

Amphibian chytrid fungus and ranaviruses in the Northwest Territories, Canada

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ABSTRACT: Pathogens can cause serious declines in host species, and knowing where pathogens associated with host declines occur facilitates understanding host-pathogen ecology. Suspected drivers of global amphibian declines include infectious diseases, with 2 pathogens in particular, *Batrachochytrium dendrobatidis* (*Bd*) and ranaviruses, causing concern. We explored the host range and geographic distribution of *Bd* and ranaviruses in the Taiga Plains ecoregion of the Northwest Territories, Canada, in 2007 and 2008. Both pathogens were detected, greatly extending their known geographic distributions. Ranaviruses were widespread geographically, but found only in wood frogs. In contrast, *Bd* was found at a single site, but was detected in all 3 species of amphibians in the survey area (wood frogs, boreal chorus frogs, western toads). The presence of *Bd* in the Northwest Territories is not congruent with predicted distributions based on niche models, even though findings from other studies at northern latitudes are consistent with those same models. Unexpectedly, we also found evidence that swabs routinely used to collect samples for *Bd* screening detected fewer infections than toe clips. Our use and handling of the swabs was consistent with other studies, and the cause of the apparent lack of integrity of swabs is unknown. The ranaviruses detected in our study were confirmed to be Frog Virus 3 by sequence analysis of a diagnostic 500 bp region of the major capsid protein gene. It is unknown whether *Bd* or ranaviruses are recent arrivals to the Canadian north. However, the genetic analyses required to answer that question can inform larger debates about the origin of *Bd* in North America as well as the potential effects of climate change and industrial development on the distributions of these important amphibian pathogens.

KEY WORDS: Ranavirus · *Batrachochytrium dendrobatidis* · Amphibian declines · *Rana sylvatica* · *Pseudacris maculata* · *Bufo boreas* · Nahanni National Park Reserve · Taiga Plains

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INTRODUCTION

Pathogens are capable of causing serious population declines in hosts (Smith et al. 2009). Two pathogens, a chytrid fungus (*Batrachochytrium dendrobatidis*; *Bd*) and ranaviruses, are among the suspected drivers of global amphibian declines (Collins & Storfer 2003, Daszak et al. 2003, Stuart et al. 2004, McCallum 2007), and both were listed as notifiable pathogens by the World Organisation for Animal Health (2008). *Bd* is linked to catastrophic declines in several parts of the world, but its origin is unclear (Rachowicz et al. 2005, Skerratt et al. 2007). In many regions, patterns of disease and declines suggest that *Bd* is a novel pathogen that sweeps through naïve host species, reducing the number of amphibian species, and then later becomes established in areas with suitable environmental conditions and sufficient host numbers (Retallick et al. 2004, Lips et al. 2006, 2008, Skerratt et al. 2007). Population genetic data for *Bd* also supports this hypothesis (Morehouse et al. 2003, Morgan et al. 2007) although this is an important area of ongoing research. Alternatively, *Bd* may be a historically widely distributed organism, and recent declines associated with *Bd* may be linked to large-scale environmental changes that increase the impact of *Bd* on host populations (Ouellet et al. 2005, Pounds et al. 2006). Regardless of the ultimate source of *Bd*, this pathogen is now on every continent where amphibians reside (Berger et al. 1998, Weldon et al. 2004, Bosch et al. 2007, Longcore et al. 2007, Kusriini et al. 2008, Lips et al. 2008).

Ranaviruses (family *Iridoviridae*) infect fish, reptiles, and amphibians, and are of considerable concern in aquaculture (see Chinchar 2002 and Williams et al. 2005 for recent reviews of ranavirus biology). Lethal amphibian ranaviruses infect a wide range of species, and die-offs have been documented world-wide, including die-offs associated with ranaculture operations (Zhang et al. 2001, Miller et al. 2007). Amphibian ranaviruses isolated from widely distributed, abundant species such as barred tiger salamanders *Ambystoma mavortium* and wood frogs *Rana sylvatica* = *Lithobates sylvaticus* can cause lethal infections in other amphibian species (Jancovich et al. 2001, Schock et al. 2008). Thus, ranaviruses may be maintained in populations of abundant species that can serve as sources of infection for rare species that share the same habitat. In addition, die-offs caused by ranaviruses can severely reduce numbers of amphibians at breeding sites and therefore may affect amphibian populations in highly fragmented habitats where recolonization is unlikely (Collins et al. 2003).

