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DEPARTMENT OF NATURAL RESOURCES

RESEARCH REPORT 81

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PRELIMINARY REPORT ON LEOPARD FROG (Rana pipiens) POPULATIONS IN WISCONSIN

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(This is a progress report on a continuing study. Results should not be cited without the consent of the authors.)

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INTRODUCTION

A decline of frogs, particularly leopard frogs (Rana pipiens), has been reported in Wisconsin and has been documented in several states throughout the country (Gibbs et al. 1971) and in England (Cooke 1972). Over the past ten years biological supply houses in Wisconsin have noted a decrease in the number of frogs collected, and a reduction in the amount of time frogs could be held alive in tanks. Outbreaks of mortality have been noticed in the field, primarily by frog collectors, and, in the fall of 1973, hundreds of dead and dying frogs were reported in several areas of the state. The Amphibian Research Facility in Ann Arbor, Michigan, reported that between 150 and 200 adult female frogs collected in the fall of 1973 from several areas in Wisconsin did not have eggs, the first time this situation had occurred (Richards 1973, pers. comm.).

Since the leopard frog plays such a valuable role in the natural food chain of countless animals, in helping to control insects and aquatic detritus, and as an experimental animal in the biological and medical laboratory, an exploratory research project was set up in the spring of 1974 to obtain preliminary data on Wisconsin frog populations and mortality. It consisted of attempts to select ponds for intensive study in which the breeding population could be estimated and monitored throughout the summer, and an extensive fall survey to determine the extent and occurrence of leopard frog die-offs at the time of freeze-up and collect specimens for analysis.

The purpose of this progress report is to (1) present the data collected in these first efforts in Wisconsin to gather objective information on leopard frog populations with a minimum of interpretation; (2) to make the data available to persons concerned with Wisconsin's situation and investigators in other states involved with similar studies; and (3) help guide continuing research and alert Wisconsin field workers to the nature of the problem and the critical need for information. A brief review of some of the information available on the leopard frog decline nationally is included in the Appendix for background reference on the scope of the frog problem.

TAXONOMY

The *Rana pipiens* complex consists of several sibling species, one of which (*Rana pipiens*) predominates in Wisconsin (Pace 1972; Vogt unpubl.).

During the course of the field collections made in connection with the fall mortality survey, a series of approximately 100 frogs taken statewide was examined to determine the possible presence of any species of *Rana* other than *pipiens*. Three individuals collected on Knapp Creek in Richland County are suspected to be *Rana blairi*, a new species described by Mechum et al. in 1973 and found in Illinois. Since *R. blairi* does not interbreed with *R. pipiens*, it is considered to be a distinct species.

Leopard frog (*Rana pipiens*)

BREEDING POPULATION STUDIES

Procedures

A search was begun in April 1974 to locate sites where a series of breeding leopard frogs could be studied in unprotected agricultural run-off areas and in protected watersheds on DNR or federal holdings. A survey in the central part of the state for habitat types to meet these requirements was centered on Mead Wildlife Area near Wisconsin Rapids, various farmlands in the same area, and parts of Monroe County, for unprotected watersheds. The areas were all share-cropped or supported intensive agriculture with the use of modern chemicals. Protected areas surveyed included: Fort McCoy, Necedah National Wildlife Refuge, and Sandhill Wildlife Area (Fig. 1). These locations fell within an area surveyed in previous years by Vogt and found to have leopard frog populations.

After observing these selected regions from 1 April to 1 June it became obvious that an additional geographic location was necessary as no breeding populations of leopard frogs could be located within these areas. During this time, however, a variety of species of frogs was observed, including pickerel, spring peeper, chorus, wood, green, and gray tree frogs, and toads. Eggs of pickerel frogs were also noted. Another search began which included the Buena Vista Wetlands, Black River, and south central and eastern portions of the state. A leopard frog population of significant numbers was finally discovered in Kewaunee County at Algoma on the Ahnapee River on 26 June. This was a less than ideal location, for it was a river situation and was periodically flooded or dried out. Also there was not a protected area nearby with which we could compare our data.

However, the basic habitat requirements for a breeding population of frogs were met by the area, and it was the only location found in which there was a high enough concentration of frogs to mark. Also, the breeding season of *Rana pipiens* was rapidly nearing the end.

The area from which frogs were collected, marked, and released was located immediately adjacent to the Ahnapee River in Kewaunee County. It included approximately 45 m of shoreline and up to .50 km inland to a series of small ponds suitable for breeding. Water levels subsided on the river front by a total of .60 m throughout the study period from June to freeze-up. Water levels in the breeding ponds varied slightly (less than 5 cm) until dry weather in late August and early September completely dried up the ponds. These ponds were located beside a bike trail on state land; they averaged less than 5 m wide by 10 m long. A rather constant supply of water was furnished by ground seepage and run-off to maintain a water depth of 10-25 cm. Both the ponds and lake front area had extensive growths of cattails (*Typha*), sedges (*Carex*), and alder (*Alnus rugosa*) growing on the drier areas.

Since it was a cool spring, the breeding season of the leopard frogs was delayed almost one month. This enabled us to capture and mark frogs before they left the breeding ponds for summer foraging sites.

Collection of frogs for marking consisted of walking through the study area and catching as many leopard frogs on each pass through the area as possible, and returning to camp with the frogs for marking and measuring. This involved wearing chest waders since the water was over 1 m deep along the river's edge. The use of 6-volt head lamp was employed to spot the frogs at night. Once frogs were spotted, every effort was made to collect them quickly, for leopard frogs do not "freeze" very long in a beam of light. Frogs were transported back to camp (a matter of 50m)

in plastic bags. Branding consisted of using 5 mm high English numerals fashioned out of 20-gauge chromium resistance wire, which were heated over a propane torch and applied to the belly of the frog. This leaves a darkened number which is visible up to two or three months afterwards, adequate time to determine population size by a mark-recapture technique (Clark 1971). Lengths and distinctive markings were recorded. They were then released in a group 5-20 m from the point of capture. The mark and recapture sequence continued day and night until the population estimate remained relatively constant.

