Conservation Status of United States Species, pp. 47–54. University of California Press, Berkeley.

———, ———, AND L. BLACKBURN. 2005. *Acris crepitans*. *In* M. J. Lannoo (ed.), Amphibian Declines: The Conservation Status of United States Species, pp. 441–443. University of California Press, Berkeley.

HAMMERSON, G. A., AND L. J. LIVO. 1999. Conservation status of the northern cricket frog (*Acris crepitans*) in Colorado and adjacent areas at the northwestern extent of the range. Herpetol. Rev. 30:78–80.

HAY, R. 1998. Blanchard's cricket frogs in Wisconsin: a status report. *In* M. J. Lannoo (ed.), Status and Conservation of Midwestern Amphibians, pp. 79–82. University of Iowa Press, Iowa City.

IRWIN, J. T. 2005. Overwintering in northern cricket frogs (*Acris crepitans*). *In* M. J. Lannoo (ed.), Amphibian Declines: The Conservation Status of United States Species, pp. 55–58. University of California Press, Berkeley.

JUNG, R. E. 1993. Blanchard's cricket frogs (*Acris crepitans blanchardi*) in southwest Wisconsin. Trans. Wisconsin Acad. Sci., Arts Lett. 81:79–87.

LANNOO, M. J. 1998. Amphibian conservation and wetland management in the upper Midwest: A catch-22 for the cricket frog? *In* M. J. Lannoo (ed.), Status and Conservation of Midwestern Amphibians, pp. 330–339. University of Iowa Press, Iowa City.

———, AND R. G. GRUNDEL. 2004. United States Northern Cricket Frog Symposium. Froglog 66:1.

———, K. LANG, T. WALTZ, AND G. S. PHILLIPS. 1994. An altered amphibian assemblage: Dickinson County, Iowa, 70 years after Frank Blanchard's survey. Am. Midl. Nat. 131:311–319.

LEHTINEN, R. M. 2002. A historical study of the distribution of Blanchard's cricket frog in southeastern Michigan. Herpetol. Rev. 33:194–197.

———, AND A. A. SKINNER. 2006. The enigmatic decline of Blanchard's cricket frog (*Acris crepitans blanchardi*): A test of the habitat acidification hypothesis. Copeia 2006:159–167.

LIPS, K. R., F. BREM, R. BRENES, J. D. REEVE, R. A. ALFORD, J. VOYLES, C. CAREY, L. LIVO, A. P. PESSIER, AND J. P. COLLINS. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. PNAS 103:3165–3170.

LONGCORE, J. R., J. E. LONGCORE, A. P. PESSIER, AND W. A. HALTEMAN. 2007. Chytridiomycosis widespread in anurans of northeastern United States. J. Wildl. Manage. 71:435–444.

LONGCORE, J. E., A. P. PESSIER, AND D. K. NICHOLS. 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 91:219–227.

MANIERO, G. D., AND C. CAREY. 1997. Changes in selected aspects of immune function in the leopard frog, *Rana pipiens*, associated with exposure to cold. J. Comp. Phys. B. 167:256–263.

MIERZWA, K. S. 1998. Status of northeastern Illinois amphibians. *In* M. J. Lannoo (ed.), Status and Conservation of Midwestern Amphibians, pp. 115–124. University of Iowa Press, Iowa City.

MOSSMAN, M. J., L. M. HARTMAN, R. HAY, J. R. SAUER, AND B. J. BHUEY. 1998. Monitoring long-term trends in Wisconsin frog and toad populations. *In* M. J. Lannoo (ed.), Status and Conservation of Midwestern Amphibians, pp. 169–198. University of Iowa Press, Iowa City.

OUELLET, M., I. MIKAELIAN, B. D. PAULI, J. RODRIGUE, AND D. M. GREEN. 2005. Historical evidence of widespread chytrid infection in North American amphibian populations. Conserv. Biol. 19:1431–1440.

PEARL, C. A., E. L. BULL, D. E. GREEN, J. BOWERMAN, M. J. ADAMS, A. HYATT, AND W. H. WENTE. 2007. Occurrence of the amphibian pathogen *Batrachochytrium dendrobatidis* in the Pacific Northwest. J. Herpetol. 41:145–149.