Knowledge of where pathogens associated with host declines occur, and under what circumstances, is essential to understanding their ecology. However,

host–pathogen systems are often complex and difficult to tease apart. The relatively low levels of biodiversity at higher latitudes create opportunities to use northern amphibian populations as a model to study the ecology of pathogens implicated in declines. We conducted surveys for *Bd* and ranaviruses in the Northwest Territories (NT), Canada, to determine the geographic distributions, prevalences, and host ranges of these pathogens. We found both pathogens in the NT and discuss the importance of our results to the ecology of these pathogens.

MATERIALS AND METHODS

Study areas and amphibian species. We focused on the western portion of the NT (Fig. 1), within the taiga plains ecoregion (Ecosystem Classification Group 2007). Three amphibian species occur in our study area: wood frogs, western toads *Bufo boreas* = *Anaxyrus boreas*, and boreal chorus frogs *Pseudacris maculata*. All 3 species have large geographic ranges that include a variety of habitats in Canada and the USA (Fournier 1997, Russell & Bauer 2000, Stebbins 2003). Western toads are federally listed as a species of Special Concern in Canada and listed as Endangered in several US states (NatureServe 2009). Boreal chorus frogs are considered stable throughout their range (NatureServe 2009). Although wood frogs appear to be stable throughout most of their range, they have been extirpated from Idaho, and populations are declining in Wyoming (NatureServe 2009).

Bd has been detected in all 3 amphibian species elsewhere in their respective ranges. In wood frogs, *Bd* has been reported from Alaska (Reeves & Green 2006, Reeves 2008), British Columbia (Ouellet et al. 2005), Colorado (Rittman et al. 2003, Green & Muths 2005, Young et al. 2007), Wyoming (Young et al. 2007), Michigan (Zellmer et al. 2008), Quebec (Ouellet et al. 2005), and Maine (Longcore et al. 2007). In western toads, *Bd* has been reported in British Columbia (Raverty & Reynolds 2001, Adams et al. 2007) and in Colorado and Wyoming, where populations have declined greatly in the past 3 decades (Green et al. 2002, Green & Muths 2005, Muths et al. 2003, Young et al. 2007). Pearl et al. (2007) did not detect *Bd* in western toads during their survey in Oregon and Washington, but only 13 toads were tested and the pathogen was found in other species in the same areas during their study. Until recently, boreal chorus frogs and western chorus frogs *Pseudacris triseriata* were considered subspecies of a larger *Pseudacris* species complex (Moriarty & Lannoo 2005, Lemmon et al. 2007). Because various reports of *Bd* have used different species names, reports of *Bd* in *P. triseriata* from western

