



# Department of Defense Legacy Resource Management Program

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**Do Frogs Still Get Their Kicks on Route 66?**

**A Transcontinental Transect for Amphibian Chytrid  
Fungus (*Batrachochytrium dendrobatidis*) Infection on U.S**

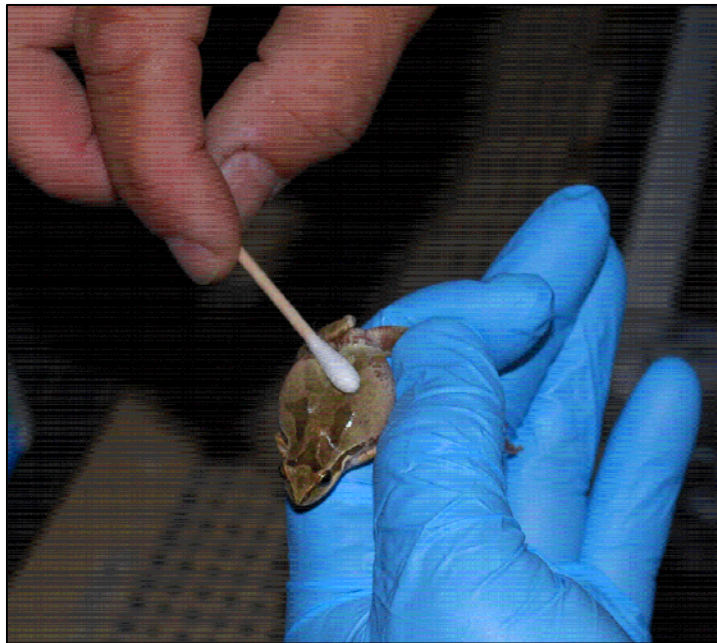
**Department of Defense Installations**

**Christopher Petersen, Robert E. Lovich, Michael J. Lannoo, Priya Nanjappa**

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**Christopher Petersen, Robert E. Lovich, Michael J. Lannoo, Priya Nanjappa**



Chris Petersen, Naval Facilities Engineering Command Atlantic, 6506 Hampton Blvd.,  
Norfolk, VA 23508

Robert E. Lovich, Naval Facilities Engineering Command Southwest, 1220 Pacific  
Highway, San Diego, CA 02132

Michael J. Lannoo, Indiana University School of Medicine, Rm. 135 Holmstedt Hall-ISU,  
Terre Haute, IN 47802, mlannoo@iupui.edu

Priya Nanjappa, Association of Fish & Wildlife Agencies, Suite 725, 444 N. Capitol Street  
NW, Washington, DC 20001

## Abstract

One fifth of the world's amphibians now face extinction. A major factor in these declines has been the spread of infection by the chytrid fungus, *Batrachochytrium dendrobatidis* (Bd); the disease it causes (chytridiomycosis) has been devastating amphibian populations globally. Two general scenarios, not mutually exclusive, have been proposed for the nature and spread of this pathogen: 1) Bd is an *epidemic*, spreading as a wave and wiping out individuals, populations, and species in its path; and 2) Bd is *endemic*, widespread throughout many geographic regions on every continent except Antarctica. Distinguishing from among these hypotheses could have impacts on amphibian management and conservation and requires, in part, broad-scale studies using standardized techniques. Towards this end, we conducted a transcontinental transect designed to sample for the presence of Bd. United States Department of Defense (DoD) installations were sampled from west to east along U.S. Highway 66 from California into central Illinois, and continuing eastward from there across to the Atlantic Seaboard along U.S. Interstate 64 (in sum from Camp Pendleton in California to Naval Air Station Oceana in Virginia, between 33° and 39° N latitude). We sampled each installation across the 2009 warm season using standardized collection and analytical techniques to address the following questions: 1) Does Bd occur in amphibian populations in these relatively undisturbed DoD environments? 2) Is there a spatial pattern to the presence of Bd? 3) Is there a temporal pattern to the presence of Bd? and 4) Do our results shed light on whether Bd is acting as an epidemic or endemic infection across North America? This study represents the single most geographically extensive survey for Bd conducted to date. Half of the amphibian species surveyed (15/30) tested positive for Bd as follows: Plethodontidae (4 species), Bufonidae (3 species), Hylidae (5 species) and Ranidae (3 species). There was a strong spatial component to our dataset. The ten eastern temperate DoD installations had higher rates of Bd infection (18.9%) than the five bases situated in the arid west (4.8%). There was also a strong temporal (seasonal) component to our dataset. In total, 78.5% of all positive samples came in the first (spring/early-summer) sampling period. These data support the conclusion of Ouellet et al. (2005) that Bd is now widespread, and argues that Bd, a pathogen that was once likely *epidemic*, can today be considered *endemic* across much of North America, extending from coast-to-coast,

with the exception of remote pockets of naïve populations. Some of the DoD installations sampled, such as Camp Pendleton, have already experienced declines of native amphibians and also tested positive for Bd. It is likely that this emerging disease will stimulate new and innovative measures to avoid further reduction of amphibian biodiversity on North American DoD installations.

## Introduction

One fifth of the world's amphibians may now be facing extinction (Stuart et al. 2004; Wake and Vredenburg 2008; <http://www.iucnredlist.org/initiatives/amphibians> [accessed 5 October, 2010]). In part these declines have been due to 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. 2006b; DiRosa et al. 2007; Skerratt et al. 2007; Jones et al. 2008; Murray et al. 2009; Wake and Vredenburg 2008; Kilpatrick et al. 2010). In the United States, this pathogen can now be found from below sea level (Lovich et al. 2008) to the highest elevations where amphibians occur (Vredenburg and Summers 2001). To date, however, most studies have been done locally, on single populations or regions 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). The result is a piecemeal picture of what is most assuredly a more contiguous and widespread problem (Blaustein et al. 2005; Kriger and Hero 2007; Zellmer et al. 2008; Goldberg et al. 2009; Tennessen et al. 2009; Hossack et al. 2010), although there have been attempts to generalize across broader geographic areas (Green et al. 2002; Garner et al. 2005; [www spatialepidemiology.net/bd-maps](http://www spatialepidemiology.net/bd-maps); [http://www.parcplace.org/Bd\\_conference.html](http://www.parcplace.org/Bd_conference.html)).

Due to the *ala carte* nature of the available datasets (a situation we do not criticize; we understand that this is how science typically reacts, especially initially, to crises such as this), there have been questions about the occurrence and spread of Bd. In response, two general scenarios have been proposed that have strong empirical support (Briggs et al. 2005; Rachowicz et al. 2006a; Fisher et al. 2009). In the first scenario, Bd is an *epidemic* (external to the population), spreading as a wave and wiping out individuals, populations, and species in its path. This has been documented, or is occurring, in Central America, in eastern Australia, and in parts of California (Berger et al. 1998; Lips 1998 1999; Lips et al. 2003, 2006; James et al. 2009; Vredenburg et al. 2010). The second scenario suggests that in certain regions of the world, such as North America, much of the spread of Bd occurred decades ago (when it was an epidemic) and that in these places it is now *endemic* (internal to the population; Ouellet et al. 2005; Vredenburg et al. 2010). Indeed, Bd is now widespread

throughout many geographic regions and is known to occur on every continent inhabited by amphibians (though some land masses appear to remain naïve); therefore, this infection may be considered global (Waldman et al. 2001; Retallick et al. 2004; Carnival et al. 2006; Adams et al. 2007, 2008; Lampo et al. 2008; Longcore et al. 2007; Pearl et al. 2007; Frías-Alvarez et al. 2008; Rothermel et al. 2008; Scalera et al. 2008; Chatfield et al. 2009; Deguise and Richardson 2009; de Queiroz Carnival et al. 2009; Fisher et al. 2009; Briggs et al. 2010). A third scenario in a sense combines the first two and has been more controversial. The Bd thermal optimum hypothesis suggests widespread benign Bd distribution has been triggered to lethality in regions by increased temperatures due to global warming (Pounds et al. 2006; but see Lips et al. 2008).

Distinguishing between the epidemic and endemic hypotheses requires, in part, broad-scale studies using standardized techniques. Further, due to the confounding factor of human disturbance on broad-scale patterns, it is best to examine low-impact (i.e. “natural”) areas. Perhaps the most widely available habitats that remain “undisturbed” (a relative term, with, perhaps the exception of the deep sea floor there are likely no longer any truly undisturbed environments left on earth; Hester and Harrison, 2007) in the United States today are United States Department of Defense (DoD) installations, which have never been completely accessible, and therefore resist the indiscriminate human traffic experienced by parks, wildlife refuges, and other public areas. Moreover, following the tragic events of September 11th, 2001, access to these installations has been further limited, and in most cases severely restricted. DoD installations encompass over 12 million ha and occur throughout the United States, making continent-wide surveys possible. DoD lands are managed differently than typical surrounding landscapes, using ecosystem management techniques. Indeed, American military lands harbor some of the greatest concentrations of endangered and threatened habitats and species in the United States (Stein et al. 2008). Insight as to which of the two hypotheses (epidemic and endemic) may be true could have significant impacts on amphibian management and conservation on military installations.

We conducted a transcontinental transect designed to assess the presence of Bd on military lands. DoD installations were sampled from west to east along U.S. Highway 66

(the “Mother Road”) 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 33° and 39 N latitude). We sampled across warm seasons, and used standardized collection and analytical techniques to address the following questions: 1) Does Bd occur in amphibian populations in these relatively undisturbed DoD environments? 2) Is there a spatial pattern to the presence of Bd? 3) Is there a temporal pattern to the presence of Bd? and 4) Do our results shed light on whether Bd is acting as an epidemic or endemic infection across North America?

## **Materials and Methods**

### **Field Samples**

In 2009, a total of 15 DoD installations were sampled as follows (Fig. 1; from west to east): Marine Corps Base Camp Pendleton in California, Camp Navajo in Arizona, Kirtland and Cannon Air Force Bases in New Mexico, Fort Sill and Camp Gruber in Oklahoma, Fort Leonard Wood in Missouri, Sparta Training Center in Illinois, Naval Support Activity Crane in Indiana, Fort Knox in Kentucky, and Radford Army Ammunitions Plant, Fort Lee, Fort A.P. Hill, Fort Belvoir, and Naval Air Station Oceana in Virginia. Each base was sampled three times: once in the spring/early summer (April, May, or the first week in June), once in mid-summer (July, August), and once in the late summer/fall (September, October). Generally, three wetland sites were sampled at each installation. An overview map of the wetland sites sampled at each installation can be found in Appendix A. Most of the sampling was done at night, when amphibians are active, using dipnets. Captured amphibians were placed in new, individual plastic bags for processing and handling. Bags were discarded after one use; boots and nets were rinsed to clean off mud and debris, and sterilized with a dilute bleach solution between wetland sites.

Amphibians are the only known animate host for Bd (Longcore et al. 1999; Pessier and Mendelson 2010; Vredenburg et al. 2010), although zoospores can survive for up to twelve weeks under favorable (cool, moist) soil conditions (Johnson and Speare 2003, 2005; Rachowicz and Vredenberg 2004; Berger et al. 2005; Mitchell et al. 2008). Therefore our survey effort focused exclusively on amphibian populations. At each installation,

postmetamorphic animals were sampled as they were encountered—because we were broadly interested in the presence of Bd we did not discriminate between salamanders and frogs. All animals were handled using sterile techniques and sampled using cotton, wooden-handled swabs. Swabs were rubbed while rolling the cotton over the body surface; five rubs each on the dorsum, flanks, ventrum, cranium, inguinal region, and the palmar/plantar surface of each foot for a total of 50 rubs (Skerratt et al. 2008; Pessier and Mendelson, 2010). The head of the swab was then broken off in an individually labeled 0.5 ml free standing polypropylene screw cap microcentrifuge tube (Fisherbrand 02-681-333) and stored and shipped cold prior to analysis.

### **Laboratory Analyses**

Swabs were analyzed for Bd using conventional PCR (polymerase chain reaction) techniques following Annis et al. (2004), Kriger and Hero (2006), and Hyatt et al. (2007). Briefly, to extract Bd DNA from field samples, one ml of 70% ethanol was added to microcentrifuge tubes containing sample swabs and stored overnight at -20 °C. Swabs were removed and the supernatant was centrifuged (16,000 x g for 10 min). ATL-PK (Qiagen) tissue lysis buffer (200 µl) was added to the pelleted fraction and incubated overnight (55 °C). To detect Bd spores, we used a nested PCR approach adapted from Gaertner et al. (2009a). Amplification products were visualized on a 3% agarose gel (Ameresco agarose 3:1 HRB). Presence or absence of a 300-bp band was compared against the EZ Load 100-bp molecular ruler (Bio-Rad) and a positive control. Negative controls were also run with each sample; samples were analyzed twice. Results were recorded on digital spreadsheets (Microsoft Excel).

