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Department of Defense Amphibian Disease Survey: Natural Resource Manager Training and Data Collection

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ABSTRACT

As an extension of previous studies conducted by the authors in 2009 and 2011 (Legacy Projects 09-423 and 11-423), a goal of this investigation was to conduct additional surveys for the chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*) on Department of Defense (DoD) lands. Unlike earlier surveys where one researcher went to multiple military sites to sample amphibians for *Bd*, in this study we trained natural resource managers at multiple military installations to collect field data simultaneously. As a result, *Bd* was sampled for at more than three times the number of military sites than in our previous surveys. In order to standardize the data collection effort, we developed an amphibian swabbing training video and datasheet and conducted three online training sessions for project volunteers. In addition, volunteers received a field swabbing kit containing all the materials need to collect field data. We mailed 71 field swabbing kits to military installations in 37 states within the continental United States (U.S.) and three countries outside the United States (Guam, Spain, and Okinawa). Fifty-two military sites returned kits containing 944 samples. Positive *Bd* results were detected in 226 samples (24.2 percent) and 70 percent of the military sites sampled contained at least one positive result for *Bd*. A total of 57 amphibian species were sampled during this investigation. Of these species, 16 tested positive for *Bd*. Results are consistent with our previous surveys, confirming that *Bd* is present on DoD installations in the continental United States extending from coast to coast. Although *Bd* is present on the majority of the military sites tested in this study, at this time, the fungus does not appear to be having a negative impact on amphibian species (zoospore levels were not at levels to become the disease chytridiomycosis). The results of this study support the hypothesis that *Bd* can today be considered endemic (likely to have spread through North America decades ago) rather than epidemic (spreading as a wave and wiping out individuals, populations, and species in its path).

INTRODUCTION

Numerous studies document the severe decline in amphibian populations worldwide and it is estimated that approximately one-third of global amphibian species have imperiled status (Stuart et al. 2004; Wake and Vrendenburg 2008; Olson et al 2013). In part, 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) caused these declines. As of June 2014, samples from 52 of 82 countries where sampling was reported detected the *Bd* pathogen, and it has been detected in 516 of 1240 (42 percent) amphibian species. (Olson et al 2013). In the United States, this pathogen is 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).

Although the distribution of amphibians with *Bd* infections is nearly global, the distribution of lethal outbreaks of *Bd*-caused amphibian declines is restricted to a few regions, notably Eastern Australia, Central America, and the western United States (Skerratt et al. 2007; Jones et al. 2008; Murray et al. 2009). The eastern three-quarters of North America reported few die-offs connected to *Bd*. This led to the hypothesis that *Bd* is endemic in amphibian populations in this region (Rachowitz et al. 2006; Kinney et al. 2011). This scenario suggests that in certain regions of the world, such as North America (exclusive of remote potions of California), 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).

STUDY OBJECTIVE

Department of Defense installations encompass approximately 28 million acres (11.3 million ha) and occur throughout the United States. These sites provide either low-impact (i.e. “natural”) or well-protected areas due to their secure borders. As a result of the restrictions to enter DoD installations, limited testing for *Bd* has occurred on military sites. The range of variation in geography, habitat types, climate, and species diversity

found on these sites led to the selection of Military installations as study sites for this investigation.

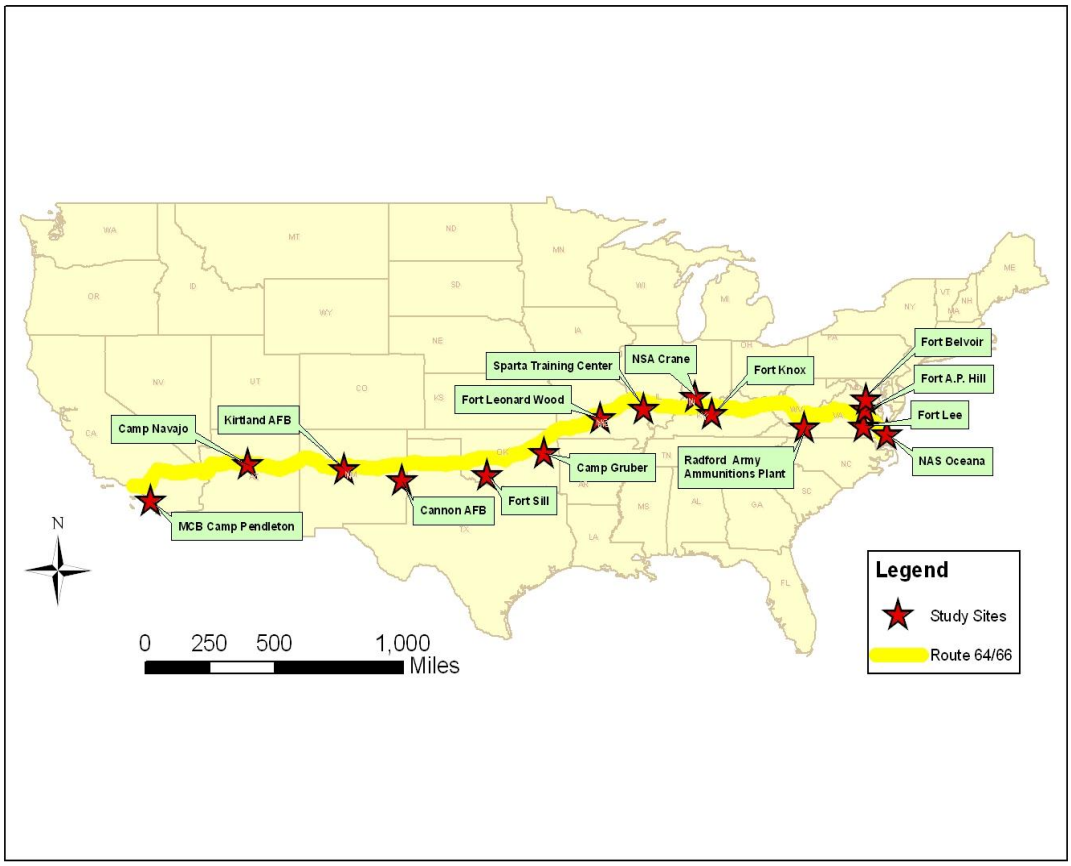
One objective of this study was to extend the work of two previous *Bd* surveys conducted by the authors on DoD lands (Legacy Projects 09-423 and 11-423). During these previous studies, we used standardized techniques to sample for *Bd* on a total of 30 DoD installations. In contrast to our previous research, the primary difference in this study was the training of many natural resource managers to collect the field data rather than the sending of one researcher/team to each site to collect data. As a result, we sampled more than three times the number of military sites than in our previous surveys.