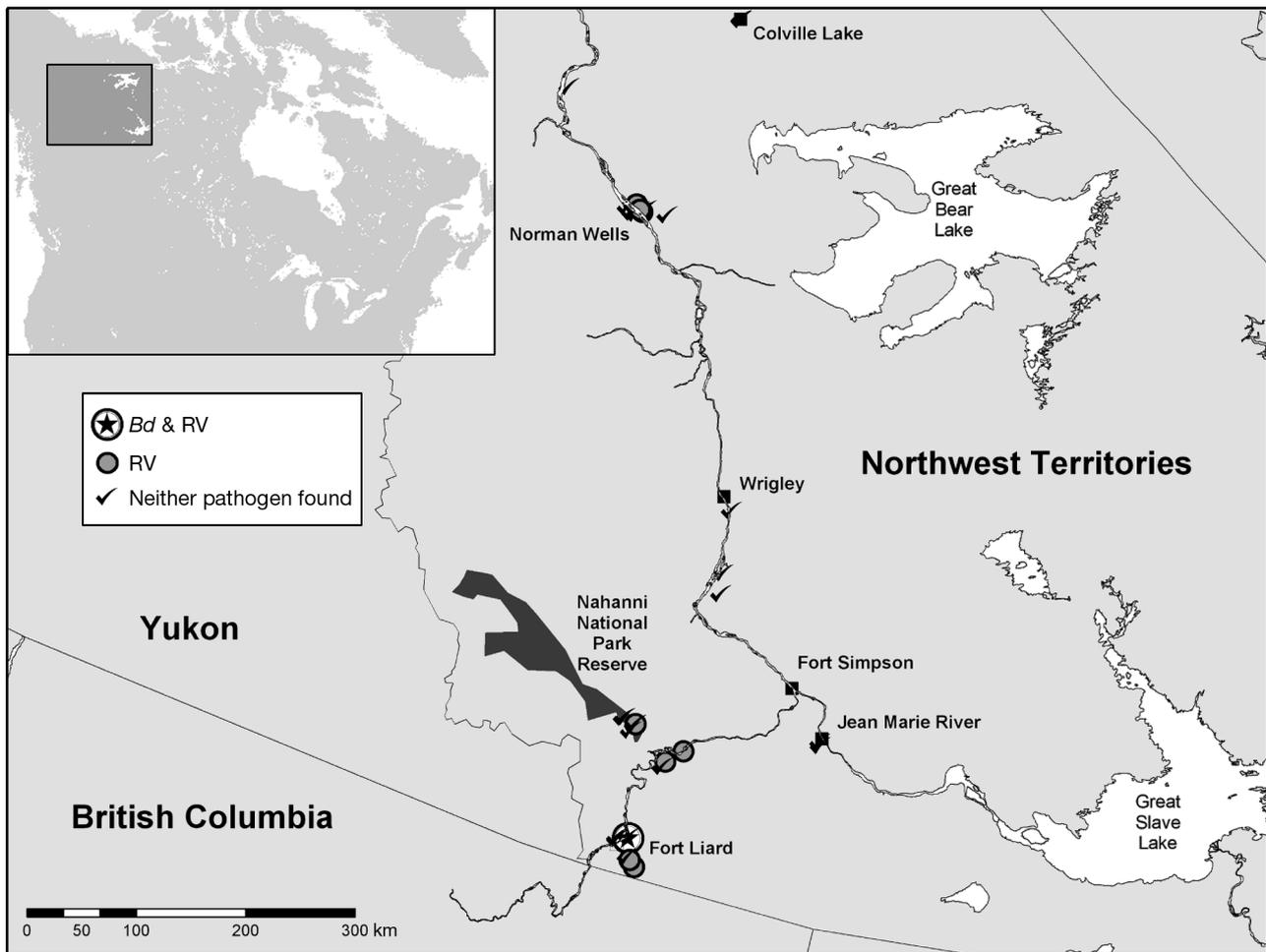


Fig. 1. Locations in the Northwest Territories, Canada, surveyed for *Batrachochytrium dendrobatidis* (*Bd*) and ranaviruses (RV) in amphibians in 2007 and 2008. Ranavirus (Frog Virus 3) was detected in wood frogs from several locations, including Norman Wells and Nahanni National Park Reserve. Chytrid fungus was detected in the Fort Liard area in western toads, boreal chorus frogs, and wood frogs. Some location indicators overlap one another due to the scale of the map

North America as well as *P. maculata* are of interest. *Bd* has been detected in *Pseudacris* sp. in Colorado (Rittman et al. 2003, Green & Muths 2005, Young et al. 2007), Arizona (Retallick & Miera 2007), and Wyoming (Young et al. 2007).

Ranaviruses have been isolated from wood frog populations, generally from die-offs, in Saskatchewan (Schock et al. 2008), Ontario (Greer et al. 2005, Duffus et al. 2008), North Carolina (Harp & Petranka 2006), and in North Dakota, Maine, and Massachusetts (Green et al. (2002). Duffus et al. (2008) reported that a ranavirus was detected in *Pseudacris* tadpoles in Ontario. However, no PCR product was sequenced in their study so the identity of the putative ranavirus is unknown. Green & Muths (2005) did not detect ranavirus infections in *Pseudacris* in their study in Colorado. Similarly, numerous *Pseudacris* tadpoles and adults from multiple sites in Saskatchewan (D. M.

Schock unpubl. data) and New York (J. L. Brunner pers. comm.) have been screened, but no *Pseudacris* individuals have tested positive thus far. We are not aware of any reports of ranaviruses in western toads.

Field surveys and collection of samples. Surveys were conducted from 25 June to 14 July in 2007 and 16 June to 5 July in 2008. We looked and listened for amphibians while walking through apparently suitable habitats (e.g. wetlands, meadows), along cut-lines near apparently suitable habitat, and while walking and dip-netting along the perimeters of apparently suitable breeding sites. In both years, most surveyed sites were accessible by road or short hikes from roads (<2 km) for logistical reasons. Some, however, were accessible only by helicopter and/or boat. The 2008 survey in Nahanni National Park Reserve was distinct in terms of accessibility, as it involved rafting down the South Nahanni River from Kraus Hot Springs to the

Reserve boundary, banking at several spots along the river where it appeared that amphibian breeding habitat might be accessible (e.g. openings in tree canopies suggestive of ponds in clearings, sandy areas with shallow ponds cut off from the river or small streams).