### **Temperature and Precipitation Data, and Statistics**

Mean annual temperature and precipitation data for a 30-yr period (1971–2000) were obtained from stations near or at each base by searching National Oceanic and Atmospheric Administration (NOAA) databases (<http://cdo.ncdc.noaa.gov/cgi-bin/climatenormals/climatenormals.pl>).



From among our three datasets (Bd infection rate [arcsine transformed], temperature, and precipitation) only temperature data were normally distributed (Shapiro-Wilk normality test, Program R) and therefore we used nonparametric Chi-square ( $\chi^2$ ) and Kruskal Wallis tests (SPSS v. 17) for our analyses. A  $\chi^2$  goodness of fit test was used to compare observed Bd infection rates across seasons (Spring/Early Summer, Mid-Summer, and Late Summer/Fall) to expected rates (based on total rate of Bd infection). Kruskal Wallis tests were used to compare Bd infection rates, temperatures, and precipitation values between arid and temperate installations. Arid installations were defined as the five western-most bases (Camp Pendleton, Camp Navajo, Kirtland Air Force Base AFB, Cannon AFB, and Ft. Sill); temperate bases were Camp Gruber, Ft. Leonard Wood, Sparta Training Center, NSA Crane, Ft. Knox, Radford AAP, Ft. A.P. Hill, Ft. Belvoir, Ft. Lee, and NAS Oceana. Significance levels were set at  $p \leq 0.05$ .

## Results

### Installations

Each base was visited three times (Table 1). In April, Camp Navajo and Fort Sill were too cold for amphibian activity, and bases were visited but no samples were collected. During the mid-summer and late-summer/fall trips the ponds at Fort Belvoir were dry and no amphibians were detected. At all other sites, during all other sampling times, animals were collected and sampled. In total, from all bases, during all visits, 1,306 amphibians were sampled for this project, 217 (16.6%) swabs tested positive for Bd. In general, the more arid the site the more difficult it was to detect amphibians, especially later in the year, and fewer animals were collected from these bases.

We did not detect Bd at two bases, Camp Navajo, AZ and Fort Sill, OK. This was not due to sample size, per se. Thirty five samples were taken at Camp Navajo; 34 in July (mid-summer), 1 in September (late-summer/fall). At Fort Sill, a total of 43 samples were taken; 12 during June (mid-summer), 31 during September (late-summer/fall). This result could have been due, in part, to a lack of samples during the spring/early summer sampling period (due to cold and snow), when the majority of positive samples at other bases were collected (see below).

Bd was detected at the remaining 13 bases (Table 1, Fig. 2). Infection rates among these sites ranged from 2% (1 of 46 samples positive) at Kirtland Air Force Base to 39% (7 of 18 samples positive) at Fort Belvoir. Other sites with high percentages of positive samples included Sparta Training Center in Illinois (31%; 55 of 180 samples positive), Camp Pendleton in California (26%; 5 of 19 samples positive), and Radford Army Arsenal in Virginia (25%; 15 of 60 samples positive). Sparta had the highest absolute number of positive samples (55), Fort Leonard Wood had the second highest (38).

### **Species**

Species infected with Bd covered a wide phylogenetic range (Table 2) including: four species of plethodontid salamanders (*Desmognathus fuscus*, *Eurycea cirrigera*, *Eurycea longicauda*, and *Pseudotriton ruber*), three species of toads (*Anaxyrus americanus*, *Anaxyrus fowleri*, *Anaxyrus woodhousii*), five hylid species (*Acris blanchardi*, *Acris crepitans*, *Hyla cadaverina*, *Hyla chrysoscelis*, and *Pseudacris crucifer*), and four ranid species (*Lithobates catesbeianus*, *Lithobates clamitans*, *Lithobates palustris*, and *Lithobates sphenoccephalus*). At no point during this study did we observe moribund amphibians. The specific species sampled at each installation can be found in Appendix A.

### **Spatial Patterns**

Aridity appeared to have an effect on Bd infection rates. Five of the six sites with the lowest infection rates (Camp Navajo, 0%; Fort Sill, 0%; Kirtland Air Force Base, 2%; Cannon Air Force Base, 6%, and Camp Gruber, 8%) occur in the arid southwest (Arizona, New Mexico) or on the Great Plains (Oklahoma); the exception was Fort Lee (7%) in Virginia. Remaining sites occur in coastal areas, or inland areas that receive higher levels of precipitation (Table 3). A second way we explored this trend was to compare the data for the western arid bases (Pendleton, Navajo, Kirtland, Cannon, and Ft. Sill) with data for the eastern temperate sites. The rate of positive samples for the arid installations was 4.8% (10/208); the rate of positive samples for the eastern temperate sites was 18.9% (207/1098). This difference was statistically significant (Kruskal-Wallis,  $p = 0.027$ ). Rates of precipitation were also different between the western arid and eastern temperate bases ( $x =$

47.9 vs. 110.6 cm annually; Kruskal-Wallis,  $p = 0.002$ ), although temperatures were not ( $x = 14.3$  vs.  $13.6^{\circ}$  C; Kruskal-Wallis,  $p = 0.3$ ).

### **Temporal Patterns (Seasonality)**

There was a strong seasonal component to our results (Fig. 3), which was statistically significant ( $\chi^2$ ,  $p = 0.031$ ). During the Spring/Early-Summer sampling period, 39.3% of all samples were positive. This number dropped to 6.1% for mid-summer samples, and to 4.5% for late-summer/fall samples. Most bases followed this pattern (Fig. 4) including Camp Pendleton, Cannon Air Force Base, Camp Gruber, Fort Leonard Wood, Sparta Training Center, Crane Naval Surface Warfare Center, Fort Knox, and Fort Lee. Camp Navajo and Fort Sill had no positive samples and Fort Belvoir had animals collected only in the spring/early summer period, and therefore these bases had no temporal pattern of infection. Radford Army Arsenal had 33.3% positive samples in mid-summer, 23.5% positive in late-summer/fall, and 15.8% positive in spring/early summer. Three East-Coast installations—Fort A.P. Hill, Fort Lee, and Naval Air Base Oceana—had higher percentages of animals infected during the late-summer/fall than in the mid-summer period.

## **Discussion**

Bd was detected in 13 of 15 DoD installations, spanning the width of the North American continent. Fifteen of 30 amphibian species sampled tested positive for Bd. There were both spatial and temporal patterns to Bd infection rates, as follows.

### **Bd is found in the highly secure environments of U.S. DoD installations**

In aggregate, the data for all bases over all three sampling periods (spring/early-summer, mid-summer, late-summer/fall) show a 16.6% rate of Bd infection. Bd was found in all but two installations, Camp Navajo in Arizona and Fort Sill in western Oklahoma. Lack of Bd detection on these bases may be the result of insufficient sampling during the first sampling period due to inclement weather (cold). Amphibians were not active during the first sampling period at either of these bases and spring and early-summer was the time when Bd was most likely to be detected (79% of our positive samples came from this first sampling period; see below).

During this study we sampled about 10% (30) of all known United States amphibians and found Bd in half of them, including four plethodontids, three bufonids, five hylids, and three ranids (Table 2). While Bd absence in the remaining species may be due to inherent resistance (Woodhams et al 2007b; Lauer et al. 2007) or ecological avoidance (Lips et al. 2003), it is most probable that in cases of no detection, individuals sampled happened to be negative, or to test negative at the time of sampling. It is likely that all amphibian species are susceptible to Bd infection, although species-specific variation in susceptibility has been shown (Woodhams et al. 2007a), as has intraspecific variation in susceptibility (Tennesen et al. 2009). 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. 2004; Hanselmann et al. 2004; Garner et al. 2006; Peterson et al. 2007).

#### **There is a spatial pattern to the presence of Bd**

The ten eastern temperate DoD installations had significantly ( $p = 0.027$ ) higher rates of Bd infection (19.6%; 207/1098) than the five bases situated in the arid western ecosystems (4%; 10/208). Bd went undetected at two of these bases (Camp Navajo and Fort Sill); two arid bases each had single-digit levels of detection (Kirtland, 2%, Cannon, 6%). Camp Pendleton was the exception. It had a 26% rate of Bd infection, but this installation is characterized by maritime Mediterranean climate, with more moisture than inland installations. Bd is known to favor cool, moist conditions (Ribas et al. 2009; Fisher et al. 2009). It therefore follows that warm and dry (i.e., arid) conditions may inhibit this pathogen. Our data are consistent with this interpretation (Fig. 2). Further, animals that bask have been shown to have an increased resistance to chytridiomycosis (Woodhams et al. 2003; Richards-Zawacki 2010).

#### **There is a temporal (seasonal) pattern to the presence of Bd**

There was a strong temporal component to our dataset (Table 1; Figure 3). In total, 78.5% of all positive samples came in the first (spring/early-summer) sampling period, and broken out by sampling period, the percent positive samples were 39.3% (168 of 427), 6.1% (29 of 477), and 4.5% (17 of 374). The data for the majority of bases (Camp Pendleton,

Cannon Air Force Base, Camp Gruber, Fort Leonard Wood, Sparta Training Center, Crane Naval Surface Warfare Center, Fort Knox, and Fort Lee) follow a temporal pattern but the data from some bases do not (Fig. 4). For example, three bases had no pattern of infection: Camp Navajo and Fort Sill had no positive samples and Fort Belvoir had animals collected only in the spring/early summer period. Other bases had infection patterns that differed seasonally: Radford Army Arsenal had 15.8% positive in spring/early summer, 33.3% positive samples in mid-summer, and 23.5% positive in late-summer/fall. Interestingly, and perhaps reflecting more favorable conditions for Bd (high moisture), three East-Coast installations—Fort A.P. Hill, Fort Lee, and Naval Air Base Oceana—had higher percentages of animals infected during the late-summer/fall than in the mid-summer period (Fig. 4). Overall, our data suggest a strong seasonal component to Bd infection, with the earliest sampling period showing the greatest infection rate (Fig. 3).

Seasonality in Bd infection rates has been previously demonstrated (Berger et al., 2004; Gaertner et al. 2009b). 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. Just as we suggest the spatial pattern of Bd presence is due to variations in moisture levels (with moisture promoting infection rates) 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. Inverting our view, our data suggest that Bd rates are lower in arid areas (arid deserts and the Great Plains) and during drier times of the year (mid- to late-summer and fall). Temperature may be a covariate, with cooler temperatures promoting the infection, although in our study, by minimizing variation in latitude among sites in the transect, we also minimized, as much as possible in a continental transect, temperature differences.

### **Our results suggest Bd is acting as endemic infection across much of the United States Middle Latitudes**

These data support the conclusion of Ouellet et al. (2005) that Bd is now widespread across much of North America (at least within areas of the United States sampled). Samples

from DoD installations located in mesic habitats show a range of Bd infection from 7% (5/67) positive samples at Fort Lee, in Virginia, to 39% positive samples at Fort Belvoir. This spatial pattern—from coast-to-coast—argues for an infection that, while once was likely *epidemic*, today is *endemic* in the United States. Further, the phylogenetic range of species that presented positive—plethodontid salamanders in three genera, bufonid toads, treefrogs in three genera, and ranid frogs—suggests that this infection has been associated with aquatic ecosystems long enough to infect numerous species.

The exceptions to this generalization based on these findings are two arid bases, Camp Navajo in Arizona and Fort Sill in Oklahoma. These installations warrant further study; there would be greater confidence with the conclusion that Bd is absent at these bases had we sampled large numbers of animals during the spring/early-summer period. Our data do not distinguish between “Bd present but not detected” and “Bd absent.” There are known to be pockets of wilderness, for example in regions of the Sierra Nevada that Bd has yet to reach (Vredenberg et al. 2010). At these places, when Bd arrives it is predicted to be an *epidemic* infection, and we suspect amphibian extirpations will follow. Camp Navajo and Fort Sill may be examples of such Bd-negative outposts, but further study is needed to confirm.

### **Management Implications**

While this study has shown spatial and temporal patterns across geographic regions, further work will be needed to determine the on-installation impacts that Bd may be having where it was found. 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 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. 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. The results of this investigation provide these data.

Amphibians play an important role in the ecosystem, and their further declines may warrant protections that hamper military training.

