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National Malformed Amphibian Study: FY 2000: Kenai National Wildlife Refuge

Annual Progress Report: WAES TR-02-01

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

National Malformed Amphibian Study

FY 2000: Kenai National Wildlife Refuge

Annual Progress Report

by

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Executive Summary:

This report summarizes findings from the first year of a multi-year study of amphibian malformations on National Wildlife Refuges in Alaska. The Kenai National Wildlife Refuge was the first refuge in Alaska to be selected as part of a nationwide study of malformed frogs. Twenty ponds were selected along Swanson River Road, Swan Lake Road and throughout the Swanson River Oil Field. These ponds were monitored throughout the summer for wood frog (*Rana sylvatica*) tadpole growth and development. Additionally, water quality parameters were measured several times during the season. Some of the ponds evaporated, thus only 13 ponds were still viable by mid-July when frog collections occurred.

The objective of the study was to capture 50-100 wood frogs at each pond as soon as they metamorphosed from tadpoles into small frogs (froglets) and examine them for physical abnormalities. Not all ponds produced the minimum number of frogs for examination. Any froglet with an anomaly was preserved and sent to the U.S. Geological Survey's National Wildlife Health Center in Madison, Wisconsin for diagnostic evaluation. Abnormalities were categorized into either malformations, deformities or "unknown cause."

We collected 348 frogs from 13 ponds. Of those, 30 or 8.6% (25 froglets; 5 late-stage tadpoles) had abnormalities visually observed in the field. This is above a predicted background rate (0-2%) of abnormalities expected in an amphibian population (Ouellet 2000). Diagnosis from the NWHC indicated that 12 (or 3.4%) of the 348 frogs actually had a malformation, an abnormality due to developmental problems (Meteyer 2000). Nevertheless, a few individual ponds had much higher rates of malformations (over 10% in one pond) and should be examined more closely.

In accordance with national guidelines and protocols, this study was designed only to investigate possible presence of malformed frogs on a National Wildlife Refuge in Alaska. Thus, no cause can be currently ascribed to the abnormalities found in frogs from the Kenai Refuge. Future research should be conducted on these same ponds to account for inter-annual variation in frog numbers and abnormalities. Additionally, toxicity testing and/or chemical analysis should be initiated on abiotic media (e.g., water and sediment) and frogs from ponds with high numbers of malformations. Finally, sampling should be expanded to include other refuges in Alaska that have wood frogs.

Introduction:

Amphibian malformations became the focus of national attention in 1995, when middle school students from Minnesota discovered large numbers of frogs with misshapen, extra, or missing limbs. Since then, reports of severely malformed frogs have increased nationally, and the North American Reporting Center for Amphibian Malformations (NARCAM) has documented amphibian abnormalities in Connecticut, Maine, Maryland, Massachusetts, New Jersey, New York, Pennsylvania, Vermont, Virginia, and West Virginia. As of 1997, Minnesota had reported the highest number of malformed frogs in the country (Helgen *et al* 1997).

Due to the increasing incidence of abnormal frog reports, the U.S. Fish and Wildlife Service (Service) began documenting these cases on several National Wildlife Refuges (NWRs) in the Northeast and Midwest regions of the United States. Ultimate causes of these anomalies have not been determined, however disease agents, increased UV radiation exposure, nutritional deficiencies, exposure to environmental contaminants or some combination of these factors may contribute to this phenomenon (Ouellet 2000). In fiscal year 2000 (FY2000), the Service's Environmental Contaminants Program received funding to investigate the prevalence of abnormal amphibians on NWRs across the country. Eventually, this investigation should help identify possible links, if any, between high incidence of abnormal frogs and potential exposure to environmental contaminants. Forty-three refuges from 31 states were selected to be sampled in 2000, including the Kenai NWR (KNWR) in Alaska.

Six species of amphibians occur in Alaska, but the wood frog (*Rana sylvatica*) is the only amphibian species which is abundant on Alaska's NWRs. Other species, such as the spotted frog (*Rana petriosa*), inhabit southeast Alaska, but are not known to occur on refuge lands. No comprehensive information had previously existed regarding the occurrence of abnormal amphibians in the state of Alaska. In 1998, an unconfirmed sighting of abnormal wood frogs was reported by a Girl Scout troop near Big Lake, Alaska, approximately 60 miles north of Anchorage. Additionally, a 3-legged wood frog was captured in Anchorage and used for educational purposes for K-12 grades (K. Simec, pers. comm.). No pathology reports are available for these early findings. In 1999, two wood frogs with missing eyes were captured from Eklutna Lake and examined by a veterinary pathologist. Eklutna Lake, the source of drinking water for the municipality of Anchorage, is located approximately 20 miles north of the city. Pathology reports indicate that one of the optic nerves was not developed in each of the two frogs. This implies that the abnormalities were true developmental malformations, because remnant optic nerves should have been present if the injuries were due to trauma (D. Mulcahy, pers. comm.).

Investigating Malformed Amphibians on the Kenai National Wildlife Refuge

Alaska has 16 NWRs totaling more than 77 million acres. Thus, several factors were considered when choosing the KNWR as the first refuge to be sampled in the state. First, biologists had ancillary information on wood frog natural history within KNWR. Since 1991, eight to ten ponds had been observed each spring for presence of wood frog egg masses, so locations of some frog-producing ponds were known (T. Bailey, pers. comm). Additionally, the KNWR is the closest refuge to the population center of the state (Anchorage) and to areas with known frog abnormalities. Finally, the KNWR has known contaminant issues (for a thorough review, see Parson 2000) and although ultimate causes of documented amphibian malformations are unknown, exposure to environmental contaminants may be a contributing factor (Ouellet 2000). Consistent with the national study design, we therefore used existence of potential contaminant sources as an additional criterion for selecting refuges

Prior to and since its establishment, the KNWR has housed a variety of activities that have introduced contaminants into the environment. For example, more than 290 oil and hazardous material spills were reported on or near the Swanson River and Beaver Creek oil and gas fields between 1957-1999. Additionally, the discovery of PCB-contaminated roads within the Swanson River oil field, in the 1980s, yielded over 107,000 tons of contaminated soil for removal and subsequent incineration (Parson 2000). Other potential contaminant sources affecting the refuge may include pesticides, formerly used defense sites (FUDS), development, mining, waste disposal, recreational uses, fires and fire retardants, underground storage tanks, aircraft accidents, biotic sources and physical transport of contaminants from sources outside refuge boundaries. Correlations between wood frog abnormalities on the KNWR and known local sources of contamination have not been examined; it is anticipated that research into the causes of wood frog abnormalities will occur during the next phase of this study.

