Department of Defense
Legacy Resource Management Program

PROJECT NUMBER (09-426)

Do Frogs Still Get Their Kicks on Route 66?

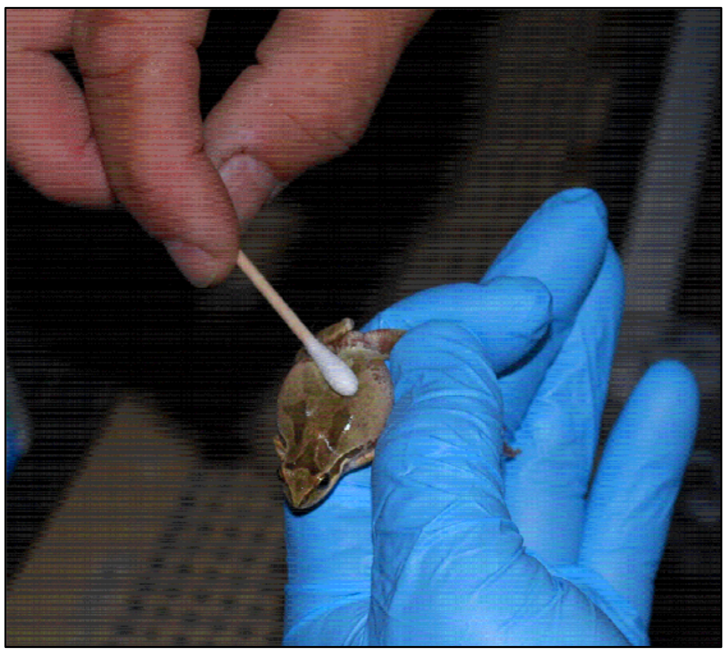
A Transcontinental Transect for Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*) Infection on U.S Department of Defense Installations

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

Final Report
February, 2011

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



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 33º and 39º 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 Vrendenburg 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 (Vredenberg 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.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 33º 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 ml) 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 (χ^2) and Kruskal Wallis tests (SPSS v. 17) for our analyses. A χ^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 sphenocephalus*). 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° C; Kruskal-Wallis, $p = 0.3$).

Temporal Patterns (Seasonality)

There was a strong seasonal component to our results (Fig. 3), which was statistically significant (χ^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 (Tennessen 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.

Acknowledgements

The authors would like to recognize Dr. Joe Mitchell and Christopher Phillips for their extensive field work and dedication on this project. We would also like to recognize Irene Macallister (USACOE) for her laboratory analysis of the field collected data. The authors recognize the following individuals, without whom this study would not have been possible: Heather Arnold, Sara Bell, John “Chip” Blackburn, Paul Block, Kurt Buhlmann, John Crawford, Megan Dinkins, Mike Dreslik, Nate Engbrecht, Jennifer Heemeyer, Stephanie Keys, Biddy Kuhns, Justin Mitchell, Bill Peterman, Sarah Shepheard, Jeremy Tiemann, Vanessa Kinney, and Dan Wylie for field collection of samples. Neil Wesslund at CERL ran the PCR reactions. Steve Andrews (Crane NWC), Jason Applegate, Terry Banks, and Timothy Southerland (Fort A.P. Hill), Bill Berry (MCB Camp Pendleton), Dana Bradshaw (Fort Lee), Mike Brandenfurg (Fort Knox), Rick Crow (Cannon AFB), Len DiIoia (Radford Army Ammunitions Plant), Carol Finley (Kirtland AFB), Jeff Howard (Camp Gruber), Kenton Lohraff (Fort Leonard Wood), John Pilcicki (Fort Belvoir), Zach Reichold (Camp Navajo), Glen Wampler and Kevin McCurdy (Fort Sill), and Michael Wright (NAS Oceana) provided access to their installations and support in the field. Jennifer Heemeyer, Vanessa Kinney, and Nate Engbrecht provided statistical consultation. Susie Lannoo proofread early drafts. Thanks also to Allan Pessier and Joe Mendelson for numerous conversations on the nuances of Bd sampling and interpretation. This project was funded through the DoD Legacy Resource Management Program (PN 09-426).

Literature Cited

Adams MJ, Galvan S, Reinitz D, Cole RA, Payre S, Hahr M, Govindarajulu P (2007) Incidence of the fungus *Batrachochytrium dendrobatidis* in amphibian populations along the Northwest Coast of North America. Herp Rev 38: 430–431.

Adams MJ, Galvan S, Scalera R, Grieco C, Sindaco R (2008) *Batrachochytrium dendrobatidis* in amphibian populations in Italy. Herp Rev 39: 324–326.

Annis SL, Dastoor FP, Ziel H, Daszak P, Longcore JE (2004) A DNA-based assay identifies *Batrachochytrium dendrobatidis* in amphibians. J Wildl Dis 40: 420–428.

Berger L, Hyatt AD, Speare R, Longcore JE (2005) Life cycle stages of the amphibian chytrid *Batrachochytrium dendrobatidis*. Dis Aquat Org 68: 51–63.

Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, Hines HB, Lips KR, Marantelli G, Parkes H (1998) Chytridiomycosis causes amphibian mortality associated with population decline in the Rain Forests of Australia and Central America. PNAS 95: 9031–9036.

Berger L, Speare R, Hines HB, Marantelli G, Hyatt AD, McDonald KR, Skerratt LF, Olsen V, Clarke JM, Gillespie G, Mahony M, Sheppard N, Williams C, Tyler MJ (2004) Effect of season and temperature on mortality in amphibians due to chytridiomycosis. Aust Vet J 82: 434–439.

