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From the Mountains to the Prairies
Seasonal *Bd* Responses Differ by Latitude and Longitude at a Continental Scale

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Abstract

The chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) severely impacts amphibians globally. To date, the majority of studies have been conducted locally, on single populations or within regions, and have often used different sampling protocols and analytical techniques producing a correspondingly diverse picture of a more widespread pattern. The resulting information has, to some extent, produced generalizations across broader geographic areas. In contrast, this study examined the prevalence and intensity of *Bd* infections by conducting cross-country transects using identical analytical techniques and controlling for season. The 2009 study, Do Frogs Still Get Their Kicks on Route 66?—A Transcontinental Transect for Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*) Infection on U.S. Department of Defense Installations, provided a transcontinental transect across the central United States, holding latitude relatively constant, and showed strong spatial and temporal trends in *Bd* prevalence and intensity. The present study, using the same analytical techniques, reports on three north-to-south, international border-to-border transects conducted at 15 Department of Defense—United States Army, Air Force, Navy, and Marine Corps—installations. These transects are: a West Coast Transect that runs from the state of Washington to southern California roughly along Interstate 5, a Midwest Transect that runs from Minnesota to southern Alabama roughly following Interstates 94 and 55, and an East Coast Transect that runs from Maine to southern Florida roughly along Interstate 95. The following questions related to the presence and intensity of *Bd* infections in North America were addressed:

1. Is there a west-to-east (longitudinal) pattern?
2. Is there a north-to-south (latitudinal) pattern?
3. Is there a temporal (seasonal) pattern?
4. To what extent are these patterns influenced by climate and geography?

In aggregate, the study results fall into two clear patterns. Installations north of latitude 40° had greater *Bd* prevalence than those installations below this latitude. In

addition, these installations had similar or greater *Bd* prevalence in the second sampling period than the first. In contrast, military installations below latitude 40° showed a pattern of increased *Bd* prevalence and intensity in the first sampling period followed by a lack of *Bd* detection in the second sampling period. These results contribute to understanding the regional differences in amphibian responses to this potentially lethal pathogen.

Introduction

One fifth of the world's amphibians may be facing extinction (Stuart et al., 2004; Wake and Vrendenburg, 2008; http://www.iucnredlist.org/initiatives/amphibians [accessed 5 October, 2012]). In part these declines have been caused by the spread of the chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*; Longcore et al., 1999), which has been devastating amphibian populations on a global scale (Daszak et al., 2003; Rachowicz et al., 2006; DiRosa et al., 2007; Wake and Vrendenburg, 2008; Jones et al., 2008; Murray et al., 2009; Kilpatrick et al., 2010). In the United States, this pathogen can be found from below sea level (Lovich et al., 2008) to the highest elevations where amphibians occur (Vredenberg and Summers, 2001; http://www.spatialepidemiology.net; Lannoo et al., 2011; Knapp et al., 2011). To date, however, most studies have been conducted locally, on single populations or within localized areas, and have often used different sampling protocols and analytical techniques (Adams et al., 2007; Frías-Alvarez, 2008; Grant et al., 2008; Deguise and Richardson, 2009; Gaertner 2009a; Goldberg et al., 2009; Sadinski et al., 2010). Although there have been attempts to generalize across broader geographic areas (www.spatialepidemiology.net; Green et al., 2002; Garner et al., 2005; Kriger and Hero, 2007; Goka et al., 2009; Murray et al., 2010; Skeratt et al., 2010; Cheng et al., 2011) the result is an overly diverse picture of what is most certainly a more widespread pattern (Blaustein et al., 2005; Kriger and Hero, 2007; Zellmer et al., 2008; Goldberg et al., 2009; Tennessen et al., 2009; Hossack et al., 2010; Savage et al., 2011).

Exploring patterns requires, in part, broad-scale studies using standardized techniques. Further, due to the confounding factor of anthropogenic disturbance, it is best to examine either low-impact (i.e. "natural") or well-protected areas. Perhaps the most widely available habitats that remain "undisturbed" (a relative term, with, perhaps the exception of the deep sea floor—it is likely there are no longer any truly undisturbed environments left on earth) in the United States today are United States Department of Defense (DoD) installations, which are secured as a matter of national interest. Military installations are protected against the indiscriminate human traffic experienced by many parks, wildlife refuges, and other public areas. Moreover, following the tragic events of

September 11, 2001, access to these installations has been further limited, and in some cases, severely restricted. Department of Defense installations encompass more 28 million acres (11.3 million ha) and occur throughout the United States, making continent-wide surveys possible. Department of Defense lands are also managed differently than typical surrounding landscapes, using ecosystem management techniques. Indeed, American military installations are shown to harbor the greatest concentrations of endangered and threatened species in the United States (Stein et al., 2008).

In 2009, we conducted a transcontinental transect designed to assess the presence of *Bd* on military lands. Fifteen DoD installations were sampled from west to east along U.S. Highway 66 from California into central Illinois, and continuing eastward from there to the Atlantic Seaboard along U.S. Interstate 64 (in sum from Camp Pendleton in California to Naval Air Station Oceana in Virginia, between 33º and 39 N latitude). The results of the investigation showed strong spatial and temporal patterns to the detection of *Bd* (Lannoo et al., 2011).

This survey reports on three north-to-south, international border-to-border transects conducted at 15 continental United States DOD installations, as follows: a West Coast Transect that runs from Washington to southern California along Interstate 5, a Midwest Transect that runs from Minnesota to southern Alabama along Interstates 94 and 55, and an East Coast Transect that runs from Maine to southern Florida along Interstate 95. The following questions related to the presence and intensity of *Bd* infections were addressed:

1. Is there a west-to-east (longitudinal) pattern?
2. Is there a north-to-south (latitudinal) pattern?
3. Is there a temporal (seasonal) pattern?
4. To what extent are these patterns influenced by climate and geography?

Materials and Methods

Ethics Statement

This research was conducted under Institutional Animal Care and Use Committee protocol number 11217 issued by the University of Illinois at Urbana-Champaign and state scientific collecting license permit numbers 2011-333 (Maine), 17496 (Minnesota), 0127112 (Mississippi), 11-SC00511 (North Carolina), SC2011062 (New Jersey), 080-11 (Oregon), 11-2011 (South Carolina), 3602 (Tennessee), 11-075 (Washington), and SCP-WCR-141-C-2011 (Wisconsin). No animals were harmed while collecting *Bd* samples.

Study Sites

In 2011, *Bd* was sampled for at 15 DoD installations along three north-south transects spanning the length and breadth of the continental United States (figure 1). The study sites were:

- **West Coast Transect** (Washington to California roughly along Interstate 5): Naval Radio Station Jim Creek and Joint Base Lewis-McChord in Washington, Camp Rilea in Oregon, Marine Corps Mountain Warfare Training Center Bridgeport and Fort Hunter Liggett in California.

- **Midwest Transect** (Minnesota to Alabama roughly along Interstates 94 and 55): Camp Ripley in Minnesota, Fort McCoy in Wisconsin, Scott Air Force Base in Illinois, Naval Support Activity Mid-South in Tennessee, and Naval Air Station Meridian in Mississippi.

