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Field Investigations of Malformed Frogs in Minnesota 1993–97

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Reports of malformed frogs were made to the Minnesota Pollution Control Agency (MPCA) from different parts of Minnesota in 1993, 1995, 1996, and 1997 and one, nine, 190, and 172 reports were received, respectively. MPCA field crews and Drs. Hoppe and McKinnell documented malformed frog locations starting in 1993. By 1997, MPCA field crews documented malformed frogs at 62 locations in Minnesota, in 29 of 87 counties. Most malformations were in young metamorphs of *Rana pipiens* but they were observed also in *R. clamitans, R. septentrionalis, R. sylvatica, Bufo americanus,* and *Hyla* spp. Frequencies of malformations varied by time of year. Most malformations occurred in rear limbs, with some in front limbs, eye, jaw or skin. Frequencies of limb duplications at the Ney Pond were higher in 1995 (11.7%) than in subsequent years. Malformations were equally likely to be left- or right-sided. A study design in which malformed frog sites were paired with 'reference' sites was attempted, although some reference sites had, or developed, malformed frogs. In some sites, body weights of malformed metamorphs were significantly lower than normal ones, while in two sites both normal and abnormal metamorphs were quite reduced in size compared with frogs from reference sites. There is the possibility of a developmental delay in some of the metamorphs.

INDEX DESCRIPTORS: malformations, Anura, *Rana pipiens*, Minnesota.

Malformed frogs have been reported in numerous areas of the United States (Reynolds and Stephens 1984, DuBois 1996, NARCAM 1999) and in other countries (Van Valen 1974, Mizgireuv et al. 1984, Borkin and Pikulik 1986, Read and Tyler 1994, Takeishi 1996, Bonin 1997, Flax and Borkin 1997, Ouellet et al. 1997). With the exception of the observations of some malformed frogs by Dr. David Merrell in 1958–63 (Merrell 1969, reviewed by Hoppe 2000), herpetologists in Minnesota have not reported observations of malformed frogs until recently (Husveth 1996, Gernes and Helgen 1997, Helgen 1997, Hoppe and McKinnell 1997, Mattson 1997, Helgen et al. 1998, Gardiner and Hoppe 1999, Hoppe 1999). In 1993, the Minnesota Pollution Control Agency (MPCA) and the Minnesota Department of Natural Resources (MDNR) first responded to a report of malformed frogs at a property 120 miles (192 km) west of the Minneapolis-St. Paul metropolitan area close to the Minnesota River and just south of the city of Granite Falls, Yellow Medicine County (Gernes and Helgen 1997). In August of 1995, Cindy Reinitz, a teacher at the Minnesota New Country School of Le Sueur, called MPCA to request assistance in investigating the strange-looking frogs discovered by her students on a nature walk at the Ney Pond in Le Sueur County. By 1996, a big upsurge in reporting of malformed frogs began, with calls coming in to MPCA from citizens, schools, biologists, and resource agency staff. During that year, MPCA received 190 reports of malformed frogs located in 50 of the 87 counties of the state.

As the extent of the malformed frog problem was unfolding (Table 1), MPCA decided to use a paired site study design to begin an investigation into the potential causes. At first, the effort was focused on locating and confirming with population surveys the sites where malformed frogs had been reported or observed by biologists. At the same time, MPCA established study sites as several pairs or clusters of wetlands in different areas of the state for a paired study design. Each pair included at least one affected site, with malformed frogs, and a nearby reference site expected to have a low frequency (<1%) of malformations. Preliminary environmental analysis was done and partnerships with other scientists and agencies were formed.

Planning was guided, in part, by the criteria for ecoepidemiology (Fox 1991) to provide a weight of evidence approach for inferring causes of biological effects in the environment (see also Gilbertson et al. 1991, Gilbertson 1996). As the problem has expanded, MPCA and several cooperators have been investigating a range of potential causes of the malformations: inorganic and organic contaminants in water, sediments and frog tissues, microbial agents and parasites, and ultraviolet light radiation. Work not reported here has gone forward in 1998 and 1999 on fractionating site water and testing fractions for malforming action on developing frogs (Fort et al. 1999a, 1999b). In addition, other research is seeking the sources and forms of contaminants such as pesticides from ground water, surface water and, preliminarily, from rainfall (Jones et al. 1999).

This report covers information on field documentation of frog malformations from 1995–97 in Minnesota, with emphasis on the data for the northern leopard frog, *Rana pipiens.* It includes the frequencies and kinds of malformations observed and comparative data on sizes of normal-appearing and malformed frogs. Other reports will give results of the analyses carried out on the frogs, the water and sediments.

Table 1. **Timeline of involvement of Minnesota Pollution Control Agency (MPCA) in malformed frog investigation 1993–97.**

1993	MPCA, MNDNR in Granite Falls, Minnesota. One site reports many deformed frogs. Preliminary frog surveys.
1994	MPCA investigates Granite Falls area, soil sampling, frog surveys.
1995	Minnesota New Country School calls MPCA for help at Ney Pond. Surveys, documentation of abnormalities, collections at four sites. Media coverage begins.
1996	MPCA receives large numbers of reports of abnormal frogs in state. Field surveys, limited chemical analysis of sites. Media coverage expands widely in fall.
1997	Intensive surveys coordinated by MPCA in Minnesota. MPCA partners with scientists and agencies. Intensive surveys and chemical sampling of paired sites, affected and reference. Verification of sites reported by citizens.

METHODS

Site Selection and Study Design

For the work in 1997, MPCA attempted to select four clusters of study sites in different areas of Minnesota based on the preliminary field sampling from 1996 and on new sites which were revealed in 1997. For each cluster or pair of study sites we sought to include a reference site with the affected site(s). Sites were designated 'affected' if they had malformed frogs, and 'reference' if they had normal frogs. The criteria for reference sites were: <1% malformations, located within the same county and river system as the affected site, in a class of wetlands similar to that of the affected site, and located on publicly owned land or having assured access and land owner permission. The affected sites were known to have significant frequencies (>5%) of malformed frogs. The study sites were selected from among those reported to MPCA from biologists, resource agency staff, from previous field surveys by MPCA or from reports from citizens. It was not the intention of this investigation to establish the proportion of wetlands in Minnesota having malformed frog populations, therefore a random or probabilistic site selection process was not used.

Frog Surveys, Description, and Analysis

By 1997, MPCA had two crews in the field. One crew was responsible for surveying frog populations at 'intensive' study sites, which were the affected and reference sites chosen for more intensive investigation. This crew collected frogs, water and sediment for analysis. At least two surveys at each site were done to document the kinds and frequency of malformations in the population. Frogs were captured using dipnets and a search and seize method in random locations in and at the edges of the breeding pond. Collection continued until at least 100 metamorphs of *Rana pipiens* were captured, or until there were no more successes in capture. The frogs were carried in clean, slightly moistened pillowcases which had been well rinsed after laundering, and placed in a clean 5 gal-pail containing a small amount of site water and covered with a pillowcase. Each frog was measured with calipers by lightly pressing down on the rear to measure the snout–vent length (SVL) to the nearest 0.1 cm, then released where found unless sent to a cooperating researcher for further analysis.

In 1997, the second field crew carried out the 'global surveys', or surveys of frogs at locations which had been reported to MPCA from citizens with the goal of confirming the reports and finding other potential sites for future study. This crew focused their efforts on six regions in the state where they conducted population surveys at sites selected from the reports received from each area. The sampling protocol differed from the intensive survey protocol because of time constraints for visiting a larger numbers of sites. Sampling of global survey sites continued until (1) five or more abnormal frogs were found in <50 frogs collected, or (2) fewer than five malformed frogs were found in a sample of >50 frogs, or (3) 2 h of collecting time had elapsed.

To avoid contamination of the sites, chest-high waders and other gear were brushed and rinsed with clean water and field nets were dedicated to each site. Frogs were collected with fresh disposable Ultra-One latex microflex gloves (VWR). Field staff used no repellents, sunscreen or other material which might contaminate samples.

Standard frog data sheets were used in 1997 for documenting the frog malformations along with written descriptions for showing the locations of the malformations on diagrams of dorsal and ventral views of a frog or a larvae (Appendix A). Three to four frogs were placed together in clean plastic bags and weighed with a 60 g Pesola Precision spring scale to within 0.2 g. In 1998 and 1999, MPCA weighed frogs individually using a digital platform type field scale and a clean hard plastic container. According to the criteria used by Dr. David Hoppe, leopard frogs were classed as adults if their SVL was >59 mm, as 'subadults' if they had an SVL of 51–59 mm, and as metamorphs if their SVL was <51 mm.

