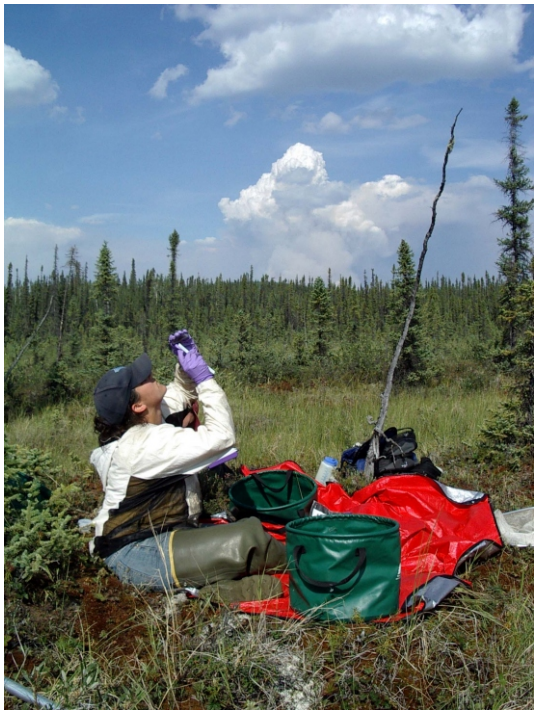
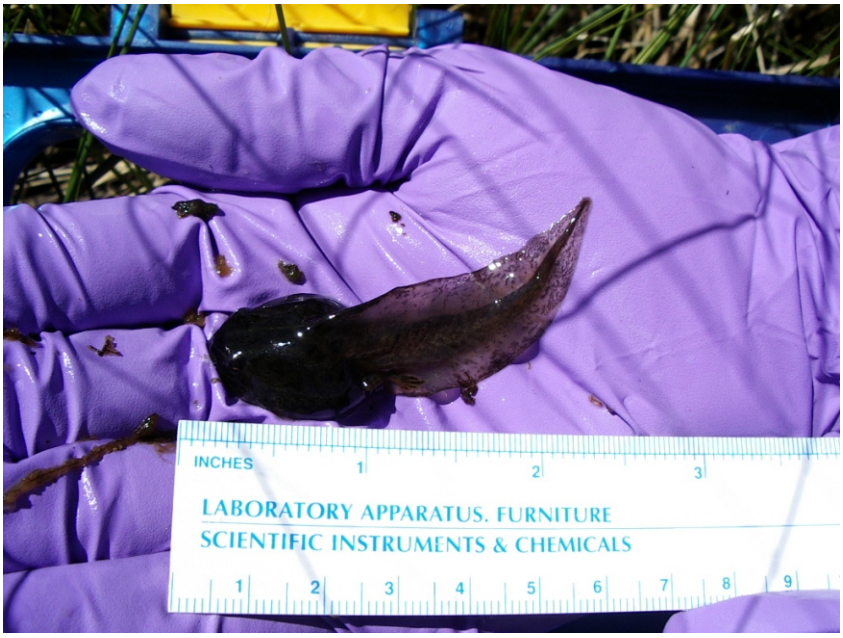
U.S. Fish & Wildlife Service

National Abnormal Amphibian Study: FY 2002: National Wildlife Refuges in Alaska

Annual Progress Report: AFWFO TR-04-02





Cite this report as: Reeves, M.K. and Trust K.A. 2004. National Abnormal Amphibian Study, FY2002: National Wildlife Refuges in Alaska, Annual Progress Report. U.S. Fish and Wildlife Service Technical Report. AFWFO-TR-04-02. 22 pp.

DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE
REGION 7

National Abnormal Amphibian Study

FY2002: National Wildlife Refuges in Alaska

Annual Progress Report

by

Mari K. Reeves
Environmental Contaminants Specialist

and

Kimberly A. Trust
Regional Amphibian Representative

Anchorage Fish and Wildlife Field Office
Anchorage, Alaska

September 27, 2004

TABLE OF CONTENTS **PAGE**

INTRODUCTION

In response to reports of abnormal amphibian sightings across the country, the U.S. Fish and Wildlife Service (Service) initiated a nation-wide study of abnormal frogs on national wildlife refuges in February 2000. The study's goals are to document those refuges with abnormal frogs and to eventually investigate what role, if any, exposure to environmental contaminants might have in causing these abnormalities.

Nationally, several species of frogs (mostly in the genus *Rana*) were selected as study animals, including the leopard frog (*Rana* spp.), the bullfrog (*Rana catesbiana*) and others. In Alaska, only the wood frog (*Rana sylvatica*) is commonly found on Refuge lands and thus, became the focus of this investigation.

Alaska has 16 national wildlife refuges totaling over 76 million acres. The Kenai National Wildlife Refuge (NWR) was the first Alaskan refuge to be assessed for abnormal frogs. Kenai NWR was chosen for a number of reasons including easy road accessibility, existing wood frog natural history information, and known contaminant sources documented in the Contaminant Assessment Process report (Parson, 2000). During the pilot year (2000) of the investigation, 30 of the 348 (or 8.6%) of the newly metamorphosed frogs (metamorphs) and late-stage tadpoles examined in the field had abnormalities. Abnormality rates in individual ponds ranged between 0 and 19%. The predicted background abnormality rate expected in amphibian populations is 0-2% (Ouellet, 2000). Of all the refuges surveyed nationally in 2000, Kenai NWR had the highest number and percentage of visibly abnormal frogs (Trust and Tangermann, 2002). Anomalies observed on the Kenai NWR included missing or shrunken hind legs, missing feet, partial limbs, and missing eyes.

Most refuges investigated nationally in 2000 were again assessed in 2001 (Trust and Reeves, 2004). In accordance with national protocols, a second year of investigations was completed on the Kenai NWR in 2001. Additionally, assessment work was conducted on ponds at four other Alaska national wildlife refuges in 2001. Ponds were surveyed on the Arctic NWR, and preliminary surveys were conducted on or near the Yukon Delta, Kanuti, and Koyukuk NWRs. These preliminary assessments were conducted to identify suitable frog ponds, monitor tadpole growth and development, and perform an opportunistic examination of newly metamorphosed wood frogs.

In 2002, investigations continued on those refuges where monitoring had been initiated in previous years (in 2000 on the Kenai NWR and in 2001 on the Arctic and Yukon Delta NWRs). Adequate numbers of frogs were successfully captured from multiple ponds on each of these refuges and from one pond on the Innoko NWR. Additionally, we provided equipment and supplies to personnel at both Koyukuk and Kanuti NWRs, where refuge staff was able to capture metamorphs from ponds near, but not on, these remote refuges. Refuge staff from the Togiak NWR was also trained in the protocols of the study, although they were unable to sample in 2002. Refuge locations are depicted in Figure 1.

OBJECTIVES

Our objectives in 2002 were to: (1) determine the incidence of abnormalities in wood frogs from a subset of water bodies on the Kenai, Arctic, and Yukon Delta NWRs; (2) collect metamorphs from ponds near Kanuti and Koyukuk NWRs; and (3) train staff at other refuges in national study protocols.

MATERIALS AND METHODS

Frog Monitoring

At each refuge, pond locations were identified with a handheld GPS unit. Pond selection was based on accessibility, opportunities to coordinate sampling or logistical support with other refuge projects in the area, and/or proximity to known sources of local contamination. Except for portions of Kenai and Tetlin NWRs, refuges in Alaska are remote and not accessible from the major road network. Therefore, access was an important consideration when choosing sampling locations.