- **East Coast Transect** (Maine to Florida roughly along Interstate 95): Naval Computer and Telecommunications Station Cutler in Maine, Fort Dix of Joint Base McGuire-Dix-Lakehurst in New Jersey, Marine Corps Base Camp Lejeune in North Carolina, Shaw Air Force Base in South Carolina, and Cape Canaveral Air Force Station in Florida.

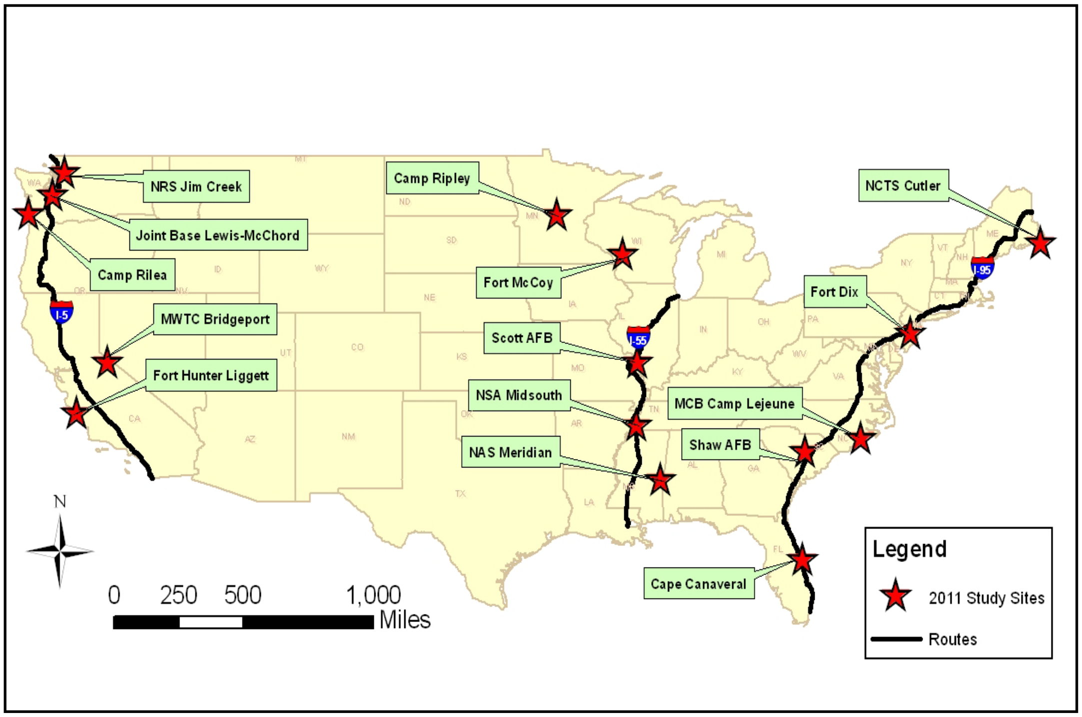

Figure 1. Department of Defense installations sampled in the present study.

Military installations were selected based on their habitat types, temperature and precipitation regimes, and resident amphibian species—looking for wide ranges in geography and environments, as well as, species diversity. Based on the installations selected, the potential existed to sample an estimated 50 species of amphibians—about one sixth of the total number of United States species (Lannoo, 2005).

Field Sampling

Field samples were collected during the northern hemisphere's warm months of 2011. Each installation was sampled twice, once during a period encompassing spring/early summer (March–June), then again during a period encompassing mid/late summer (July–September). Maps of the sample sites for each military installation are in Appendix A. Samples were taken both day and night. Between sites, mud and other debris were cleaned off the gear and the gear was disinfected with a dilute bleach solution.

The study goal was to sample at least 20 amphibians at each installation during each sampling period. To achieve this, on average three wetland sites were generally sampled at each installation. Sites were recorded using a Global Positioning System. Post-metamorphic animals were sampled when they were available; tadpoles were used if they were not.

Amphibians were captured by hand or by using a dip net. Animals were handled with nitrile gloves and placed individually in plastic bags for processing. Gloves and bags were discarded after one use. All animals were sampled using sterile cotton, plastic-handled swabs (Medical Wire & Equipment Co., Corsham, England). For post-metamorphic animals, the swabs were rolled over the body surface a total of 50 times as follows: five rubs each on the back, sides, belly, and head; between the thighs; and on the bottom of each foot. For tadpoles, the mouthparts and oral area were swabbed. Following swabbing, the head of the swab was broken off into a 0.6 ml microcentrifuge tube (Fisherbrand 05-407-01; Pessier and Mendelson, 2010). Samples were stored at 4 ºC and shipped to a laboratory on ice packs prior to analysis (below). Following processing, animals were released at their site of capture. Field notes documenting sample tubes numbers, installation, wetland name, coordinates, date, time, species, sex, and age class (larva or adult) were recorded.

Temperature and Precipitation Data

Mean annual temperature and precipitation data for a 30-year period (1971–2000) was obtained from weather stations near or at each installation from National Oceanic and Atmospheric Administration (NOAA) databases (http://cdo.ncdc.noaa.gov/cgi-bin/climatenormals/climatenormals.pl).

Elevation Data

The approximate elevation for each wetland site on each military installation was obtained from Google Earth Imagery (http://www.google.com/earth/index.html). The elevation of the sample sites ranged from approximately 4 feet at Cape Canaveral to approximately 8,300 feet at MWTC Bridgeport.

Laboratory Analyses

A real-time TaqMan PCR technique (Boyle et al., 2003; Hyatt et al., 2007) was used to analyze *Bd* swabs. Briefly, DNA template was prepared with PrepMan Ultra (Applied Biosystems) and an exogenous internal positive control labeled with TaqMan VIC (Applied Biosystems) was used for each sample to detect PCR inhibitors. Reactions used the TaqMan Environmental Mastermix 2.0 (Applied Biosystems). Assays were run in triplicate on an ABI/Applied Biosystems 7900HT thermocycler using 384 well plates. Samples that amplified at a Ct of<50 in 2 or more wells were considered positive. Quantification standards were created by growing *Bd* isolate JEL 197 on 1 percent Tryptone Agar and harvested of zoospores by rinsing plates with 1x phosphate buffered saline. After collection, zoospores were counted three times on a hemocytometer to determine the range of zoospores ml $^{-}$1. Standard curves were generated with ten-fold serial dilutions (range 1×10^{6} to 1×10^{-2} zoospores). In addition to positive controls (quantification standards), each plate included a negative control (TaqMan Mastermix and no sample DNA), as well as four positive and negative quality assurance controls consisting of swabs either inoculated with *Bd* zoospores or sham-inoculated. The intensity of infection in the positive samples was expressed as the number of zoospore equivalents per swab (Vredenburg et al., 2010).