Blaustein AR, Romansic JM, Scheessele EA, Han BA, Pessier AP, Longcore JE (2005) Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus *Batrachochytrium dendrobatidis*. Cons Biol 19: 1460–1468.

Briggs CJ, Vredenburg VT, Knapp RA, Rachowicz LJ (2005) Investigating the population-level effects of chytridiomycosis: An emerging infectious disease of amphibians. Ecology 86: 3149–3159.

Briggs CJ, Knapp RA, Vredenburg VT (2010) Enzootic and epizootic dynamics of the chytrid fungus pathogen of amphibians. PNAS. pnas.0912886107.

Carnival ACOQ, Puschendorf R, Peixoto OL, Verdade VK, Rodrigues MT (2006) Amphibian chytrid fungus broadly distributed in the Brazilian Atlantic Rain Forest. EcoHealth 3: 41–48.

Chatfield MWH, Rothermel BB, Brooks CS, Kay JB (2009) Detection of *Batrachochytrium dendrobatidis* in amphibians from the Great Smoky Mountains of North Carolina and Tennessee, USA. Herp Rev 40: 176–179.

Daszak P, Cunningham AA, Hyatt AD (2003) Infectious disease and amphibian population declines. Divers Dist 9: 141–150.

Daszak P, Strieby A, Cunningham AA, Longcore JE, Brown CC, Porter D (2004) Experimental evidence that the bullfrog (*Rana catesbeiana*) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. Herp J 14: 201–207.

de Queiroz Carnival ACO, Puschendorf R, Peixoto OL, Verdade VK, Rodrigues MT (2009) Amphibian chytrid fungus broadly distributed in the Brazilian Atlantic Rain Forest. EcoHealth 3: 41–48.

Deguise I, Richardson JS (2009) Prevalence of the chytrid fungus (*Batrachochytrium dendrobatidis*) in Western Toads in southwestern British Columbia, Canada. Northwest Nat 90: 35–38.

DiRosa I, Simoncelli F, Fagotti A, Pascolini R (2007) The proximate cause of amphibian declines? Nature 447: E4–E5.

Fisher, MC, Garner TWJ, Walker SF (2009) Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomcosis in space, time, and host. Annu Rev Microbiol 63: 291–310.

Frías-Alvarez P, Vredenburg VT, Familiar-López M, Longcore JE, González-Bernal E, Santos-Barrera G, Zambrano L, Parra-Olea G (2008) Chytridiomycosis survey in wild and captive Mexican amphibians. EcoHealth 5: 18–26.

Gaertner JP, Forstner MRJ, O'Donnell L, Hahn D (2009a) Detection of *Batrachochytrium dendrobatidis* in endemic salamander species from Central Texas. EcoHealth 6: 20–26.

Gaertner JP, Gaston MA, Spontak D, Forstner MR, Hahn D (2009b) Seasonal variation in the detection of *Batrachochytrium dendrobatidis* in a Texas population of Blanchard's Cricket Frog (*Acris crepitans blanchardi*). Herp Rev 40: 184–187.

Garner TWJ, Walker S, Bosch J, Hyatt AD, Cunningham AA, Fisher MC (2005) Chytrid fungus in Europe. Emerg Infect Dis 11: 1639–1641.

Garner TWJ, Walker S, Bosch J, Hyatt AD, Cunningham AA, Fisher MC (2006) The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American Bullfrog, *Rana catesbeiana*. Biol Lett 2: 455–459.

Goldberg CS, Hawley TJ, Waits LP (2009) Local and regional patterns of amphibian chytrid presence on the Osa Peninsula, Costa Rica. Herp Rev 40: 309–311.

Grant EHC, Bailey LL, Ware JL, Duncan KL (2008) Prevalence of the amphibian pathogen *Batrachochytrium dendrobatidis* in stream and wetland amphibians in Maryland, USA. Appl Herp 5: 233–241.

Green DE, Converse KA, Schrader AK (2002) Epizootiology of sixty-four amphibian morbidity and mortality events in the USA, 1996–2001. Ann New York Acad Sci 969: 323–339.

Hanselmann R, Rodríguez A, Lampo M, Fajardo-Ramos L, Aguirre AA, Kilpatrick AM, Rodríguez JP, Daszak P (2004) Presence of an emerging pathogen of amphibians in introduced bullfrogs *Rana catesbeiana* in Venezuela. Biol Cons 120: 115–119.

Hester RE, Harrison RM (2007) Biodiversity Under Threat. Royal Society of Chemistry, London.

Hossack BR, Adams MJ, Grant EHC, Pearl CA, Bettaso JB, Barichivich WJ, Lowe WH, True K, Ware JL, Corn PS (2010) Low prevalence of chytrid fungus (*Batrachochytrium dendrobatidis*) in amphibians of U.S. headwater streams. J Herp 44: 253–260.

Holland, D.C., and Goodman, R.H. A guide to the amphibians and reptiles of MCB Camp Pendleton, San Diego County, California. Report prepared for AC/S Environmental Security.

Hyatt AD, Boyle DG, Olsen V, Boyle DB, Berger L, Obendorf D, Dalton A, Krieger K, Hero M, Hines H, Phillott R, Campbell R, Marantelli G, Gleason F, Colling A (2007) Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Dis Aquat Org 73: 175–192.