In accordance with national protocols, the goal was to inspect 50 to 100 newly metamorphosed frogs at each productive pond. Ponds were swept for metamorphs by one to five people using hand-nets. Metamorphs were examined for developmental status (Gosner, 1960) as well as for anomalies including missing, extra, or misshapen limbs, missing or abnormal eyes, or any other abnormality of the body. Abnormal metamorphs and late-stage tadpoles (Gosner stages 42-45) were collected and anesthetized using MS-222 (1 g l^{-1} water) or clove oil. The abnormal frogs were then placed on paraffin blocks (approximately 8 cm x 4 cm x 4 cm) in a 7 cm deep sealable Tupperware container to limit fumes. Surgical tape and map pins were used to position the frogs, and 100% reagent-grade ethanol was poured into the tray until it covered the metamorphs. Approximately 12 hours later, metamorphs were transferred to plastic jars containing 70% ethanol and stored until examination by personnel at the U.S. Geological Survey Biological Resources Division (USGS-BRD) National Wildlife Health Center (NWHC) in Madison, WI.

For some ponds, the minimum of 50 frogs could not be collected in one day, so collections occurred over several days. In these instances, frogs (Gosner stage 42 or greater) were collected and placed in a container with site vegetation and water. The frogs were kept in a cool place for up to three days, or until at least 50 frogs were captured. Consistent with national protocols, metamorphs were then measured, observed for abnormalities, and either retained for further analysis or returned to their natal ponds. If 50 frogs were not collected by the third day, all frogs were released.

Due to a change in the national protocols for frog investigations, water quality parameters were not measured at any of the refuges during 2002.

Kenai National Wildlife Refuge

Twenty ponds were originally investigated in the 2000 pilot study on the Kenai NWR (Trust and Tangermann, 2002) (Figure 2). Two of the original ponds were hydrologically connected, and were treated as one pond in 2002. Another pond sampled in 2000 had water flowing through it during spring thaw, and neither eggs nor frogs were observed after the initial visit. It was consequently removed from the study. Environmental contaminants biologists and/or refuge personnel initiated searches for egg masses on the remaining 18 ponds in May. Tadpole development on the Kenai NWR was monitored on each pond once in May and once in June, then with greater frequency in July; collections occurred in mid to late July. Although 18 ponds were monitored for tadpole growth in 2002, eight of the ponds dried up completely before metamorphosis occurred. Therefore, only 10 of the ponds originally monitored in 2000 were sampled for metamorphs in 2002.

Arctic National Wildlife Refuge

The ponds sampled in the Arctic NWR were located near the Porcupine River approximately 100 miles northeast of Fort Yukon, AK on the south side of the Brooks Range (Figure 3). The remote location requires access by either plane or boat. In the Arctic NWR, tadpole development was not monitored prior to collections, due to logistical constraints; the previous year's study information was used to determine timing of collection, which began in mid-July. Sampling in 2001 also offered insight into timing and progression of wood frog development within this part of the Arctic NWR. Collection of metamorphs was conducted from July 17-26, 2002. Two of the original 13 ponds were found to be hydrologically connected and thus were combined. Metamorphs were found in five of the remaining 12 ponds surveyed on this refuge in 2002.

Yukon Delta National Wildlife Refuge

Eight ponds on the Yukon Delta NWR were initially examined for egg masses beginning in late May, 2002 (Figure 4). Ponds were assessed again in late June. Two of these ponds were hydrologically connected, and were therefore treated as a single pond. In June, tadpoles ranged from Gosner stage 39-41, and we initiated collections earlier than expected, during the week of July 4. Wood frog metamorphs were ultimately collected from five of the seven ponds. One of the ponds was large, which made locating metamorphs difficult, resulting in collection of fewer than 50 individuals. One of the ponds had dried out prior to metamorphosis, and one of the ponds had three egg masses that failed to hatch.

Kanuti National Wildlife Refuge

Two ponds were monitored in Bettles, AK, just outside the refuge's northern boundary and the site of the refuge field station (Figure 5). We are reporting the data because this initial reconnaissance effort provides preliminary information about timing of egg laying and frog

metamorphosis in this geographic area and because we intend to continue to provide technical assistance to refuge personnel in support of frog monitoring activities.

Ponds near the Kanuti NWR were monitored in early June and in early July. No metamorphs were collected this year because the monitored ponds dried out by July 11, probably before the tadpoles metamorphosed. Three ponds were sampled in 2001, but Pond 2 was dropped in 2002 because its shallow, vegetated end dried early in the summer the previous year and any surviving tadpoles migrated through a channel into a deep bog lake where collection was not possible. Although egg masses were located in the other two ponds monitored, all proposed sampling locations dried up before tadpoles metamorphosed.

Koyukuk National Wildlife Refuge

Koyukuk NWR ponds monitored in 2002 were near, but not within the refuge boundaries. Refuge personnel hope to expand the frog investigations to include ponds within the refuge in the future. A pond near Galena, AK, was monitored for egg masses on June 7 and 21, and July 2, 13 and 24 (Figure 6). Fewer than 50 metamorphs were collected between July 26 and 29, 2002.

Innoko National Wildlife Refuge

Personnel from Koyukuk NWR had the opportunity to sample one pond within the Innoko NWR while in the area working on other projects. The pond is just south of Nulato, AK (Figure 7). The pond was monitored on June 23 and 26, 2002. Eighty-two metamorphs were collected from this pond on July 7.

RESULTS AND DISCUSSION

Kenai National Wildlife Refuge

Of the 543 metamorphs inspected from eight ponds, 54 had visible abnormalities in 2002 (Table 1). Abnormality rates in individual ponds ranged from 6-15%. Types of abnormalities observed in the field are described in Table 2. Over the last three years, the observed abnormality rate in a subset of Kenai NWR ponds has consistently been elevated above the rate predicted for amphibian populations (Ouellet, 2000). The percentage of abnormal metamorphs collected at all ponds on Kenai NWR was 8.6% (range: 0-19%), 5.5% (range: 4-11%), and 9.9% (range: 6-15%) for 2000, 2001, and 2002, respectively. No single pond from which metamorphs were captured appears to show a consistent prevalence of high abnormality rates; rather, abnormalities are distributed fairly evenly in all of the sampled ponds in the study area (Figure 8). Nevertheless, it is important to note that all ponds sampled are along the road system and this area represents a small portion of the entire Kenai NWR (Figure 9).

Arctic National Wildlife Refuge

Of the 266 metamorphs collected, seven individuals were abnormal (Table 1). This overall abnormality rate of 2.6% is identical to that found on these Arctic NWR ponds in 2001 (Trust and Reeves 2004). Abnormality rates in specific ponds ranged from 0-6%. Ponds AR005 and AR006 were combined due to close proximity and likelihood of a shared water source. Eight of the 13 ponds dried up prior to collections and could not be sampled. Descriptions of visual abnormalities are presented in Table 3.

Yukon Delta National Wildlife Refuge

None of the 280 frogs collected at the Yukon Delta NWR were abnormal. One tadpole with only one eye was found early in the season, but because tadpole collection is not within national protocols, this animal was released. Also, one of the ponds, Pond YKD02, had three dead egg masses in it early in the season. A drainpipe from the Women's Prematernal Home drains into the pond, leaving discolored sediment at the outfall. No egg masses from pond YKD02 survived, and this pond did not produce any tadpoles.

Kanuti National Wildlife Refuge

No metamorphs were collected from the Kanuti NWR. All ponds dried out before metamorphs could be collected. Tadpoles had reached Gosner stages 36-37 by the time the ponds were nearly dry, on July 11. No further sampling was conducted in 2002. The ponds in Bettles, AK will not be sampled in the future; if any surveys are conducted, they will occur on the refuge.