### **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. Sterile 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 off or on the installation
- Prevent the release of exotic amphibian pets on DoD installations
- Increase the awareness of the disease to military personnel and base residents

### **Conclusions**

This study represents the most geographically extensive survey for Bd conducted to date. Half of the amphibian species surveyed (15/30) tested positive for Bd as follows: Plethodontidae (4 species), Bufonidae (3 species), Hylidae (5 species) and Ranidae (3 species). There was a strong spatial component to our dataset. The ten eastern temperate DoD installations had higher rates of Bd infection (18.9%) than the five bases situated in the arid southwest or Great Plains ecosystems (4.8%). There was also a strong temporal (seasonal) component to our dataset. In total, 78.5% of all positive samples came in the first (spring/early-summer) sampling period. These data support the conclusion of Ouellet et al. (2005) that Bd is now widespread across much of North America and suggests that Bd, an infection that, while once was likely *epidemic*, today can be considered *endemic*, occurring from coast-to-coast, even on the relatively undisturbed lands managed by the DoD.

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**Table 1.** Summary of percent Bd positive amphibians detected at each DoD installation for each of the three 2009 sampling periods.

Bases are arranged geographically, as they occur from west to east (Fig. 1).

Sampling Period	Base																		
	MCB Camp Pendleton	Camp Navajo	Kirtland AFB	Cannon AFB	Fort Sill	Camp Gruber	Fort Leonard Wood	Sparta	NSA Crane	Fort Knox	Radford Amy Plant	Fort A.P. Hill	Fort Belvoir	Fort Lee	NAS Oceana				
<b>Spring/ Early Summer</b>	5 15 33%		0 4 0%	4 21 19%		5 27 18.50%	35 60 58.30%	40 60 66.70 %	28 60 46.70 %	16 60 26.70%	3 19 15.80%	14 47 29.80%	7 18 38.90%	3 28 10.70%	11 36 30.60%				
<b>Mid- Summer</b>	0 2 0%	0 34 0%	1 36 2%	0 31 0%	0 12 0%	0 32 0%	2 60 3.30%	10 60 16.70 %	0 60 0%	5 60 8.30%	8 24 33.30%	2 20 10%		1 24 4.20%	0 22 0%				
<b>Late Summer/ Early Fall</b>	0 2 0%	0 1 0%	0 6 0%	0 13 0%	0 31 0%	0 2 0%	1 60 1.70%	5 60 8.30%	0 60 0%	0 60 0%	4 17 23.50%	3 16 23%		1 15 6.70%	3 31 9.70%				

**Table 2.** A list of species sampled for the presence of Bd, organized by families; salamanders followed by frogs. Boldface type indicates at least one specimen tested positive. Thirty species were tested, which represents about 10% of the species found in North America. Frogs are disproportionately represented.

Ambystomatidae

*Ambystoma maculatum*  
*Ambystoma mavortium*  
*Ambystoma tigrinum*

Plethodontidae

***Desmognathus fuscus***  
***Eurycea cirrigera***  
***Eurycea longicauda***  
***Pseudotriton ruber***

Salamandridae

*Notophthalmus viridescens*

Bufonidae

***Anaxyrus americanus***  
***Anaxyrus fowleri***  
*Anaxyrus punctatus*  
*Anaxyrus terrestris*  
***Anaxyrus woodhousii***

Hylidae

***Acris blanchardi***  
***Acris crepitans***  
***Hyla cadaverina***,  
***Hyla chrysoscelis***  
*Hyla cinerea*  
*Hyla femoralis*  
*Hyla squirella*  
*Hyla versicolor*  
***Pseudacris crucifer***  
*Pseudacris regilla*  
*Pseudacris triseriata*

## Microhylidae

*Gastrophryne carolinensis*

## Ranidae

*Lithobates blairi*

***Lithobates catesbeianus***

***Lithobates clamitans***

*Lithobates palustris*

***Lithobates sphenoccephalus***

**Table 3.** Mean annual precipitation (cm) and temperature (° C) at each of the fifteen DoD installations sampled. These data are plotted against Bd infection rates at each base in Figure 2.

<b>Installation</b>	<b>Mean Annual Precipitation</b>	<b>Mean Annual Temperature</b>
Camp Pendleton	33.6	16.9
Camp Navajo	54.3	9.7
Kirtland AFB	24.1	13.8
Cannon AFB	47	14.3
Fort Sill	80.4	16.6
Camp Gruber	111.2	15.6
Fort Leonard Wood	113.1	13.1
Sparta	108.6	12.6
Crane	123	12.9
Fort Knox	112.6	12.5
Radford	92.7	10.7
Fort A.P. Hill	113.1	13.6
Fort Belvoir	103.4	13.8
Fort Lee	115	15.7
Oceana	113	15.5

## Figure Legends

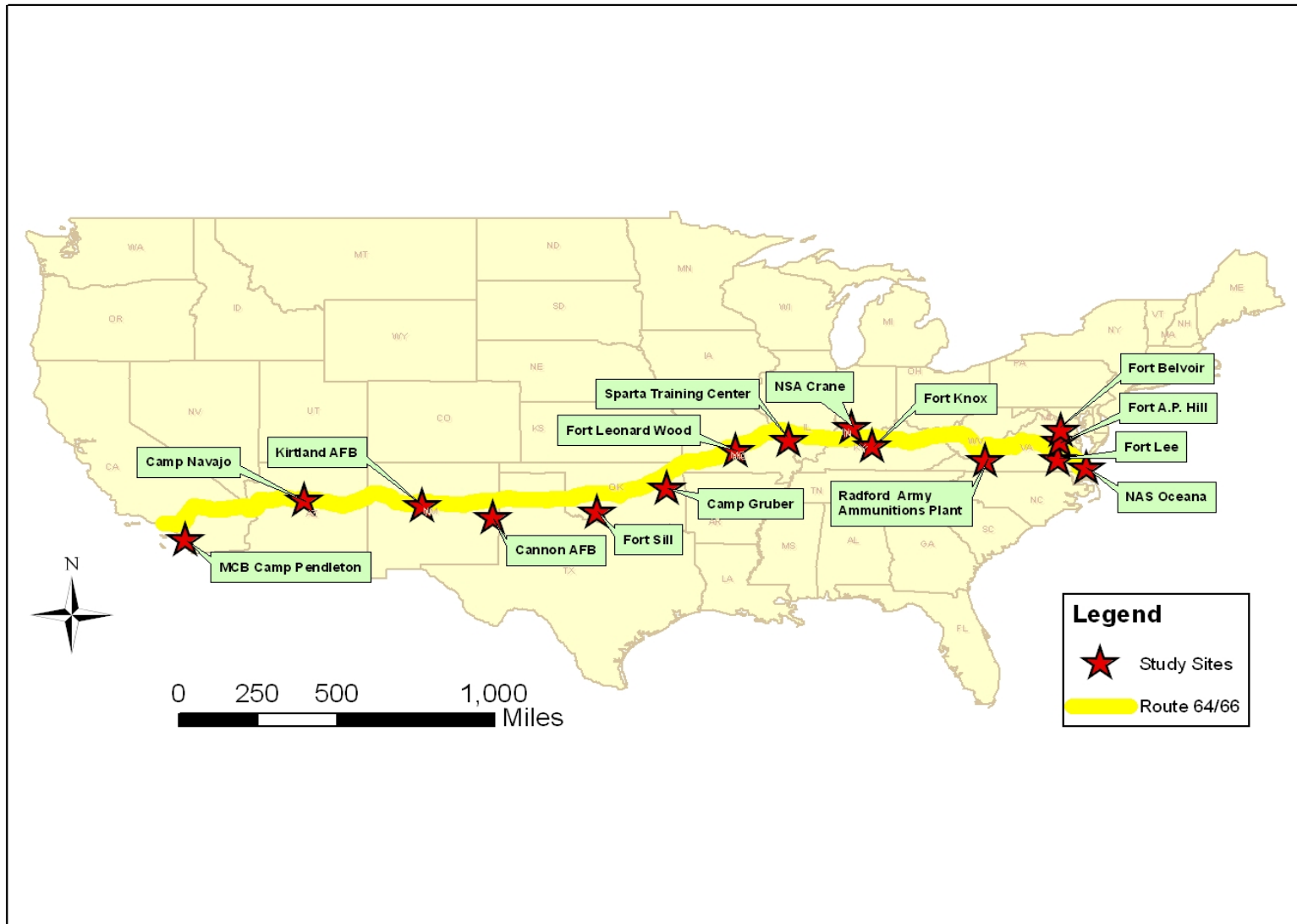
Figure 1. Department of Defense installations participating in the present study. From California to Illinois, bases were located near Route 66; from Illinois east to the coast, sites were chosen near Interstate 64 to hold latitude relatively constant (between 33° and 39° N).

Figure 2. Percentage of Bd positive samples by installation. Bases are arranged from west to east, in the order they appear in Figure 1. Left side y-axis indicates both mean annual temperature (° C, red line) and mean annual precipitation (cm, blue line). Note the low percentage of positive samples from the arid western installations, although the relationship between annual precipitation levels and Bd infection rates was not statistically significant.

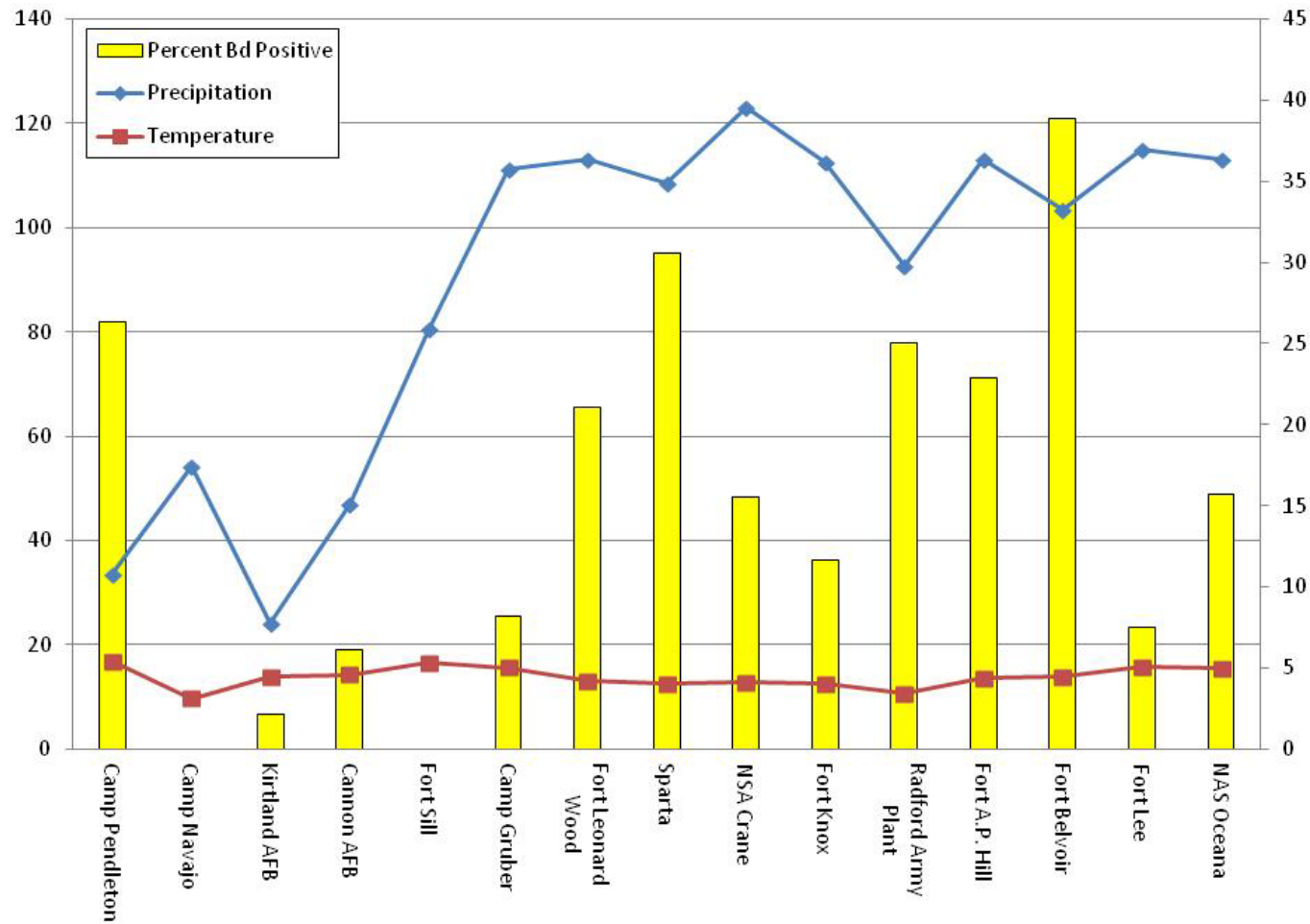
Figure 3. Temporal pattern (seasonality) of Bd infection rates across all installations. Note the strong tendency for the highest infection rates to occur during the spring/early summer sampling period, followed by a precipitous drop off during the mid- to late-summer and fall.