REEDER, A. L., M. O. RUIZ, A. PESSIER, L. E. BROWN, J. M. LEVENGOOD, C. A. PHILLIPS, M. B. WHEELER, R. E. WARNER, AND V. R. BEASLEY. 2005. Intersexuality and the cricket frog decline: Historic and geographic trends. Environ. Health Perspect. 113:261–265.

RETALLICK, R. W. R., H. MCCALLUM, AND R. SPEARE. 2004. Endemic infection of the amphibian chytrid fungus in a frog community post-decline. PLoS Biol. 2:e351.

RUSSELL, R. W., G. J. LIPPS, S. J. HECNAR, AND G. D. HAFFNER. 2002. Persistent organic pollutants in Blanchard's cricket frogs from Ohio. Ohio J. Sci. 102:119–122.

WOODHAMS, D., R. A. ALFORD, AND G. MARANTELLI. 2003. Emerging disease of amphibians cured by elevated body temperature. Diseases Aquat. Org. 55:65–67.

———, AND ———. 2005. Ecology of chytridiomycosis in rainforest stream frog assemblages of tropical Queensland. Conserv. Biol. 19:1449–1459.

———, L. ROLLINS-SMITH, C. CAREY, L. REINART, M. TYLER, AND R. ALFORD. 2006. Population trends associated with skin peptide defenses against chytridiomycosis in Australian frogs. Oecologia 146:531–540.

ZIPPEL, K. C., AND C. TABAKA. 2008. Amphibian chytridiomycosis in captive Acris crepitans blanchardi (Blanchard's cricket frog) collected from Ohio, Missouri, and Michigan, USA. Herpetol. Rev. 39:192–193.

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Low Prevalence of *Batrachochytrium dendrobatidis* Across *Rana sylvatica* Populations in Southeastern Michigan, USA

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The emerging infectious disease chytridiomycosis has been implicated in the decline and extinction of numerous amphibian species worldwide (Berger et al. 1998; Lips et al. 2006; Skerratt et al. 2007). The fungus causing this disease, *Batrachochytrium dendrobatidis* (*Bd*), has been present in North American amphibian populations since at least the 1960s (Ouellet et al. 2005); however, in many areas of North America, there is little evidence of negative effects of the disease on amphibian population persistence. Understanding how environmental factors affect infection prevalence is thus important for determining under what conditions chytridiomycosis is likely to have a devastating impact on populations.

We conducted a preliminary assessment of the role of season and habitat quality on chytridiomycosis infection prevalence in populations of the Wood Frog, *Rana sylvatica*, in southeastern Michigan, USA. In laboratory studies, *Bd* appears to be limited by temperatures outside the range of 4–25°C (Piotrowski et al. 2004). Several studies also have noted that the prevalence and severity of infections in wild populations tend to vary seasonally (Berger et al. 2004; Kriger and Hero 2006, 2007; Retallick et al. 2004; Woodhams and Alford 2005). Given this, we predicted that levels of infection would be higher in the spring as opposed to the summer because the warmer temperatures experienced during the summer months in southeastern Michigan should limit *Bd* infection rates (Berger et al. 2004; Kriger and Hero 2006, 2007; Ouellet et al. 2005; Retallick et al. 2004; Woodhams and Alford 2005; Woodhams et al. 2003). Additionally, habitat quality may affect

infection rates because the higher stress levels associated with low quality habitats may make individuals more susceptible to infection (Carey and Bryant 1995). Ponds that are exposed to high levels of agricultural and urban runoff may be particularly stressful for amphibians. For example, both pesticides (Relyea 2005) and road de-icing salt (Sanzo and Hecnar 2006) affect larval Wood Frog survivorship. Thus, we predicted that Wood Frog (*Rana sylvatica*) populations in ponds surrounded by agricultural or urban areas would show higher levels of infection than populations surrounded by intact, forested habitat.

Methods.—To assess whether season affects infection prevalence, breeding adults and metamorphs were tested for the presence of *Bd* DNA, since breeding adults often experience colder temperatures than metamorphs. Adults were sampled from eight populations in March 2007 and metamorphs were sampled from five populations in June 2007. All adults and metamorphs were sampled from populations on the University of Michigan's Edwin S. George Reserve (Fig. 1). Temperatures during the 30 days prior to sampling ranged from -16–23°C (mean temperature = 2°C) for the adults and 5–33°C (mean temperature = 20°C) for the metamorphs. The dorsum, venter, and feet of adults and metamorphs were swabbed with a sterile cotton swab. Swabs were stored in 95% ethanol until extraction.

