AMPHIBIAN POPULATION AND PATHOGEN SURVEYS IN THE DEHCHO AND SAHTU, NORTHWEST TERRITORIES, 2007 AND 2008

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ABSTRACT

Five species of amphibians, all anurans, are known to occur in the Northwest Territories (NT): wood frogs (*Rana sylvatica* = *Lithobates sylvaticus*; Family Ranidae), northern leopard frogs (*Rana pipiens* = *Lithobates pipiens*; Family Ranidae), western toads (*Bufo boreas* = *Anaxyrus boreas*; Family Bufonidae), Canadian toads (*Bufo hemiophrys* = *Anaxyrus hemiophrys*; Family Bufonidae) and boreal chorus frogs (*Pseudacris maculata*; Family Hylidae). Of these five, wood frogs, western toads, and boreal chorus frogs, are known from the Dehcho whereas wood frogs and boreal chorus frogs are known from the Sahtu.

Baseline information on species distribution and abundance is key to identifying and then addressing declines on regional and local scales. The amphibians that occur in the NT, and those that potentially occur in the NT, tend to be widespread species at the northern edges of their ranges. While some information exists on these species elsewhere in their respective ranges, information for NT populations is incomplete. This is of immediate concern for two species in the NT, northern leopard frogs and western toads because they require multi-jurisdictional management plans under the federal *Species at Risk Act*.

There were four objectives for the project:

• Improve our understanding of the distribution and abundance of all amphibian species found in the Dehcho and Sahtu Regions.

• Collect recent data on western toads and northern leopard frogs to aid in developing NT information for implementation of the federal *Species at Risk Act*.

• Collect information on amphibians for the Protected Areas Strategy by confirming species presence in proposed protected areas in the Dehcho.

• Provide information on the presence of infectious diseases in NT populations of amphibians, particularly chytrid fungus and ranaviruses, which are implicated in amphibian population declines worldwide.

A total of 47 sites were visited in 2007 (28 in the Dehcho, 19 in the Sahtu). No amphibians were encountered at 11 sites (4 in the Dehcho, 7 in the Sahtu). Or, stated otherwise, 77% (36/47) of sites were inhabited by amphibians overall, with 86% (24/28) of sites inhabited in the Dehcho and 58% (11/19) inhabited in Sahtu. In 2008, a total of 40 sites in Dehcho were visited. Of these 40 sites, no amphibians were encountered at 7 sites; or, 83% (33/40) sites were inhabited by amphibians. No calculations were made for the Sahtu in 2008 due to the nature of the site visits. Wood frogs were found at several sites in the Dehcho and Sahtu, including Nahanni National Park Reserve (NPR), and were detected at all sites where amphibians were detected. Western toads were detected at two sites near Ft. Liard, including one site where tadpoles were found both years. The breeding site is within a gravel pit areas that is frequented by all-terrain-vehicle users. Western toads were not found in Nahanni NPR despite focused efforts to

verify reports of the species in the Yohin Lake area. Boreal chorus frogs were found at several sites in the Dehcho although not in Norman Wells, as suggested they would be by widely available distribution maps.

During surveys in 2007 and 2008, a total of 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 chytrid fungus and ranaviruses. Both chytrid fungus and ranaviruses were detected in amphibian populations in the NT, greatly extending the known range of both pathogens. Although ranaviruses were found widely, including in Norman Wells, they were detected only in wood frogs. In contrast, chytrid fungus was found in only the Ft. Liard area but was detected in all three species of amphibians. Detection of chytrid fungus in the Dehcho is not congruent with current predicted distribution ranges based on niche models, and further research is needed to determine what is permitting the pathogen to persist in the area. The identity of the ranavirus detected in our study was confirmed to be Frog Virus 3 by sequence analysis of the major capsid protein gene and shares 99%+ similarity in that area of the genome to ranaviruses isolated from wood frogs elsewhere in North America. As well as chytrid fungus and ranaviruses, a single wood frog was found with a malformed hind foot, and several wood frog tadpoles were found in Norman Wells that appeared to have a white fungal growth associated with the cloaca. The identity of the infectious agent is not resolved but does not appear to be *Saprolegnia*.

Several recommendations arise from the findings of this project. Among the recommendations are an investigation into the feasibility of a coordinated,

long-term monitoring program at even a modest number of locations – Ft. Liard, Ft. Simpson and Norman Wells, for example. A program would be valuable in terms of understanding the biology of northern amphibian populations. In addition to monitoring populations over the longer term, thereby providing important baseline information, these programs could also resolve whether or not boreal chorus frogs occur in the Norman Wells area. Second, concrete or rock obstacles should be installed in and around western toad breeding habitats to prevent destruction due to ATV's and other motorized vehicles. Third, until visual proof of northern leopard frogs is obtained from the Dehcho, its occurrence there should be classified as unconfirmed. If northern leopard frogs are eventually located in the Dehcho, genetic work is recommended to test whether they are endemic to the area or if they may have been translocated from elsewhere, possibly as a result of use as fish bait. Fourth, any future pathogen screening that involves the widely used protocol swabs should store swabs in the freezer as soon as possible or put the swabs directly into a lysis buffer that is thermally stable at the temperatures likely to be encountered in the field. Finally, field hygiene protocols should be adopted that prevent the incidental translocation of pathogens among sites. These protocols should not be limited to workers directly involved with amphibians because pathogens can be spread by anyone.

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INTRODUCTION

Of the approximately 6000 species of amphibians on earth, at least 2000 are declining at rates that threaten them with extinction (Stuart et al. 2004). The rate and taxonomic scope of these declines are unparalleled among vertebrates (Stuart et al. 2004; McCallum 2007). Identified and suspected drivers of amphibian declines are diverse and may act synergistically (e.g., habitat destruction, infectious diseases, global climate changes, environmental contamination) and readers are directed to works such as Blaustein and Johnson (2003), Collins and Storfer (2003), Linder et al. (2003), Semlitsch (2003) for in-depth reviews.

Baseline information on species distribution and abundance is key to identifying and then addressing declines on regional and local scales. The amphibians that occur in the Northwest Territories (NT), and those that potentially occur in the NT, tend to be widespread species at the northern edges of their ranges. While some information exists on these species elsewhere in their respective ranges, information for NT populations is incomplete. This is of immediate concern for two species in the NT, northern leopard frogs (*Rana pipiens* = *Lithobates pipiens*[1]) and western toads (*Bufo boreas* = *Anaxyrus*

[1] The phylogenetics and taxonomy of North American amphibians have recently been re-evaluated and as a result, several changes in nomenclature adopted by authorities.

boreas) in-part because they require multi-jurisdictional management plans under the federal *Species at Risk Act*.

More generally, amphibian populations in the north provide unique opportunities to study the biology of populations, including populations of amphibian pathogens, at the edge of their respective ranges. Populations at the edge of a species' range frequently exhibit specialized adaptations and distinctive ecological traits that shed light on ecological and evolutionary dynamics of the species as a whole (Yakimowski and Eckert 2007; Hampe and Petit 2005). Northern amphibian populations may also be an instructive model system for understanding the impacts of climate changes because of the accelerated rate at which changes are occurring in higher latitudes, the relatively short life cycle of amphibians, and the tight coupling of amphibian population ecology with climatic variables.

Amphibian species known to occur in the Northwest Territories

Five species of amphibians, all anurans, are known to occur in the NT:

• wood frogs (*Rana sylvatica* = *Lithobates sylvaticus*; Family Ranidae)

Nomenclature changes were not proposed for Columbia spotted frogs or long-toed salamanders. Readers are directed to Frost et al. (2006) and Crother (2008) for additional information.

• northern leopard frogs (*Rana pipiens* = *Lithobates pipiens*; Family Ranidae)

• western toads (*Bufo boreas* = *Anaxyrus boreas*; Family Bufonidae)

• Canadian toads (*Bufo hemiophrys* = *Anaxyrus hemiophrys*; Family Bufonidae)

• boreal chorus frogs (*Pseudacris maculata*; Family Hylidae)

Of these five, wood frogs (Figures 1 – 4, 8), western toads (Figures 5 – 8), and boreal chorus frogs (Figures 9 – 11), are known from the Dehcho whereas wood frogs and boreal chorus frogs are known from the Sahtu. Wood frogs are found in a variety of habitats across northern North America south of the tree line, and are the only North American amphibian species found north of the Arctic Circle (Russell and Bauer 2000). Populations appear to be stable throughout most of their range although the species has been extirpated from Idaho, and populations are declining in Wyoming (NatureServe 2009). Boreal chorus frogs are also found in a variety of habitats across much of western North America. They are considered stable throughout their range (NatureServe 2009). Western toads are federally listed as a species of Special Concern in Canada and are listed as Endangered in several US states (NatureServe 2009). Although western toads are known from the southern part of the Dehcho, there are unconfirmed reports of western toads in the south-east portion of Nahanni National Park Reserve (Douglas P. Tate, personal communications).

Canadian toads and northern leopard frogs are known from the south-east part of the Territory, south of Great Slave Lake.

Readers are directed to Fournier (1997) and Hodge (1976) for reviews of historical records of amphibians in the NT, and to Corkran and Thoms (2006), Russell and Bauer (2000), and Stebbins (2003) for species identification guides. Natural history information for all five amphibian species is considerable, particularly as it pertains to southern populations, and readers are encouraged to consult synthetic volumes such as Lannoo (2005).

Wood frogs and boreal chorus frogs sometimes begin breeding activities before ice has melted from breeding ponds whereas western toads begin breeding activities slightly later in the spring, sometimes as late as early June. In all three species, males call[2] to attract mates during their brief mating season but are rarely heard at other times. Eggs are generally laid in shallow, fishless ponds and tadpoles metamorphose before fall (Moriarty and Lannoo 2005; Muths and Nanjappa 2005; Redmer and Trauth 2005; Russell and Bauer 2000). Wood frogs and boreal chorus frogs overwinter on land, buried in leaf litter and vegetation, by employing physiological mechanisms that allow them to tolerate freezing and the associated cellular anoxia and dehydration (Storey and Storey 1996). However, these mechanisms only allow the frogs to freeze to approximately -3^{o}C, and like all amphibians in the NT, their overwinter survival is tied to adequate insulation from snow cover. Western toads overwinter on land by moving below the frost

[2] Recordings of all anuran species found in the NT can be heard by visiting www.carcnet.ca and navigating the links to the species of interest.

line through the use of burrows, rotted tree roots and other pre-existing cavities (Brown and Symes 2007; Muths and Nanjappa 2005). By way of comparison, Canadian toads also overwinter by burrowing below the frost line whereas northern leopard frogs overwinter at the bottom of well oxygenated ponds or lakes that don't freeze to the bottom, either because the water is sufficiently deep or because it is otherwise prevented from freezing to the bottom (e.g., spring fed pond) (Russell and Bauer 2000; Ewert and Lannoo 2005; Rorabough 2005).

Additional amphibian species that may occur in the Dehcho

Columbia spotted frogs (*Rana luteiventris*; Family Ranidae) could potentially occur in the southern Dehcho based on their known range (Figure 12). They are associated with mountainous regions of western North America, from Alaska and the Yukon Territory (YT) south-eastward into Oregon, Idaho, and Montana, with fragmented populations in northern Nevada and Utah. Populations have been found at elevations in excess of 3000 m in Montana and Nevada (Reaser and Pilliod 2005; Stebbins 2003). The northern range of Columbia spotted frogs is poorly resolved, as reflected in the variability of published range maps (e.g., Corkran and Thoms 2006; Green et al. 1997; Stebbins 2003) and data available from NatureServe (accessed 12 March 2009). Slough and Mennell (2006) describe several sightings, including recent ones, near Watson Lake, YT, in areas associated with the Liard River basin. Generally, the frogs were encountered at water's edge in and around pond outlets.

Columbia spotted frogs breed in fishless shallow ponds associated with permanent lakes, ponds and wetlands, and deposit eggs in areas with sun exposure (Reaser and Pilliod 2005; Slough and Mennell 2006). Some sources suggest that young-of-the-year may overwinter as tadpoles, particularly in high elevation or northern locations (e.g., Russell and Bauer 2000; Slough and Mennell 2006) although other sources dispute this claim (e.g., Reaser and Pilliod 2005). Columbia spotted frogs overwinter underwater at the bottom of ponds, but overwintering locations are not necessarily the same water bodies as breeding ponds (Reaser and Pilliod 2005). Slough and Mennell (2006) suggest that the range of Columbia spotted frogs in northern BC and YT is associated with areas that receive early, deep snow cover, a feature that likely prevents thick ice formation and therefore aids in successful overwintering of metamorphosed individuals, and possibly tadpoles. Environment Canada climate normals based on data 1971-2000 (www.climate. weatheroffice.ec.gc.ca/climate_normals /index_e.html; accessed 12 February 2008) indicate that Watson Lake and Ft. Liard are similar in terms of average temperatures, snowfall, and several other climatic variables. Assuming that the Watson Lake and Ft. Liard weather stations are equally representative of their respective regions, these climate normals support the plausibility of *R. luteiventris* occurring in the Dehcho.

The long-toed salamander (*Ambystoma macrodactylum;* Family Ambystomatidae) could also potentially occur in the Dehcho based on its known distribution (Figure 13) and its life history characteristics. Long-toed salamanders occur in a variety of habitats across western North America, including alpine

meadows, semiarid grasslands and coniferous forests, and populations are found at elevations up to 2800 m in AB (Pilliod and Fronzuto 2005; Russell and Bauer 2000). They are primarily terrestrial and/or subterranean, spending the majority of their lives in mammal burrows, rotting logs and under debris and rocks, but rarely more than 100 m from water (Pilliod and Fronzuto 2005). In AB and BC, breeding takes place in ponds in early spring, often before breeding ponds are free of ice. Eggs are typically laid in shallow areas of fishless permanent or semi-permanent ponds and slow moving streams, and hatch in 2-3 weeks depending on temperature. In high elevation populations, larvae may take up to three years to metamorphose and leave their natal ponds. Because this species is associated with rivers and drainages in northern BC that connect with rivers in the Dehcho (e.g., Petitot River), and the ability of this species to thrive at high elevations, it is plausible that long-toed salamanders may also occur in the Dehcho where snowfall is sufficient to allow overwintering.

Amphibian pathogens and links with population declines

Pathogens (e.g., viruses, bacteria, parasitic worms and protozoans, etc.) are normal, integral components of healthy functioning ecosystems (Kuris et al. 2008; Hudson et al. 2006). However, pathogens can become a conservation issue when their dynamics threaten the persistence of host species. Such situations have arisen primarily as a result of anthropogenic changes in host-pathogen dynamics. For example, changes in agricultural practices or urban development can inadvertently “improve” the habitat used by pathogen vectors like mosquitoes (Warner 1968).

Linking specific pathogens to declines in amphibian species can be difficult. Most amphibians are secretive and cryptic, with the exception of calling males of many anurans during breeding season. This is coupled with the fact that our understanding of amphibian diseases is in its infancy relative to other vertebrate diseases. Making links between pathogens and population trends is further complicated because most amphibians in Canada have boom-and-bust population cycles. Many species live 5+ years and may forego breeding activities altogether in years when rain or other environmental triggers are insufficient or occur too late in the year. As a result, detection of steady downward declines, rather than fluctuations associated with usual boom-bust cycles, may require decades of monitoring (Alford and Richards 1999). Despite these inherent difficulties with linking amphibian population declines and infectious diseases, two amphibian pathogens, chytrid fungus and ranaviruses, have been implicated in amphibian declines and both pathogens were listed as notifiable by the OIE in 2008 (World Organization for Animal Health 2008).

The pathogens discussed here are ones that have garnered recent attention as potential drivers of abnormal population declines. However, this is not a comprehensive review of pathogens known to affect amphibians. Rather, it is intended to provide context for the Project Objectives.

Chytrid fungus

The amphibian pathogen *Batrachochytrium dendrobatidis* (*Bd*), a chytrid fungus, is linked to catastrophic amphibian declines around the world (Skerratt et al. 2007). In addition to a rapidly growing body of scientific literature, the gravity of the situation has prompted a collaborative global mapping project of *Bd*-positive locations and the species from which *Bd* has been isolated at each location (http://www.spatialepidemiology.net/bd/information/).

The origin of *Bd* is unclear (Rachowicz et al. 2005). In many regions of the world, patterns of disease and declines suggest *Bd* is a novel pathogen that sweeps through naïve host species, decimating amphibian diversity, and then later becomes established in areas with suitable environmental conditions and sufficient host numbers (Retallick et al. 2004; Skerratt et al. 2007; Lips et al. 2008). Available population genetic data for *Bd* also supports this hypothesis (Moorehouse 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 widespread and wreaking havoc in many places. Further, because the lifecycles of *Bd* and amphibians are both tightly coupled with temperature and humidity, it is reasonable to anticipate that changes in climate will lead to changes in disease dynamics. For example, transmission dynamics may change as a result of changes in host assemblages at breeding

sites due to reduced availability of breeding habitats, or range expansions of *Bd* into previously uninhabitable areas, directly, or through the range expansions of carrier species (Garner et al. 2006).

Ouellet et al. (2005) argue that *Bd* is not linked with recent amphibian declines in eastern North America because they detected high prevalences of *Bd* as far back as 1960 in species that have not declined. Longcore et al. (2007) make a similar argument based on high infections rates in road-killed specimens of several species that have not declined in Maine, USA. Unlike eastern North America, there is strong evidence linking *Bd* to the declines of species in western North America including mountain yellow-legged frogs (*Rana muscosa*) in California (Briggs et al. 2005); boreal toads (*Anaxyrus boreas boreas*) in Colorado (Muths et al. 2003); and several native Ranid frogs in Arizona (Bradley et al. 2002; Schlaepfer et al. 2007).

Bd has been detected in all three amphibian species in the Dehcho, elsewhere in their respective ranges. *Bd* was detected in wood frogs in Alaska from Kenai National Wildlife Refuge (Reeves and Green 2006; Reeves 2008) although not in Tetlin or Innoko National Wildlife Refuges (Reeves 2008), or in Denali National Park (Chestnut et al. 2008). In a large study of opportunistically collected museum specimens, Ouellet et al. (2005) detected *Bd* by histology in two wood frogs collected from Williams Lake, BC in 1976. Further abroad, *Bd* has been detected in wood frogs in Rocky Mountain National Park, Colorado (Rittman et al. 2003; Green and Muths 2005), south-central Wyoming/north-

central Colorado (Young et al. 2007), Michigan (Zellmer et al. 2008), Quebec (Ouellet et al. 2005), and Maine (Longcore et al. 2007).