Our studies differ from many other previous research efforts to sample for *Bd* due to the use of volunteers/citizen science and broad geographic scale. Broad-scale studies are important when investigating the various factors (temperature, precipitation, elevation, and infection rate) influencing the *Bd* pathogen. To date, most studies are conducted locally, on single populations or within localized areas, and often use different sampling protocols and analytical techniques (Adams et al. 2007; Frías-Alvarez 2008; Grant et al. 2008; Deguise and Richardson 2009; Goldberg et al. 2009; Sadinski et al. 2010). The use of the volunteers allowed us to sample at many sites simultaneously across the continental United States.

BACKGROUND

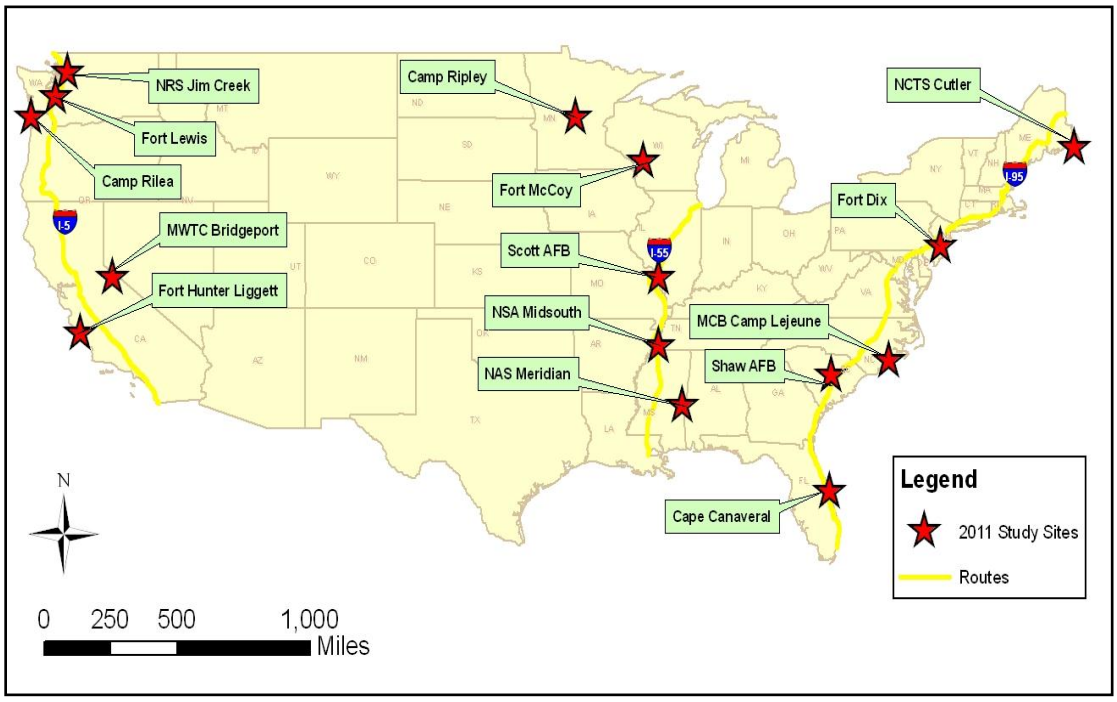
In 2009, we conducted a transcontinental transect designed to assess the presence of *Bd* on military lands. Fifteen DoD installations were sampled from west to east along U.S. Highway 66 from California into central Illinois, and continuing eastward to the Atlantic Seaboard along U.S. Interstate 64 (in sum from Camp Pendleton in California to Naval Air Station Oceana in Virginia, between 33º and 39º N latitude; figure. 1). The results of the investigation show strong spatial and temporal patterns to the detection of *Bd* (Lannoo et al. 2011). The ten eastern temperate DoD installations have higher rates of *Bd* infection than the five bases situated in the arid west. There is a strong temporal (seasonal) component to our dataset. In total, 78.5 percent of all positive samples came in the first (spring/early-summer) sampling period.