Data Analysis

To test for patterns in *Bd* prevalence and intensity, Akaike's information criterion (AIC) was applied, specifically AIC_C for overdispersed data and small sample sizes (Burnham and Anderson, 1998), to compare models (general linear models; SPSS v. 17). Models were designed to fit the pattern of *Bd* presence to five variables: (geographic region [G]—east, central, and west; season [S]—spring, summer, and mean; seasonal rainfall [R]; mean seasonal temperature [T]; and latitude [L]). Twenty-four candidate models were selected to test and six of those were the top candidate models for a previous *Bd* study (Lannoo et al., 2011). Under the ideal model selection framework, one model would be the best fit. Significance levels were set at $p \leq 0.05$.

Results

Field Sampling

Each of the 15 installations was sampled twice—one time during the spring/early summer (March–June), the second time during the mid/late summer (July-September). During the spring/early summer, a total of 321 samples were collected that yielded 311 unequivocal results. Of these, 70 (22.5 percent) tested *Bd* positive. The number of animals sampled at each site during this period ranged from zero (MWTC Bridgeport, where it was too cold for amphibians to be active) to 39 (NCTAMS Cutler). Percent positive samples per installation ranged from zero (NRS Jim Creek, Fort McCoy, NSA Mid-South, Cape Canaveral) to 71.4 percent (Fort Dix).

During the mid/late summer, a total of 361 samples were collected that returned 358 unequivocal results. Of these, 67 (18.7 percent) tested *Bd*-positive. The number of samples from each site ranged from 19 (NRS Jim Creek and MCB Camp Lejeune) to 30 (Fort Hunter Liggett). Percent positive samples per installation ranged from zero (MWTC Bridgeport, Fort Hunter Liggett, Scott AFB, NSA Mid-South, NAS Meridian, MCB Camp Lejeune, Shaw AFB, and Cape Canaveral) to 68 (Camp Ripley). In total, from all installations during the course of this study, 682 amphibians of 28 species were sampled producing 669 unequivocal results. Of these, 137 (20.5 percent) tested positive for *Bd*.

Installations

Batrachochytrium dendrobatidis was detected at 12 of the 15 installations sampled. *Bd* was detected at:

- **West Coast Transect**: Naval Radio Station Jim Creek, Joint Base Lewis-McChord, Camp Rilea, and Fort Hunter Liggett

- **Midwest Transect**: Camp Ripley, Fort McCoy, Scott Air Force Base, Naval Air Station Meridian
- **East Coast**: Naval Computer and Telecommunications Station Cutler, Fort Dix, Marine Corps Base Camp Lejeune, Shaw Air Force Base

Combined infection prevalence over both sampling periods (spring/early summer, mid/late summer) ranged from 3.8 percent (2 of 53 samples positive) at Fort Hunter Liggett to 58.7 percent (27 of 46 samples positive) at Fort Dix. Camp Rilea (56.8 percent), Camp Ripley (38.0 percent), and MCB Camp Lejeune (33.3 percent) also had high infection rates (figure 2).

Batrachochytrium dendrobatidis was not detected at three military installations—MWTC Bridgeport, NSA Mid-South, and Cape Canaveral. It is not believed that this was the result of sample size. Forty-nine samples were taken at Cape Canaveral—25 in spring/early summer and 24 in mid/late summer. Thirty-nine samples were taken at NSA Mid-South—14 in spring/early summer and 25 in August mid/late summer. Twenty-one samples were taken at MWTC Bridgeport—none during the spring/early summer period and 21 during July mid/late summer.

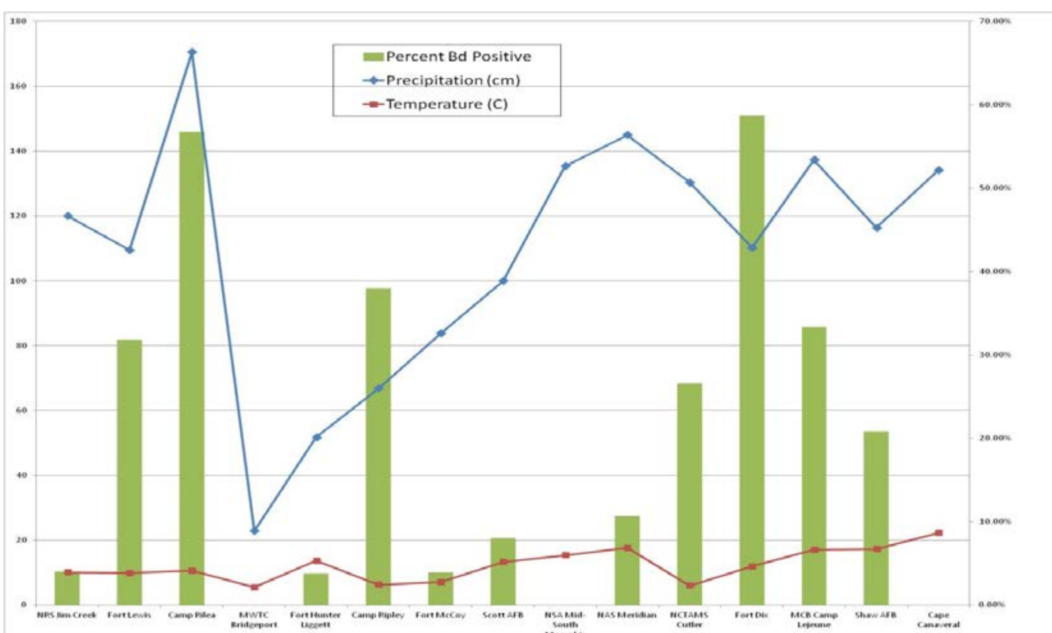

Figure 2. *Bd* infection prevalences in relation to mean annual temperatures and mean annual precipitation at sample installations.

Seasonal Results

Overall, 22.5 percent of the spring/early summer samples tested positive for *Bd* (figure 3). Infection rates ranged from zero (Cape Canaveral, NSA Mid-South, NRS Jim Creek, and Fort McCoy) to 71.4 percent (Fort Dix) (figure 4). A total of 18.7 percent of the mid/late summer samples tested positive for *Bd*. Infection rates for the mid/late summer period ranged from zero (Cape Canaveral, Shaw AFB, MCB Camp Lejeune, NSA Mid-South, NAS Meridian, Scott AFB, Fort Hunter Liggett, and MWTC Bridgeport) to 68.0 percent (Camp Ripley) (figure 4).