Logistical considerations also were a factor in our decision to sample the KNWR. Most of Alaska's refuges are not on the main road system, and funding limitations in FY2000 limited our ability to sample remote refuges. Portions of the KNWR were road-accessible, which was critical since exact timing of frog development and metamorphosis were poorly understood. Frequent revisits allowed us to monitor these parameters throughout the summer.

The goal during the initial phase of the national amphibian study was to perform a field reconnaissance of various NWRs and determine whether malformed amphibians were present and to begin documenting the prevalence of these malformations. Investigation of potential causative factors such as contaminant analysis of abiotic media associated with frog habitat, including pond water and sediment were beyond the scope of this initial pilot study. For this project, our objectives were to: 1. Determine the incidence of abnormalities in wood frogs from a subset of water bodies on the KNWR; 2. Determine the type of abnormality (malformation vs deformation) from preserved, abnormal frogs; and 3. Correlate water quality parameters from collection ponds with numbers of abnormalities.

Methods:

Sampling Efforts

Sampling for abnormal frogs was conducted in accordance with national guidelines using standard operating procedures established by the U.S. Fish and Wildlife Service, Chesapeake Bay Field Office. In May of 2000, staff from the Division of Environmental Contaminants, Ecological Services-Anchorage and KNWR identified 20 ponds on the refuge that contained wood frog egg masses. A crew of 6 people spent three days locating suitable ponds. Pond selection criteria included prior survey of ponds for egg masses by refuge biologists, inspection of aerial photos and topographic maps to find other probable wood frog ponds, and review of a recent contaminants assessment report (Parson 2000) to identify areas that had potential contaminant influences. We selected 5 ponds in the Swanson River Oil Field, 8 ponds near Swan Lake road and 7 ponds near Swanson River Road for further monitoring (Figure 1). Ponds were located in a range of habitats, some were adjacent to potential contaminant sources, such as drill pads or roads, and others were up to one mile away from any known or suspected source of local contamination. Pond locations were selected using the criteria described above, and did not reflect a treatment/control study design.

Pond locations were recorded with a military GPS-PLGR® unit using a UTM coordinate system and WGS84 datum information. A survey flag was used to mark egg masses in each pond and coordinates were recorded for each of those locations. A metal survey marker was attached to a tree either adjacent the pond or on the road closest to the pond, and the tree was marked with two colors of survey flagging. A compass bearing was recorded from the marked tree towards the first egg mass flag to assist with relocating the pond as the summer progressed and vegetation became thicker.

From June 7 to August 2, 2000, ponds were monitored weekly for egg and tadpole development using stages described in Gosner (1960). Metamorphs were inspected at 13 of these 20 ponds starting in mid-July. Ponds were swept by 1-3 people using handnets with the intent of catching 50-100 metamorphs per pond. Froglets were examined for physical anomalies including missing limbs, extra limbs, deformed limbs, and missing eyes. Frogs lacking abnormalities were inspected and returned to their natal ponds. Abnormal froglets and late-stage tadpoles ($\geq$ Gosner stage 44-45) were collected and anesthetized using MS222 (1:5 dilution with water). Frogs were then placed in a tray with 2 cm of paraffin lining the bottom. 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, froglets were transferred to ICHEM® glass jars containing 100% ethanol for 1-2 weeks. They were then transferred to 70% ethanol until examination by personnel at the National Wildlife Health Center, Madison, WI (NWHC).