Koyukuk National Wildlife Refuge

Seventeen metamorphs were collected from the pond in Galena, AK. It was not possible to capture 50 metamorphs at this location. Thirty-six adult frogs were also collected from the area around this pond. No abnormalities were detected.

Innoko National Wildlife Refuge

Eighty-two metamorphs were collected from the pond on Innoko NWR on July 7, 2002. All appeared to be normal.

CONCLUSIONS AND RECOMMENDATIONS

Kenai National Wildlife Refuge

The abnormality rates in sampled areas at the Kenai NWR have been consistently elevated during the past three years of monitoring. We recommend that ponds included in the study continue to be monitored to further evaluate annual variability and confirm repeatability of these observations. We also recommend increasing the number of sites sampled to include more

remote locations that are not accessible by road. By comparing data from these relatively undeveloped areas to that from more developed sites in proximity to human activities, biologists will gain a better understanding of factors that may affect the incidence of abnormalities among refuge amphibian populations. Based on national protocols, Kenai NWR should progress to Phase II of the investigation process, including more complete characterization of this phenomenon and investigation of multiple stressors to the wood frog population that may be responsible for these abnormalities.

Arctic National Wildlife Refuge

During the last two years of sampling, abnormality rates in individual ponds sampled in the Arctic NWR have ranged between 0-6%. In 2001, AR002 and AR010 had abnormality rates greater than or equal to 3%. In 2002, two ponds (AR002 and AR007) had abnormality rates greater than 3%. Because 3% of the frogs sampled in pond AR002 were abnormal in 2001 and 6% were abnormal in 2002, further monitoring is recommended at this pond.

ACKNOWLEDGMENTS

We would like to acknowledge the following people for their assistance with the project: Fred Broerman, Richard Carroll and family, Joseph Connor, Paul Cotter, Maureen Dezeeuw, Chadd Fitzpatrick, Philip Johnson, Brian McCaffery, Tiffany Parson, Jordan Stout, and Rebecca Viray, In addition, we would also like to thank Jennifer Bryant, Lisa Saperstein, David Payer, Roger Kaye, and Beverly Reitz, for their review of the document and assistance in the field.

LITERATURE CITED

Gosner, K. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica. 16:183-190.

Ouellet, M., B. Joel, R. Jean, D.G. Jean-Luc, and L. Stephanie. 1997. Hindlimb deformities (Ectromelia, Ectrodactyly) in free-living anurans from agricultural habitats. J. Wildlife Diseases 33:95-103.

Ouellet, M. 2000. Amphibian deformities: Current state of knowledge. In *Ecotoxicology of Amphibians and Reptiles.* D.W. Sparling, G. Linder, and C.A. Bishop, eds. Society of Environmental Toxicology and Chemistry (SETAC) Press, Pensacola, FL. 904 pp.

Parson, T.A.S. 2000. Kenai National Wildlife Refuge Contaminant Assessment. U.S. Fish and Wildlife Service, Region 7, Anchorage, AK. 123 pp.

Trust, K.A. and M.K. Reeves. 2004. National Abnormal Amphibian Study, FY 2001: National Wildlife Refuges in Alaska. Annual Progress Report. U.S. Fish and Wildlife Service Technical Report. AFWFO-TR-04-01. 24 pp.

Trust, K.A. and H. Tangermann. 2002. National Malformed Amphibian Study, FY 2000: Kenai National Wildlife Refuge, Annual Progress Report. U.S. Fish and Wildlife Service Technical Report. WAES-TR-02-01. 19 pp.

FIGURES

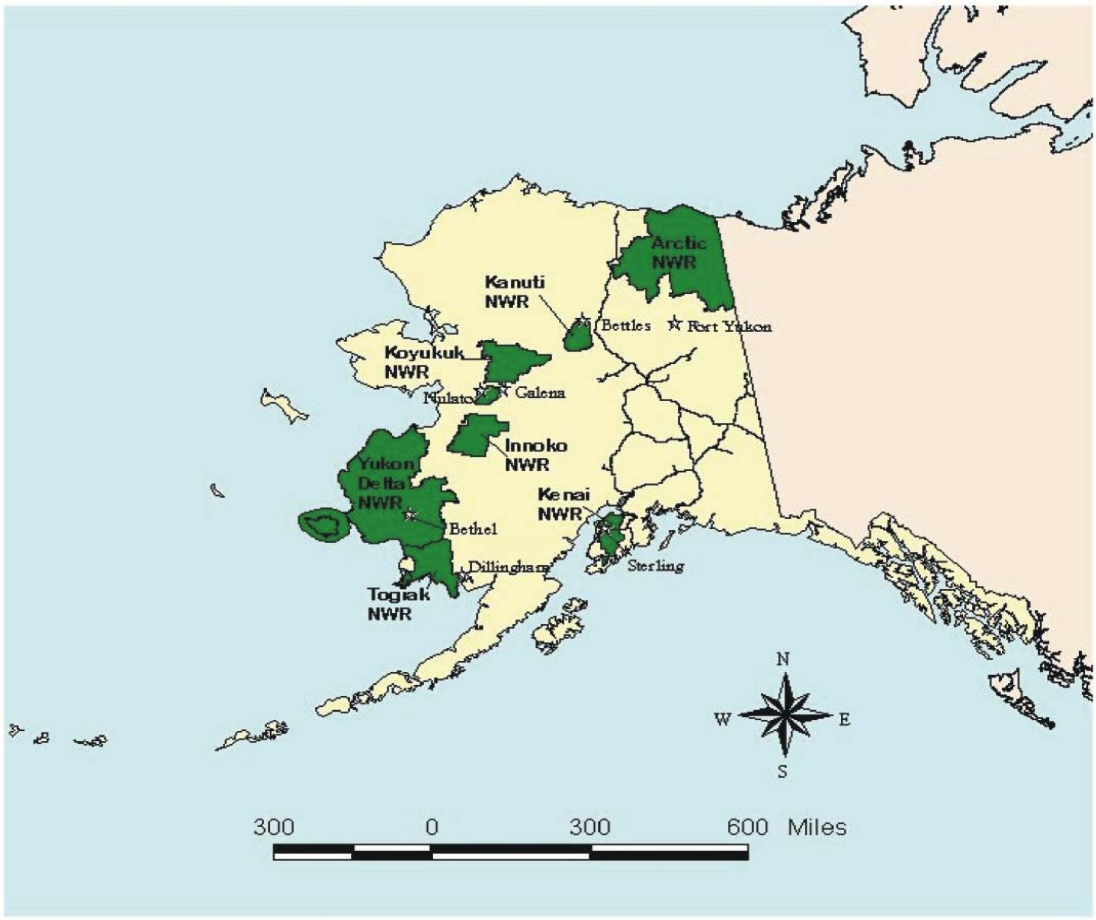


Figure 1. Location of National Wildlife Refuges in Alaska involved with abnormal amphibian monitoring in 2002.