Bd has been reported from several populations of western toads: Adams et al. (2007) detected *Bd* in western toads in several places along the coast of British Columbia and Raverty and Reynolds (2001) detected *Bd* in a western toad that originated from north-eastern British Columbia. In the USA, *Bd* has been detected in populations in Colorado and Wyoming that have declined dramatically in the past three decades (Green et al. 2002; Green and 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 and Lannoo 2005, Lemmon et al. 2007). Because various reports of *Bd* used different species names, reports of *Bd* in *P. triseriata* from western North America as well as *P. maculata* are of interest. *Bd* has been detected in *Pseudacris sp.* in Colorado (Rittman et al. 2003; Green and Muths 2005), Arizona (Retallick and Miera 2007), and south-central Wyoming/north-central Colorado (Young et al. 2007).

Ranaviruses

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). In a review of 64 amphibian mortality events in the USA, Green et al. (2002) report that ranaviruses affected only widespread and abundant amphibian species. However, amphibian ranaviruses isolated from abundant species such as barred tiger salamanders (*Ambystoma mavortium*) and wood frogs can cause lethal infections in multiple amphibian species (Jancovich et al. 2001; Schock et al. 2008). Thus, the potential exists for ranaviruses maintained in resilient populations of abundant species to serve as sources of infection to vulnerable rare species in the same habitat. Further, die-offs caused by ranaviruses can severely reduce numbers of amphibians at breeding sites and may affect amphibian populations in highly fragmented habitats where re-colonization is unlikely (Collins et al. 2003).

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 and Petranka 2006), and in North Dakota, Maine, and Massachusetts (Green et al. (2002). Green and Muths (2005) did not detect ranavirus in wood frogs in a study in Colorado although only 13 animals were tested. The author is not aware of any reports of ranavirus

from western toads although Green and Muths (2005) screened for ranavirus in Colorado and did not detect virus. Duffus et al. (2008) report that 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 and Muths (2005) did not detect ranavirus in *Pseudacris* in their study in Colorado. Similarly, numerous *Pseudacris* tadpoles and adults from multiple sites in Saskatchewan (DM Schock, unpublished data) and New York (JL Brunner, personal communication) have been screened but no *Pseudacris* individuals thus far have tested positive, even at sites where wood frogs have tested positive for ranavirus.

Apparently opportunistic pathogens

Some microbes such as *Saprolegnia sp* (Oomycetes fungus) and *Aeromonas sp* (bacterium) are frequently isolated from immuno-compromised, stressed and otherwise ill amphibians (Wright and Whitaker 2001). Although not currently linked with amphibian declines, apparently opportunistic pathogens such as *Saprolegnia* and *Aeromonas* may be of greater consequence than currently recognized. The biology of most pathogens in wild amphibian populations is not well understood and it is unclear to what extent some microbes are strictly opportunistic pathogens versus being the primary cause of mortality. For example, *Saprolegnia* attacks amphibian egg masses and may be an under-appreciated cause of mortality prior to metamorphosis (Kiesecker and Blaustein 1997; Ruthig 2008). Further, the impacts opportunistic pathogens, are likely to increase because of interactions with other changes that degrade the

quality of amphibian habitat. For example, Kieseker et al. (2001) argue that reduced water depth at breeding ponds, such as those that might accompany El Nino patterns, increases the amount of UV-B to which amphibian eggs and embryos are exposed, which in turn increases their vulnerability to *Saprolegnia*.

Physical abnormalities

Deformities, malformations and other physical abnormalities in amphibians has been the subject of numerous studies and substantive reviews (Ouellet 2000; Blaustein and Johnson 2003; Lannoo 2008). While frogs with extra limbs and other gross abnormalities have been documented for centuries (cited in Ouellet 2000), the large numbers of malformed individuals at some "hotspots", that is sites where abnormality rates >5%, (e.g. Minnesota – Vandenlangenberg et al. 2003), have raised concerns. Deformities interfere with activities such as locomotion, foraging, and predator avoidance and therefore have the potential to affect recruitment at local and regional scales. Setting aside abnormalities caused by physical trauma (e.g., failed depredation attempts), understanding the causes of high abnormality rates is an active area of research, with two potential causes receiving the majority of attention: chemical contamination of amphibian habitats (Gardiner et al. 2003; Taylor et al. 2005), and infections by the trematode *Ribeiroia ondatrae* (Johnson et al. 1999; 2008). While both of these causes may independently generate malformations in amphibians, there also appear to be links between various sources of contamination and trematode infections (Kiesecker 2002; Blaustein and Johnson 2003). For example, nutrient rich run-off from cropland increases population

sizes of snails that are intermediate hosts of the trematode. This in turn increases trematode infection rates in amphibian larvae, the host that the trematode enters after it leaves the snail (Johnson and Chase 2004).

Malformations have been documented in all five species of amphibians that occur in the Northwest Territories (reviewed in Ouellet 2000; Carey et al. 2003; Lannoo 2008). The author is not aware of any reports of malformed amphibians from the Northwest Territories. However, Chestnut et al. (2008) and Reeves et al. (2008) report on sites in Alaska where high numbers of malformed wood frogs were found.

Project Objectives

There were four objectives for the project:

• Improve our understanding of the distribution and abundance of all amphibian species found in the Dehcho and Sahtu Regions.

• Collect recent data on western toads and northern leopard frogs to aid in developing NT information for implementation of the federal *Species at Risk Act*.

• Collect information on amphibians for the Protected Areas Strategy by confirming species presence in proposed protected areas in the Dehcho.

• Provide information on the presence of infectious diseases in NT populations of amphibians, particularly chytrid fungus and ranaviruses. Tissue sampling and pathogen detection methods also provided amphibian DNA that can potentially be incorporated into future population genetic or phylogenetic analyses.

Based on the known distributions of amphibians that occur in the NT, AB, and BC (Hodge 1976; Corkran and Thoms 2006; Russell and Bauer 2000), boreal chorus frogs, western toads, and wood frogs were expected in the Dehcho while wood frogs and boreal chorus frogs were expected in the Sahtu. The known ranges of spotted frogs and long-toed salamanders suggested the possibility of encountering the species in the Dehcho. Based on the known host species ranges of ranaviruses and chytrid fungus, it was anticipated that one or both pathogens could be detected in the NT.

METHODS

Field Surveys

Surveys were conducted from 25 June - 14 July in 2007, and 16 June – 5 July in 2008 (see Appendices 1 and 2 for detailed accounts). In 2007, work in the Dehcho generally proceeded from south to north, from Ft. Liard to Ft. Simpson, and then west to Nahanni Butte Village and Nahanni National Park Reserve (NPR), particularly in the Yohin Lake area. Work in the Sahtu was primarily focussed in the Norman Wells area. In 2008, plans to spend considerable time in the Ft. Liard area were hampered by chronic flood-related road closures that prevented travel between Ft. Simpson and Ft. Liard. However, in 2008, surveys were conducted in several areas of the Dehcho including Ft. Liard, Ft. Simpson, Jean Marie, and Wrigley. The 2008 surveys in Nahanni NPR involved rafting down the South Nahanni River from Kraus Hot Springs to the Reserve boundary. We banked 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 streams). A specific trip was also made from the river to the cabin area at Yohin Lake to search again for western toads.

Survey time in the Sahtu in 2008 was very short and was qualitatively different from the rest of the amphibian surveys: In the Norman Wells area, only sites where amphibians were encountered in 2007 were visited in 2008, and of those, only a subset of ponds were revisited. Only the Colville Lake vicinity was

newly explored in 2008. The trip to Colville Lake was only 24 hours long and there was no access to a vehicle. As a result, the geographic area that could be covered was relatively small.

In both years, the majority of sites surveyed were accessible by road, or short hikes from roads (< 2 km). Several, however, were accessible only by helicopter and/or boat, and as mentioned above, the 2008 survey in Nahanni NPR was distinct in terms of accessibility from the river.

Amphibians were looked and listened for while walking through apparently suitable habitats (wetlands, meadows), along cut-lines near apparently suitable habitat, and walking and dip-netting along the perimeters of apparently suitable breeding sites. In an attempt to facilitate comparisons of abundances among sites, which were highly variable in multiple ways (e.g., accessibility, vegetation, amount of apparently suitable habitat, water depth, amount of time available to conduct surveys), abundance was quantified in terms of numbers of individuals encountered per unit-time spent searching (Heyer et al. 1994). Ultimately, this method of standardization was only useful for frog-stage wood frogs for reasons discussed in the Results section. Search time did not include time spent collecting tissue samples (see below).

Some sites were visited multiple times because there wasn't enough time to collect tissue samples on the first visit. Other sites were visited more than once because notable initial findings warranted follow-up visits, to the extent that

time and logistics would allow. As a result, some sites appear in Appendices 1 and 2 more than once.

Non-Lethal Tissue Collection

In addition to visual and auditory surveys, up to 61 individuals of each life stage of each species at each site were captured to collect basic information (e.g., length, weight, stage of development; Figure 15), and to non-lethally collect tissue and/or swab samples for pathogen screening (Figures 16 & 17). Sample collections were always done after the encounter surveys. Not all individuals that were encountered were captured, and not all captured individuals were sampled. Tissue samples were collected by cutting a single hind-toe from metamorphosed animals, or a small (5mm or less) piece of tail tip from tadpoles, using a new blade for each animal. As well, most sampled individuals were also swabbed for *Bd* following the protocol described by Hyatt et al. (2007). Briefly, swabbing involved gently running a sterile swab (MW100 tube dry swabs, Medical Wire & Equipment Company, UK) across the animal's ventral surface and toe-webbing, or in the case of tadpoles, the mouthparts were gently swabbed with a swirling motion of the swab. These are areas of the body most likely to be infected with *Bd* zoosporangia, and consequently, exhibit hyperkeratosis and other pathology associated with chytridiomycosis, the disease caused by *Bd* (Berger et al. 2005; Pessier 1999).

Concerted efforts were made to prevent accidental spread of pathogens within and among sites. First, animal that were captured for processing were individually held in new plastic Ziploc® bags or individual plastic containers (Figure 14) from time of capture until release a short time later. Bags were full of air plus sufficient moisture/water for the life stage, and all temporary enclosures were kept in the shade throughout the duration. 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 when dealing with tadpoles) was bleached, rinsed and usually dried between sites. Multiple sets of nets and buckets were carried at all times. Nets, buckets, boots, etc., were generally bleached at night when access to running water (preferably hot) greatly facilitated the process. The use of powdered bleach (16% dry bleach, Zep Manufacturing Company, Canada) was also 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 disinfected. Then the equipment was rinsed using either tap 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 ever came into contact with bleach water and no bleach water ever drained into ponds.

PCR-Based Pathogen Detection and Identification

DNA was extracted from tissue (toe, tail) samples using a salt extraction method (Sambrook and Russell 2001) whereas DNA was extracted from swabs using PrepMan Ultra® (Applied Biosystems Inc., USA) according to manufacturer specifications. These DNA extractions were kept separate and used separately in screening assays, thus, for most animals, there were two separate DNA extractions – one from the swabs and one from the tissue samples.

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 Inc., USA). Animals were scored as positive if the reactions in both wells amplified the target DNA sequence prior to 40 amplification cycles. Negative samples exhibited no amplification in either well. Samples with amplification in only one well, or evidence of amplification after 40 cycles, were re-run. In most instances, the results of the re-run were conclusive and animals could reliably be scored as positive or negative, but in the small number of instances where there was any doubt, animals were scored as negative.

When confirming all *Bd* positives and several negatives by re-running a subset of samples, some animals that tested negative for *Bd* when the swabs were screened tested positive when the DNA from tissues were screened. As a consequence, all animals from the Ft. Liard region (both years), Norman Wells (both years), and 20 additional animals/year from elsewhere were screened

multiple times, using DNA from tissues as well as from swabs. Disagreement between sources of DNA occurred for three animals from the Ft. Liard region – in these cases, swabs consistently tested negative while toe tissues consistently tested positive. These three animals were scored are positive for *Bd*.

Animals were assayed for ranaviruses using the DNA extracted from tissues and the PCR methods described in 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. 1997). PCR products were treated with SYBR Green nucleic acid stain (Invitrogen, USA) according to manufacturer specifications and then visualized by electrophoresis on 1.5% agarose gels using an ultraviolet transilluminator (BioRad, USA). A PCR product of ~ 500 bp indicated 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 Corporation, USA) according to manufacturer specifications and sequenced using methods described by Sanger et al. (1977) on an ABI 3730 automated capillary column sequencer at Arizona State University (Applied Bio-Systems, USA). Sequences were aligned by eye and analyzed using MEGA version 4.0 (Tamura et al. 2007) and then compared to other reported ranavirus MCP sequences using the by BLAST tool from the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analyses

Stata 10 (StataCorp, College Station, Texas) was used for statistical analyses. Confidence intervals for pathogen prevalences were calculated using the binomial-exact platform. Most population data were strictly descriptive, and for reasons discussed below, no attempt was made to statistically compare across populations.

RESULTS

Amphibian encounter surveys

A total of 47 sites were visited in 2007 (28 in the Dehcho, 19 in the Sahtu). No amphibians were encountered at 11 sites (4 in the Dehcho, 7 in the Sahtu). Or, stated otherwise, 77% (36/47) of sites were inhabited by amphibians overall, with 86% (24/28) of sites inhabited in the Dehcho and 58% (11/19) inhabited in Sahtu. In 2008, a total of 40 sites in Dehcho were visited. Of these 40 sites, no amphibians were encountered at 7 sites; or, 83% (33/40) sites were inhabited by amphibians. No calculations were made for the Sahtu in 2008 due to the nature of the site visits (see Methods section).

Western toads were encountered at two sites in the Ft. Liard area (Table 1). At one site, Muskeg River gravel pit, hundreds of tadpoles were found in 2007 and 2008 (Figure 6). At the other site, a large adult was seen but not captured. During visits to the gravel pit site in 2008, all-terrain-vehicle (ATV) tracks were seen that criss-crossed the breeding ponds. Unfortunately, photographs of the tracks were lost with the field camera that was lost to the Nahanni River. While the author was taking tadpole measurements at the site, a young couple and a small child drove up on an ATV. They drove through the breeding pond, stopped on a muddy area and produced a small plastic container they had brought with them. During casual conversation with the group, they indicated to the author that they had come collect some more tadpoles as the ones they had collected earlier had died. During casual conversation, the author suggested that rearing

tadpoles is actually a lot of work and it would be best if they brought the tadpoles back to the pond at the end of the afternoon so they wouldn't have to care for them. They ultimately left with a couple of wood frog tadpoles, identified for them by the author. The fate of the collected tadpoles is unknown.

Despite specific survey efforts in the Yohin Lake area of Nahanni NPR, and elsewhere in the south-east portion of the Reserve, no western toads were located. However, the area covered relative to the size of the Reserve was small. Continued amphibian monitoring and reporting of incidental sightings may increase the known amphibian diversity of the Reserve beyond wood frogs.

Boreal chorus frogs were encountered in several places in the Dehcho including the Ft. Liard, Blackstone, Nahanni Butte Village, Ft. Simpson, Jean Marie, and Wrigley areas (Table 2). Tadpoles were exclusively associated with water bodies < 50 cm deep, or that had extensive shallow areas < 50 cm deep (Figures 6 & 10). No boreal chorus frogs were encountered at any of the places visited in the Sahtu.

Wood frogs were widespread in the Dehcho and Sahtu (Table 3) and were encountered at all sites where amphibians were encountered; that is, no sites were found where only boreal chorus frogs and / or western toads were encountered. Wood frogs were the only amphibian species found in Nahanni NPR. Only frog-stage individuals were encountered at Nahanni NPR and no breeding ponds were found although opportunities to examine ponds were limited relative to other areas that were surveyed. Wood frogs were also the only

amphibian species encountered in the Sahtu although there were no opportunities to survey areas south of Norman Wells.

Wood frog tadpoles were found in many types of water bodies (Figures 3, 6, 10) although these water bodies generally had shallow areas and frequently had emergent vegetations several meters away from the shore. Tadpoles were not evenly distributed throughout water bodies, or even along the edges of water bodies, but rather, they were generally clustered.

Coloration of frog-stage wood frogs was highly variable and individuals with spotted colouration were found in the same ponds as the non-spotted individuals (Figure 1). The spotted individuals are found more often in northern populations.

No eggs of any amphibian species were found, which was expected given the time of year of the surveys (June and July). However tadpoles of all three species expected during the survey were located, thereby confirming at least one breeding location for each species. Further, tadpoles of different species co-occurred at many breeding sites in the Dehcho (Tables 1 - 3), indicating the species of amphibians in the Dehcho may share breeding sites in areas where ranges overlap.

A northern leopard frog may have been heard calling in the Ft. Liard area (K29 Road) in 2007 but attempts to located and visually confirm the identity of the animal that made the sounds were not successful. A similar situation is reported in an ecological assessment of the Edéhzhíe Candidate Protected Area

(EBA Engineering Consultants Ltd. 2005). A return trip to the K29 road in 2008 to look for northern leopard frogs resulted in locating only more wood frogs. Initial planning for the 2008 surveys included extended time for follow-up work in the area. Unfortunately, road closures due to flooding, combined with limited time available to survey other areas, ultimately allowed for only a single day to revisit the K29 road. While it is not improbable that northern leopard frogs could occur in the Dehcho, finding them near Ft. Liard would constitute an expansion of the known range by several hundred kms. Until visual proof of the species is obtained from the Dehcho, its occurrence there should be classified as unconfirmed. If northern leopard frogs are eventually located in the Dehcho (Figure 18), genetic work is recommended to test whether they are endemic to the area or if they may have been translocated from elsewhere, possibly as a result of use as fish bait.

Long-toed salamanders nor Columbia spotted frogs were encountered. However, there is apparently suitable habitat in the southern Dehcho, and the species are associated with rivers and drainages in northern British Columbia that connect with rivers in the Dehcho.

Abundances were not inferred or estimated based on unit-effort of time spent searching for western toads or boreal chorus frogs. In the case of western toads, the species was encountered at only two sites, one of which with 100’s of tadpoles that were immediately visible upon arrival and a small number of frog-stage individuals encountered shortly thereafter, and one site where only a large adult was encountered, that individual having been seen within 5 minutes of

arriving at the pond. No reasonable comparisons between sites can be made with so few data points. However, based on the relatively small number of sites at which they were found, western toads do not appear to be as abundant in the Dehcho as boreal chorus frogs or wood frogs. For somewhat analogous reasons, abundances were not estimated or inferred for boreal chorus frogs: frog-stage individuals were encountered at only a small number of sites, and tadpoles, when present, were generally immediately visible and often numbered in the hundreds. Based on the number of sites at which boreal chorus frogs were found in the Dehcho, they are intermediate in abundance between western toads and wood frogs.