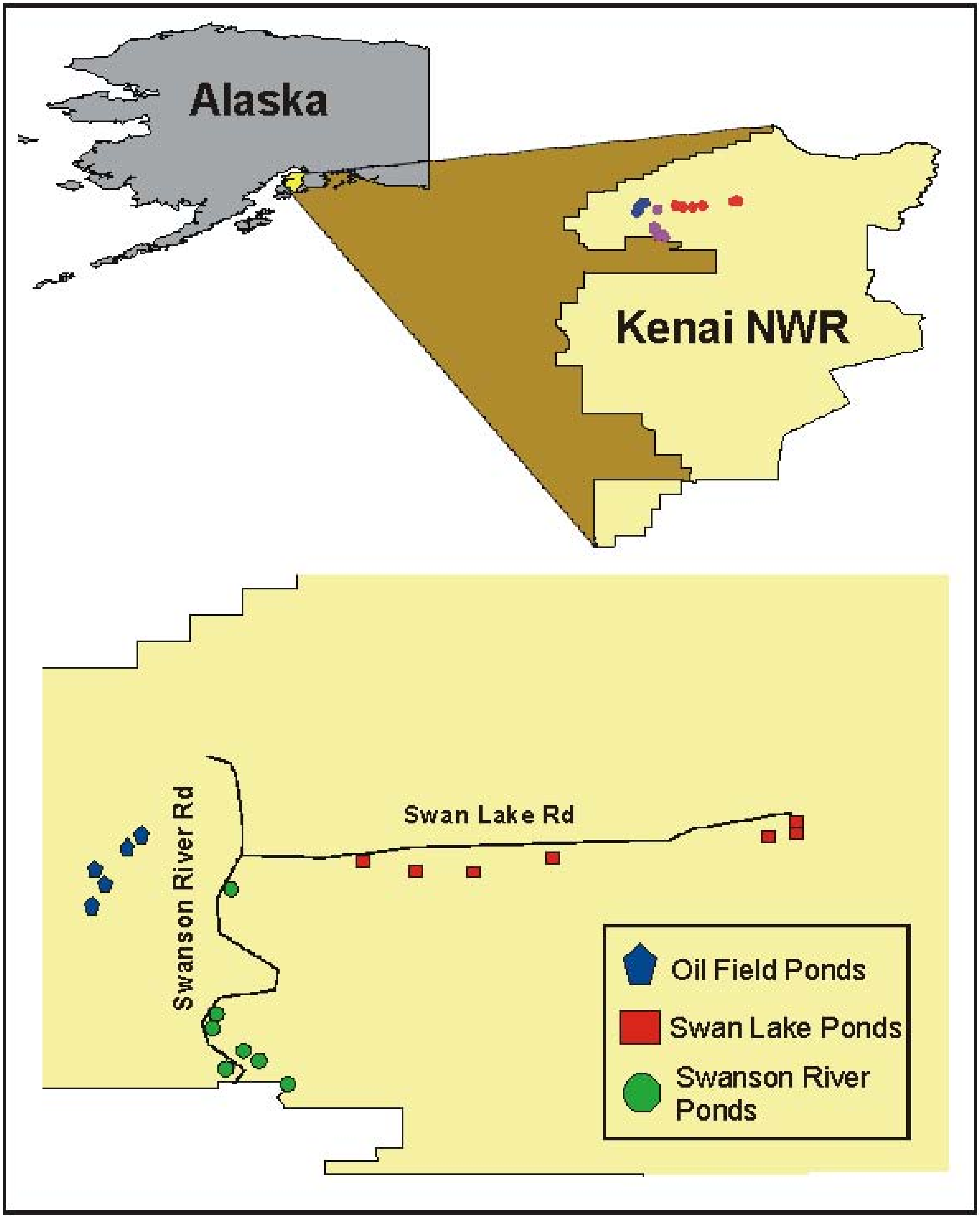

Figure 1: Locations of ponds on the Kenai National Wildlife Refuge monitored during the 2000 Malformed Amphibian Survey.

Several other techniques were employed for catching froglets with limited success. A 2 m x 1 m wood and wire-mesh cage was constructed and placed in one of the ponds. The top was covered with screen, but it did not have a bottom so it could be pushed down into the mud to hold it in place. About one-half of the cage was filled with water. Approximately 50 tadpoles were placed inside to observe development. Nevertheless, when the pond was checked 3-4 days later, few tadpoles were found. Pit-fall traps were deployed at one pond in attempts to catch froglets as they left their natal ponds on their way to the forest. Coffee cans were buried in two designs (a straight line and a W-shape) on opposite sides of Swanson River pond #3 (SR0003). These traps were monitored daily from June 21 to July 24, 2000. A few adults and froglets were found in the traps late in the season. Unfortunately, shrews and mice also fell into the traps and were usually deceased by the time the collector arrived.

Water Quality Measurements

Several water quality parameters (pH, temperature, conductivity and dissolved oxygen) were measured weekly in the field using a Hydrolab®. Prior to each measurement series, two-buffer calibrations were performed using pH buffers accurate to ± 0.02 pH units which bracketed the pH of the samples. Conductivity standards were used to check meter performance prior to the measurement series in the field. The dissolved oxygen membrane was changed twice during the season.

Other water quality parameters (total alkalinity, total hardness, conductivity, turbidity, ammonia, nitrate, nitrite and phosphate) were measured in all 20 ponds once during the egg mass stage in May. Of the 20 original ponds, 16 ponds were still viable (i.e., had not dried up) by mid-July, therefore the additional water quality measurements were conducted once more on 16 ponds when tadpoles were beginning to metamorphose in July. To measure these parameters, triplicate 1-L water quality samples were collected from each pond in plastic bottles. Water quality sample containers were triple-rinsed in the water to be sampled prior to collection. Water samples were surface grabs and each sample bottle was extended in front of the collector to avoid contamination due to resuspension of sediment or from the collector. Sample bottles were filled to the top to minimize gaseous exchange. Each sample bottle was labeled prior to collection and placed in a cooler for transport to a field laboratory for analysis.

Hardness and alkalinity were determined using a Hach digital titrator and Hach (1992) methods. Turbidity was measured using a Hach Model 2100P Portable Turbidity Meter calibrated with Gelex secondary standards for 1, 10, and 100 nephelometric turbidity units (NTU) which were calibrated immediately prior to the sampling trip and checked in the field laboratory. Ammonia, nitrate/nitrite, and phosphate were determined using Hach methods (1992) and a Hach DR2010 spectrophotometer with a pour-through cell in the field laboratory.

Water quality parameters collected only twice were statistically analyzed using Systat 9 (SPSS Inc.). Included in these analyses were dissolved oxygen, pH, temperature and conductivity measurements taken when water quality grab samples were collected. For this document, we did not statistically analyze the water quality variables measured weekly. We grouped ponds by areas: Oil field = OF; Swan Lake = SL ponds and Swanson River = SR, and looked at differences among parameters by sampling event. Sampling events occurred in May and July. Multiple analysis of variance (MANOVA) was used to examine the differences among all variables by Pond Site and Sampling Event. If appropriate, analysis of variance (ANOVA) was then used to examine the differences among variables by main effects, separately. Pairwise comparisons among those variables that were significantly different were made using Bonferroni's test. Differences were considered significant if $p < 0.05$.

Results and Discussion:

Field sampling produced data regarding the incidence of abnormal frogs determined by visual observation in the field. Subsequent diagnostic evaluations from NWHC provided information about the incidence of malformations in these abnormal frogs. Field results were evaluated at two geographic scales; at the refuge level and at the individual pond level.

Field Results:

Refuge Scale

Of the 348 metamorphosed frogs inspected, 30 or 8.6% had abnormalities. Five of these were abnormal late-stage tadpoles that were also collected and sent to NWHC. Etiology of abnormalities found in tadpoles could not be determined, however they are included in the results for this study (Table 1).

Table 1. Numbers of abnormalities and malformations found in wood frogs on the Kenai NWR, 2000.

Date Collected	Pond ID	Metamorphs examined	Abnormalities	Malformations
7/25/00	OF0001	59	11	6
7/18-26/00	OF0002	28	1	0
7/26-8/1/00	OF0003	16	2	1
7/12/00	OF0004	49	4	2
7/26/00	OF0005	8	0	0
7/22/00	SL0002	53	0	0
7/24-8/1/00	SL0005	16	1	1
7/25-8/1/00	SL0007	28	2	0
7/25/00	SR0002	19	0	0
7/17-24/00	SR0003	58	7	2
7/13-25/00	SR0004	12	1	0
7/25/00	SR0006	1	1	0
7/25/00	SR0007	1	0	0
	Total	**348**	**30**	

Individual Pond Comparisons

Our goal was to inspect at least 50 metamorphs per pond to calculate a "per pond" abnormal percentage. We only had three ponds that produced 50 metamorphs or more. Therefore, for comparison and because our number of ponds that met the >50-frog criteria was so low, we also included one pond that produced 49 metamorphs in our individual pond percentage calculation. In two of the oil field ponds, OF0001 and OF0004, the percentage of abnormal metamorphs was 18.6% and 8.2%, respectively. On one of the Swan Lake ponds (SL0002), all of the 53 metamorphs examined were apparently normal, but on one of the Swanson River ponds (SR0003), 12.1% of the froglets were abnormal (Table 1).