Analysis for differences in the mean SVL of metamorphs was done using the Student's t-test ($\alpha = 0.05$) on means with unequal variances to compare malformed frogs from affected sites to normal frogs from reference sites in the pairs of sites because the tissues of malformed frogs were compared with those of metamorphs from reference sites. Also, it appeared that even normal frogs in some affected sites were depressed in size (see below). In most cases, frogs were collected from the paired sites on the same sampling date. Differences between mean body weights and mean SVL of malformed vs. normal-appearing frogs within sites was done using Student's t test ($\alpha = 0.05$) for samples with unequal variances (Zar 1974).

Frog Preservation

Predetermined numbers of frogs were collected and sent for additional analysis by five biologists who were partners in MPCA's workplan in 1997 (given below). Where possible, the same frogs were used by two different biologists. Depending on the analysis to be done, larvae and frogs were either kept and shipped live to cooperating researchers (e.g., for chromosome and micronuclei studies), or were preserved in 10% formalin after anaesthetization with MS-222 (tricaine methanesulfonate, Sigma Corp., Schoettger and Julin 1967) for histological analysis. Live frogs were shipped in plastic containers with holes for air and lined with sterile sphagnum moss moistened with water from the collection site. These were shipped in large, hard-sided coolers with ice packs. If the frogs had to be held before shipping, they were kept at ca. 10°C. For tissue analysis, frogs were put in level 1 chemically clean jars with teflon-lined lids (Eagle-Picher) and frozen on dry ice in the field.

Partnerships With Other Scientists

A partnership with the National Institute of Environmental Health Sciences (NIEHS) was formed in 1997 for the analysis of

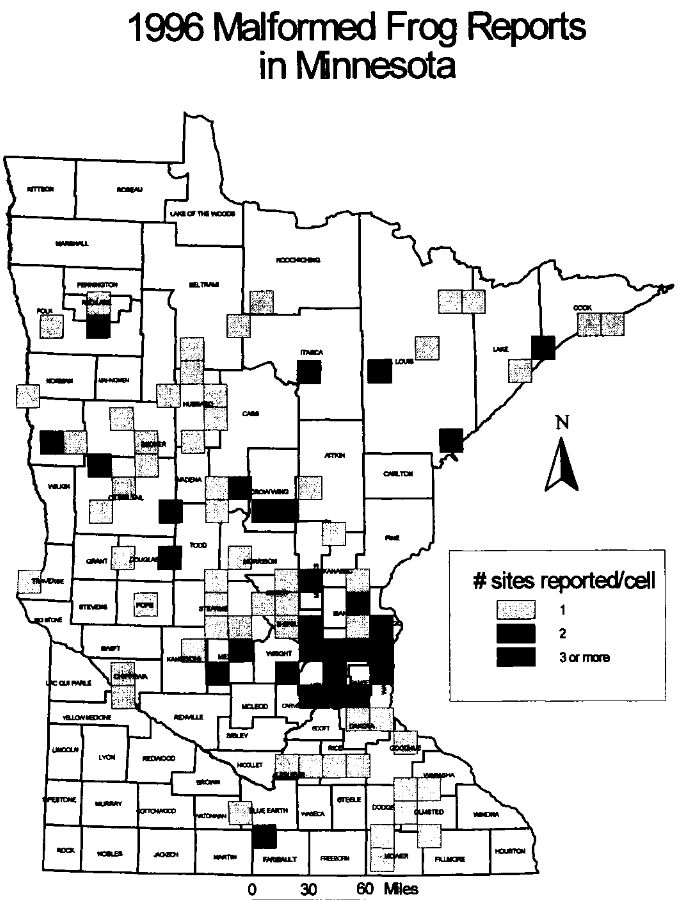


Fig. 1. Reports to the Minnesota Pollution Control Agency of malformed frogs from different locations in Minnesota during 1996. Reports were received from school children, citizens, biologists and resource agency staff. Squares or cells are based on 12 × 12 mile grids. The number of sites reported within the cell is coded by shading.

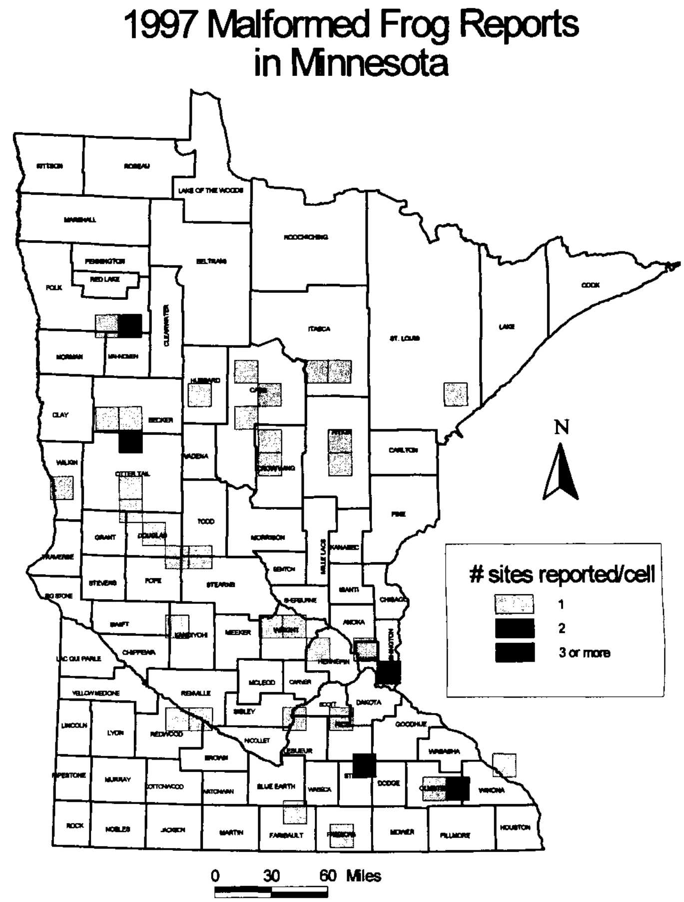


Fig. 2. 1997 reports of malformed frogs in Minnesota to the Minnesota Pollution Control Agency. Reports were received from school children, Hamline University, citizens, biologists and resource agency staff. Squares or cells are based on 12 × 12 mile grids. The number of sites reported within the cell is coded by shading.

environmental samples, water and sediments, from the intensive survey sites. Cooperative arrangements were made with several biologists for analysis of frogs: Dr. Robert McKinnell for analysis of frog gonads, Dr. Michael Lannoo for histological work, Drs. Steven Goldberg and Charles Bursey for analysis of parasites in frogs, Dr. Jeanne M. Lust for work on micronuclei, and Dr. Debra Carlson for frog chromosome studies. In addition, a collaboration began with Dr. Carol Meteyer at the National Wildlife Health Center Laboratory of the USGS Biological Resources Division, Madison, Wisconsin for diagnostic analysis of frogs for microbial agents and parasites and for characterization of the malformations. Dr. E. Michael Thurman of the USGS analytical laboratory in Kansas sampled and analyzed some of the study sites, as did the Minnesota Department of Agriculture. Eggs of frogs and water samples were delivered to the US Environmental Protection Agency Laboratory in Duluth, Minnesota, for analysis. Some of this work will be reported in separate publications.

In addition, a project named "The Thousand Friends of Frogs" was initiated in 1996 with Hamline University's Center for Global Environmental Education (CGE). CGE produced materials for schools to assist with identification and reporting of malformed frogs, and, more recently, has greatly expanded its role in providing a frog reporting hotline in 1998 and 1999 by creating environmental education on amphibians for the internet (see Hamline University, CGE in Literature Cited), and producing an educational play about frogs.

RESULTS

Overall, MPCA received one report of malformed frogs in 1993, none in 1994, nine in 1995, 190 in 1996 (Fig. 1), and 172 in 1997 (Table 2, Fig. 2). The number of sites confirmed by biologists to have malformed frogs was one in 1993, four in 1995, 21 in 1996, and 64 in 1997 (Fig. 3). Information has been relayed to NARCAM. In 1997, MPCA's global survey crew verified 62 sites with one or more malformed frogs in 29 counties. Of these, 32 sites occurring in 16 counties had three or more malformed frogs. Also, surveys of larvae in 1997 showed a range of malformations from a frequency of 2.5–7.5% in affected sites. Very few malformations have been seen in adult frogs. By far the greatest numbers have occurred in the size class of frogs <50 mm SVL. In 1997, only seven out of 457 frogs classed as adults (>60 mm) showed malformations (Table 5), and these were often less extreme. It appears that the malformed metamorphs are not surviving the winter in Minnesota.

When MPCA first responded to the call from the Minnesota New Country School (MNCS), its staff (Helgen) did photographic documentation of frogs collected at that site. Some of these photographs can be seen on the MNCS and the MPCA web sites (see Literature

Table 2. Reports of malformed frogs to MPCA 1993–97 received from citizens, biologists and resource staff. Numbers of sites, counties and malformed frogs confirmed and documented are given.