In addition, to assess the relationship between habitat quality and *Bd* distribution, we collected larvae of *R. sylvatica* from 16 populations across southeastern Michigan (Fig. 1) during June 2005 and 2006. Aerial images (Michigan Department of Natural Resources 1998) were used to select ponds with varying degrees of surrounding forest and wetland fragmentation. Larvae were stored in 95% ethanol until extraction. The oral discs of six individuals from each population were excised in the lab using sterilized razor blades and forceps.

Extraction of *Bd* DNA was completed following the methodology of Hyatt et al. (2007). DNA from larval samples was extracted from the oral discs, whereas DNA from the metamorphs and adults was extracted from the swabs. DNA extracted from larvae was pooled in groups of three for each population. The pooled-larval samples and both the adult and metamorph samples were then diluted 1:10 with double deionized water. Taqman diagnostic quantitative PCR (Boyle et al. 2004) was used to detect the presence of *Bd* DNA. Quantitative Taqman PCR assays were performed in triplicate using an Applied Biosystems Prism 7700 Sequence Detection System following the protocol of Boyle et al. (2004). VIC$_{TM}$ Exogenous Internal Positive Control reagents were used for the detection of PCR inhibitors (Applied Biosystems following Hyatt et al. 2007). Inhibitors did not appear to be present in any of the samples. A sample was only considered positive for *Bd* if all three replicates indicated the presence of the fungus. Samples testing positive in one or two replicates were re-assayed once. If the second assay produced a consistent negative or positive result for all three replicates the sample was considered negative or positive, respectively. Samples testing positive in one or two replicates of the second assay were considered "suspicious." Prevalence rates were calculated by dividing the number of infected individuals by the total number of sampled individuals, and 95% confidence intervals were calculated based on a binomial distribution (Stata Intercooled v. 10.0).

The percentage of combined agricultural and urban land cover

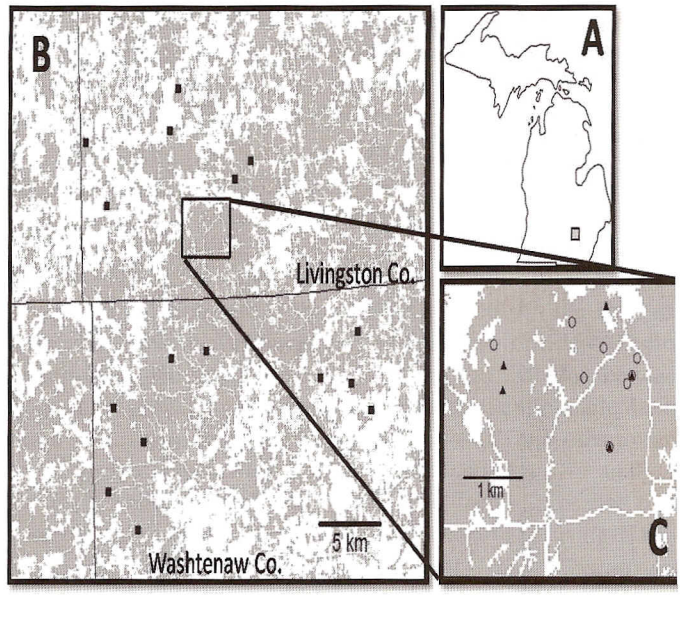

FIG. 1. Wood Frog (*Rana sylvatica*) sampling locations in southeastern Michigan, USA, showing areas sampled for *Batrachochytrium dendrobatidis* in adults (open circles), metamorphs (triangles), and larvae (squares). Agricultural and urban areas are white. Forests, wetland rivers, and lakes are gray.

within 1 km (estimated genetic neighborhood size of *R. sylvatica* Berven and Grudzien 1990) of each of the 16 ponds sampled for larvae was calculated in ArcGIS v. 9.2 using the 2001 National Land-Cover Database (Homer et al. 2004). This percentage ranged from 6.18 to 78.55 (Table 1).

