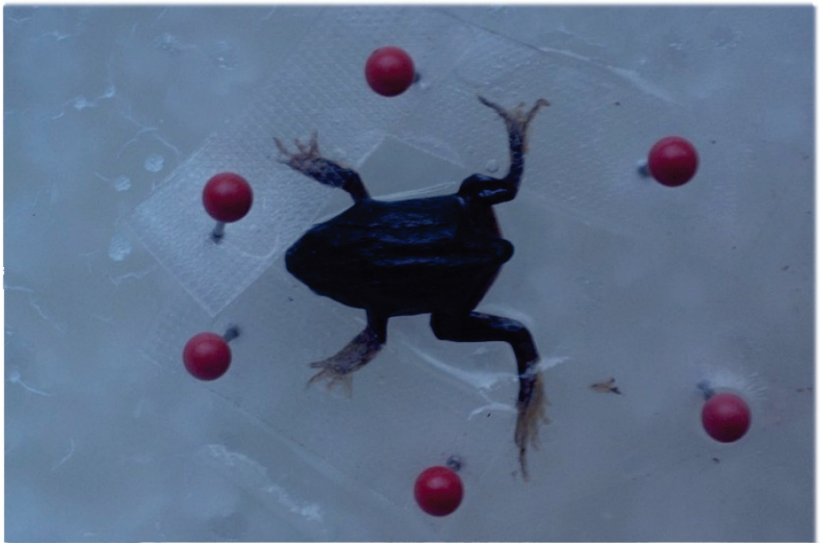
U.S. Fish & Wildlife Service

National Abnormal Amphibian Study: FY 2001: National Wildlife Refuges in Alaska

Annual Progress Report: AFWFO TR-04-01



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

National Abnormal Amphibian Study

FY 2001: National Wildlife Refuges in Alaska

Annual Progress Report

by

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INTRODUCTION

In response to reports of abnormal amphibian sightings across the country, in February 2000, the U.S. Fish and Wildlife Service (Service) initiated a nation-wide survey of abnormal frogs on National Wildlife Refuges. 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 refuges totaling over 76 million acres. The Kenai National Wildlife Refuge (NWR) was the first Alaska refuge to be surveyed 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 of the newly metamorphosed frogs and late-stage tadpoles examined in the field had abnormalities. Pond-specific abnormality rates ranged between 0% and 19%. This was above the predicted abnormality rate (0-2%) expected in amphibian populations (Ouellet, 2000). Of all the refuges monitored in 2000, Kenai NWR had the highest number and percentage of visibly abnormal frogs. Anomalies observed on the Kenai NWR included missing or shrunken hind limbs, missing feet, abnormal musculature, partial limbs, and missing irises or eyes.

Most refuges investigated nationally in 2000 were again assessed in 2001, including the Kenai NWR. Additionally, ponds were surveyed in 2001 on the Arctic NWR south of the Brooks Range, and preliminary information was collected from ponds on or near the Yukon Delta, Kanuti, and Koyukuk NWRs (Figure 1). Information gathered in these preliminary assessments included identification of suitable frog ponds, limited monitoring of tadpole growth and development, and opportunistic examination of newly metamorphosed wood frogs.

OBJECTIVES

Our objectives in 2001 were to: (1) determine the prevalence of abnormalities in wood frogs from a subset of water bodies on the Kenai and Arctic NWRs, and (2) identify suitable frog habitat on other refuges in order to expand investigations in subsequent years.

MATERIALS AND METHODS

Frog Monitoring

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

Environmental contaminants biologists and Refuge personnel initiated searches for egg masses on selected ponds in May and early June on or near each of the five refuges. Thereafter, frequency of pond monitoring varied. Except for the Kenai NWR, timing of metamorphosis was unknown. Therefore, to track tadpole development, ponds were monitored at least one time before the estimated start of metamorphosis. The ponds on the Kenai NWR were monitored for tadpoles weekly in June and July, and the ponds on the Arctic NWR were monitored one time in early July. The other three refuges were sampled at various intervals from June to August.

Nationally, a goal was established to inspect 50 to 100 newly metamorphosed frogs (also referred to as metamorphs) at each productive pond. Ponds were swept for metamorphs by one to five people using hand-nets starting in mid-July. For some ponds, the target number of 50 frogs could not be collected in one day, so collections occurred over several days to meet the 50-frog goal. In these instances, frogs 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.

Metamorphs were examined for developmental status (Gosner, 1960) and inspected for anomalies including missing, extra, or misshapen limbs and eyes. After visual inspection, metamorphs that appeared normal were released in their natal pond. Abnormal metamorphs and late-stage tadpoles (Gosner stages 42-45) were retained and anesthetized using MS-222 (1 g l^{-1} of water) or clove oil. Abnormal frogs were then placed on paraffin blocks (approximately 8 cm x 14 cm) in a 5 cm deep tray. 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 ICHEM® glass jars containing 70% ethanol and stored for radiography by personnel at the U.S. Geological Survey – Biological Resources Division (USGS-BRD) National Wildlife Health Center (NWHC), Madison, WI.

Kenai National Wildlife Refuge

Twenty ponds were originally monitored in the 2000 pilot study on the Kenai NWR (Trust and Tangermann, 2002) (Figure 2). For consistency and to evaluate annual variation, the same ponds were revisited in 2001. Two of the original ponds were hydrologically connected in 2001, and were thus considered one pond. Another pond sampled in 2000 had water flowing through it during the spring thaw of 2001, and neither eggs nor frogs were seen after the initial visit. This pond was consequently removed from the study. Therefore, 18 of the ponds monitored in 2000 were included in the search for egg masses in May of 2001. Subsequently, these ponds were monitored weekly during June for tadpole development, and collections of metamorphosed frogs were conducted in July/August of 2001.