James TY, Litvintseva AP, Vilgalys R, Morgan JAT, Taylor JW, Fisher MC, Berger L, Weldon C, du Preez L, Longcore JE (2009) Rapid global expansion of the fungal disease chytridiomycosis into declining and healthy amphibian populations. PLoS Path 5: e1000458.

Johnson ML, Speare R (2003) Survival of *Batrachochytrium dendrobatidis* in water: quarantine and disease control implications. Emerg Infect Dis 9: 922–925.

Johnson, ML, Speare R (2005) Possible modes of dissemination of the amphibian chytrid *Batrachochytrium dendrobatidis* in the environment. Dis Aquat Org 65: 181–186.

Jones, KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P (2008) Global trends in emerging infectious diseases. Nature 451: 990–993.

Kilpatrick AM, Briggs CJ, Daszak P (2010) The ecology and impact of chytridiomycosis: an emerging disease of amphibians. Trends Ecol Evol 25: 109–118.

Kriger KM, Hero J-M (2006) Survivorship in wild frogs infected with chytridiomycosis. EcoHealth 3: 171–177.

Kriger KM, Hero J-M (2007) The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. Divers Dist 13: 781–788.

Lampo M, Sánchez D, Nicolás A, Márquez M, Nava-González F, García CZ, Rinaldi M, Rodríguez-Contreras A, León F, Han BA, Chacón-Ortiz A (2008) *Batrachochytrium dendrobatidis* in Venezuela. Herp Rev 39: 449–454.

Lauer A, Simon MA, Banning JL, André E, Duncan K, Harris RN (2007) Common cutaneous bacteria from the eastern red-backed salamander can inhibit pathogenic fungi. Copeia 2007: 630–640.

Lips KR (1998) Decline of a tropical montane amphibian fauna. Cons Biol 12: 106–117.

Lips KR (1999) Mass mortality and population declines of anurans at an upland site in western Panama. Cons Biol 13: 117–125.

Lips KR, Brem F, Brenes R, Reeve JD, Alford RA, Voyles J, Carey C, Livo L, Pessier AP, Collins JP (2006) Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Ecology 103: 3165–3170.

Lips KR, Diffendorfer J, Mendelson III JR, Sears MW (2008) Riding the wave: reconciling the roles of disease and climate change in amphibian declines. PLoS Biol 6: 441–454.

Lips KR, Green DE, Papedick R (2003a) Chytridiomycosis in wild frogs from southern Costa Rica. J Herp 37: 215–218.

Lips KR, Reeve JD, Witters LR (2003b) Ecological traits predicting amphibian declines in Central America. Cons Biol 17: 1078–1088.

Longcore JR, Longcore JE, Pessier AP, Halteman WA (2007) Chytridiomycosis widespread in anurans of northeastern United States. J Wildl Manage 71: 435–444.

Longcore J, Pessier A, Nichols DK (1999) *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 91: 219–227.

Lovich R, Ryan MJ, Pessier AP, Claypool B (2008) Infection with the fungus *Batrachochytrium dendrobatidis* in an non-native *Lithobates berlandieri* below sea level in the Coachilla Valley, California, USA. Herp Rev 39: 315–317.

Mitchell KM, Churcher TS, Garner TWJ, Fisher MC (2008) Persistence of the emerging pathogen *Batrachochytrium dendrobatidis* outside the amphibian host greatly increases the probability of host extinction. Proc Royal Soc Biol Sci B 275: 329–334.

Murray KA, Skerratt LF, Speare R, McCallum H (2009) Impact and dynamics of disease in species threatened by the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*. Cons Biol 32: 1242–1252.

Ouellet M, Mikaelian I, Pauli BD, Rodrigues J, Green DM (2005) Historical evidence of widespread chytrid infection in North American amphibian populations. Cons Biol 19: 1431–1440.

Pearl CA, Bull EL, Green DE, Bowerman J, Adams MJ, Hyatt A, Wente WH (2007) Occurrence of the amphibian pathogen *Batrachochytrium dendrobatidis* in the Pacific Northwest. J Herp 41: 145–149.

Pessier, AP, Mendelson III, JR (2010) A manual for control of infectious diseases in amphibian survival assurance colonies and reintroduction programs. Proceedings from a Workshop 16–18 Feburary 2009, San Diego Zoo, San Diego, California, USA.

Peterson JD, Wood MB, Hopkins WA, Unrine JM, Mendonca MT (2007) Prevalence of *Batrachochytrium dendrobatidis* in American Bullfrog and Southern Leopard Frog larvae from wetlands on the Savannah River site, South Carolina. J Wildl Dis 43: 450–460.

Piotrowski JS, Annis SL, Longcore JE (2004) Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96: 9–15.

Pounds JA, Bustamante MR, Coloma LA, Consuegra JA, Viteri MP, Puschendorf R, Ron SR, Sánchez-Azofeifa GA, Still CJ, Young BE (2006) Widespread amphibian extinctions from epidemic disease driven by global warming. Nature 439: 161–167.

Rachowicz LJ, Hero J-M, Alford RA, Taylor JW, Morgan JAT, Vredenburg VT, Collins JP, Briggs CJ (2006a) The novel and endemic pathogen hypotheses: competing explanations for the origin of emerging infectious diseases of wildlife. Cons Biol 19: 1441–1449.

Rachowicz LJ, Knapp RA, Morgan JAT, Stice MJ, Vredenburg VT, Parker JM, Briggs CJ (2006b) Emerging infectious disease as a proximate cause of amphibian mass mortality. Ecology 87: 1671–1683.

