EcoHealth 13, 368–382, 2016
DOI: 10.1007/s10393-016-1101-4

EcoHealth

Original Contribution

Prevalence and Seasonality of the Amphibian Chytrid Fungus *Batrachochytrium dendrobatidis* Along Widely Separated Longitudes Across the United States

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Abstract: The chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) has been implicated in amphibian declines on almost all continents. We report on prevalence and intensity of *Bd* in the United States amphibian populations across three longitudinally separated north-to-south transects conducted at 15 Department of Defense installations during two sampling periods (late-spring/early summer and mid to late summer). Such a standardized approach minimizes the effects of sampling and analytical bias, as well as human disturbance (by sampling restricted military bases), and therefore permits a cleaner interpretation of environmental variables known to affect chytrid dynamics such as season, temperature, rainfall, latitude, and longitude. Our prevalence of positive samples was 20.4% (137/670), and our mean intensity was 3.21 zoospore equivalents (SE = 1.03; range 0.001–103.59). Of the 28 amphibian species sampled, 15 tested positive. Three sites had no evidence of *Bd* infection; across the remaining 12 *Bd*-positive sites, neither infection prevalence nor intensity varied systematically. We found a more complicated pattern of *Bd* prevalence than anticipated. Early season samples showed no trend associated with increasing temperature and precipitation and decreasing (more southerly) latitudes; while in late season samples, the proportion of infected individuals decreased with increasing temperature and precipitation and decreasing latitudes. A similar pattern held for the east–west gradient, with the highest prevalence associated with more easterly/recently warmer sites in the early season then shifting to more westerly/recently cooler sites in the later season. *Bd* intensity across bases and sampling periods was comparatively low. Some of the trends in our data have been seen in previous studies, and our results offer further continental-level *Bd* sampling over which more concentrated local sampling efforts can be overlaid.

Keywords: amphibians, chytrid fungus, department of defense, *Batrachochytrium dendrobatidis*

Introduction

Worldwide, declines in amphibian populations are occurring at a rate several times faster than decreases in birds or

Published online: March 2, 2016

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mammals, with an estimated 42% of amphibian species in decline (Stuart et al. 2004; IUCN Red List 2014). In addition to global threats, such as habitat degradation and the impacts of invasive species that all organisms face, amphibians are also disproportionately affected by emerging infectious diseases. One of these, a chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*; Longcore et al. 1999), is considered a leading cause of amphibian declines (Berger et al. 1998; DiRosa et al. 2007; Skerratt et al. 2007; Briggs et al. 2010). Although the distribution of amphibians with *Bd* infections is nearly global, the distribution of lethal outbreaks of *Bd*-caused amphibian declines has to date been restricted to a few regions, notably Eastern Australia, Central America, and the western United States (Lips et al. 2006; Skerratt et al. 2007; Jones et al. 2008; Murray et al. 2009). As research on *Bd* continues, the complexity of this fungus and factors that affect it continue to be revealed. At the global scale, *Bd* detection is impacted by pressure factors such as trade and the introduction of alien host species (Liu et al. 2013) and associated with fundamental niche factors such as climate (Rohr and Raffel 2010; Rohr et al. 2011; Olson et al. 2013). Because of the preference of *Bd* for cool, moist environments, microhabitat characteristics such as temperature, moisture, and vegetation cover were empirically found to be important drivers of *Bd* infection (Raffel et al. 2010). Models also support these findings and indicate that mean diurnal temperature range and annual precipitation were important predictors of *Bd* occurrence (Murray et al. 2011), and natural vegetation and host species richness can be key factors associated with *Bd* occurrence (Becker and Zamudio 2011). Previous studies also suggest that *Bd* prevalence is predicted by host traits and host diversity (Venesky et al. 2013; Becker et al. 2014).

Other complexities of factors that affect *Bd* are known. For example, few die-offs connected to *Bd* have been reported in the eastern three-quarters of North America (east of the Rocky Mountains). This has led to the hypothesis that *Bd* is endemic in amphibian populations in this region (Rachowitz et al. 2006; Kinney et al. 2011). This scenario also suggests that in certain regions of the world, such as the majority of North America, much of the spread of *Bd* occurred decades ago (when it was epidemic) and that in these places it is now endemic (arising within the population). Lannoo et al. (2011) indicated that further testing of the endemic hypothesis involving surveys over broad geographic scales is warranted.

In regions where *Bd* is considered endemic, it can impact populations when environmental conditions are favorable (Retallick et al. 2004; Ouellet et al. 2005; Longo et al. 2010; Savage et al. 2011; Terrell et al. 2014). As a result, when sampling for *Bd*, it is important to distinguish between prevalence (percentage of individuals infected divided by number sampled) and intensity (strength of the infection, measured in zoospore equivalents). In populations where *Bd* is acting as an endemic disease, prevalence and intensity can be coupled (Briggs et al. 2010; Kinney et al. 2011); where *Bd* is acting in an epidemic fashion, prevalence and intensity can be uncoupled (Terrell et al. 2014). Both prevalence and intensity vary seasonally in endemic infections. Kinney et al. (2011) reported both *Bd* prevalence and intensity dropped from spring to summer in a population of crawfish frogs (*Lithobates areolatus*) located in southern Indiana—adults sampled in mid-summer showed no signs of infection. These same animals emerged from winter senescence and entered breeding wetlands with an infection prevalence of about 25%, and emerged from breeding wetlands with an infection rate of over 50%. Prevalence and intensity were linked, and chytrid-related deaths occurred following breeding. Post-breeding adults that returned to their burrows subsequently cleared the infection, presumably by basking.

When interested in natural rates of disease occurrence and transmission, it becomes useful to sample sites with a minimal disturbance history. Military installations are landscapes secured and protected in the interest of national security. These protections also extend to the natural resources contained on military bases—landscapes that have been shown to harbor the greatest density of threatened and endangered species and habitats of any federally owned lands in the United States (Stein et al. 2008).