Arctic National Wildlife Refuge

Beginning June 9, 2001, 13 ponds were searched for egg masses in the Arctic NWR (Figure 3). The ponds were located near the Porcupine River approximately 100 miles northeast of Fort Yukon, AK on the south side of the Brooks Range. Ponds on the Porcupine River were chosen because they were within the geographic range of wood frogs and the boundaries of the Arctic NWR, and according to local residents, contained enough wood frogs to make a sampling effort in the area feasible (Richard Carroll, pers. comm.). Data regarding numbers of wood frogs and distribution on the arctic coastal plain are scarce, and wood frogs are believed to be rare or absent north of the Brooks Range.

These same ponds were monitored for tadpole growth and development again on July 4-6, 2001, and were surveyed for a third time on July 18-26, 2001. During this latter visit, metamorphs were collected and examined.

Water Quality Sampling at Arctic National Wildlife Refuge

In accordance with national protocols, water quality parameters were measured at each refuge multiple times during the 2000 sampling season, including the ponds sampled during the pilot year on the Kenai NWR (Trust and Tangermann, 2002). For 2001, the national protocols changed, and less emphasis was placed on water quality sampling. In July 2001, limited water quality parameters were measured one time at nine ponds in the Arctic NWR during frog collections. Water quality data were not collected at any other refuges in 2001.

Koyukuk National Wildlife Refuge

For the Koyukuk NWR, this initial reconnaissance year was coordinated closely with on-going refuge projects. Many of these projects involved outreach and education in remote Alaskan communities that were located near, but not within refuge boundaries. Therefore the ponds monitored in 2001 were near, but not in the refuge. We are reporting the data because the initial reconnaissance efforts provided preliminary information about timing of egg laying and frog metamorphosis in these geographic areas and because we intend to continue providing technical

assistance to refuge personnel in support of frog monitoring activities. Personnel at Koyukuk NWR hope to expand frog monitoring to include ponds within the refuge, in addition to those ponds sampled near villages during 2001, if resources allow.

Three ponds near Galena, AK, were monitored for egg masses beginning May 29, 2001. Follow-up monitoring of tadpole development occurred on June 20 and July 13, 2001. Upon returning to the ponds in July (at the estimated time of metamorphosis), biologists found only dead tadpoles. They sent four samples to NWHC for diagnostic evaluation. Three of the tadpoles were too decomposed for proper examination, but a *Rana* virus was isolated from one tadpole. The cause of death was attributed to a viral infection. Past evidence of tadpole die-offs in other states has been attributed to a similar *Rana* virus, thus it is possible that a viral infection caused the death of the tadpoles in the ponds near the Koyukuk NWR (David Green, NWHC, pers. comm.). On August 8, 2001, biologists from the refuge organized a wetlands appreciation day with school children from Galena. During their field trip to a near-by pond (locally known as "Duck" pond), the group opportunistically collected metamorphosing wood frogs (Figure 4). Abnormal frogs were sent to NWHC for radiography.

Kanuti National Wildlife Refuge

For the Kanuti NWR, this initial reconnaissance year was coordinated closely with on-going refuge projects. Many of these projects involved outreach and education in remote Alaskan communities that were located near, but not within refuge boundaries. Therefore the ponds monitored in 2001 were near, but not in the refuge. Seven ponds near Kanuti NWR were monitored for egg masses from June 4-7, 2001 (Figure 5); egg masses were found in three of the seven ponds. Two of the three ponds with egg masses were again monitored for tadpoles from June 19-20, 2001. Both ponds had dried considerably since the previous visit, but it appeared that sufficient water remained to support the tadpoles until metamorphosis. Kanuti NWR staff received a report that the ponds were mostly dry on July 17. By the time they arrived in Bettles, AK on July 19, the tadpoles were dead and decomposing. Water was present in the deepest portion of a third pond, but collections were not possible from this area.

Yukon Delta National Wildlife Refuge

Seven ponds on the Yukon Delta NWR were initially monitored for egg masses beginning on June 12, 2001. Ponds were monitored again on July 6, 2001. Wood frog metamorphs were obtained from only one of the seven ponds, with only four individuals inspected (Figure 6). Of the six remaining ponds, two had completely dried up, three had late-stage tadpoles but no metamorphs, and the last pond lacked both tadpoles and metamorphs.

Ancillary Studies

Additional investigations were conducted in 2001 to supplement the abnormal amphibian study. Two ancillary studies were initiated on the Kenai NWR: (1) an invertebrate catalog was assembled for seven of the original 20 ponds sampled in 2000, to identify species and investigate

the presence of predaceous insects and their larvae, and (2) limited toxicity tests were initiated on organic compounds extracted from two of the ponds investigated in 2000 and 2001. The toxicity tests were completed in 2002. Additionally, water quality data were collected from ponds in the Arctic NWR. Methods for these ancillary studies are presented below.

Invertebrate Monitoring - Kenai National Wildlife Refuge

The University of Alaska Anchorage's Environment and Natural Resources Institute (ENRI) was contracted by the Service to collect and describe invertebrate fauna and their general ecological characteristics from seven of the study ponds on the Kenai NWR. The purpose of the study was to quantify invertebrate predator presence and density, and begin to explore the hypothesis that these predacious insects could serve as a source of physical trauma to frog tadpoles.