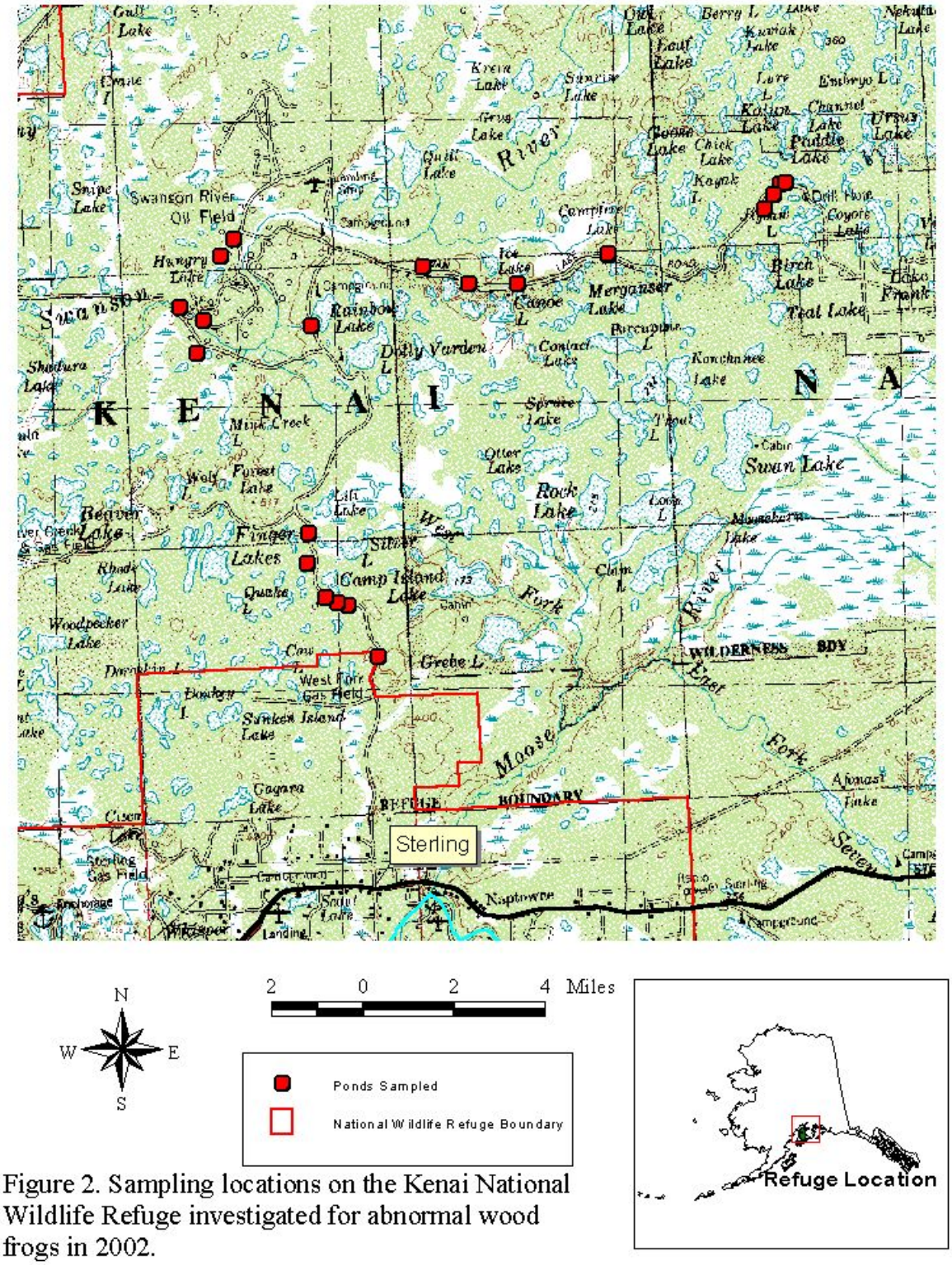


Figure 2. Sampling locations on the Kenai National Wildlife Refuge investigated for abnormal wood frogs in 2002.

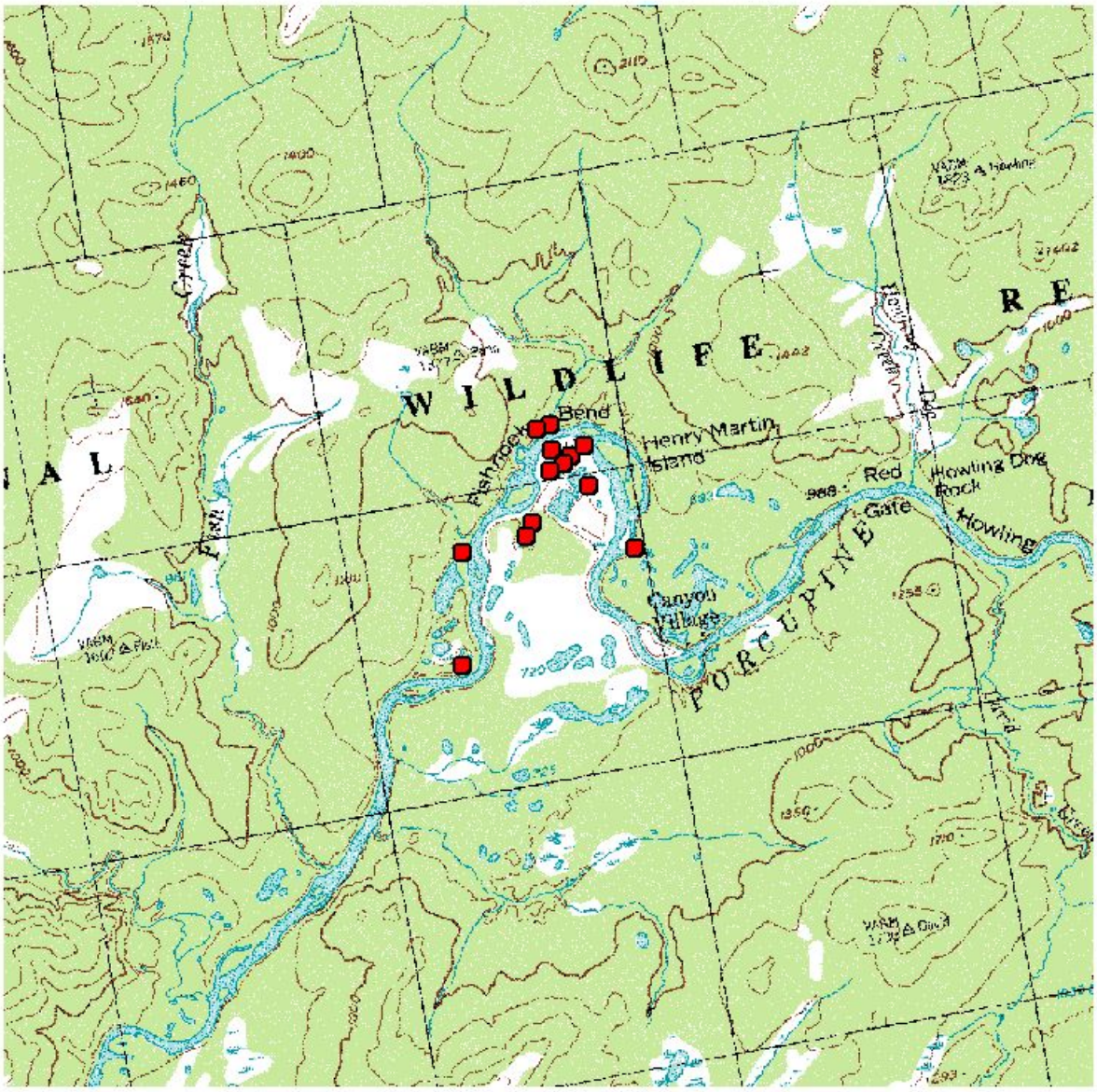


Figure 3. Sampling locations on the Arctic National Wildlife Refuge investigated for abnormal wood frogs in 2002.