Figure 1. Department of Defense installations participating in the 2009 study.



In 2011, we conducted a second survey to assess the presence of *Bd* following the same standardized methodology as the 2009 survey and sampled for *Bd* at an additional 15 DoD installations along three north-south transects spanning the length and breadth of the continental United States (figure. 2). The results of this study also show *Bd* prevalence rates increase from west to east across the United States and early season intensities on average are higher than late-season intensities. These results suggest animals exposed to warm and dry summer conditions can clear the infection (Woodhams et al. 2003; Kinney et al. 2011).

Figure 2. Department of Defense installations participating in the 2011 study.



MATERIALS AND METHODS

In the winter of 2012, ZooAtlanta developed a training video specifically for this study that demonstrates a protocol for skin swabbing amphibians to detect for *Bd* fungus. The video discusses techniques to prevent the spread the fungus, if present, from one wetland site to another. The DoD Legacy Program approved this video for public release and uploaded it to YouTube (http://www.youtube.com/watch?v=a5CtPrGOK8c). The researchers developed a field datasheet to assist with standardizing the data collection effort (appendix A).

In March 2013, the researchers sent a request to the DoD Services asking for volunteers to participate in the study. This request was distributed through the DoD Natural Resources Program, the National Military Fish and Wildlife Association (NMFWA), and the Department of Defense-Partners in Amphibian and Reptile Conservation (DoD PARC) group. Interested individuals were asked to email the researchers requesting to participate in the investigation. In total, 71 military installations,

including three outside the continental U.S (Naval Station Rota, Spain; Commander Fleet Activities Okinawa; and Naval Base Guam) indicated they would participate in the study.

Between March and April 2013, the researchers conducted three online webinar training sessions for the volunteers from the military sites. The training sessions provided background information on the previous amphibian disease surveys conducted by the researchers on DoD lands in 2009 and 2011, featured the swabbing video by ZooAtlanta, and allowed for the group to ask questions regarding the data collection effort.

Figure 3. Cooler filled with field supplies.

The researches purchased field supplies needed to conduct the skin swabbing procedure, packaged them into small coolers for shipping (figure 3), and mailed them to the volunteers at each military site. A pre-paid shipping label was provided so volunteers could return the coolers after they had collected their data. The coolers contained: 25 cotton swabs (wooden handled), 25 microcentrifuge tubes and caps, nitrile rubber gloves, datasheet, sterile wipes, a permanent marker (fine-tipped Sharpie), and an ice pack.

Field data collection occurred between March and August of 2013. It was up to the discretion of the volunteers to choose when samples were collected, where samples were collected, and which species were sampled. The only limitations were that at least 20 samples had to be collected at each military site and that samples were returned no later than September 2013.

The field protocol followed by the volunteers was as follows: volunteers captured Amphibians by hand or by using a dip net. Animals were handled with nitrile rubber gloves and placed individually in plastic bags for processing. Nitrile rubber 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, volunteers rolled the 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, the mouthparts and oral area were swabbed. Following swabbing, volunteers broke the head of the swab 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. 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.

STUDY SITES

As indicted above, 71 field swabbing kits were mailed to military installations within 37 U.S. states and three countries outside the United States (Guam, Spain, and Okinawa; table 1). Of the installations mailed kits, 6 were Air Force, 24 were Army, 14 were Army National Guard, 4 were joint bases, 2 were Marine Corps, and 21 were Navy installations.

Table 1. Seventy one military installations sent *Bd* kits. Bold text indicates the 50 military installations that returned samples for analysis (sheet 1 of 4).

DoD Service	Military Installation	State
Air Force	**Beale Air Force Base**	**California**
Air Force	**Columbus Air Force Base**	**Mississippi**
Air Force	**Shaw Air Force Base**	**South Carolina**
Air Force	US Air Force Academy	Colorado
Air Force	Vandenberg Air Force Base	California
Air Force	Wright Patterson Air Force Base	Ohio
Army	**Aberdeen Proving Ground**	**Maryland**
Army	**Camp Atterbury**	**Indiana**
Army	Camp Guernsey	Wyoming
Army	**Camp McCain**	**Mississippi**

Table 1. Seventy one military installations sent *Bd* kits. Bold text indicates the 50 military installations that returned samples for analysis (sheet 2 of 4).