The first objective of this study was to extend our previous work (Lannoo et al. 2011) by surveying for *Bd* prevalence (proportion of individuals infected) and intensity (strength of the infection, measured as zoospore equivalents) over three north-to-south transects at widely separated longitudes across the United States. To maintain comparability to the results of Lannoo et al. (2011), we used the same methodology and sampled United States Department of Defense (DOD) installations. Furthermore, for this study the same team of researchers following the same protocol collected field samples at all 15 study sites during the two sampling periods. This consistency reduces the confounding factors of sampling and analysis bias, as well as human disturbance on the dynamics of *Bd* infection transforming into the disease chytridiomycosis (Lannoo et al. 2011). Following the conclusions of Lannoo et al. (2011), our first hypothesis is that because hot, dry, sparsely vegetated habitats limit this

Figure 1. Department of Defense installations sampled in the present study. Note that bases were selected to form three North–south transects along both east and west coasts and through the middle of the North American continent.

fungus, *Bd* would be more often present and more intense at bases located in the north and east—that is, in the northern portion of the western transect, and throughout the mid-western and eastern transects (see below).

Our second objective was to assess the relationship of seasonality to the prevalence and intensity of *Bd* in the United States. Seasonality in *Bd* prevalence has been previously demonstrated (Berger et al. 2004; Gaertner et al. 2009; Kinney et al. 2011; Savage et al. 2011). As summer proceeds, the prevalence of *Bd* in amphibian populations decreases (Lannoo et al. 2011) as *Bd*-positive frogs clear their infection (Johnson and Speare 2005; Piotrowski et al. 2004; Woodhams et al. 2003, 2005; Kinney et al. 2011). This pattern is tied to the life history and physiological ecology of *Bd*, which thrives in cool, moist conditions—temperatures >28 °C are lethal (Fisher et al. 2009; Stevenson et al. 2013). We felt a study design consisting of three north-to-south transects would provide a wide range of both temporally and spatially mediated temperatures and precipitation levels, providing a further test of the relationship of *Bd* prevalence to temperature and precipitation. Our second hypothesis is that *Bd* would be more often present and more intense during our first sampling period (spring/early summer [March–June]), than our second (mid/late summer [July–September]). Our third hypothesis, building on the first, is that at any point in time, *Bd* prevalences and intensities would be lowest at our low-latitude sites, because temperatures are more likely to exceed the thermal maximum of *Bd* for longer portions of the year.

Materials and Methods

Ethics Statement

This research was conducted under Institutional Animal Care and Use Committee protocol number 11,217 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, we sampled for *Bd* at 15 DOD installations along three north–south transects spanning the length and breadth of the continental United States (Fig. 1). Transects were as follows.

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

Midwest Transect (Minnesota to Mississippi 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 (NSA Mid-South), and Naval Air Station Meridian (NAS Meridian) in Mississippi.

East Coast Transect (Maine to Florida roughly along Interstate 95): Naval Computer and Telecommunications Area Master Station Cutler in Maine, Fort Dix 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.

We selected these military installations to maximize the range of variation in geography, habitat types, climate, and species diversity. Based on the installations selected and the distribution of United States amphibians, 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

We collected field samples 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). Samples were taken exclusively during the day and individual sites were not re-sampled during each field period. Between sites, we cleaned mud and other debris from gear, and disinfected gear with a dilute bleach solution.

Our goal was to sample at least 20 amphibians at each installation during each sampling period. To achieve this, three (range one to seven) wetland sites were generally sampled at each installation. We recorded sample sites using a Global Positioning System (GPS). In most cases, we sampled post-metamorphic animals (adults and juveniles), but we sampled tadpoles when no adults or juveniles were encountered.

We captured amphibians by hand or using a dip net. To prevent the spread of disease, we secured animals wearing nitrile gloves and placed animals individually in plastic bags for processing. We discarded gloves and bags after one use. We sampled all animals using sterile cotton, plastic-handled swabs (Medical Wire & Equipment Co., Corsham, England). For post-metamorphic animals, we rolled swabs 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, we swabbed mouthparts and oral disks. Following swabbing, we broke the head of the swab into a 0.6 ml microcentrifuge tube (Fisherbrand 05-407-01; Pessier and Mendelson 2010). We stored samples at 4°C and shipped them on ice packs prior to analysis (described below). Following processing, we released animals at their site of capture.

Temperature and Precipitation Data

We obtained maximum daily temperature and precipitation data for a 30-day period prior to the sampling time from weather stations near or at each installation using National Oceanic and Atmospheric Administration (NOAA) databases (http://cdo.ncdc.noaa.gov/cgi-bin/climatenormals/climatenormals.pl). We then averaged all maximum daily temperatures and summed the daily precipitation for use as covariates in our analyses.

Laboratory Analyses

We used a real-time TaqMan PCR technique (Boyle et al. 2004; Hyatt et al. 2007) to analyze *Bd* swabs. Briefly, we prepared a DNA template with PrepMan Ultra (Applied Biosystems) and used an exogenous internal positive control labeled with TaqMan VIC (Applied Biosystems) for each sample to detect PCR inhibitors. For reactions, we used the TaqMan Environmental Mastermix 2.0 (Applied Biosystems). We ran assays in triplicate on an ABI/Applied Biosystems 7900HT thermocycler using 384 well plates. We considered samples that amplified at a Ct of <50 in 2 or more wells positive (Ct is the cycle number at which the fluorescent-labeled *Bd* probe crosses the threshold to indicate signal). We considered samples that amplified at a Ct of <50 in 1 well equivocal. For positive samples, we created quantification standards by growing *Bd* isolate JEL 197 on 1% Tryptone Agar and harvested zoospores by rinsing plates with 1x phosphate buffered saline. After collection, we counted zoospores three times on a hemocytometer to determine the range of zoospores ml $^{-}1$. We

generated standard curves 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.

We expressed the intensity of infection in the positive samples as the mean (averaged over the three reactions) number of zoospore equivalents (INT) per swab (Vredenburg et al. 2010). We calculated mean INT as the sum of all swab INT means divided by the number of positive swabs. We calculated *Bd* prevalence (PREV) as the number of positive swabs divided by the number of unequivocal swabs (equivocal swabs were eliminated).