Rachowicz LJ, Vredenburg VT (2004) Transmission of *Batrachochytrium dendrobatidis* within and between amphibian life stages. Dis Aquat Org 61: 75–83.

Retallick RWR, McCallum H, Speare R (2004) Endemic infection of the amphibian chytrid fungus in a frog community post-decline. PLoS Biol 2: 1965–1971.

Ribas L, Li M-S, Doddington BJ, Robert J, Seidel JA, Kroll JS, Zimmerman LB, Grassly NC, Garner TWJ, Fisher MC (2009) Expression profiling the temperature-dependent amphibian response to infection by *Batrachochytrium dendrobatidis*. PLoS One 4: e8408.

Richards-Zawacki CL (2010) Thermoregulatory behaviour affects prevalence of chytrid fungal infection in a wild population of Panamanian Golden Frogs. Proc Royal Soc Biol Sci B 277: 519–528.

Rothermel BB, Walls SC, Mitchell JC, Dodd CK, Irwin LK, Green DE, Vazquez VM, Petranka JW, Stevenson DJ (2008) Widespread occurrence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in the southeastern USA. Dis Aquat Org 82: 3–18.

Sadinski W, Roth M, Treleven S, Theyerl J, Dummer P (2010) Detection of the chytrid fungus, *Batrachochytrium dendrobatidis*, on recently metamorphosed amphibians in the North-Central United States. Herp Rev 41: 170–175.

Scalera R, Adams MJ, Galvan SK (2008) Occurrence of *Batrachochytrium dendrobatidis* in amphibian populations in Denmark. Herp Rev 39: 199–200.

Skerratt LF, Berger L, Hines HB, McDonald KR, Mendez D, Speare R (2008) Survey protocol for detecting chytridiomycosis in all Australian frog populations. Dis Aquat Org 80: 85–94.

Skerratt LF, Berger L, Speare R, Cashins S, McDonald KR, Phillott AD, Hines HB, Kenyon N (2007) Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4: 125–134.

Stein BA, Scott C, Benton N (2008) Federal Lands and Endangered Species: The Role of Military and Other Federal Lands in Sustaining Biodiversity. BioScience 58: 339-347.

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller, RW (2004) Status and trends of amphibians and extinctions worldwide. Science 306: 1783–1786.

Tennessen JA, Woodhams DC, Chaurand P, Reinert LK, Billheimer D, Shyr Y, Caprioli RM, Blouin MS, Rollins-Smith LA (2009) Variations in the expressed antimicrobial peptide repertoire of Northern Leopard Frog (*Rana pipiens*) populations suggest intraspecies differences in resistance to pathogens. Develop Comp Immunol 33: 1247–1257.

Vredenburg VT, Knapp RA, Tunstall TS, Briggs CJ (2010) Dynamics of an emerging disease drive large-scale amphibian population extinctions. PNAS. pnas.0914111107.

Vredenburg, VT, Summers AT (2001) Field Identification of chytridiomycosis in *Rana muscosa*. Herpetological Review 32: 151–152.

Wake DB, Vredenburg VT (2008) Colloquium paper: are we in midst of the sixth mass extinction? A view from the world of amphibians. PNAS 105: 11466–11473.

Waldman B, van de Wolfshaar KE, Klena JD, Andjic V, Bishop PJ, de Norman RJB (2001) Chytridiomycosis in New Zealand frogs. Surveillance 28: 9–11.

Woodhams DC, Hyatt AD, Boyle DG, Rollins-Smith LA (2007a) The Northern Leopard Frog *Rana pipiens* is a widespread reservoir species harboring *Batrachochytrium dendrobatidis* in North America. Herpetol Rev 39: 66–68.

Woodhams DC, Alford RA, Marantelli G (2003) Emerging disease of amphibians cured by elevated body temperature. Dis Aquat Org 55: 65–67.

Woodhams DC, Ardipradja K, Alford RA, Harris R, Marantelli G, Reinert LK, Rollins-Smith LA (2007b Resistance to chytridiomycosis varies among amphibian species and is correlated with skin peptide defenses. Anim Cons 10: 409–417.

Zellmer AJ, Richards CL, Martens LM (2008) Low prevalence of *Batrachochytrium dendrobatidis* across *Rana sylvatica* populations in southeastern Michigan, USA. Herp Rev 39: 196–199.

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**
Spring / Early Summer	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%
Mid-Summer	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%
Late Summer / Early Fall	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 sphenocephalus

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.

Installation	Mean Annual Precipitation	Mean Annual Temperature
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 33º and 39º 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.