DoD Service	Military Installation	State
Army	**Camp Ripley**	**Minnesota**
Army	**Camp Swift Training Center**	**Texas**
Army	**Fort A.P. Hill**	**Virginia**
Army	Fort Devens USAG	Massachusetts
Army	**Fort Drum**	**New York**
Army	**Fort Hood**	**Texas**
Army	**Fort Huachuca**	**Arizona**
Army	**Fort Lee**	**Virginia**
Army	**Fort Leonard Wood**	**Missouri**
Army	**Fort Polk**	**Louisiana**
Army	**Fort Riley**	**Kansas**
Army	**Fort Stewart**	**Georgia**
Army	Fort Wainwright	Alaska
Army	**JBLM Yakima Training Center**	**Washington**
Army	**Milan Army Ammunition Plant**	**Tennessee**
Army	**Picatinny Arsenal**	**New Jersey**
Army	**Redstone Arsenal**	**Alabama**
Army	**White Sands Missile Range**	**New Mexico**
Army National Guard	**Camp Maxey**	**Texas**
Army National Guard	**Camp Rilea**	**Oregon**
Army National Guard	**Camp Blanding Joint Training Center**	**Florida**
Army National Guard	**Camp Butner**	**North Carolina**
Army National Guard	**Camp Grafton Training Center**	**North Dakota**
Army National Guard	Camp Roberts	California

Table 1. Seventy one military installations sent *Bd* kits. Bold text indicates the 50 military installations that returned samples for analysis (sheet 3 of 4).

DoD Service	Military Installation	State
Army National Guard	**Stones Ranch Military Reservation, Connecticut**	**Connecticut**
Army National Guard	**Fort Custer Training Center**	**Michigan**
Army National Guard	**Fort Indiantown Gap**	**Pennsylvania**
Army National Guard	**Orchard Combat Training Center**	**Idaho**
Army National Guard	Marseilles Training Area	Illinois
Army National Guard	**Fort Jackson/McCrady Training Center**	**South Carolina**
Army National Guard	Missouri Army National Guard, Macon Training Site	Missouri
Army National Guard	**Najaf Training Center**	**Oregon**
Army National Guard	**Sparta Training Center**	**Illinois**
Army National Guard	**Sparta Training Center– Training Area**	**Illinois**
Joint Base	**Joint Base McGuire-Dix-Lakehurst**	**New Jersey**
Joint Base	Joint Base Pearl Harbor-Hickam	Hawaii
Joint Base	Joint Base San Antonio	Texas
Joint Base	**Joint Base Langley-Fort Eustis**	**Virginia**
Marine Corps	Marine Corps Base Quantico	Virginia
Marine Corps	MCAS Miramar	California
Navy	Commander,Fleet Activities Okinawa	Okinawa
Navy	**Great Pond**	**Maine**
Navy	Naval Base Guam	Guam
Navy	**Manchester Fuel Department**	**Washington**

Table 1. Seventy one military installations sent *Bd* kits. Bold text indicates the 50 military installations that returned samples for analysis (sheet 4 of 4).

DoD Service	Military Installation	State
Navy	**Naval Air Station Fallon**	**Nevada**
Navy	Naval Air Station Key West	Florida
Navy	Naval Air Station Lemoore	California
Navy	**Naval Air Station Whidbey Island (Ault Field)**	**Washington**
Navy	**Naval Air Station, Patuxent River**	**Maryland**
Navy	**Naval Base Ventura County, Point Mugu**	**California**
Navy	**Naval Base Kitsap-Bangor**	**Washington**
Navy	**Naval Radio Station Jim Creek**	**Washington**
Navy	Naval Undersea Warfare Center	Rhode Island
Navy	Naval Weapons Station Seal Beach Detachment Fallbrook	California
Navy	NAVFAC Southwest	California
Navy	**NAVMAG Indian Island**	**Washington**
Navy	NAVSTA ROTA, Spain	Spain
Navy	**NIROP Santa Cruz**	**California**
Navy	**NSF Indian Head**	**Maryland**
Navy	**NVWC Key Port**	**Washington**
Navy	**Smokey Point**	**Washington**

LABORATORY ANALYSES

A real-time TaqMan PCR technique (Boyle et al. 2004; Hyatt et al. 2007) was used to analyze Bd swabs. Briefly, a 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 1×106 to $1\times10_{-2}$ 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).