Abundances, as determined per numbers encountered per unit time, were calculated for frog-stage wood frogs for 64 sites (Figure 19). Wood frogs were encountered at an average rate of 4 frogs per 30 minutes in both the Dehcho and the Sahtu. However, at some sites in the Dehcho, encounter rates were very high. The most striking outlier in this respect was the visit to Nahanni Butte in 2007. The survey happened to coincide with young of the year leaving the ponds and warm and wet weather conditions, both of which presumably contributed to the large number of frogs encountered in a very short period of time. In contrast, another outlier was the survey at the Yohin Lake cabin area in Nahanni NPR. The area was extensively searched in 2007 for the purposes of investigating reports of western toads in the area. Despite the substantially greater amount of time spent searching, more wood frogs were not encountered. In general, the majority of points lie within a cluster and there is no evidence of increased

numbers detected with increased time spent searching beyond 120 minutes of search time.

Abundances for wood frog tadpoles were not calculated because of the high degree of clustering of tadpoles with ponds and the variability among ponds in terms of size and access. What is apparent from the surveys, however, is that wood frogs readily use many types of water bodies for breeding and that they could readily be detected using the methods employed here.

Amphibian body measurements and stages of development

Body sizes and masses for all three species (Table 4, Figure 20) were consistent with data published elsewhere (Russell and Bauer 2000; Stebbins 2003; Corkran and Thoms 2006; Moriarty and Lannoo 2005; Muths and Nanjappa 2005; Redmer and Trauth 2005). The data collected here will contribute to a database of information that will help track the health and resilience of amphibian populations in the NT.

A finding worth noting in this report is the degree to which stage of development of tadpoles could vary drastically among water bodies that are relatively close together. The most striking example encountered was in Norman Wells in 2007. The wood frogs at the Loomis Green House pond were smaller (average snout-vent length 14.6 mm; stdev = 1.7mm; n = 37) and less developed (Gosner stages 30 – 35) whereas less than 1.5 km away, wood frog tadpoles from a pond near the airstrip had an average snout-vent length of 22.0 mm

(stdev = 1.7mm; n = 5) and were at Gosner stages 41 – 44. These differences are likely due to microhabitat differences between the sites: the pond at the Loomis Green house site was shaded by trees and buildings, small, and exceptionally densely populated, requiring only a single dipnet swipe to collect more tadpoles than required for sampling. In contrast, the pond near the airstrip was mostly in full sun, and the tadpoles were located in a small area of the pond but presumably used the entire pond as they sought out food and optimal temperatures over the course of a day.

Amphibian pathogens and physical abnormalities

During surveys in the Dehcho and Sahtu, NT, in 2007 and 2008, a total of 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 5).

Bd was detected at one site near Ft. Liard, where at least one individual of all three amphibian species tested positive. This site was among those that were visited in 2007 and 2008, and *Bd* was detected both years. The author did not notice any gross signs of *Bd* infection such skin sloughing in frogs (Pessier et al. 1999; Berger et al. 2005) or abnormal mouth parts in tadpoles (Knapp and Morgan 2006 and references therein, but see Padgett-Flohr and Goble 2007).

Ranavirus was detected in wood frogs at three sites near Ft. Liard, one site near Blackstone, one site in Nahanni National Park Preserve, and four sites in the Norman Wells vicinity (Figure 21). At the site near Ft. Liard where *Bd* was detected, ranavirus was also detected, although no individual tested positive for both pathogens. Ranavirus was 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 infections such as red hemorrhages on the legs and tail and abdominal swelling (Greer et al. 2005) when they were sampled in the field. However, most individuals that tested positive did not outwardly appear infected.

The MCP sequences of all ranavirus detected in this study were 99%+ identical to one another (576 or 577 bp / 577 bp). BLAST searches were used to compare the MCP sequences obtained in our study to those reported elsewhere. The MCP sequences from our study were 99%+ identical (576 or 577 bp / 577 bp) to the ranavirus MCP sequence of Frog Virus 3 (FV3; GenBank™ Accession number AY548484.1), the type isolate of amphibian ranaviruses (Tan et al. 2004). Previous studies have shown 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) are 98%+ identical to the FV3 MCP sequence.

In two vicinities, Norman Wells and Ft. Liard, samples sizes were sufficiently large to allow for meaningful 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. Confidence intervals overlapped when *Bd* prevalences in each vicinity were compared between years (data not shown) so data from both years were pooled for each vicinity. The prevalence of *Bd* in the Ft. Liard vicinity was 14% (7/51, 95% CI = 6-26%) while the prevalence of *Bd* in the Norman Wells vicinity was 0% (0/59, 97.5% one-tailed CI = 0 – 6%). The difference in *Bd* prevalences between Norman Wells and Ft. Liard was significant (Fisher's Exact Test, $\chi^2_{1df} = 7.5738$, 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. Confidence intervals of ranavirus prevalences overlapped between years within each vicinity (not shown) so data for each vicinity were pooled across years. In both vicinities, ranavirus prevalence in wood frogs was 7% (Norman Wells = 9/131, 95% CI = 3 - 13%; Ft. Liard = 10/135, 95% CI = 4 - 13%). There was no significant difference in ranavirus prevalence between Norman Wells and Ft. Liard (Fisher's Exact Test, $\chi^2_{1df} = 0.0251$, P = 0.874).

While working in Norman Wells in 2008, several wood frog tadpoles were observed to have white "fuzz" associated with their cloaca (Figure 22). Grossly, these observations are consistent with fungal infections by *Saprolegnia* or closely related members of Oomycetes (Wright and Whittaker 2001). Three tadpoles were humanely, instantly euthanized by decapitation with sterile scalpel

blades and tissue samples were collected for PCR-based diagnostics. Attempts to amplify genetic markers diagnostic for *Saprolegnia* (Petrisko et al. 2008) were unsuccessful and at the time this report is being prepared, the identity of the organisms remains unresolved.

On 19 June 2008, a wood frog (snout-vent length 38 mm, mass 5.5 g) was encountered at the Muskeg River Gravel Pit near Ft. Liard that had a hind foot that was entirely bilaterally, symmetrically duplicated. Unfortunately, photos of the frog were lost when the field camera was lost in the Nahanni River a few days later. The frog was observed by both DM Schock and DG Allaire (Wildlife Technician, ENR Dehcho Region). No other gross physical abnormalities were observed in 2007 or 2008.

DISCUSSION

Amphibian population surveys

Wood frogs populations appear to be large and the species is widespread in the Dehcho and Sahtu. Western toads have a limited range in the NT, appear to have low abundances relative to boreal chorus frogs and wood frogs, and may be vulnerable to extirpation due to stochastic events or habitat destruction. It is possible that the encounter survey methods used in this study were inadvertently biased against detecting western toads and/or biased towards finding wood frogs. However, particularly in the Ft. Liard and the Norman Wells areas, most water bodies that could be accessed were investigated. With that in mind, it may be that western toads don't use water bodies that are readily accessible, for whatever reason. The fact that tadpoles were found in ponds in an actively used gravel pit argues against this, however. Additional information, acquired incidentally or through directed research, will be beneficial in determining what the habitat uses of western toads are in the Dehcho.

The actual distribution of the boreal chorus frogs in the Sahtu may require additional study. Based on numerous widely available range maps (e.g., Russell and Bauer 2000; Stebbins 2003; Fisher et al. 2007), it was anticipated that boreal chorus frogs would be encountered in Norman Wells. One possible explanations for this unexpected absence of boreal chorus frogs in Norman Wells is that the surveys somehow missed detecting the species. While possible, this seems unlikely since the same techniques detected boreal chorus frogs at

several sites in the Dehcho. A second explanation may be that boreal chorus frogs don't actually occur in the area. Fournier (1997) discusses that historical records of the species in the Sahtu, including the Norman Wells area, may be inaccurate. On an encouraging note, the presence or absence of the species in the Norman Wells area is an issue that can be easily resolved: simple auditory surveys conducted for a few springs will detect the species if it is there. Since there are only two possible species occurring in the area – wood frogs and boreal chorus frogs – and their calls are not readily confused, involving local non-biologist natural history enthusiasts in the auditory surveys would require minimal training. Involvement of local enthusiasts can also improve spatial and temporal coverage of the area and therefore improve the chances of detecting boreal chorus frogs if present.

Although attempts were made to permit general comparisons among sites by standardizing counts of amphibians encountered per unit time of search effort, this ultimately proved uninformative for western toads and boreal chorus frogs. Adults of both species were encountered infrequently, and tadpoles, when present, were immediately obvious due to the very shallow water and often numbered in the hundreds.

Special care must be taken when considering the "abundances" of tadpoles and how that information is used to infer population abundances. Females of the amphibian species in the NT can lay several hundred eggs when they breed, and consequently, it is possible that a water body that contains a large number of tadpoles may actually be the offspring of a single breeding pair.

As well, the majority of tadpoles that hatch do not survive to metamorphosis and high rates of attrition continue throughout post-metamorphic maturation prior to sexual maturity. Thus, numbers of tadpoles in a breeding pond are not a reliable indicator of breeding adult population size. Even in areas where multiple adults are encountered and it is reasonable to assume that tadpoles in a breeding pond are the offspring of multiple breeding pairs, tadpole abundances can be misleading because tadpoles are not evenly distributed within water bodies, generally being clumped in specific areas of the pond. As a consequence, considerable time may be spent examining a water body before locating any tadpoles, regardless of actual numbers present. The problem can be especially pronounced when breeding sites vary greatly in size, vegetation, clarity, and access on all sides, as was the case with ponds where wood frog tadpoles were found. A final aspect of amphibian biology that must be kept in mind when using tadpole numbers as an indication of overall population size and structure is the widely recognized (Alford and Richards 1999; Heyer et al. 1994) variability among years in terms of breeding success and recruitment.

Amphibian pathogens and physical abnormalities

Both *Bd* and ranaviruses were detected in amphibian populations in the NT, greatly extending the known range of both pathogens. Although ranaviruses were found widely, they were detected only in wood frogs. In contrast, *Bd* was

found in only the Ft. Liard area but was detected in all three species of amphibian.

Detection of *Bd* in the Dehcho is not congruent with niche models developed by Ron (2005). The study by Ron (2005) predicted the geographic distribution of *Bd* using several variables related to elevation, precipitation and temperature from locations where *Bd* was reported in the literature. The models did not predict *Bd* would occur in the NT, with the closest predicted areas > 450 km south or west of where *Bd* was detected near Ft. Liard. 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 and Green 2006; Reeves 2008), but not in Tetlin or Innoko National Wildlife Refuges (Reeves 2008), or Denali National Park (Chestnut et al. 2008). Similarly, Adams et al. (2007) and Pearl et al. (2007) detected *Bd* at several locations along western North America, as predicted by Ron (2005).

Further research is needed to identify the underlying reason(s) for the discrepancy between predictions made by Ron (2005) and detection of *Bd* in the NT. However, one interesting possibility is that the presence of *Bd* in the NT may be linked to host species composition 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 in the winter. In areas where only wood frogs exist, persistence of *Bd* may be dependent upon on suitable environmental conditions. This explanation is consistent with the results of surveys in Alaska (Reeves and 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 and Nanjappa 2005; Brown and Symes 2007). It is plausible that *Bd* is persisting at this northern location by overwintering on western toads.

Ranavirus was detected in wood frogs in several locations in our study, including Norman Wells, which is the most northerly record of ranavirus in North America. The identity of the ranavirus detected in our study was confirmed to be FV3 by sequence analysis of the MCP gene. We did not encounter wood frog die-offs at any of the sites although it is probable 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 and 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. 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. For example, it would be informative to examine the effect of host species assemblages on ranavirus

disease dynamics at sites where multiple species of ranid frog occur (e.g., Greer et al. 2005, Duffus et al. 2008), to those in the NT where only wood frogs occur (e.g., Norman Wells, this study).

It is unknown whether *Bd* or ranaviruses are recent arrivals to the Northwest Territories. However, future genetic analyses of northern strains of *Bd* will undoubtedly inform the debate about the origin of *Bd* in North America, and to what extent the range of *Bd* is shifting northward as a result of anthropomorphic changes such as global warming and industrial development. More thorough characterization of northern strains of ranavirus may shed light on regions of the genome that affect host range, a key trait affecting the threat ranaviruses pose to amphibian populations.

It is worth considering that *Bd* and amphibian ranaviruses may have been in the north for considerable periods of evolutionary time. The existence of long-term, locally adapted host-pathogen relationships in the north are not routinely addressed in discussions about the potential links between climate change and disease dynamics. Rather than considering the consequences of new host species moving into areas and affecting existing disease dynamics, attention is most often focused on the consequences of new pathogens moving into new areas. In the event that *Bd* and/or ranaviruses have been evolutionarily stable [3]

[3] Note that this does not mean that pathogens that are long-standing members of an ecosystem are necessarily avirulent or benign. Virulence and pathogenicity are characteristics of host-

components of northern ecosystems, it is possible that threats posed by these pathogens to the long-term persistence of their amphibian hosts in the north may arise from the movement of additional host species into northern areas that affect transmission and other disease dynamics. Without limits on migration, amphibians are expected to expand into higher latitudes more so than other vertebrates (Araújo et al 2006; Lawler et al 2009) and so it would seem that an unfortunate natural experiment is anticipated in the Canadian north as additional amphibian species shift northward.

Unexpectedly, it appears that at least some swab samples likely degraded prior to DNA extraction. The author 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. Further, not a single tadpole tested positive at the *Bd* positive site. This is unusual as *Bd* infects tadpoles in numerous other systems, frequently being detected using swabs. Because *Bd* causes external infections of mouthparts in tadpoles, tail clips from tadpoles are 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. Instead, it seems likely that the lack of tadpoles that tested positive

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pathogen relationships that are shaped by ecological and evolutionary forces such as transmission dynamics and whether a pathogen routinely infects and is transmitted among multiple host species.

for *Bd* is due to degradation of swab samples and the reduced likelihood of detecting *Bd* in tadpoles with tissue samples. Recently, another study has come to light where storage and handling conditions appear to impact the integrity of swabs (Van Sluys et al. 2008). Although it is 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 impact the integrity of samples collected on swabs.

Despite the large number of sites visited, and individual amphibians handled, only a single physical abnormality was found, which involved the hind foot of a 5.5 g wood frog from the Ft. Liard area. Thus, the rate of physical abnormalities encountered in this study is less than 1 %. Chestnut et al. (2008) and Reeves et al. (2008) report high rates of malformed wood frogs at some sites in Alaska, and Reeves et al. (2008) in particular argue that there is a correlation between deformity rates and proximity to roads. The majority of the sites visited in the Dehcho and Sahtu during this study were immediately adjacent to, or within 2 km, of a road. Additional research would be needed to identify the underlying mechanisms that generate such high deformity rates in some northern wood frog populations but not in others. However, it does not appear at this time to be related to proximity to roads.

RECOMMENDATIONS

Several recommendations arise from the findings of this project. First, the feasibility of a coordinated, long-term monitoring program at even a modest number of locations – Ft. Liard, Ft. Simpson and Norman Wells, should be investigated. Such a program would be valuable in terms of understanding the biology of northern amphibian populations. Such programs could also be developed with an eye towards public education and outreach. For example, these programs could readily include local natural history enthusiasts. In addition to monitoring populations over the longer term, thereby providing important baseline information, these programs could also resolve whether or not boreal chorus frogs occur in the Norman Wells area and other parts of the Sahtu.

Second, concrete or rock obstacles should be installed in and around western toad breeding habitats to prevent destruction due to ATV's and other motorized vehicles.

Third, until visual proof of northern leopard frogs is obtained from the Dehcho, its occurrence there should be classified as unconfirmed. If northern leopard frogs are eventually located in the Dehcho, genetic work is recommended to test whether they are endemic to the area or if they may have been translocated from elsewhere, possibly as a result of use as fish bait.

Fourth, any future pathogen screening that involves the widely used protocol swabs should store swabs in the freezer as soon as possible or put the swabs directly into a lysis buffer that is thermally stable at the temperatures likely

to be encountered in the field. Molecular grade ethanol may also be an option for swab storage although some reports indicate that valuable DNA may be lost during the subsequent extraction process (Hyatt et al. 2007).

Finally, field hygiene protocols should be adopted that prevent the incidental translocation of pathogens among sites. These protocols should not be limited to workers directly involved with amphibian as pathogens can be spread by anyone. Field workers often work at several sites chosen for their diversity, abundance, or ecological significance. This makes field workers a highly likely route by which pathogens can be spread within and among sites. To prevent the spread of pathogens, including those not yet recognized, it is imperative that field workers incorporate field hygiene into their standard protocols. Basic hygiene should be employed to minimize transmission of pathogens among individuals at a given site: using disposable gloves between animals, holding animals individually until they are processed and released, disinfecting rulers, callipers etc between animals. Equipment such as boots, seines, dipnets, buckets, etc. should be thoroughly disinfected between sites. These precautions will also minimize the spread of other species (e.g. the microscopic larvae of invasive invertebrates, seed pods of invasive plants). Just because a researcher is not working on, or even aware of, a particular pathogen does not mean they cannot effectively spread it among sites.

Table 1. Locations in the Dehcho, NT, where western toads (*Anaxyrus boreas* = *Bufo boreas*) were confirmed present in 2007 and/or 2008.

Vicinty	Latitude / longitude	Site name	Location within site (if applicable)
Ft. Liard	N60 18 17.2; W123 19 22.3	Muskeg River Gravel Pit	Pond 1
Ft. Liard	N60 18 07.7; W123 20 02.1	Muskeg River Gravel Pit	Pond 3
Ft. Liard	N60 18 12.9; W123 19 39.2	Muskeg River Gravel Pit	Pond 4
Ft. Liard	N60 09 28.0; W123 14 30.9	Roadside Pond 3	

Table 2. Locations in the Dehcho and Sahtu, NT, where boreal chorus frogs (*Pseudacris maculata*) were confirmed present in 2007 and/or 2008.