Data Analysis

We excluded all individuals for which indeterminate results were obtained from all three PCR runs. Next we conducted a principal component analysis in R (R Core Team 2015) on the variables of latitude, longitude, average maximum 30-day temperature, and 30-day precipitation. We retained all principal components with an eigenvalue greater than

Table 1. Summary of *Bd* Prevalence by Species and Life Stage for Amphibians Sampled at Department of Defense Installations During 2011

Species	Sites		Adult & Juvenile		Larvae	
	#	*Bd* Pos.	#	*Bd* Pos.	#	*Bd* Pos.
Ambystoma gracile	1	0	2	0	–	–
Ambystoma laterale	1	0	0	–	13	0
Ambystoma macrodactylum	1	0	1	0	–	–
Dicamptodon tenebrosus	1	0	0		8	0
Plethodon vehiculum	1	0	4	0	–	–
Notophthalmus viridescens	1	0	1	0	–	–
Taricha granulosa	1	1	2	1	–	–
Anaxyrus americanus	2	1	2	1	–	–
Anaxyrus fowleri	2	0	5	0	–	–
Anaxyrus terrestris	3	0	8	0	–	–
Acris crepitans	3	2	120	16	–	–
Acris gryllus	2	2	91	15	–	–
Hyla cinerea	1	0	25	0	18	0
Pseudacris crucifer	1	0	1	0	–	–
Pseudacris regilla	2	2	3	3	–	–
Pseudacris ocularis	1	0	1	0	–	–
Pseudacris ornata	1	1	2	2	–	–
Pseudacris sierra	2	1	48	1	–	–
Gastrophryne carolinensis	3	0	3	0	6	0
Lithobates catesbeianus	4	2	5	3	–	–
Lithobates clamitans	3	3	95	39	22	0
Lithobates palustris	1	1	10	3	–	–
Lithobates pipiens	1	1	2	1	–	–
Lithobates septentrionalis	1	1	2	2	–	–
Lithobates sphenocephalus	1	1	2	1	–	–
Lithobates sylvaticus	2	1	22	14	36	2
Rana aurora	3	2	76	30	1	0
Rana muscosa	1	0	6	0	–	–
Totals		22	539	132	104	2

Early and late season sampling combined and the summary does not include tadpoles that were swabbed, but could not be identified to species.

Table 2. Summary Data for *Bd* Prevalence Data for Amphibians Sampled at Department of Defense Installations During 2011 for the Early Season, Late Season, and Overall Sampling Period Combined

Base	Early season				Late season				Overall			
	Total	Unequiv.	# pos.	% pos	Total	Unequiv.	# pos.	% pos	Total	Unequiv.	# pos.	% pos
NCTAMS Cutler	39	39	4	10.3	25	25	13	52.0	64	64	17	26.6
Fort Dix	23	21	15	71.4	26	25	12	48.0	49	46	27	58.7
Camp Lejeune	28	26	15	57.7	19	19	0	0.0	47	45	15	33.3
Shaw AFB	25	23	10	43.5	25	25	0	0.0	50	48	10	20.8
Cape Canaveral	25	25	0	0.0	24	24	0	0.0	49	49	0	0.0
Camp Ripley	25	25	2	8.0	25	25	17	68.0	50	50	19	38.0
Fort McCoy	26	26	0	0.0	25	25	2	8.0	51	51	2	3.9
Scott AFB	26	25	4	16.0	25	25	0	0.0	51	50	4	8.0
NSA Mid-South	15	15	0	0.0	25	25	0	0.0	40	40	0	0.0
NAS Meridian	22	22	5	22.7	25	25	0	0.0	47	47	5	10.6
NRS Jim Creek	6	6	0	0.0	19	19	1	5.3	25	25	1	4.0
Fort Lewis	25	24	8	33.3	22	20	6	30.0	47	44	14	31.8
Camp Rilea	12	12	5	41.7	25	25	16	64.0	37	37	21	56.8
MWTC Bridgeport	21	21	0	0.0	–	–	–	–	21	21	0	0.0
Fort Hunter Liggett	23	23	2	8.7	30	30	0	0.0	53	53	2	3.8
Total	341	333	70	21.0	340	337	67	19.9	681	670	137	20.4

Data include the total number of swabs taken, the number of unequivocal swabs, number of swabs positive for *Bd*, and the percent of swabs positive for *Bd* per base.

one and conducted a Kaiser–Meyer–Olkin (KMO) test of sampling adequacy and Bartlett's test of sphericity to determine if variable reduction was warranted and variances were equal. We then used a varimax rotation to determine which variables were associated with which components and retained the pc-scores as our new covariates.

To determine if the probability of *Bd* infection in amphibians varied by season, latitude, longitude, and with recent temperature, and rainfall, we conducted a series of mixed-effect binary logistic regressions using the R package lme4 (Bates et al. 2015). We used the species sampled as the random effect and season and principal component scores (see below) as the main effects. Next, we established a set of candidate models including the null (intercept only), global (all main effects and two-way interactions), all main effects models, all two-way main effects models, and all two-way main effects models with interaction. We assessed the candidate models using an information theoretic approach (Burnham and Anderson 2002) using the R package AICcmodavg (Mazerolle 2015). We then used the R package effects (Fox 2003) to determine how the presence/absence of *Bd* was affected by our main effects.

To analyze the intensity data, we first eliminated all *Bd* negative individuals from the dataset, then z-transformed our intensity estimates so they were centered and scaled by standard deviation units. We then followed the same methods for establishing and assessing candidate models as with the probability of infection analysis, except we used general linear mixed-effects models. For both analyses, if our candidate set of models (0.95 cumulative akaike weights) contained multiple models (i.e., low resolution), we performed model averaging of the parameters using AICcmodavg (Mazerole 2015). We then assessed the parameter effects by examining whether or not confidence intervals bounded zero.