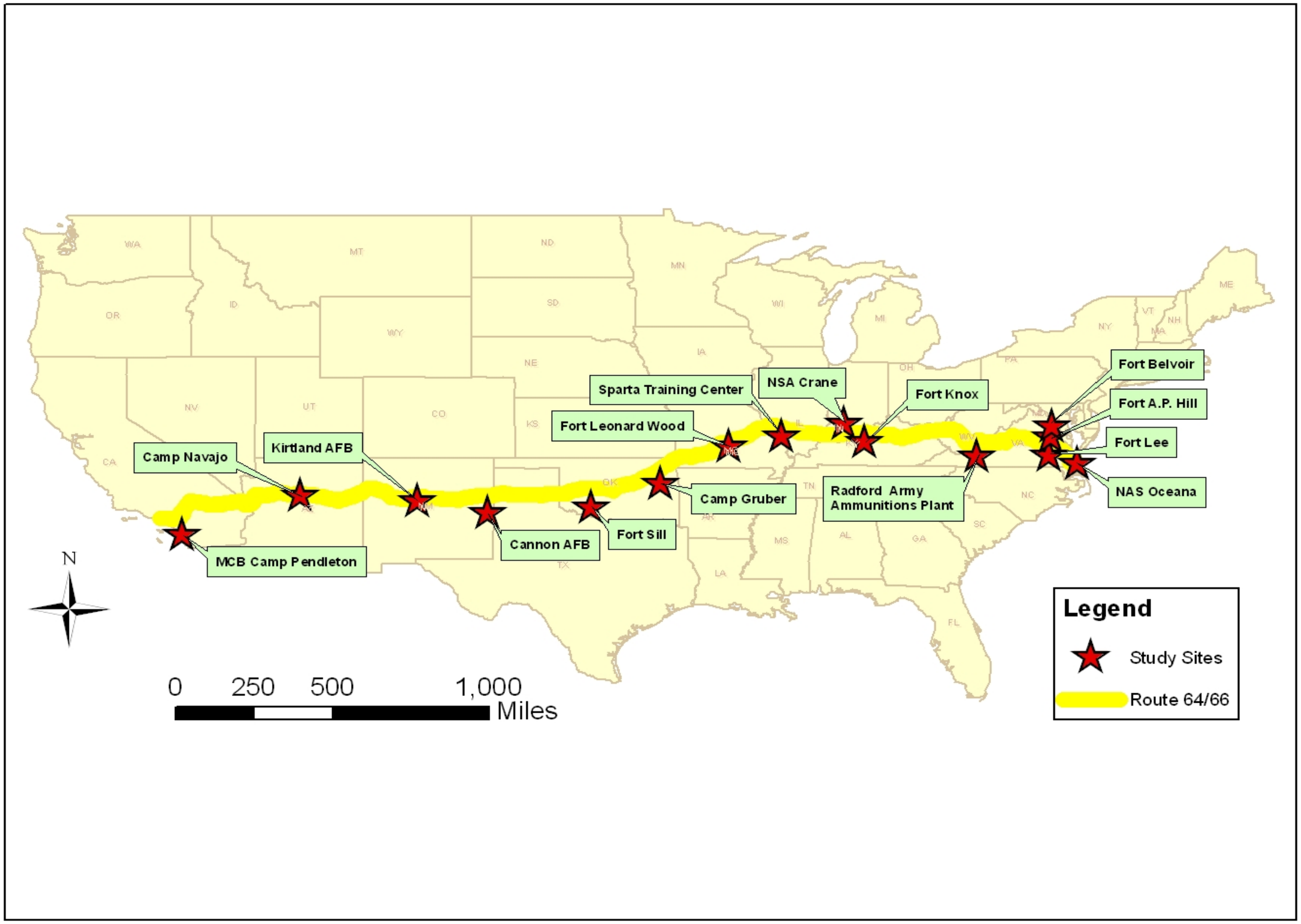


Figure 2. Percentage of Bd positive samples, mean annual precipitation and mean annual temperature by installation.