Vicinity	Latitude / longitude	Site name	Location within site (if applicable)
HWY 1 btwn Ft. Providence and Ft. Simpson	N61 08 39.0; W119 13 56.6	Roadside Pond 10; Wallace Creek rest area	
HWY 3 btwn Yellowknife and Ft. Providence	N61 54 55.2; W116 30 21.5	roadside rest area ~2hrs W of Yellowknife	
Blackstone	N60 56 55.1; W123 05 41.1	Nahanni Butte Winter Road Pond 1	
Blackstone	N60 57 13.7; W123 06 57.0	Nahanni Butte Winter Road Pond 2	
Ft. Liard	N60 18 17.2; W123 19 22.3	Muskeg River Gravel Pit	Pond 1
Ft. Liard	N60 18 11.7; W123 19 54.8	Muskeg River Gravel Pit	Pond 2
Ft. Liard	N60 18 07.7; W123 20 02.1	Muskeg River Gravel Pit	Pond 3
Ft. Liard	N60 18 12.9; W123 19 39.2	Muskeg River Gravel Pit	Pond 4
Ft. Liard	N60 09 28.0; W123 14 30.9	Roadside Pond 3	
Ft. Simpson	N62 02 27.9 W122 01 22.9	518 DOT Gravel Pit	Stop 1
Ft. Simpson	N62 02 34.9 W122 01 04.6	518 DOT Gravel Pit	Stop 2
Ft. Simpson	N61 46 39.9; W121 17 25.5	Gravel Pits ~10km E of Ft. Simpson	
Ft. Simpson	N61 46 53.1; W121 19 36.7	Roadside Pond 12	
Jean Marie	N61 29 57.2; W120 37 54.5	Roadside Pond 13	
Jean Marie	N61 28 14.7 W120 36 52.9	Roadside Pond 14	
Nahanni Butte	N61 01 51.5; W123 23 39.0	Nahanni Butte Village	sloughs near gas station being built
Nahanni Butte	N61 01 06.3; W123 22 09.4	Nahanni Butte Village	gravel drop just off the road
Wrigley	N62 27 29.0 W123 00 31.3	Roadside Pond 15	
Wrigley	N63 08 39.9 W123 15 38.1	Roadside Pond 16	
Wrigley	N62 37 51.0 W123 04 19.4	Roadside Pond 17	

Table 3. Locations in the Dehcho and Sahtu, NT, where wood frogs (*Lithobates sylvaticus* = *Rana sylvatica*) were confirmed present in 2007 and/or 2008.

Region	Latitude / longitude	Site name	Location within site (if applicable)
Ft. Liard	N60 15 21.5; W123 29 06.0	K29 Road	Stop 1
Ft. Liard	N60 16 32.2; W123 29 11.0	K29 Road	Stop 2
Ft. Liard	N60 16 53.0; W123 29 14.9	K29 Road	Stop 3
Ft. Liard	N60 17 16.5; W123 29 45.2	K29 Road	Stop 4 (= 5 km marker)
Ft. Liard	N60 19 20.3; W123 30 42.0	K29 Road	Stop 5
Ft. Liard	N60 17 34.2; W123 29 56.0	K29 Road	Stop 6
Ft. Liard	N60 16 35.5 W123 29 13.5	K29 Road	Stop 7
Ft. Liard	N60 16 37.4; W123 29 11.9	K29 Road	between 3 and 4 km marker
Ft. Liard	N60 17 16.2; W123 29 45.5	K29 Road	5 km marker
Ft. Liard	N60 18 06.2; W123 30.1 5.4	K29 Road	7 km marker
Ft. Liard	N60 20 26.6 W123 30 32.9	K29 Road	11 km marker
Ft. Liard	N60 19 16.3; W123 18 16.5	Muskeg Demonstration Forest	along trail
Ft. Liard	N60 18 17.2; W123 19 22.3	Muskeg River Gravel Pit	Pond 1
Ft. Liard	N60 18 11.7; W123 19 54.8	Muskeg River Gravel Pit	Pond 2
Ft. Liard	N60 18 07.7; W123 20 02.1	Muskeg River Gravel Pit	Pond 3
Ft. Liard	N60 18 12.9; W123 19 39.2	Muskeg River Gravel Pit	Pond 4
Ft. Liard	N60 18 14.9; 123 19 29.1	Muskeg River Gravel Pit	Pond 5
Ft. Liard	N60 11 31.0; W123 20 09.4	Roadside Pond 1	
Ft. Liard	N60 05 36.6; W123 05 34.7	Roadside Pond 2	
Ft. Liard	N60 09 28.0; W123 14 30.9	Roadside Pond 3	
Ft. Liard	N60 08 17.6; W123 11 37.7	Roadside Pond 4	

Table 3 continued. Wood frog locations in the Dehcho and Sahtu.

Region	Latitude / longitude	Site name	Location within site (if applicable)
HWY 7	N60 27 42.9; W123 22 07.4	Big Island Creek Bridge on HWY 7	
HWY 7	N60 26 06.3; W123 21 13.0	Roadside Pond 5	
HWY 7	N60 43 20.7; W123 21 12.9	Whistler's Landing	area from pond to boat launch
Blackstone	N61 07 33.3; W122 51 01.8	Lindberg Bed & Breakfast	
Blackstone	N60 56 55.1; W123 05 41.1	Nahanni Butte Winter Road Pond 1	
Blackstone	N60 57 13.5; W123 06 56.8	Nahanni Butte Winter Road Pond 2	
Nahanni Butte	N61 01 06.3; W123 22 09.4	Nahanni Butte Village	gravel drop just off the road
Nahanni Butte	N61 01 47.9; W123 23 10.5	Nahanni Butte Village	walking along roads
Nahanni Butte	N61 01 51.5; W123 23 39.0	Nahanni Butte Village	sloughs near gas station being built
Nahanni NPR	N61 09 43.7; W123 48 49.3	upper Jackfish River area	
Nahanni NPR	N61 13 24.2 W123 46 15.2	Yohin Lake Cabin area	
Nahanni NPR	N61 14 0.3; W123 45 29.7	Trail from Yohin Lake trailhead Cabin - Stop 1	
Nahanni NPR	N61 14 46.8; W123 59 43.1	Nahanni River Stop 1	S side of the river (river right)
Nahanni NPR	N61 15 27.3; W123 58 14.0	Nahanni River Stop 2	S side of the river (river right)
Ft. Simpson	N62 02 27.9 W122 01 22.9	518 DOT Gravel Pit	Stop 1
Ft. Simpson	N62 02 34.9 W122 01 04.6	518 DOT Gravel Pit	Stop 2
Ft. Simpson	N61 46 39.9; W121 17 25.5	Gravel Pits ~10km E of Simpson	
Jean Marie	N61 29 57.2; W120 37 54.5	Roadside Pond 13	
Jean Marie	N61 28 14.7 W120 36 52.9	Roadside Pond 14	
Wrigley / Ft. Simpson	N62 08 48.4 W122 31 35.7	Stop Point for N side of Ferry Crossing	
Wrigley	N62 27 29.0 W123 00 31.3	Roadside Pond 15	
Wrigley	N63 08 39.9 W123 15 38.1	Roadside Pond 16	

Table 3 continued. Wood frog locations in the Dehcho and Sahtu.

Region	Latitude / longitude	Site name	Location within site (if applicable)
Norman Wells	N65 17 25.6; W126 52 34.4	Bosworth Creek - Upper Bridge	
Norman Wells	N65 15 07.1; W126 39 53.2	DOT pond	
Norman Wells	N65 17 47.4; W126 49 53.1	Honey Bucket Road Slough	slough opposite sewage lagoon
Norman Wells	N65 16 30.1; W126 47 20.5	Loomis Greenhouse	small pond in yard
Norman Wells	N65 17 35.3; W126 36 21.6	Marshland btwn Jackfish Lake and Lake to the east	
Norman Wells	N65 18 11.5; W126 11 00.6	NW edge of Jackfish Lake	origin of Bosworth Creek
Norman Wells	N65 15 47.1 W126 43 35.3	VOR tower road	slough on side of road to tower
Norman Wells	N65 15 36.1; W126 41 52.1	W end of DOT Lake	"North-Wright Airstrip"
Norman Wells	N65 13 33.2; W126 51 08.2	W side of Mackenzie River	Stop 1
Norman Wells	N65 13 37.2; W126 46 23.5	W side of Mackenzie River	Stop 2
Norman Wells	N65 15 17.9; W126 58 26.4	W side of Mackenzie River	Canol Trail Head
Between Ft. Good Hope & Norman Wells	N66 00 23.4; W128 56 14.9	swampy area in the middle of nowhere	
Colville Lake	N67 02 31.5; W126 05 49.2	Colville Stop 2	
Colville Lake	N67 02 04.2; W126 05 58.2	Lily Pond	

Table 4. Snout-vent length and Gosner stages of development of tadpoles in the Dehcho and Sahtu, NT, in 2007 and 2008. Western toad tadpoles were encountered at a single site - Muskeg River Gravel Pit, near Ft. Liard - and the numbers of tadpoles encountered both years allowed for meaningful comparison between years. Measurements were combined across years for boreal chorus frogs and wood frogs. Wood frogs were the only amphibians encountered in both the Dehcho and Sahtu. Because of the large geographic distances between the southern-most and northern-most sites, wood frog tadpole measurements were grouped according to Region.

Species	Gosner Stage	Snout-vent length mean ± STD, number measured	Range of snout - vent lengths	Notes
western toad tadpoles (found at a single site in the Dehcho)				
2007	Stages 40 - 42	12.1 mm ± 0.7 mm , n = 12	11 - 13 mm	In 2007, 61 tadpoles were examined for developmental stage - all were stages 40 - 42. Twelve tadpoles were measured for snout-vent length.
2008	< Stage 26	8.7 mm ± 1.5 mm, n = 3	7 - 10 mm	
	Stages 26 - 30	no tadpoles encountered at these stages		
	Stages 31 - 35	no tadpoles encountered at these stages		
	Stages 36 - 39	11.9 mm ± 0.8 mm, n = 26	10 - 13 mm	
	Stages 40 - 41	13 mm ± 0 mm, n = 5	13 mm	
boreal chorus frog tadpoles (this species found only in the Dehcho)				
	< Stage 26	8.2 mm ± 3.0 mm, n = 23	3 - 12 mm	
	Stages 26 - 30	9.6 mm ± 1.9 mm, n = 11	7 - 13 mm	
	Stages 31 - 35	11.8 mm ± 1.1 mm, n = 6	10 - 13 mm	
	Stages 36 - 40	10.9 mm ± 0.9 mm, n = 12	9 - 12 mm	
	Stages 41 - 43	no tadpoles encountered at these stages		
	Stages 44 - 46	13.5 mm ± 1.3 mm, n = 4	12 - 15 mm	
wood frogs (Dehcho sites)				
	< Stage 26	9 mm, n =1	9 mm	
	Stages 26 - 30	no tadpoles encountered at these stages		
	Stages 31 - 35	16.2 mm ± 2.7 mm, n = 24	12 - 23 mm	
	Stages 36 - 40	19.7 mm ± 3.5 mm, n = 57	13 - 24 mm	92 tadpoles were in these stages of development, 57 of which were measured for snout-vent lengths.
	Stages 41 - 45	19.5 mm ± 2.1 mm, n = 10	17 - 24 mm	12 tadpoles were in these stages of development, 10 of which were measured for snout-vent lengths.
wood frogs (Sahtu sites, southern-most Sahtu sites in Norman Wells vicinity)				
	< Stage 26	no tadpoles encountered at these stages		
	Stages 26 - 30	12.9 mm ± 2.1 mm, n = 10	10 - 16 mm	
	Stages 31 - 35	16.4 mm ± 2.0 mm, n = 44	13 - 20 mm	
	Stages 36 - 40	20.2 mm ± 2.5 mm, n = 21	15 - 25 mm	
	Stages 41 - 44	22.0 mm ± 1.6 mm, n = 6	19 - 23 mm	

Table 5. Results of 2007 and 2008 surveys for chytrid fungus and ranavirus in amphibians in the Dehcho and Sahtu, NT. PCR-based diagnostics were used to screen samples for the pathogens. *Due 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 since tadpole tail clips are much less likely to be positive for chytrid than swabs, these results may reflect false negatives.

						Chytrid screen		Ranavirus screen	
Year	Region	Vicinity	Latitude / Longitude	Site name	Species	tadpoles	frogs	tadpoles	frogs
2007									
	Dehcho	Ft. Liard	N60 17 16; W123 29 46	K29 road	wood frog	--	0/5	--	0/5
	Dehcho	Ft. Liard	N60 19 16; W123 18 16	Muskeg River Demonstration Forest	wood frog	--	0/4	--	0/4
	Dehcho	Ft. Liard	N60 18 17; W123 19 22	Muskeg River Gravel Pit Ponds	wood frog	0/21	2/7	5/21	0/7
					western toad	0/61	0/1	0/61	0/1
	Dehcho	Ft. Liard	N60 05 37; W123 05 33	Roadside Pond 2	wood frog	0/25	--	2/25	--
	Dehcho	Ft. Liard	N60 09 28; W123 14 31	Roadside Pond 3	wood frog	0/7	0/10	0/7	0/10
	Dehcho	Ft. Liard	N60 08 18; W123 11 38	Roadside Pond 4	wood frog	0/12	0/2	3/12	0/2
	Dehcho	Blackstone	N61 07 33; W122 51 02	Lindberg Landing	wood frog	--	0/3	--	1/3
	Dehcho	Blackstone	N60 56 55; W123 05 39	Nahanni Butte Winter Road Pond 1	wood frog	0/12	--	1/12	--
					chorus frog	0/13	0/4	0/13	0/4
	Dehcho	Blackstone	N60 57 14; W123 06 57	Nahanni Butte Winter Road Pond 2	wood frog	0/5	0/7	0/5	0/7
	Dehcho	Nahanni NPR	N61 09 44; W123 48 49	near where Jackfish River enters South Nahanni River	wood frog	--	0/6	--	0/6
	Dehcho	Nahanni NPR	N61 13 24; W123 46 15	Yohin Lake	wood frog	--	0/5	--	1/5
	Sahtu	Norman Wells	N65 15 36; W126 41 52	Airstrip area	wood frog	0/5	--	0/5	--
	Sahtu	Norman Wells	N65 17 26; W126 52 34	Bosworth Creek - upper bridge	wood frog	--	0/13	--	0/13
	Sahtu	Norman Wells	N65 18 12; W126 11 1	Bosworth Creek - origin	wood frog	--	0/7	--	0/7
	Sahtu	Norman Wells	N65 15 18; W126 58 26	W side of Mackenzie River - Canol trailhead	wood frog	--	0/10	--	0/10
	Sahtu	Norman Wells	N65 13 33; W126 51 08	W side of Mackenzie River - stop 1	wood frog	--	0/3	--	0/3
	Sahtu	Norman Wells	N65 13 37; W126 46 24	W side of Mackenzie River - stop 2	wood frog	--	0/8	--	0/8
	Sahtu	Norman Wells	N65 15 8; W126 39 53	DOT Pond	wood frog	0/1	0/6	1/1	0/6
	Sahtu	Norman Wells	N65 17 47; W126 49 53	Honey Bucket Road Slough	wood frog	--	0/4	--	1/4

Table 5 continued. Results of amphibian pathogen screens.

Year	Region	Vicinity	Latitude / Longitude	Site name	Species	Chytrid screen		Ranavirus screen	
						tadpoles	frogs	tadpoles	frogs
	Sahtu	Norman Wells	N65 17 35; W126 36 22	Jackfish Lake - east end	wood frog	--	0/1	--	0/1
	Sahtu	Norman Wells	N65 16 30; W126 47 21	Loomis Greenhouse pond	wood frog	0/37	--	2/37	--
	Sahtu	Norman Wells	N65 15 47; W126 43 35	VOR tower road ponds	wood frog	0/1	--	0/1	--
	Sahtu	Norman Wells/ Ft. Good Hope	N66 00 23; W128 56 15	Middle of nowhere swamp	wood frog	--	0/2	--	0/2
2008									
	Dehcho	Jean Marie	N61 29 57; W120 37 55	Roadside Pond 13	wood frog	--	0/4	--	0/4
					chorus frog	0/30*	--	0/30	--
	Dehcho	Jean Marie	N61 28 15; W120 36 53	Roadside Pond 14	wood frog	0/8	0/23	0/8	0/23
					chorus frog	--	0/2	--	0/2
	Dehcho	Wrigley	N62 27 29; W123 00 31	Roadside Pond 15	wood frog	--	0/2	--	0/2
	Dehcho	Wrigley	N63 08 40; W123 15 38	Roadside Pond 16	wood frog	--	0/4	--	0/4
					chorus frog	--	0/3	--	0/3
	Dehcho	Wrigley	N62 37 51; W123 04 19	Roadside Pond 17	chorus frog	0/4	--	0/4	--
	Dehcho	Ft. Liard	N60 18 17; W123 19 22	Muskeg River Gravel Pit Ponds	wood frog	0/10	3/8	0/10	0/8
					chorus frog	0/4	1/1	0/4	0/1
					western toad	0/34	1/3	0/34	0/3
	Dehcho	Ft. Liard	N60 16 36; W123 29 14	K29 Road	wood frog	0/5	0/9	0/5	0/9
	Dehcho	Ft. Liard	N60 05 37; W123 05 33	Roadside Pond 2	wood frog	0/5	--	0/5	--
	Dehcho	Ft. Liard	N60 09 28; W123 14 31	Roadside Pond 3	wood frog	--	0/1	--	0/1
	Dehcho	Nahanni NPR	N61 14 47; W123 59 43	River Stop 1	wood frog	--	0/3	--	0/3
	Dehcho	Nahanni NPR	N61 15 27; W123 58 14	River stop 2	wood frog	--	0/2	--	0/2
	Dehcho	Nahanni NPR	N61 13 08; W123 46 26	Yohin Lake Cabin	wood frog	--	0/5	--	0/5
	Dehcho	Nahanni NPR	N61 14 0; W123 45 30	Yohin Trail Stop 1	wood frog	--	0/2	--	0/2
	Dehcho	Blackstone	N60 56 55; W123 05 39	Nahanni Butte Winter Road Pond 1	wood frog	0/16	--	0/16	--
	Dehcho	Blackstone	N60 57 14; W123 06 57	Nahanni Butte Winter Road Pond 2	wood frog	0/16	--	0/16	--

Table 5 continued. Results of amphibian pathogen screens.