Results

Since the work on the Algoma area began fairly late in the spring, our work was confined largely to exploring the marking technique and making a population estimate. The results, calculated by the Schnabel method of population estimation, indicated a breeding population of 51.2 individuals in the study area (Table 1).

Observations in the vicinity of the study area and the immediate surroundings during the late spring and early summer revealed a preponderance of small frogs approximately 2 inches (5 cm) in length. During the last two weeks of July, pit traps were set to estimate the number of leopard frog young emerging from breeding ponds. They were checked four times a day, but only toads (*Bufo*) were captured. The adults at this time were generally found away from the ponds.

During the latter part of October, frogs were sparse throughout the Algoma Study Area. About 15 live frogs and 25 road-killed frogs were observed. On October 30, approximately 500 leopard frogs were observed at a site a mile upstream. One week later, 4 sick frogs were observed at this site. Subsequently, over 50 dead frogs were counted in the Ahnapee River near Forestville. Dead frogs could be found every 9 m lying on the river bottom. No frogs were observed on the banks.

TABLE 1. Population estimate of leopard frogs on the 4.5-acre Ahnapee Study Area.

Date	(A) No. Trapped	No. Marked	(B) Marked Animals in Area	No. marked Recaptures	(C) Sum of marked Recaptures	Population Estimate*
26 June	13	13				
27 June	28	18	13	10		
6 July	3	2	31	1	11	41.6
7 July	7	4	33	3	14	49.1
8 July	12	8	37	4	18	62.9
TOTAL	63	45		18		51.2

$$* \frac{\Sigma\,[(A) \times (B)]}{C}$$

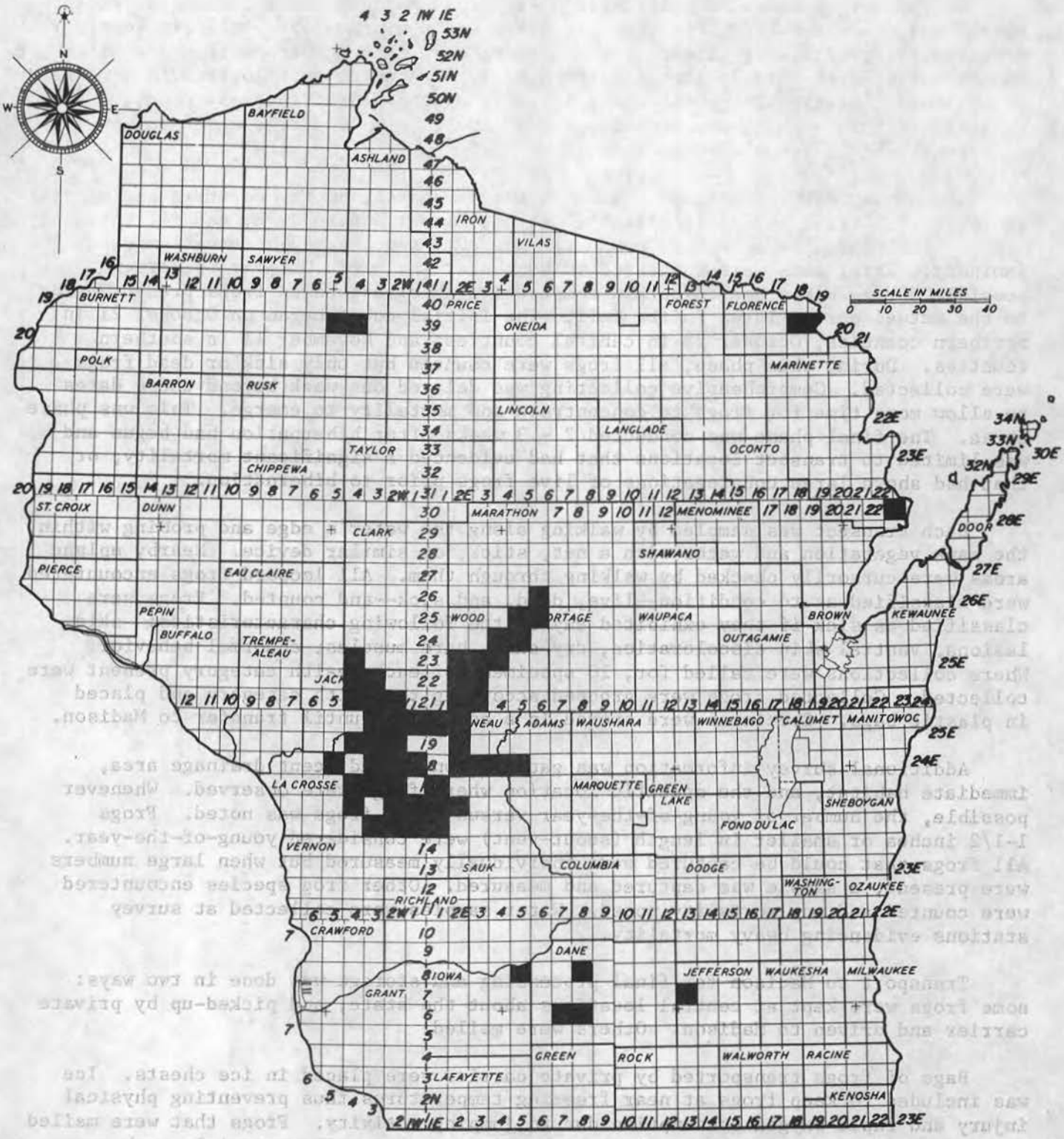

FIGURE 1. Areas intensively searched during the spring of 1974 without finding breeding populations of leopard frogs.