	Reports		Confirmations		
Year	# Reports	# Counties	# Sites	# Counties	Number Malformed
1993	1	1	1	1	3
1994	0	0	0	0	0
1995	9	8	4	3	259
1996	190	50	21	14	368
1997	172	46	64	29	517

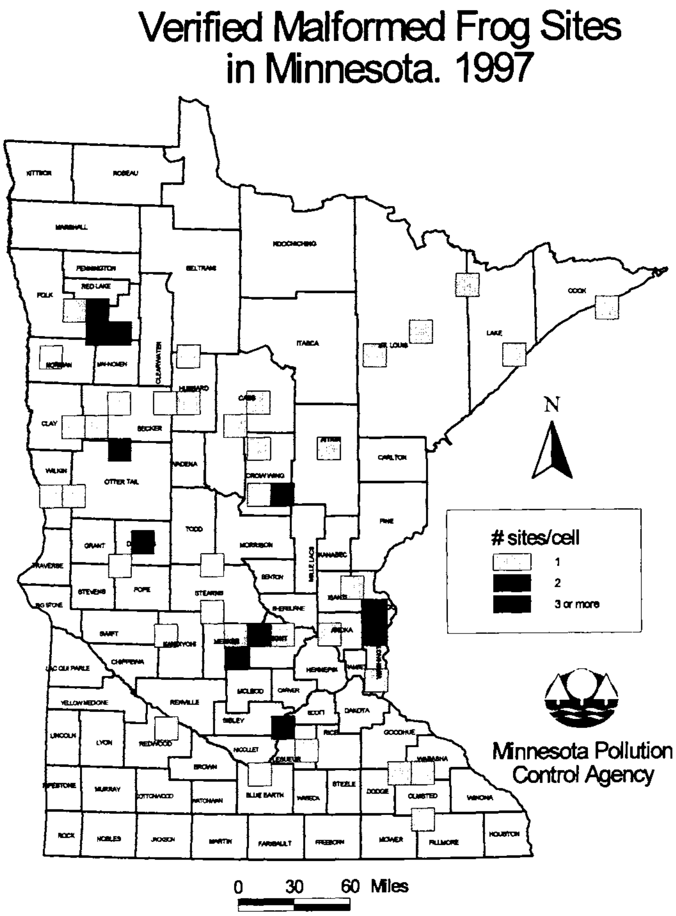


Fig. 3. Location of sites with malformed frogs confirmed by frog surveys by MPCA staff or Dr. Hoppe in 1997. Sites were selected for surveys in 1997 from the sites reported to the Minnesota Pollution Control Agency during 1996 and 1997. Squares or cells are based on 12 × 12 mile grids. The number of sites reported within the cell is coded by shading.

Cited) and have been widely distributed to scientists and the media. In 1995, there was no standard reporting form available, so written descriptions were made of the malformations. An example of the descriptions made from the first malformed frogs documented by MPCA at the Ney pond in 1995 is given in Table 3.

In 1995, MPCA documented malformed frogs at the Ney site in northwestern Le Sueur County on the bluff above the Minnesota River, the Boe site in Sibley County south of Henderson, Minnesota near the Minnesota River on the side opposite to the Ney pond, and at the Kra site in Meeker County about 90 miles (144 km) west of the Minneapolis-St. Paul metropolitan area and north of Litchfield, Minnesota. The Kra site was close to the North Fork of the Crow River which drains into the Mississippi River.

In 1996, MPCA began seeking sites for the paired site study. MPCA surveyed the Ney, Tou, and Boe sites, plus the Gel site as a potential reference site in Le Sueur County. The affected sites Dor and Hyde in Becker County, and the Cum site designated as a potential reference site in northwestern Minnesota, were surveyed. In Meeker County, MPCA surveyed Roi and Kra as affected sites, and Man as a potential reference site. David Hoppe surveyed the CWB affected site and the Kal site as potential reference site in Crow Wing County, about 120 miles (192 km) north/northwest of the St. Paul metropolitan area.

By 1997, it was apparent there might be difficulties in finding reference sites for a paired site study. The Win site in the northwest was added as a candidate to reference Dor and Hyd, and the LMS site was added as a candidate reference site for Roi. The intensive study sites for 1997 (Fig. 4) were located in the northwestern part of Minnesota (Dor, Win, and Hyd as affected sites, Cum as reference site), in the north-central part of Minnesota in Crow Wing County (CWB, Kal as reference site), in the central part of the state about 80 miles (128 km) west of the Minneapolis-St. Paul metropolitan area (Kra and Roi, Man and LMS as references), and about 40 miles (64 km) south of St. Paul (Ney, Boe, Tou, with Gel as reference in Le Sueur County, Fig. 4). The distance between the sites in Le Sueur County and the northwest sites was approximately 300 miles (480 km).

Malformation frequencies differed between and within sites depending on time of year. In 1996 (Table 6), the Ney pond had almost no malformations in late July, yet the numbers increased towards the end of summer and early fall (Table 6). In contrast, at the Roi site in 1996 the number of malformations was very high in July (66%) and diminished to 20% by October. At other sites, the frequencies were more stable. In 1997, frequencies did not shift as dramatically during the season in the study sites. An example of the seasonal data is given in Table 7.

The kinds of malformations observed have been similar across the state and nation (see NARCAM 1900), although they do show local differences in the frequencies of the types of malformations in space and time. At the Ney pond in 1995, there was a high percentage of extra limbs (11.7%) and either anteverted, meaning folded or bent back on itself, or rotated limbs (17.6%), but in 1996 and 1997 these kinds of malformations were hardly present. In 1996, the combined frequency of rotated and extra limbs at the three affected sites accounted for only 3.0 % and in 1997 for 6% of the malformations (Table 4). In addition, in 1995 two other sites located on both sides of the Minnesota River not far from the Ney site showed malformations similar to those from the Ney Pond. At the same time, a pond (Kra) in central Minnesota north of Litchfield in a watershed

Table 3. Original descriptions of a subset of 1995 malformed frogs at the second visit to Ney Pond by MPCA, August 18, 1995. Photographs of many of these frogs are widely available. All references to 'leg' are to rear leg. Snout-vent length (SVL) is given in mm..

No.	SVL	Description of malformation
1.	38	leg twisted, foot folded towards rear
2.	37	three legs
3.	38	leg extends straight out, as if no muscle, paralyzed, very thin
4.	35	right eye completely missing, skin covers eye socket
5.	45	left leg twisted and close to body, with an extra projecting thin stump
6.	36	leg severely contorted at base, shortened, cannot see leg, juts (left only)
7.	40	left leg missing, no stump, cannot feel pelvis, just muscle
8.	38	three legs, two on left side, abnormal leg anterior to the normal left leg
9.	35	left leg contorted at base, shortened
10.	42	right leg very thin most of length except for a small widening of muscle at base
11.	42	skin-covered, conical projection from ventral midline towards right side, 12 mm
12.	39	left leg very thin, no toes (or 1 stump), no webbing, as if toes had atrophied
13.	40	right leg thin, skin too tight, as if webbed across leg, leg cannot extend fully
14.	48	right leg 'folded' in at knee, has two protruding pieces of skin-covered bone in knee area
15.	46	left leg very thin with little muscle
16.	44	right leg—lower leg contorted, thigh ok, foot splays out with 2–3 feet, one full foot (bent), one less complete, one partial, they make a 'basket'
17.	44	left leg 'webbed' in thigh area which is contorted, leg cannot extend much
18.	43	right leg contorted, has two 'feet', second one is small, 3 toes
19.	40	left leg juts straight posteriorally, cannot flex, very thin (no muscle)
20.	44	left leg contorted, a short stump with no foot
21.	35	right leg very thin, useless, with a bone at the tail site
22.	43	both legs are 'webbed', cannot extend
23.	33	left leg webbed, a second 'foot' coming out below the knee
24.	38	right leg extended, does not flex, is thin
25.	42	no right eye, skin covers eye socket, legs are ok
26.	37	extra left leg anterior of the left knee, webbed to upper thigh (three legs)
27.	40	left leg looks raw, almost shrunken, bone projects above knee, as if leg has atrophied
28.	39	left leg webbed, shortened, foot 'dissolved', contorted back, almost no lower leg, just a thin stick-like piece
29.	37	right leg has bone projecting out at knee, leg bends back then extends towards foot, almost no lower leg length
30.	37	webbed right leg, lower leg tight to the body and leg cannot extend
31.	38	thin leg, contorted and webbed, has black speckle skin (saw on others), knee area deformed, blue spot dorsally between an anterior of eyes
32.	34	very long tail, not resorbed (BL = 34 with tail = 59 mm)

of the Mississippi River had high numbers of malformed *Rana pipiens* with missing limbs or very shortened femurs, but no duplications or anteversions in 1995 (Table 4). The frogs were very diminished in size when they were observed at the end of September 1995, appearing to have less mass than mature larvae would have.