Diagnostic Results

Of the 30 metamorphs sent to the NWHC, 12 were malformed (40%), 12 were deformed due to trauma (40%) and six (20%) were undetermined (Table 2). Five of the six abnormal animals in the "unknown" category were late-stage tadpoles. At this life-stage, the causes of visible anomalies are difficult to diagnose because the skeletons have not calcified sufficiently for proper x-ray analysis.

Table 2. Diagnostic results of 30 abnormal frogs from the Kenai NWR sent to the National Wildlife Health Center, Madison, Wisconsin in 2000.

Pond	**# Malformed**	**% Malformed**	**# Trauma**	**% Trauma**	**# Unknown**	**% Unknown**
OF0001	6	10.2	3	5.1	2	3.4
OF0002	0	0	1	3.6	0	0.0
OF0003	1	6.3	0	0	1	6.3
OF0004	2	4.1	2	4.1	0	0.0
OF0005	0	0	0	0	0	0
SL0002	0	0	0	0	0	0
SL0005	1	6.3	0	0	0	0
SL0007	0	0	2	7.1	0	0
SR0002	0	0	0	0	0	0
SR0003	2	3.4	3	5.2	2	3.4
SR0004	0	0	0	0	1	8.3
SR0006	0	0	1	100	0	0
SR0007	0	0	0	0	0	0
Totals	**12**		**12**		**6**	

Out of the 348 metamorphs inspected, 8.6% exhibited some type of visual abnormality in the field and 3.4% had confirmed developmental malformations. In normal frog populations, morphological abnormalities and trauma-related deformities occur in 0-2% of the individuals (as reviewed by Ouellet, 2000). Types of malformations observed in KNWR frogs are summarized in Table 3 and photographs of specific abnormalities can be found in Appendix B.

Table 3. Diagnosis of malformations from frogs of the Kenai National Wildlife Refuge.

Pond ID	Sample ID	Diagnosis*
OF0001	40	Amelia of left hind limb (LHL)
OF0001	2	Brachygnathia
OF0001	3	Brachygnathia
OF0001	31	Amelia of LHL with missing ischium; Luxation of left sarco-iliac joint
OF0001	52	Bone bridge of left tibiafibula
OF0001	44	Brachygnathia
OF0003	6	Micromelia of LHL; Ectrodactyly of LHL; Brachydactyly of 4 left toes (II-V)
OF0004	32	Scoliosis (or kyphosis)
OF0004	18	Amelia of right front limb
SL0005	10	Amelia of left front limb; Abnormal left otolith
SR0003	55	Microcephaly with skin fold on dorsal head
SR0003	37	Amelia of LHL

*Glossary of terms located in Appendix A.

Although the percentage of malformations for all ponds was 3.4%, certain ponds had much higher percentages of malformed frogs and warrant further investigation (Table 2). For example, from one of the ponds (OF0001), six of the 11 abnormal frogs collected were malformed. We had examined 59 frogs from that pond, thus 10.2% of the metamorphs from OF0001 were malformed. This was the highest percentage of malformations from any of the ponds. Nevertheless, sample sizes from several of the other ponds were below the 50-frog minimum, and in some instances individual pond abnormality percentages could not be calculated. For example, only one metamorph was caught at SR0006, and it was abnormal. Clearly, a sample size of one is insufficient to determine the percentage of abnormal frogs from this pond.

Water Quality

For statistical analysis, we removed three outliers from the conductivity data in the July sampling event. Usually, the value of each variable used for analysis was the average value of the triplicate samples measured from the Hydrolab sonde, which was placed directly in the pond. In ponds SR0001, SR0002 and SR0007, the first triplicate value for conductivity was an order of magnitude higher than the other two values in the triplicate (or other conductivity values measured in any of the ponds). The abnormally high value was always the first value measured after the sonde was placed in the water. Because it was so high, we reasoned that it could have been due to incomplete mixing of the water prior to the value being measured. Therefore, we were unsure that the measurement was taken correctly and those three data points were removed. The average for conductivity was then calculated on the remaining two samples. Additionally, we did not include nitrite in our statistical analyses, because values from the first sampling event were below measurable concentrations, thus no temporal comparisons could be made.

Table 4. Mean and (standard deviation) of water quality parameters included in statistical analysis. Data are separated by Sampling Event (1= May; 2 = July) and grouped by Pond Area (Oil Field, Swan Lake and Swanson River).

	EVENT 1			**EVENT 2**		
	Oil Field	Swan Lake	Swanson River	Oil Field	Swan Lake	Swanson River
N*	5	8	7	5	5	6
Alkalinity (mg/L)	7.8 (5.2)	6.4 (7.4)	5.8 (4.3)	7.9 (4.5)	3.8 (1.2)	6.4 (4.1)
Ammonia (mg/L)	0.02 (0.01)	0.02 (0.02)	0.01 (0.01)	0.02 (0.02)	0.05 (0.11)	0.04 (0.03)
Dissolved O_2 (mg/L)	8.1 (1.1)	8.7 (1.8)	7.9 (1.7)	4.7 (1.7)	4.2 (2.1)	3.0 (1.8)
Hardness (mg/L)	11.5 (6.1)	7.0 (7.5)	8.7 (4.2)	9.8 (7.3)	4.8 (2.7)	11.0 (5.0)
Nitrate (mg/L)	0.68 (0.35)	0.34 (0.32)	0.71 (0.35)	0.68 (0.54)	0.63 (0.98)	0.89 (0.46)
pH	7.7 (0.1)	5.6 (0.4)	6.5 (0.4)	5.7 (0.3)	5.6 (0.6)	5.6 (0.2)
Phosphate (mg/L)	0.02 (0.01)	0.04 (0.05)	0.06 (0.06)	0.03 (0.01)	0.14 (0.3)	0.06 (0.08)
Conductivity (µS/cm)	19.2 (10.7)	11.7 (10.7)	16.1 (10.5)	25.8 (13.7)	8.4 (6.2)	41.3 (11.4)
Temp (^{0} C)	12.5 (.94)	12.9 (1.9)	11.1 (1.8)	22.8 (1.2)	20.8 (2.3)	17.5 (1.8)
Turbidity (NTU)	0.95 (0.31)	1.03 (0.36)	1.93 (0.89)	1.7 (1.3)	6.8 (11.0)	10.3 (15.2)

*Sample size (N) equals the number of ponds per area per sampling event.