Year	Region	Vicinity	Latitude / Longitude	Site name	Species	Chytrid screen		Ranavirus screen	
						tadpoles	frogs	tadpoles	frogs
	Sahtu	Norman Wells	N65 15 8; W126 39 53	DOT pond	wood frog	0/5	--	0/5	--
	Sahtu	Norman Wells	N65 17 47; W126 49 53	Honey Bucket Road Slough	wood frog	0/11	0/4	1/11	1/4
	Sahtu	Norman Wells	N65 15 47; W126 43 35	VOR tower road ponds	wood frog	0/12	0/1	3/12	0/1
	Sahtu	Colville Lake	N67 02 32; W126 05 49	Colville Stop 2	wood frog	--	0/1	--	0/1
	Sahtu	Colville Lake	N67 02 04; W126 05 58	Colville Lily Pond	wood frog	0/9	0/1	0/9	0/1

Figure 2. Highly variable coloration of three juvenile wood frogs from a single pond in the Ft. Liard area (27 June 2007). Wood frogs exhibit a remarkable

range of colours and patterns. In the Northwest Territories, the white upper lip stripe and dark brown eye mask (usually present) can be useful for identification, though not necessarily elsewhere in this species' range. Spotted individuals (lowest panel) are found more frequently in the northern parts of the range.

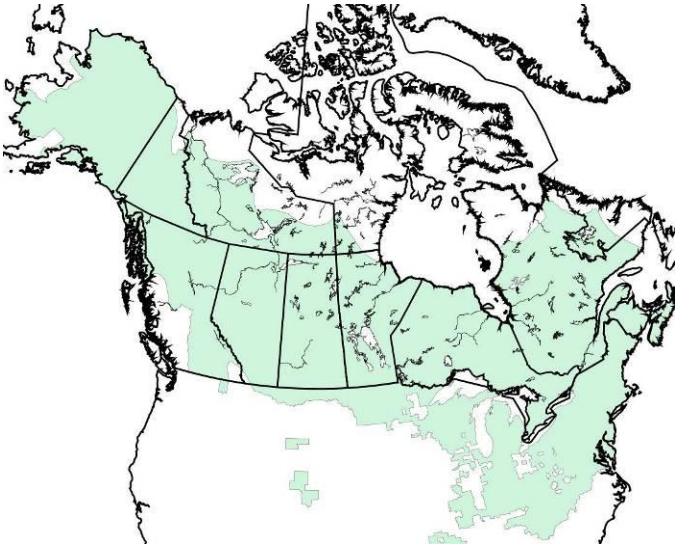

Figure 3. Geographic distribution of wood frogs. Wood frogs are the only North American amphibian found north of the Arctic Circle. The true distribution of wood frogs north of the Arctic Circle is not well defined and the north-most areas of maps should be interpreted cautiously. Map adapted from NatureServe (accessed 12 March 2009).

Figure 4. Examples of ponds where wood frog tadpoles were found. Top panel is a pond near the airstrip in Norman Wells, near, but separated from, the DOT Lake (background). Tadpoles were clustered in a 2 m x 1 m pocket on the left hand side of the pond. The middle panel is a pond in Colville Lake. Note that the

aquatic vegetation emerges several metres from the edge of the pond, indicative of shallow – and therefore relatively warm – water. The bottom panel is a road side pond south of Ft. Liard. Approximately 40% of the perimeter of the pond was accessible for survey. Within that area, tadpoles were clustered in two 2 m x 1 m pockets.

Figure 5. A wood frog that is metamorphosing. This individuals is at Gosner Stage 42 – 45. It was found on 13 July 2007 in the Norman Wells area pond mentioned in Figure 3.

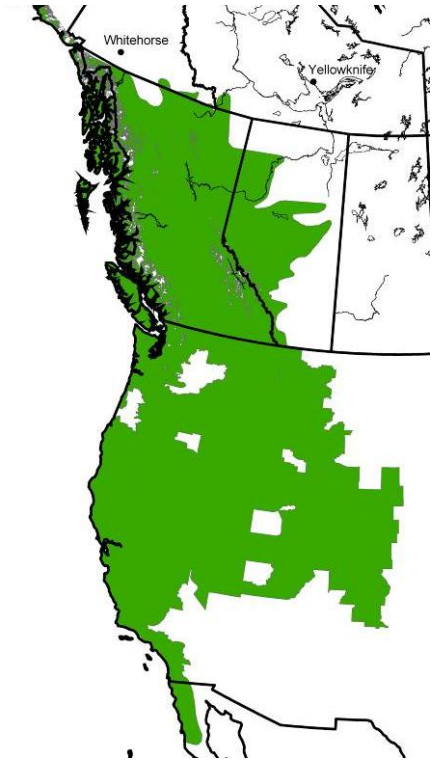

Figure 6. Geographic distribution of western toads. Map adapted from NatureServe (accessed 12 March 2009).

Figure 7. Site near Ft. Liard where western toad tadpoles were found in 2007 and 2008. This site is at the Muskeg River gravel pit and is frequented by ATVs and other motorized vehicles. Other western toad breeding sites likely exist in the vicinity although no others were found during the surveys despite concerted efforts to locate additional sites. Wood frog and boreal chorus tadpoles were also

found at this pond and nearby ponds in the gravel pit. Bottom panel is of western toad tadpoles that can be seen as black dots in the upper panel.

Figure 8. A western toad that has nearly completed metamorphosis. This individual is at Gosner Stage ~ 42 and was found at the Muskeg River gravel pit near Ft. Liard on 26 June 2007.

Figure 9. Western toad tadpoles (left) and a wood frog tadpole (right), shown together for comparison of size, colour, and general morphology. Notice that on the wood frog, the tailfin is high, rounded and originates mid-way up the body whereas the boreal toad tailfin is much lower in profile and originates at the base of the tail. These three tadpoles were found in the same pond within the Muskeg River gravel pit in the Ft. Liard area on 26 June 2007

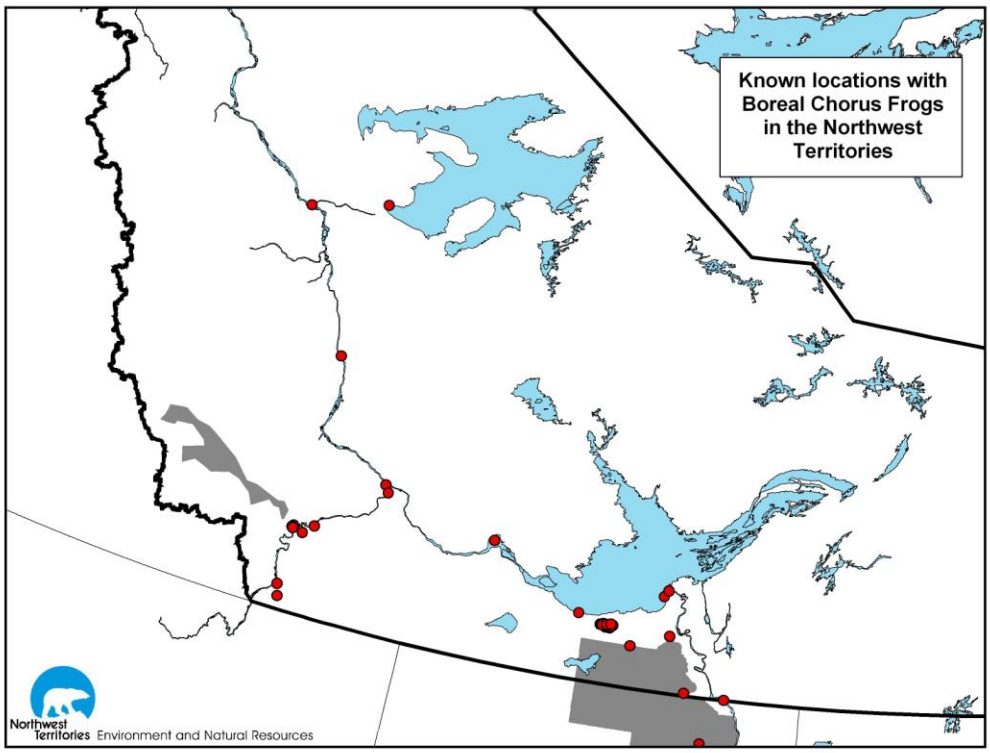

Figure 10. Known locations of boreal chorus frogs in the NWT. The distribution of boreal chorus frogs across North America is being resolved in light of recent taxonomic re-evaluations of the *Pseudacris* species complex.

Figure 11. Examples of ponds where boreal chorus frog tadpoles were found. The upper two panels are from the Blackstone area. Wood frog as well as boreal chorus frog tadpoles were found at these sites in 2007 and 2008. The lower panel is from the road side stop just east of Wallace Creek on Highway 1.

Hundreds of boreal chorus frog tadpoles were found in the ponds on either side of the culvert in 2008.

Figure 12. A boreal chorus frog that has nearly completed metamorphosis. This individual is at Gosner Stage 43 – 45 and was found at a pond in the Blackstone area on 28 June 2007.

Figure 13. Geographic distribution of Columbia spotted frogs, based on data from NatureServe (accessed 12 March 2009) and locations listed in Slough and Mennell (2006).

Figure 14. Geographic distribution of long-toed salamanders. Map adapted from NatureServe (accessed 12 March 2009).

Figure 15. A wood frog held separately in a new plastic bag with air and moist vegetation from the time of capture until release after processing to reduce the risk of spreading pathogens among individuals at a site.

Figure 16. Taking the snout-vent length measurement of a wood frog. Photo credit DG Allaire.

Figure 17. Swabbing the underside and legs of a wood frog for *Bd*. Swabbing collects cells on the surface of the frog that can then be tested for the presence of *Bd*. Photo credit DG Allaire.

Figure 18. Collecting a tail clip from a tadpole. A small piece of tissue was taken from the tip of the tail and then placed into vials containing 70% molecular grade ethanol. Tail and toe clips were screened for ranavirus and Bd. Photo credit DG Allaire.

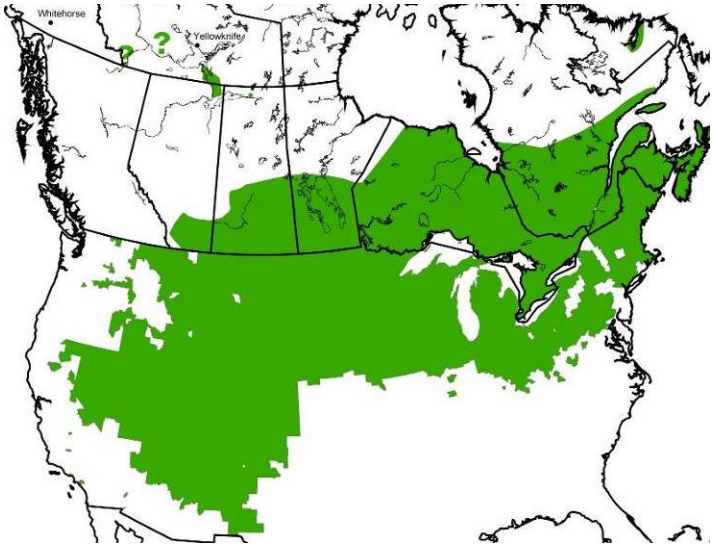

Figure 19. Geographic distribution of northern leopard frogs. The reports of northern leopard frogs in the Dehcho are unconfirmed. Map adapted from NatureServe (accessed 12 March 2009).

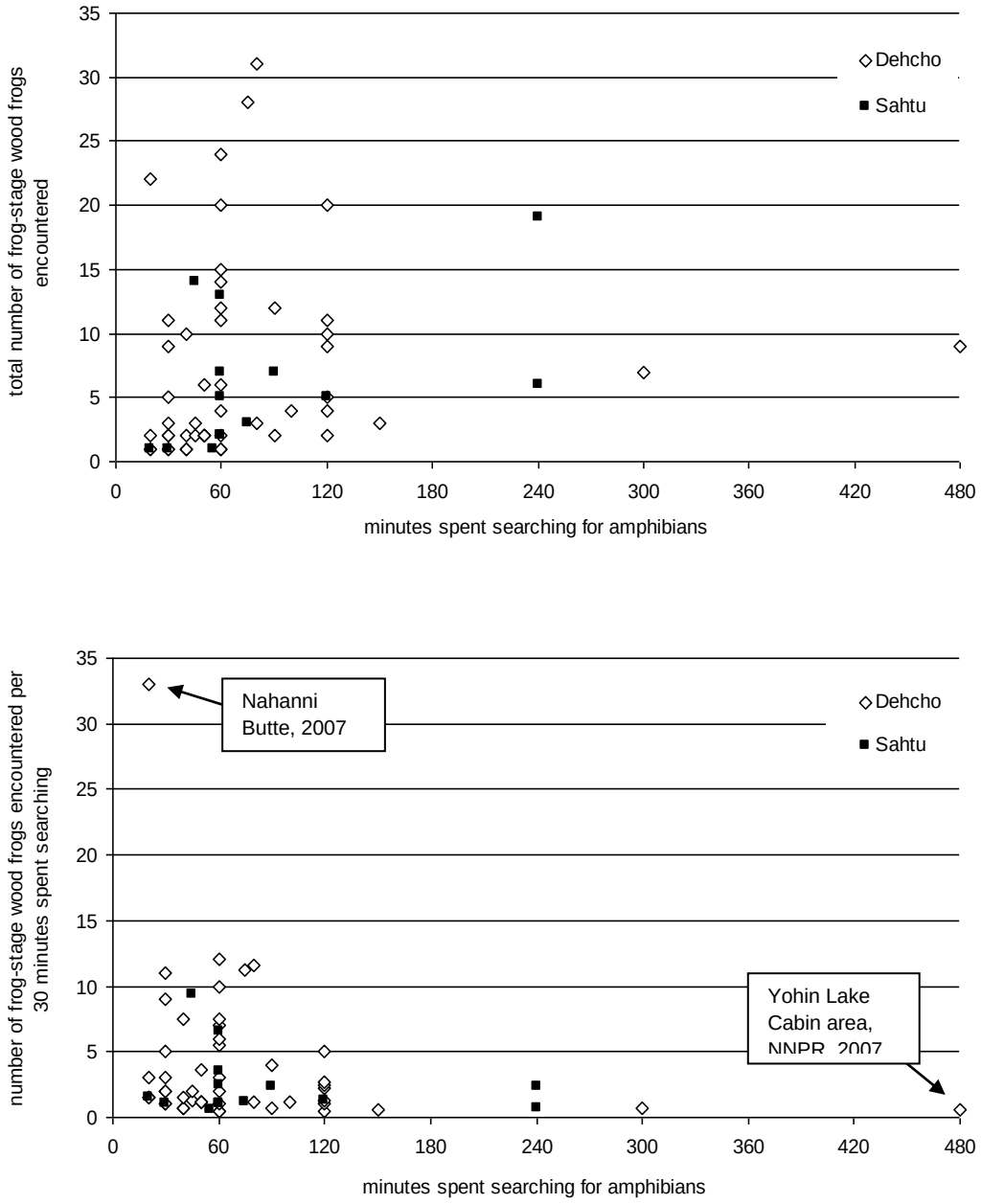

Figure 20. Returns on time investments during encounter surveys – numbers of frog-stage wood frogs encountered does not improve with time after 120 minutes of search time. A total of 64 site visits were conducted where comparisons of the number of frog-stage wood frog encountered are appropriate. The upper panel is

the total number of wood frogs encountered over time. The lower panel displays the same 64 data points standardized per 30 minutes of search time.

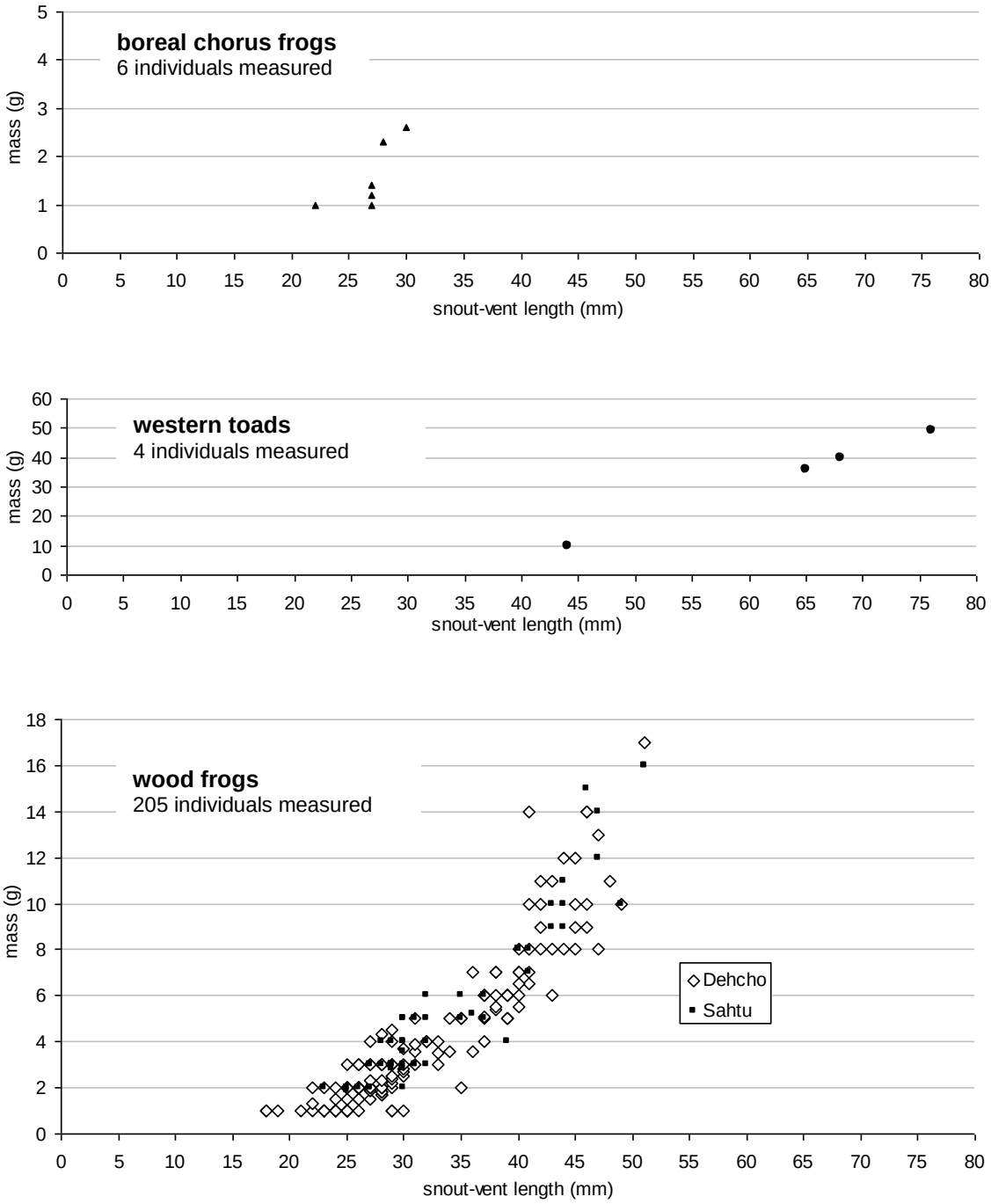

Figure 21. Scatter plots of snout-vent length versus mass of frog-stage boreal chorus frogs, western toads and wood frogs in the Dehcho and Sahtu, NT in 2007 and 2008. Not all wood frogs that were captured were measured. Note Y-axis in each panel differs to facilitate visualization of data. Mass measurements <

1 g were not reliable therefore the very smallest individuals were rounded up to 1 g.