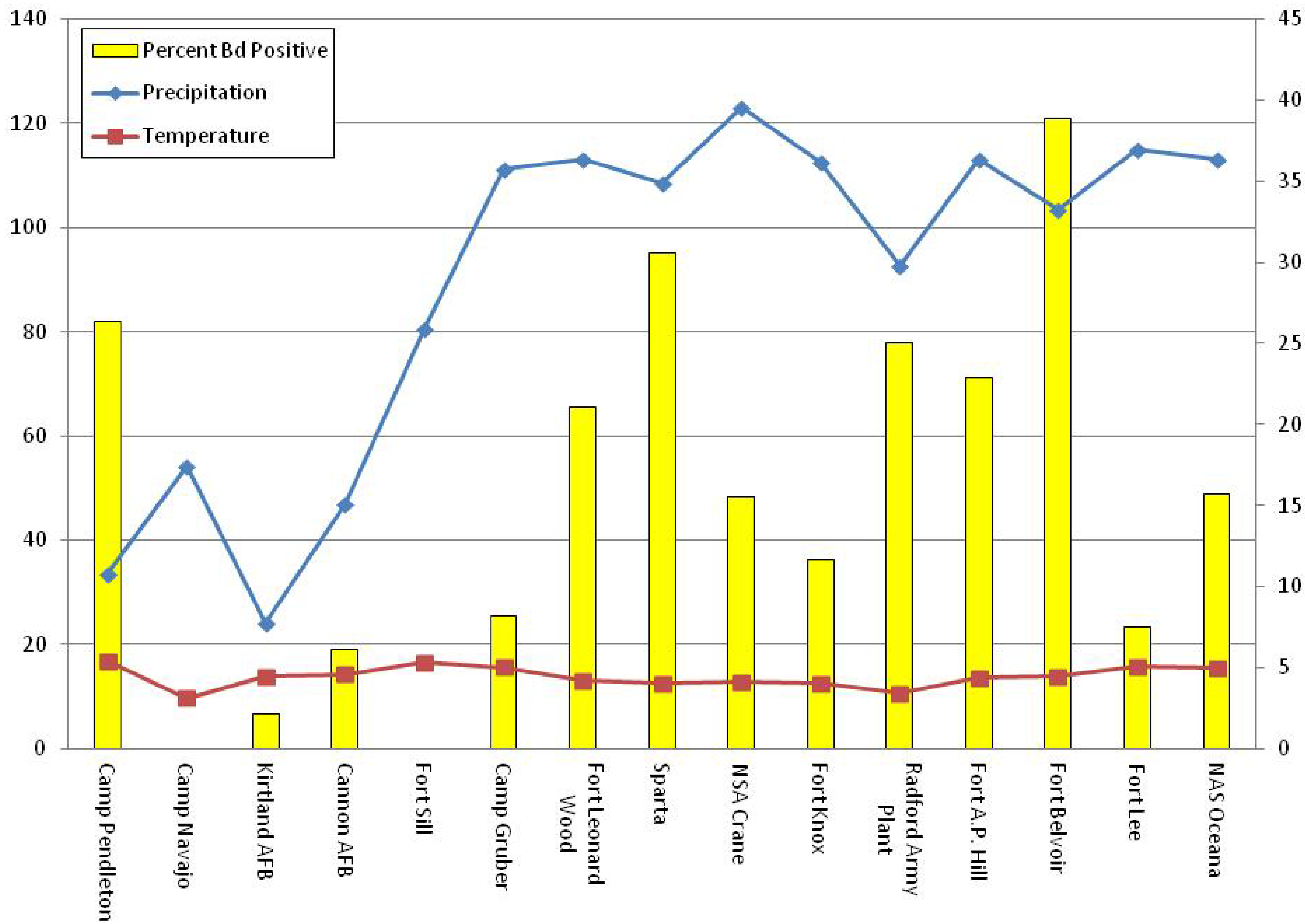
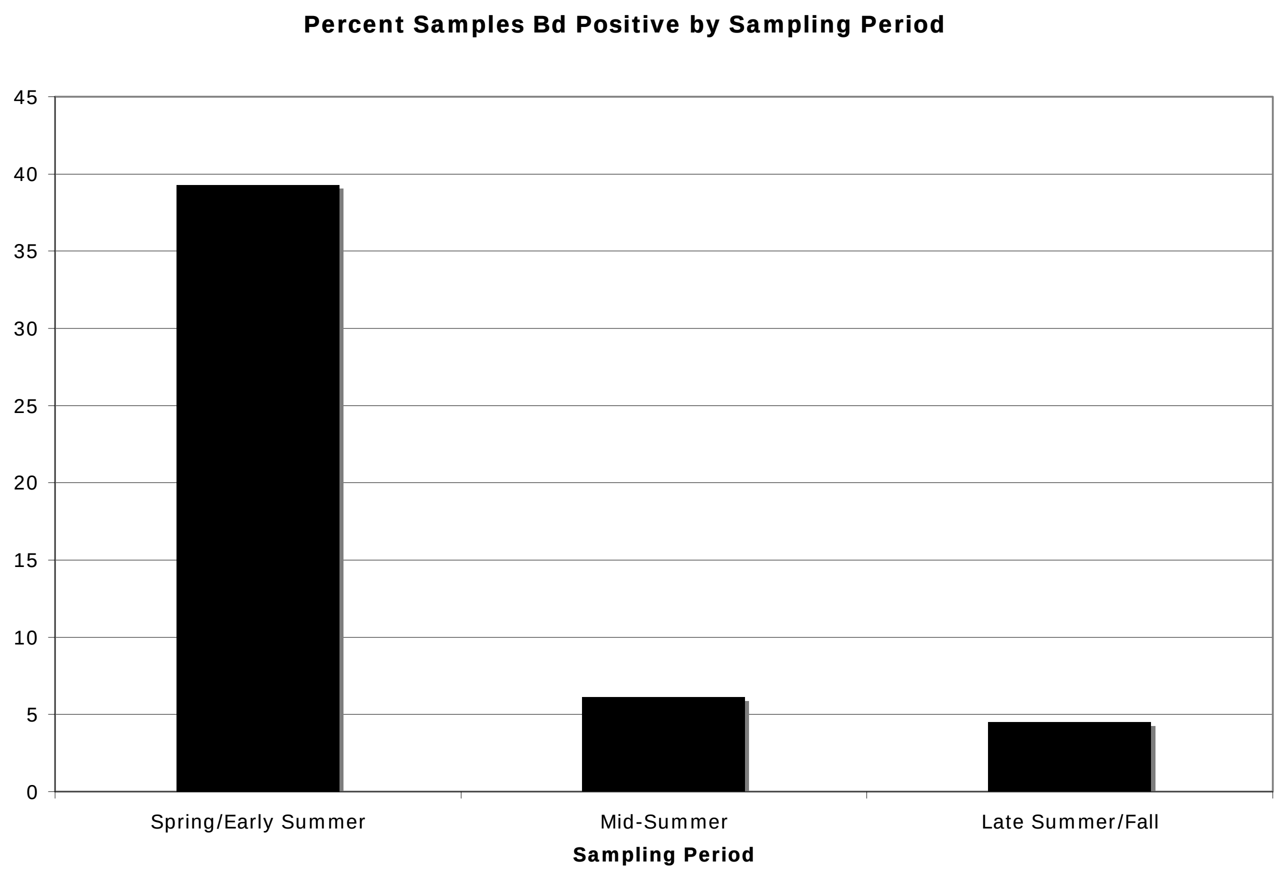
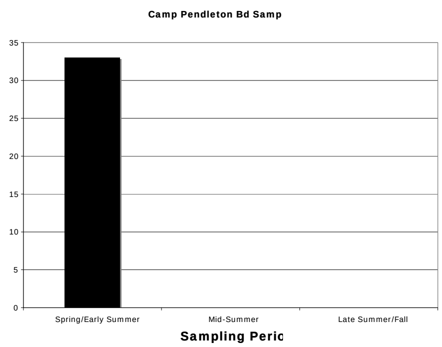
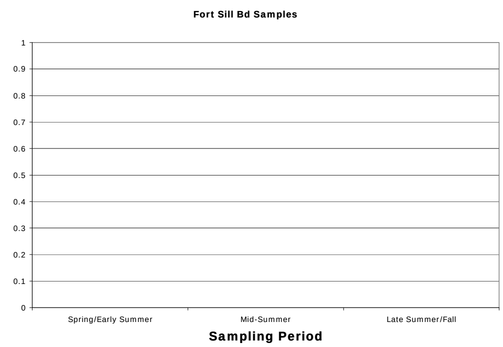
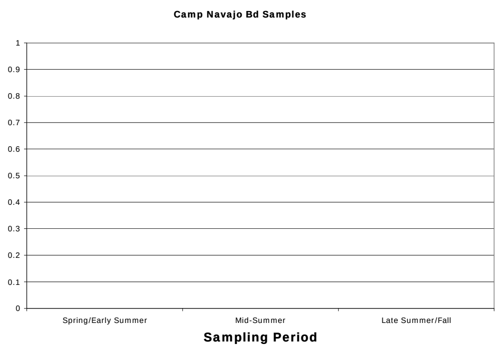