Results.—Two of 239 (prevalence = 0.83%; 95% confidence interval = 0.1–3.0%) samples tested positive for the presence of *Bd*. One of 70 (1.4%) adults, zero of 73 metamorphs, and 1–3 of 96 (1.0–3.1%) larvae tested positive in each of three replicates. The range surrounding the number of infected larvae arises from pooling the larvae into groups of three for the analyses. As a result, a positive sample indicates that at least one of the three individuals was positive for *Bd*. In addition, one of 70 (1.4%) adults tested positive in two out of three replicates and thus was classified as suspicious.

Discussion.—We found a very low level of *Bd* infection in populations of *R. sylvatica* in southeastern Michigan (0.83%). Other studies of North American *R. sylvatica* populations have found much higher rates of infection (15.5%, Longcore et al. 2007; 6.6% Ouellet et al. 2005). We calculated 95% confidence intervals for each of these studies to assess the extent to which our results differed from these previous studies and found that our confidence intervals did not overlap (Longcore et al. 2007: 6.4–29.4%; Ouellet et al. 2005: 3.4–11.1%). Our results are consistent with the idea that the quality of the habitat and the season may be important predictors of infection rates. For the effects of season, we found one adult that tested positive for *Bd*, while no metamorphs tested positive. Temperatures during the adult breeding period remained at or below the optimal temperature range for *Bd*, whereas during the metamorph sampling period, temperatures exceeded the maximum temperature at which *Bd* can survive in the laboratory (Piotrowski et al. 2004). Similarly, we detected *Bd* in larvae in a pond exposed to one of the largest areas of anthropogenic distur-

Table 1. Wood Frog (*Rana sylvatica*) larval infection rates in relation amount of agricultural and urban habitat surrounding ponds in southastern Michigan, USA.

Percent Agricultural/ Urban Land Cover	Number Infected/ Sample Size
6.18	0/6
8.73	0/6
14.61	0/6
15.35	0/6
17.06	0/6
17.09	0/6
18.09	0/6
25.42	0/6
35.28	0/6
54.07	0/6
55.49	0/6
57.57	0/6
59.24	0/6
62.82	0/6
65.39	(1–3)/6
78.55	0/6

ance in this study (Table 1). However, the low level of infection e found prevents us from testing our hypotheses statistically. uture research on the effects of habitat quality and seasonality on *d* infection prevalence remains a priority.

Two hypotheses may explain the low infection levels detected this study, given the high prevalence of *Bd* both worldwide and other areas of the Wood Frog's range. First, the more terrestrial fe-history of *R. sylvatica* may help prevent infection in this spees (Longcore et al. 2007). *Rana sylvatica* is an explosive breeder at breeds in early spring. Larvae develop and metamorphose in pproximately 6 weeks, and juveniles then move into the terresal habitat for foraging (Regosin et al. 2003). In comparison to her co-occurring species, wood frogs are in the ponds for a shorter nount of time. These results are consistent with Lips et al. (2003) ypothesis that the probability of decline as a result of *Bd* infecon is positively related to the amount of time the species spends aquatic habitats. However, while rates of infection in *R. sylvatica* e typically lower than in other co-occurring species (Longcore al. 2007; Ouellet et al. 2005), the levels of infection in *R. sylvatica* en in this study are much lower than in other studies, suggesting at other factors may have contributed to the low prevalence of *d* across southeastern Michigan.

Second, it is possible that habitat differences between southstern Michigan and other parts of *R. sylvatica*'s range could acount for the low infection prevalence seen in our study, as comared with other studies. Differences in climate, for instance, in mperature or the amount of rainfall, are associated with differces in infection rates (Kriger and Hero 2007), and thus may ctate the range over which *Bd* is viable. However, this seems likely, because projections from an ecological niche model (Ron 005) suggest that the habitat of southeastern Michigan is more matically suitable for *Bd* than other areas where *Bd* prevalence in wood frogs has been found to be higher (Longcore et al. 2007). Similarly, differences in the structure of the landscape separating populations may contribute to the variation in infection prevalence across *R. sylvatica*'s range. A fragmented landscape, resulting in reduced connectivity among amphibian populations, may hinder the spread of *Bd* and thus keep regional infection rates low. Further research at a broader geographic scale will be necessary for evaluating whether such habitat differences contribute to the observed patterns of infection in *R. sylvatica*.