We captured up to 61 individuals of each life stage of each species at each site to collect basic information (e.g. length, weight, stage of development; D. M. Schock unpubl. data), and to non-lethally collect tissue and/or swab samples for pathogen screening. Not all individuals that were encountered were captured, thus numbers presented here do not reflect total numbers encountered. Tissue samples were collected by cutting a single hind toe from frogs, or a small (≤ 5 mm) piece of tail tip from tadpoles, using a new blade and new gloves for each animal. Tissue samples were stored individually in 1 ml of 70% chemical grade ethanol until processing at the lab. Most sampled individuals were also swabbed for *Bd* following Hyatt et al. (2007). A sterile swab (MW100 tube dry swabs, Medical Wire & Equipment) was gently and repeatedly run across the animal's ventral surface, legs, and toe webbing, or in the case of tadpoles, the mouthparts were gently swabbed with a swirling motion. These are areas of the body most likely to be infected with *Bd* zoospores (Pessier et al. 1999, Berger et al. 2005). The swabs were stored in sealed freezer bags, grouped according to site, in the dark, at 4 to 22°C for 4 mo (2007) or 1 mo (2008) until they could be shipped to the lab, where they were frozen at -20°C for 8 mo (2007) or <1 mo (2008) when they were screened.

Concerted effort was made to prevent accidental spread of pathogens within and among sites. First, amphibians were individually held in new plastic Ziploc® bags or individual plastic containers from the time of capture until release a short time later. Bags were full of air plus sufficient moisture/water for the life stage, and all containers and bags were kept in the shade. Second, new latex gloves, blades, and swabs were used for each animal when taking samples. Finally, equipment that came into contact with animals (e.g. buckets and nets) was bleached, rinsed, and usually dried between sites. Multiple sets of nets and buckets were carried at all times. The use of powdered bleach (16% dry bleach, Zep Manufacturing) was instrumental in the disinfection process, particularly in situations where weight or bulk of gear was of concern. The bleach powder was mixed with a bucket of pond water and the equipment was disinfected. Equipment was rinsed using either potable water brought in containers, or in some instances, with water from the next site visited—that is, bleached equipment was taken to the next site and then doused with buckets of water from that site. In this manner, animals were only ever exposed to water from their own sites. In all instances

of use in the field, bleach water was disposed of on the road or on flat rocky areas devoid of vegetation where it could evaporate and degrade. No animals came into contact with bleach water and no bleach water drained into ponds.

PCR-based pathogen detection and identification.

DNA was extracted from tissue samples (toe, tail) using a salt extraction method (Sambrook & Russell 2001), whereas DNA was extracted from swabs using PrepMan Ultra® (Applied Biosystems) in accordance with the manufacturer's instructions. Tissue and swab DNA extractions were kept separate and used separately in screening assays; thus, for most animals, there were 2 separate DNA sources.

To screen samples for *Bd*, we used quantitative PCR (qPCR) following the protocol of Boyle et al. (2004). Samples were run in duplicate on a 384-well optical PCR plates on an ABI Prism 7900 Sequence Detection system (Applied Biosystems). Animals were scored as positive if the reactions in both wells amplified the target DNA sequence prior to 40 amplification cycles. Samples where there was no amplification in either well were scored as negative for *Bd*. Samples were re-run in duplicate if there was amplification in only 1 well, or evidence of amplification after 40 cycles. In most instances, the results of the re-run were conclusive and animals could reliably be scored as positive or negative. In the small number of instances where there was any doubt, animals were scored as negative.

While confirming all *Bd* positives and several negatives by re-running a subset of samples, it was noticed that some animals tested negative for *Bd* when DNA from swab extractions was used whereas they tested positive when DNA from tissue extractions was used. As a consequence, all animals from the Fort Liard region (both years), Norman Wells (both years), and 20 additional animals per year from elsewhere were screened multiple times, using DNA from tissues as well as from swabs. Disagreement between sources of DNA occurred for 3 animals from the Fort Liard region—in these cases, swabs consistently tested negative while toe tissues consistently tested positive. These 3 animals were scored as positive for *Bd*.

Animals were assayed for ranaviruses using the DNA extracted from tissues and the PCR methods described by Schock et al. (2008). Briefly, genetic markers were used that target a conserved ~500 base pair (bp) region of the major capsid protein (MCP) gene, the MCP4/5 markers (Mao et al. 1996). PCR products were treated with SYBR Green nucleic acid stain (Invitrogen) according to the manufacturer's directions and then visualized by electrophoresis on 1.5% agarose gels using an ultraviolet transilluminator (BioRad). A PCR product of ~500 bp indicated that an individual was positive for ranavirus, whereas no PCR band indicated no infection.