Results

Across all bases and combining the two sampling periods, the 681 swabs collected (from 28 species) produced 670 unequivocal results. Of the 28 species sampled, 15 tested positive for *Bd* (Table 1). Among salamanders, only one individual, a *Taricha granulosa* sampled at NRS Jim Creek, tested positive. *Bd*-positive frog species included one bufonid (*Anaxyrus americanus*), four hylids (*Acris crepitans*,

Table 3. Summary Statistics of *Bd* Intensity Data for Amphibians Sampled at Department of Defense Installations During 2011 for the Early Season, Late Season, and Overall Sampling Period Combined

Base	Early season					Late season					Overall					Lat.	Long.
	N	Mean	SE	Min	Max	*N*	Mean	SE	Min	Max	*N*	Mean	SE	Min	Max		
NCTAMS Cutler	4	25.91	25.9	0.001	103.59	13	0.49	0.26	0.01	3.45	17	6.47	6.07	0.001	103.59	44.657	−67.294
Fort Dix	15	2.46	1.42	0.01	18.95	12	0.28	0.1	0.01	1.24	27	1.49	0.81	0.01	18.95	40.013	−74.573
Camp Lejeune	15	3.9	1.82	0.01	22.03	–	–	–	–	–	15	3.9	1.82	0.01	22.02	34.627	−77.318
Shaw AFB	10	2.59	1.28	0.01	11.91	–	–	–	–	–	10	2.6	1.28	0.01	11.91	33.836	−80.512
Cape Canaveral	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	28.473	−80.534
Camp Ripley	2	0.1	0.09	0.01	0.2	17	0.5	0.38	0.01	6.61	19	0.47	0.36	0.01	6.61	46.082	−94.336
Fort McCoy	0	0.05	0.01	0.04	0.05	2	0.05	0.07	0.04	0.05	2	0.05	0.01	0.04	0.05	44.062	−90.637
Scott AFB	4	2.1	1.91	0.08	7.84	–	–	–	–	–	4	2.1	1.91	0.08	7.84	38.550	−89.842
NSA Mid-South	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	35.323	−89.873
NAS Meridian	5	5.56	3.5	0.02	18	–	–	–	–	–	5	5.56	3.5	0.02	18	32.551	−88.612
NRS Jim Creek[†]	–	–	–	–	–	1	28.22	–	–	–	1	28.2	–	–	–	48.172	−121.945
Fort Lewis	8	1.58	1.03	0.02	8.44	6	0.14	0.07	0.01	0.45	14	0.96	0.61	0.01	8.44	47.028	−122.513
Camp Rilea	5	4.03	2.04	0.04	10.74	16	0.82	0.55	0.01	8.16	21	1.58	0.68	0.01	10.74	46.115	−123.941
MWTC Bridgeport	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	38.399	−119.480
Fort Hunter Liggett	2	42.88	42.83	0.05	85.71	–	–	–	–	–	2	42.88	42.8	0.05	85.71	35.966	−121.312
Overall	70	5.43	1.95	0.001	103.59	67	0.9	0.45	0.01	8.16	137	3.21	1.03	0.001	103.59		

Data include the overall number of samples submitted, the mean zoospore equivalents with the associate standard error, minimum, and maximum per base.
[†]Jim Creek was excluded for the late season because it had only one *Bd* positive individual.

Table 4. Results of Principal Components Analysis on the Variables of Latitude, Longitude, Average Maximum 30-day Temperature, and 30-day Precipitation for *Bd* Sample Data Taken at 14 Military Installations Across the Continental United States in 2011

	PC1	PC2	PC3	PC4
Overall results				
Eigenvalues	1.757	1.037	0.736	0.470
SD	1.325	1.018	0.858	0.685
Proportion of variance	0.439	0.259	0.184	0.117
Cumulative proportion	0.439	0.699	0.883	1.000

Variable	Loading		Varimax rotation	
	PC1	PC2	PC1	PC2
Latitude	**−0.631**	0.111	**0.540**	0.246
Longitude	**0.500**	0.238	−0.473	**0.841**
Avg. max. 30-day temp	**0.540**	**−0.474**	0.325	**0.439**
30-day precipitation	0.246	**0.841**	**−0.615**	-0.200

High loading values and rotated values are bolded.

Table 5. Model Selection Results for the Eleven Candidate Mixed-Effects Binary Logistic Regression Models and General Linear Mixed-Effects Models Used to Determine the Prevalence of *Bd* Infection and Intensity with Season, Latitude/Precipitation Component, and Longitude/Temperature Component

Model	K	−2LL	AIC_c	ΔAIC_c	w_i	Σw_i
Probabilty if *Bd* infection						
Global	**8**	**−264.97**	**546.17**	**0.00**	**1.00**	**1.00**
Season + lat./precip. + inter.	5	−290.32	590.74	44.57	0.00	1.00
Lat./precip. + long./temp. + inter.	5	−297.29	604.66	58.49	0.00	1.00
Lat./precip. + long./temp.	4	−302.70	613.46	67.29	0.00	1.00
Lat./precip.	3	−304.20	614.45	68.28	0.00	1.00
Season + lat./precip.	4	−304.09	616.25	70.08	0.00	1.00
Null	2	−306.12	616.27	70.10	0.00	1.00
Season	3	−305.13	616.30	70.13	0.00	1.00
Long./temp.	3	−305.26	616.56	70.39	0.00	1.00
Season + long./temp.	5	−303.25	616.59	70.42	0.00	1.00
Season + long./temp. + inter.	4	−304.82	617.71	71.54	0.00	1.00
Bd intensity						
Null	**3**	**−144.48**	**295.14**	**0.00**	**0.27**	**0.27**
Season	**4**	**−143.76**	**295.83**	**0.69**	**0.19**	**0.47**
Long./temp.	**4**	**−144.01**	**296.33**	**1.19**	**0.15**	**0.62**
Lat./precip.	**4**	**−144.46**	**297.22**	**2.08**	**0.10**	**0.72**
Season + long./temp.	**5**	**−143.67**	**297.80**	**2.66**	**0.07**	**0.79**
Season + lat./precip.	**5**	**−143.76**	**297.97**	**2.83**	**0.07**	**0.86**
Lat./precip. + long./temp.	**5**	**−143.89**	**298.24**	**3.10**	**0.06**	**0.92**
Season + long./temp. + inter.	6	−143.14	298.93	3.80	0.04	0.96
Season + lat./precip. + inter.	6	−143.74	300.13	4.99	0.02	0.98
Lat./precip. + long./temp. + inter.	6	−143.88	300.41	5.27	0.02	1.00
Global	9	−143.05	305.52	10.39	0.00	1.00

Results include number of parameters (K), −2 log-likelihood (−2LL), AIC_c, ΔAIC_c, akaike weights (w_i), and cumulative akaike weights (Σw_i). The candidate set of models are bolded. Main effects were season, latitude/climate component, and longitude component, and the mixed-effect was species.

