. *Xenopus laevis*: A possible vector of ranavirus infection? J Wildl Dis 43:645–52.

Rollins-Smith LA, Ramsey JP, Pask JD, Reinert LK, Woodhams DC. 2011. Amphibian immune defenses against chytridiomycosis: Impacts of changing environments. Integ Comp Biol 51:552–62.

Romero LM. 2004. Physiological stress in ecology: Lessons from biomedical research. Trend Ecol Evol 19:249–55.

Rowley JJ, Alford RA. 2013. Hot bodies protect amphibians against chytrid infection in nature. Scientific Reports 3.

Sagarin RD, Gaines SD, Gaylord B. 2006. Moving beyond assumptions to understand abundance distributions across the ranges of species. Trend Ecol Evol 21:524–30.

Sagarin RD, Gaines SD. 2002. The 'abundant centre' distribution: To what extent is it a biogeographical rule? Ecol Lett 5:137–47.

Sapolsky RM, Romero LM, Munch AU. 2000. How do glucocorticoids influence stress responses? Integrating perimissing, suppressive, stimulatory and adaptive actions. Endocr Rev 21:55–89.

Schock DM, Ruthig GR, Collins JP, Kutz SJ, Carrière S, Gau RJ, Veitch AM, Larter NC, Tate DP, Guthrie G, et al.. 2010. Amphibian chytrid fungus and ranaviruses in the Northwest Territories, Canada. Dis Aquat Organ 92:231–40.

Searle CL, Gervasi SS, Hua J, Hammond JI, Relyea RA, Olson DH, Blaustein AR. 2011. Differential host susceptibility to *Batrachochytrium dendrobatidis*, an emerging amphibian pathogen. Conserv Biol 25:965–74.

Skerratt LF, Berger L, Speare R, Cashins S, McDonald KR, Phillott AD, Hines HB, Kenyon N. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4:125–34.

Somero GN. 2005. Linking biogeography to physiology: Evolutionary and acclimatory adjustments of thermal limits. Front Zool 2:1.

St-Amour V, Wong WM, Garner TWJ, Lesbarreres D. 2008. Anthropogenic influence on prevalence of two amphibian pathogens. Emerg Infect Dis 14:1175–6.

Stephen C. 2014. Toward a modernized definition of wildlife health. J Wildl Dis 50:427–30.

Swierk L, Graham SP, Langkilde T. 2014. The stress of scramble: sex differences in behavior and physiological stress response in a time-constrained mating system. Beh Ecol Sociobiol 68:1761–8.

U.S. Fish and Wildlife Service. 2015. National wetlands inventory website. Washington, DC: U.S. Department of the Interior, Fish and Wildlife Service. Available online at: http://www.fws.gov/wetlands/.

Weir LA, Mossman MJ. 2005. North American amphibian monitoring program (NAAMP). In: Lannoo M, editor. Amphibian declines: The conservation status of United States species. Berkeley, CA: University of California Press. p. 307–13.

Wiens JJ. 2011. The niche, biogeography and species interactions. Phil Trans R Soc B 366:2336–50.

Wikelski M, Cooke SJ. 2006. Conservation physiology. Trend Ecol Evol 21:38–46.

Wisz MS, Hijmans RJ, Li J, Peterson AT, Graham CH, Guisan A. 2008. Effects of sample size on the performance of species distribution models. Divers Distrib 14:763–73.

Wolf K, Bullock GL, Dunbar CE, Quimby MC. 1968. Tadpole edema virus: A viscerotropic pathogen for anuran amphibians. J Infect Dis 118:253–62.

Woodhams DC, Ardipradja K, Alford RA, Marantelli G, Reinert LK, Rollins-Smith LA. 2007. Resistance to chytridiomycosis varies among amphibian species and is correlated with skin peptide defenses. Anim Conserv 10:409–17.

Zuk M. 1996. Disease, endocrine–immune interactions, and sexual selection. Ecology 77:1037–42.