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Literature Cited

Berger, L., R. Speare, P. Daszak, D. E. Green, A. A. Cunningham, C. L. Goggin, R. Slocombe, M. A. Ragan, A. D. Hyatt, K. R. McDonald, H. B. Hines, K. R. Lips, G. Marantelli, and H. Parkes. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proc. Natl. Acad. Sci. 95:9031–9036.

———, ———, H. B. Hines, G. Marantelli, A. D. Hyatt, K. R. McDonald, L. F. Skerratt, V. Olsen, J. M. Clarke, G. Gillespie, M. Mahony, N. Sheppard, C. Williams, and M. J. Tyler. 2004. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. Aust. Vet. J. 82:434–439.

Berven, K. A., and T. A. Grudzien. 1990. Dispersal in the wood frog (*Rana sylvatica*)—implications for genetic population structure. Evolution 44:2047–2056.

Boyle, D. G., D. B. Boyle, V. Olsen, J. A. T. Morgan, and A. D. Hyatt. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Dis. Aquat. Org. 60:141–148.

Carey, C., and C. J. Bryant. 1995. Possible interrelations among environmental toxicants, amphibian development, and decline of amphibian populations. Environ. Health Persp. 103:13–17.

Homer, C., C. Q. Huang, L. M. Yang, B. Wylie, and M. Coan. 2004. Development of a 2001 National Land-Cover Database for the United States. Photogramm. Eng. Rem. Sens. 70:829–840.

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

Kriger, K. M., and J. M. Hero. 2006. Survivorship in wild frogs infected with chytridiomycosis. EcoHealth 3:171–177.

———, and ———. 2007. Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. J. Zool. 271:352–359.

Lips, K. R., F. Brem, R. Brenes, J. D. Reeve, R. A. Alford, J. Voyles, C. Carey, L. Livo, A. P. Pessier, and J. P. Collins. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proc. Natl. Acad. Sci. 103:3165–3170.

———, J. D. Reeve, and L. R. Witters. 2003. Ecological traits predicting amphibian population declines in Central America. Conserv. Biol. 17:1078–1088.

Longcore, J. R., J. E. Longcore, A. P. Pessier, and W. A. Halteman. 2007. Chytridiomycosis widespread in anurans of northeastern United States. J. Wildlife Manage. 71:435–444.

OUELLET, M., I. MIKAELIAN, B. D. PAULI, J. RODRIGUE, AND D. M. GREEN. 2005. Historical evidence of widespread chytrid infection in North American amphibian populations. Conserv. Biol. 19:1431–1440.

PIOTROWSKI, J. S., S. L. ANNIS, AND J. E. LONGCORE. 2004. Physiology of *Bd*, a chytrid pathogen of amphibians. Mycologia 96:9–15.

REGOSIN, J. V., B. S. WINDMILLER, AND J. M. REED. 2003. Terrestrial habitat use and winter densities of the wood frog (Rana sylvatica). J. Herpetol. 37:390–394.

RELYEA, R. A. 2005. The lethal impact of roundup on aquatic and terrestrial amphibians. Ecol. Appl. 15:1118–1124.

RETALLICK, R. W. R., H. MCCALLUM, AND R. SPEARE. 2004. Endemic infection of the amphibian chytrid fungus in a frog community post-decline. Plos Biol. 2:1965–1971.

RON, S. R. 2005. Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the New World. Biotropica 37:209–221.

SANZO, D., AND S. J. HECNAR. 2006. Effects of road de-icing salt (NaCl) on larval wood frogs (*Rana sylvatica*). Environ. Pollut. 140:247–256.

SKERRATT, L. F., L. BERGER, R. SPEARE, S. CASHINS, K. R. MCDONALD, A. D. PHILLOTT, H. B. HINES, AND N. KENYON. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4:125–134.

WOODHAMS, D. C., AND R. A. ALFORD. 2005. Ecology of chytridiomycosis in rainforest stream frog assemblages of tropical Queensland. Conserv. Biol. 19:1449–1459.

———, ———, AND G. MARANTELLI. 2003. Emerging disease of amphibians cured by elevated body temperature. Dis. Aquat. Org. 55:65–67.