FALL MORTALITY SURVEY

Procedures

Survey transects were established by DNR fish managers in all counties except Monroe and Menominee (Fig. 2). Each transect was a minimum of .5 mile in length measured, in the case of streams, on one side only. The number of transect sites per county varied from 3 to 7 with an average of 5. Transects were located in a variety of habitats including large lakes and streams, spring-fed ditches and ponds, and marshes. Within each county an attempt was made to sample different drainages and different habitat types within drainages.

The survey was run in four phases on most transects in the northern and central sectors: (1) trial run, (2) initial count, (3) count and collect, and (4) mortality check. In the southern sector, phase (2) was ommitted because hibernation was imminent. Trial runs were conducted in September and early October to screen possible survey sites and to obtain comparative observations on frogs present prior to the actual survey later in the fall. The initial count began on October 21 in northern counties, October 28 in central counties, and November 11 in southern counties. During this phase, all frogs were counted but only sick or dead frogs were collected. Comprehensive collecting was delayed one week beyond these dates to allow more time for frogs to concentrate and mortality to emerge. This was phase three. The final phase was conducted 2 - 3 weeks after hibernation had begun and was limited to transect locations that had evidenced a significant mortality, or that had shown large congregations of live frogs prior to hibernation.

Each transect was sampled by walking along the water's edge and probing within the bank vegetation and water with a net, stick, or similar device. Nearby upland areas were cursorily checked by walking through them. All leopard frogs encountered were classified as to condition--live, dead, and sick--and counted. Frogs were classified as sick if they exhibited any of the following characteristics: skin lesions, ventral skin discoloration, dry skin, hard muscles, abnormal behavior. Where collections were called for, 10 specimens of each health category present were collected. Collected frogs were grouped according to health category and placed in plastic bags. Specimens were stored in a cool place until transfer to Madison.

Additional survey information was gathered on the adjacent drainage area, immediate habitat, and the specific location where frogs were observed. Whenever possible, the number of young-of-the-year versus older frogs was noted. Frogs 1-1/2 inches or smaller in length (snout-vent) were considered young-of-the-year. All frogs that could be captured were individually measured but when large numbers were present, a sample was captured and measured. Other frog species encountered were counted and any mortality noted. Water samples were collected at survey stations evidencing heavy mortality.

Transport to Madison for final processing and storage was done in two ways: some frogs were kept at central locations about the state, and picked-up by private carrier and driven to Madison. Others were mailed.

Bags of frogs transported by private carrier were placed in ice chests. Ice was included to keep frogs at near freezing temperatures thus preventing physical injury and rapid oxygen consumption due to jumping activity. Frogs that were mailed were placed in cardboard boxes along with moisture-conserving materials such as spagnum moss or wet paper towelling. Delivery in Madison was usually 2-3 days after mailing.

Most frogs mailed were received in good condition, although a few died from oxygen depletion caused by warm conditions during transport. Some frogs collected early in the survey and maintained in storage 2-4 weeks in plastic bags before pick-up could be arranged died or became so weakened that they did not survive transport. Approximately 150 frogs were lost in this way. In general, frogs that were mailed were received in better condition than those received by private carrier because storage time after collection was minimized.

Once in Madison the frogs were separated into three groups: those to be delivered fresh for various tests, those to be preserved by formalin injection, and those to be stored by freezing. Fresh specimens were delivered within one or two days to those conducting various tests. Frogs to be preserved were injected and stored in standard 10% buffered formalin. Frogs to be frozen were wrapped in aluminum foil and placed in plastic bags and frozen.

Frogs collected for tests were weighed and measured (snout to vent), sexed, and examined for presence of eggs in the laboratory at Madison. A sample of speciments from the University of Wisconsin Zoology Museum and the Milwaukee Public Museum was examined for comparative information.

Results

Pattern of Mortality

The fall survey was completed for 70 counties. Transects were laid out in 378 sites, and were run a total of 625 times (Table 2). Observers counted 4,086 live and 958 sick or dead leopard frogs, and collected 582 live and 462 sick or dead animals. The geographic areas used to classify the results are shown in Figure 3. Further refinements of these data are presented for reference purposes in the Appendix: survey results by county; and transects evidencing mortality of 5 or more frogs, and less than 5 frogs. Some mortality occurred in species other than the leopard frog, although apparently to a far lesser extent (Table 3).

The numbers of leopard frogs observed in the survey are minimum and cannot be used as indicators of the actual number of frogs present in an area. Large numbers of frogs can concentrate in a very small area and finding concentrations can be an elusive pursuit. Weather influenced the sighting of frogs with cold weather driving them under protective bank vegetation or into the water. Once hibernation had begun, the observation of dead or sick frogs was limited to clear water or water shallow enough for net sampling. In the north, icing over of survey sites hampered or eliminated final mortality checks. Furthermore, no attempt was made to document the extent of mortality by repeated monitoring.

However, the numbers have value as comparative indicators of abundance and the occurrence of mortality. Most surveys were conducted in three stages--preliminary, count, and count/collect--and many stations were checked at least once again for mortality. Thus, some portion of any frogs present on a transect was probably sighted at some point of the survey.

The pattern of the locations of live and sick or dead frogs, and of those transects on which no frogs were observed, are shown in Figures 4-6, plotted by civil town. The map showing transect locations with no frogs represents areas of good frog habitat surveyed under suitable weather conditions (Fig. 4). Information on all the transects was pared down and data from those transects were eliminated

where absence of frogs could be attributed to poor time of observation (e.g. early morning hours at 32°F) or location of transect in poor wintering habitat (e.g. edges of forested streams).

Shown in relation to the occurrence of live frogs are areas where sick or dead frogs were observed (Fig. 5). Areas where more than 5 frogs were found sick or dead are shown in Figure 6. While mortality occurred in several areas throughout the state, there was a definite concentration of die-offs in east central counties, with transects in Dodge and Calumet Counties showing the greatest observed mortality, 316 and 253 sick or dead frogs, respectively.