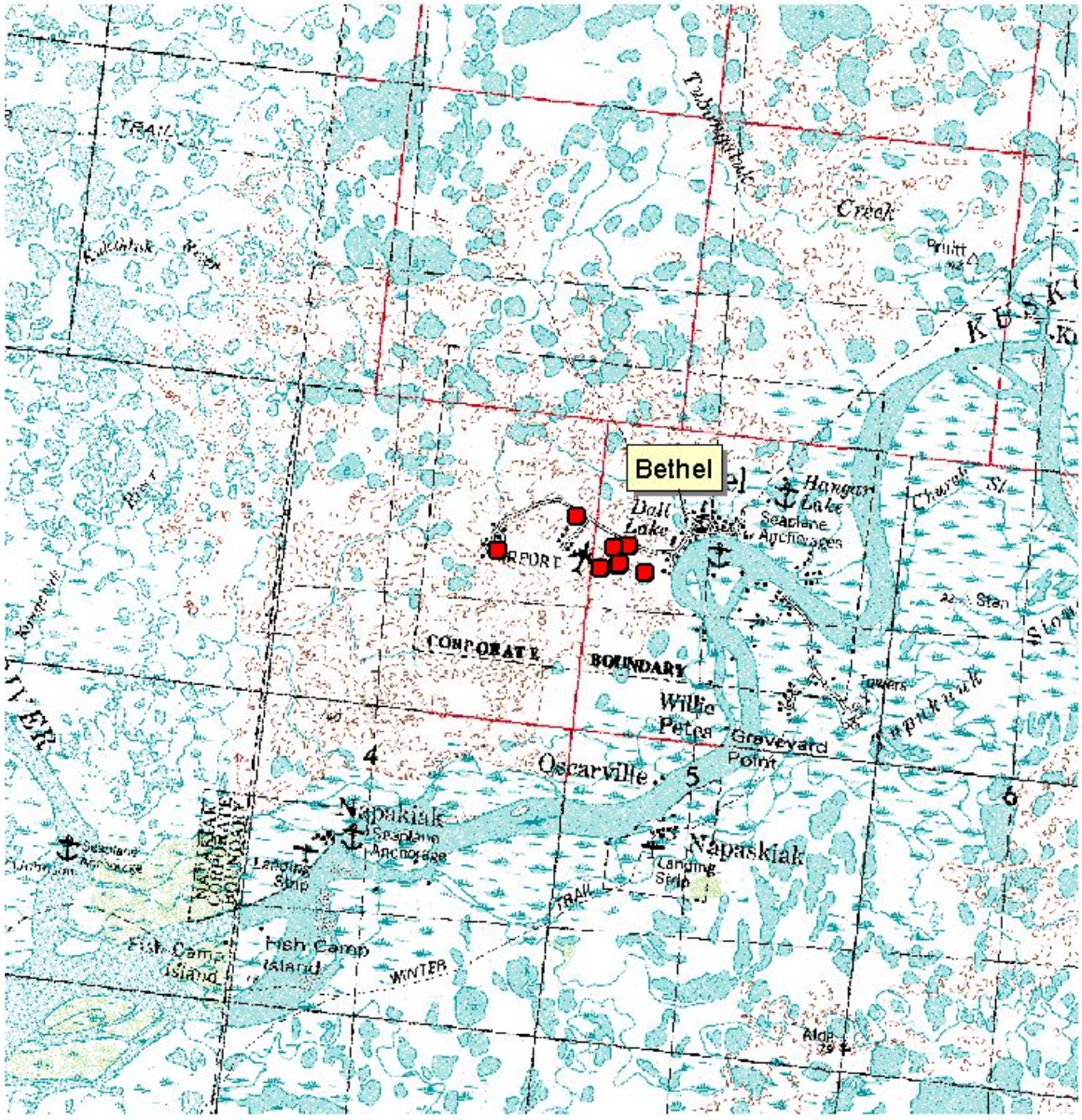


Figure 4. Sampling locations on the Yukon Delta National Wildlife Refuge investigated for abnormal wood frogs in 2002.

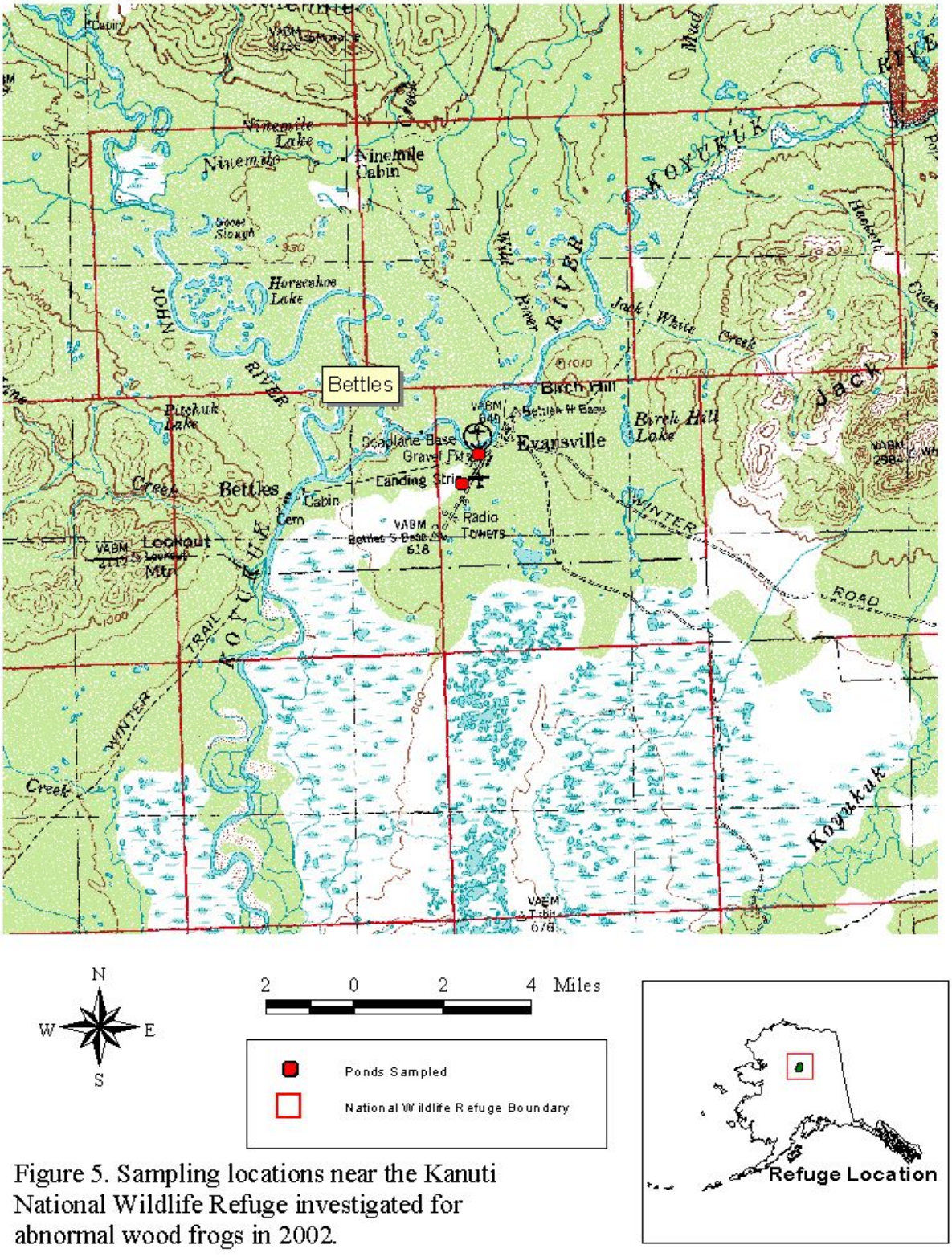


Figure 5. Sampling locations near the Kanuti National Wildlife Refuge investigated for abnormal wood frogs in 2002.