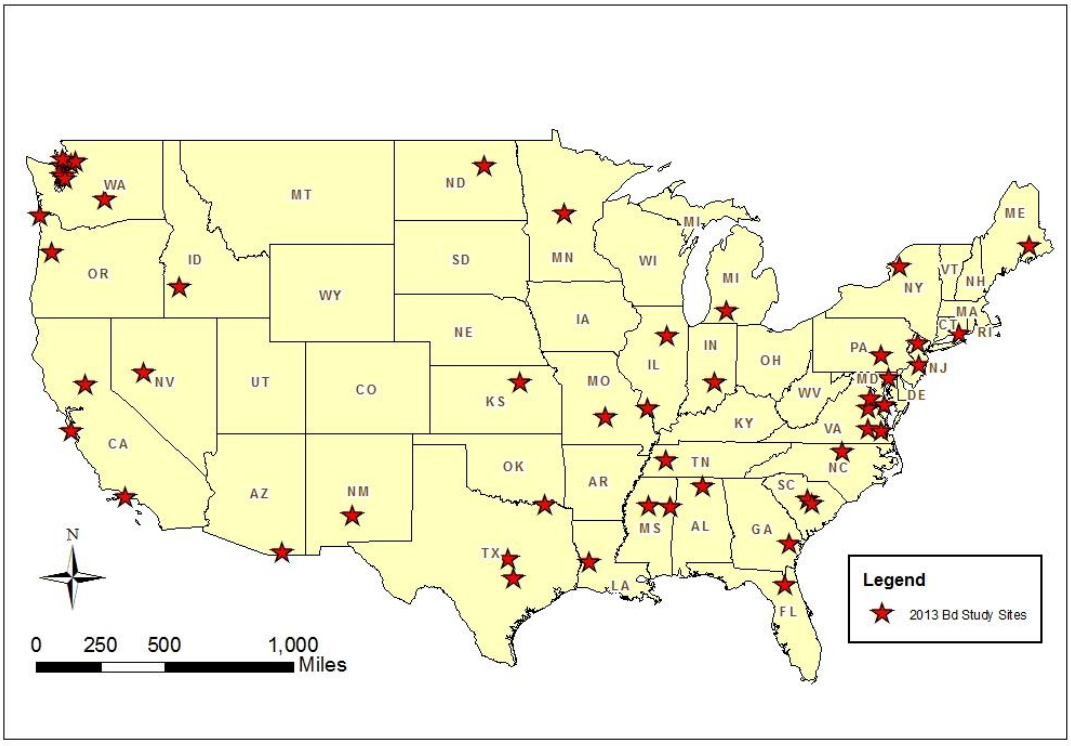
RESULTS

Of the 71 field swabbing kits mailed to military installations, 52 were returned containing 944 samples (figure 4). Joint Base McGuire-Dix-Lakehurst and Patuxent River Naval Air Station each returned two field swabbing kits. We received an average of 18.2 samples per cooler (92.5 percent). Field data was collected between March and September 2013, with the majority of the samples collected during the month of June. Typically, samples were recorded from multiple wetland sites on a military installation.

Of the 944 samples returned, 932 produced unequivocal results. *Bd* prevalence for all the samples was 24.2 percent (226 samples). The average zoospore equivalent for these positive samples was 11.0 (note: for a *Bd* infection to be considered the disease chytridiomycosis, zoospore equivalents must be greater than 10,000).

Of the 50 military installations that returned samples, 35 (70 percent) had at least one positive result for *Bd* (table 2). The following military sites did not have any *Bd*-positive samples: Columbus Air Force Base, Shaw Air Force Base, Camp Atterbury, Camp McCain, Fort Huachuca, Fort Polk, Fort Riley, Fort Stewart, White Sands Missile Range, Camp Butner, Orchard Combat Training Center, Fort Jackson/McCrady Training

Figure 4. Location of military sites that returned samples in 2013.



Center, Sparta Training Area, Naval Base Ventura County, Point Mugu, and NIROP Santa Cruz.

Percent positive samples per installation range from 5.0 percent (Fort Hood, Great Pond Outdoor Adventure Center, and NVWC Key Port) to 81.1 percent (Naval Air Station Fallon). There does not appear to be any spatial pattern to the military sites containing positive samples throughout the U.S. (figure 5). Positive samples were detected at military installations on the east and west coasts of the U.S.in addition to central sites. In addition, positive *Bd* samples were detected at latitudes spanning the continental United States. The eight military sites that had *Bd* prevalence rates above 50 percent were not restricted to a particular geographic area or latitude (figure 6).

Table 2. Results of *Bd* analysis for each of the 50 military installations arranged by ascending *Bd* percentage.

Military Installation	Positive No.	Negative No.	Total	Percent Positive
Columbus Air Force Base	NA	20	20	0
Shaw Air Force Base	NA	20	20	0
Camp Atterbury	NA	9	9	0
Camp McCain	NA	11	11	0
Fort Huachuca	NA	20	20	0
Fort Polk	NA	18	18	0
Fort Riley	NA	20	20	0
Fort Stewart	NA	20	20	0
White Sands Missile Range	NA	11	11	0
Camp Butner	NA	8	8	0
Orchard Combat Training Center	NA	19	19	0
Fort Jackson/McCrady Training Center	NA	4	4	0
Sparta Training Area	NA	20	20	0
Naval Base Ventura County, Point Mugu	NA	20	20	0
NIROP Santa Cruz	NA	20	20	0
Fort Hood	1	19	20	5
Great Pond Outdoor Adventure Center	1	19	20	5
NVWC Key Port	1	19	20	5
Aberdeen Proving Ground	1	17	18	5.5
Camp Blanding Joint Training Center	1	17	18	5.5
Fort Lee	1	17	18	5.6
Redstone Arsenal	1	15	16	6.3
Najaf Training Center	2	18	20	10
Beale Air Force Base	2	18	20	10

Military Installation	Positive No.	Negative No.	Total	Percent Positive
Join Base Langley-Fort Eustis	2	13	15	13.3
Smokey Point	3	17	20	15
Stone Ranch Military Reservation, Connecticut	4	13	17	23.5
Picatinny Arsenal	5	14	19	26.3
Fort Custer Training Center	5	14	19	26.3
Sparta Training Center	5	14	19	26.3
Fort A.P. Hill	6	14	20	30
Naval Air Station Whidbey Island (Ault Field)	6	14	20	30
NSF Indian Head	6	14	20	30
Joint Base McGuire-Dix-Lakehurst	13	27	40	32.5
Naval Base Kitsap-Bangor	7	13	20	35
Camp Swift Training Center	7	11	18	38.9
Camp Rilea	8	12	20	40
Milan Army Ammunition Plant	8	12	20	40
Fort Indiantown Gap	8	12	20	40
Fort Leonard Wood	9	11	20	45
Camp Grafton Training Center	9	11	20	45
NAVMAG Indian Island	9	11	20	45
Naval Air Station, Patuxent River	20	19	39	51.3
Camp Maxey	10	9	19	52.6
Manchester Fuel Department	11	8	19	57.9
Camp Ripley	11	7	18	61.1
JBLM Yakima Training Center	12	7	19	63.1
Naval Radio Station Jim Creek	8	4	12	66.7
Fort Drum	13	6	19	68.4
Naval Air Station Fallon	9	2	11	81.8

Figure 5. Military sites with positive and negative results for the *Bd* pathogen.