Figure 4. Temporal pattern (seasonality) of Bd infection rates for each installation. Note the variation, with most bases showing and early-summer to late-summer reductions. Bases near or on the East Coast had a tendency towards higher rates in the late-summer/fall than in the early summer.

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

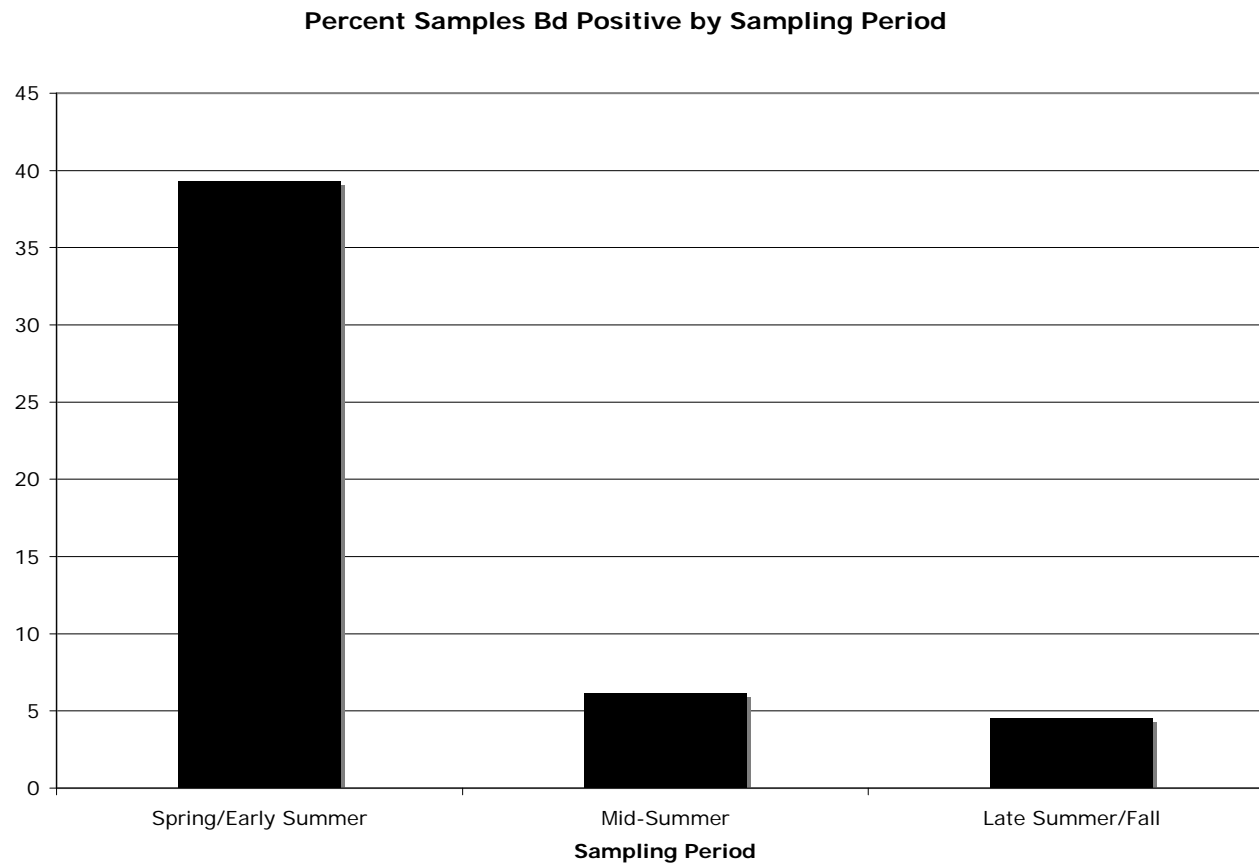


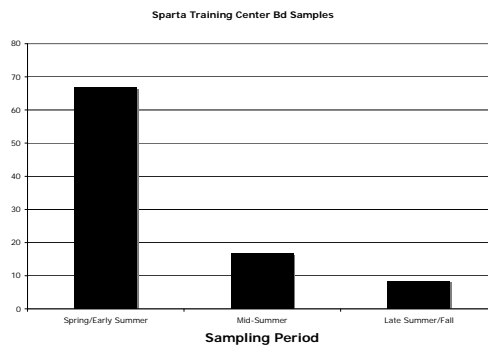
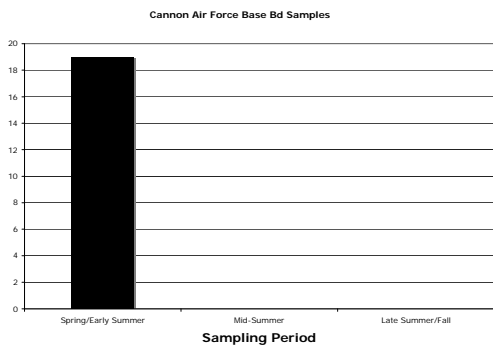
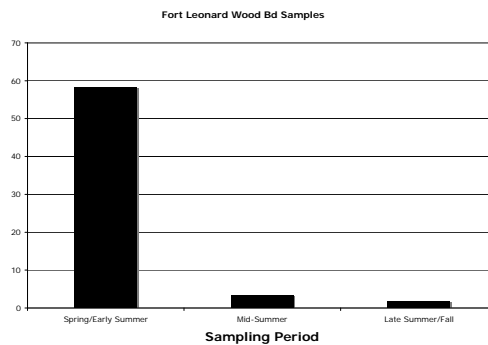
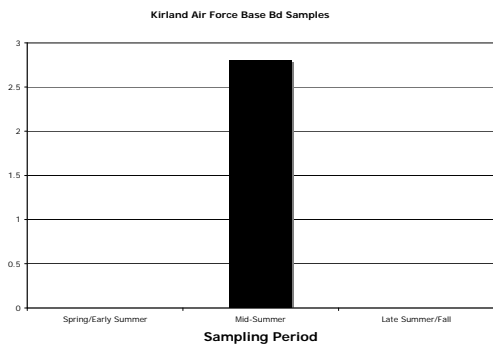
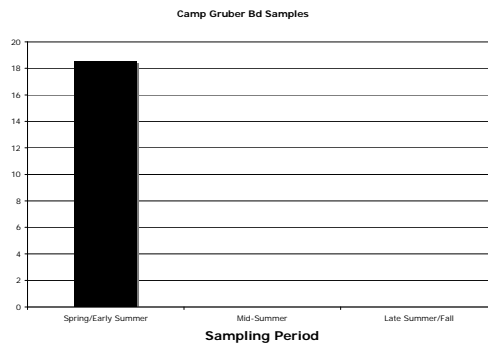
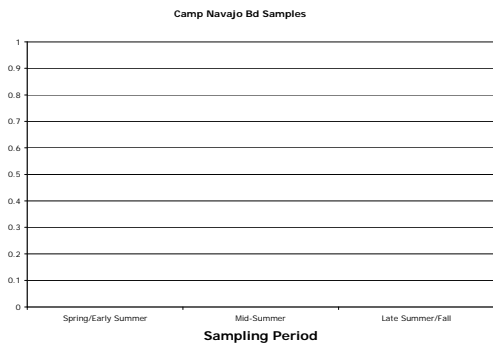
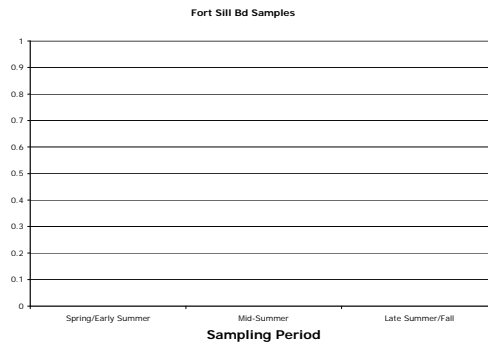
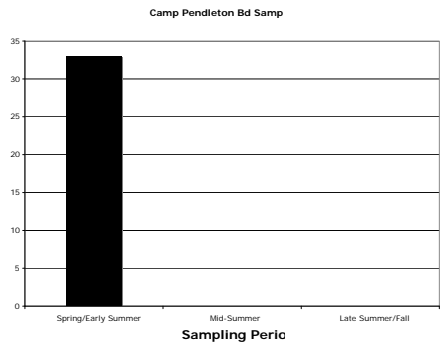
**Figure 2. Percentage of Bd positive samples, mean annual precipitation and mean annual temperature by installation.**

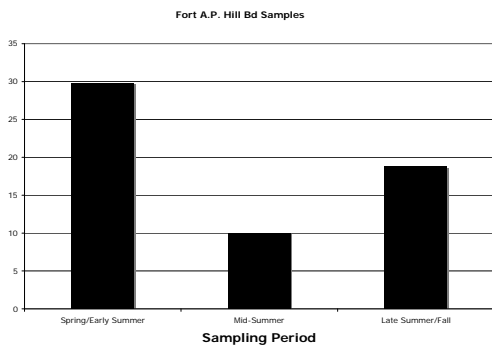
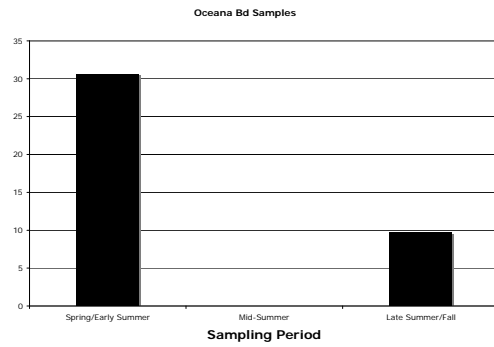
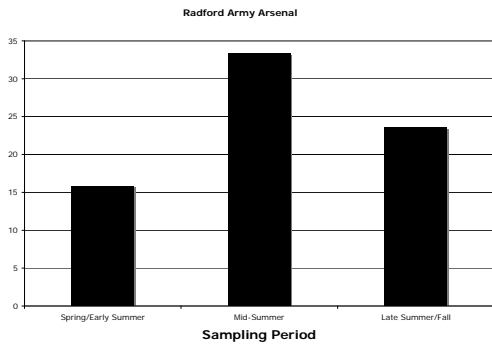
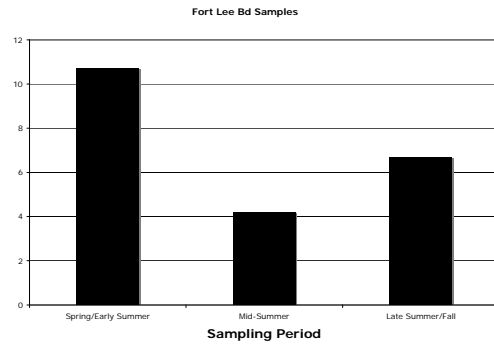
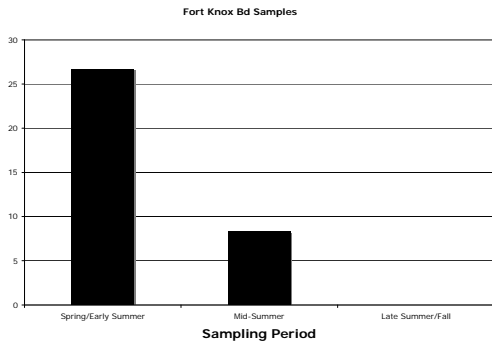
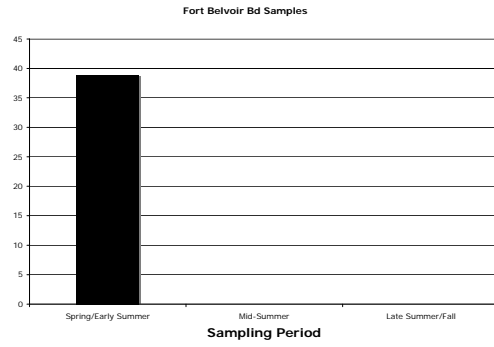
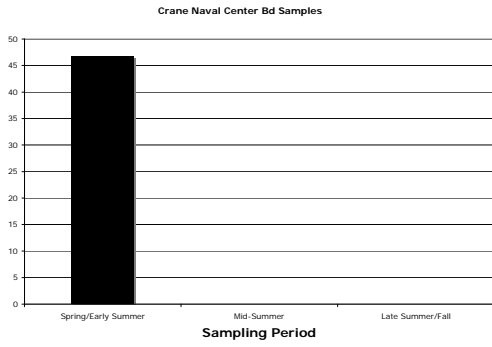