Ratios between the number of live and sick or dead frogs observed tend to substantiate the heavier mortality occurrence in the central counties compared to the north (Table 2). Twenty-three live frogs were observed to one sick or dead animal in the north, while in the central counties six live were observed to one sick or dead animal. Collections in the southern part of the state are probably not valid for this type of comparison, since they were made much later in the fall and live frogs in particular were difficult to see in deep sometimes turbid water.

A seasonal progression of mortality developed from the survey data (Table 4). Mortality apparently began at a low level and increased with time. At a given site, for example, one or two sick or dead frogs might initially be observed, whereas the number would increase to 50 or more within a few weeks. Most sick or dead frogs were observed in the water on the lake or stream bed although some cases of sick and dead frogs out of water were reported. The most noticeable die-offs began to occur from two-three weeks after the frogs entered the water for hibernation.

Freeze-up halted most observations although indications are that mortality was still occurring during the winter under the ice. Sampling under ice in November in Dunn County (Muddy Creek) and in Burnett County (Grettum Flowage) yielded dead frogs. Dead frogs have also been observed through January and February in trout streams in southwestern counties.

Unusual behavior occurred at several sites: frogs were observed crawling out of water onto ice, and dead frogs were noted on the ice of partially frozen waters in November and December.

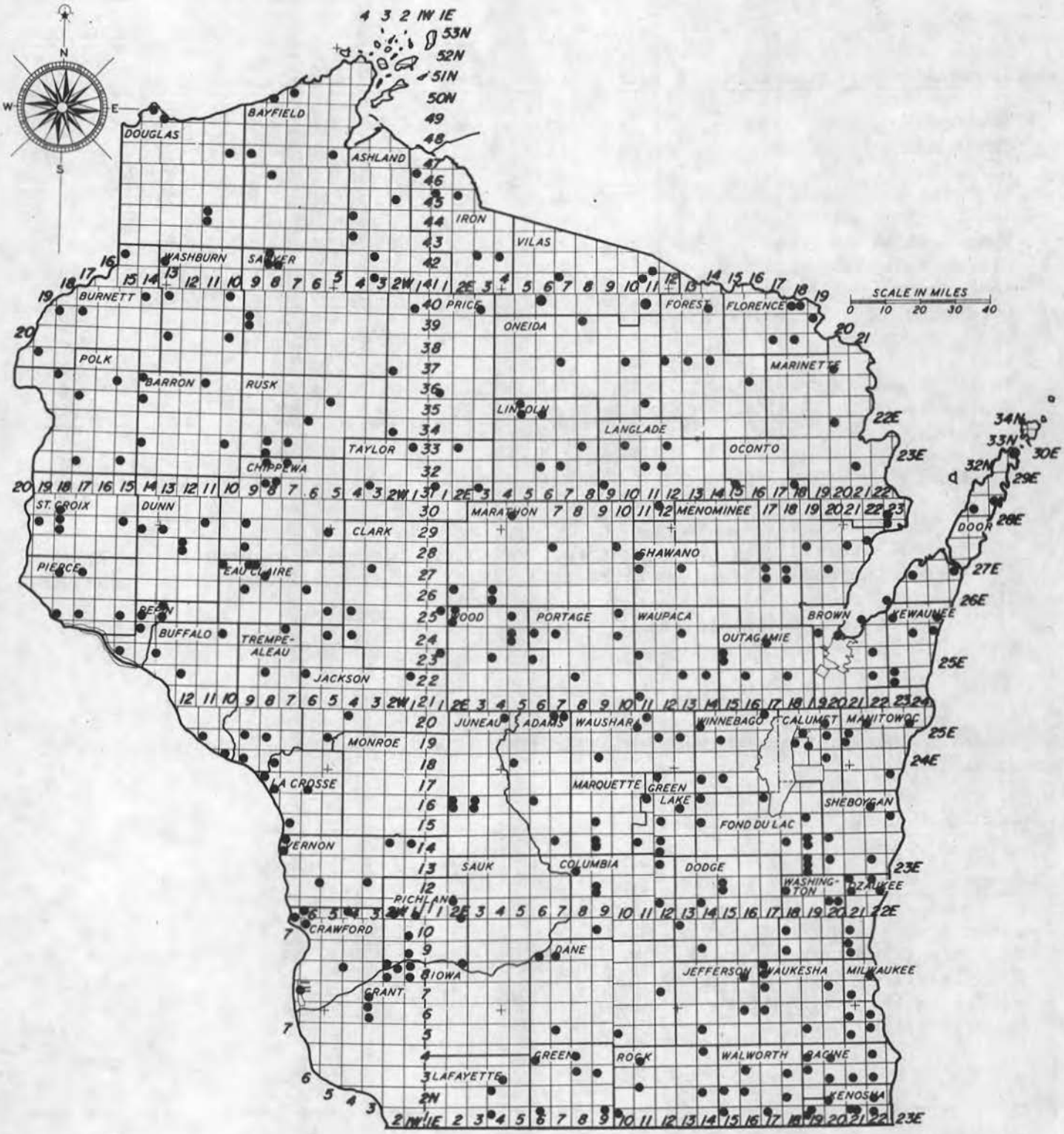

FIGURE 2. Transects established in 70 counties for mortality survey, October-December, 1974.

TABLE 2. Fall mortality survey results by geographic area.*

Location	No. Transects	No. Runs**	No. Observed: Live	No. Observed: Sick or Dead	No. Observed: Live: Sick or Dead	No. Collected: Live	No. Collected: Sick or Dead
Northwest	40	77	597	35	17:1	79	27
Northcentral	36	79	151	5	30:1	60	3
Northeast	32	64	299	5	60:1	83	2
North	108	220	1,047	45	23:1	222	32
West Central	49	94	230	14	16:1	18	10
Midcentral	53	70	274	82	3:1	98	56
East Central	58	107	2,385	408	6:1	193	279
Central	160	271	2,889	504	6:1	309	345
Southwest	34	50	72	5	14:1	31	4
Southcentral	31	39	41	347	1:8	6	68
Southeast	45	45	37	57	1:2	14	13
South	110	134	150	409	1:3	51	85
TOTAL	378	625	4,086	958	4:1	582	462

* Numbers are minimum. Frogs were not recounted for every observation date; the highest number observed on a transect at any one time was used in the tabulation.