In July 2001, invertebrates were collected from seven ponds using standard methods (Prussian *et al.*, 2001). Briefly, a 0.3 m X 0.3 m D-frame (350 μm mesh net) was held at a depth of 1 m and was continuously swept at constant depth through a 10 m long, 1 m wide transect. Each transect began 10 m perpendicularly from the shore and ended at the shore. Sampling was conducted from a canoe at ten evenly spaced transects across each pond. Each 10 m transect sampled a volume of water approximately equal to 3 m^3. Therefore, approximately 30 m^3 of water was sampled from the littoral zone of each pond. Samples were removed from the net, placed in pre-labeled whirl-pak® bags, preserved with 70% ethanol, and returned to the ENRI laboratory for identification.

Each sample was sorted in its entirety under 2X magnification. Invertebrates were placed in glass vials with fresh 70% ethanol and stored until identification. All individuals were identified and counted using standard taxonomic keys.

Several invertebrate metrics were calculated to compare functional, taxonomic, and diversity attributes among sites. Number of taxa is a commonly used richness metric that is one measure of biological diversity and in some studies reduced taxonomic richness has been linked to water quality impairment. Percentage metrics describe the density of predators relative to other functional feeding groups. Percent predacious Coleoptera and Odonata are metrics that include organisms capable of preying on small vertebrates. The number of predacious individuals is the sum of all predacious individuals found in each sample. Invertebrate density is the number of individuals per m^3 and was calculated by dividing the total number of individuals collected from all ten transects by the approximate volume of water sampled (30 m^3).

A more complete description of sampling methods and a summary of results is found in a technical report prepared by ENRI. Copies of this report are available by contacting the Anchorage Fish and Wildlife Field Office at (907) 271-2888.

Water Toxicity Study - Kenai National Wildlife Refuge

To determine whether organic contaminants in the ponds could cause mortality or abnormalities, a water toxicity study was conducted by personnel at the USGS-BRD, Columbia Laboratory, on two of the ponds (OF0001 and OF0003) that had an elevated incidence of abnormalities observed visually during the 2000 field surveys (Trust and Tangermann, 2002).

Semi-permeable membrane devices (SPMDs) were deployed from July 11 to August 8, 2001, at each of the two ponds. Five SPMDs were placed in each of three replicate stainless-steel canisters and were attached to a stainless steel cable anchored to shore. The SPMDs were hung in mid-water and allowed to absorb organic compounds for 28 days. They were retrieved and frozen immediately at -4^0C until they could be extracted. Field blanks were collected simultaneously upon retrieval of the canisters. Organic compounds absorbed by the SPMDs were extracted and used to run chronic toxicity tests on spring peeper (*Pseudacris crucifer*) tadpoles from the SPMD extracts at two levels of UV-B radiation. Endpoints evaluated were time to metamorphosis, mass at metamorphosis, and presence/absence of visible abnormalities.

The USGS-BRD prepared a technical report which describes study design and results in greater detail. A copy of this report can be obtained by contacting the Anchorage Field Office at (907) 271-2888.

Water Quality Measurements - Arctic National Wildlife Refuge

Using a Hydrolab®, pH, temperature, conductivity and dissolved oxygen data were collected along the shorelines of 9 ponds in the Arctic NWR. Prior to each measurement series, two buffer calibrations, which bracketed the pH of the samples, were performed using pH buffers accurate to ± 0.02 pH units. Conductivity standards were used to check meter performance prior to the measurement series in the field. The dissolved oxygen membrane was changed one time before the entire series was initiated.

RESULTS AND DISCUSSION

Frog Monitoring

Kenai National Wildlife Refuge

Of the 397 metamorphs inspected from eight ponds, 22 had visible abnormalities (Table 1). The range of visible abnormalities in individual ponds was 0% to 10.5%. Types of abnormalities observed in the field are described in Table 2. Six ponds at the Kenai NWR exceeded the baseline abnormality percentage of 0-2% predicted in amphibian populations (Ouellet, 2000).

Arctic National Wildlife Refuge

Of the 352 metamorphs collected, 9 were abnormal (Table 3). The range of abnormalities at individual ponds was 0% to 6%. Ponds ARC05 and ARC06 were combined due to proximity and likelihood of a shared water source. Eight of the 13 ponds dried up prior to collections and could not be sampled. The measured abnormality rate at two of the ponds sampled exceeded the predicted 2% rate of amphibian abnormalities (Ouellet, 2000). Descriptions of visual abnormalities are presented in Table 4.

Koyukuk National Wildlife Refuge

Fifty frogs were examined at one pond near the Koyukuk NWR, and five appeared abnormal and were sent to NWHC for radiography. Abnormalities visually observed in the field are described in Table 5. This rate of visual abnormalities exceeded the predicted 0-2% abnormality rate (Ouellet, 2000).

Kanuti National Wildlife Refuge

No metamorphs were collected from the three ponds surveyed near the Kanuti NWR. A total of 56 tadpoles (26 from Pond 1, 6 from Pond 2, and 24 from Pond 3) were collected. Gosner stage, total length and body length were recorded. Tadpoles were then released. No abnormalities were observed.

Yukon Delta National Wildlife Refuge

Four metamorphs of the desired developmental stage were collected from one pond on the Yukon Delta NWR. Morphometric data were collected and the metamorphs were released. All were normal.