**Figure 3. Temporal pattern (seasonality) of Bd infection rates across all installations.**

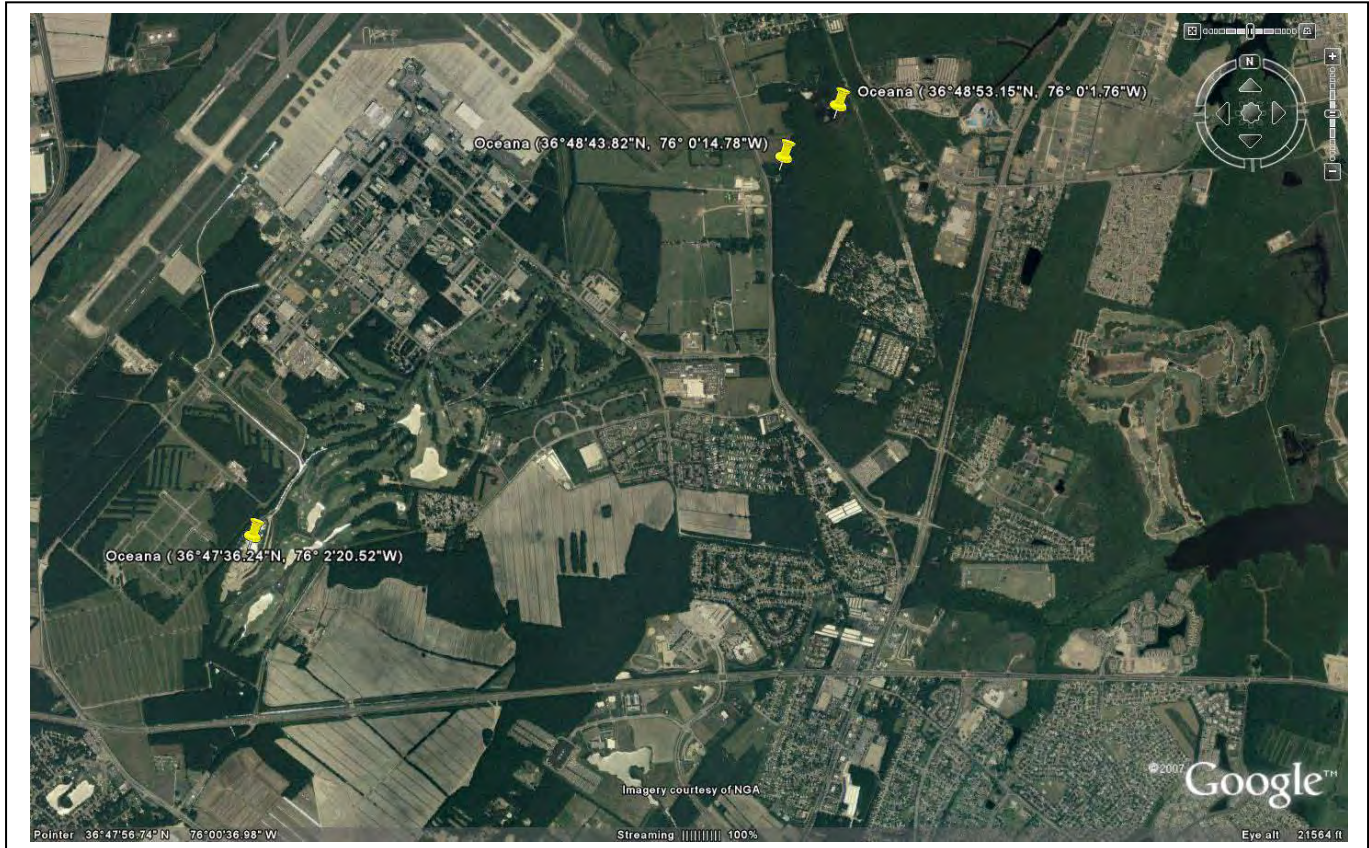






# Naval Air Station Oceana, Virginia

## Wetland Survey Sites



## Species Sampled

**American Toad - *Anaxyrus americanus***

Southern Toad - *Anaxyrus terrestris*

**Southern Leopard Frog - *Lithobates sphenoccephalus***

Bullfrog - *Lithobates catesbeianus*

Green Frog - *Lithobates clamitans*

Squirrel Treefrog - *Hyla squirella*

Green Treefrog - *Hyla cinerea*

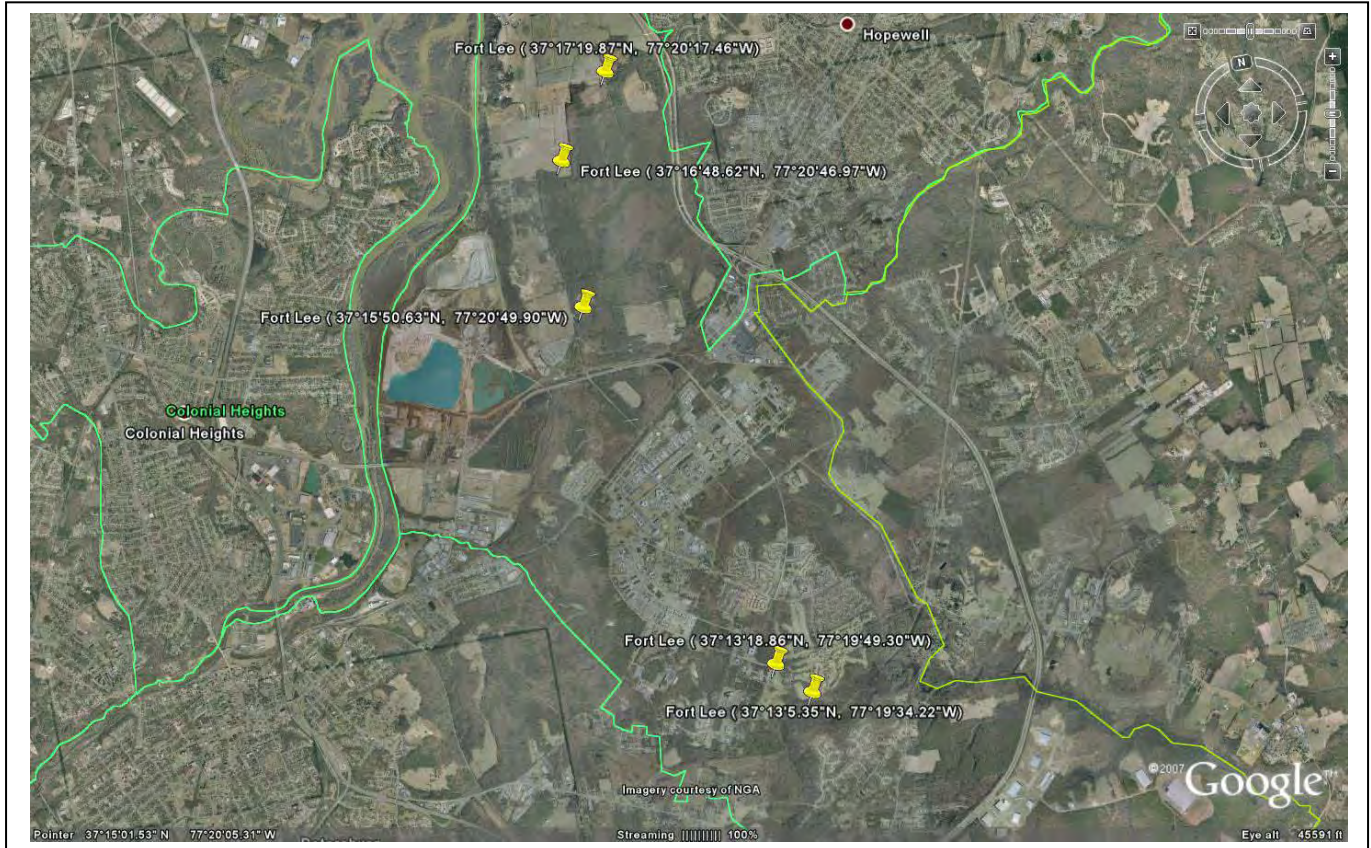
Pine Woods Treefrog - *Hyla femoralis*

Narrow-mouthed Toad - *Gastrophryne carolinensis*

(Boldface type indicates at least one specimen tested positive for Bd.)

# Fort Lee, Virginia

## Wetland Survey Sites



## Species Sampled

Fowler's Toad - *Anaxyrus fowleri*

**Southern Leopard Frog - *Lithobates sphenoccephalus***

**Bullfrog - *Lithobates catesbeianus***

Green Frog - *Lithobates clamitans*

Pine Woods Treefrog - *Hyla femoralis*

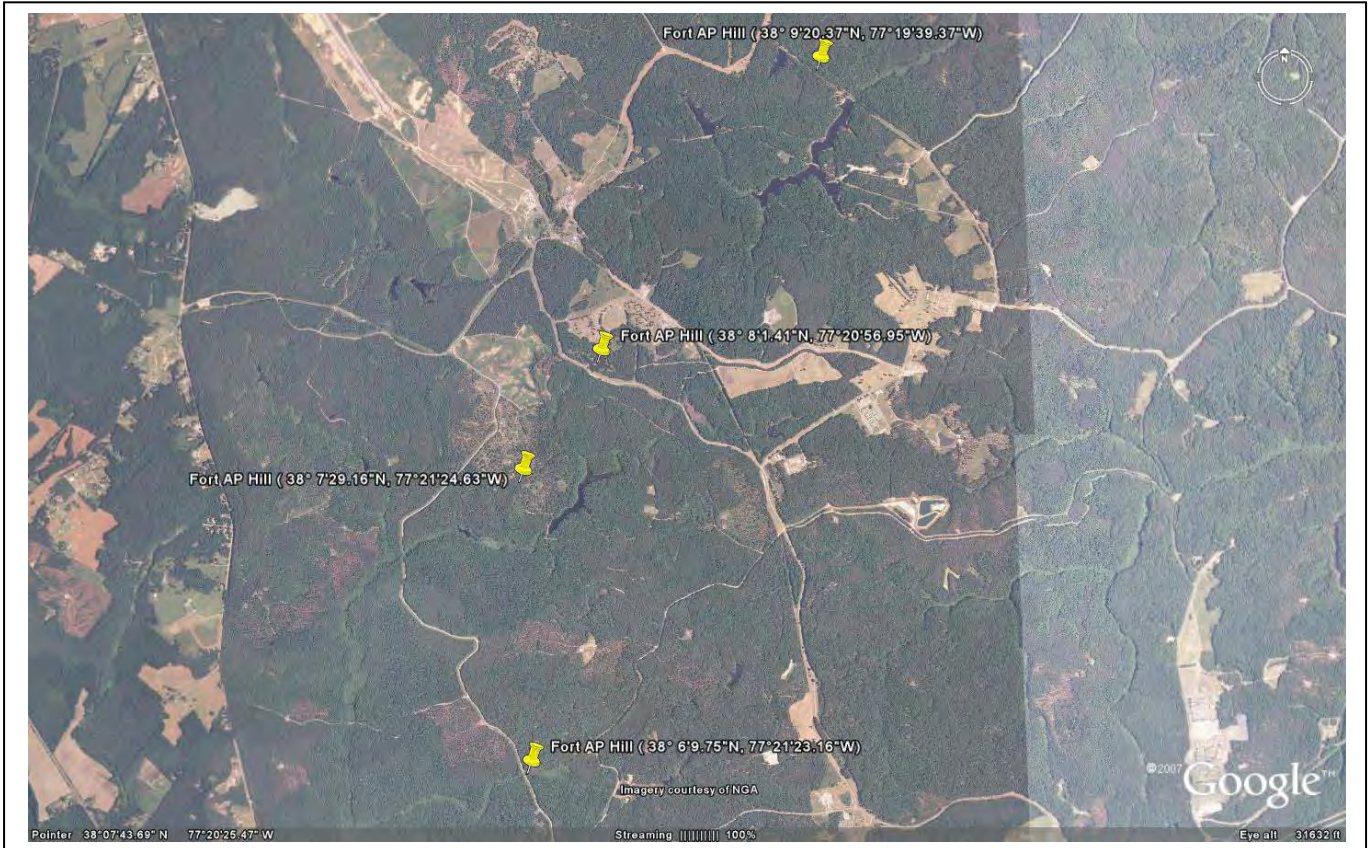
**Cope's Gray Treefrog - *Hyla chrysoscelis***

**Northern Cricket Frog - *Acris crepitans***

(Boldface type indicates at least one specimen tested positive for Bd.)