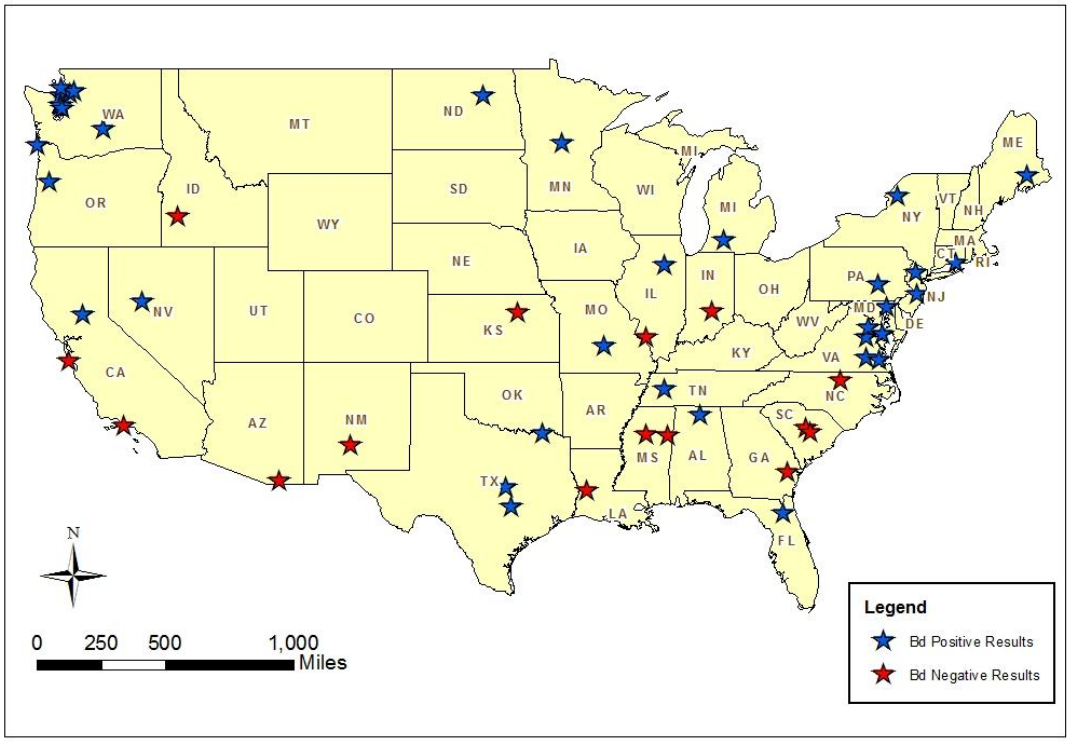
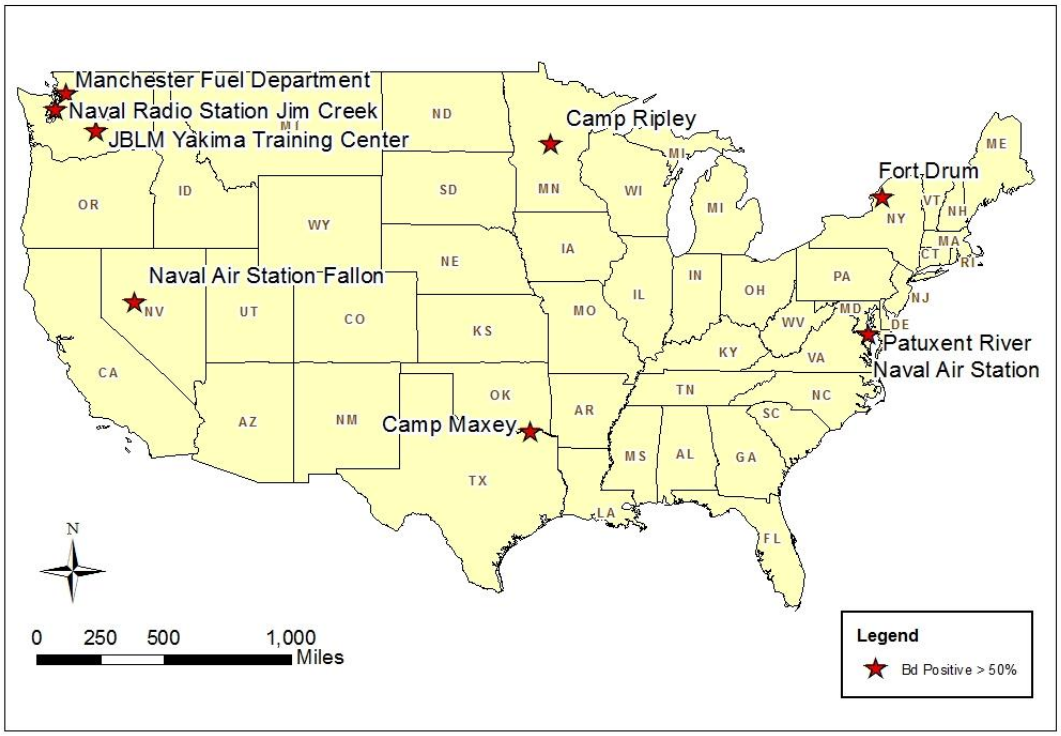


Figure 6. Military sites with *Bd* prevalence rates greater than 50 percent.