A. gryllus, *Pseudacris regilla*, and *P. ornata*), and nine ranids (*L. catesbeianus*, *L. clamitans*, *L. palustris*, *L. pipiens*, *L. septentrionalis*, *L. sphenocephalus*, *L. sylvaticus*, *Rana aurora,* and *R. sierrae*).

The number of animals sampled per site ranged from 21 (MWTC Bridgeport) to 64 (NCTAMS Cutler). The overall PREV was 20.4% (137 positives) and ranged from zero to 58.7% across installations (Table 2). Mean INT was 3.21 (SE = 1.03) and ranged from 0.001 to 103.59 across swabs (Table 3).

For the early season sampling period, the number of animals sampled per site ranged from 6 (NRS Jim Creek) to 39 (NCTAMS Cutler). Of the 341 early season swabs collected, 333 produced unequivocal results. The overall early season PREV was 21% (70 positives) and ranged from zero to 71.4% across installations (Table 2). Mean INT was 5.43 (SE = 1.95) and ranged from 0.001 to 103.59 across swabs (Table 3).

For the late season sampling period, the number of animals sampled at each site ranged from 0 (MWTC Bridgeport) to 30 (Fort Hunter Liggett). Of the 340 late season swabs we collected, 337 produced unequivocal results. The overall late season PREV was 19.9% (67 positives) and ranged from 0% to 68% across installations (Table 2). Mean INT was 0.90 (SE = 0.45) and ranged from 0.05 to 28.22 (Table 3) across swabs. We did not include MWTC Bridgeport in the analysis because no samples could be obtained in the late season period.

Variable Reduction

Our results for the KMO test of sampling adequacy (score = 0.574) and Bartlett's tests of sphericity

Table 6. Estimates, Standard Errors, and 95% Confidence Intervals for All Non-redundant Parameters in the Global Mixed-Effects Binary Logistic Regression Model of *Bd* Prevalence and the Model Average Estimates of the 95% Confidence Set of Mixed-Effects General Linear Models for *Bd* Intensity

Parameter	$\beta_{est.}$	SE	95% confidence interval	
			Lower	Upper
Probability of *Bd* infection				
β_I	**−1.458**	**0.724**	**−2.877**	**−0.039**
β_{Late}	−0.380	0.413	−1.189	0.429
β_{PC1}	**2.321**	**0.632**	**1.081**	**3.560**
β_{PC2}	0.249	0.421	−0.575	1.074
$\beta_{Late:PC1}$	**−3.285**	**0.521**	**−4.306**	**−2.263**
$\beta_{Late:PC2}$	**1.918**	**0.633**	**0.678**	**3.158**
$\beta_{PC1:PC2}$	**−0.743**	**0.218**	**−1.171**	**−0.315**
Parameter	β	Uncond SE	95% confidence interval	
			Lower	Upper
Bd intensity				
Intercept	0.823	0.561	−0.276	1.921
Season	−0.145	0.132	−0.404	0.113
Latitude/precip.	−0.023	0.125	−0.268	0.222
Longitude/temp.	0.062	0.076	−0.087	0.211

All parameter estimates that do not have a confidence interval that bound zero are bolded.

($\chi^2 = 298.67$, $df = 6$, $p \ll 0.001$) suggested variable reduction was warranted among latitude, longitude, average maximum 30-day temperature, and 30-day precipitation. Two components were retained which explained 66.9% of the cumulative variance (Table 4). Latitude and 30-day precipitation were best explained in PC1 with a positive association for latitude and a negative for precipitation when rotated (Table 4). Longitude and average maximum 30-day temperature were best explained in PC2 with positive associations when rotated and because we designated longitudes west of the prime meridian as negatives, more easterly latitudes represented a higher PC score (Table 4).

Bd Infection Prevalence

Of the eleven binary logistic mixed-effects models examined, the global model carried the lowest ΔAIC_c score and had an akaike weight of 1.00, suggesting it was the best model (Table 5). The strongest predictors in the global model were the parameters associated with the latitude/precipitation component and all interaction of effects and the intercept (Table 6). When examining the effects from the global model, we found season and the longitude/temperature components alone did not provide predictive power in discriminating the probability of *Bd* infection (Table 6; Fig. 2). Our latitude/precipitation component had strong resolution suggesting higher latitude with less recent precipitation had a greater probability of *Bd* infection (Table 6; Fig. 3). For the longitude/temperature component, we found more easterly sites with recent warmer temperatures had a higher probability of infection (Table 6; Fig. 3).

Predictive power greatly increased when accounting for the interactions among season, latitude/precipitation, and longitude/temperature (Table 6). There was a shift in the probability of *Bd* infection between seasons along a latitudinal/precipitation gradient (Table 6; Fig. 4). In the early season, a higher probability of infection was associated with higher latitudes with less recent precipitation (Table 6; Fig. 4). By the late season, a higher probability of infection was associated with lower latitudes and increased recent precipitation (Table 3; Fig. 4). A similar pattern held true for an east–west gradient with the highest probability of

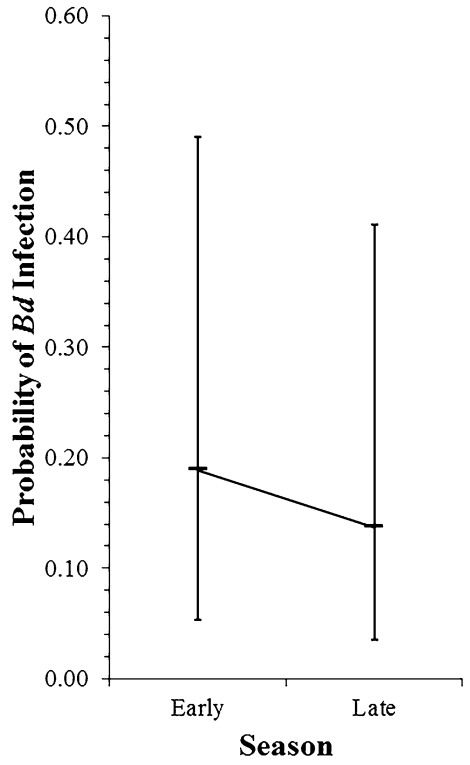

Figure 2. The mean effect of sampling season (and 95% confidence intervals) on the probability of *Bd* infection in amphibians sampled from 14 military installations across the continental United States in 2011.

infection being associated with more easterly/recently warmer sites in the early season to more westerly/recently cooler sites in the later season (Table 6; Fig. 4).