Figure 22. A wood frog tadpole from Norman Wells (9 July 2007) with stereotypic gross pathology associated with ranavirus infections. The tadpole is in a plastic bag with pond water. Note the bloated body and the red blotches on the hind limbs and tail fin. The ranavirus from this tadpole, as well as all other the ranaviruses from amphibians in the NT during this study, were identified as Frog Virus 3 (FV3) based on their major capsid protein gene sequences.

Figure 23. A wood frog tadpole from Normal Wells (2 July 2008) with an unidentified infection of the cloaca. The tadpole is in a small plastic container with pond water. The white growth in the cloaca area contrasts with the black feces.

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APPENDICES

Appendix 1. Summary of the 2007 amphibian field survey in the Dehcho and Sahtu, NT.

Region	Vicinity	Site name	Location within site	GPS coordinates	Date of visit	Method examined	Species	Number physically observed		Number heard calling	Survey effort (time)	Weather conditions	Temp	Time of day	Samples collected	Sample ID (if any collected)	Field surveyors	Additional notes
								Frog	Tadpole									
Dehcho	Ft. Liard	Muskeg River Demonstration Forest	walked along trail	N60 19 16.3; W123 18 16.5	25-Jun-07	walk	wood frog	9			120 min total	mostly sunny turned to sudden down-pour	15 - 20°C	evening	4 toe clips	NT1 - NT4	Allaire, Schock	
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 1	N60 18 17.3; W123 19 22.2	25-Jun-07	walk, dipnet	wood frog	1	10 - 30		60 min total	mostly sunny to mostly cloudy	15 - 20°C	evening	1 toe, 2 tail	NT7 - NT9	Allaire, Schock	
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 1	N60 18 17.3; W123 19 22.2	25-Jun-07	walk, dipnet	western toad		200 - 300		60 min total	mostly sunny to mostly cloudy	15 - 20°C	evening	2 tail clips	NT5, NT6	Allaire, Schock	
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 2	N60 18 11.7; W123 19 54.8	25-Jun-07	walk, dipnet	wood frog	1			30 min total	mostly sunny to mostly cloudy	15 - 20°C	evening	1 toe	NT10	Allaire, Schock	
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 2	N60 18 11.7; W123 19 54.8	25-Jun-07	walk, dipnet	boreal chorus frog			2	30 min total	mostly sunny to mostly cloudy	15 - 20°C	evening			Allaire, Schock	
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 1	N60 18 17.3; W123 19 22.2	26-Jun-07	walk, dipnet	wood frog	1	1		NA	mostly sunny	20 - 25°C	morning - afternoon	1 toe, 1 tail	NT11, NT72	Allaire, Schock	Didn't "survey" this site this morning- instead, collected boreas tadpoles and spent time processing them
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 1	N60 18 17.3; W123 19 22.2	26-Jun-07	walk, dipnet	western toad		200 - 300		NA	mostly sunny	20 - 25°C	morning - afternoon	60 tail clips	NT12 - NT71	Allaire, Schock	Didn't "survey" this site this morning- instead, collected B boreas tadpoles and spent time processing them
Dehcho	Ft. Liard	Roadside Pond 1		N60 11 31.0; W123 20 09.4	26-Jun-07	walk, dipnet	wood frog		20 - 30		30 min total	mostly sunny	20 - 25°C	late afternoon			Allaire, Schock	
Dehcho	Ft. Liard	Roadside Pond 2		N60 05 36.5; W123 05 33.4	26-Jun-07	walk, dipnet	wood frog		25		120 min total	mostly sunny	20 - 25°C	late afternoon to evening	25 tail clips	NT73 - NT97	Allaire, Schock	Tadpoles found in two "pockets"; not evenly distributed throughout the pond
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 3	N60 18 06.5; W123 20 05.9	27-Jun-07	walk, dipnet	wood frog	4	18		120 min total	mostly sunny	15 - 20°C	morning	4 toe, 18 tail	NT104 - NT125	Allaire, Schock	Went back to the gravel pit to better explore the south area.
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 3	N60 18 06.5; W123 20 05.9	27-Jun-07	walk, dipnet	western toad	1			120 min total	mostly sunny	15 - 20°C	morning	1 toe	NT103	Allaire, Schock	Went back to the gravel pit to better explore the south area.
Dehcho	Ft. Liard	K29 Road	between 3 and 4 km marker	N60 16 37.4; W123 29 11.9	27-Jun-07	walk	wood frog	2			20 min total	mostly sunny	15 - 20°C	early afternoon			Allaire, Schock	
Dehcho	Ft. Liard	K29 Road	5 km marker	N60 17 16.2; W123 29 45.5	27-Jun-07	walk	northern leopard frog			1	20 min total	mostly sunny	15 - 20°C	early afternoon			Allaire, Schock	UNCONFIRMED - heard calls <50m into bush. Attempts to visually confirm identity not successful.
Dehcho	Ft. Liard	K29 Road	5 km marker	N60 17 16.2; W123 29 45.5	27-Jun-07	walk	wood frog	9			30 min total	mostly sunny	15 - 20°C	early afternoon	3 toe clips	NT100 - NT102	Allaire, Schock	
Dehcho	Ft. Liard	K29 Road	7 km marker	N60 18 06.2; W123 30.1 5.4	27-Jun-07	walk	wood frog	1			20 min total	mostly sunny	15 - 20°C	early afternoon	1 toe	NT98	Allaire, Schock	
Dehcho	Ft. Liard	K29 Road	15 km marker	N60 22 42.5; W123 31 18.5	27-Jun-07	walk	none				30 min total	mostly sunny to mostly cloudy	15 - 20°C	early afternoon			Allaire, Schock	
Dehcho	Ft. Liard	K29 Road	11 km marker	N60 20 26.6 W123 30 32.9	27-Jun-07	walk	wood frog	1			20 min total	light rain	15°C	late afternoon	1 toe	NT99	Allaire, Schock	
Dehcho	Ft. Liard	K29 Road	gravel pit pond adjacent to river edge at start of road	N60 15 16.2; W123 27 39.8	27-Jun-07	walk	none				20 min total	light rain	15°C	late afternoon			Allaire, Schock	
Dehcho	Ft. Liard	Roadside Pond 3		N60 09 28.0; W123 14 30.9	27-Jun-07	walk, dipnet	wood frog	10	3		120 min total	partly sunny	15 - 20°C	evening	10 toe, 3 tail clips	NT126-NT135, NT138, NT139, NT142	Allaire, Schock	
Dehcho	Ft. Liard	Roadside Pond 3		N60 09 28.0; W123 14 30.9	27-Jun-07	walk, dipnet	boreal chorus frog		4			partly sunny	15 - 20°C	evening	4 tail clips	NT136, NT137, NT140, NT141	Allaire, Schock	
Dehcho	Ft. Liard	Roadside Pond 3		N60 09 28.0; W123 14 30.9	28-Jun-07	walk	western toad	1			NA	mostly sunny	20°C	late morning			Allaire, Carriere, Schock, Stacey	Toad saw while returning the WF and CF processed night of 27 June 07. Unable to catch the toad.
Dehcho	Ft. Liard	Roadside Pond 4		N60 08 17.6; W123 11 37.7	27-Jun-07	walk, dipnet	wood frog	2	22		90 min total	mostly sunny	20°C	evening	2 toes, 12 tail clips	NT143 - NT156	Allaire, Carriere, Schock, Stacey	

Appendix 1 continued. Summary of the 2007 amphibian field survey in the Dehcho and Sahtu, NT.

Region	Vicinity	Site name	Location within site	GPS coordinates	Date of visit	Method examined	Species	Number physically observed		Number heard calling	Survey effort (time)	Weather conditions	Temp	Time of day	Samples collected	Sample ID (if any collected)	Field surveyors	Additional notes
								Frog	Tadpole									
Dehcho	HWY 7	Big Island Creek Bridge on HWY 7		N60 27 42.9; W123 22 07.4	28-Jun-07	walk	wood frog	2			50 min total	mostly sunny	15 - 20°C	mid-afternoon			Allaire, Carriere, Schock, Stacey	
Dehcho	HWY 7	Roadside Pond 5		N60 26 06.3; W123 21 13.0	28-Jun-07	walk	wood frog	5	4		120 min total	mostly sunny	20°C	mid-afternoon			Allaire, Carriere, Schock, Stacey	
Dehcho	HWY 7	Roadside Pond 6		N60 40 21.2; W123 26 54.9	28-Jun-07	walk	none				60 min total	partly sunny	20°C	late afternoon			Allaire, Carriere, Schock, Stacey	Pond full of small yellowish minnow like fish - photographed by S. Carriere.
Dehcho	HWY 7	Whistler's Landing	area from pond to boat launch	N60 43 20.7; W123 21 12.9	28-Jun-07	walk	wood frog	2			120 min total	partly sunny becoming rainy then downpour	15 - 20°C	late afternoon			Allaire, Carriere, Schock, Stacey	
Dehcho	Blackstone	Lindberg Bed & Breakfast		N61 07 33.3; W122 51 01.8	28-Jun-07	walk	wood frog	3			150 min total	light rain	15 - 20°C	evening	3 toe clips	NT157 - NT159	Allaire, Carriere, Schock, Stacey	
Dehcho	Blackstone	Nahanni Butte Winter Road Pond 1		N60 56 55.3; W123 05 39.2	29-Jun-07	walk	wood frog	11	1		120 min total	partly sunny	15 - 20°C	late morning	11 toe, 1 tail	NT172 - NT183	Allaire, Schock	
Dehcho	Blackstone	Nahanni Butte Winter Road Pond 1		N60 56 55.3; W123 05 39.2	29-Jun-07	walk	boreal chorus frog	4	21	1	120 min total	partly sunny	15 - 20°C	late morning	13 tail; 4 toe clips	NT184 - NT200	Allaire, Schock	
Dehcho	Blackstone	Nahanni Butte Winter Road Pond 2		N60 57 13.5; W123 06 56.8	29-Jun-07	walk	wood frog	12	5		60 min total	partly sunny	15 - 20°C	afternoon	7 toe clips; 5 tail	NT160 - NT171	Allaire, Schock	
Dehcho	Ft. Simpson	Snye W of landing strip		N61 51 39.5; W121 22 18.6	2-Jul-07	walk	none				60 min total	overcast	15 - 20°C	morning			Schock	"Pond" intermittently connected with larger water bodies, saw a pike in the water.
Dehcho	Nahanni Butte	Nahanni Butte Village	sloughs near gas station being built	N61 01 51.5; W123 23 39.0	3-Jul-07	walk	wood frog	15			60 min total	light rain	15°C	late morning			Hardisty, Matou, Schock, Tsetso	
Dehcho	Nahanni Butte	Nahanni Butte Village	sloughs near gas station being built	N61 01 51.5; W123 23 39.0	3-Jul-07	walk	boreal chorus frog			2	60 min total	light rain	15°C	late morning			Hardisty, Matou, Schock, Tsetso	
Dehcho	Nahanni Butte	Nahanni Butte Village	gravel pit on side of the road	N61 01 27.9; W123 23 2.6	3-Jul-07	walk	wood frog	24			60 min total	light rain	15°C	late morning			Hardisty, Matou, Schock, Tsetso	
Dehcho	Nahanni NPR	Nahanni NPR - cabin at Yohin Lake	Yohin Lake	N61 13 24.2 W123 46 15.2	3-Jul-07	walk	wood frog	9			480 min total	showers off and on all day	15°C	afternoon	5 toe clips	NT201 - NT205	Hardisty, Matou, Schock, Tsetso	
Dehcho	Nahanni NPR	Nahanni NPR - upper Jackfish River area	where Jackfish River enters the South Nahanni River	N61 09 43.7; W123 48 49.3	4-Jul-07	walk	wood frog	7			300 min total	clear	20°C	late afternoon and evening	6 toe clips	NT206 - NT211	Hardisty, Matou, Schock, Tsetso	Chose this general area from the air based on sandy soil plus trees near ponds that appear ephemeral and warm. The recent heavy flooding in the area may have affected the outcome of our survey and area should be checked again in the future.
Dehcho	Nahanni Butte	Nahanni Butte Village	gravel drop just off the road	N61 01 06.3; W123 22 09.4	5-Jul-07	walk	wood frog	28			75 min total	partly sunny	20°C	late morning	28 toe clips	NT212 - NT 239	Hardisty, Matou, Schock, Tsetso	
Dehcho	Nahanni Butte	Nahanni Butte Village	gravel drop just off the road	N61 01 06.3; W123 22 09.4	5-Jul-07	walk	boreal chorus frog			2	75 min total	partly sunny	20°C	late morning			Hardisty, Matou, Schock, Tsetso	
Dehcho	Nahanni Butte	Nahanni Butte Village	gravel pit on side of the road	N61 01 27.9; W123 23 2.6	5-Jul-07	walk	wood frog	22			20 min total	mostly sunny	20°C	afternoon			Hardisty, Matou, Schock, Tsetso	Stopped here again today to get a second site to allow comparisons of the abundance of frogs.
Sahtu	Norman Wells	VOR tower road ponds	slough on side of road to tower	N65 15 47.1 W126 43 35.3	7-Jul-07	walk, dipnet	wood frog		1		20 min total	mostly cloudy	20°C	afternoon	1 tail clip	NT240	Schock, Veitch	
Sahtu	Norman Wells	DOT Lake		N65 15 8.4; W126 39 53.0	7-Jul-07	walk, dipnet	wood frog	1			20 min total	mostly cloudy	20°C	afternoon			Schock, Veitch	

Appendix 1 continued. Summary of the 2007 amphibian field survey in the Dehcho and Sahtu, NT.

Region	Vicinity	Site name	Location within site	GPS coordinates	Date of visit	Method examined	Species	Number physically observed		Number heard calling	Survey effort (time)	Weather conditions	Temp	Time of day	Samples collected	Sample ID (if any collected)	Field surveyors	Additional notes
								Frog	Tadpole									
Sahtu	Norman Wells	Bosworth Creek - Upper Bridge		N65 17 25.6; W126 52 34.4	8-Jul-07	walk, dipnet	wood frog	19			240 min total	sunny	20 - 25°C	mid-morning	13 toe clips	NT241 - NT253	Guthrie, Meulenbroek, Meulenbroek, Schock	
Sahtu	Norman Wells	Jackfish Lake	campground & day use area	N65 17 38.5; W126 38 47.0	8-Jul-07	walk	none				60 min total	sunny	25°C	mid-afternoon			Guthrie, Meulenbroek, Meulenbroek, Schock	
Sahtu	Norman Wells	DOT Pond		N65 15 8.4; W126 39 53.0	9-Jul-07	walk, dipnet	wood frog	5	1		60 min total	sunny	20°C	morning	5 toe, 1 tail clip	NT 254 - NT259	Guthrie, Schock	
Sahtu	Between Ft. Good Hope and Norman Wells	swampy area in the middle of nowhere		N66 00 23.4; W128 56 14.9	9-Jul-07	walk, dipnet	wood frog	6			240 min total	sunny	20 - 25°C	afternoon until early evening	2 toe clips	NT 261, NT 262	Schock	
Sahtu	Between Ft. Good Hope and Norman Wells	a different swampy area in the middle of nowhere		N65 57 30.2; W128 55 51.2	9-Jul-07	walk, dipnet	none				60 min total	clear	20°C	evening			Schock	
Sahtu	Norman Wells	Marshland btwn Jackfish Lake and Lake to the east		N65 17 35.3; W126 36 21.6	10-Jul-07	walk, dipnet	wood frog	2			60 min total	sunny	25°C	early afternoon	1 toe clip	NT263	Guthrie, Kivy, Schock	
Sahtu	Norman Wells	NW edge of Jackfish Lake	origin of Bosworth Creek	N65 18 11.5; W126 11 00.6	10-Jul-07	walk, dipnet	wood frog	7			90 min total	sunny then storm moved in	20°C	afternoon	4 toe clips	NT260, NT264 - NT269	Guthrie, Kivy, Schock	
Sahtu	Norman Wells	Loomis Greenhouse	small pond in yard	N65 16 30.1; W126 47 20.5	11-Jul-07	dipnet	wood frog		100 - 200		NA	partly sunny	20°C	morning	37 tailclips	NT270 - NT306	Fowler, Lessie, Schock	Just put the dip net in a couple times and caught more tadpoles than needed for a sample.
Sahtu	Norman Wells	W side of Mackenzie River	Stop 1	N65 13 33.2; W126 51 08.2	11-Jul-07	walk	wood frog	2			60 min total	cloudy	15 - 20°C	early afternoon	2 toe clips	NT307, NT308	Fowler, Lessie, Popko, Schock	
Sahtu	Norman Wells	W side of Mackenzie River	Stop 2	N65 13 37.2; W126 46 23.5	11-Jul-07	walk	wood frog	13			60 min total	cloudy	15 - 20°C	mid-afternoon	9 toe clips	NT309 - NT317	Fowler, Lessie, Popko, Schock	
Sahtu	Norman Wells	W side of Mackenzie River	Canol Trail Head	N65 15 17.9; W126 58 26.4	11-Jul-07	walk	wood frog	14			45 min total	cloudy, storm moved in	15 - 20°C	late afternoon	10 toe clips	NT318 - NT327	Fowler, Lessie, Popko, Schock	
Sahtu	Norman Wells	DOT Lake		N65 15 09.8; W126 39 51.6	13-Jul-07	walk	wood frog	3			75 min total	sunny	20°C	late morning	1 toe clip	NT328	Lessie, Popko, Schock	Went back to this site to check for any additional sick/dead amphibians but none found.
Sahtu	Norman Wells	W end of DOT Lake	"North-Wright Airstrip"	N65 15 36.1; W126 41 52.1	13-Jul-07	walk, dipnet	wood frog		30		90 min total	sunny	20°C	early afternoon	5 tail clips	NT329 - NT 333	Lessie, Popko, Schock	Tadpoles all clustered in one pocket within one pond that was about 1 m deep.
Sahtu	Norman Wells	Roadside Pond 7	just S of VOR	N65 15 42.8; W126 43 13.1	13-Jul-07	walk, dipnet	none				45 min total	sunny	25°C	early afternoon			Lessie, Popko, Schock	Apparently suitable CF habitat - good candidate pond for "frog watch".
Sahtu	Norman Wells	Honey Bucket Road Slough	slough opposite of sewage lagoon	N65 17 47.4; W126 49 53.1	13-Jul-07	walk	wood frog	7			60 min total	sunny	25°C	afternoon	4 toe clips	NT334 - NT337	Lessie, Popko, Schock	
Sahtu	Norman Wells	Dodo Lake	W of Norman Wells	N64 53 57.9; W127 10 44.8	14-Jul-07	walk	none				75 min total	clear, few high clouds	15°C	morning			Kearney, Popko, Schock	
Sahtu	Norman Wells	Linton Lake	W of Norman Wells	N64 50 0.3; W127 14 45.8	14-Jul-07	walk	none				30 min total	clear, few high clouds	15°C	morning			Kearney, Popko, Schock	
Sahtu	Norman Wells	Washout Bridge Lake	W of Norman Wells	N64 43 01.3; W127 06 23.9	14-Jul-07	walk	none				45 min total	overcast	15°C	morning			Kearney, Popko, Schock	Fish seen surfacing in pools; R. Popko guessed some sort of trout. Also appears there may be a spring feeding the pools.
Sahtu	Norman Wells	Grotto Creek Area	W of Norman Wells	N64 44 30.6; W126 41 39.5	14-Jul-07	walk	none				60 min total	overcast	20°C	late morning			Kearney, Popko, Schock	

Appendix 2. Summary of the 2008 amphibian field survey in the Dehcho and Sahtu, NT.