# Fort A.P. Hill, Virginia

## Wetland Survey Sites



## Species Sampled

American Toad - *Anaxyrus americanus*

**Fowler's Toad - *Anaxyrus fowleri***

**Bullfrog - *Lithobates catesbeianus***

**Green Frog - *Lithobates clamitans***

Pickerel Frog - *Lithobates palustris*

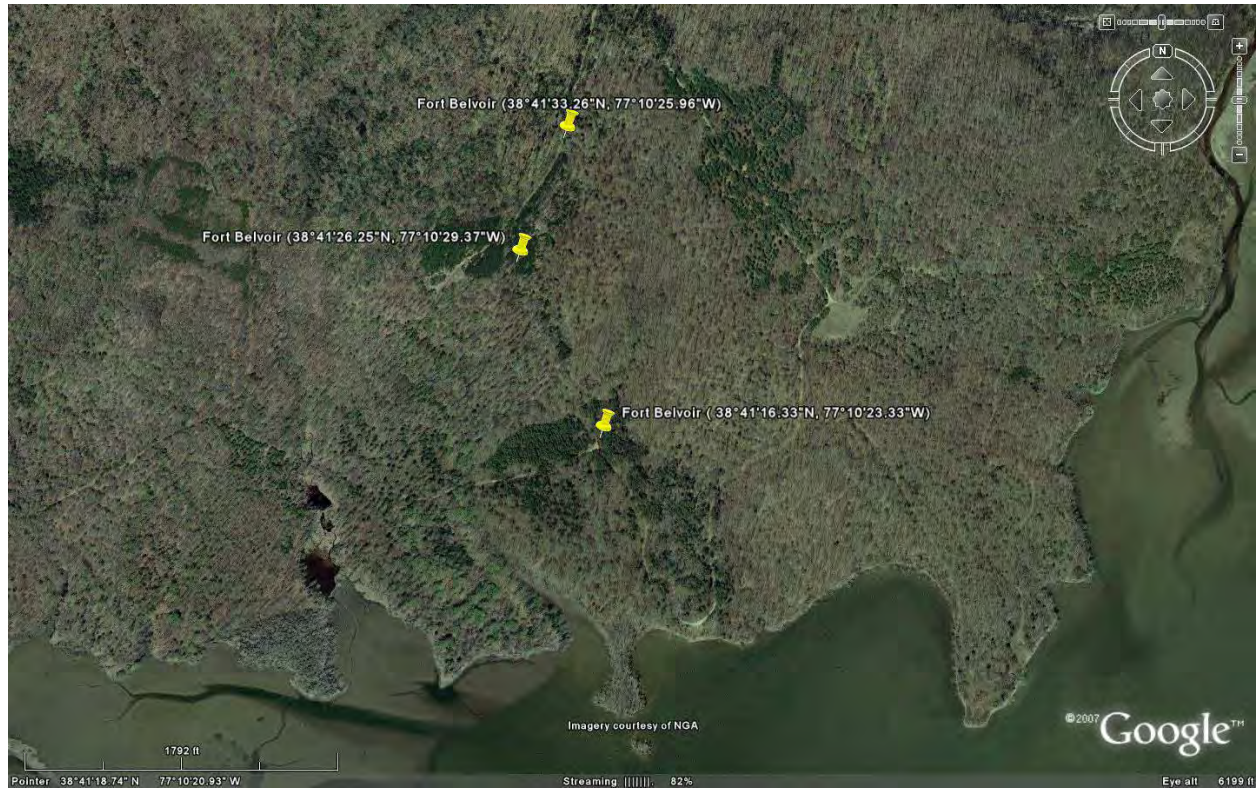
**Northern Cricket Frog - *Acris crepitans***

Red-spotted Newt - *Notophthalmus viridescens*

(Boldface type indicates at least one specimen tested positive for Bd.)

# Fort Belvoir, Virginia

## Wetland Survey Sites



## Species Sampled

Fowler's Toad - *Anaxyrus fowleri*

**Bullfrog - *Lithobates catesbeianus***

**Green Frog - *Lithobates clamitans***

Green Treefrog - *Hyla cinerea*

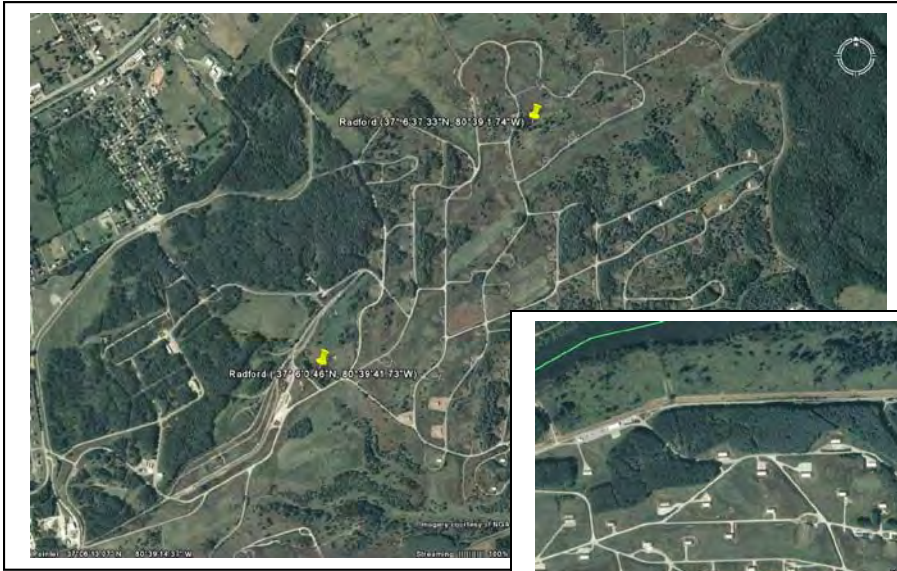
Cope's Gray Treefrog - *Hyla chrysoscelis*

**Northern Cricket Frog - *Acris crepitans***

(Boldface type indicates at least one specimen tested positive for Bd.)

# Radford Army Ammunitions Plant, Virginia

## Wetland Survey Sites



## Species Sampled

**Bullfrog - *Lithobates catesbeianus***

**Green Frog - *Lithobates clamitans***

Gray Treefrog - *Hyla versicolor*

**Spring Peeper - *Pseudacris crucifer***

Chorus Frog - *Pseudacris triseriata*

**Northern Dusky Salamander - *Desmognathus fuscus***

Southern Two-lined Salamander - *Eurycea cirrigera*

**Long-tailed Salamander - *Eurycea longicauda***

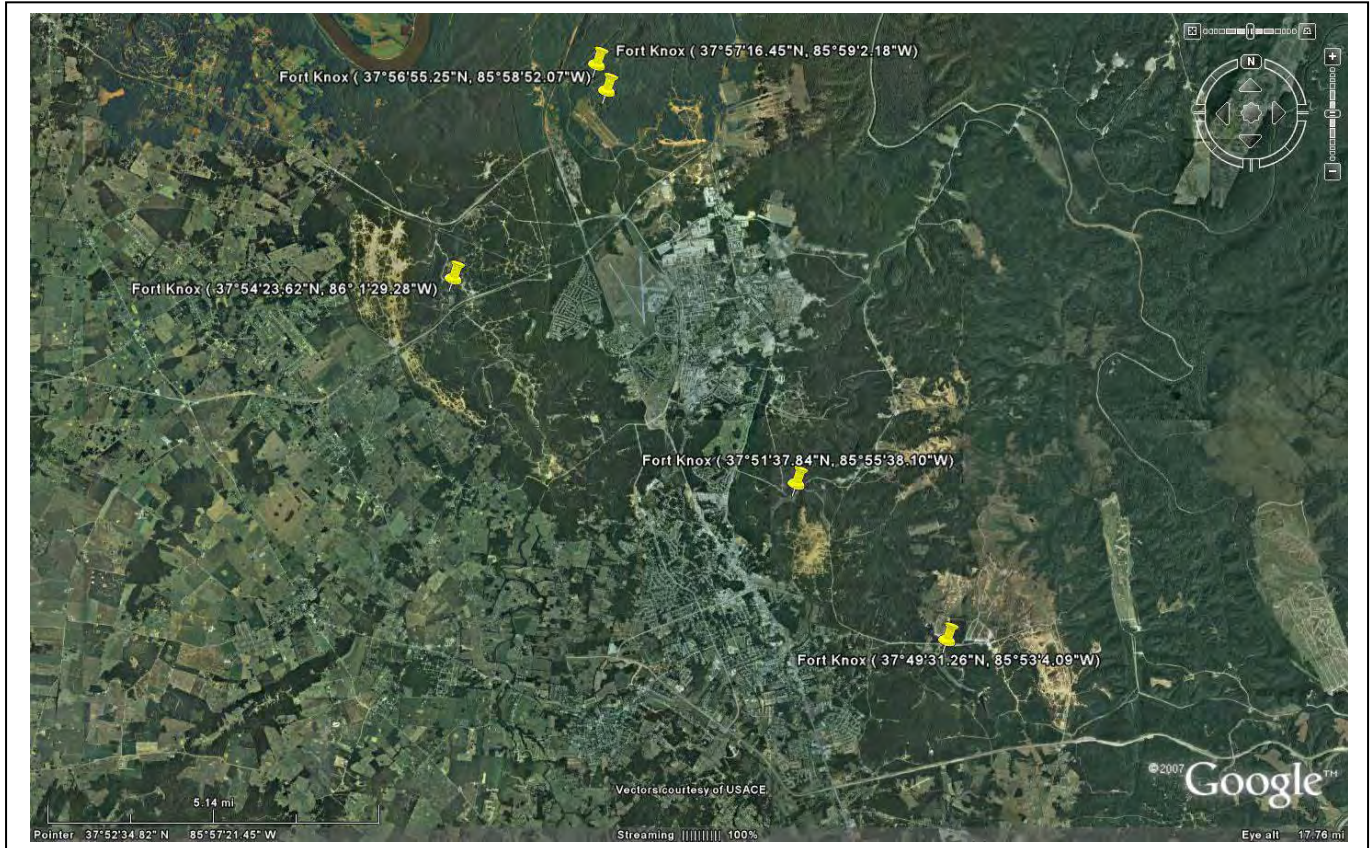
**Northern Red Salamander - *Pseudotriton ruber***

(Boldface type indicates at least one specimen tested positive for Bd.)



# Fort Knox, Kentucky

## Wetland Survey Sites



## Species Sampled

Bullfrog - *Lithobates catesbeianus*

Southern Leopard Frog - *Lithobates sphenoccephalus*

Green Frog - *Lithobates clamitans*

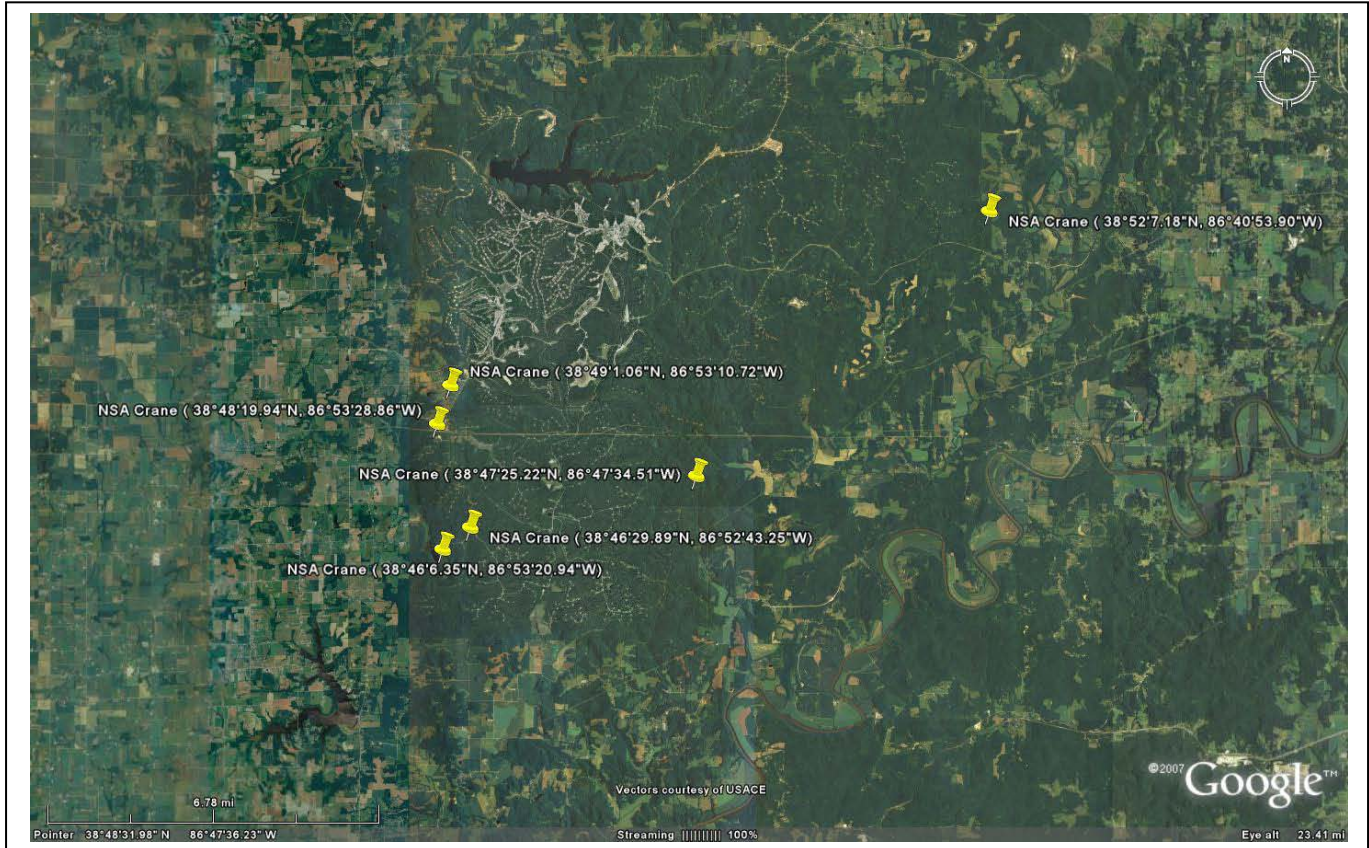
**Blanchard's Cricket Frog – *Acris blanchardi***

Red-spotted Newt - *Notophthalmus viridescens*

(Boldface type indicates at least one specimen tested positive for Bd.)