All PCR products that produced a 500 bp band were prepared for sequencing with ExoSAP-IT® (USB) according to manufacturer directions and sequenced on an ABI 3730 automated capillary column sequencer (Applied Bio-Systems). Sequences were aligned by eye and analyzed using MEGA version 4.0 (Tamura et al. 2007) and then compared to the MCP sequences of other ranaviruses using the BLAST tool from the National Center for Biotechnology Information (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Stata 10 (StataCorp) was used for statistical analyses. Confidence intervals (CIs) were calculated using the binomial-exact platform.

RESULTS

In total, 396 wood frogs (223 tadpoles, 173 frogs), 61 boreal chorus frogs (51 tadpoles, 10 frogs), and 99 western toads (95 tadpoles, 4 frogs) were screened for *Bd* and ranaviruses (Table 1). Western toads were encountered only in the Fort Liard area. Only wood frogs were encountered at any of the sites north of Wrigley.

Bd was detected at 1 site near Fort Liard (Table 1; Fig. 1), and was detected in all 3 amphibian species. This site was visited in 2007 and 2008, and *Bd* was detected in both years. We did not notice any gross signs of *Bd* infection such as skin sloughing in frogs (Pessier et al. 1999, Berger et al. 2005) or abnormal mouth parts in tadpoles (Knapp & Morgan 2006 and references therein, but see Padgett-Flohr & Goble 2007).

Ranaviruses were detected in wood frogs at 3 sites near Fort Liard, 1 site near Blackstone, 1 site in Nahanni National Park Preserve, and 4 sites in the Norman Wells area (Table 1; Fig. 1). Ranavirus-positive frogs were detected at the same site where *Bd* was detected, although no individuals tested positive for both pathogens. Ranaviruses were not detected in boreal chorus frogs or western toads. In some instances, tadpoles that tested positive for ranavirus by PCR had exhibited stereotypic gross signs of ranavirus infections such as red hemorrhages on the legs and tail and abdominal swelling (Greer et al. 2005). However, most individuals that tested positive for ranavirus did not outwardly appear infected.

The MCP sequences of all ranaviruses detected in our study were 99%+ identical to one another (576 or 577 bp / 577 bp). BLAST searches indicated that the MCP sequences from our study were >99% identical (576 or 577 bp / 577 bp) to the MCP sequence of Frog Virus 3 (FV3; GenBank accession number AY548484.1), the type isolate of amphibian ranaviruses (Tan et al. 2004). Previous studies showed that the MCP sequence

of ranaviruses detected in wood frogs and northern leopard frogs in Saskatchewan (Schock et al. 2008) and Ontario (Greer et al. 2005) were >98% identical to the FV3 MCP sequence. Representative MCP sequences from our study were submitted to GenBank under accession numbers GQ144407 and GQ144408.

In 2 areas, Norman Wells and Fort Liard, samples sizes were sufficiently large to allow for statistical comparisons of prevalences. Because of the potential bias in *Bd* detection in tadpoles, only frog-stage individuals were included in comparisons of *Bd* prevalence. CIs overlapped when *Bd* prevalence in each area was compared between years (data not shown) so data from both years were pooled for each area. The prevalence of *Bd* in the Fort Liard area was 14% (7/51, 95% CI = 6 to 26%) while the prevalence of *Bd* in the Norman Wells area was 0% (0/59, 97.5% 1-tailed CI = 0 to 6%). The difference in *Bd* prevalence between Norman Wells and Fort Liard was significant (Fisher's exact test, χ^2 1 df = 7.5738, $p = 0.006$).

Ranaviruses were only detected in wood frogs, and therefore only wood frogs were included in statistical comparisons. Both tadpoles and frog-stage wood frogs were included in calculations since there were no concerns about swab-related sampling biases between life stages; ranaviruses cause internal infections and therefore tissues, not swabs, were screened for ranavirus. CIs of ranavirus prevalences overlapped between years within each area (not shown) so data for each area were pooled across years. In both areas, ranavirus prevalence in wood frogs was 7% (Norman Wells = 9/131, 95% CI = 3 to 13%; Fort Liard = 10/135, 95% CI = 4 to 13%). There was no significant difference in ranavirus prevalence between Norman Wells and Fort Liard (Fisher's exact test, χ^2 1 df = 0.0251, $p = 0.874$).

DISCUSSION

We detected *Bd* and ranaviruses, 2 pathogens associated with amphibian declines, in the NT, thereby extending the known range of both pathogens. Although ranaviruses were found widely, they were detected only in wood frogs. In contrast, *Bd* was found only in the Fort Liard area but was detected in all 3 amphibian species.