SPECIES

A total of 57 amphibian species were sampled during this investigation. Of these species, 16 tested positive for *Bd* (table 3). Among salamanders, two species (Eastern Newt [*Notophthalmus viridescens*] and the Rough-skinned Newt [*Taricha granulose*]) tested positive for *Bd*. *Batrachochytrium dendrobatidis*-positive frog species included one bufonid (*Anaxyrus flowleri*), four hylids (*Acris crepitans*, *Psuedoacris crucifer*, *Pseudacris feriarum*, and *Pseudacris regilla*) and nine ranids (*Lithobates catesbeianus, Lithobates clamitans, Lithobates palustris, Lithobates pipiens, Lithobates septentrionalis, Lithobates sphenocephalus*, *Lithobates sylvaticus*, *Rana aurora*, and *Rana luteiventris*). Percent positive samples per frog species ranged from 2.6 percent (Mink Frog [*Lithobates septentrionalis*]) to 100 percent (Upland Chorus Frog [*Pseudacris feriarum*] (note: only one individual Upland Chorus Frog was sampled in this investigation, and it tested positive for *Bd*). Several of the species that tested positive have been documented as *Bd* positive in other studies; salamanders and ranids, including Bullfrogs, may be carriers of this infection (Daszak et al. 2003; Hanselmann et al. 2004; Olson et al 2013; Peterson et al. 2007).

Table 3. Species sampled for the presence of *Bd*.
Bold text indicates at least one specimen tested positive for *Bd* (sheet 1 of 2).

***Ambystomatidae*—Mole Salamanders**
Ambystoma gracile (Northwestern Salamander)
Ambystoma macrodactylum (Long-toed Salamander)
Ambystoma maculatum (Spotted Salamander)
Ambystoma opacum (Marbled Salamander)
Ambystoma talpoideum (Mole Salamander)
Ambystoma tigrinum (Eastern Tiger Salamander)
***Dicamptodontidae*—Pacific Giant Salamanders**
Dicamptodon ensatus (California Giant Salamander)
Dicamptodon tenebrosus (Coastal Giant Salamander)
***Plethodontidae*—Lungless Salamanders**
Ensatina eschscholtzii (Ensatina)
Plethodon cinereus (Eastern Red-backed Salamander)
Plethodon glutinosus (Northern Slimy Salamander)
***Salamandridae*—Newts and "True Salamanders"**
Desmognathus fuscus (Northern Dusky Salamander)
Eurycea bislineata (Northern Two-lined Salamander)
***Notophthalmus viridescens* (Eastern Newt)**
***Taricha granulosa* (Rough-skinned Newt)**
***Bufonidae*—True Toads**
Anaxyrus americanus (American Toad)
Anaxyrus boreas (Western Toad)
Anaxyrus cognatus (Great Plains Toad)
Anaxyrus debilis (Chihuahuan Green Toad)
***Anaxyrus woodhousii* (Woodhouse's Toad)**
Anaxyrus punctatus (Red-spotted Toad)
Anaxyrus quercicus (Oak Toad)
Anaxyrus terrestres (Southern Toad)
***Hylidae*—Tree Frogs**
***Acris crepitans* (Northern Cricket Frog)**
Acris gryllus (Southern Cricket Frog)
Hyla andersonii (Pine Barrens Treefrog)
Hyla arenicolor (Canyon Treefrog)
Hyla chrysoscelis (Cope's Gray Treefrog)

Table 3. Species sampled for the presence of *Bd*.
Bold text indicates at least one specimen tested positive for *Bd* (sheet 2 of 2).

Hyla cinérea (Green Treefrog)
Hyla femoralis (Pine Woods Treefrog)
Hyla gratiosa (Barking Treefrog)
Hyla squirella (Squirrel Treefrog)
Hyla versicolor (Gray Treefrog)
Hyla wrightorum (Arizona Treefrog)
***Pseudacris crucifer* (Spring Peeper)**
***Pseudacris feriarum* (Upland Chorus Frog)**
Pseudacris maculata (Boreal Chorus Frog)
Pseudacris ocularis (Little Grass Frog)
***Pseudacris regilla* (Pacific Treefrog)**
***Microhylidae*—Narrow-Mouthed Frogs**
Gastrophryne carolinensis (Eastern Narrow-mouthed Toad)
***Scaphiopodidae*—American Spadefoot**
Scaphiopus couchii (Couch's Spadefoot)
Scaphiopus holbrooki (Eastern Spadefoot)
Spea bombifrons (Plains Spadefoot)
Spea hammondii (Western Spadefoot)
Spea multiplicata (Mexican Spadefoot)
***Ranidae*—Pool/True Frogs**
Lithobates blairi (Plains Leopard Frog)
***Lithobates catesbeianus* (American Bullfrog)**
***Lithobates clamitans* (Green Frog)**
Lithobates grylio (Pig Frog)
***Lithobates palustris* (Pickerel Frog)**
***Lithobates pipiens* (Northern Leopard Frog)**
***Lithobates septentrionalis* (Mink Frog)**
Lithobates sevosus (Dusky Gopher Frog)
***Lithobates sphenocephalus* (Southern Leopard Frog)**
***Lithobates sylvaticus* (Wood Frog)**
***Rana aurora* (Northern Red-legged Frog)**
***Rana luteiventris* (Columbia Spotted Frog)**