Ancillary Studies

Invertebrate Monitoring - Kenai National Wildlife Refuge

The purpose of the invertebrate study was to investigate the presence of invertebrate tadpole predators, and determine whether they existed in numbers sufficient to cause trauma, which could account for a portion of the visible amphibian abnormalities observed in the Kenai NWR ponds. A total of 19 to 21 taxa per pond were collected from six of the seven ponds surveyed and 13 taxa were collected from the remaining pond. Invertebrate densities ranged from 7.6 to 27.4 individuals m^{-3}. The majority of the taxa collected from each pond were predacious; predacious invertebrates made up 66% to 100% of each sample. The majority of these individuals were of the order Odonata (dragonflies and damselflies). Coleoptera (beetles) were the second most abundant predators, followed by the Hemiptera (waterbugs).

This report established that invertebrate predators do exist in large numbers in several Kenai NWR ponds. Interactions between invertebrates and tadpoles are probable because of the high density of predators and the small size of early-stage tadpoles. Therefore, it is plausible that depredation can account for at least a portion of the gross abnormalities observed in the Kenai NWR metamorph population we examined.

Water Toxicity Study – Kenai National Wildlife Refuge

Tadpoles reared in the SPMD extract from pond OF0003 took significantly longer to reach metamorphosis and were smaller upon metamorphosis than tadpoles from any other treatment. Tadpoles reared in the SPMD extract from OF0001 were not significantly different from the water control or any of the field blank controls. Responses from the field blanks were not significantly different than the controls. None of the endpoints examined were significantly affected by UV-B alone or by an interaction between SPMD extracts and UV-B. No physical abnormalities were noted during the experiment.

Water Quality Measurements - Arctic National Wildlife Refuge

Water quality constituents were measured in 9 of the 13 ponds (Table 6). The time of day in which measurements were taken varied.

CONCLUSIONS AND RECOMMENDATIONS

Kenai National Wildlife Refuge

Since surveys for abnormal amphibians on National Wildlife Refuges were initiated nationally in 2000, biologists have documented rates of abnormal frogs in sampled areas of the Kenai NWR that exceed national thresholds of concern (abnormality rates $\geq$ 3%). Based on this finding, we recommend that ponds included in the study continue to be monitored. 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, biologists will have an opportunity to examine the extent to which proximity to human activities and development may affect abnormality rates among refuge amphibian populations. Finally, we recommend that more detailed studies be conducted to identify possible stressors that may contribute to the observed abnormalities.

Arctic National Wildlife Refuge

In 2001, one pond at Arctic NWR (ARC10) had an abnormality rate greater than 3%. Based on national protocols, any pond with $\geq$3% abnormal metamorphs for two out of three years should continue to be monitored. Therefore, we recommend that ponds in the Arctic NWR be monitored for a second year.