Detection of *Bd* in our study area is not congruent with niche models developed by Ron (2005). Ron (2005) predicted the geographic distribution of *Bd* using several abiotic variables related to elevation, precipitation, and temperature based on locations where *Bd* was reported in the literature. The models did not predict that *Bd* would occur in the NT, with the closest predicted areas >450 km south or west of

Table 1. 2007 and 2008 surveys for chytrid fungus and ranaviruses in amphibians in the Northwest Territories, Canada. PCR-based diagnostics were used to screen samples for the pathogens. Values indicate the number of individuals that tested positive out of the number screened. NPR: National Park Reserve

Latitude / longitude	General area	Site name	Species	Chytrid screen Tadpoles Frogs	Ranavirus screen Tadpoles Frogs
2007					
60° 17' 16" N, 123° 29' 46" W	Ft. Liard	K29 road	Wood frog	–	–
60° 19' 16" N, 123° 18' 16" W	Ft. Liard	Muskeg River demonstration forest	Wood frog	–	–
60° 18' 17" N, 123° 19' 22" W	Ft. Liard	Muskeg River gravel pit ponds	Wood frog	0/21	5/21
			Western toad	0/61	0/61
60° 05' 37" N, 123° 05' 33" W	Ft. Liard	Roadside Pond 2	Wood frog	0/25	2/25
60° 09' 28" N, 123° 14' 31" W	Ft. Liard	Roadside Pond 3	Wood frog	0/7	0/7
60° 08' 18" N, 123° 11' 38" W	Ft. Liard	Roadside Pond 4	Wood frog	0/12	3/12
61° 07' 33" N, 122° 51' 02" W	Blackstone	Lindberg Landing	Wood frog	–	–
60° 56' 55" N, 123° 05' 39" W	Blackstone	Nahanni Butte Winter Road Pond 1	Wood frog	0/12	1/12
			Chorus frog	0/13	0/13
60° 57' 14" N, 123° 06' 57" W	Blackstone	Nahanni Butte Winter Road Pond 2	Wood frog	0/5	0/5
61° 09' 44" N, 123° 48' 49" W	Nahanni NPR	Near where Jackfish River enters South Nahanni River	Wood frog	–	–
61° 13' 24" N, 123° 46' 15" W	Nahanni NPR	Yohin Lake	Wood frog	–	–
65° 15' 36" N, 126° 41' 52" W	Norman Wells	Airstrip area	Wood frog	0/5	0/5
65° 17' 26" N, 126° 52' 34" W	Norman Wells	Bosworth Creek – upper bridge	Wood frog	–	–
65° 18' 12" N, 126° 11' 1" W	Norman Wells	Bosworth Creek – origin	Wood frog	–	–
65° 15' 18" N, 126° 58' 26" W	Norman Wells	W side of Mackenzie River – Canal trailhead	Wood frog	–	–
65° 13' 33" N, 126° 51' 08" W	Norman Wells	W side of Mackenzie River – Stop 1	Wood frog	–	–
65° 13' 37" N, 126° 46' 24" W	Norman Wells	W side of Mackenzie River – Stop 2	Wood frog	–	–
65° 15' 8" N, 126° 39' 53" W	Norman Wells	DOT Pond	Wood frog	0/1	1/1
65° 17' 47" N, 126° 49' 53" W	Norman Wells	Honey Bucket Road slough	Wood frog	–	–
65° 17' 35" N, 126° 36' 22" W	Norman Wells	Jackfish Lake – east end	Wood frog	–	–
65° 16' 30" N, 126° 47' 21" W	Norman Wells	Loomis Greenhouse pond	Wood frog	–	–
65° 15' 47" N, 126° 43' 35" W	Norman Wells	VOR tower road ponds	Wood frog	0/37	2/37
66° 00' 23" N, 128° 56' 15" W	Norman Wells/ Ft. Good Hope	Middle of nowhere swamp	Wood frog	0/1	0/1
			Wood frog	–	–
2008					
61° 29' 57" N, 120° 37' 55" W	Jean Marie River	Roadside Pond 13	Wood frog	–	–
			Chorus frog	0/30 ^a	0/30
61° 28' 15" N, 120° 36' 53" W	Jean Marie River	Roadside Pond 14	Wood frog	0/8	0/8
			Chorus frog	–	–
62° 27' 29" N, 123° 00' 31" W	Wrigley	Roadside Pond 15	Wood frog	–	–
63° 08' 40" N, 123° 15' 38" W	Wrigley	Roadside Pond 16	Wood frog	–	–
			Chorus frog	–	–
62° 37' 51" N, 123° 04' 19" W	Wrigley	Roadside Pond 17	Chorus frog	0/4	0/4
60° 18' 17" N, 123° 19' 22" W	Ft. Liard	Muskeg River gravel pit ponds	Chorus frog	0/10	0/10
			Wood frog	0/4	0/4
			Chorus frog	1/1	0/1
			Western toad	0/34	0/34
60° 16' 36" N, 123° 29' 14" W	Ft. Liard	K29 Road	Wood frog	0/5	0/5