** Does not include trial runs.

TABLE 3. Observations on species other than *Rana pipiens* during the fall mortality survey.

Species	Total Number Observed: Live	Total Number Observed: Sick-Dead	Ratio: Live: Sick - Dead
Green frog (*Rana clamitans*)	928	15	62:1
Bullfrog (*Rana catesbeiana*)*	128	0	128:0
Pickerel frog (*Rana palustris*)	65	1	65:1
Wood frog (*Rana sylvatica*)	28	0	28:0
Unclassified species	112	2	56:1
TOTAL	1,261	18	70:1

* Included green frogs in many instances.

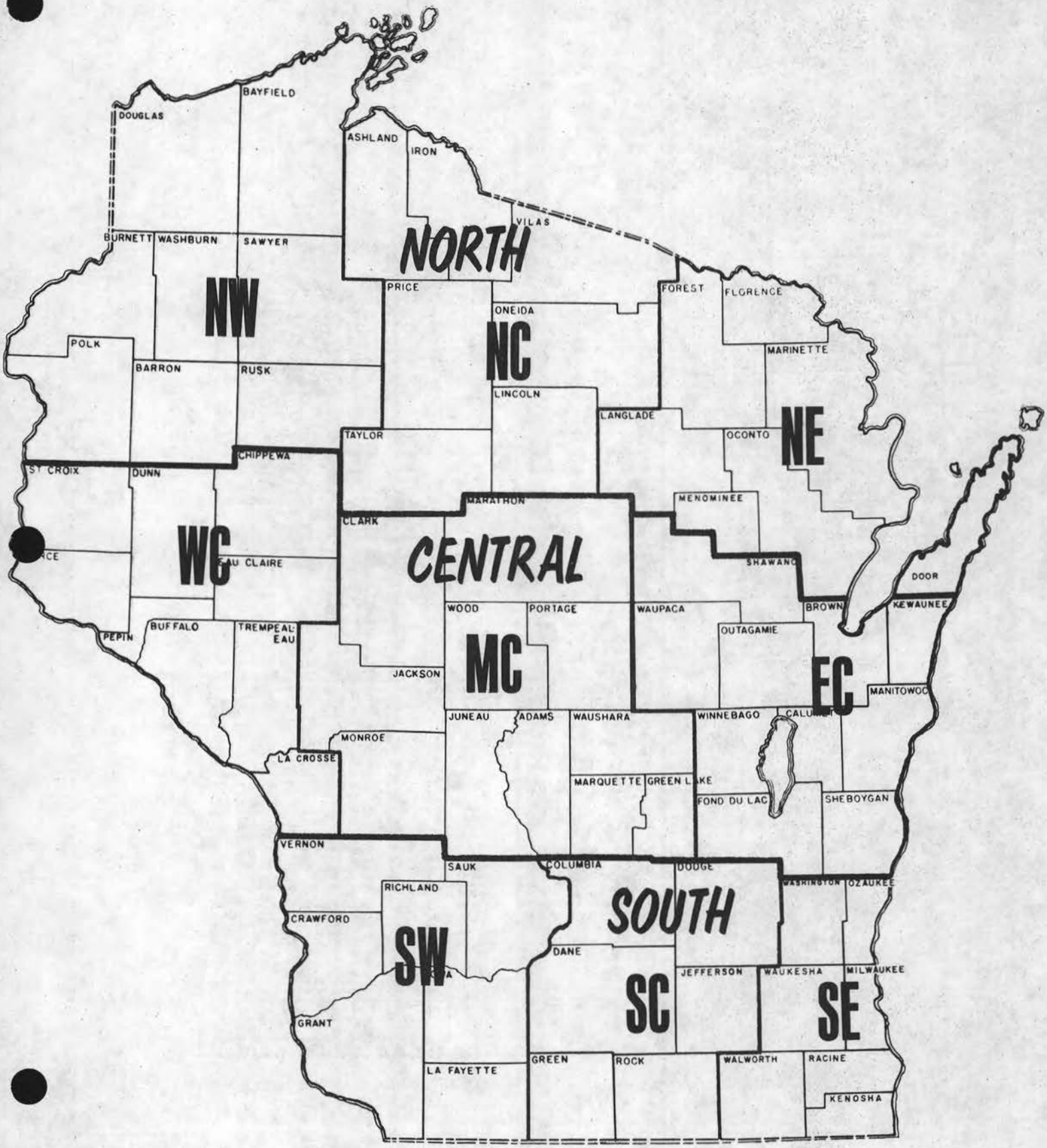

FIGURE 3. Geographic areas used as the basis for the compilation of results in the fall mortality survey.

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FIGURE 4. Transects on which no frogs were observed during the fall mortality survey, October-December, 1974.