Region	Vicinity	Site name	Location within site	GPS coordinates	Date of visit	Method examined	Species	Number physically observed		Number heard calling	Survey effort (time)	Weather conditions	Temp	Time of day	Samples collected	Sample ID (if any collected)	Field surveyors	Additional notes
								Frog	Tadpole									
	HWY 3 btwn Yellowknife and Ft Providence	roadside rest area ~2hrs W of Yellowknife		N61 54 55.2; W116 30 21.5	16-Jun-08	auditory	boreal chorus frog			2-5	N/A	light rain; been raining in the area for several hours; wind averaging 10km/hr	10°C	late morning			Schock	Heard the frogs calling on the N side of the HWY but precise pond not located. Elevation 230m.
Dehcho	HWY 1 btwn Ft. Providence and Ft. Simpson	Roadside Pond 10; Wallace Creek rest area		N61 08 39.0; W119 13 56.6	16-Jun-08	walk	boreal chorus frog		~200		20 min total	mostly cloudy, wind light	15°C	late afternoon			Schock	Shallow pond in the ditch between the outhouse and the road and the pond connected via culvert on the E side of the E approach into the pit stop. Some water 50cm deep but most water less than 30cm deep. Most tadpoles with SVL less than 10mm and no hind limbs (Gosner stage <30). Elevation 263m.
Dehcho	Ft. Simpson	Roadside Pond 11		N61 47 53.5; W121 19 19.2	16-Jun-08	walk, dipnet	none				30 min total	mostly sunny, wind 10km/hr	15 - 20°C	early evening			Schock	Swampy area on N and S side of HWY, just E of the Wriggley turn off. Only vertebrate encountered was small fish (~10cm total length). Elevation 165m.
Dehcho	Ft. Simpson	Roadside Pond 12		N61 46 53.1; W121 19 36.7	16-Jun-08	walk, dipnet	boreal chorus frog			2-5	15 min total	mostly sunny, wind 10km/hr	15 - 20°C	evening			Schock	Pond on S side of HWY, near airport turnoff. Water very dark with tanins. Pond extends at least 50m into swampy area with deciduous trees and areas that are only occasionally flooded whereas other areas appear to be ususally filled with water. Elevation 183m
Dehcho	Jean Marie	Roadside Pond 13		N61 29 57.2; W120 37 54.5	17-Jun-08	walk, dipnet	boreal chorus frog		1000's	2	60 min total	mostly sunny, calm	15 - 20°C	early afternoon	30 tail clips	NT404 - 433	Allaire, Schock	Surveyed on both sides of the road. Elevation 153m
Dehcho	Jean Marie	Roadside Pond 13		N61 29 57.2; W120 37 54.5	17-Jun-08	walk, dipnet	wood frog	6			60 min total	mostly sunny, calm	15 - 20°C	early afternoon	4 toe clips	NT400 - 403	Allaire, Schock	Surveyed on both sides of the road. Elevation 153m
Dehcho	Jean Marie	Roadside Pond 14		N61 28 14.7 W120 36 52.9	17-Jun-08	walk, dipnet	boreal chorus frog	2	200		80 min total	sunny, calm	20°C	late afternoon	2 toe clips	NT465-NT466	Allaire, Schock	Surveyed on both sides of the road, back towards Jean Marie from GPS point, but roadside ditches ran for at least 100m in both directions from GPS location. Counted the CF tadpoles for the encounter survey but didn't collect any for tissue samples since a good sample was collected at Roadside Pond 13. Focussed on collecting WF tissue samples. The CF tadpoles were very small (svl ~5mm).

Appendix 2 continued. Summary of the 2008 amphibian field survey in the Dehcho and Sahtu, NT.

Region	Vicinity	Site name	Location within site	GPS coordinates	Date of visit	Method examined	Species	Number physically observed		Number heard calling	Survey effort (time)	Weather conditions	Temp	Time of day	Samples collected	Sample ID (if any collected)	Field surveyors	Additional notes
								Frog	Tadpole									
Dehcho	Jean Marie	Roadside Pond 14		N61 28 14.7 W120 36 52.9	17-Jun-08	walk, dipnet	wood frog	31	13		80 min total	sunny, calm	20°C	late afternoon	8 tail clips, 23 toe clips	NT434 - NT464	Allaire, Schock	Surveyed on both sides of the road, back towards Jean Marie from GPS point, but roadside ditches ran for at least 100m in both directions from GPS location. Counted the CF tadpoles for the encounter survey but didn't collect any for tissue samples since a good sample was collected at Roadside Pond 13. Focussed on collecting WF tissue samples. The CF tadpoles were very small (svl ~5mm).
Dehcho	Wrigley	Roadside Pond 15		N62 27 29.0 W123 00 31.3	18-Jun-08	walk, dipnet	boreal chorus frog		200 - 500		40 min total	sunny, calsm	20°C	noon			Allaire, Schock	~80km S of Wrigley. Borrow Pit with sandy bottom on E side of road. Deep in middle but lots of emergent veg along shores. Clean clear water. 2 huge (15cm+ long) leeches encountered. Surveyed primarily 50m in either direction of where the "driveway" comes to the water. Most CF tadpoles Gosner <26 and svl 5-10mm. Elevation 174m
Dehcho	Wrigley	Roadside Pond 15		N62 27 29.0 W123 00 31.3	18-Jun-08	walk, dipnet	wood frog	1			40 min total	sunny, calsm	20°C	noon			Allaire, Schock	~80km S of Wrigley. Borrow Pit with sandy bottom. Deep in middle but lots of emergent veg along shores. Clean clear water. 2 huge (15cm+ long) leeches encountered. Surveyed primarily 50m in either direction of where the "driveway" comes to the water. Most CF tadpoles Gosner <26 and svl 5-10mm. Elevation 174m
Dehcho	Wrigley	Wrigley float plane base		N63 12 30.9 W123 24 35.6	18-Jun-08	walk	none				10 min total	sunny, calm	20°C	early afternoon			Allaire, Schock	Lake immediately E of the community. Unlikely frog breeding habitat due to depth, size of lake (=cold). Elevation 130m
Dehcho	Wrigley	Roadside Pond 16		N63 08 39.9 W123 15 38.1	18-Jun-08	walk	boreal chorus frog	3			60 min total	sunny, mostly calm but wind gusts sometimes to 10km/hr	20 - 25°C	mid afternoon	3 toe clips	NT467 - NT469	Allaire, Schock	~20km S of Wrigley. Swampy areas with moist sandy soil, horsetails on either side of road; surveyed within 100m in either direction of the "676" road marker. All frogs were found in short veg (<30cm tall), primarily horsetail, and were not at a pond.
Dehcho	Wrigley	Roadside Pond 16		N63 08 39.9 W123 15 38.1	18-Jun-08	walk	wood frog	4			60 min total	sunny, mostly calm but wind gusts sometimes to 10km/hr	20 - 25°C	mid afternoon	4 toe clips	NT470 - NT473	Allaire, Schock	~20km S of Wrigley. Swampy areas with moist sandy soil, horsetails on either side of road; surveyed within 100m in either direction of the "676" road marker. All frogs were found in short veg (<30cm tall), primarily horsetail, and were not at a pond.

Appendix 2 continued. Summary of the 2008 amphibian field survey in the Dehcho and Sahtu, NT.

Region	Vicinity	Site name	Location within site	GPS coordinates	Date of visit	Method examined	Species	Number physically observed		Number heard calling	Survey effort (time)	Weather conditions	Temp	Time of day	Samples collected	Sample ID (if any collected)	Field surveyors	Additional notes
								Frog	Tadpole									
Dehcho	Wrigley	Roadside Pond 17		N62 37 51.0 W123 04 19.4	18-Jun-08	walk, dipnet	boreal chorus frog		4		50 min total	light rain, wind calm	15 - 20°C	early evening	4 tail clips	NT474 - NT477	Allaire, Schock	Roadside pond on E side of road. Steep side against the road. Elevation 151m
Dehcho	Wrigley	Roadside Pond 17		N62 37 51.0 W123 04 19.4	18-Jun-08	walk, dipnet	boreal chorus frog			2	50 min total	light rain, wind calm	15 - 20°C	early evening			Allaire, Schock	Roadside pond on E side of road. Steep side against the road. Elevation 151m
Dehcho	Wrigley	Roadside Pond 15		N62 27 29.0 W123 00 31.3	18-Jun-08	walk	wood frog	3			N/A	overcast, calm	15 - 20°C	evening	2 toe clips	NT478 - NT479	Allaire, Schock	Stopped at Roadside Pond 15 again on way back to ferry to increase wood frog samples. Heard 5-10 CF calling and all the CF tadpoles again.
Dehcho	Wrigley/ Ft. Simpson	Stop Point for N side of Ferry Crossing		N62 08 48.4 W122 31 35.7	18-Jun-08	walk	wood frog	1			NA	mostly cloudy, wind light	15 - 20°C	evening			Allaire, Schock	Found the large wood frog in wet area associated with the culvert on the W side of the road where vehicles wait. No time to catch or process the frog since ferry was pulling in. Estimate 10g, 40mm svl. Elevation 108m
Dehcho	Ft. Simpson	518 DOT Gravel Pit	Stop 1	N62 02 27.9 W122 01 22.9	18-Jun-08	walk	wood frog	3			30 min total	lightly overcast, calm	20°C	late evening			Allaire, Schock	DOT Gravel Pit at ~ marker 518 N of Simpson. Encounter surveys only, no tissue samples. This particular area of ponds D. Allaire estimates is 5-7 years old. Scarce emergent veg, water 2+ deep in middle of some ponds. Elevation 124m.
Dehcho	Ft. Simpson	518 DOT Gravel Pit	Stop 1	N62 02 27.9 W122 01 22.9	18-Jun-08	walk	boreal chorus frog		10		30 min total	lightly overcast, calm	20°C	late evening			Allaire, Schock	DOT Gravel Pit at ~ marker 518 N of Simpson. Encounter surveys only, no tissue samples. This particular area of ponds D. Allaire estimates is 5-7 years old. Scarce emergent veg, water 2+ deep in middle of some ponds. Elevation 124m.
Dehcho	Ft. Simpson	518 DOT Gravel Pit	Stop 2	N62 02 34.9 W122 01 04.6	18-Jun-08	walk	wood frog	2			30 min total	lightly overcast, calm	20°C	late evening			Allaire, Schock	DOT Gravel Pit at ~ marker 518 N of Simpson. Encounter surveys only, no tissue samples. Oldest, most N and E area of ponds. Water <2m deep, most only 1m deep, lots of emergent veg. Elevation 124m.
Dehcho	Ft. Simpson	518 DOT Gravel Pit	Stop 2	N62 02 34.9 W122 01 04.6	18-Jun-08	walk	boreal chorus frog		12		30 min total	lightly overcast, calm	20°C	late evening			Allaire, Schock	DOT Gravel Pit at ~ marker 518 N of Simpson. Encounter surveys only, no tissue samples. Oldest, most N and E area of ponds. Water <2m deep, most only 1m deep, lots of emergent veg. Elevation 124m.

Appendix 2 continued. Summary of the 2008 amphibian field survey in the Dehcho and Sahtu, NT.

Region	Vicinity	Site name	Location within site	GPS coordinates	Date of visit	Method examined	Species	Number physically observed		Number heard calling	Survey effort (time)	Weather conditions	Temp	Time of day	Samples collected	Sample ID (if any collected)	Field surveyors	Additional notes
								Frog	Tadpole									
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 1	N60 18 17.2; W123 19 22.3	19-Jun-08	walk, dipnet	western toad		300 - 500 tadpoles		20 min total	sunny, wind mostly calm but up to 15km/hr	20°C	late afternoon			Allaire, Schock	Same pond where 100's of boreas tadpoles found in 2007. Tadpoles not sampled on 19 June; sampled on 21 June when there was more time. Elevation 198m
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 1	N60 18 17.2; W123 19 22.3	19-Jun-08	walk, dipnet	boreal chorus frog	1			20 min total	sunny, wind mostly calm but up to 15km/hr	20°C	late afternoon	1 toe clip	NT480	Allaire, Schock	Same pond where 100's of boreas tadpoles found in 2007. Tadpoles not sampled on 19 June; sampled on 21 June when there was more time. Elevation 198m
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 1	N60 18 17.2; W123 19 22.3	19-Jun-08	walk, dipnet	wood frog		10		20 min total	sunny, wind mostly calm but up to 15km/hr	20°C	late afternoon			Allaire, Schock	Same pond where 100's of boreas tadpoles found in 2007. Tadpoles not sampled on 19 June; sampled on 21 June when there was more time. Elevation 198m
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 3	N60 18 07.7; W123 20 02.1	19-Jun-08	walk, dipnet	western toad	1			30 min total	sunny, wind mostly calm but up to 15km/hr	20°C	early evening	1 toe clip	NT481	Allaire, Schock	Tadpoles not sampled on 19 June due to time constraints. Elevation 210 m
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 3	N60 18 07.7; W123 20 02.1	19-Jun-08	walk, dipnet	boreal chorus frog		2		30 min total	sunny, wind mostly calm but up to 15km/hr	20°C	early evening			Allaire, Schock	Tadpoles not sampled on 19 June due to time constraints. Elevation 210 m
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 3	N60 18 07.7; W123 20 02.1	19-Jun-08	walk, dipnet	wood frog	2	1		30 min total	sunny, wind mostly calm but up to 15km/hr	20°C	early evening	2 toe clips	NT482, NT483	Allaire, Schock	Tadpoles not sampled on 19 June due to time constraints. Elevation 210 m
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 4	N60 18 12.9; W123 19 39.2	19-Jun-08	walk, dipnet	western toad	2	3		50 min total	mostly sunny, calm	20°C	early evening	2 toe clips, 3 tail clips	NT484, NT485, NT492 - NT494	Allaire, Schock	This pond within Muskeg Gravel Pit not surveyed in 2007. Elevation 206m.
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 4	N60 18 12.9; W123 19 39.2	19-Jun-08	walk, dipnet	boreal chorus frog	6			50 min total	mostly sunny, calm	20°C	early evening	6 toe clips	NT487 - NT491	Allaire, Schock	This pond within Muskeg Gravel Pit not surveyed in 2007. Elevation 206m.
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 4	N60 18 12.9; W123 19 39.2	19-Jun-08	walk, dipnet	wood frog		4		50 min total	mostly sunny, calm	20°C	early evening	4 tail clips	NT495 - NT498	Allaire, Schock	This pond within Muskeg Gravel Pit not surveyed in 2007. Elevation 206m.

Appendix 2 continued. Summary of the 2008 amphibian field survey in the Dehcho and Sahtu, NT.

Region	Vicinity	Site name	Location within site	GPS coordinates	Date of visit	Method examined	Species	Number physically observed		Number heard calling	Survey effort (time)	Weather conditions	Temp	Time of day	Samples collected	Sample ID (if any collected)	Field surveyors	Additional notes
								Frog	Tadpole									
Dehcho	Ft. Liard	K29 Road	Stop 1	N60 15 21.5; W123 29 06.0	20-Jun-08	walk	wood frog	10			40 min total	mostly sunny, calm	15°C	mid morning			Allaire, Schock	Encounter survey along the trail. No tissue samples taken in the interest of time - priority given to the encounter survey. 9/10 WF were recent metas whereas 1 was an adult with estimated weight ~10 g. Elevation 236m.
Dehcho	Ft. Liard	K29 Road	Stop 2	N60 16 32.2; W123 29 11.0	20-Jun-08	walk	wood frog	5	1		30 min total	mostly sunny, calm	15°C	late morning			Allaire, Schock	Encounter survey along W side of road (sandy+muddy ditch and swampy area up to 20m W of road). No tissue samples taken in the interest of time - priority given to the encounter survey. 2/5 frogs were recent metas, the other 3 were adult sized. Elevation 255 m.
Dehcho	Ft. Liard	K29 Road	Stop 3	N60 16 53.0; W123 29 14.9	20-Jun-08	walk	wood frog	2			60 min total	mostly sunny, calm	20°C	late morning			Allaire, Schock	Bridge at 4 km marker. "Walked" along stream over which the bridge crosses but thick veg made it difficult to cover much distance - only about 50m in either direction of the bridge surveyed in any meaningful way. The WF were encountered near the road. Elevation 319 m.
Dehcho	Ft. Liard	K29 Road	Stop 4 = 5 km marker	N60 17 16.5; W123 29 45.2	20-Jun-08	walk	wood frog	20			120 min total	mostly sunny, calm	20°C - 25°C	early afternoon			Allaire, Schock	This is the vicinity where I (Schock) thought I heard a northern leopard frog in 2007. We searched the area thoroughly for an hour, including flipping logs and bush-whacking. No evidence of northern leopard frogs whatsoever. The 2007 encounter remains unconfirmed. No tissue samples taken in the interest of time - priority given to the encounter survey. Elevation 352m.
Dehcho	Ft. Liard	K29 Road	Stop 5	N60 19 20.3; W123 30 42.0	20-Jun-08	walk	wood frog	2			50 min total	mostly sunny, calm	20°C - 25°C	afternoon			Allaire, Schock	Near the 9km marker. Walked along small stream that goes under road, then followed the streams that feeds it for another 50m or so. Lots of caddisfly larvae on the underside of flat rocks in the streams. No tissue samples taken in the interest of time - priority given to the encounter survey. Elevation 330m.
Dehcho	Ft. Liard	K29 Road	Stop 6	N60 17 34.2; W123 29 56.0	20-Jun-08	walk	wood frog	11			30 min total	mostly sunny, calm	25°C	afternoon			Allaire, Schock	Surveyed slough and then followed stream on W side of road for ~100m (NW-ward direction). No tissue samples taken in the interest of time - priority given to the encounter survey. Elevation 328m.