Table 1 (continued)

Latitude / longitude	General area	Site name	Species	Chytrid screen		Ranavirus screen	
				Tadpoles	Frogs	Tadpoles	Frogs
2008							
60° 05' 37" N, 123° 05' 33" W	Ft. Liard	Roadside Pond 2	Wood frog	0/5	-	0/5	-
60° 09' 28" N, 123° 14' 31" W	Ft. Liard	Roadside Pond 3	Wood frog	-	0/1	-	0/1
61° 14' 47" N, 123° 59' 43" W	Nahanni NPR	River Stop 1	Wood frog	-	0/3	-	0/3
61° 15' 27" N, 123° 58' 14" W	Nahanni NPR	River Stop 2	Wood frog	-	0/2	-	0/2
61° 13' 08" N, 123° 46' 26" W	Nahanni NPR	Yohin Lake cabin	Wood frog	-	0/5	-	0/5
61° 14' 0" N, 123° 45' 30" W	Nahanni NPR	Yohin Trail Stop 1	Wood frog	-	0/2	-	0/2
60° 56' 55" N, 123° 05' 39" W	Blackstone	Nahanni Butte Winter Road Pond 1	Wood frog	0/16	-	0/16	-
60° 57' 14" N, 123° 06' 57" W	Blackstone	Nahanni Butte Winter Road Pond 2	Wood frog	0/16	-	0/16	-
65° 15' 8" N, 126° 39' 53" W	Norman Wells	DOT pond	Wood frog	0/5	-	0/5	-
65° 17' 47" N, 126° 49' 53" W	Norman Wells	Honey Bucket Road slough	Wood frog	0/11	0/4	1/11	1/4
65° 15' 47" N, 126° 43' 35" W	Norman Wells	VOR tower road ponds	Wood frog	0/12	0/1	3/12	0/1
67° 02' 32" N, 126° 05' 49" W	Colville Lake	Colville Stop 2	Wood frog	-	0/1	-	0/1
67° 02' 04" N, 126° 05' 58" W	Colville Lake	Colville Lily Pond	Wood frog	0/9	0/1	0/9	0/1

^aDue to logistical problems, these boreal chorus frog tadpoles could not be swabbed. The chorus frog tadpoles were tail clipped and the tissues assayed for both pathogens, but because tadpole tail clips are much less likely to be positive for chytrid than swabs, these results may reflect false negatives

where we detected *Bd* near Fort Liard. Further research is needed to identify the underlying reason(s) for the discrepancy between predictions made by Ron (2005) and our finding of *Bd* in the NT. One explanation may be related to information that was available at the time Ron (2005) developed his models—niche models extrapolate from available data, and *Bd* is now known from many more locations than when Ron (2005) developed his projections. However, other studies that have searched for *Bd* at northern latitudes have been consistent with predictions made by Ron (2005): *Bd* was detected in Kenai National Wildlife Refuge, Alaska (Reeves & Green 2006, Reeves 2008), but not in Tetlin or Innoko National Wildlife Refuges (Reeves 2008), Alaska, or Denali National Park, Alaska (Chestnut et al. 2008). Similarly, Adams et al. (2007) and Pearl et al. (2007) detected *Bd* at several locations in western North America, as predicted by Ron (2005).

An alternate explanation for *Bd* in the NT may be linked to host species assemblages at a location and each species' uses of microhabitats that protect *Bd* from inhospitable conditions. For example, the extent to which *Bd* can withstand freezing in nature is unknown (Daszak et al. 2003, but see Seimon et al. 2007). *Bd* may be able to persist in areas with otherwise unsuitable habitats as a result of the overwintering strategy of host species that avoid freezing. Wood frogs and boreal chorus frogs overwinter on land, buried under leaf litter and snow cover. Both of these species freeze to -3°C, a feat made possible by physiological cryoprotectants that control the freezing process and mitigate the physiological stresses associated with freezing (Storey & Storey 1996). In areas where only wood frogs exist, persistence of *Bd* may depend on suitable environmental conditions. This explanation is consistent with the results of surveys in Alaska (Reeves & Green 2006, Chestnut et al. 2008, Reeves 2008): *Bd* was only detected in areas where the environmental conditions are conducive to *Bd* survival, as predicted by Ron (2005). In contrast, western toads do not freeze during winter, but overwinter below the frost line by burrowing and using existing cavities such as those associated with decayed root channels and abandoned beaver lodges (Muths & Nanjappa 2005, Brown & Symes 2007). It is plausible that *Bd* is persisting near Fort Liard by overwintering on western toads.