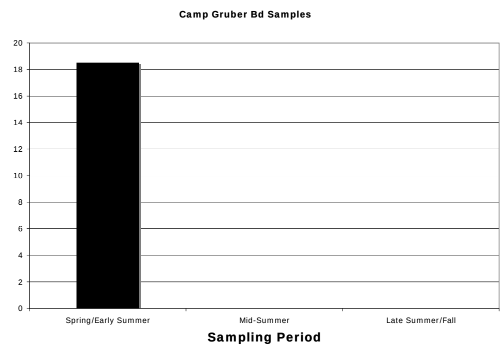
Figure 3. Temporal pattern (seasonality) of Bd infection rates across all installations.

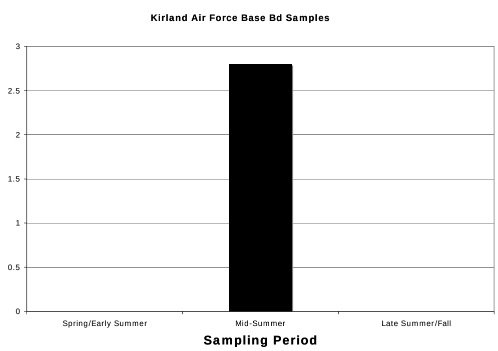


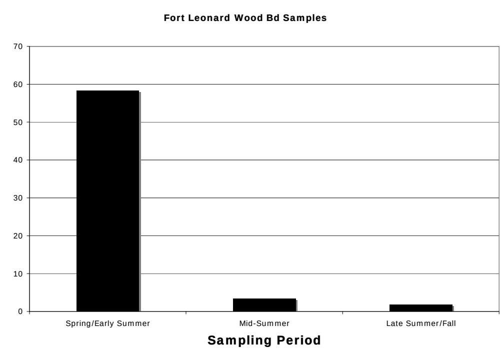

Camp Pendleton Bd Samp
0 5 10 15 20 25 30 35
Spring/Early Summer
Mid-Summer
Late Summer/Fall
Sampling Peric

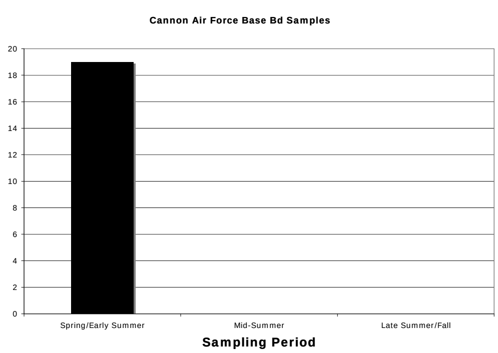

Fort Sill Bd Samples
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
Spring/Early Summer
Mid-Summer
Late Summer/Fall
Sampling Period

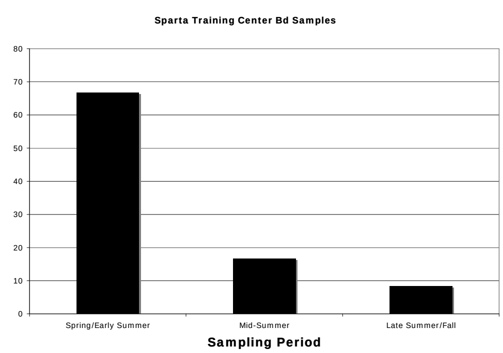

Camp Navajo Bd Samples
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
Spring/Early Summer
Mid-Summer
Late Summer/Fall
Sampling Period

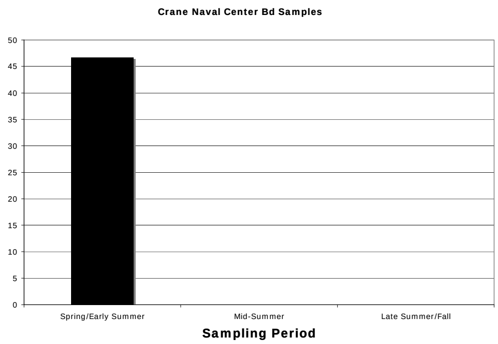

Camp Gruber Bd Samples
0 2 4 6 8 10 12 14 16 18 20
Spring/Early Summer
Mid-Summer
Late Summer/Fall
Sampling Period