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FIGURES

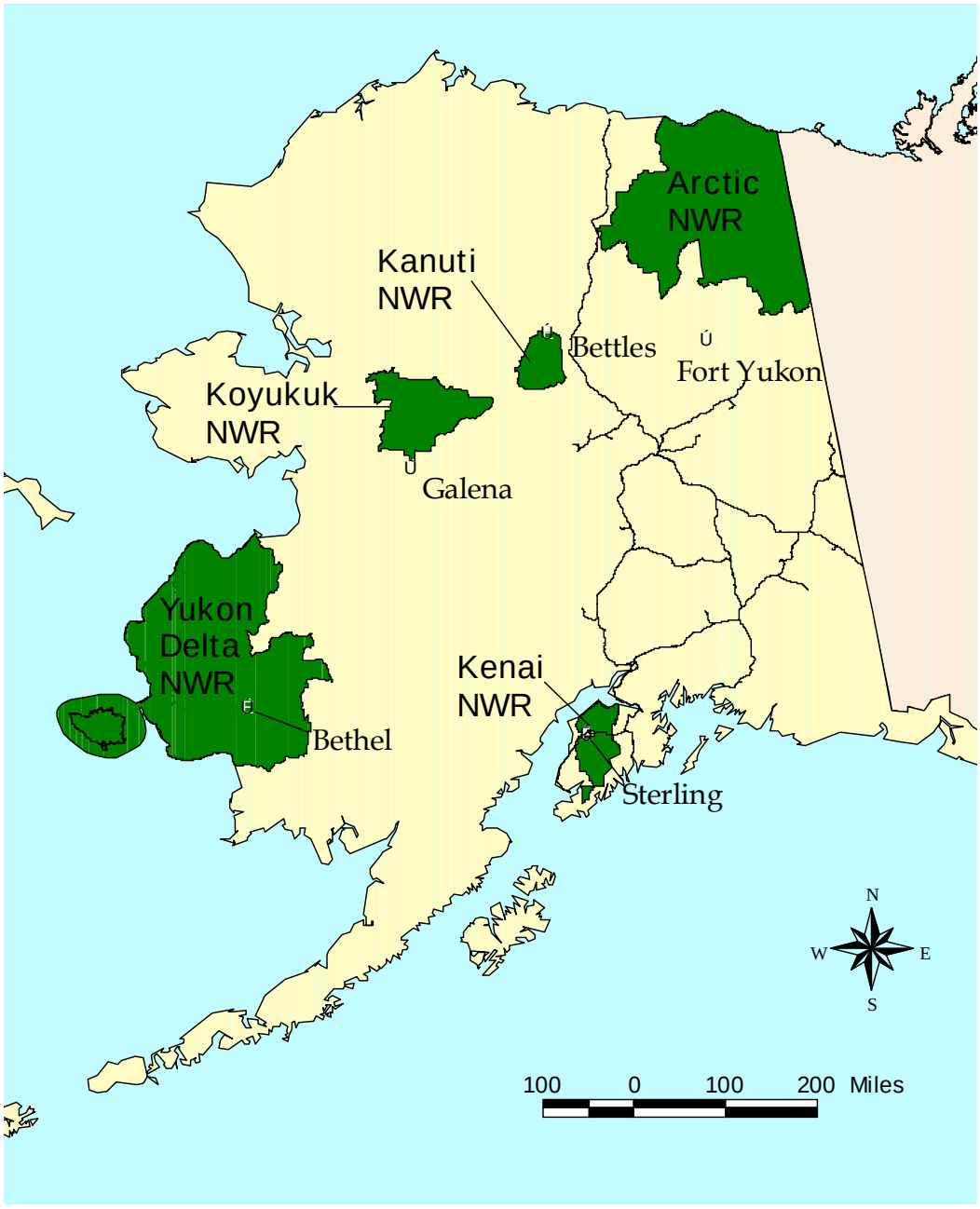

Figure 1. Locations of National Wildlife Refuges in Alaska Monitored for Abnormal Wood Frogs in 2001.

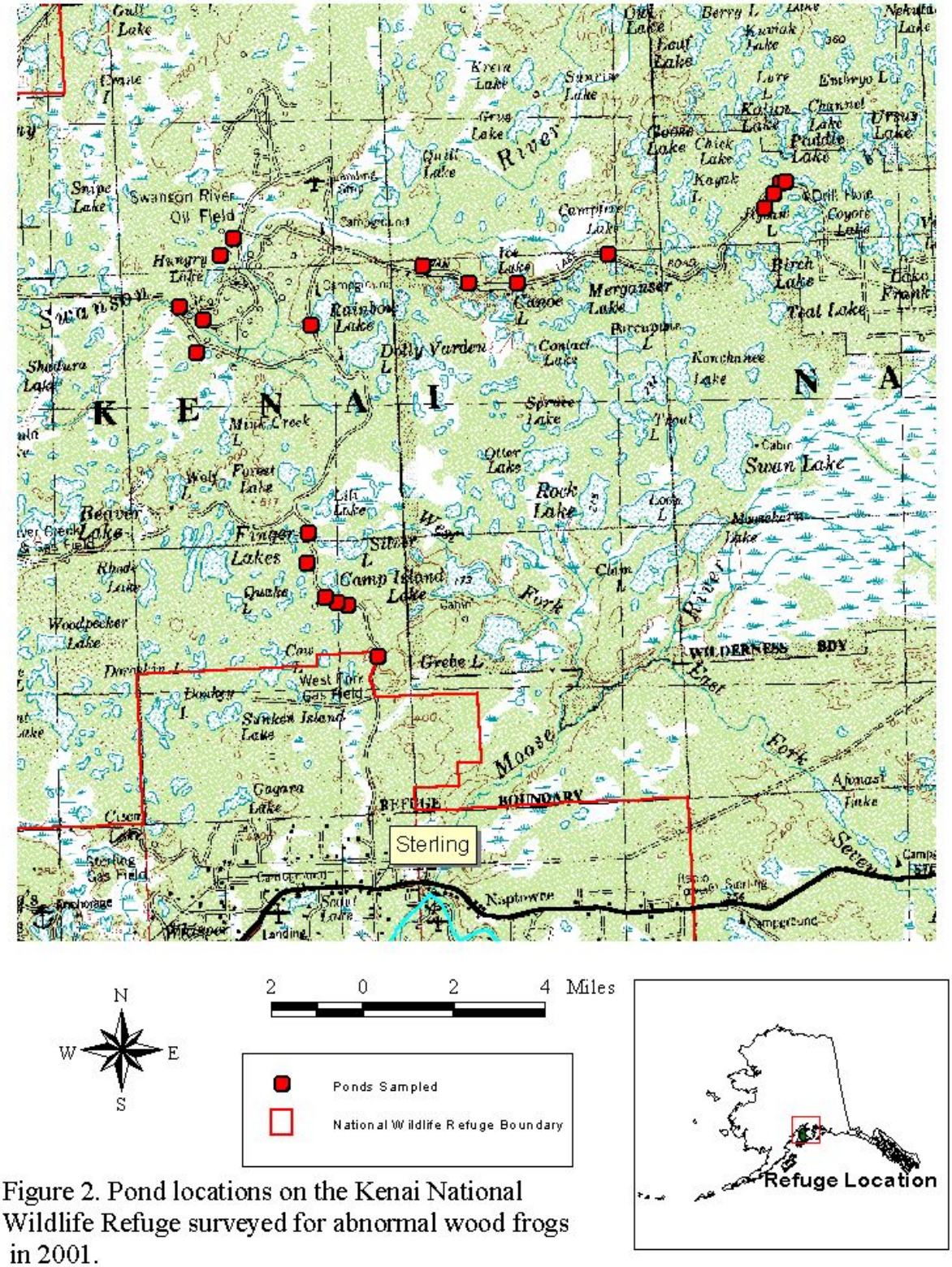

Figure 2. Pond locations on the Kenai National Wildlife Refuge surveyed for abnormal wood frogs in 2001.

