tion; name of collaborative laboratory or researcher conducting histological sections or PCR analyses; and names of cooperative land owners or land management agencies. We aim to expedite the review and publication process! Please e-mail submissions directly to Associate Editor, Dr. Dede Olson: dedeolson@fs.fed.us.

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The Northern Leopard Frog *Rana pipiens* is a Widespread Reservoir Species Harboring *Batrachochytrium dendrobatidis* in North America

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The Northern Leopard Frog, *Rana pipiens*, is wide-ranging across North America and commonly harvested for research and teaching (Gibbs et al. 1971; Moriarty 1998). *Rana pipiens* were harvested on the order of 100,000 specimens per year in the early 1980s in Quebec (Gilbert et al. 1994) and 100,000 pounds per year in the early 1970s in Minnesota (Moriarty 1998). Carey et al. (1999) suggested that population declines observed in this species in the late 1960s or early 1970s in some areas (Gibbs et al. 1971; Rorabaugh 2005) may have been caused by the emergence of chytridiomycosis, a disease caused by infection with the fungus *Batrachochytrium dendrobatidis* (*Bd*). A histological survey of *Bd* infections in *R. pipiens* collected in Quebec province of Canada between 1960 and 2001 revealed a prevalence of 10–20% (Ouellet et al. 2004). Because of their widespread commercial use and transport, we sampled several populations of this species to test for the potential of adult *R. pipiens* to act as a reservoir for *Bd* that could potentially facilitate the spread of disease.

Rana pipiens from Minnesota and Vermont were obtained from commercial suppliers (Table 1) and sampled by swabbing 10 times on each of ventral surface, thighs, and feet upon receipt at Vanderbilt University or after several months in the laboratory. Frogs arrived in boxes with damp sphagnum moss and were in contact with one another. Frogs were then housed in groups of 4–5 in polypropylene (opaque) plastic tanks with Plexiglas® covers (45.7 × 25.4 × 20.3 cm), sterilized with bleach before use, and tilted to one side to allow access to both water and the dry bottom of the container. In addition, *R. pipiens* were sampled in the field at a site near Plainwell, Michigan (42°19'26.7"N, 85°47'01.2"W). At the field site, individuals of six other species were sampled concurrently. Diagnosis of infection status, including the number of zoospore equivalents per swab, was performed at the Australian Animal Health Laboratory, CSIRO, Geelong, Victoria, Australia. All samples were analyzed in triplicate by Taqman real-time PCR assay according to Boyle et al. (2004). Sampling was different to that described by Hyatt et al. (2007) for groups containing multiple tadpoles or juveniles. Animals were rinsed in 50 ml HPLC-grade water for 15 min and the solution absorbed onto swabs as described by Boyle et al. (2004), perhaps underestimating infection loads.

We found that *R. pipiens* obtained from Minnesota and Vermont by two commercial suppliers were positive for *Bd*, indicating infection, but clinical signs of disease were absent. It is unknown whether the frogs became infected in the wild or after collection by suppliers. In general, infection intensity was low, typically ranging from 0 to 651 zoospore equivalents. A swab from one frog contained 90,599 zoospore equivalents. Although some animals died in captivity, there were no significant die-offs observed after housing frogs in the laboratory for several years, indicating that some *R. pipiens* may have resistance to chytridiomycosis. All 55 individuals and seven species sampled in the field in Michigan in 2004 were negative for *Bd*, indicating infection prevalence at the site between 0 and 6.49% with 95% confidence. The data imply that the Michigan population was probably not infected in the field. After exposure to water housing *R. pipiens* from commercial suppliers, at least one and up to four of six frogs collected from Michigan developed low-level *Bd* infections (Table 1).