There appears to be no significant difference ($P < 0.01$) in whether the malformations are located on the left or the right side of the animals. Of 689 malformations recorded in 1997, 321 were left-sided, 344 were right-sided and 24 were on both sides.

In 1996, comparisons of mean SVL between affected and companion reference sites showed significant differences. Frogs from the designated reference sites showed overall greater mean SVL (Table 8). In 1997, both SVL and body weight were measured. Data from the sites designated as potential pairs of affected and reference sites are shown in Table 9. Within-site comparisons of the mean body weights of normal and abnormal metamorphosed *Rana pipiens* collected in September 1997 showed significant differences at the Dor and Win sites, and in one collection date from the Ney site, but not at Hyd or Roi sites, or at Ney on another collection date (Table 10). In 1997, frogs from the Roi site had a significant difference between mean SVL of normal and abnormal metamorphs within the site, but Ney frogs did not in 1997, nor did the Hyd site. However, both the Ney site and Hyd site normal and malformed frogs were dramatically smaller in September compared with the other sites. Hyd normal metamorphs averaged only 34 mm, the abnormals only 33 mm on 9/9/97. Ney normal and abnormal metamorphs averaged 40 mm on 9/11 and just 38 mm on 9/29/97.

Comparison between the normal frogs from the reference sites with the malformed frogs from the affected sites, however, resulted in significant differences. The pairs of sites where the mean body weight was significantly greater in the reference site compared to the affected site were Cum/Dor, Win/Hyd and Gel/Ney. The pairs which showed significantly greater body length in the reference compared to the affected site are Win/Hyd, LMS/Roi and Gel/Ney.

DISCUSSION

The upsurge in reports of malformed frogs from 1993–96 is probably not just the result of the attention of the media, although the intensity of media coverage in 1996 undoubtedly helped to direct people to MPCA with reports of malformed frogs. Herpetologists and other resource biologists and most of the observers who called into MPCA also reported that they had not seen malformed frogs in

Table 4. Types of abnormalities recorded in 1995, 1996, and 1997 in juvenile *Rana pipiens* from sites in Minnesota. Ney and Boe are near Minnesota River, Kra is a site in Meeker County. For 1996 and 1997, the overall percentages of types of malformations are given.

	% of total malformations				
	by site, 1995				
Description	Ney	Boe	Kra	1996 Overall	1997 Overall
Limb anteverted or rotated	17.6	6.3		1.8	3.4
Extra limb	11.7	37.5		1.2	2.6
Extra digits or foot	1.2	12.5		0	1.2
Limb deficiencies					
Missing limb	9.9		10.8	15.2	4.3
Partial limb	9.4	25	81.4	50.5	26.5
Missing foot	3.5			1.2	0.4
Partial foot				2.4	5.9
Missing digits	0.6			3.3	17.1
Abnormal limb			7.3	0.1	
Abnormal foot or digits	2.9	4.9	4	18.3	
Limb thin, atrophy	13.5	1	1.5	6.7	
Limb straightened			0.3	0.3	
Cutaneous fusion of skin, rear limbs	12.3			4.3	
Spine projecting from posterior end	11.1			0	0.3
Missing eye	1.8				2.3
Abnormal eye				3.6	5.7
Bony spur or cyst	1.8		1	1.2	
Cranial abnormality				0.3	3.9
other1	4.2		1	6	7.0
Total abnormalities	173	16	102	329	742.0

Table 5. Numbers of malformations in size classes of *Rana pipiens* surveyed in 1997 in Minnesota. Malformations are grouped by major location. More than one malformation can occur in one individual.

	Metamorphs <50 mm	Subadult 50–60 mm	Adult >60 mm
Eye	46	3	2
Front limb	25	3	0
Hind limb	544	20	4
Jaw	23	2	2
Spine	4	1	0

the past. Some had been collecting frogs for years: the family in Granite Falls had collected many young frogs for fishing each year in several holes dug in their yard and had not seen malformations before the summer of 1993. An older man from Little Falls, Minnesota said he collected the small "frisky" frogs for fishing each fall and had never seen bad frogs before. A natural resource agency staff person's family had been collecting frogs for bait dealers at their property in northwestern Minnesota for years and the first malformed frogs appeared in 1997.

In 1997, the frequencies of malformations at three intensive study sites ranged from 4.5–24% which meant the problem of malformed frogs had not diminished in Minnesota through 1997. Malformed frogs have been observed by MPCA in the following species: *Bufo americanus*, *Hyla* sp., *Rana clamitans*, *R. pipiens*, *R. septentrionalis*, and *R. sylvatica*. Malformed frogs have been observed through many areas of the state in many different wetlands. The malformations are predominantly in the rear limbs, with the majority as missing or partial limbs, but malformations in eyes, cranium, skin and other structures have also been recorded. In some cases, it is truly amazing the frogs have been able to capture food and grow at least to some extent.

The distribution of the kinds of malformations can differ between sites, but differences have also been observed within a specific site during one season or from year to year. Also, overall malformation frequencies observed can changed dramatically in some sites, up or down, from July to late September or early October. For these reasons, it is important to survey the populations of metamorphs throughout the season (July–early October at a minimum). A single population survey in one season could be misleading. Even when some malformed frogs are removed from the population, it is important to continue surveys because biologically, if not statistically, significant numbers of malformed frogs may appear in subsequent surveys. Different types of malformations at one site could result from exposure of different early stages in development. Several factors could be affecting the changes in frequency of malformations: malformed frogs may have higher mortality rates, not just from predation. Malformed metamorphs might be surveyed more often if they tend to disperse less and remain closer to the natal wetland than the normal metamorphs. The degree of early stage mortality of malformed frogs prior to metamorphosis is an unknown. There may be delayed development with malformed larvae metamorphosing later in the season; or there could be variability in the estimates from field surveys.

We have debated what a background frequency of abnormalities might be. The working hypothesis is that anything higher than a 1–2% malformation rate is abnormal. To some biologists (e.g., David Hoppe) even 1% is high, but others prefer a rate closer to 2% reflecting the rate for human birth defects. In one study, background rates of congenital anomalies in humans at birth during 1989–92 were reported as 1.8% in urban and forest areas, 2.1% in corn/

Table 6. Surveys of *Rana pipiens* (Rapi) metamorphs in 1996 at several sites in Minnesota*. Cty—county; Abn—abnormal. Data for CWB and Man sites from D. Hoppe.

Date	Site	*n*	# Abn	% Abn
04.30.96	Boe	3	0	0
06.20.96	Boe	4	0	0
07.24.96	Boe	178	1	0.6
08.27.96	Boe	137	1	0.7
08.14.96	Cum	148	0	0
05.21.96	Cwb	17	0	0
06.19.96	Cwb	18	0	0
07.06.96	Cwb	5	2	40.0
07.11.96	Cwb	16	0	0
07.19.96	Cwb	411	36	8.8
08.01.96	Cwb	77	14	18.2
09.12.96	Cwb	86	9	10.5
08.14.96	Dor	191	45	23.6
09.27.96	Dor	40	17	43.0
07.29.96	Fis	117	11	9.4
09.12.96	Gel	116	1	0.9
07.30.96	Hyd	239	37	15.5
08.21.96	Kal	192	2	1.0
09.12.96	Kal	112	1	0.9
08.27.96	Man	114	0	0
07.25.96	Ney	117	1	0.9
09.12.96	Ney	104	8	7.7
09.30.96	Ney	70	32	46.0
08.15.96	Pem	46	0	0
07.02.96	Roi	102	66	64.7
08.15.96	Roi	96	35	36.5
10.11.96	Roi	70	14	20.0
07.25.96	Tou	27	0	0

* Overall total 2853,333 malformed, 11.7% malformed.