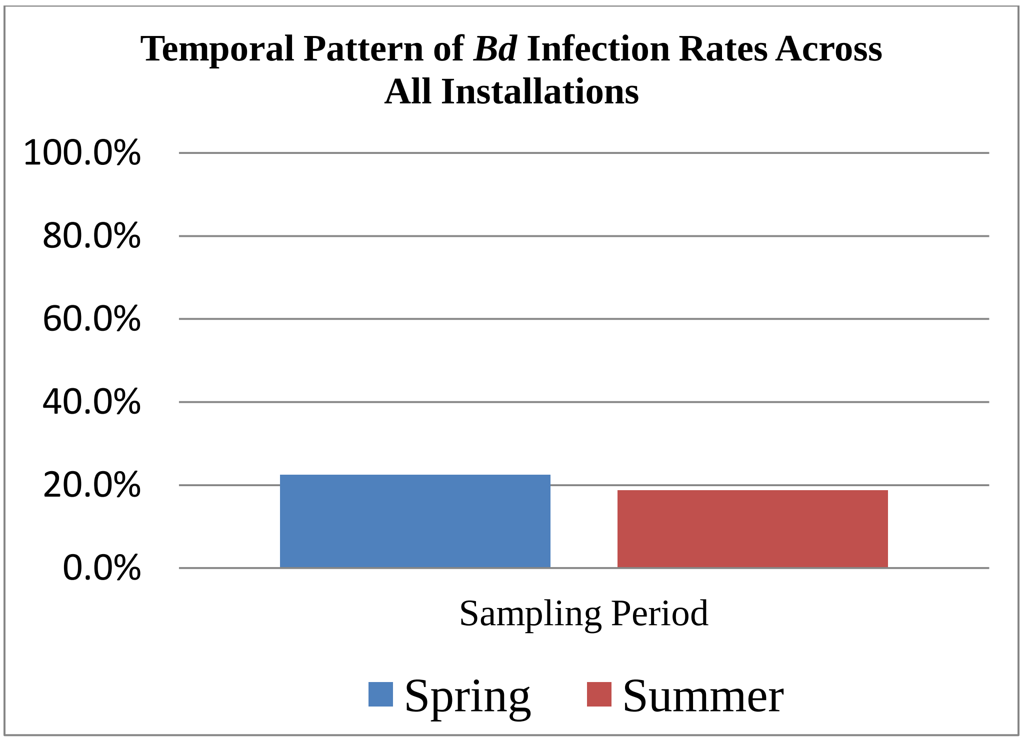

Figure 3. Seasonal (temporal) pattern of *Bd* infection rates for all military installations.

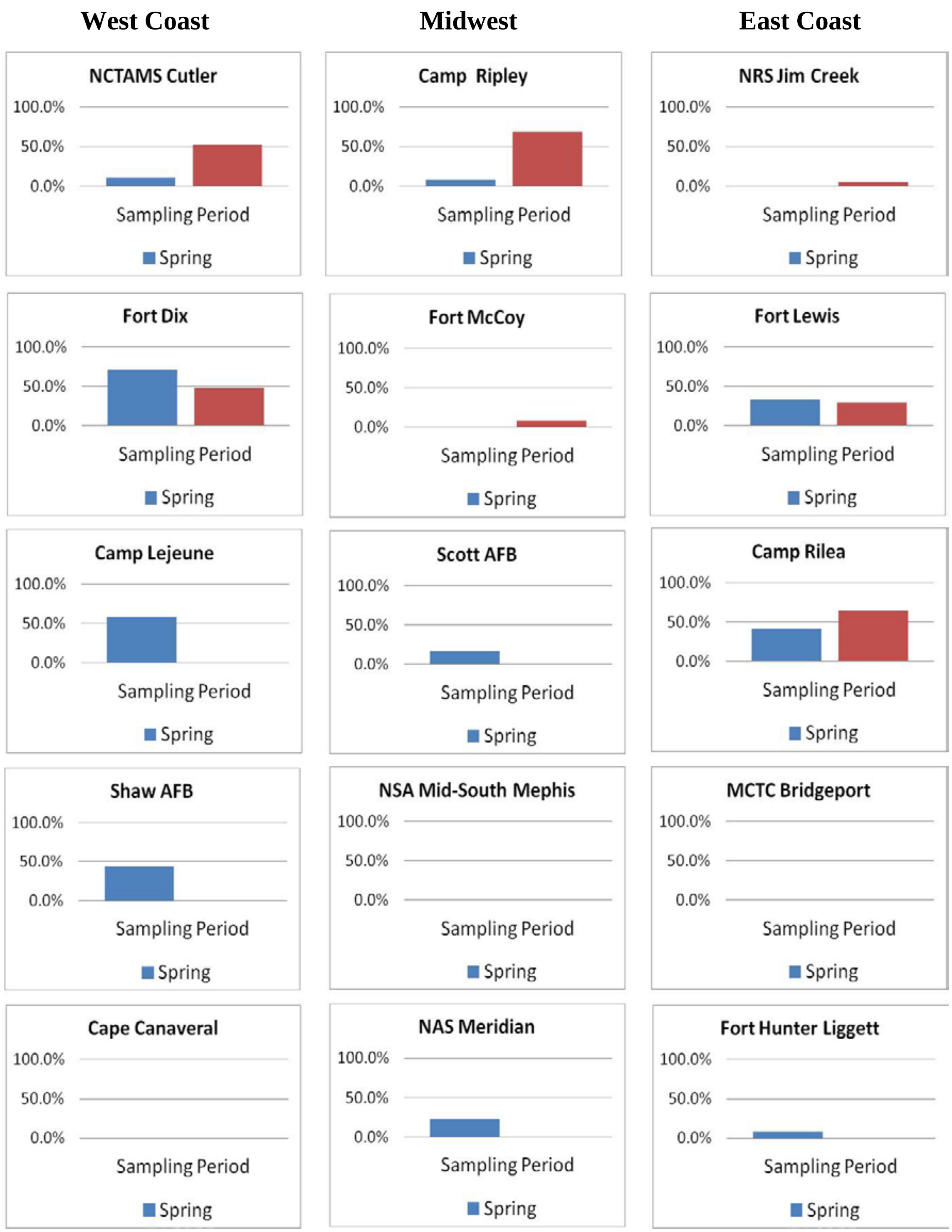

Figure 4. Bar graphs of *Bd* prevalence for 15 military installations sampled in this study arranged in columns from west to east, and rows from north to south.

Species

A total of 28 amphibian species were sampled during this investigation. Of these species, 15 tested positive for *Bd* (table 1). Among salamanders, only one individual, a *Taricha granulosa* sampled at NRS Jim Creek, tested positive. *Batrachochytrium dendrobatidis*-positive frog species included one bufonid (*Anaxyrus americanus*), four hylids (*Acris crepitans*, *Acris gryllus*, *Pseudacris regilla*, and *Pseudacris ornata*) and nine ranids (*Lithobates catesbeianus*, *Lithobates clamitans*, *Lithobates palustris*, *Lithobates pipiens*, *Lithobates septentrionalis*, *Lithobates sphenocephalus*, *Lithobates sylvaticus*, *Rana aurora*, and *Rana sierrae*).