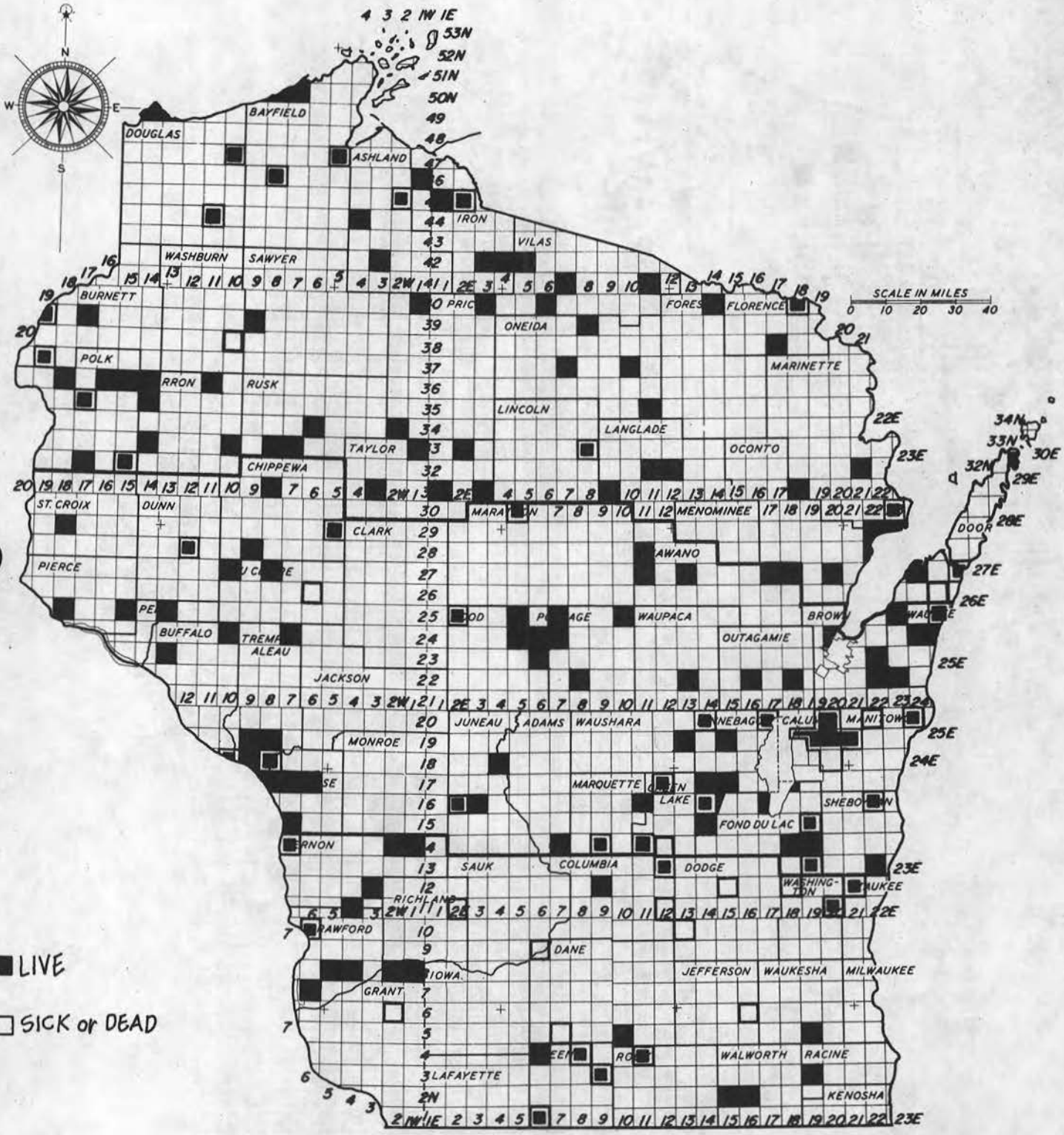

FIGURE 5. Transects on which live and sick or dead frogs were observed, October-December, 1974.