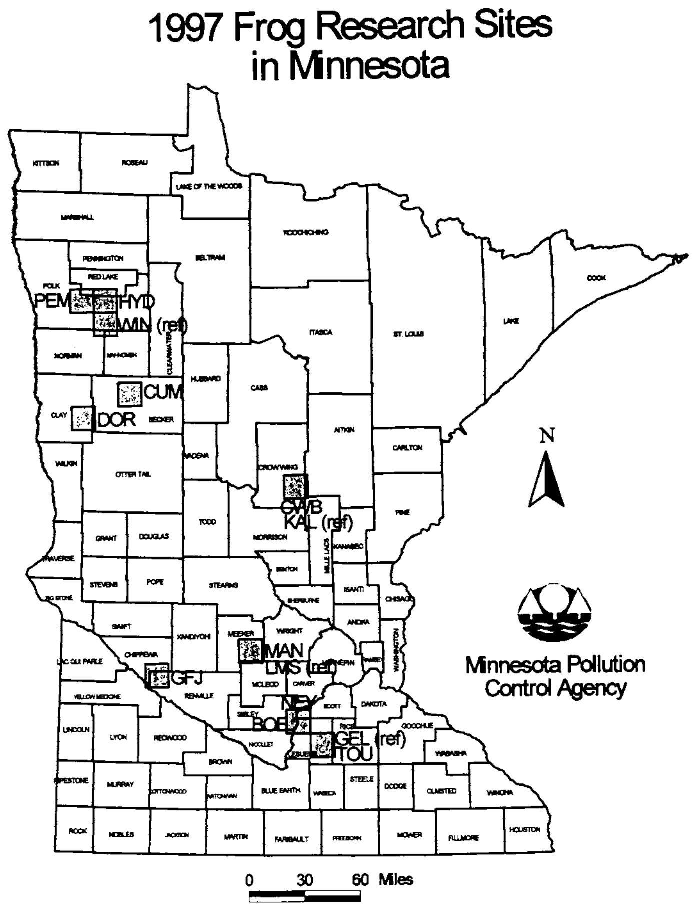


Fig. 4. Locations of wetlands in Minnesota for research on malformed frogs by MPCA in 1997. Sites designated as reference (ref) are indicated.

soybean crop areas, and 2.7% in a wheat/corn/soybean/potato crop area in Minnesota (Garry et al. 1996). Making a distinction between 0.5% and 2% by using the current frog survey protocol with 100 metamorphs is not possible. This protocol is used to identify areas where there are very significant levels of malformed frogs (with frequencies of ≥5%). There has been no difficulty identifying sites in Minnesota with such frequencies of malformed frogs.

One of the greater difficulties has been finding sites that do not have malformed frogs, especially sites that do not have them consistently over time. In the paired study design it has been a challenge to identify appropriate reference sites using the operational definition that such sites would have only normal, or <1%, malformed metamorphosing frogs. A couple of the reference sites had almost no malformations in 1996 (Gel 0.9%, Cum 0%). The Cum site had a history of collections by the property owners' children, who had not seen any malformed frogs for a few years prior to 1997. Yet in 1997, each of these sites had significant numbers of malformed frogs: Gel had 5.4% in July; Cum site had 0% in July and 8% in August 1997. These observations may be indicative of the idea that these sites are in transition. Staff from resource agencies have assisted in suggesting potential reference sites, based on sites they knew were 'good frog sites' on publicly owned lands. Yet one new candidate reference site in 1997, the Win site, had significant malformed frogs in the surveys: 5% malformations in larvae, 6% in the metamorphs.

Table 7. Frequencies of malformations in *Rana pipiens* metamorphs in some research sites in Minnesota in 1997. # mal/*n* is number malformed/total number (*n*) collected.

	Site									
	Cum		Dor		Hyd		Ney		Gel	
Month	#mal/*n*	% mal	#mal/*n*	% mal	#mal/*n*	% mal	#mal/*n*	% mal	#mal/*n*	% mal
July	0/106	0	14/128	10.9	18/136	13.2	5/108	4.6	6/111	5.4
August	-	-	25/106	23.6	38/666	5.7	16/237	6.7	-	
Sept.	7/87	8.0	14/165	8.5	8/176	4.5	45/423	10.6	2/106	1.9

Table 8. Comparison of snout–vent lengths (SVL, mm) of *Rana pipiens* metamorphs from Minnesota in 1996. Paired reference (Ref) and affected (Aff, having abnormal frogs) site data are compared. t-test is for difference between means with unequal variance.

Site	Type	Date	Mean SVL	Critical t	t-test	*P*
Cum	Ref	8/14/96	48.2	1.975	16.35	<0.001
Dor	Aff	8/14/96	42.0			
Cum	Ref	8/14/96	48.2	2.000	14.27	<0.001
Hyd	Aff	9/26/96	38.1			
Gel	Ref	9/12/96	46.1	2.012	18.26	<0.001
Ney	Aff	9/12/96	34			
Man	Ref	8/27/96	45.6	1.982	13.22	<0.001
Roi[a]	Aff	8/15/96	37.8			

[a] This pair of sites was nor sampled on the same data. At the estimated growth rate of increase, mean at Roi would have increased to 39.9

Table 9. Average snout–vent lengths (SVL) and body weights (Wt) for *Rana pipiens* metamorphs at four pairs of study sites in 1997. Ref—designated as reference site, Aff—site with known population of malformed frogs, Abn—abnormal, Nor—normal; $\bar{x}_{Wt}$—mean weight in mg ± standard deviation, n_{Wt} or n_{SVL}—number of frogs measured; $\bar{x}_{SVL}$–average Length, mm. The Win site was new in 1997, and turned out to have malformed frogs.

Type	Site	Date	Abn/Nor	$\bar{x}_{Wt}$	n_{Wt}	$\bar{x}_{SVL}$	n_{SVL}
Ref	CUM	7/22/97	Nor	7.365 ± 0.7292	100	4.395 ± 0.2491	100
Ref	CUM	9/8/97	Abn	10.733 ± 0.6351	3	4.733 ± 0.2082	3
Ref	CUM	9/8/97	Nor	9.741 ± 0.8513	44	4.709 ± 0.21	44
Aff	DOR	7/22/97	Abn	7.643 ± 0.8635	14	4.193 ± 0.2786	14
Aff	DOR	7/22/97	Nor	7.72 ± 0.4684	101	4.142 ± 0.2192	101
Aff	DOR	7/23/97	Abn	5.917 ± 0.4491	6	4.133 ± 0.1633	6
Aff	DOR	8/13/97	Abn	7.687 ± 1.535	38	4.603 ± 0.2908	38
Aff	DOR	9/8/97	Abn	9.235 ± 1.038	13	4.769 ± 0.2898	13
Aff	DOR	9/8/97	Nor	10.639 ± 1.221	83	4.917 ± 0.2403	83
Ref	WIN	7/23/97	Abn	10.25 ± 0.3536	2	4.7 ± 0.1414	2
Ref	WIN	7/23/97	Nor	11.044 ± 0.6655	98	4.526 ± 0.2193	98
Ref	WIN	9/9/97	Abn	9.6 ± 0.207	8	4.7 ± 0.2878	8
Ref	WIN	9/9/97	Nor	11.646 ± 0.7152	61	4.725 ± 0.2292	61
Aff	HYD	7/22/97	Abn	2 ± 0.6374	17	3.035 ± 0.32	17
Aff	HYD	7/22/97	Nor	2.229 ± 0.2458	85	2.961 ± 0.244	85
Aff	HYD	8/12/97	Abn	2.722 ± 0.6691	18	3.211 ± 0.3104	18
Aff	HYD	8/12/97	Nor	2.25 ± 0.3536	2	2.85 ± 0.0707	2
Aff	HYD	9/9/97	Abn	2.825 ± 0.1035	8	3.35 ± 0.414	8
Aff	HYD	9/9/97	Nor	3.282 ± 0.3865	95	3.439 ± 0.3036	95
Ref	LMS	7/21/97	Abn	9.467 ± 5.7839	3	4.3 ± 0.39994	3
Ref	LMS	7/21/97	Nor	7.723 ± 1.1008	99	4.098 ± 0.19639	99
Ref	LMS	10/1/97	Abn	7.1667 ± 0.2887	3	4.2738 ± 0.2517	3
Ref	LMS	10/1/97	Nor	7.986 ± 1.941	29	4.713 ± 0.1875	31
Aff	ROI	7/15/97	Abn	3.729 ± 0.6473	7	3.443 ± 0.0976	7
Aff	ROI	7/15/97	Nor	3.391 ± 0.45589	92	3.3119 ± 0.16025	92
Aff	ROI	9/23/97	Abn	7.478 ± 0.2635	9	4.4778 ± 0.2906	9
Aff	ROI	9/23/97	Nor	8.035 ± 0.6985	51	4.612 ± 0.2754	51
Ref	GEL	7/17/97	Abn	3.4 ± 0.4183	5	3.58 ± 0.2387	5
Ref	GEL	7/17/97	Nor	3.519 ± 0.8724	94	3.542 ± 0.3011	94
Ref	GEL	9/11/97	Abn	6 ± 3.1213	2	4.1 ± 0.2828	2
Ref	GEL	9/11/97	Nor	8.024 ± 1.1467	97	4.557 ± 0.2715	97
Aff	NEY	7/18/97	Abn	4	4	3.7 ± 0.0816	4
Aff	NEY	7/18/97	Nor	3.668 ± 0.8571	95	3.542 ± 0.3124	95
Aff	NEY	9/11/97	Abn	5.288 ± 0.5239	16	4.019 ± 0.2401	16
Aff	NEY	9/11/97	Nor	6.1207 ± 0.8426	106	4.032 ± 0.4219	106
Aff	NEY	9/29/97	Abn	3.957 ± 0.1535	21	3.738 ± 0.4248	21
Aff	NEY	9/29/97	Nor	4.722 ± 1.5919	91	3.771 ± 0.45443	91

Table 10. Comparison of body weights (Wt) and snout-vent lengths (SVL) in *Rana pipiens* metamorphs from Minnesota 1997. (A) Results of t-tests run for differences between the means of normal and abnormal metamorphs within sites are given. Ney comparisons for two different sampling dates are given. (B) Results for differences between the normal frogs from reference (Ref) sites and abnormal frogs from paired affected (Aff) sites are given. Affected sites were known to have malformed frogs. ns = no significance.