Ambystomatidae	
	Ambystoma gracile (Northwestern Salamander) *Ambystoma laterale* (Blue-spotted Salamander) *Ambystoma macrodactylum* (Long-toed Salamander)
Dicamptodontidae	
	Dicamptodon tenebrosus (Coastal Giant Salamander)
Plethodontidae	
	Plethodon vehiculum (Western Redback Salamander)
Salamandridae	
	Notophthalmus viridescens (Eastern Newt) ***Taricha granulose* (Rough-skinned Newt)**
Bufonidae	
	***Anaxyrus americanus* (American Toad)** *Anaxyrus fowleri* (Fowler's Toad) *Anaxyrus terrestres* (Southern Toad)
Hylidae	
	***Acris crepitans* (Northern Cricket Frog)** ***Acris gryllus* (Southern Cricket Frog)** *Hyla cinérea* (Green Treefrog) *Pseudacris crucifer* (Spring Peeper) ***Pseudacris regilla* (Northern Pacific Chorus Frog)** *Pseudacris ocularis* (Little Grass Frog) ***Pseudacris ornate* (Ornate Chorus Frog)**
Microhylidae	
	Gastrophryne carolinensis (Eastern Narrow-mouthed Toad)
Ranidae	
	***Lithobates catesbeianus* (American Bullfrog)** ***Lithobates clamitans* (Green Frog)** ***Lithobates palustris* (Pickerel Frog)** ***Lithobates pipiens* (Northern Leopard Frog)** ***Lithobates septentrionalis* (Mink Frog)** ***Lithobates sphenocephalus* (Southern Leopard Frog)** ***Lithobates sylvaticus* (Wood Frog)** ***Rana aurora* (Northern Red-legged Frog)** *Rana muscosa* (Mountain Yellow-legged Frog) ***Rana sierrae* (Sierra Nevada Yellow-legged Frog)**
Note: Species are organized by families—salamanders followed by frogs. Bold indicates at least one specimen tested positive. Twenty-eight species were tested. Frogs are disproportionately represented.	

Table 1. Species sampled for the presence of *Bd*

Model Selection

Of the 24 models chosen, the first 7 models had similar Akaike weights (w_i), therefore there was little resolution as to which one was the best fit (table 2). Although the seven best models share the variables season, temperature, and latitude, they explained little variance in the data (table 2). The best model had an r^2 of 0.287 and included the season, the mean seasonal temperature, and their interaction (table 2). Although this model only explained a small amount of the variance, overall it was significant (F=3.22, p=0.041).

There was also a significant difference in the prevalence of *Bd* between early and late samples (F=6.37, p=0.019), as well as, a significant relationship with temperature (F=4.31, p=0.049). Finally, there was a significant interaction of season with temperature (F=5.92, p=0.023). Initially, there does not appear to be a difference in mean *Bd* prevalence based on season; however, when controlling for mean seasonal temperature, mean *Bd* actually increases later in the season (figure 5). Military installations with a cooler mean seasonal temperature have a higher rate of *Bd* prevalence later in the season, whereas those with a warmer mean seasonal temperature have a lower *Bd* prevalence later in the season (figure 6). Conversely, early in the season there appears to be no association with *Bd* prevalence and mean seasonal temperature (figure 6). The shift occurs at approximately 22 °C where *Bd* prevalence drops to 0 (figure 6).

Models	r^2	AIC_C	ΔAIC_C	w_i	Evidence Ratio
S+T+S×T	0.29	14.35	0.00	0.16	1.00
L+T	0.20	14.83	0.48	0.13	1.27
G+L	0.26	15.48	1.13	0.09	1.76
L	0.10	15.63	1.28	0.09	1.89
T	**0.10**	**15.69**	**1.34**	**0.08**	**1.95**
R+L	0.25	15.82	1.47	0.08	2.09
S+L+S×L	0.24	16.11	1.76	0.07	2.41
R+T	0.21	17.33	2.98	0.04	4.44
L+T+L×T	0.20	17.57	3.22	0.03	5.01
R	0.03	17.67	3.32	0.03	5.27
S+T	**0.11**	**17.78**	**3.43**	**0.03**	**5.55**
L+R	0.10	18.01	3.66	0.03	6.24
S+L	0.10	18.05	3.70	0.03	6.36
T+R	**0.10**	**18.07**	**3.72**	**0.03**	**6.43**
S	**0.00**	**18.47**	**4.12**	**0.02**	**7.85**
R+L+T	0.25	18.65	4.30	0.02	8.60
G	0.08	18.75	4.40	0.02	9.02
S+R+L+T	0.32	19.23	4.88	0.01	11.49
S+R	**0.04**	**20.08**	**5.73**	**0.01**	**17.57**
S+T+R	**0.11**	**20.48**	**6.13**	**0.01**	**21.42**
L+R+L×R	0.11	20.60	6.25	0.01	22.79
R+L+R×L	0.26	21.74	7.39	0.00	40.25
G+L+G×L	0.26	21.74	7.39	0.00	40.25
S+R+S×R	0.04	22.72	8.37	0.00	65.58

Note: Variables for the models are the categorical variables of: S–season (early/late) and G–geographic location (East Coast, Midwest, and West Coast Transects), and the covariates of T–temperature, R–rainfall, and L–latitude.

Bold entries represent the top six models from a previous study (Lannoo et al., 2011).

Table 2. Results of AIC analysis on the sets of candidate models for *Bd* prevalence for 14 military installations during the study including model structure, r^2, AIC_C, ΔAIC_C, Akaike weights (w_i), and the evidence ratio for the model.