# NSA Crane, Indiana

## Wetland Survey Sites



## Species Sampled

American Toad - *Anaxyrus americanus*

Fowler's Toad – *Anaxyrus fowleri*

Green Frog - *Lithobates clamitans*

Southern Leopard Frog - *Lithobates sphenoccephalus*

Cope's Gray Treefrog - *Hyla chrysoscelis*

**Blanchard's Cricket Frog – *Acris blanchardi***

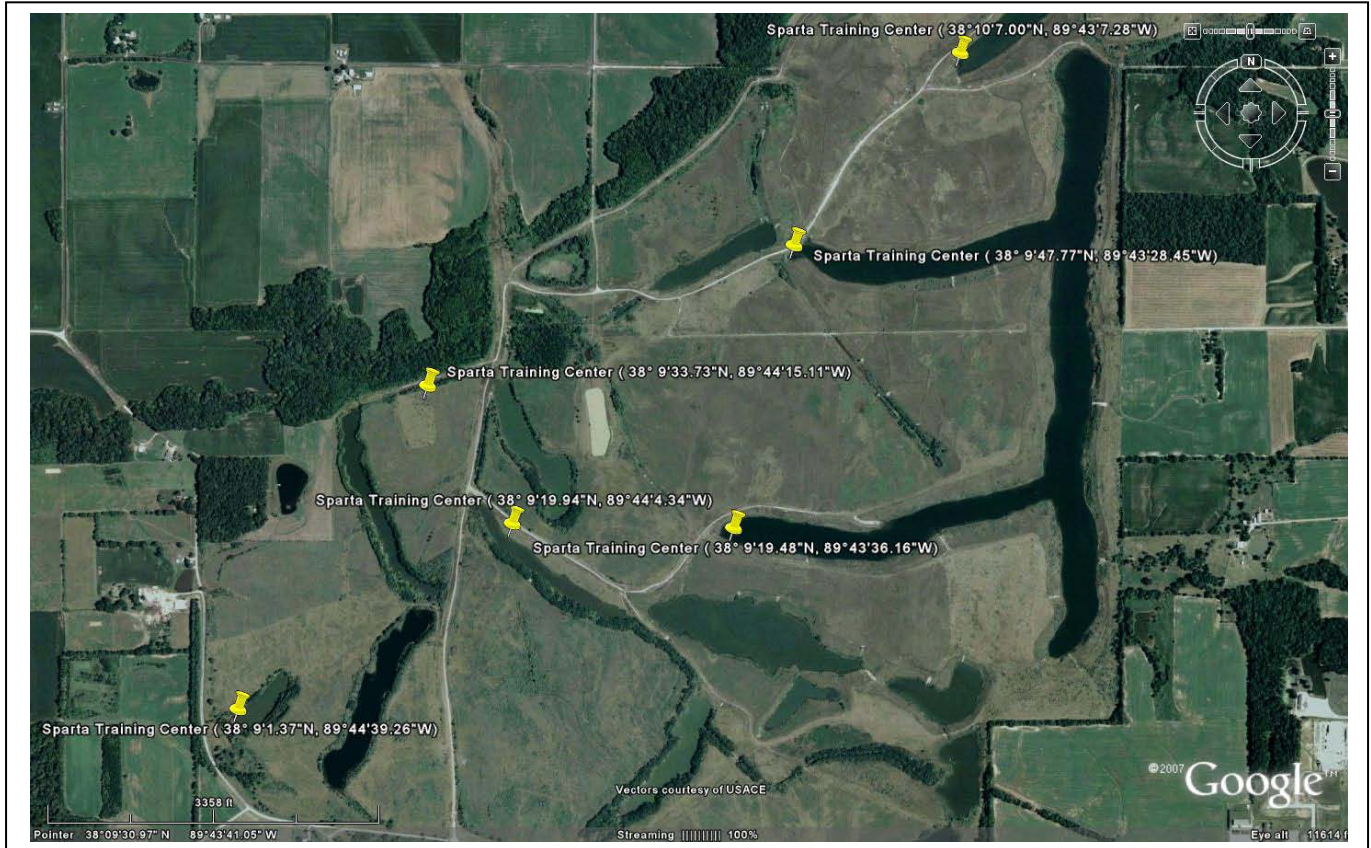
Western Chorus Frog – *Pseudacris triseriata*

Red-spotted Newt - *Notophthalmus viridescens*

(Boldface type indicates at least one specimen tested positive for Bd.)

# Sparta Training Center, Illinois

## Wetland Survey Sites



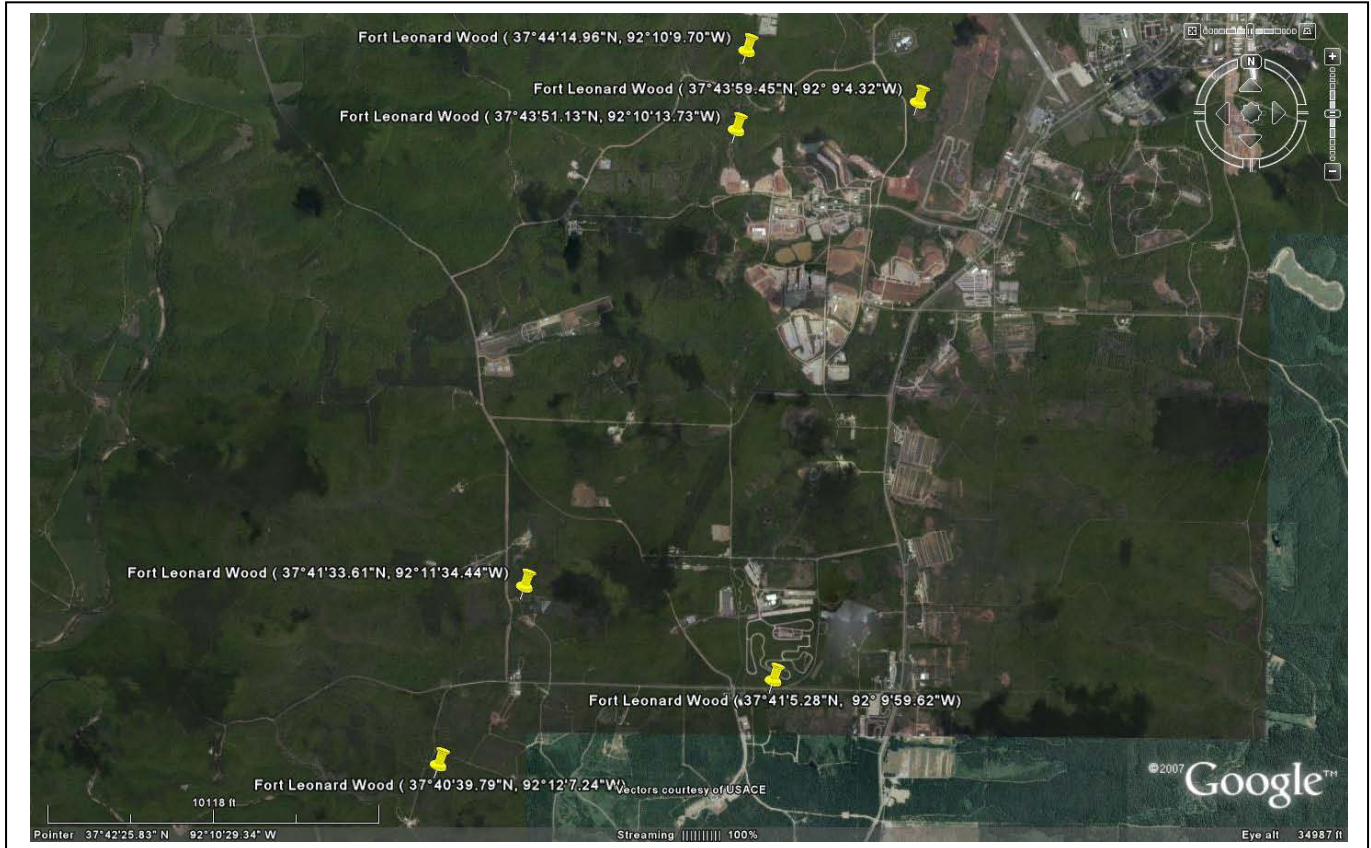
## Species Sampled

**Northern Cricket Frog - *Acris crepitans***

(Boldface type indicates at least one specimen tested positive for Bd.)

# Fort Leonard Wood, Missouri

## Wetland Survey Sites



## Species Sampled

Southern Leopard Frog - *Lithobates sphenoccephalus*

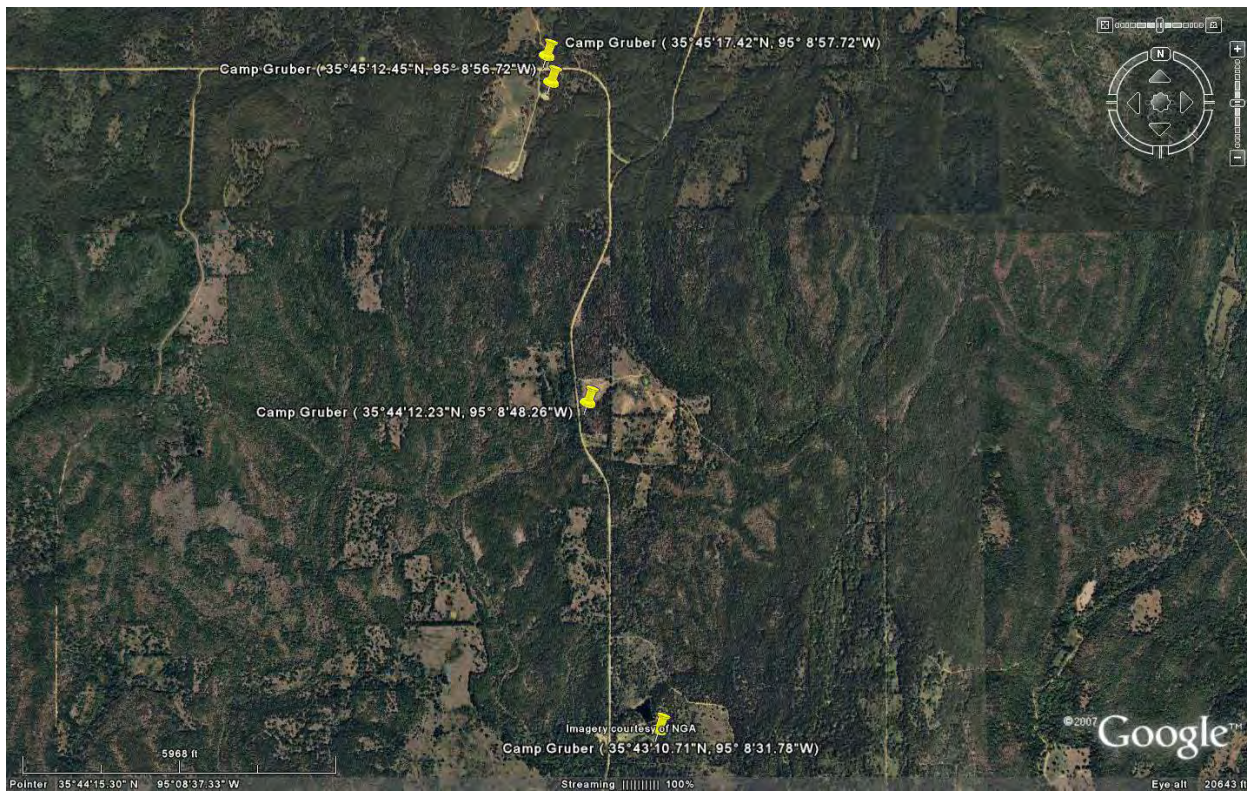
Eastern Gray Treefrog - *Hyla versicolor*

**Blanchard's Cricket Frog - *Acris blanchardi***

(Boldface type indicates at least one specimen tested positive for Bd.)