We were primarily interested in the differences in water quality parameters among pond areas within each sampling event. Some parameters, such as temperature inevitably change as the summer progresses, air temperature increases and the ponds evaporate. This evaporative process may also concentrate some nutrients, thus changes between sampling events are inevitable. Therefore, we separated the data by Sampling Event and looked at the differences among variables by Pond Area (Table 4). For the May sampling event, pH and turbidity were significantly different among Pond Areas ($p < 0.001$ and $p = 0.014$, respectively). Regarding turbidity, all three areas were different from each other ($p < 0.001$) with Swanson River > Swan Lake > Oil Field. At all three areas, pH was significantly different ($p = 0.03$), with Oil Field > Swanson River > Swan Lake. For the July sampling event, conductivity and temperature were significantly different among Pond Areas ($p = 0.001$). Conductivity decreased from Swanson River > Oil Field > Swan Lake, but the only significant difference was between Swan Lake and Swanson River ($p =0.001$). Temperature was highest in the Oil Field area, followed by Swan Lake and finally the Swanson River area. Temperatures in all areas were significantly different from each other ($p \leq 0.02$).

Most nutrient values were low in all ponds for both sampling events and variability was high for most constituents. This, coupled with small sample sizes and a significant interaction, may have masked some important differences. For example, pH was significantly higher in the oil field sites during the first sampling event, but not statistically different during the second sampling period, even though the absolute value was still higher than the other two areas. Due to the small number of malformed frogs (per pond), statistical correlations between percent malformity and water quality parameters were not performed.

Constraints and Recommendations:

Because wood frogs are the only species of frog known to occur on Alaska refuges and because we collect froglets as soon as they leave their natal ponds, timing of metamorphosis is critical. Unlike other ranid species, wood frogs leave their natal ponds as soon as they metamorphose and seek shelter in the detrital layer of nearby vegetation. We assumed that if we missed peak metamorphosis at a pond, there would not be another one later in the season. This assumption seemed to hold true, however metamorphosis did not occur simultaneously in all ponds, as we had anticipated. We missed peak metamorphosis on several ponds because we had about a 3-day check schedule. Because of problems encountered this year (e.g., eruptive metamorphosis in areas where few tadpoles had been found), we believe it is wise to continue to monitor ponds on a weekly basis beginning in mid-June and switching to daily visits closer to anticipated metamorphosis dates.

We recommend collecting data on invertebrates, vegetation and weather, none of which were collected this year. From personal observation (H. Tangermann), there seems to be a correlation between the number of metamorph abnormalities and high invertebrate populations.

Flagging the egg masses was not necessary. Taking notes on the general area where the masses were found should suffice, as they disintegrate fairly rapidly after the tadpoles hatch, the flags are hard to find at the end of the season when the vegetation is tall and thick, and egg mass location did not reliably indicate where tadpoles or metamorphs could be found. The field crew should take a net to look for tadpoles in early May when the first search for egg masses is conducted. Tadpole development in individual ponds was highly varied and some ponds may have tadpoles very early in the season, possibly tadpoles that have over-wintered.

We recommend using pitfall traps again because they can help determine the timing of peak metamorphosis. We suggest placing pitfall traps in at least two ponds, mid-season, to estimate the beginning and end of metamorphosis. Traps should be placed in one pond that has early-stage tadpoles and one that has later stage tadpoles. It is imperative that the traps be checked twice daily. A straight line formation is adequate but a W-shaped trap line worked better on our trial pond.

Finally, late-stage tadpoles should only be examined if all four limbs are present and the mouth is developed between the nostril and the eye. This will ensure that the mouth, head and pelvis region are more complete and bones are visible on x-rays.

Conclusions:

The overall percentage (8.6%) of abnormal frogs collected from KNWR was greater than the percentage (0-2%) of total abnormalities expected in wild populations, and even the percentage of malformed frogs (3.4%) exceeded this conservative estimate. Furthermore, some individual ponds had much higher numbers of malformed frogs and warrant further investigation. This study was designed only to investigate the presence of abnormalities in frogs from a National Wildlife Refuge in Alaska. Thus, no cause can be currently ascribed to these abnormalities. Several theories have been postulated regarding the etiology of malformations in frogs from other geographic regions including disease agents, increased UV radiation exposure, nutritional deficiencies, exposure to environmental contaminants or some combination of these factors (Ouellet 2000).

Future research should be conducted on these same ponds to account for inter-annual variation in frog numbers and abnormalities. Subsequent sampling will also increase sample sizes, thus making the data more robust to analysis. Additionally, an invertebrate catalog (e.g., species/distribution) should be created for ponds with high incidences of trauma-related deformities. Finally, toxicity testing and/or chemical analysis should be initiated on abiotic media (e.g., water and sediment) and frogs from ponds with high numbers of malformed frogs and reference ponds.

Acknowledgments:

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Literature Cited

Gosner, K. 1960. A Simplified Table for Staging Anuran Embryos and Larvae with Notes on Identification. Herpetologica. 16:183-190.

Hach. 1992. Water Analysis Handbook. Hach Company, Loveland, CO. 831 pp.

Hellgen, J.C., M. Gernes, D. Bowers, J. Burkhart, K. Gallagher, and D. Hoppe. 1997. Field investigations of abnormal and normal frog sites in MN from 1995-1997. 18th Annual Meeting, Society of Environmental Toxicology and Chemistry (SETAC), November 16-20, 1997, San Francisco, CA.

Meteyer, C.U. 2000. Field guide to malformations of frogs and toads with radiographic interpretations. Biological Science Report USGS/BRD/BSR-2000-0005.