When examining the latitude/precipitation and longitude/temperature components, interaction was complex and shifted along both gradients (Table 6; Fig. 5). At low latitudes with less recent precipitation, we found that the probability of *Bd* infection decreased with more easterly sites with warmer recent temperatures (Table 6; Fig. 5). At higher latitudes with less recent precipitation, the pattern inverted where the highest probability of *Bd* infection increased with more westerly sites with cooler recent temperatures (Table 6; Fig. 5).

Bd Infection Intensity

Of the eleven mixed-effects general linear models, the global model performed the worst, although all models including an interaction term also performed poorly (Table 5). The null model performed the best. However, the resolution in discriminating between all other models that had combinations of season, latitude/climate, and longitude was low (Table 5). All of these models comprised the candidate set, and after model averaging of parameters, we found none of the main effects had any predictive power in determining the intensity of *Bd* infection (i.e., they all bounded zero; Table 6).

Discussion

Given the number of scientists studying *Bd*, as well as the individual and lab-based variations in swabbing techniques and sample transportation, storage, and analyses, it becomes useful to conduct frequent surveys by the same collaborators within a long-term monitoring framework and across large geographical areas to provide a template for comparison (Kriger et al. 2007). In this study, we surveyed for *Bd* prevalence and intensity on DoD installations over three north-to-south transects at widely separated longitudes across the United States using the same team of researchers and following the same protocol. Similar to the findings of Lannoo et al. (2011), we found *Bd* to be widespread spatially, with restricted military installations not naïve to *Bd*. Only three installations had no *Bd*-infected amphibians (NSA Mid-South, MWTC Bridgeport, Cape Canaveral); however, *Bd* is known from these regions in general (www.bd.maps.net; Olson et al. 2013).

One-fifth (20.4%) of the 670 amphibians we swabbed across the United States tested positive for the presence of *Bd*. Early season (21.0%) and late season (19.9%) prevalences were essentially identical. In contrast, overall infection intensities varied, averaging 3.21, with early season intensities being, on average, higher (5.93) than late season intensities (0.90). While these differences were not significant, they are consistent with our previous continental survey (Lannoo et al. 2011), and with the observation that animals exposed to warm and dry summer conditions can clear the infection (Woodhams et al. 2003; Kinney et al. 2011).

All of the species that tested positive for *Bd* in this investigation have tested positive in other studies (www.bd.maps.net; Lannoo et al. 2011). Similarly, other trends in our data have been seen in our previous research. For example, *Bd* prevalence rates increased from west to east on the North American continent (Lannoo et al. 2011). Interestingly, the relationship between the mean proportion of *Bd* infections differed depending on season. In the early season, there was no trend associated with increasing temperature and precipitation and decreasing (more

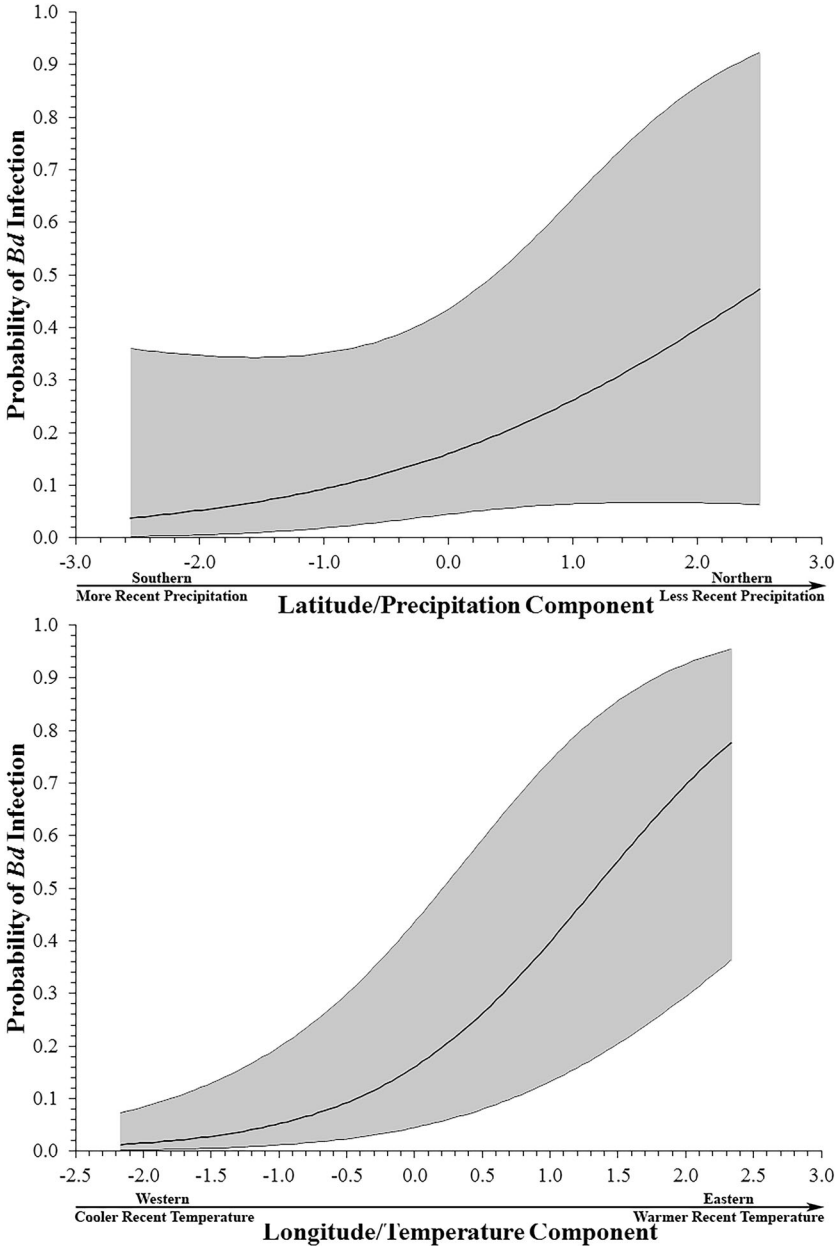

Figure 3. The effects of the latitude/climate component and longitude component on the probability of *Bd* infection in amphibians sampled from 14 military installations across the continental United States in 2011. The *shaded* area represents the 95% confidence interval.

southerly) latitudes; in contrast, during the late season, the mean proportion of infected individuals decreased with increasing temperature and precipitation and decreasing latitudes.