# Camp Gruber, Oklahoma

## Wetland Survey Sites



## Species Sampled

**Bullfrog - *Lithobates catesbeianus***

Southern Leopard Frog - *Lithobates sphenoccephalus*

Blanchard's Cricket Frog – *Acris blanchardi*

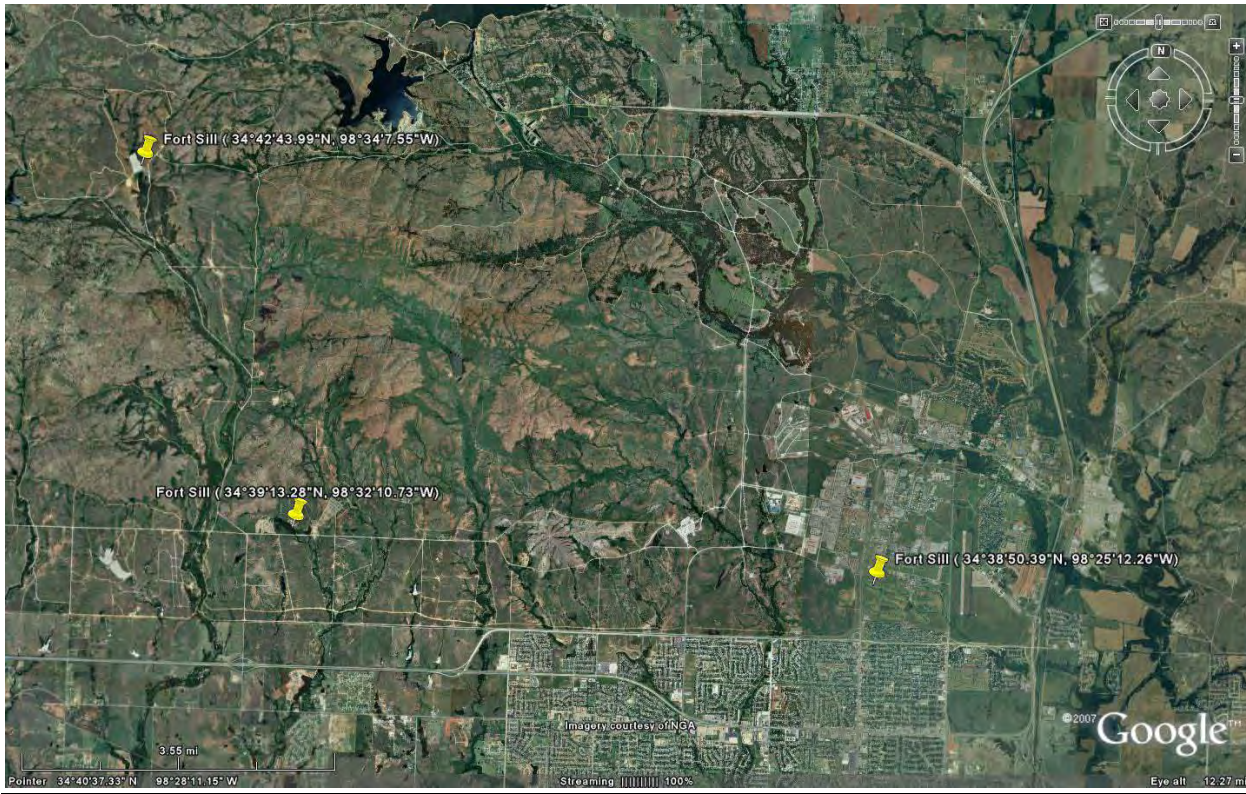
**Cope's Gray Treefrog - *Hyla chrysoscelis***

Green Treefrog - *Hyla cinerea*

(Boldface type indicates at least one specimen tested positive for Bd.)

# Fort Sill, Oklahoma

## Wetland Survey Sites



## Species Sampled

Southern Toad - *Anaxyrus terrestris*

Blanchard's Cricket Frog – *Acris blanchardi*

Plains Leopard Frog – *Lithobates blairi*

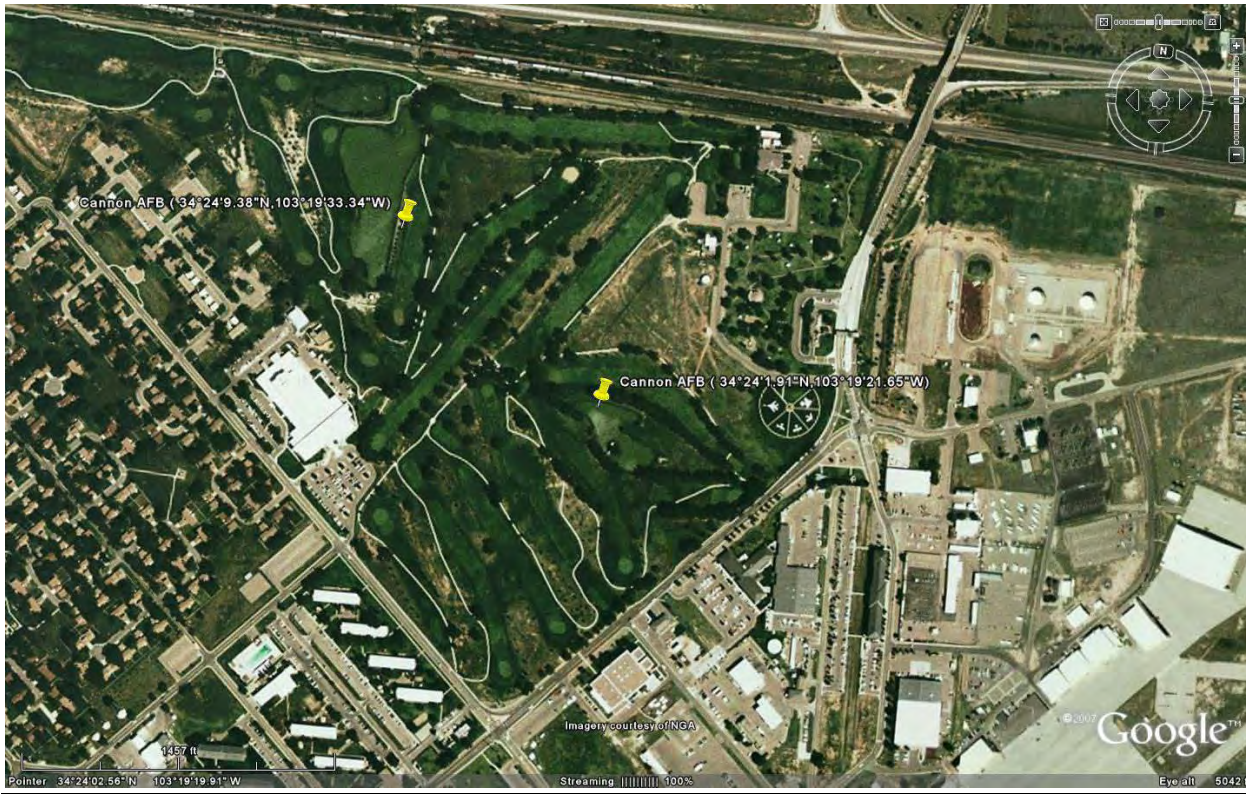
Bullfrog - *Lithobates catesbeianus*

Green Frog - *Lithobates clamitans*

(Boldface type indicates at least one specimen tested positive for Bd.)

# Cannon AFB, New Mexico

## Wetland Survey Sites



## Species Sampled

**Woodhouses's Toad - *Anaxyrus woodhousii***

(Boldface type indicates at least one specimen tested positive for Bd.)

# Kirtland AFB, New Mexico

## Wetland Survey Sites



## Species Sampled

**Woodhouses's Toad - *Anaxyrus woodhousii***

Red-spotted Toad - *Anaxyrus punctatus*

Spotted Salamander – *Ambystoma maculatum*

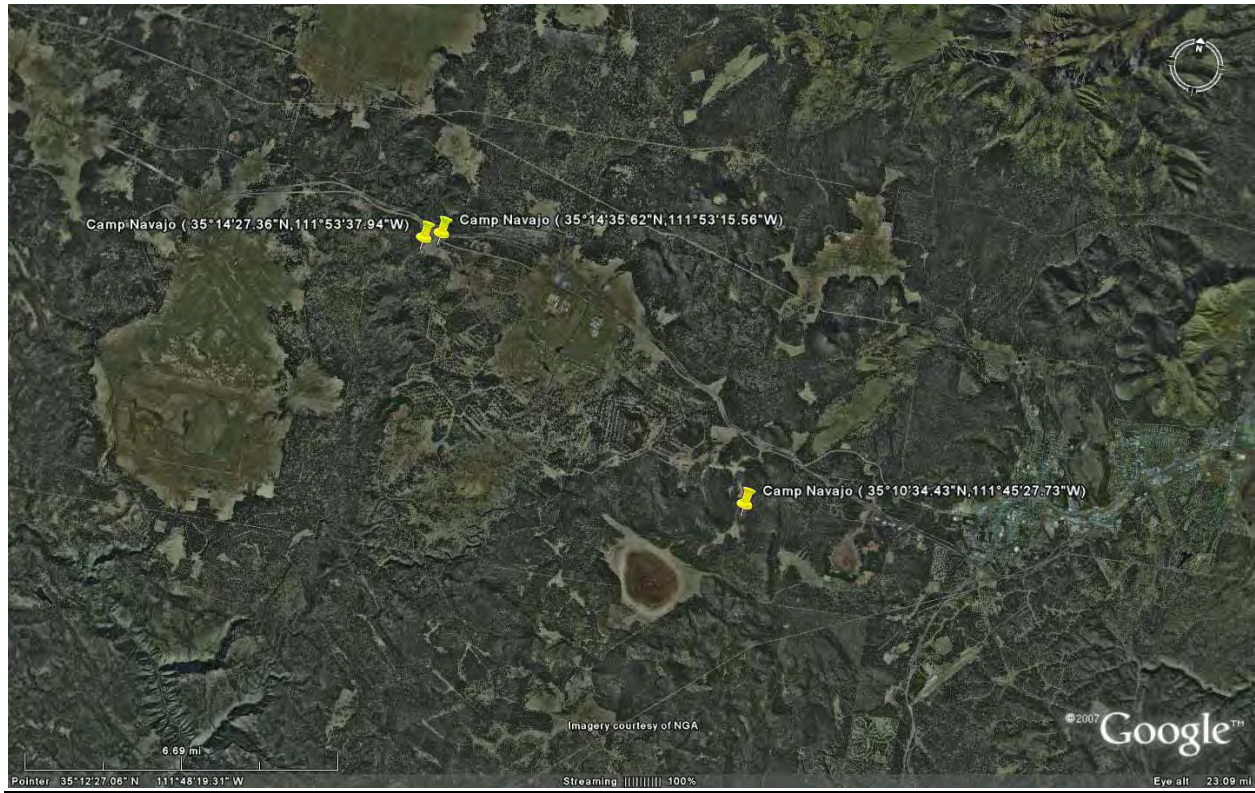
Tiger Salamander – *Ambystoma tigrinum*

(Boldface type indicates at least one specimen tested positive for Bd.)



# Camp Navajo, Arizona

## Wetland Survey Sites



## Species Sampled

Striped Chorus Frog – *Pseudacris triseriata*

Tiger Salamander – *Ambystoma tigrinum*

Barred Tiger Salamander – *Ambystoma mavortium*

(Boldface type indicates at least one specimen tested positive for Bd.)

# MCB Camp Pendleton, California

## Wetland Survey Sites



## Species Sampled

**Bullfrog - *Lithobates catesbeianus***

Northern Pacific Treefrog – *Pseudacris regilla*

**California Treefrog – *Hyla cadaverina***

(Boldface type indicates at least one specimen tested positive for Bd.)