Ranaviruses were detected in wood frogs in several locations in our study, including Norman Wells (65° 17' N), which is the most northerly record of amphibian ranaviruses in North America. In all instances, the ranaviruses we detected were FV3-like based on their MCP gene sequences. However, Schock et al. (2008) demonstrated with restriction enzyme analyses that amphibian ranaviruses whose MCP sequences are

>99% identical to FV3 can differ elsewhere in their genome. Detailed characterizations required to adequately compare northern amphibian ranaviruses to those found elsewhere await further study.

We did not encounter any wood frog die-offs, but we suspect that ranavirus related die-offs occur in wood frogs in the NT as they do elsewhere (Green et al. 2002, Greer et al. 2005, Harp & Petranka 2006, Schock et al. 2008). It is possible that our surveys occurred too early in the year, and therefore too early in epidemic curves, to detect large die-offs. Furthermore, our study likely underestimates the prevalence of ranaviruses in northern populations of wood frogs because we screened toe/tail tissues. Amphibian ranaviruses attack internal organs, especially liver, kidney, and gastro-intestinal tissues (Bollinger et al. 1999, Greer et al. 2005). However, testing these organs requires lethal sampling. By testing toe/tail tissues, our methods could only detect infections where virus was circulating in the blood in sufficiently high titres. Future studies that facilitate repeated visits to sites over the entire course of the amphibian active season will be invaluable to understanding ranavirus disease dynamics in general.

An unexpected finding we encountered was evidence that some of our swab samples may have degraded prior to DNA extraction. We became aware of the situation when positive results were obtained when screening frog toes but negative results were obtained from swabs of those same individuals. Because *Bd* causes external infections of mouthparts in tadpoles, tail clips from tadpoles are inherently less likely to collect zoospores than swabs, and therefore are more likely to give false negative results. As a consequence, failure to detect *Bd* in tail clips is not informative. The handling and storage of our samples were consistent with those described by Hyatt et al. (2007), wherein they report no decrease in zoospore recovery in samples stored at room temperature for 18 mo. Recently, another study has shown that storage and handling conditions appear to impact the integrity of swabs (Van Sluys et al. 2008). Although we do not know which aspect(s) of handling may have affected the swabs in our study, prolonged exposure to high temperatures, the topic addressed by Van Sluys et al. (2008), did not occur. This suggests that other aspects of storage or handling may also affect the integrity of samples collected on swabs in field settings.

It is unknown whether *Bd* or ranaviruses are recent arrivals to the Canadian north or if they have been components of northern ecosystems for considerable periods of evolutionary time. This important question can be explored through further genetic analyses. The results of such analyses can also inform the larger debate about the origin of *Bd* in North America as well

as the extent to which the ranges of these pathogens may be shifting as a result of climate change or rapid industrial development. If *Bd* and/or ranaviruses have historically been components of northern ecosystems, disease dynamics will have been influenced by local selection pressures on hosts and pathogens alike. Amphibian ranaviruses and *Bd* infect multiple host species and therefore transmission dynamics will likely be affected by changes in northern host species assemblages. Without limits on migration, amphibians are predicted to expand into higher latitudes to a greater extent than other vertebrates as our climate changes (Araújo et al. 2006, Lawler et al. 2009). As a result, threats posed by ranaviruses or *Bd* to the long-term persistence of northern amphibian populations may arise from altered disease dynamics due to the movement of additional host species into northern areas. Regardless of any changes that may occur in host or pathogen geographic ranges, climate change in the north will likely lead to changes in *Bd* disease dynamics because its lifecycle is tightly coupled with both temperature and humidity (Woodhams et al. 2003, Pounds et al. 2006). Similarly, temperature alone can profoundly affect mortality rates due to ranavirus infections (Rojas et al. 2005).

Although logistical issues involved with working in the north can be considerable, the biological tractability of the ecosystem creates opportunities to understand complex questions surrounding the role of infectious diseases in global amphibian declines. Lessons learned from watching the north may be instrumental in managing the effect of infectious diseases on amphibian populations much farther south.

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