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. Ed. D.W. Sparling, G. Linder and C.A. Bishop. 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.

Appendix A: Glossary of Diagnostic Terms

Amelia: No evidence of a limb, the hip region is smooth and the pigment pattern is not disrupted.

Brachydactyl: Short toe; The normal number of metatarsal bones are present but the number of phalanges (bones in the toe) are reduced.

Brachygnathia: Abnormal shortness of lower jaw; same as mandibular micrognathia.

Bone Bridge: A bone structure that spans the space between two margins of bent bone. This bone structure appears radiographically as a plane of linear rays of bone that extend from the margins of bent bone and fill the angle between bone margins.

Ectrodactyly: Missing toe; Distinguished from brachydactyly and refers to a completely missing digit including the metatarsal bone and phalanges.

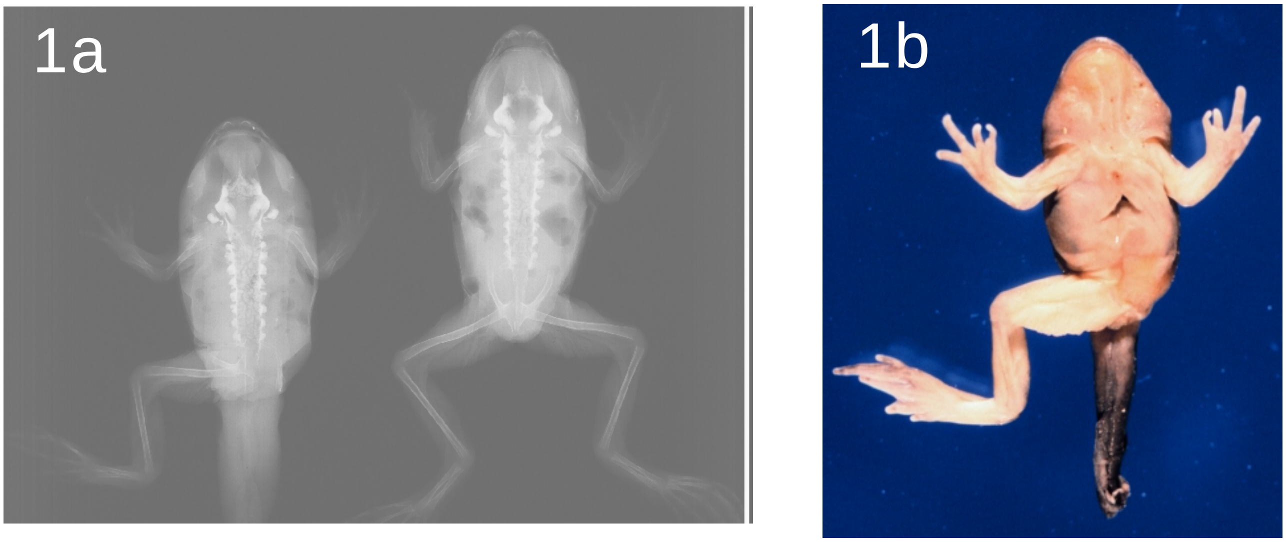
Luxation: Dislocation of an anatomical part (as a bone at a joint or the lens of the eye).

Microcephaly: Small head, blunt snout.

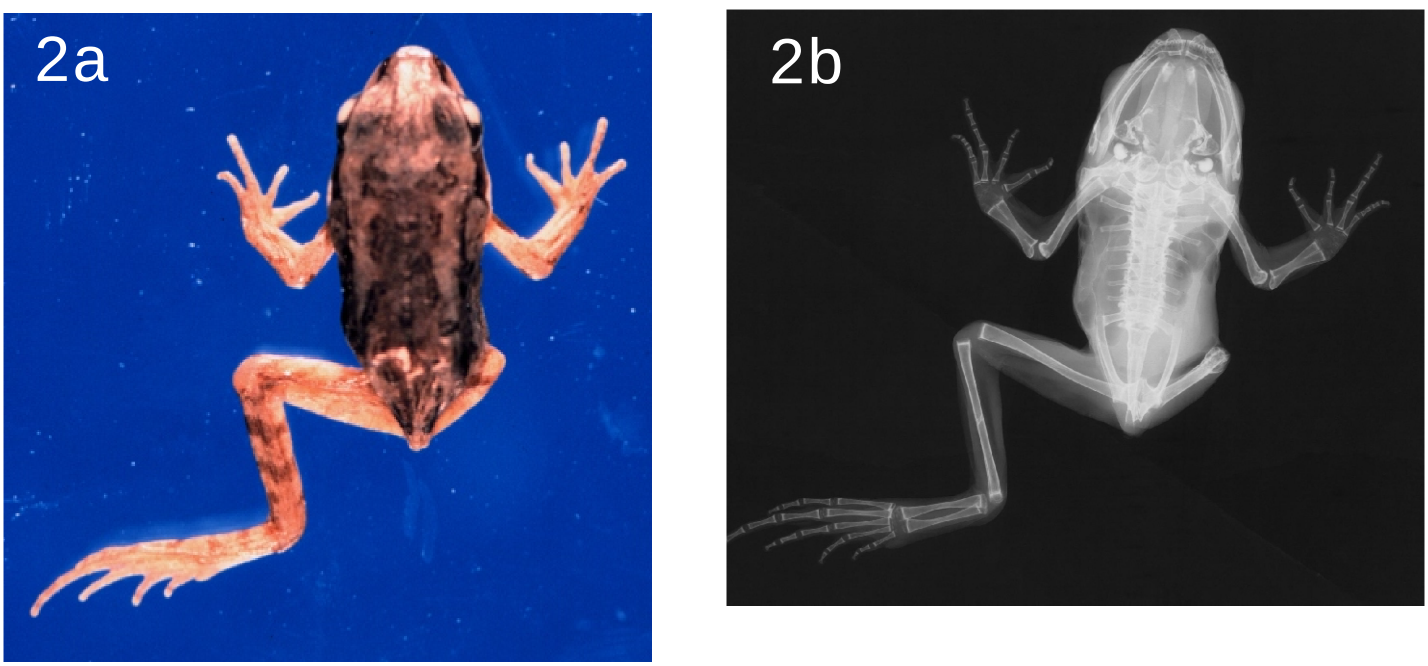
Micromelia: Proportionally small or short limb.