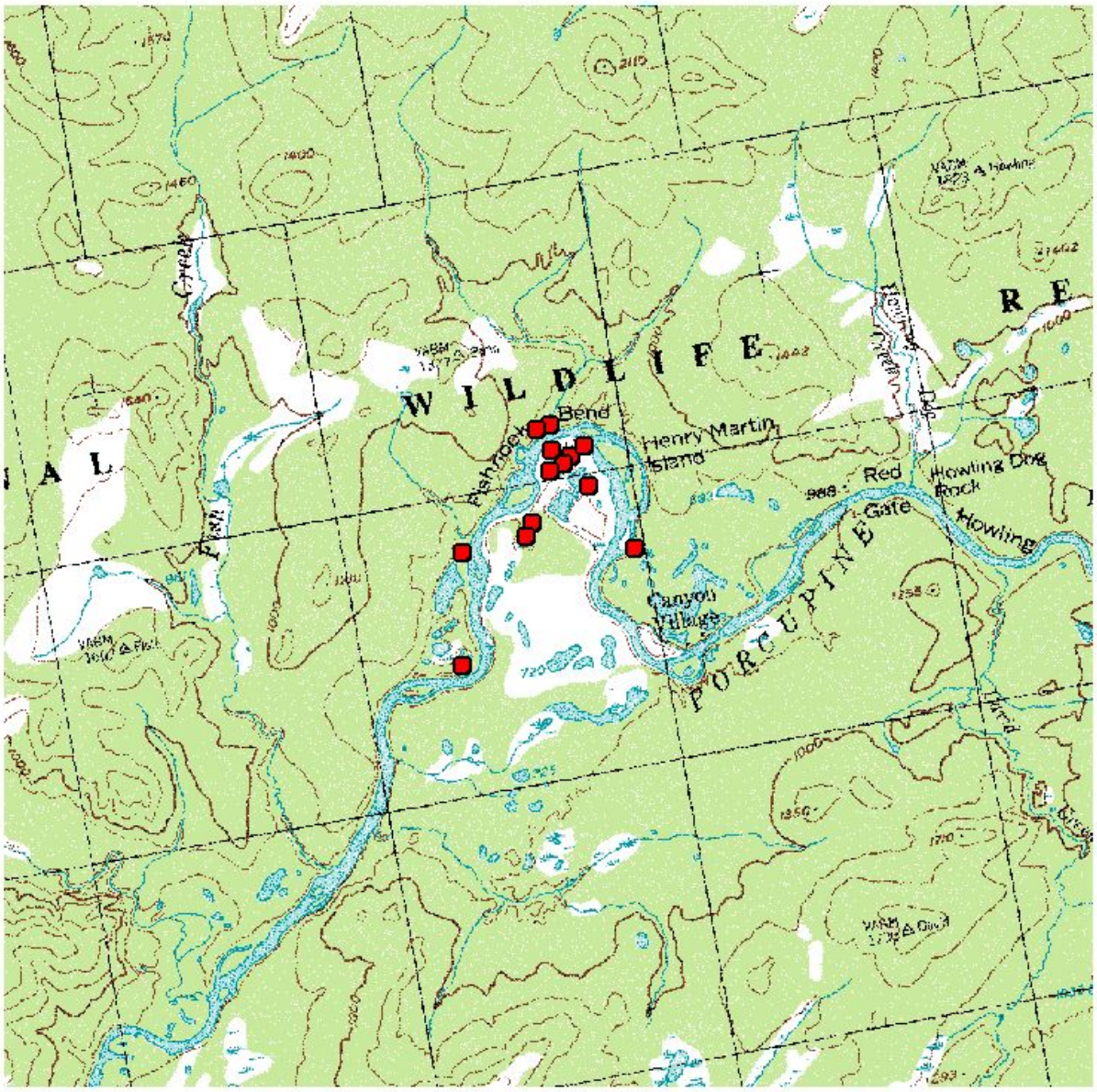

Figure 3. Pond locations on the Arctic National Wildlife Refuge surveyed for abnormal wood frogs in 2001.

Figure 4. Pond locations near the Koyukuk National Wildlife Refuge surveyed for abnormal wood frogs in 2001.