A. Comparisons of normal minus abnormal frogs within sites

Site	Ref/Aff	Probability of calculated t-value: Wt	SVL
Dor	Aff	$P < 0.01$	ns
Win	Ref	$P < 0.001$	ns
Hyd	Aff	ns	ns
LMS	Ref	ns	$P < 0.05$
Roi	Aff	ns	$P < 0.001$
Gel	Ref	ns	$P < 0.001$
Ney	Aff	$P < 0.05$	ns
Ney	Aff	ns	ns

B. Comparisons between paired sites: of normals (Ref) minus abnormals (Aff)

Sites	Probability of calculated t-value: Wt	SVL
Cum Ref Dor Aff	$P < 0.01$	ns
Win Ref Hyd Aff	$P < 0.001$	$P < 0.001$
LMS Ref Roi Aff	ns	$P < 0.05$
Gel Ref Ney Aff	$P < 0.001$	$P < 0.001$

Biologists in Minnesota would not accept these rates of malformation as normal.

We do not know what the long term consequences may be for frog populations in wetlands where there are malformed frogs. Both normal and malformed metamorphs may be compromised in some way. At one site (CWB), heavy mortality of normal and malformed metamorphosing frogs was observed in 1997. We have recorded malformations in some of the larvae collected in 1997; otherwise almost all of the malformations have been observed in the newly metamorphosed frogs, with very few in the adults. This suggests that the malformed frogs are not surviving the winter into the next season of life. They would not reach sexual maturity, let alone breed and transfer their genetic material to the next generation. Because of the sudden upsurge in numbers, the widespread distributions, and the large number of species of amphibians involved, it seems doubtful that the malformations are genetically transmitted to offspring. Transfer of a maternal burden of contaminants to the yolky eggs which the females produce in the fall for the next spring could be an important factor, especially if a potential causative agent is lipid soluble, as seen in fish, reptiles and birds (Russell et al. 1999). This is still considered one important way there could be exposure to the causative agent(s), particularly because early life stages tend to be more sensitive to toxicological agents than adults.

Whether the populations of *Rana pipiens* in wetlands in Minnesota with malformed frogs will be dramatically reduced or even disappear is presently unknown and would need to be carefully documented. The potential for impairments to normal-appearing metamorphs and the fact that few of the malformed young of the year frogs survive to the next spring suggests that in sites with high rates of malformations there could be effects on the population of frogs.

Our data suggest that there may be a developmental delay or inhibition of growth in the malformed frogs in some study sites where statistically significant reductions in body weight or body length in the malformed frogs compared to the normal metamorphs were observed. In two of the affected study sites for 1997, all the metamorphs, both abnormal and normal, showed sharply reduced growth compared with other sites. In one site (Hyd) this might have resulted from crowding of the larvae in the breeding pond, where over 100 eggs masses were observed. Frogs with mean SVL of 38 mm (Ney) and 34 mm (Roi) in September are very unlikely to have the stamina to migrate to overwintering sites and to survive the rigors of Minnesota winters. If they metamorphose late in the season, they would not have had the time for feeding as young adults on protein rich invertebrates.

To conclude, MPCA will continue to investigate what this new phenomenon indicates about the health of frog populations specifically and of wetlands generally in Minnesota. Our work will continue to focus on the life cycle of the frogs and on their habitats to investigate the most likely stages and sources of exposures to frogs from pesticides, heavy metals, endocrine-disrupting organics, microbial agents, parasites, and UVB. Potential causative agents could be in the landscape and in the water as the frogs develop from egg to herbivorous tadpole into insect-eating adults which disperse and migrate from shallow breeding ponds to deeper waters for the winter. Much more on the ground investigation coupled with laboratory analysis of amphibian foods, water and sediments of the habitars is needed. Fractionation studies, combined with laboratory bioassays (Fort et al. 1999a, 1999b) and exposures of site water with and without UVB are promising approaches. These approaches will elucidate whether there is something new in the ponds, or new combinations of chemicals present during the time when the frogs are laying their eggs, or perhaps activation by ultraviolet light of humanmade or natural chemicals is taking place in the ponds. We do not understand what kind of body burdens the adult females may be carrying which could affect earliest egg development. At the same time, there is a great need for more extensive laboratory research to document which of the chemical agents or other stressors to which the frogs are exposed can cause malformations of the types that have been documented in Minnesota and other states. Laboratory work needs to be closely linked to what is in the environments of the frogs. Finally, there is a need for better understanding of the hormonal mechanisms which control development, especially which specific manmade or natural chemical agents which are likely to occur in the frog habitats can affect hormonal controls of development.

Although many researchers feel there may be multiple, possibly interacting causes of the malformations in frogs, the chronology of the appearance of high frequencies of malformations in several species of anurans in many states and countries in the 1990s has some parallel to the dramatic changes in bird populations and egg-shell thickness in different countries after the introduction of DDT in the mid-1940s (Cooke 1973, Ratcliffe 1967, Hickey and Anderson 1968). In that case, the causative agent was persistent, lipid-soluble and bioaccumulated in the food chain. If whatever is causing the malformations in frogs lacks these characteristics, it will be more difficult to pinpoint. Alternatively, if several agents that are capable of causing malformations, possibly via the same mechanism, have appeared in the 1990's, finding the solution to the malformed frog problem could be far more difficult than linking DDE to a depression of calcium deposition in eggs of birds.

LITERATURE CITED

BONIN, J., M. OUELLET, J. RODRIGUE, J.-L. DESGRANGES, F. GAGNÉ, T.F. SHARBEL, and L.A. LOWCOCK. 1997. Measuring the health of frogs in agricultural habitats subjected to pesticides. Pages 246–257. *In* Amphibians in decline: Canadian studies of a global problem. D.M. Green, ed. Herpetological Conservation Vol. 1. Society for the Study of Amphibians and Reptiles, St. Louis, Missouri.

BORKIN, L.J., and M.M. PIKULIK. 1986. The occurrence of polymely and polydactyly in natural populations of anurans of the USSR. Amphibia-Reptilia 7:205–216.

COOKE, A.S. 1973. Shell thinning in avian eggs by environmental pollutants. Environmental Pollution 4:85–152.

DUBOIS, R.B. 1996. Recent observations of deformed anurans in Wisconsin. A short report. Wisconsin Department of Natural Resources, Brule, Wisconsin.

FLAX, N.L., and L.J. BORKIN. 1997. High incidence of abnormalities in anurans in contaminated industrial areas (eastern Ukraine). Herpetologia Bonnensis 1997:119–123.

FORT, D.J., R.L. ROGERS, H.F. COPLEY, L.A. BRUNING, E.L. STOVER, J.C. HELGEN, and J.G. BURKHARDT. 1999a. Progress toward identifying causes of maldevelopment induced in *Xenopus* by pond water and sediment extracts from Minnesota, U.S.A. Environmental Toxicology and Chemistry 198:2316–2324.

FORT, D.J., T.L. PROPST, E.L. STOVER, J.C. HELGEN, R.B. LEVEY, K. GALLAGHER, and J.G. BURKHARDT. 1999b. Effects of pond water, sediment, and sediment extracts from Minnesota and Vermont, U.S.A., on early development and metamorphosis of *Xenopus*. Environmental Toxicology and Chemistry 18:2305–2315.

GARDINER, D.M., and D.M. HOPPE. 1999. Environmentally induced limb malformations in mink frogs (*Rana septentrionalis*). Journal of Experimental Zoology 284:207–216.

GARRY, V.F., D. SCHREINEMACHERS, M.E. HARDINS, and J. GRIFFITH. 1996. Pesticide appliers, biocides and birth defects in rural Minnesota. Environmental Health Perspectives 104:394–399.

GERNES, M., and J. HELGEN. 1997. Problem investigation: deformed frogs in Granite Falls, MN in 1993 and 1994. Minnesota Pollution Control Agency. Unpubl. report to U.S. EPA under grant no. X995749-01, June 30 1997.

Hamline University, Center for Global Environmental Education. St. Paul, Minnesota. http://cgee.hamline.edu/frogs.

HELGEN, J. 1997. The frogs of Granite Falls: frogs as biological indicators. Pages 55–57. *In* Minnesota's amphibians and reptiles, their conservation and status. Moriarty, J.J., and D. Jones, eds. Minnesota Herpetological Society.