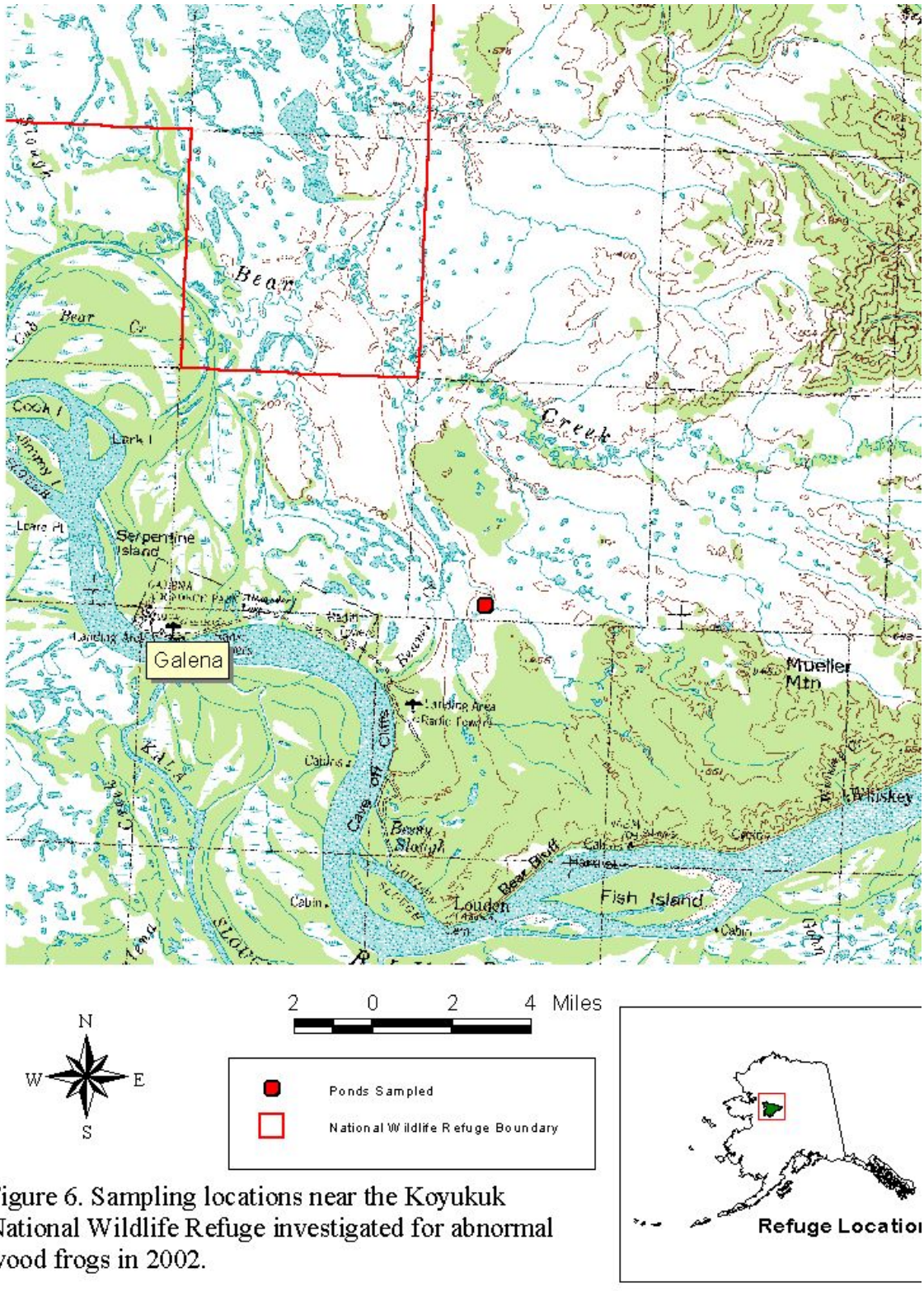


Figure 6. Sampling locations near the Koyukuk National Wildlife Refuge investigated for abnormal wood frogs in 2002.

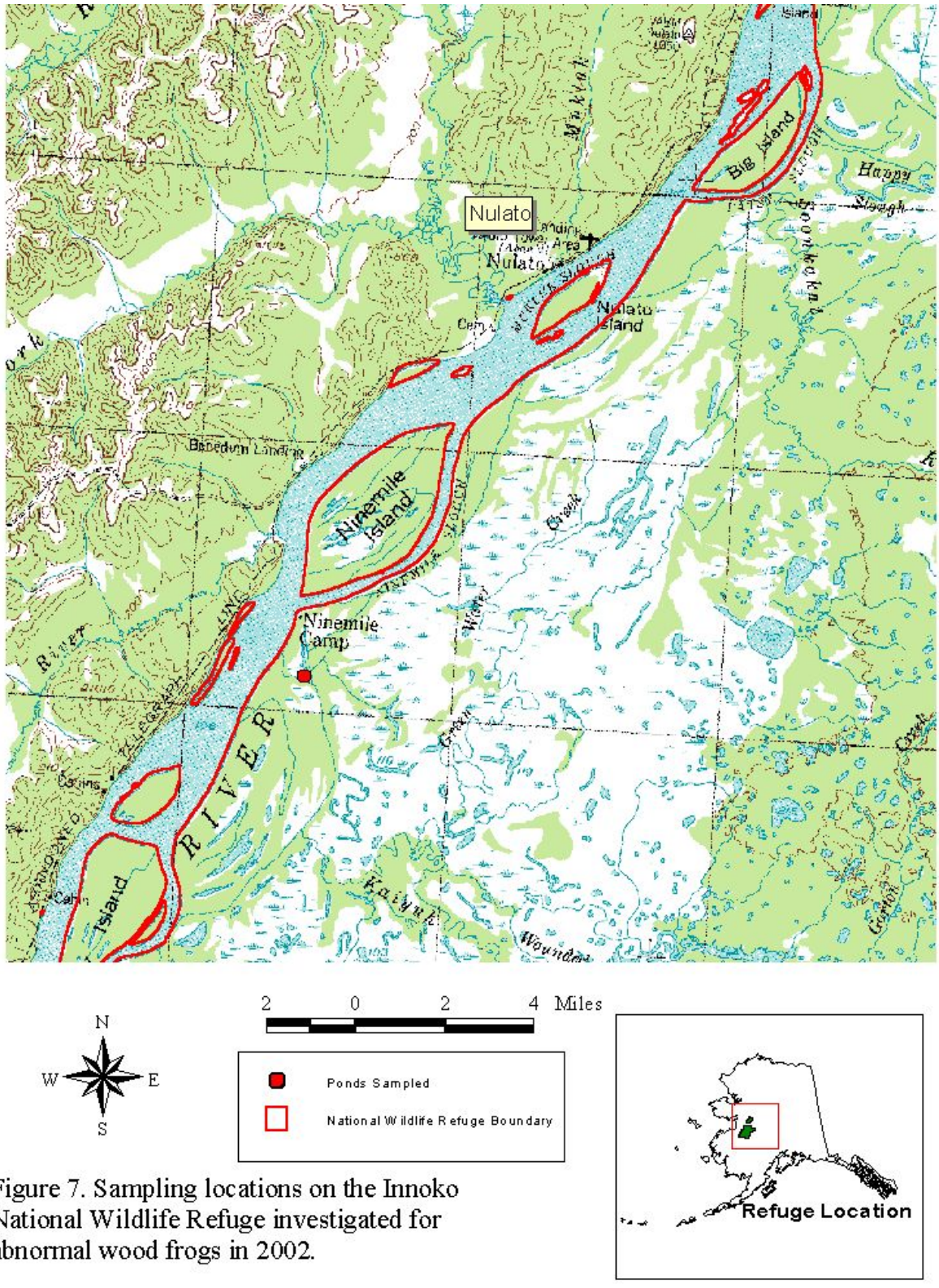


Figure 7. Sampling locations on the Innoko National Wildlife Refuge investigated for abnormal wood frogs in 2002.