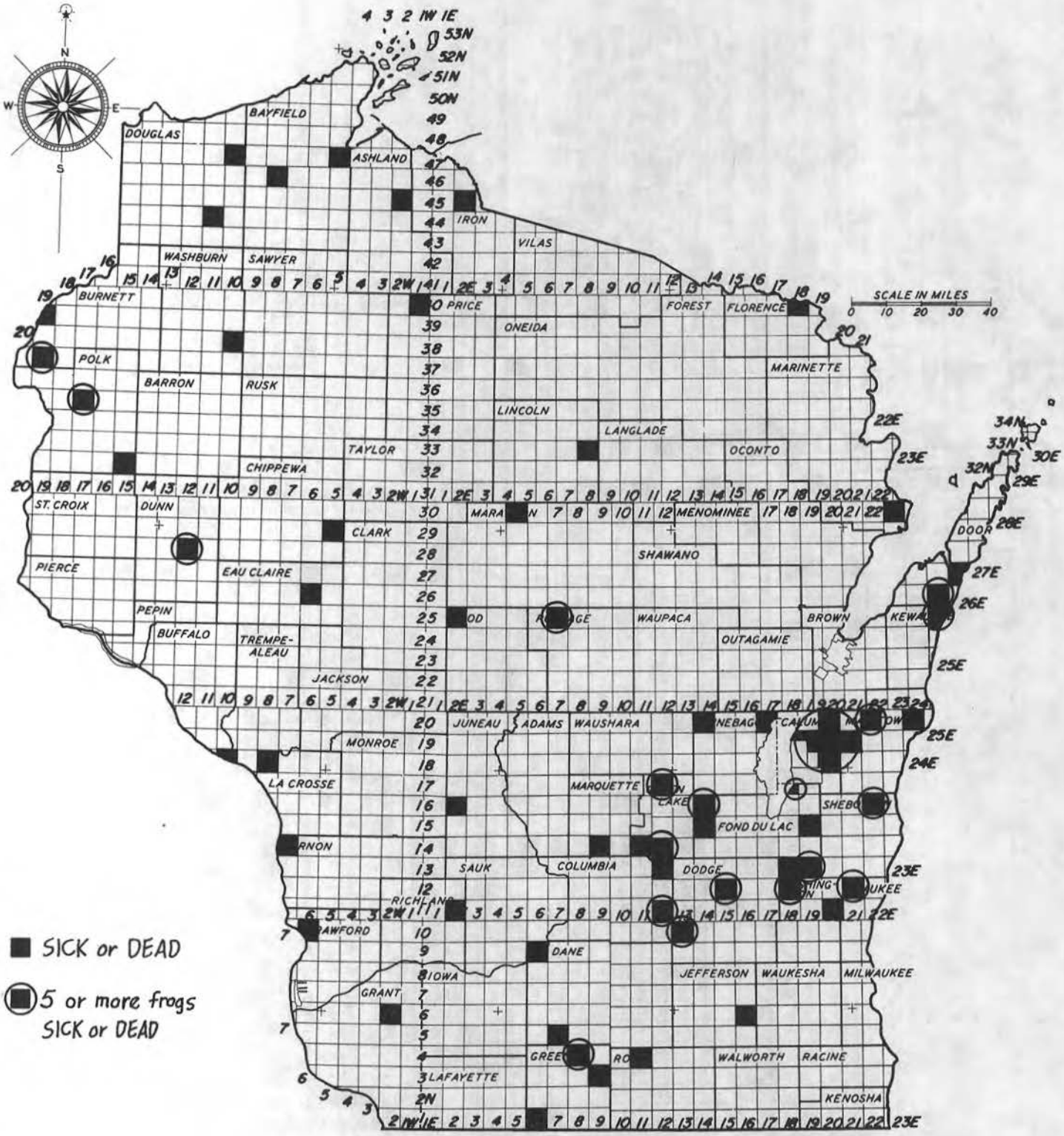

FIGURE 6. Transects on which five or more sick or dead frogs were observed, October-December, 1974.

TABLE 4. Number of leopard frogs observed per survey month grouped by geographic area.

Location	No. Live Frogs Observed				No. Sick/Dead Frogs Observed			
	Sept.	Oct.	Nov.	Dec.	Sept.	Oct.	Nov.	Dec.
Northwest	549	481	21	--*	0	41	6	--
Northcentral	19	132	0	--	1	4	0	--
Northeast	--	322	7	0	0	3	0	--
North	568	935	28	0	1	48	6	--
Westcentral	61	155	42	--	0	2	11	--
Midcentral	--	102	109	51	--	3	5	71
Eastcentral	--	1,333	1,177	10	--	0	230	172
Central	61	1,590	1,328	61	0	5	246	243
Southwest	30	4	30	8	0	0	3	2
Southcentral	--	--	51	5	--	--	50	296
Southeast	--	--	37	--	--	--	57	--
South	30	4	118	13	0	0	110	298
TOTAL	659	2,529	1,474	74	1	53	362	541

* Transects not run.

Population Characteristics

Sex ratio. Specimens collected during the fall mortality survey were sexed in the laboratory. The major criterion used was the enlarged thumb pad of the male. Sex could not be externally differentiated in frogs less than 5 cm in length (snout-vent). Of 151 frogs 5 cm and above in length, 51 percent were male and 49 percent female (Table 5).

Age ratio and size classes. Fish managers conducting the fall survey were asked to classify frogs observed into two size classes: larger and smaller than 1-1/2 inches (3.8 cm). On this basis 53 percent of 2,250 frogs observed were larger than 1-1/2 inches and 47 percent smaller (Table 6). Collected specimens were also measured, using 5 cm (2 inches) as the dividing point. Of 218 frogs, 69 percent were larger than 2 inches (5 cm). This higher percentage undoubtedly reflects the tendency to collect the larger frogs in spite of the slightly higher minimum measurement of 5 cm. Because of these disparities, it is not possible to determine a meaningful age ratio at this time. However, these data do provide a minimum field estimate of the proportion of young-of-the-year in the fall population (47%).

Length and weight data obtained from 278 specimens collected during the fall mortality survey provided some interesting information on size classes and size at sexual maturity. Those frogs less than 5 cm in length were classified as young of the year. They averaged 8 g in weight and 4.2 cm in length (Table 7). Males averaged 22 g in weight and 6.0 cm in length.

All females over 5 cm in length were examined internally for pigmented eggs. This showed generally that females between 5 and 6 cm did not have pigmented eggs present (Fig. 7) (one exception was a gravid female which was 5.0 cm long). A few females at 6 cm in length had eggs present, and all 6.5 cm and above were gravid.

Egg production. Presence of pigmented eggs in the females collected in the fall throughout the state coincided almost exactly with size. Fifty-three percent of the females examined did not have pigmented eggs. They averaged 13 g in weight and 5.4 cm in length, while 47 percent had pigmented eggs and averaged 35.5 g and 7.2 cm (Table 8). A small sample of museum specimens was examined for comparative purposes and this information parallels that found in the larger field sample (Table 8).

The threshold of egg production in females appeared to be at 6 cm in length, with all frogs examined above 6.5 cm being sexually mature and those below this length generally being sexually immature (Fig. 7).

TABLE 5. Sex ratios of frogs* collected during the fall mortality survey, October-December, 1974.

Location	Total Frogs	No. Males	No. Females
North	44	26 (59%)	18 (41%)
Central	97	46 (47%)	51 (53%)
South	10	5 (50%)	5 (50%)
TOTAL	151	77 (51%)	74 (49%)

* 5 cm and above in length.

TABLE 6. Percentage of leopard frogs larger and smaller than 1-1/2 inches (3.8 cm) observed in the field, October-December, 1974.

Location	No. Stations	Total No. Frogs Sampled	Frogs >1-1/2 inches* No.	Percent	Frogs <1-1/2 inches* No.	Percent
North	23	582	308	53	274	47
Central	21	1,619	864	53	755	47
South	7	59	20	34	39	66
TOTAL	51	2,260	1,192	Avg. 53%	1,068	Avg. 47%

* Snout-vent measurement.

TABLE 7. Weight and length of immature and male leopard frogs collected during fall mortality survey, October-December, 1974.

	Total No.	Weight (g) Avg.	Weight (g) Range	Length (cm) Avg.	Length (cm) Range
Immatures*	68	8	2-13	4.2	3.0-5.0
Males	77	22	11-41	6.0	5.0-7.5

* Less than 5 cm in length (sex could not be differentiated externally).