DISCUSSION

The results of this investigation are consistent with those recorded during our two previous *Bd* surveys on military lands in 2009 and 2011 (Lannoo et al. 2011, Petersen et al. In Prep). Despite the security that maintains the fence lines and boundaries of military installations, this survey found *Bd* to be prevalent across the DoD installations in the continental United States extending from coast to coast. In this investigation, we detected *Bd* at 35 of the 50 (70 percent) installations sampled with a total *Bd* prevalence of 24.2 percent. In comparison, in 2009 we detected *Bd* at 13 of 15 (87 percent) military installations with an overall infection rate of 16.6 percent (Lannoo et al. 2011). In 2011, 12 of 15 (80 percent) military sites tested positive for *Bd* with an overall infection rate of 22.5 percent (Petersen et al. In Prep.). These results confirm that *Bd* is present on many of the military installations in the United States.

Although *Bd* is present on the majority of the military sites tested in this study, at this time the fungus does not appear to have a negative impact on amphibian species. As reported above, the average zoospore equivalent for positive samples was 11. For a *Bd* infection to be considered the disease chytridiomycosis, zoospore equivalents must be greater than 10,000. No samples had zoospore equivalents that reached this threshold. In addition, no observations of dead or dying amphibians were reported by the military installation biologists, and no die-offs were previously reported. Therefore, even though *Bd* is present on many military installations across the United States, it is not reaching the levels of the disease chytridiomycosis and does not appear to be negatively impacting amphibian populations on those sites. The results of this study support the hypothesis that *Bd* can today be considered endemic (likely have been spread through North American decades ago) rather than epidemic (spreading as a wave and wiping out individuals, populations, and species in its path).

No spatial pattern to the military sites exists with *Bd* positive samples. Sites with *Bd* positive samples were found on both the east and west coasts of the U.S. in addition to the mid-west region. The *Bd* fungus is known to be well established on the east and west coasts of the U.S. Although four of the eight military sites with *Bd* prevalence rates above

50 percent (Manchester Fuel Department, Naval Radio Station Jim Creek, JBLM Yakima Training Center, and Naval Air Station Patuxent River) occurred on the east and west coasts of the U.S., four additional sites (Naval Air Station Fallon, Camp Maxey, Camp Ripley, and Fort Drum) with rates above 50 percent were located in interior regions of the United States. Therefore, no spatial pattern was clearly identified. It is likely that site-specific temperature and moisture combinations play a role determining the spatial distribution of *Bd* across large landscapes. *Bd* is known to favor cool, moist conditions and that warm, dry conditions from arid regions may inhibit this pathogen (Ribas et al. 2009; Fisher et al. 2009; Piotrowski et al. 2004).

Since only one sampling event occurred at any particular military installation during both the spring and summer, the impact of seasons on *Bd* rates could not be investigated in this study. However, Berger et al. (2004), Gaertner et al. (2009), and Lannoo et al (2011) previously demonstrated seasonality in *Bd* infection rates. As summer proceeds, *Bd*-positive frogs appear to lose their infection (Woodhams et al. 2003; Piotrowski et al. 2004; Pessier and Mendelson 2010; Richards-Zawacki 2010). It is also true that infected animals can develop chytridiomycosis and die, and thus be lost to later surveys. We suggest the temporal (seasonal) pattern is due to moisture availability, with *Bd* present at the highest rates during the wettest times of the year. Temperature may be a covariate with cooler temperatures promoting the infection.

A total of 57 amphibian species were sampled during this investigation. Of these species, 16 tested positive for *Bd* (two salamander species and 14 frog/toad species). Of the species that tested positive for *Bd* in this investigation, all species except the Upland Chorus Frog (*Pseudoacris feriarum*) have been documented to be *Bd* positive in other studies (http://www.fs.fed.us/pnw/lwm/aem/docs/olson/2013_supp_txt_tables_olson_aanensen_ronnenberg_et_al_plosone_bdmaps.pdf).

MANAGEMENT IMPLICATIONS

At this time, environmental managers can take only limited steps 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 impacted the loss of the red-legged frog. The results of this investigation and the similar 2009 and 2011 surveys 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.

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 is used in wetlands off the installation.

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

- Do not allow the collection 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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APPENDIX A

Bd Sampling Data Sheet

Installation Name:	
Wetland Name:	
Date:	Time:
GPS: Latitude	GPS: Longitude
Observer:	

Notes:

Vial Number	Species	Sex (M/F)	Age Class (Juvenile/Adult)	Snout-vent Length (mm)