Scoliosis: Lateral deviation (either left or right) in the normally straight line of the spine.

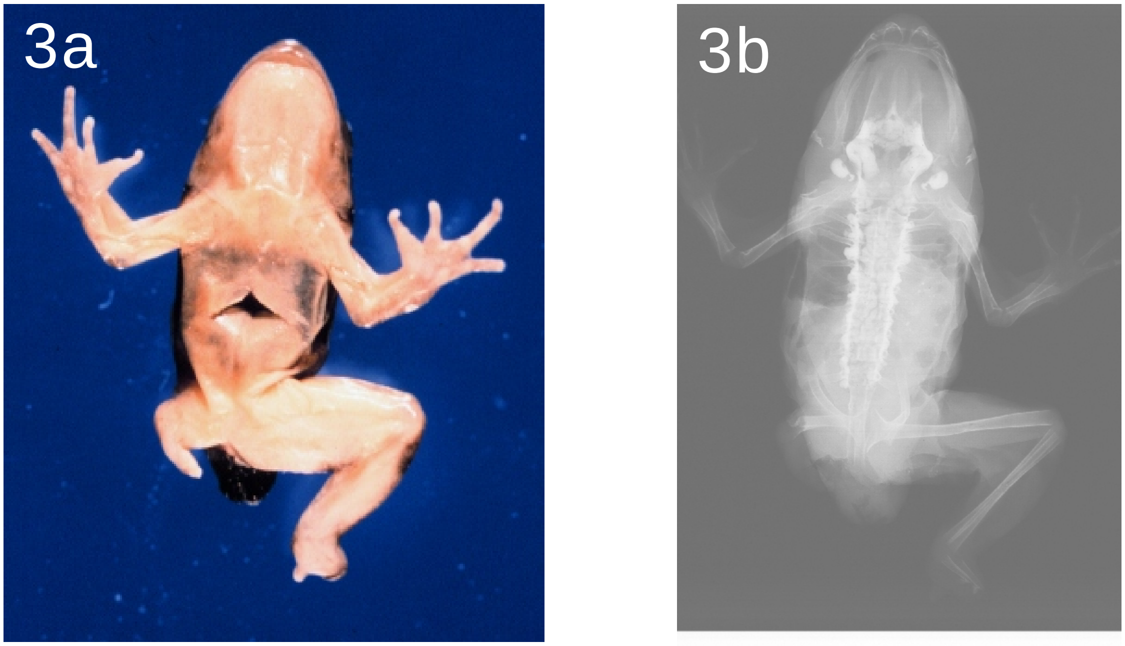
Appendix B: Photos and corresponding x-rays of select abnormal frogs from the Kenai NWR.



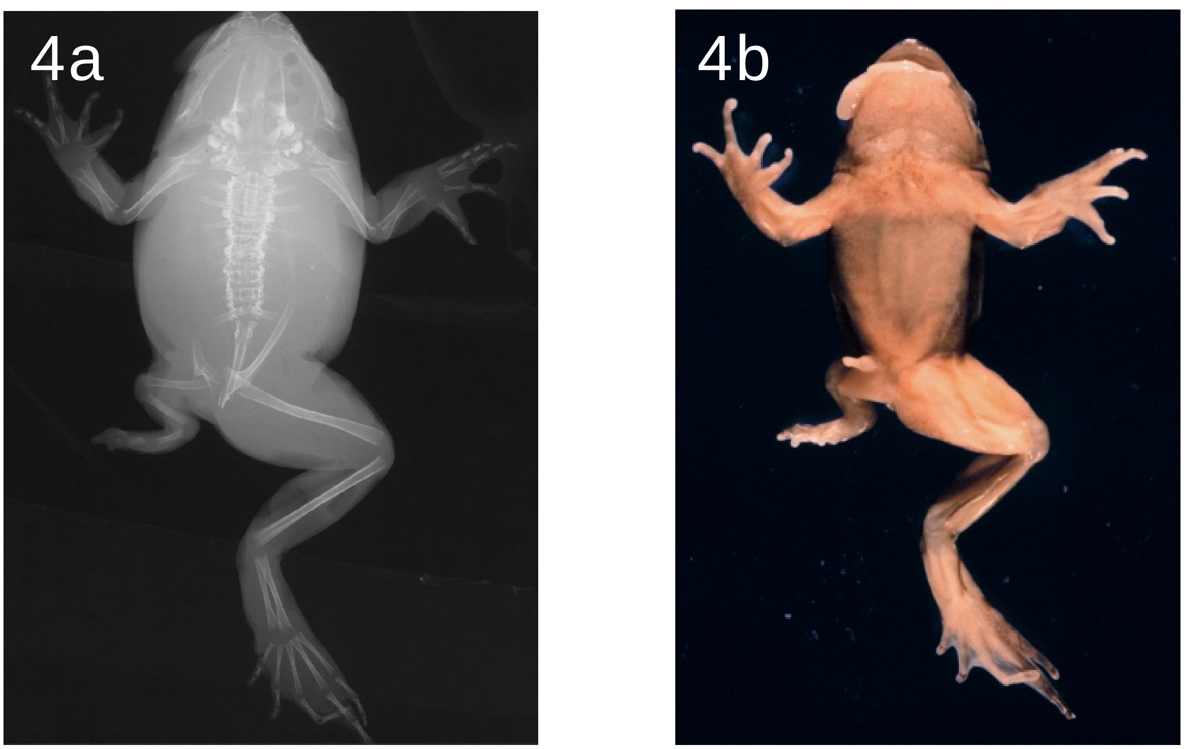
1 a) X-ray of malformed frog from pond OFOOO1 on left; normal frog x-ray on right. Notice missing pelvic bones in frog on left b) Photograph of malformed frog corresponding to x-ray on left in 1a.