These results suggest that *R. pipiens* can function as a reservoir or carrier species for *Bd*. This places *R. pipiens* in a category with other wide-ranging and introduced species used for food, fish bait, research, teaching, and pets (Fisher and Garner 2007). Current regulations do not require amphibian suppliers or breeders to deliver uninfected frogs or to check infection status. However, unless frogs escape or are released into new habitats, or their waste water is not treated, they do not necessarily put susceptible amphibians at risk. Unfortunately, disease emergence caused by introduced species that are *Bd* reservoirs is increasingly detected (Fisher and Garner 2007), for example in Britain (Cunningham et al. 2005) and Uruguay (Mazzoni et al. 2003). We recommend increased monitoring and stricter biosecurity to prevent the spread of chytridiomycosis. The overall health of amphibians obtained from commercial suppliers has improved since the report by Gibbs et al. (1971), and more rigorous distribution procedures will surely benefit research and conservation.

It seems unlikely that all populations of *R. pipiens* carry *Bd*. Therefore, measures should be taken to limit human-mediated exposure to the pathogen. Populations that already carry *Bd* may still be at risk. Different strains of *Bd* may differ in pathogenicity (Berger et al. 2005; Retallick et al. 2007). Hence, exposure of susceptible amphibians to new strains of *Bd* carried by *R. pipiens* and other reservoir species may continue to threaten populations with disease.

Of interest for conservation management, population level variation in susceptibility may exist, and this awaits exposure experiments and immune defense characterization of different populations. Since carrier species are expected to survive *Bd* exposure, susceptible controls may be used in exposure experiments as in Daszak et al. (2004).

This study was not extensive and is comprised of information

TABLE 1. Amphibians sampled for diagnosis of infection with *Batrachochytrium dendrobatidis*. Frogs obtained by commercial suppliers were sampled in the laboratory at Vanderbilt University. Samples that returned a low number of zoospore equivalents in only one well* or two wells** (from a total of three) were defined as "indeterminate."

Date sampled (month/yr)	Location	Number swabbed	Species	Life-history stage	*Bd* diagnosis (no. zoospore equivalents)
3/04	BioCorporation, Alexandria, MN; frogs harvested in Minnesota, swabbed upon receipt	1	*Rana pipiens*	adult	0
		1	*Rana pipiens*	adult	0.2*
		1	*Rana pipiens*	adult	0.3*
		1	*Rana pipiens*	adult	1**
		1	*Rana pipiens*	adult	2**
		1	*Rana pipiens*	adult	6**
		2	*Rana pipiens*	adult	1
		1	*Rana pipiens*	adult	3
		3	*Rana pipiens*	adult	4
		1	*Rana pipiens*	adult	5
		1	*Rana pipiens*	adult	7
		1	*Rana pipiens*	adult	9
		2	*Rana pipiens*	adult	10
		1	*Rana pipiens*	adult	11
		1	*Rana pipiens*	adult	12
		2	*Rana pipiens*	adult	14
		1	*Rana pipiens*	adult	27
9/04	BioCorporation, Alexandria, MN; frogs harvested in Minnesota and kept in laboratory	3	*Rana pipiens*	adult	0
		1	*Rana pipiens*	adult	28
		1	*Rana pipiens*	adult	59
		1	*Rana pipiens*	adult	268
		1	*Rana pipiens*	adult	491
		1	*Rana pipiens*	adult	651
		1	*Rana pipiens*	adult	90599
12/04	BioCorporation, Alexandria, MN; frogs harvested in Minnesota and kept in laboratory	1	*Rana pipiens*	adult	0
		1	*Rana pipiens*	adult	0.46*
		1	*Rana pipiens*	adult	1*
		1	*Rana pipiens*	adult	13
		1	*Rana pipiens*	adult	18
		1	*Rana pipiens*	adult	288
12/04	Connecticut Valley Biological Supply Co., Southhampton, MA; frogs harvested in Vermont, swabbed upon receipt	2	*Rana pipiens*	adult	0
		2	*Rana pipiens*	adult	1
		1	*Rana pipiens*	adult	0.08*
		1	*Rana pipiens*	adult	3
		1	*Rana pipiens*	adult	248
7/04	Plainwell, Michigan	1	*Bufo americanus*	adult	0
		1 group of 5	*Bufo americanus*	juvenile	0
		2 groups of 5	*Hyla sp.*	tadpole	0
		1 group of 5	*Pseudacris crucifer*	juvenile	0
		1 group of 2	*Pseudacris triseriata*	juvenile	0
		1 group of 5	*Rana sylvatica*	juvenile	0
		1	*Rana sp.*	tadpole	0
		1 group of 2	*Rana clamitans*	juvenile	0
		2	*Rana clamitans*	juvenile	0
		3	*Rana clamitans*	adult male	0
		7	*Rana pipiens*	juvenile	0
9/04	Plainwell, Michigan	6	*Rana pipiens*	subadult	0
12/04	Laboratory (collected from Plainwell, Michigan, 9/04)	2	*Rana pipiens*	subadult	0
		1	*Rana pipiens*	subadult	0.13*
		1	*Rana pipiens*	subadult	0.73*
		1	*Rana pipiens*	subadult	1**
		1	*Rana pipiens*	subadult	17