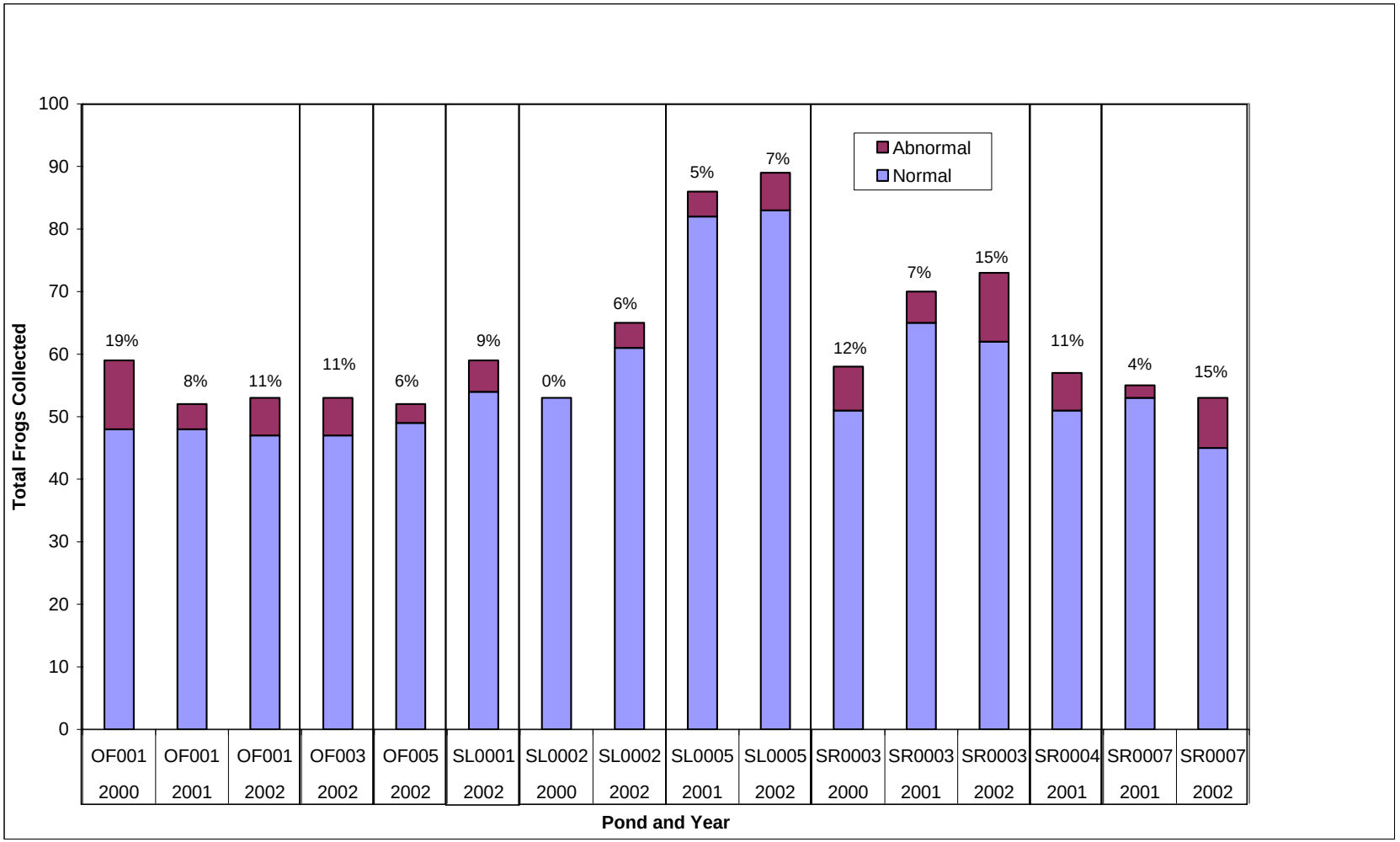


Figure 8. Numbers of Normal and Abnormal Frogs collected at Kenai NWR Ponds, 2000-2002. Only ponds from which greater than 50 metamorphs were examined are shown.