Our prevalence average of 20.4% aligns with other multispecies *Bd* surveys across the United States. For example, in the Pacific Northwest, Adams et al. (2007) reported a *Bd* prevalence of 21.5%. Working in the East, Tupper et al. (2011) found 18% prevalence in anurans on Cape Cod; Davidson and Chambers (2011) found an 18% *Bd* prevalence in Virginia; and Huang and Wilson (2013) found a 19% prevalence in the Piedmont and Blue Ridge ecoregions of northern Georgia. In the Midwest, Krynak et al. (2012) found a 20.2% *Bd* prevalence in Ohio, while Rodriguez et al. (2009) found a 22% prevalence working in northern Minnesota. Of course, other studies find higher or lower *Bd* prevalences depending on species sampled [e.g., Red-spotted Newts seem to have high prevalences (Groner and Relyea 2010; Bletz and Harris 2013)], region of the country (Chestnut et al. 2008; Saenz et al. 2010; Tatarian and Tatarian 2010; Gaertner et al. 2012), altitude (Hasken et al. 2009), life history stage (Kinney et al. 2011), season sampled (Kinney et al. 2011; Savage et al. 2011), and hydrologic regime (Terrell et al. 2014).

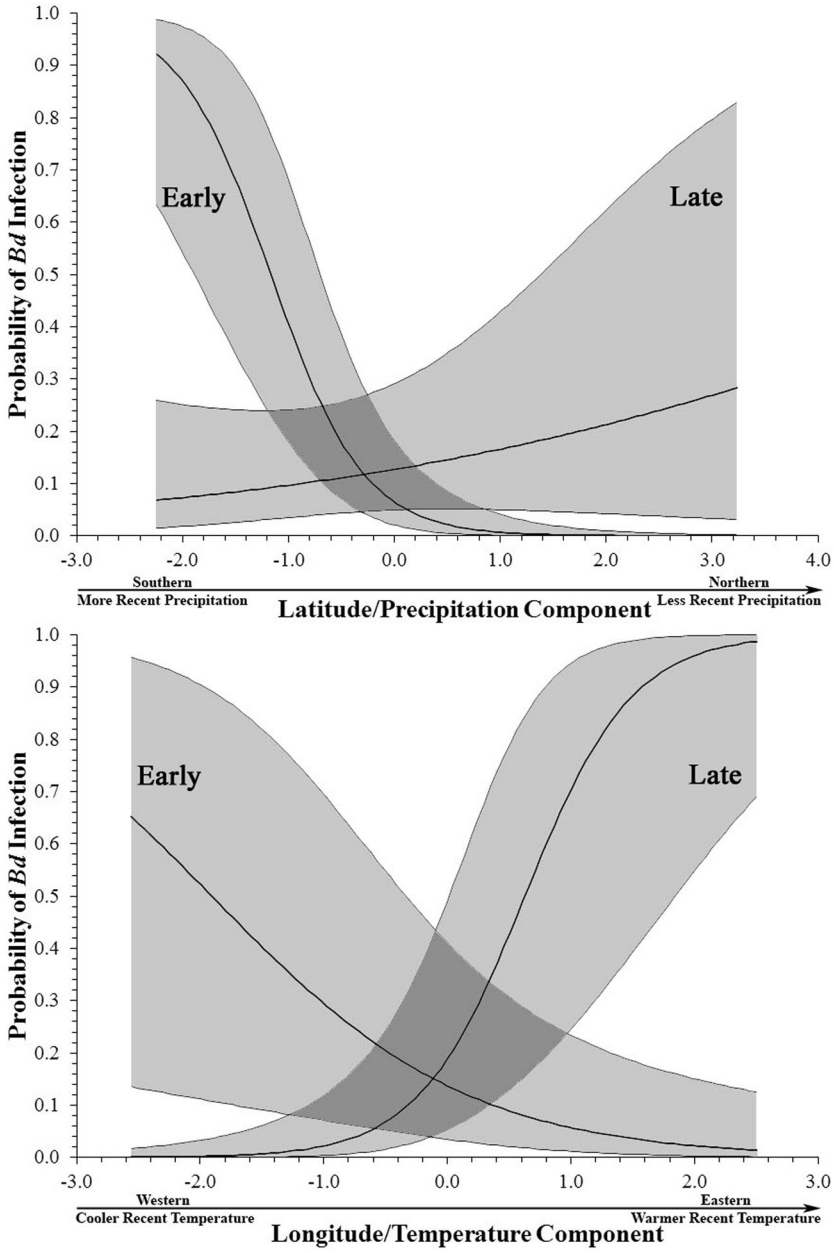

Figure 4. The interactive effects of season and the latitude/climate component and longitude component on the probability of *Bd* infection in amphibians sampled from 14 military installations across the continental United States in 2011. The *lighter shaded* area represents the 95% confidence interval and the *darker shaded* area represents where confidence intervals overlap.