Appendix 2 continued. Summary of the 2008 amphibian field survey in the Dehcho and Sahtu, NT.

Region	Vicinity	Site name	Location within site	GPS coordinates	Date of visit	Method examined	Species	Number physically observed		Number heard calling	Survey effort (time)	Weather conditions	Temp	Time of day	Samples collected	Sample ID (if any collected)	Field surveyors	Additional notes
								Frog	Tadpole									
Dehcho	Ft. Liard	K29 Road	Stop 7	N60 16 35.5 W123 29 13.5	20-Jun-08	walk	wood frog	14	25		60 min total	mostly sunny, calm	25°C	afternoon	9 toe clips, 5 tail clips	NT499 - NT512	Allaire, Schock	Slough and pond ~20m W of road, through the willows. Max depth <1m, lots of dead wood and moss. Collected samples from only 5 tadpoles because of time constraints. Elevation 332m.
Dehcho	Ft. Liard	K29 Road	Stop 8	N60 15 13.7; W123 27 41.2	20-Jun-08	walk	none				15 min total	partly sunny	20°C	late afternoon			Allaire, Schock	The relatively new gravel pit right near the river. Deep water, very little veg. Maybe in a few years some frogs will move in. Elevation 219m.
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 5	N60 18 14.9; 123 19 29.1	21-Jun-08	walk, dipnet	wood frog	2			40 min total	mostly sunny	15°C	morning			Schock	This pond within Muskeg Gravel Pit not surveyed in 2007. Logs and assorted debris at NE end of this pond would make potentially good garter snake habitat if they were in the area. Elevation 209m.
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 1	N60 18 17.2; W123 19 22.3	21-Jun-08	walk, dipnet	western toad		31		NA	mostly sunny becoming cloudy. Ultimately chased out of the area by downpour.	20°C	afternoon	31 tail clips	NT524 - NT554	Schock	This trip to Muskeg River Gravel Pit specifically to collect tissue samples. Boreas tadpoles in distinct clumps in the shallowest, clearest part of the pond. Wood frog tadpoles found exclusively in the deeper (~30cm) water with more vegetation.
Dehcho	Ft. Liard	Muskeg River Gravel Pit	Pond 1	N60 18 17.2; W123 19 22.3	21-Jun-08	walk, dipnet	wood frog		11		NA	mostly sunny becoming cloudy. Ultimately chased out of the area by downpour.	20°C	afternoon	11 tail clips	NT513 - NT523	Schock	This trip to Muskeg River Gravel Pit specifically to collect tissue samples. Boreas tadpoles in distinct clumps in the shallowest, clearest part of the pond. Wood frog tadpoles found exclusively in the deeper (~30cm) water with more vegetation.
Dehcho	Ft. Liard	Roadside Pond 2		N60 05 36.6; W123 05 34.7	21-Jun-08	walk, dipnet	wood frog		5		40 min total	overcast, mostly calm	10 - 15°C	late evening	5 tail clips	NT555 - NT559	Schock	elevation 405m
Dehcho	Ft. Liard	Roadside Pond 3		N60 09 34.0 W123 14 17.5	22-Jun-08	walk, dipnet	wood frog	1			**40 min total	overcast, occassional light rain, wind gusts	10 - 15°C	late morning	1 toe clip	NT 560	Schock	** weather conditions severely impeded survey; not representative, do not include in calculations. Two black bears seen along road within 1 km of the turn in to this pond in the past 24 hours. elevation 394m
Dehcho	Nahanni NPR	Kraus Hot Springs		N61 15 18.5; W124 03 36.9	25-Jun-08	walk	none				120 min total	mostly sunny	20°C	afternoon			Okrainec, Schock	River very high so the hot springs actually underwater. Upland, water in the immediate vicinity (~200-500m radius of the campsite) highly mineralized and not likely to support amphibians. Attempts to survey further upland from river severely hindered by the dense vegetation. Elevation 244m

Appendix 2 continued. Summary of the 2008 amphibian field survey in the Dehcho and Sahtu, NT.

Region	Vicinity	Site name	Location within site	GPS coordinates	Date of visit	Method examined	Species	Number physically observed		Number heard calling	Survey effort (time)	Weather conditions	Temp	Time of day	Samples collected	Sample ID (if any collected)	Field surveyors	Additional notes
								Frog	Tadpole									
Dehcho	Nahanni NPR	Nahanni River Stop 1	S side of the river (river right)	N61 14 46.8; W123 59 43.1	26-Jun-08	walk	wood frog	4			100 min total	mostly sunny	20°C	early afternoon	3 toe clips	NT561 - NT563	Deneyoua, Okrainec, Schock, Tate	Location = where a fast moving stream enters the river (S side of river); several little pools in the sandy areas of the stream's "flood plain", Equisetum. The wood frogs were found in the upland areas where there was conifer as well as deciduous cover. Elevation 233m.
Dehcho	Nahanni NPR	Nahanni River Stop 2	S side of the river (river right)	N61 15 27.3; W123 58 14.0	26-Jun-08	walk	wood frog	3			45 min total	sunny	25°C	afternoon	2 toe clips	NT564 - NT 565	Deneyoua, Okrainec, Schock, Tate	Recent metas, just wee, ~1.5g, natal pond must be somewhere nearby. Elevation 231m.
Dehcho	Nahanni NPR	Nahanni River Stop 3	N side of the river (river left)	N61 15 40.1; W123 50 43.2	26-Jun-08	walk	none				60 min total	sunny	25°C	late afternoon			Deneyoua, Okrainec, Schock, Tate	Explored the area in the stream's flood plain, much of which with well established willows, as well as some of the surrounding upland as best as could be accomplished. A small, shallow, clear pool near mouth of a small snye (log pile at W-most end) looks like possible toad breeding habitat although the pond may be fully connected to river much of the year. Other small ponds along the stream, closer to the river, with schools of small sivery fish (<4cm long) and it appeared the fishes had recently become stranded. Elevation 221m.
Dehcho	Nahanni NPR	Nahanni River Stop 4 - Yohin Lake Trail Head	S side of the river (river right)	N61 14 12.4; W123 44 54.8	26-Jun-08	walk	none				60 min total	clear, calm	20°C	late evening			Tate, Schock	No amphibians encountered in the immediate vicinty of our campsite, which was at the established site at the trail head. The bank had washed away a lot and the entrance to the trail head was tough to spot from the river. Elevation 237m.
Dehcho	Nahanni NPR	Trail from trailhead to Yohin Lake - Stop 1	~600m from campsite, 2km from Yohin Lake Cabin	N61 14 0.3; W123 45 29.7	27-Jun-08	walk	wood frog	3			80 min total	sunny, calm, but in the trees	20°C	noon	2 toe clips	NT567 - NT568	Deneyoua, Okrainec, Schock, Tate	Not far from a slough with lots of emergent vegetation. Elevation 214m.
Dehcho	Nahanni NPR	Trail from trailhead Stop 1 en route to Yohin Lake Cabin		Start: N61 14 0.3; W123 45 29.7 Stop: N61 13 07.9; W123 46 26.4	27-Jun-08	walk	wood frog	11			**60 min total	partly sunny, calm	25°C	early afternoon			Deneyoua, Okrainec, Schock, Tate	**Not consistent methodology with encounter surveys at other locations: Visual encounter while walking quickly as possible to Yohin Lake along the established trail. Kept a tally of frogs seen by any of us. 8/11 wood frogs encountered were within the last 1/3 of the trail, that is, closest to Yohin Lake.
Dehcho	Nahanni NPR	Yohin Lake Cabin area		N61 13 07.9 W123 46 26.4	27-Jun-08	walk, dipnet	wood frog	12			90 min total	partly sunny, calm	25°C	early afternoon	5 toe clips	NT569 - NT573	Deneyoua, Okrainec, Schock, Tate	No obvious toad breeding habitat in the cabin area, and, as in 2007, pike seen in Yohin Lake as well as the smaller sloughs in the vicinity.

Appendix 2 continued. Summary of the 2008 amphibian field survey in the Dehcho and Sahtu, NT.

Region	Vicinity	Site name	Location within site	GPS coordinates	Date of visit	Method examined	Species	Number physically observed		Number heard calling	Survey effort (time)	Weather conditions	Temp	Time of day	Samples collected	Sample ID (if any collected)	Field surveyors	Additional notes
								Frog	Tadpole									
Dehcho	Just E of Nahanni NPR boundary	Nahanni River Stop 5	N side of the river (river left)	N61 03 57.6; W123 33 18.5	27-Jun-08	walk	none				NA	clear, calm	15°C	late evening			Schock	This is where we made camp on night of 27 June - Schock looked around the vicinity of the campsite (~200m, as veg/water/energy would allow).
Dehcho	Nahanni Butte	Nahanni Butte Village	walking along roads	N61 01 47.9; W123 23 10.5	28-Jun-08	walk	wood frog	1			60 min total	mostly sunny, wind gusts up to 15km/hr	15°C - 20°C	evening			Deneyoua, Schock, Tate	Unlike in 2007, we had very limited time to look around Nahanni Butte, and no vehicle to cover distance. Surveys in 2007 and 2008 not comparable. The WF encountered in 2008 was a large adult, estimated weight ~10-12g. No time to collect tissue sample due to water taxi departure time.
Dehcho	Blackstone	Nahanni Butte Winter Road Pond 1		N60 56 55.1; W123 05 41.1	29-Jun-08	walk, dipnet	boreal chorus frog		200 - 500		45 min total	sunny, wind gusts up to 20km/hr	20°C - 25°C	evening			Schock	In the interest of time, especially making the ferry back across to Simpson, sampled only 16 of the WF tadpoles. Elevation 216m.
Dehcho	Blackstone	Nahanni Butte Winter Road Pond 1		N60 56 55.1; W123 05 41.1	29-Jun-08	walk, dipnet	wood frog	2	200 - 500		45 min total	sunny, wind gusts up to 20km/hr	20°C - 25°C	evening	16 tail clips	NT590 - NT605	Schock	In the interest of time, especially making the ferry back across to Simpson, sampled only 16 of the WF tadpoles. Elevation 216m.
Dehcho	Blackstone	Nahanni Butte Winter Road Pond 2		N60 57 13.7; W123 06 57.0	29-Jun-08	walk, dipnet	boreal chorus frog			1	NA	sunny, wind gusts up to 20km/hr	25°C	afternoon			Schock	This chorus frog called multiple times while I was processing the wood frog tadpoles (took ~ 2 hours). Elevation 208m
Dehcho	Blackstone	Nahanni Butte Winter Road Pond 2		N60 57 13.7; W123 06 57.0	29-Jun-08	walk, dipnet	wood frog		>100		40 min total	sunny, wind gusts up to 20km/hr	25°C	afternoon	15 tail clips	NT574 - NT589	Schock	Surveyed pond on N side of the road. All tadpoles at ~ same stage of development (Gosner 39-40). Tadpoles highly clumped in space; more than 80% were encountered along the N and W edges (water ~30cm deep, clear but lots of veg). Deepest part of this pond appears to be >2m. Elevation 208m
Dehcho	Ft. Simpson	Gravel Pits ~10km E of Ft. Simpson, near "Mexican Hat" VOR site.		N61 46 39.9; W121 17 25.5	30-Jun-08	walk, dipnet	boreal chorus frog		10		60 min total	partly sunny, calm	15°C - 20°C	early afternoon			Tate, Schock	Walked and dipped along the edges of ponds in the middle of the pits. No tissue samples due to time contraints. Ferocious "bulldogs"; serious blood loss by Danna. Doug photographed a CF tadpole and a dragonfly that had caught a bulldog. Elevation 166m.
Dehcho	Ft. Simpson	Gravel Pits ~10km E of Ft. Simpson, near "Mexican Hat" VOR site.		N61 46 39.9; W121 17 25.5	30-Jun-08	walk, dipnet	wood frog	20			60 min total	partly sunny, calm	15°C - 20°C	early afternoon			Tate, Schock	Walked and dipped along the edges of ponds in the middle of the pits. No tissue samples due to time contraints. Ferocious "bulldogs"; serious blood loss by Danna. Doug photographed a CF tadpole and a dragonfly that had caught a bulldog. Elevation 166m.

Appendix 2 continued. Summary of the 2008 amphibian field survey in the Dehcho and Sahtu, NT.

Region	Vicinity	Site name	Location within site	GPS coordinates	Date of visit	Method examined	Species	Number physically observed		Number heard calling	Survey effort (time)	Weather conditions	Temp	Time of day	Samples collected	Sample ID (if any collected)	Field surveyors	Additional notes
								Frog	Tadpole									
Dehcho	HWY 1 btwn Ft. Providence and Ft. Simpson	Roadside Pond 10; Wallace Creek pit stop		N61 08 39.0; W119 13 56.6	30-Jun-08	walk	boreal chorus frog		100 - 200		NA	partly sunny, wind gusty.	20°C - 25°C	late afternoon			Schock	CF tadpoles at Gosner stages 36 - 45. Water dropped a bit since 18 June 08, but water still 30 - 50cm deep on both sides of the culvert. Elevation 258m.
Sahtu	Norman Wells	DOT pond		N65 15 07.1; W126 39 53.2	1-Jul-08	walk, dipnet	wood frog		2		30 min total	bright with high overcast	20°C	mid afternoon	2 tail clips	NT606, NT607	Veitch, Schock	This site positive for ranavirus 2007. Elevation 63m.
Sahtu	Norman Wells	Honey Bucket Road Slough		N65 17 46.9; W126 49 54.2	2-Jul-08	walk, dipnet	wood frog	5	11		120 min total	partly sunny, calm	15°C - 20°C	mid morning	4 toe clips, 11 tail clips; 3 whole tadpoles	NT608 - NT622	Schock	Spent several hours here to do a thorough job since ranavirus detected from this site in 2007. Total area surveyed ~ 150m E of bridge and ~ 100 m S of bridge; didn't go further S because water gets increasingly deeper and with less emergent veg (i.e less tadpole-friendly). Tadpoles very clumped: 10 of the 11 caught in a single 10m x 10m section of the ditch that runs E from the bridge. 3 whole tadpoles collected (also tail clipped) to better examine the white growth associated with their cloacas (=NT609, NT616, NT617). Photographed at ENR office. Elevation 64m
Sahtu	Norman Wells	VOR tower road ponds	ponds on either side of driveway to tower, just off the HWY.	N65 15 47.1; W126 43 35.3	2-Jul-08	walk, dipnet	wood frog	2	12		60 min total	partly sunny, calm	20°C - 25°C	evening	1 toe clips, 12 tail clips	NT623 - NT635	Schock	This is the vicinity where Phil Spencer, ENR - GIS specialist, indicated he had heard chorus frogs in mid-May 2008. However, I did not encounter any chorus frogs.
Sahtu	Colville Lake	Colville Stop 1		N67 02 12.5; W126 05 30.0	3-Jul-08	walk, dipnet	none				30 min total	partly cloudy, wind 5-15km/hr	15°C	late afternoon			Veitch, Schock	Upland swampy area near bridge. Elevation 259m.
Sahtu	Colville Lake	Colville Stop 2		N67 02 31.5; W126 05 49.2	3-Jul-08	walk, dipnet	wood frog	1			30 min total	partly cloudy, wind 5-15km/hr	15°C	early evening	1 toe clip	NT636	Veitch, Schock	"Pond" N of gas station - can see the Coop. Is about 15m x 10m total size. The children from the community said they'd seen frogs here in the past. No evidence of breeding at this pond - seems more likely that migrating frogs travel through this part of the community. Elevation 252m.
Sahtu	Colville Lake	Colville Stop 3		N67 02 37.4; W126 05 33.5	4-Jul-08	walk, dipnet	none				55 min total	clear, wind up to 15km/hr	10°C	evening			Veitch, Schock	Pond S of water treatment plant, the E and N sides of the pond. Time of day, temperature and/or wind may have factored into the survey but we only had 24 hrs in Colville Lake so did what we could with the time we had. Elevation 250m.
Sahtu	Colville Lake	Colville Stop 4		N67 02 43.1; W126 05 31.5	4-Jul-08	walk, dipnet	none				40 min total	clear, wind up to 15km/hr	10°C	morning			Veitch, Schock	Elevation 249m.
Sahtu	Colville Lake	Colville Stop 5		N67 02 40.4 W126 05 54.0	4-Jul-08	walk, dipnet	none				30 min total	clear, wind up to 15km/hr	10°C - 15°C	morning			Veitch, Schock	The W edge of pond described for Colville Stop 3.

Appendix 2 continued. Summary of the 2008 amphibian field survey in the Dehcho and Sahtu, NT.

Region	Vicinity	Site name	Location within site	GPS coordinates	Date of visit	Method examined	Species	Number physically observed		Number heard calling	Survey effort (time)	Weather conditions	Temp	Time of day	Samples collected	Sample ID (if any collected)	Field surveyors	Additional notes
								Frog	Tadpole									
Sahtu	Colville Lake	Colville Lily Pond		N67 02 04.2; W126 05 58.2	4-Jul-08	walk, dipnet	wood frog	1	14		55 min total	sunny, wind <5km/hr.	$15^{o}C$ - $20^{o}C$	morning	1 toe clip, 9 tail clips	NT637 - NT646	Veitch, Schock	N edge of this pond, which is at the S edge of the community. Lots of emergent veg, including water lilies. Tadpoles within a ~20m stretch of the shore-line, all within ~3m of the shore. Elevation 250m.
Sahtu	Norman Wells	DOT pond		N65 15 07.1; W126 39 53.2	5-Jul-08	walk, dipnet	wood frog		3		40 min total	partly sunny, wind up to 10km/hr	$15^{o}C$	morning	3 tail clips	NT647 - NT649	Schock	Returned to increase tissue sample sizes from this pond.