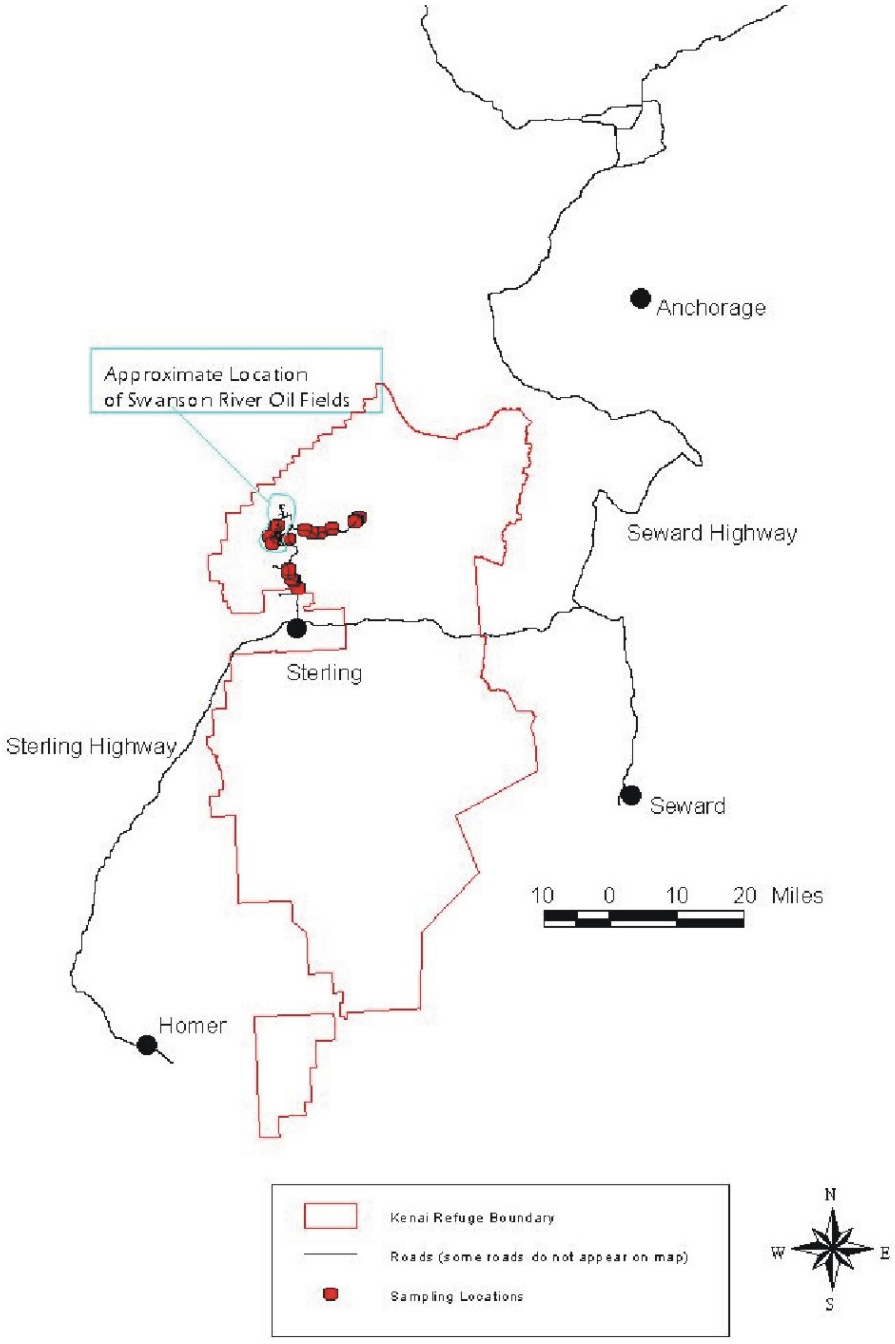


Figure 9. Kenai Refuge Boundary with Sampling Locations and Roads

TABLES

Table 1. Total number of wood frog metamorphs collected and number abnormal frogs per pond on Alaska national wildlife refuges in 2002.

Refuge	Sample Date	Pond ID	# Collected	# Abnormal	% Abnormal
Kenai NWR	7/24/02	OF001	53	6	11.3%
Kenai NWR	7/25/02	OF003	53	6	11.3%
Kenai NWR	7/20/02	OF005	52	3	5.8%
Kenai NWR	7/18/02	SR003	73	11	15.0%
Kenai NWR	7/29/02	SR004	17	3	17.6%
Kenai NWR	7/24/02	SR006	29	2	6.9%
Kenai NWR	7/12/02	SR007	53	8	15.1%
Kenai NWR	7/29/02	SL005	89	6	6.7%
Kenai NWR	7/19/02	SL001	59	5	8.5%
Kenai NWR	7/19/02	SL002	65	4	6.2%
Arctic NWR	7/20/02-7/26/02	AR001	17	0	0%
Arctic NWR	7/22/02	AR002	50	3	6.0%
Arctic NWR	7/20/02-7/21/02	AR005/6	56	1	1.8%
Arctic NWR	7/21/02	AR007	91	3	3.3%
Arctic NWR	7/24/02	AR010	52	0	0%
Yukon Delta NWR	7/18/02	YKD03	55	0	0%
Yukon Delta NWR	7/5/02	YKD04/ YKD05	68	0	0%
Yukon Delta NWR	7/5/02	YKD06	60	0	0%
Yukon Delta NWR	7/18/02	YKD07	13	0	0%
Yukon Delta NWR	7/14/02	YKD08	86	0	0%
Innoko NWR	7/7/02	IN001	82	0	0%
Koyukuk NWR (Off-refuge)	7/26/02-7/29/02	KO001	17	0	0%

Table 2. Visual observations of abnormal wood frogs collected on the Kenai Refuge in 2002.

Pond ID	Frog ID	Body Part Affected	Abnormality Description
OF001	7KEOF00113	Right hind limb	Ectromelia
OF001	7KEOF00139	Left hind limb	Micromelia
OF001	7KEOF00142	Right eye; left hind limb	Iris lacks pigmentation, Ectromelia
OF001	7KEOF00151	Left hind limb	Ectromelia
OF001	7KEOF0016	Right hind limb	Ectromelia
OF001	7KEOF0017	Left eye	Iris lacks pigmentation
OF003	7KEOF00318	Torso	Lump in throat
OF003	7KEOF00323	Right hind limb	Syndactyly
OF003	7KEOF00325	Left hind limb, Right fore limb	Ectromelia; Amelia
OF003	7KEOF00351	Right hind limb	Ectromelia
OF003	7KEOF00353	Torso	Herniated intestines
OF003	7KEOF0036	Left hind limb	Amelia
OF005	7KEOF00511	Right hind limb	Micromelia
OF005	7KEOF00512	Left hind limb; Right hind limb	Ectromelia
OF005	7KEOF0055	Right hind limb	Ectromelia
SL001	7KESL0013	Torso	Lump in throat
SL001	7KESL00136	Torso	Skin discoloration
SL001	7KESL0014	Left hind limb	Ectromelia
SL001	7KESL00148	Tail	Lump, growth, or cyst
SL001	7KESL00149	Left hind limb	Ectromelia
SL002	7KESL0021	Left hind limb	Micromelia
SL002	7KESL00212	Left hind limb	Amelia
SL002	7KESL00218	Right hind limb	Ectromelia
SL002	7KESL00220	Torso	Cut
SL005	7KESL00517	Left hind limb	Micromelia
SL005	7KESL00538	Left eye	Iris lacks pigmentation
SL005	7KESL00566	Right hind limb; Torso	Ectromelia; wound
SL005	7KESL00574	Right eye	Iris lacks pigmentation
SL005	7KESL00581	Left hind limb	Micromelia
SL005	7KESL0059	Left eye	Iris lacks pigmentation
SR003	7KESR0031	Right hind limb	Ectromelia
SR003	7KESR00318	Left fore limb	Ectrodactyly
SR003	7KESR00321	Left hind limb	Injury
SR003	7KESR00324	Left hind limb	Amelia
SR003	7KESR00327	Right hind limb	Ectromelia

Pond ID	Frog ID	Body Part Affected	Abnormality Description
SR003	7KESR0033	Jaw	Cut
SR003	7KESR00335	Right hind limb	Ectromelia
SR003	7KESR00346	Left hind limb; Right hind limb	Micromelia
SR003	7KESR0035	Right eye	Iris lacks pigmentation
SR003	7KESR00372	Right hind limb	Ectromelia
SR003	7KESR00344	Left hind limb	Amelia
SR004	7KESR00414	Right hind limb	Amelia
SR004	7KESR00417	Right eye	Iris lacks pigmentation
SR006	7KESR00610	Right eye	Iris lacks pigmentation
SR006	7KESR00622	Right fore limb	Ectromelia
SR007	7KESR00715	Left hind limb	Ectromelia
SR007	7KESR00716	Right hind limb	Other
SR007	7KESR00720	Left eye; mouth	Iris lacks pigmentation; bleeding
SR007	7KESR00726	Left hind limb; Right eye	Ectromelia; Iris lacks pigmentation
SR007	7KESR00728	Left eye	Iris lacks pigmentation
SR007	7KESR00739	Left eye	Iris lacks pigmentation
SR007	7KESR00753	Left hind limb	Amelia
SR007	7KESR0076	Torso	Injury, internal bleeding
SR004	7KESR00412	Left eye	Iris lacks pigmentation

Table 3. Description of abnormalities found in wood frogs from Arctic Refuge in 2002.

Pond ID	Frog ID	Body Part Affected	Abnormality Description
AR002	7AR02001	Left hind limb	Polydactlyl
AR002	7AR02002	Left hind limb	Injury
AR002	7AR02003	Right hind limb	Injury
AR005	7AR05001	No description provided by field crew	No description provided by field crew
AR007	7AR07001	Torso	Herniated intestines
AR007	7AR07002	Left hind limb	Micromelia
AR007	7AR07003	Jaw	Swollen

U.S. Department of the Interior
U.S. Fish and Wildlife Service

http://www.fws.gov
September 2004