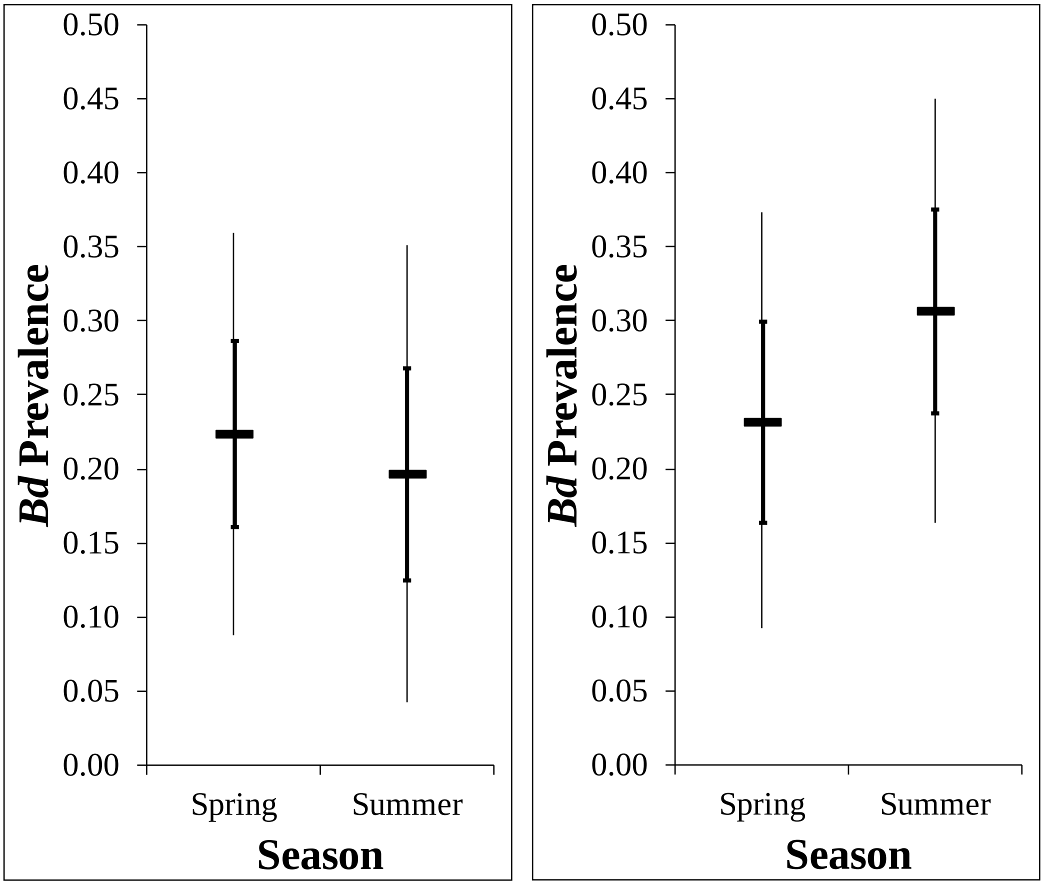

Figure 5. Means, standard error (thick line), and 95 percent confidence interval (thin line) for the raw (left) and estimated marginal means (right) of *Bd* prevalence for 14 military installations during the study under the best fit model selected using AIC_C. Means were evaluated at a temperature of 19.04 °C.

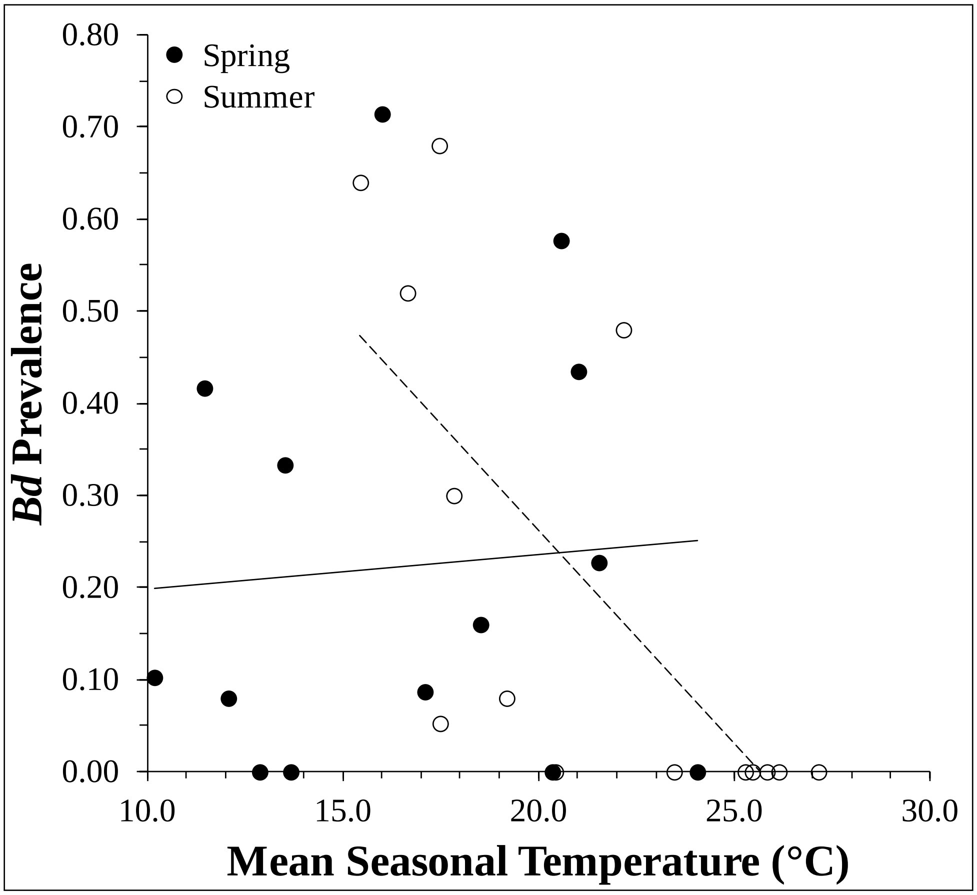

Figure 6. Relationship of *Bd* prevalence with mean seasonal temperature between early and late seasons for 14 military installations during the study under the best fit model selected using AIC_C.

Discussion

While military installations are utilized for training and testing, they are also secured and protected in the interest of national security. These protections also extend to the natural resources contained on military lands which have been shown to harbor the greatest density of threatened and endangered species of any federally-owned land in the United States (Stein et al., 2008). Despite the security that maintains the fence lines and boundaries of military installations, in keeping with prior studies (Lannoo et al., 2011), this survey found *Bd* to be prevalent across the DoD installations in the continental United States.

In this investigation, standardized collection and analytical techniques were used to address the following questions related to the presence and intensity of *Bd* infections:

1. Is there a west-to-east (longitudinal) pattern?
2. Is there a north-to-south (latitudinal) pattern?
3. Is there a temporal (seasonal) pattern?
4. To what extent are these patterns influenced by climate and geography?

Batrachochytrium dendrobatidis was detected at 12 of the 15 installations sampled with a total prevalence of *Bd* infection of 20.5 percent (for both sampling periods and all installations). Of the 28 species sampled, 15 tested positive for *Bd*. Many of the species that tested positive for *Bd* in this investigation have tested positive in other studies (summarized in part in Lannoo et al., 2011).

Is there a longitudinal pattern to the presence of *Bd*?

Study data show a strong west-to-east pattern in the presence of *Bd* infection among each of the three transects (West Coast, Midwest, and East Coast). In total, for all installations and sampling periods, 21.1 percent of the positive samples were from installations along the West Coast Transect, 12.7 percent were at installations along the Midwest Transect, and 27.4 percent were at installations along the East Coast Transect (figure 7). The two installations with the highest prevalence of *Bd* infection were Camp Rilea—the most western installation—with 56.8 percent and Fort Dix—second to last most eastern site—with 58.7 percent. The data support what has been reported in other studies on the distribution of *Bd*; this fungus species is widespread throughout North America, particularly along the east and west coasts of the United States (Lannoo et al., 2011).