HELGEN, J., M.C. GERNES, D. HOPPE, R.G. MCKINNELL, and D.L. CARLSON. 1998. Investigation of deformed frogs in Minnesota 1996. Unpubl. Report to the Legislative Commission on Minnesota Resources from the Minnesota Pollution Control Agency, May 1 1998.

HELGEN, J., R.G. MCKINNELL, and M.C. GERNES. 1998. Investigation of malformed northern leopard frogs in Minnesota. Pages 288–300. *In* Status and conservation of midwestern amphibians. M.J. Lannoo, ed. University of Iowa Press, Iowa City.

HICKEY, J.J., and D.W. ANDERSON. 1968. Chlorinated hydrocarbons and eggshell changes in raptorial and fish-eating birds. Science 162:271–273.

HOPPE, D.M. 2000. History of Minnesota frog abnormalities: do recent findings represent a new phenomenon? *In* Investigating amphibian declines. Proceedings of the 1998 midwest amphibian conference. H. Kaiser, and G.S. Casper, eds. Journal of the Iowa Academy of Science 107: 86–89.

HOPPE, D.M., and R.G. MCKINNELL. 1997. Observations on the status of Minnesota leopard frog populations. Pages 38–42. *In* Minnesota's amphibians and reptiles, their conservation and status. J.J. Moriarty, and D. Jones, eds. Minnesota Herpetological Society.

HUSVETH, J.J., LEHTINEN, R., and M. KIRWIN. 1996. The occurrence of deformed wood frogs (*Rana sylvatica*) in Itasca State Park [Clearwater County, MN] and adjacent areas. Unpubl. report, University of Minnesota Lake Itasca Forestry and Biological Station.

JONES, P.M., E.M. THURMAN, E. LITTLE, S. KERSTEN, J. HELGEN, and E.A. SCRIBNER. 1999. Pesticide and metabolite concentrations in sediments and surface and ground water found at sites where frog malformations are present in Minnesota. Seventh Symposium on the Chemistry and Fate of Modern Pesticides (abstract). University of Kansas, Lawrence.

MATTSSON, J.P. 1997. Amphibian monitoring on national wildlife refuges in Region 3 1997: final report. U.S. Fish and Wildlife Service, Washington, D.C.

MERRELL, D.J. 1969. Natural selection in a leopard frog population. Journal of the Minnesota Academy of Science 35:86–89.

METEYER, C.U., I.K. LOEFFLER, J. BURKHART, K. CONVERSE, E. GREENE, J. HELGEN, S. KERSTEN, R. LEVEY, L. EATON-POOLE, and J.F. FALLON. 2000. *In* Investigating amphibian declines. Proceedings of the 1998 midwest amphibian conference. H. Kaiser, and G.S. Casper, eds. Journal of the Iowa Academy of Science 107:72–78.

MINNESOTA NEW COUNTRY SCHOOL. http://www.mncs.k12.mn.us/html/projects/frog/frog.html. Le Sueur, Minnesota.

Minnesota Pollution Control Agency. http://www.pca.state.mn.us/hot/frogs.html. St. Paul, Minnesota

MIZGIREUV, I.V., N.L. FLAX, L.J. BORKIN, and V.V. KHUDOLEY. 1984. Dysplastic lesions and abnormalities in amphibians associated with environmental conditions. Neoplasma. 31:175–181.

NARCAM. 1999. http://www.npsc.nbs.gov/narcam/reports. North American Reporting Center for Amphibian Malformations. Northern Prairie Science Center. U.S. Geological Survey, Biological Resources Division, Washington, D.C.

OUELLET, M., J. BONIN, J. RODRIGUE, J.-L. DESGRANGES, and S. LAIR. 1997. Hindlimb deformities (ectromelia, ectrodactyly) in free-living anurans from agricultural habitats. Journal of Wildlife Diseases 33:95–104.

RATCLIFFE, D.A. 1967. Decrease in eggshell weight in certain birds of prey. Nature 215:208–210.

READ, J.L., and M.J. TYLER. 1994. Natural levels of abnormalities in the trilling frog (*Neobactrachus centralis*) at the Olympic Dam Mine. Bulletin of Environmental Contamination and Toxicology 53:25–31.

REYNOLDS, T.D., and T.D. STEPHENS. 1984. Multiple ectopic limbs in a wild population of *Hyla regilla.* Great Basin Naturalist 44:166–169.

RUSSELL, R.W., F.A.P.C. GOBAS, and G.D. HAFFNER. 1999. Maternal transfer and in ovo exposure of organochlorines in oviparous organisms: a model and field verification. Environmental Science and Technology 33: 416–420.

SCHOETTER, R.A., and A.M. JULIN. 1967. Efficacy of MS-222 as an anaesthetic on four salmonids. U.S. Bureau of Sport Fisheries and Wildlife Investigations in Fish Control, Washington, D.C.

TAKEISHI, M. 1996. On the frog, *Rana ornativentris,* with supernumerary limbs found at Yamada Greenery Area in Kitakyushu City, Fukuoka Prefecture, Japan. Bulletin of the Kitakyushu Museum of Natural History 15:119–131.

ZAR, J.H. 1974. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, New Jersey.

Frog/Toad Abnormality Sheet
Minnesota Pollution Control Agency

Frog #_____ **Site**______________________ **Observers**______________________________ **Date**____________ **Time**___________

Species______________________ **Collected/released** **Preservation**______________ **Destination**______________

Front limbs:

L R
- ☐ ☐ entire limb missing
- ☐ ☐ radio-ulna partially missing (*describe*)______________________
- ☐ ☐ humerus partially missing (*describe*)______________________
- ☐ ☐ hand missing
- ☐ ☐ complete radio-ulna present, abnormal musculature
  - ☐ enlarged
  - ☐ atrophied
- ☐ ☐ digits missing from hand (*specify digits*)______________________
- ☐ ☐ digits shortened, fused or clubbed
- ☐ ☐ other (*describe*)______________________

Hind limb:

L R
- ☐ ☐ limb present, unusual angle (bent or antiversion, etc.) (*describe*)______________________
- ☐ ☐ limb present, unusual angle (twisted, rotated, etc.) (*describe*)______________________
- ☐ ☐ entire limb present, abnormal size (atrophied, enlarged) (*describe*)______________________
- ☐ ☐ digits missing from foot (*specify digits*)______________________
- ☐ ☐ digits shortened, fused or clubbed______________________
- ☐ ☐ digits in abnormal location (*describe*)______________________
- ☐ ☐ extra digits (*describe*)______________________
- ☐ ☐ tarsal bones missing
- ☐ ☐ tarsal bones partially missing (*proportion present*)______________
- ☐ ☐ metatarsals missing
- ☐ ☐ complete tibiofibula present, abnormal musculature
  - ☐ enlarged
  - ☐ atrophied
- ☐ ☐ portion of tibiofibula missing (*proportion of normal or length of tibiofibula present*)__________
- ☐ ☐ entire tibiofibula missing
- ☐ ☐ complete femur present, abnormal musculature
  - ☐ enlarged
  - ☐ atrophied
- ☐ ☐ portion of femur missing (*proportion of normal or length of femur present*)__________
- ☐ ☐ entire limb missing

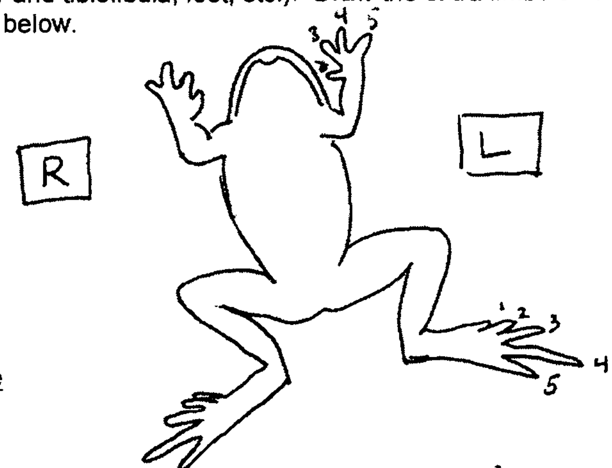
FROGTOAD.XLS

Extra Limbs:

How many extra limbs present?______________________

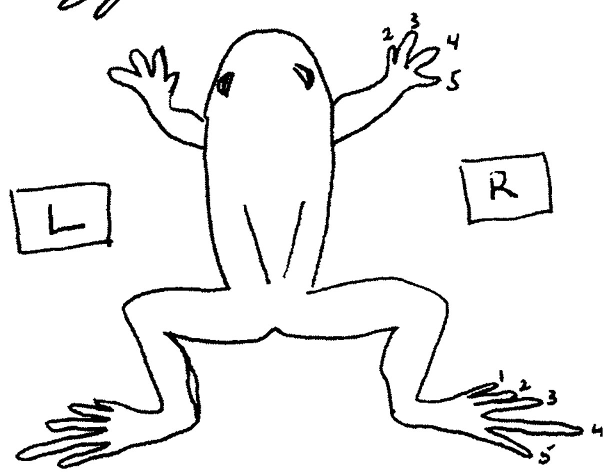
For each extra limb, describe location of origin (left or right, hip, knee, spine, etc.). Also specify musculature (larger or smaller than normal limb), and completeness (entire limb present or portion of limb). If only part of an extra limb is present, try tospecify which part is present (femur, femur and tibiofibula, foot, etc.). Draw the extra limbs on the frog diagram below.