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Occurrence of *Batrachochytrium dendrobatidis* in Amphibian Populations in Denmark

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Amphibian decline is a global phenomenon with multiple causes (Stuart et al. 2004). Some declines have been attributed to the disease chytridiomycosis that affects the skin of amphibians (Skerratt et al. 2007). The agent responsible for chytridiomycosis is the fungus *Batrachochytrium dendrobatidis* (Berger et al. 1998). There is evidence that the spread of *B. dendrobatidis* around the world occurred in the last half century (Ouellet et al. 2005), and there is a need for detailed information on its current spatial extent. In Europe, *B. dendrobatidis* has been reported in several amphibian species in multiple countries, such as Spain, Portugal, Italy, Switzerland, France, Germany and the UK (Cunningham et al. 2005; Garner et al. 2005, 2006; Mutschmann et al. 2000; Simoncelli et al. 2005; Stagni et al. 2004). No comprehensive surveys have occurred in Denmark but a single record of *B. dendrobatidis* for *Rana* kl. *esculenta* on the island of Bornholm is reported (www.spatialepidemiology.net) and confirmed by Trent Garner (pers. comm. to R. Scalera, 2007). Here, we report the results surveys carried out at four sites in Denmark (Fig. 1) on two nat amphibians: *Rana temporaria* and *Rana* kl. *esculenta*.

In summer 2007, we hand captured individual amphibians a sampled them for *B. dendrobatidis* by rubbing a cotton-tipped sw over the body of each individual. Frogs were held separately pr to swabbing and technicians wore a new pair of gloves for ea individual handled. The sampling is harmless and was carried c *in-situ* so as to release the sampled animals within just a few mi utes at the location where they were captured. As the frog w restrained, the swab was firmly rubbed back and forth 25–30 tim targeting the drink patch, the mouth, and the webbing betwe each toe. The swab was immediately inserted, cotton side dow into a 2 ml screw-cap tube containing 1 ml of 70% ethanol a stored upright. Vials were shipped to the laboratory for analysi and each swab was analyzed individually for the presence of *dendrobatidis*. Swabs were qualitatively analyzed using a PC assay (45 amplification cycles). Presence of *B. dendrobatidis* w determined by presence of PCR product visualized on agarose ge (30–90 minute electrophoresis) containing positive controls. Fra ments were sized using a molecular weight marker (Pisces M lecular LLC, Boulder, Colorado, USA (Annis et al. 2004; J. Woo pers. comm.). All field gear was cleaned with a brush and wat and then sterilized using a dilute bleach solution between eac sampling location.

Two of the 13 amphibians we swabbed were positive for *E dendrobatidis* (Table 1). We found *B. dendrobatidis* on individu als from both species and at 2 of the 4 study areas we examine One of the positive results was for an adult of *Rana* kl. *esculent* captured in Vestamager. The other positive result was for a juve nile of *Rana temporaria* captured in Egense. We did not find an frogs that were dead or that appeared to be sick.

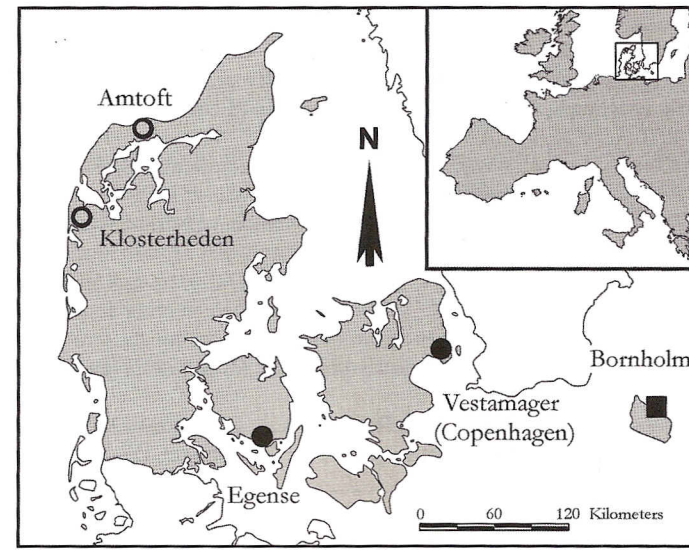

FIG. 1. Locations of study areas in Denmark where amphibians were sampled for the presence of *Batrachochytrium dendrobatidis* in 2007. Circles are filled at locations where we found *B. dendrobatidis*. The square symbol indicates the location of the positive record reported by Trent Garner (see text). Vestamager is located on the island of Zealand, close to Copenhagen, Egense is on Fyn Island, and both Amtoft and Klosterheden are on the Jutland Peninsula.