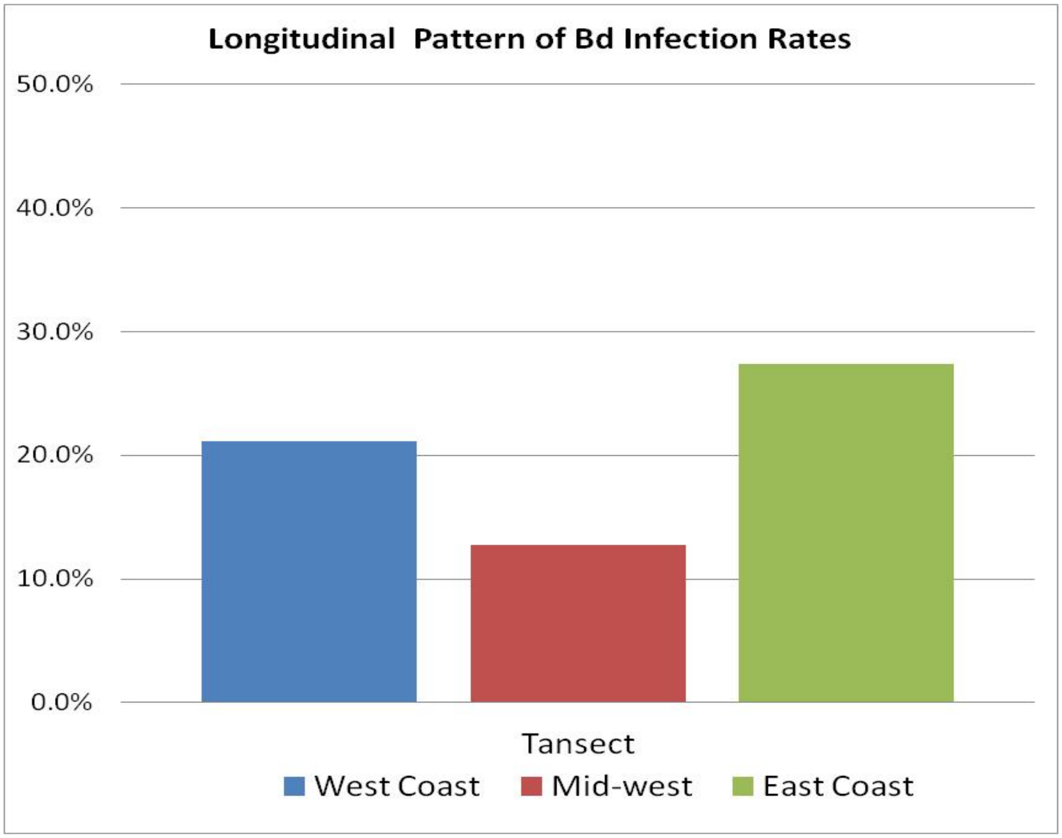

Figure 7. Longitudinal spatial pattern of Bd infection rates

Is there a latitudinal pattern to the presence of *Bd*?

Study data also show a strong north-to-south pattern to the presence of *Bd* infection. The installations north of approximately latitude 40° (NRS Jim Creek, Joint Base Lewis-McChord, Camp Ripley, Camp Rilea, NCTAMS Cutler, Fort McCoy, and Fort Dix) typically had higher *Bd* infection than those located below this latitude (Scott AFB, MWTC Bridgeport, Fort Hunter Liggett, NSA Mid-South, MCB Camp Lejeune, Shaw AFB, NAS Meridian, and Cape Canaveral). The combined infection prevalence for those installations north of latitude 40° was 31.9 percent whereas that for the installations below the prevalence was 10.2 percent (figure 8). The three installations where no *Bd*-positive samples were recorded (MWTC Bridgeport, NAS Mid-South, and Cape Canaveral) were all below latitude 40°.

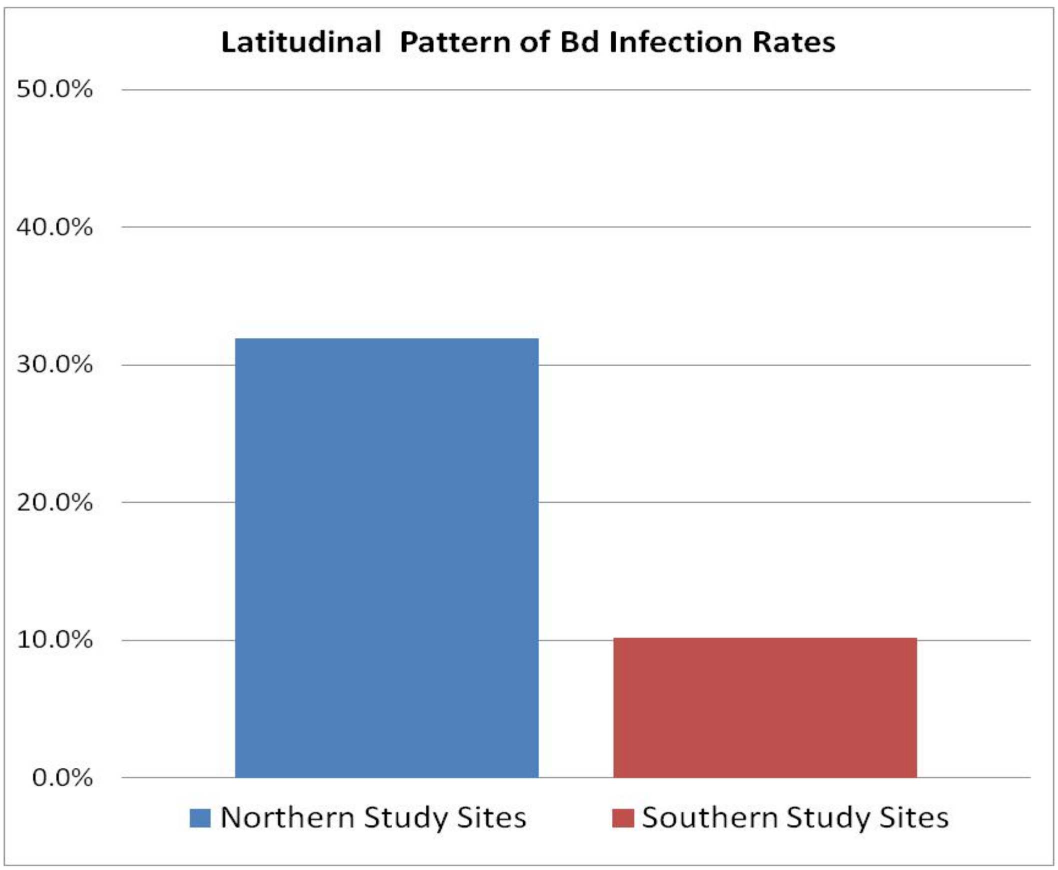

Figure 8. Latitudinal spatial pattern of *Bd* infection rates

Is there a temporal (seasonal) pattern to the presence of *Bd*?

There was not a strong temporal component to the study dataset when combining all the military installations and transects together. In total, 22.5 percent of the spring/early summer samples tested positive for *Bd* and 18.7 percent of the mid/late summer samples tested positive for *Bd* (figure 3). However, a temporal trend was observed within the data when considering the latitudinal (north/south) position of the installations. In general, the northern installations (greater than latitude 40°) had similar or greater *Bd* prevalence in the second sampling period (mid/late summer; 39.6 percent) than the first sampling period (spring/early summer; 14.4 percent). This was observed at NRS Jim Creek, Camp Rilea, Camp Ripley, Fort McCoy, and NCTAMS Cutler. Joint Base Lewis McChord was the exception to this observation, however, the infection prevalence was similar for both the spring/early summer sampling period (33.3 percent) and the mid/late summer sampling period (30.0 percent). In comparison, the installations below latitude 40° (Fort Dix, Scott AFB, MWTC Bridgeport, Fort Hunter Liggett, NSA Mid-South, MCB Camp Lejeune, Shaw AFB, NAS Meridian, and Cape Canaveral) had the greatest percent of *Bd*-positive samples during the first sampling period (spring/early summer; 28.5 percent) than the second sampling period (mid/late summer; 5.5 percent). These observations may be the result of mean seasonal temperatures. Military installations with a cooler mean seasonal temperature have a higher rate of *Bd* prevalence later in the season, whereas those with a warmer mean seasonal temperature have a lower *Bd* prevalence later in the season. These observations are consistent with those reported in previous studies (Lannoo et al., 2011).