Origin



Musculature

Completeness



Frog/Toad Abnormality Sheet
Minnesota Pollution Control Agency

Eyes:

L R
- [ ] [ ] eye absent
- [ ] [ ] eye smaller than normal
- [ ] [ ] pupil abnormally shaped
- [ ] [ ] pupil reduced in size
- [ ] [ ] abnormal pupil
- [ ] [ ] abnormal iris
- [ ] [ ] cloudy eye
- [ ] [ ] abnormal eye membrane (*describe*)______________________
- [ ] [ ] pigment mutation (*describe*)______________________
- [ ] [ ] eye in unusual position (*describe*)______________________
- [ ] [ ] extra eye(s) (*describe*)______________________
- [ ] [ ] other (*describe*)______________________

Jaws:

- [ ] lower jaw shortened
- [ ] upper jaw shortened
- [ ] jaw curved (*describe*)______________________
- [ ] other deformity (*describe*)______________________

Spine:

- [ ] curved (scoliosis)
- [ ] extended spine (*describe/length*)______________________
- [ ] other (*describe*)______________________

Bones:

L R
- [ ] [ ] cyst on bone, bone growth (*describe*)______________________
- [ ] [ ] bony spur (*describe*)______________________

Webbing (cutaneous fusion):

L R
- [ ] [ ] between femur and tibiofibula
- [ ] [ ] absent between digits (*describe*)______________________
- [ ] [ ] extra webbing (*describe*)______________________
- [ ] [ ] other (*describe degree*)______________________

- [ ] **Retained Tail** *length present*______________

- [ ] **Abnormal color or pattern**
(*describe and locate*)______________________

- [ ] **Emaciation**
(*Describe*)______________________

- [ ] **Red leg**

- [ ] **Hemorrhaging or Bruising**
(*Describe*)______________________

- [ ] **Skin growth**
(*Describe*)______________________

- [ ] **Any bleeding or fresh injuries?**
(*describe*)______________________

- [ ] **Other abnormalities**
(*please describe*)______________________

Appendix A. Continued

Frog/Toad Tally Sheet
Minnesota Pollution Control Agency

Page ___ of ___

Site________________ Observers______________________________________ Date_______

Air Temp.__________ % Abnormal_________ Time______

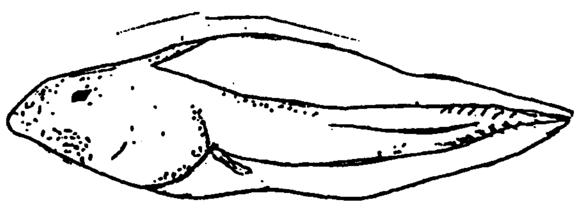
Frog #	Species	Adult/ Metamorph	Normal/ Abnormal	Weight (g)	Snout-vent length (cm)	Tail (cm)	Comments
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							
23							
24							
25							
26							
27							
28							
29							
30							
31							
32							
33							
34							

Appendix A. Continued

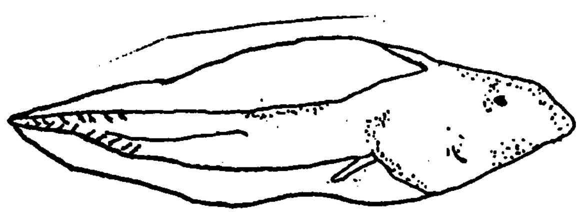
Larvae Abnormality Sheet
MPCA Deformed Frog Investigation

Axial malformations
- tail curved/flexed (indicate apex of curve(s) on diagram)
  - dorsal
  - ventrally   right   *USE S-severe (>45 degrees)
  - laterally-   left   M- mild (<45 degrees)
- tail coiled
  - dorsally
  - other ____________
- tail wavy
- tail reduced
- extra tail(s)-specify total number of tails____________
- tail forked
- tail damaged (specify)______________________________

Edema
- facial
- cephalic
- optic
- cardiac
- abdominal



Head/Face
- microcephaly
- flattened face
- rounded brow/head
- sloping forehead
- distended mouth
- two heads



Gut
- coiling absent
- coiling incomplete
- coiling abnormal

Eyes L R
- microophthalmia
- incomplete closure of choroid fissure
- complete failure of choroid fissure to close
- pigmentation
  - apigmentation
  - depigmentaion
  - eccentric accumulation
  - capping
- dislocation of lens
- two lenses
- rupture of optic cup
- irregular eye shape
- displaced eyes ____________
  - narrowing of eyes
  - diencephalon
  - cyclops

Blisters
- tail-dorsal
  - ventral
- other______

Other Abnormalities
- Emaciated
- Abnormal pigmentation
  - Central ventral spot:
    - number:________________________
    - color:________________
  - Other (specify)______________________________
- Trapping damage (specify)
- Hemorrhage (describe type and location)
- Red Leg

Appendix A. Continued

Larvae Abnormality Sheet
MPCA Deformed Frog Investigation

Polliwog #

SITE ____________ Observers ____________ DATE ____________ TIME ____________

SPECIES ________ Collected/ Released Preservation ____________ Destination ____________

Front limbs:

☐ Front limbs not emerged

L R
☐ ☐ One front limb emerged, other limb present but not emerged
☐ ☐ entire limb missing at shoulder
☐ ☐ limb partially missing (*describe*)____________
☐ ☐ foot missing
☐ ☐ complete tibiofibula present, abnormal musculature
☐ enlarged
☐ atrophied
☐ ☐ digits missing from foot (*specify digits*)____________
☐ ☐ digits fused or clubbed
☐ ☐ other (*describe*)____________

Extra Limbs:

☐ No extra limbs

How many extra limbs present?____________

For each extra limb, describe location of origin (left or right, hip, knee, spine, etc.). Also specify musculature (larger or smaller than normal limb), and completeness (entire limb present or portion of limb). If only part of an extra limb is present, try to specify which part is present (femur, femur and tibiofibula, foot, etc.). Draw the extra limbs on the frog diagram below.

Hind limb:

☐ Too small to see digits
☐ Too small to see tibiofibula
☐ Too small to see femur

L R
☐ ☐ entire limb present, unusual angle(twisted, rotated, etc.) (*describe*)____________
☐ ☐ entire limb present, abnormal size (atrophied, enlarged) (*describe*)____________
☐ ☐ digits missing from foot (*specify digits*)____________
☐ ☐ digits shortened, fused or clubbed____________
☐ ☐ digits in abnormal location (*describe*)____________
☐ ☐ extra digits (*describe*)____________
☐ ☐ tarsal bones missing
☐ ☐ complete tibiofibula present, abnormal musculature
☐ enlarged
☐ atrophied
☐ ☐ portion of tibiofibula missing
(*length of tibiofibula present*)____________
☐ ☐ entire tibiofibula missing
☐ ☐ complete femur present, abnormal musculature
☐ enlarged
☐ atrophied
☐ ☐ portion of femur missing
(*length of femur present*)____________
☐ ☐ entire limb missing
☐ ☐ other (describe)____________

Appendix A. Continued

Tadpole Lengths cm (30 Total)

#	Body Length	Total Length	Stage	#	Body Length	Total Length	Stage
1				16			
2				17			
3				18			
4				19			
5				20			
6				21			
7				22			
8				23			
9				24			
10				25			
11				26			
12				27			
13				28			
14				29			
15				30			

Appendix A. Continued

Site Visit Sheet
MPCA Deformed Frog Investigation

Site:________ **Observers:**________________________ **Date:**________

County:______________ **Arrival Time:**___________ **Departure time:**__________

SAMPLES COLLECTED:

Samples Collected for	Sample Type	Site #	# JARS	Total # JARS	JAR Type & Volume	Ship Date

# FROGS	SPECIES	N/A	AGE*	# JARS	JAR TYPE	PRESERVATION	DESTINATION	Ship Date

GPS FILE	TIME	SATILITES	PDOP	PTS	DESCRIPTION

FROGS CAUGHT:

# FROGS	SPECIES	N/A	AGE*

FROGS OBSERVED:

#FROGS	SPECIES	N/A	AGE*	Dd/Aliv	HRD/SN

*E-egg mass L- larvae M-metamorph S-subadult A-adult

Location metamorphs caught:

Water Temperature			Dissolved Oxygen	
Air Temperature			pH	
Sp. Conductivety				

Observations:

Vist2 Revised: 7/15/99

Appendix A. Continued