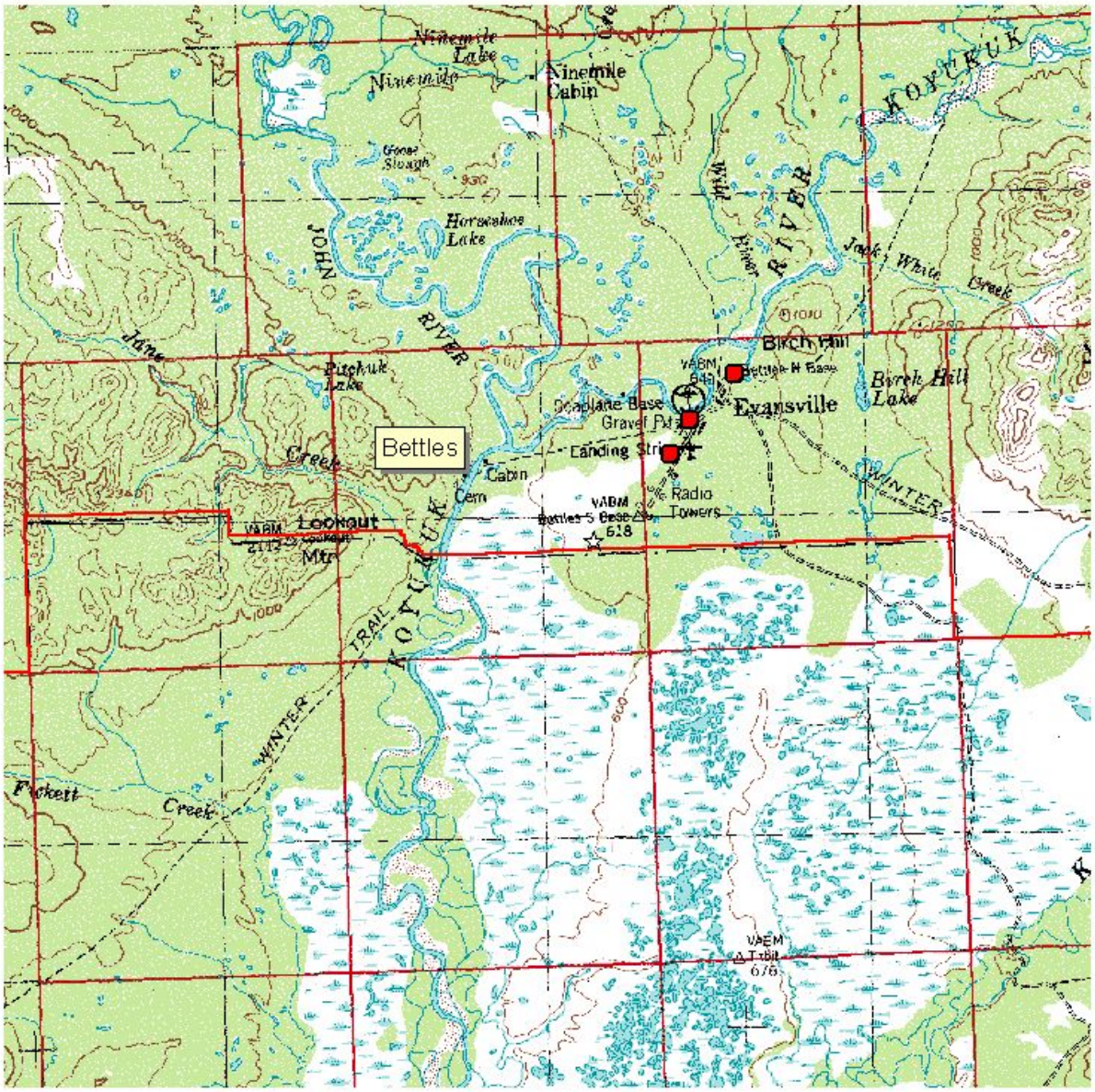

Figure 5. Pond locations near the Kanuti National Wildlife Refuge surveyed for abnormal wood frogs in 2001.

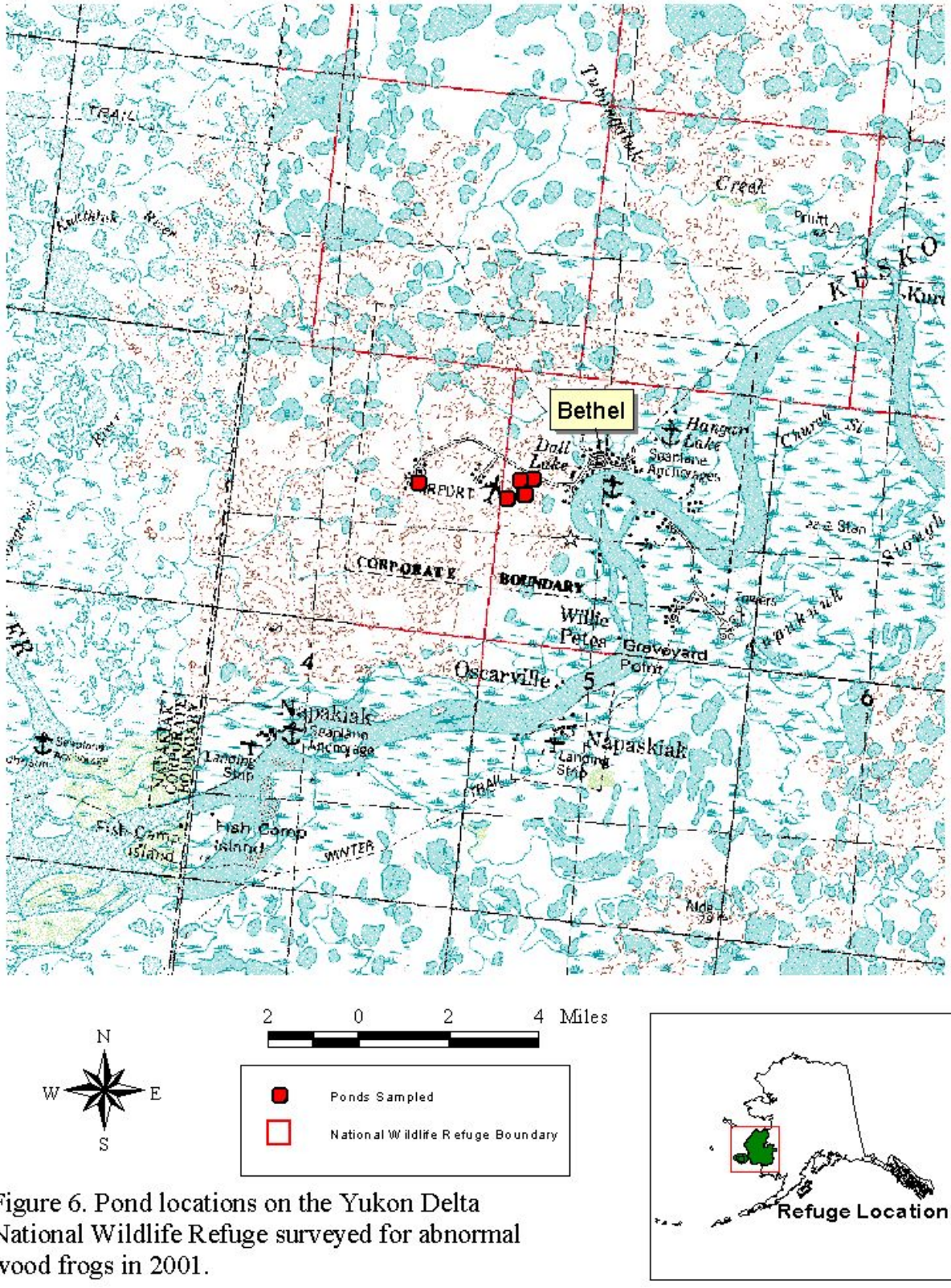

Figure 6. Pond locations on the Yukon Delta National Wildlife Refuge surveyed for abnormal wood frogs in 2001.