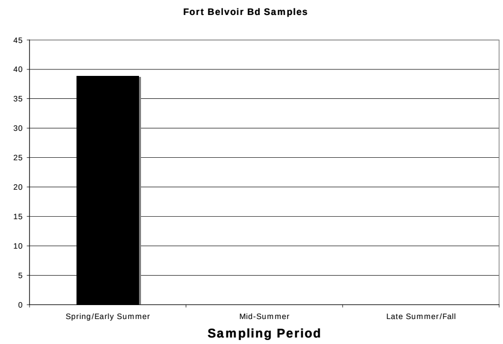

Kirland Air Force Base Bd Samples
0 0.5 1 1.5 2 2.5 3
Spring/Early Summer
Mid-Summer
Late Summer/Fall
Sampling Period

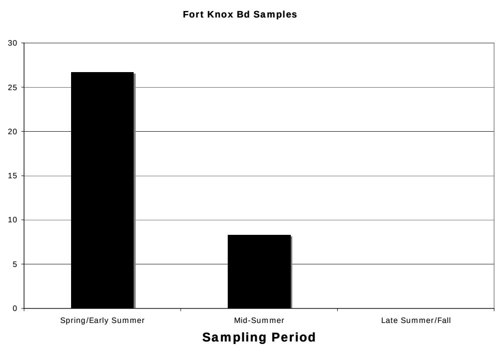

Fort Leonard Wood Bd Samples
0 10 20 30 40 50 60 70
Spring/Early Summer
Mid-Summer
Late Summer/Fall
Sampling Period

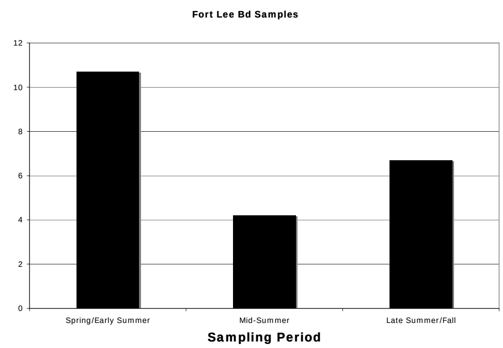

Cannon Air Force Base Bd Samples
0 2 4 6 8 10 12 14 16 18 20
Spring/Early Summer
Mid-Summer
Late Summer/Fall
Sampling Period

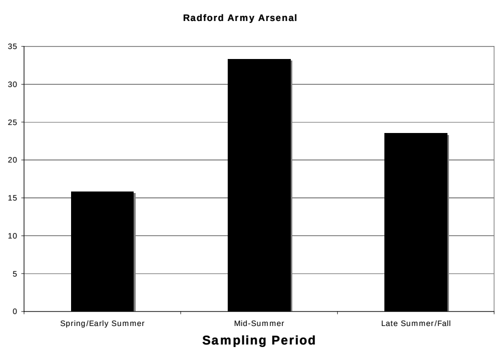

Sparta Training Center Bd Samples
0 10 20 30 40 50 60 70 80
Spring/Early Summer
Mid-Summer
Late Summer/Fall
Sampling Period

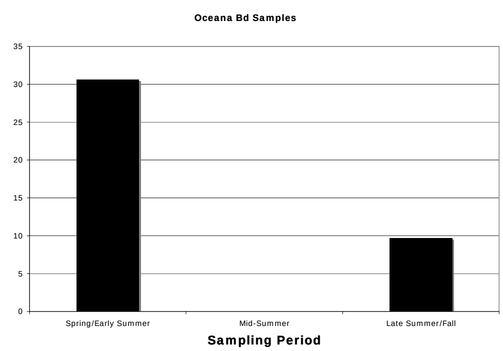

Crane Naval Center Bd Samples
0
5
10
15
20
25
30
35
40
45
50
Spring/Early Summer
Mid-Summer
Late Summer/Fall
Sampling Period

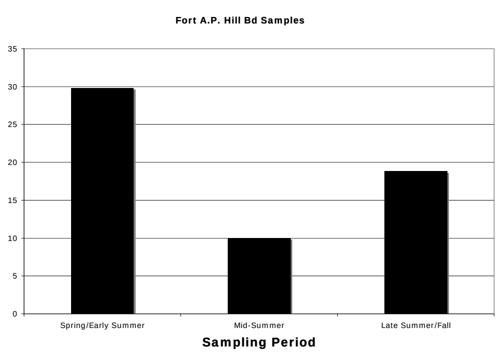

Fort Belvoir Bd Samples
0
5
10
15
20
25
30
35
40
45
Spring/Early Summer
Mid-Summer
Late Summer/Fall
Sampling Period


Fort Knox Bd Samples
0
5
10
15
20
25
30
Spring/Early Summer
Mid-Summer
Late Summer/Fall
Sampling Period


Fort Lee Bd Samples
0
2
4
6
8
10
12
Spring/Early Summer
Mid-Summer
Late Summer/Fall
Sampling Period


Radford Army Arsenal
0
5
10
15
20
25
30
35
Spring/Early Summer
Mid-Summer
Late Summer/Fall
Sampling Period


Oceana Bd Samples
0
5
10
15
20
25
30
35
Spring/Early Summer
Mid-Summer
Late Summer/Fall
Sampling Period


Fort A.P. Hill Bd Samples
0
5
10
15
20
25
30
35
Spring/Early Summer
Mid-Summer
Late Summer/Fall
Sampling Period