Our results suggest a pattern much more complicated than our hypotheses presupposed. Instead of prevalences and intensities being linked and varying in geographically and seasonally predictable ways, prevalences and intensities were uncoupled (probably because intensities were so low), and the predictive power of our prevalence models greatly increased when accounting for the interactions among season, latitude/precipitation, and longitude/temperature. In particular, we observed a shift in the probability of *Bd* infection between seasons along a latitudinal/precipitation gradient (Table 6; Fig. 4), as follows. In the early season, an infection prevalence was associated with higher latitudes with less recent precipitation (Table 6: Fig. 4), supporting hypothesis one. By the late season, however, prevalence was associated with lower latitudes and increased recent precipitation (Table 3; Fig. 4), refuting hypotheses one and three. A similar pattern held for the east–west gradient, with the highest prevalences associated with more easterly/recently warmer sites in the early season, supporting hypothesis one, then shifting to more westerly/recently cooler sites in the later season (Table 6; Fig. 4), refuting hypothesis one. Prevalences were equivalent between early (20.4%) and late (21%) season samples, refuting hypothesis two, while

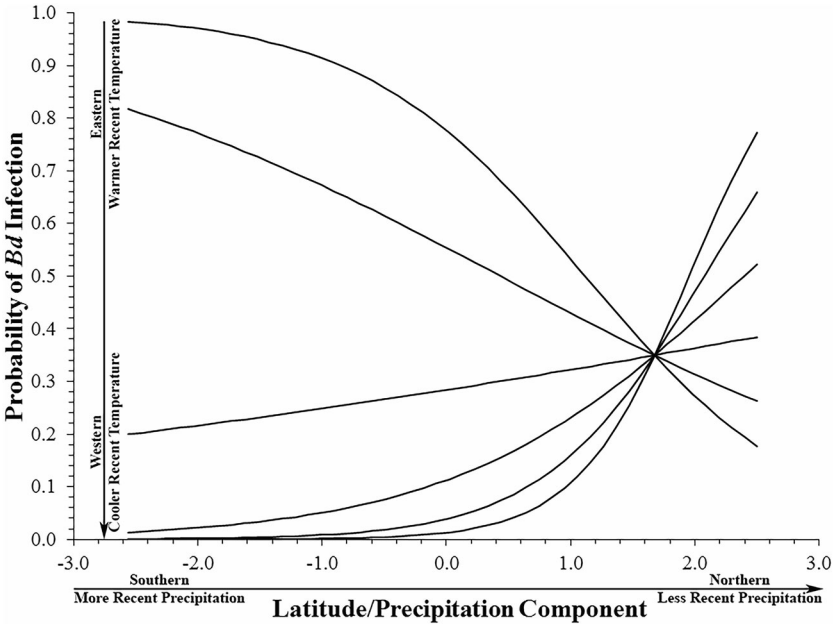

Figure 5. The interactive effects of the latitude/climate component and longitude component on the probability of *Bd* infection in amphibians sampled from 14 military installations across the continental United States in 2011. Lines represent the mean value of the latitudinal component evaluated at −2.16664, −1.24878, −0.33093, 0.586925, 1.50478, and 2.33085 (from *top* to *bottom*)

intensities increased from 3.21 to 5.43 (SE = 1.95 (Tables 1 and 2), also refuting hypothesis two.

We found *Bd* intensity to be comparatively low (Table 1). Further, we found that after model averaging, none of the main effects had predictive power (i.e., they all bounded zero; Table 6). The lack of effects on Bd intensity is likely the result of a combination of low observed intensity values and little variation among samples. Vredenburg et al. (2010) have suggested that at an intensity of about 10,000 zoospore equivalents, *Bd* shifts from being an infection to the disease, chytridiomycosis (see also Kinney et al. 2011). Therefore, our sampling did not reveal infection intensities high enough to trigger die-offs. However, with an average of one in five amphibians at these bases infected (the mean PREV was 20.4%), the potential exists for chytridiomycosis flare-ups should environmental conditions be conducive. Terrell et al. (2013) have shown that, in the face of varying climactic conditions, *Bd* prevalences remain relatively constant while intensities vary. Under the cool, wet conditions favored by the fungus, *Bd* infections can intensify and become fatal.

It has been suggested that habitat type may be an important factor potentially influencing *Bd* prevalence or intensity that certain aquatic habitats, especially cooler, shaded lentic ones, might be more conducive to *Bd* survival and therefore transmission among amphibians (Woodhams et al. 2003; Berger et al. 2004; Rohr and Raffel 2010; Lannoo et al. 2011). Although the scope of our project did not address this complicated, multifaceted environmental question, we do acknowledge that some of the variation in our results could be attributable to sampling amphibians in a variety of habitats.

This study provides important insight into the latitudinal and seasonal precipitation factors which affect *Bd*. Military installations in the United States have a tremendous density of native amphibians and have provided an important network of outdoor laboratories to sample for *Bd* across the North American continent. In the face of continuing environmental alterations brought about by habitat-independent factors such as climate change, disease dynamics, and invasive species, military installations may provide valuable controls to assess the effects of these factors on populations, communities, and ecosystems.

Acknowledgements

Support for this project (10-426) came from the Department of Defense Legacy Resource Management Program https://www.dodlegacy.org.legacy/index.aspx. The authors thank Chris Bucciantini, David Davis, Jackie Hancock, Ethan Kessler, John Maile, Jeff Phillips, and Dan Wylie for collecting specimens. For providing access to their installations and support in the field, we thank David Beckmann, Fort McCoy; Jay Brezinka, Camp Ripley; Chris Bucciantini, Naval Air Station Meridian; Angy Chambers, Cape Canaveral Air Force Station; Liz Clark, Fort Hunter Liggett; David Davis, Shaw Air Force Base; Chad Garber, Marine Corps Base, Camp Lejeune; Jackie Hancock, Fort Hunter

Liggett; Jim Heide, Naval Support Activity Mid-South; Andrew Irvine, Marine Corps Mountain Warfare Training Center; Jim Lynch, Fort Lewis; Jeff Mach, Camp Rilea Armed Forces Training Facility; John Maile, Camp Ripley; John Miller, Jim Creek Naval Radio Station; Kari Moore, Naval Computer and Telecommunications Station, Cutler; Cindy Nolan, Scott Air Force Base; John Richardson, Fort Lewis; Bill Rogers, Marine Corps Base, Camp Lejeune; Kristen Sharp, Fort Dix; Roger Smith, Fort Dix; Linda Wagoner, Jim Creek Naval Radio Station; Rob Williamson, Naval Support Activity Mid-South.

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