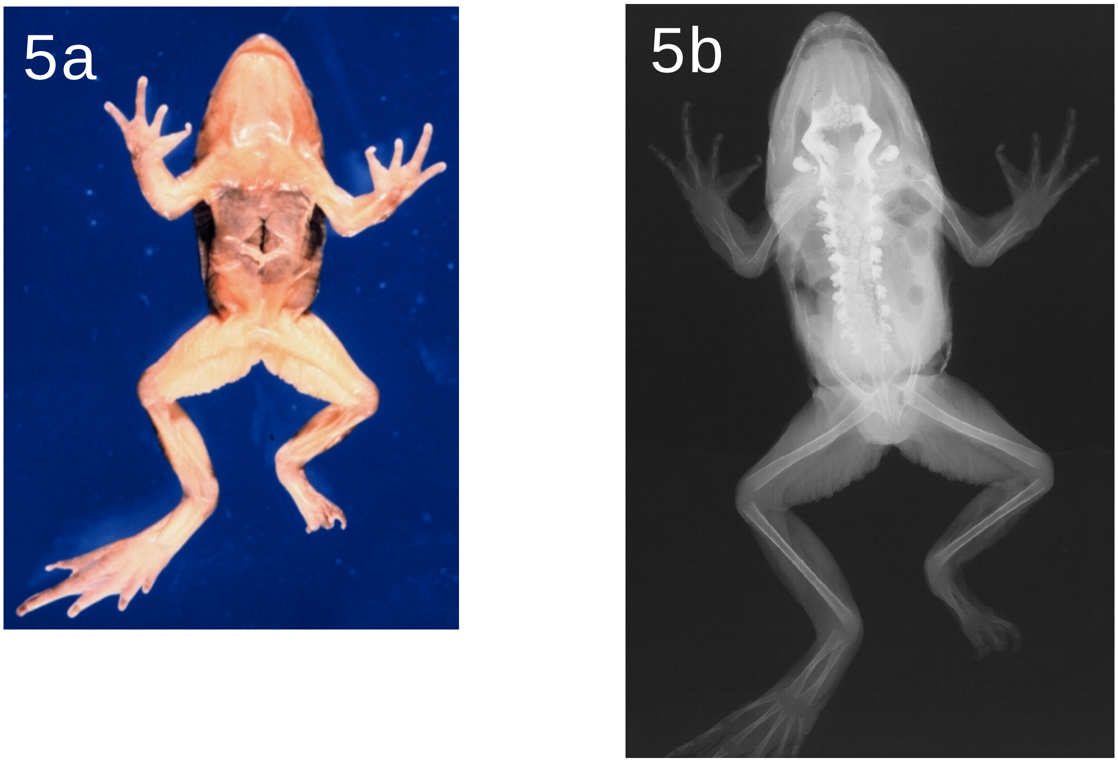
2 a) Photograph of deformed frog from pond SROOO3 b) X-ray of deformed frog corresponding to 2a. Notice development of pelvis and femur: Suspected etiology of missing lower hind leg is trauma.



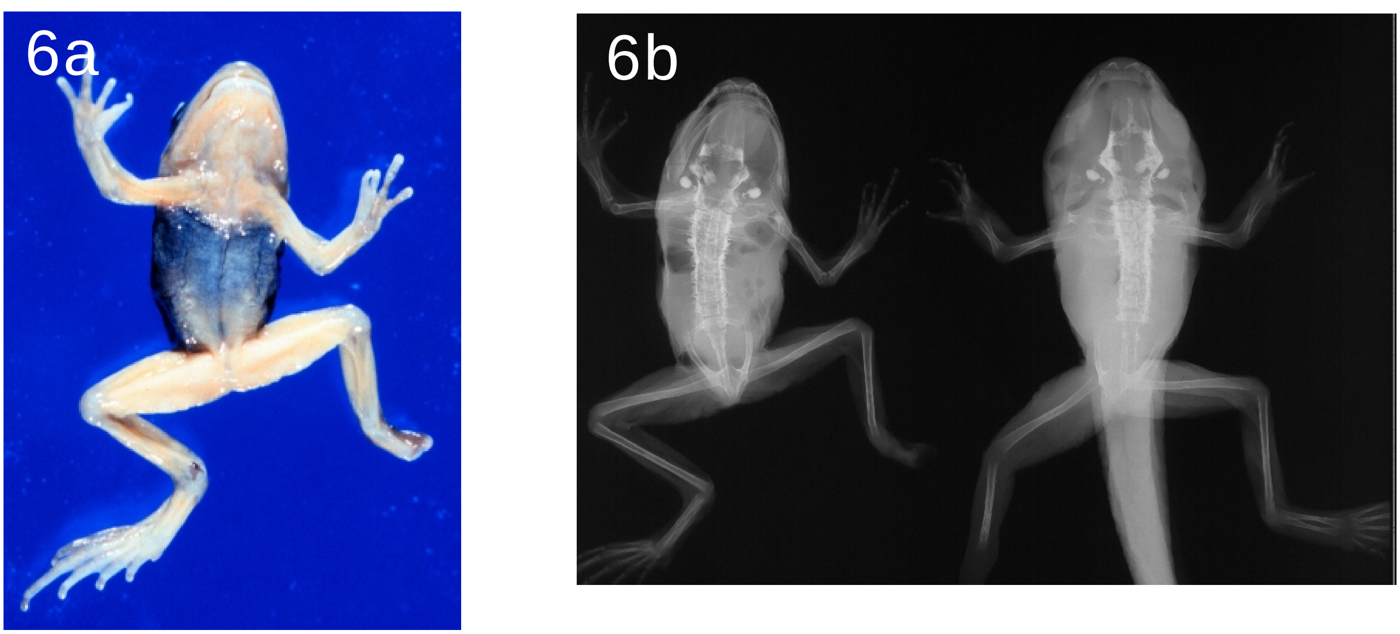
3 a) Photograph of deformed frog from pond SROOO6 b) Corresponding x-ray to photo 3a. Suspected etiology of missing hind leg is trauma.



4 a) X-ray of deformed frog from pond SROOO3. Notice shrunken left hind leg, but also broken bones in pelvic region and digits of right hind limb b) Photograph of deformed frog corresponding x-ray in 4a.



5 a) Photograph of a frog with both deformities and malformations from pond OFOOO1
b) X-ray of frog corresponding with 5a. Abnormalities associated with right hind limb ascribed to trauma-related events. However, there is mild shortness of the jaw which is considered a malformation.



6 a) Photograph of another frog from an oil field pond, OFOOO4, with both deformities and malformations
b) X-ray of frog on left corresponds to photograph in 6a. The missing right hind foot is a suspected deformity caused by trauma, while the scoliosis (curvature of the spine) is a suspected malformation.

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