accumulated while undertaking other studies. The data, however, are important because in addition to identifying *R. pipiens* as a carrier of *Bd*, they highlight the infectivity of *Bd* and the requirement for stringent biosecurity.

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Batrachochytrium dendrobatidis in Wood Frogs (*Rana sylvatica*) from Three National Wildlife Refuges in Alaska, USA

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Batrachochytrium dendrobatidis (*Bd*) is a fungal pathogen implicated in recent amphibian declines (Pounds et al. 2006). It was first documented in Alaska, USA, in 2002, in a single dead subadult Wood Frog (*Rana sylvatica*) in the Kenai National Wildlife Refuge (Reeves and Green 2006). Since then, there have only been two other studies of *Bd* in Alaska. In one, *Bd* was found in Boreal Toads (*Bufo boreas*) and Red-legged Frogs (*Rana aurora*) in western Canada and southeast Alaska (Adams et al. 2007). In the other, *Bd* was not found in wood frogs in Denali National Park (Chestnut et al. 2008). *Bd* distribution in other parts of Alaska is unknown. In summer of 2006, we sampled adult Wood Frogs from three Alaskan National Wildlife Refuges to screen them for *Bd*.

Methods.—Wood Frogs from the Innoko, Kenai, and Tetlin refuges were sampled (Fig. 1). From these refuges, we tested 48 opportunistically-encountered adult frogs from 29 breeding ponds between 11 May and 21 July 2006 (Table 1). At Kenai, four ponds were road-accessible and six were in remote areas, 1–10 km from any road (Fig. 1). All ponds at Innoko (N = 9) and Tetlin (N = 10) were in remote areas, 35–125 km from any road. All animals were alive when sampled and appeared healthy. Frogs were swabbed

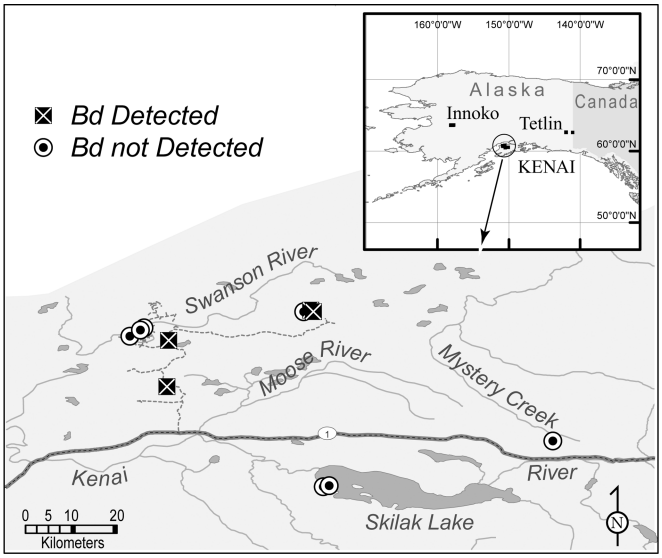

Fig. 1. Wood Frog (*Rana sylvatica*) *Bd* sampling locations in Alaska, USA, and Kenai Refuge sampling ponds and *Bd* detection locations. Dashed lines are gravel roads.