Is there an elevation pattern to the presence of *Bd*?

Our data do not show a clear pattern for the incidence of *Bd* based on elevation. The sites with the lowest and highest elevation (Cape Canaveral and MWTC Bridgeport, respectively) had no detection of *Bd* (figure 9). The installation with the second to highest elevation (Camp Ripley) had the third highest incidence of *Bd*. A notable trend was observed in the annual precipitation, which generally decreases as elevation increases.

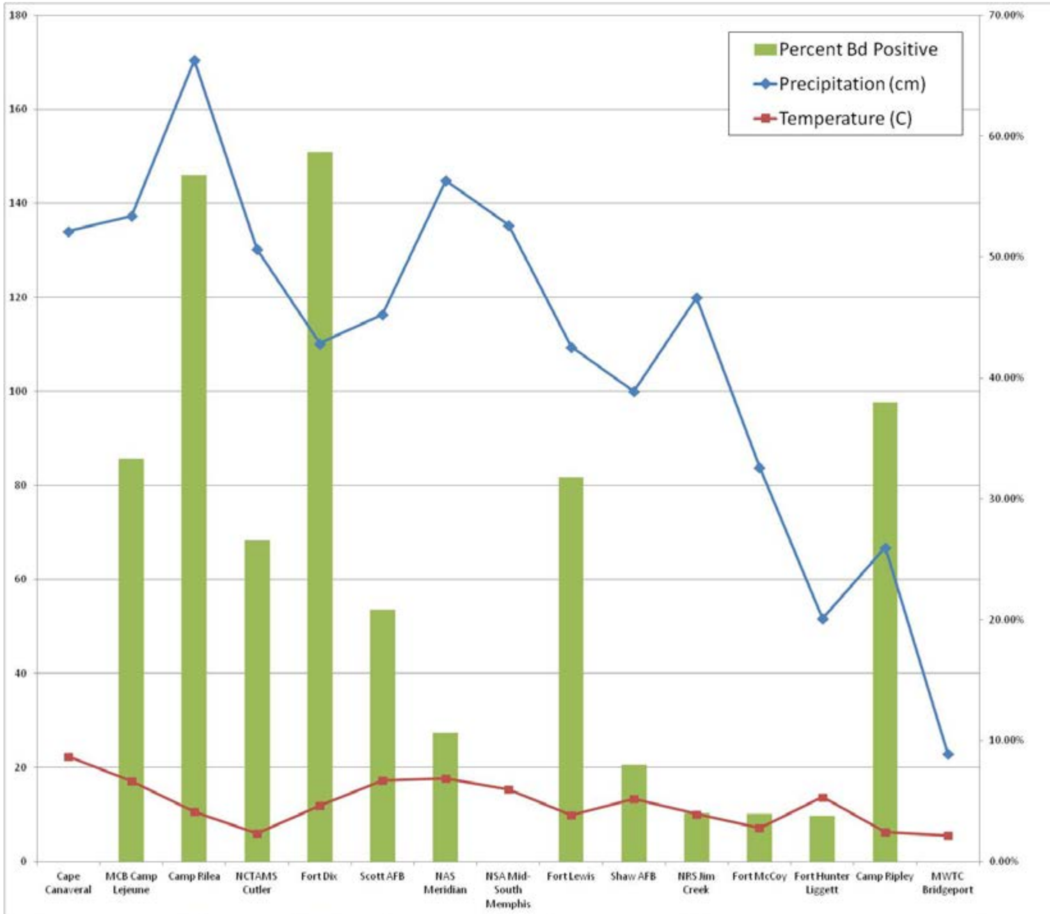

Figure 9. Percent of *Bd*-positive samples, annual precipitation, and temperature by military installation. Installations arranged in order of ascending elevation.

Comparison to the 2009 Military Installation *Bd* Survey

The results of this investigation support the findings of the 2009 military installation *Bd* survey (Lannoo et al., 2011). As with the 2009 study, this investigation found *Bd* present in the highly secure environments of U.S. military installations. Additionally, both studies confirmed that there is a spatial pattern to the presence of *Bd*. Department of Defense installations located on the east and west coasts of the United States had higher prevalences of *Bd* infection than installation situated in the midwest ecosystems. Lastly these data support the conclusion that *Bd* is now widespread, and promote the idea that *Bd* can today be considered endemic extending from coast-to-coast across much of North America, with the exception of remote pockets of native populations.

To date, the 2009 military installation *Bd* survey and this follow-up investigation represent the most comprehensive survey of *Bd* on DoD lands. In total, from all installations and field visits, 1,975 amphibians from 45 species were sampled. *Batrachochytrium dendrobatidis* was detected at the majority of the DoD sites sampled (25 of 30 military installations).

Management Implications

At this time, there are only limited steps that environmental managers can take to prevent the introduction and spread of *Bd* on an installation. In some cases, *Bd* may have already impacted populations of amphibians on military installations. For example, the red-legged frog (*Rana draytonii*) used to be found at Marine Corps Base Camp Pendleton, but has been extirpated since at least the 1990's (Holland and Goodman 1998) from Los Angeles to northern Baja California, México. This species is now federally - listed as endangered. While the impacts of *Bd* were not fully understood in the 1990's, it may have had an impact on the loss of the red-legged frog. The results of this investigation and the similar 2009 survey conclude that understanding if *Bd* is present or absent on an installation, and what species it is impacting is important to the overall management of natural resources on each site. Amphibians play an important role in the ecosystem, and their further declines may warrant protections that hamper military training.

Additionally, it has been demonstrated in a laboratory that crayfish exposed to *Bd* got sick and around a third died within seven weeks (McMahon et al., 2012). This research also revealed that infected crayfish in water with tadpoles—separated by mesh, so the crustaceans wouldn't eat the baby frogs—also became infected. These results suggest that crayfish can probably act as a reservoir for the disease. Additional research is needed to study the crayfish-frog- Bd relationship in natural environments; however it is important to be aware that *Bd* may not only be impacting amphibian species, but also nonamphibian hosts. There are several federally-listed and Species at Risk (SAR) crayfish species on DoD installations that could be impacted by the *Bd* fungus.

Tips on Preventing the Introduction and Spread of *Bd* on Military Installations

- Wet or muddy boots, fishing, and camping equipment may be contributing to the spread of the disease. Sterilize equipment with a solution of diluted bleach if the equipment has been used in wetlands off the installation.

- Monitor wetland sites in the spring for dead/dying frogs. A high mortality rate of amphibians may indicate they are infected with *Bd*.

- Do not allow collecting or translocation of amphibian species on or off the installation.

- Prevent the release of exotic amphibian pets on DoD installations.

- Increase the awareness of military personnel and installation residents about the disease.

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