TABLES

Table 1. Numbers of abnormalities from wood frog studies on the Kenai NWR in 2001.

Pond ID	**# of Frogs Collected**	**#Abnormal**	**% Abnormal**
OF0001	52	4	7.7
OF0003	21	0	0
SL0001	21	1	4.8
SL0002	35	0	0
SL0005	86	4	4.7
SR0003	70	5	7.1
SR0004	57	6	10.5
SR0007	55	2	3.6
Totals	**397**	**22**	**5.5**

Table 2. Visual observations of abnormal wood frogs collected on the Kenai NWR in 2001.

Pond ID	Frog ID	Type of Abnormality	Body Part Affected
OF0101	OF01-001	Abnormal musculature	Right hind limb
OF0101	OF01-002	Ectromelia	Left hind limb
OF0101	OF01-003	Amelia	Right hind limb
OF0101	OF01-004	Amelia	Left hind limb
SL0101	SL01-001	Calf twisted or deformed	Left hind limb
SL0105	SL05-001	Amelia	Right hind limb
SL0105	SL05-002	Ectromelia	Left hind limb
SL0105	SL05-003	Micromelia	Left hind limb
SL0105	SL05-004	Ectromelia	Right hind limb
SR0103	SR03-001	Ectromelia	Right hind limb
SR0103	SR03-002	Ectromelia	Left hind limb
SR0103	SR03-003	Amelia	Left hind limb & right hind limb
SR0103	SR03-004	Ectromelia	Left hind limb
SR0103	SR03-005	Ectromelia	Right hind limb
SR0104	SR04-001	Ectromelia	Right hind limb
SR0104	SR04-002	Irregular spacing of LH digits; missing portion of tail; small thread of tissues connecting LH foot to pelvic region	Left hind limb
SR0104	SR04-003	Amelia	Right hind limb
SR0104	SR04-004	Micromelia; 6 digits on RH foot	Right hind limb
SR0104	SR04-005	Ectromelia	Right hind limb
SR0104	SR04-006	Ectromelia	Right hind limb
SR0107	SR07-001	Ectromelia	Right hind limb
SR0107	SR07-002	Ectromelia	Left hind limb

Table 3. Numbers of abnormal wood frogs found during studies on the Arctic NWR in 2001.

Pond ID	**# Collected**	**# Abnormal**	**% Abnormal**
ARC02	66	2	3.0%
ARC04	50	0	0.0%
ARC05/6	58	1	1.7%
ARC07	51	1	2.0%
ARC10	67	4	6.0%
ARC13	60	1	1.7%
Totals	**352**	**9**	**2.6%**

Table 4. Description of abnormalities found in wood frogs from Arctic NWR in 2001.

Pond ID	Frog ID	Type of Abnormality	Body Part Affected
ARC02	7AR0102-001	Micromelia	Left hind limb
ARC02	7AR0102-002	Ectromelia	Right hind limb
ARC05	7AR0105-001	Micromelia	Left hind limb
ARC07	7AR0107-001	Micromelia; polyphalangy	Right fore limb
ARC10	7AR0110-001	Micromelia	Right fore limb
ARC10	7AR0110-002	Micromelia; brachygnathia	Right fore limb and jaw
ARC10	7AR0110-003	Polyphalangy; micromelia	Right hind limb
ARC10	7AR0110-004	Ectromelia	Right hind limb
ARC13	7AR0113-001	White nodule at tail tip	Tail

Table 5. Description of abnormalities found in wood frogs from one pond near the Koyukuk NWR in 2001.

Pond ID	Frog ID	Type of Abnormality	Body Part Affected
Duck Pond	7-KO-001	Back hind feet curled towards each other	Both hind limbs
Duck Pond	7-KO-002	Digits of RHF curled inwards	Right hind limb
Duck Pond	7-KO-003	Digits of LFH curled inwards	Left hind limb
Duck Pond	7-KO-004	Digits of RHF curled inwards	Right hind limb
Duck Pond	7-KO-005	Ectromelia	Right hind limb

Table 6. Water quality measurements from ponds on the Arctic NWR in 2001.

Pond ID	Date Sampled	Temperature ^{0}C	Dissolved O_2 mg/L	Specific Conductivity *u*S/cm	pH
ARC02	7/19/01	19.83	3.4	200.4	6.21
ARC03	7/19/01	19.34	2.49	225.3	6.81
ARC04	7/19/01	21.60	2.6	219.31	6.76
ARC05	7/20/01	22.58	11.45	185.5	10.4
ARC06	7/20/01	21.75	9.38	135	10.12
ARC07	7/20/01	21.02	9.54	126.7	7.27
ARC08	7/21/01	20.87	4.64	398.3	7.39
ARC10	7/19/01	17.97	0.97	277.6	6.57
ARC11	7/24/01	18.87	4.01	247.8	7.08

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