FIGURE 7. Relationship between weight and length in females with and without eggs, October-December, 1974 (logarithimic scale)

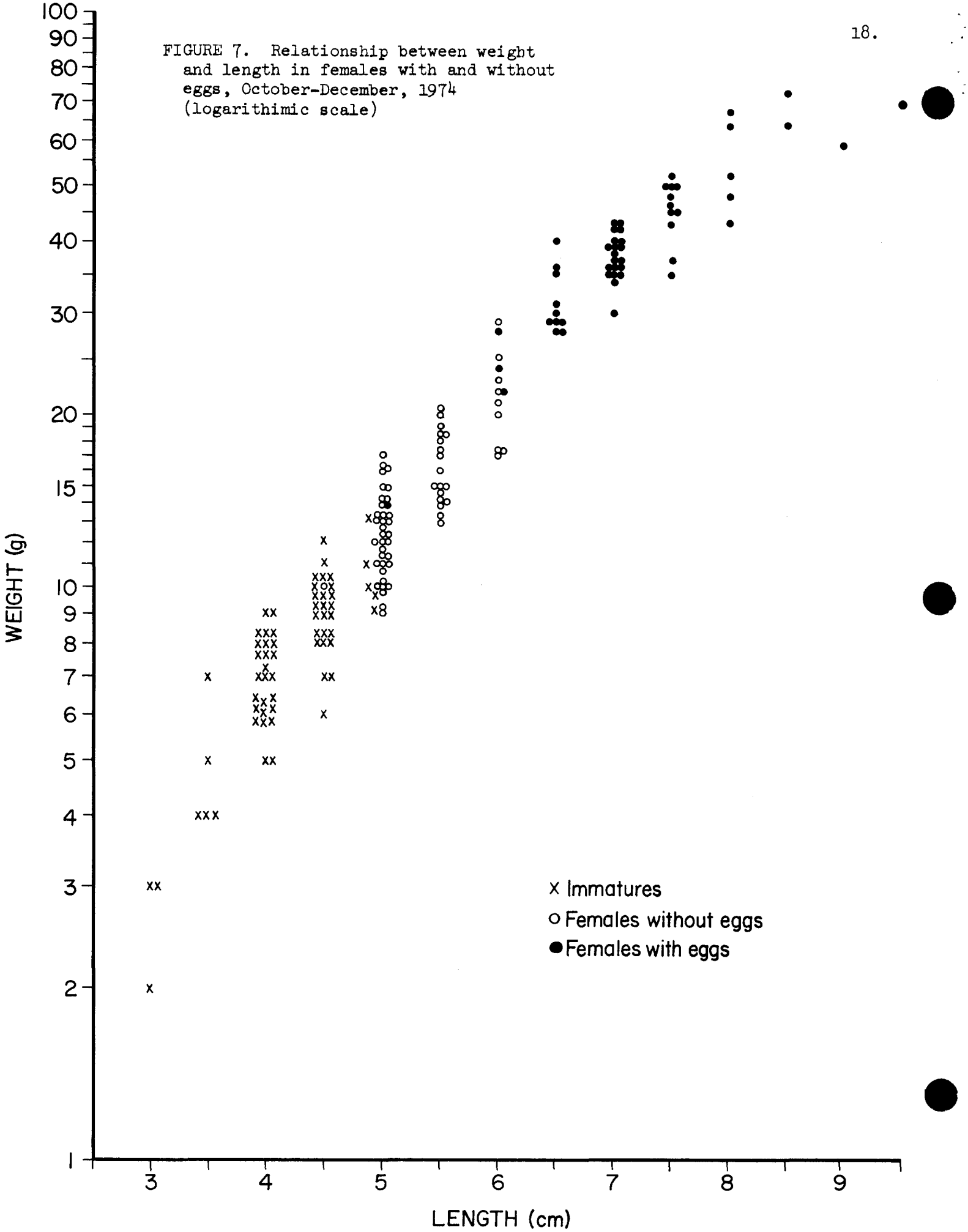

TABLE 8. Weight, length and presence of eggs in females collected during the fall mortality survey, October-December, 1974.

		With Eggs				Without Eggs			
Location	Total No.	No.	Percent	Avg. Wt. (g)	Avg. Lg. (cm)	No.	Percent	Avg. Wt. (g)	Avg. Lg. (cm)
North	19	5	26	54	8.1	14	74	14	5.3
Central	109	54	50	35	7.1	55	50	13.4	5.4
South	5	3	--	(21)	(5.8)	2	--	--	(5.3)
TOTAL	133	62	47	35.5	7.2	71	53	13.0	5.4
(Range)				(14-70)	(5.0-9.5)			(9-29)	(4.5-6.0)
Museum specimens	7	6		42	7.4	1		13	5.5

Pathology

Sick frogs were characterized by a variety of cutaneous abnormalities including ventral skin discoloration, hemorrhages, rigid hard muscles, and erosion of toes and feet. These are the classic symptoms of the disease in frogs commonly known as "redleg". Although redleg is caused by bacteria, this disease is not usually pathogenic and outbreaks are generally conceded to occur as a result of some type of environmental stress. Contacts were made with several investigators who kindly volunteered to undertake a variety of tests on samples of live, sick and dead frogs.

Bacteria. Twenty-two frogs were submitted for bacteriological examination. Ninteen of these evidenced one or more of the following symptoms: slight to strong lividity of the hindquarters, and gray or mottled livers with petechial hemorrhages. Some spleenomegaly was observed. Positive cultures for *Aeromonas* were obtained on five of these frogs (including one "shakily positive"), collected in the following locations: Two specimens from Langlade Co. (Peters Marsh, transect #5, live), and one each from Sawyer Co. (Musky Bay, Lac Court d'Oreilles, transect #1, live), Winnebago Co. (Fountain Pond, transect #3, live), and Kewaunee Co. (E. Alaska Lake, transect #2, sick).

One pooled sample (4-5 frogs) from Winnebago Co. run separately from the above indicated presence primarily of *Aeromonas*. A number of species of *Aeromonas* were found in addition to *hydrophila*, the one associated with redleg. Other bacterial organisms found were *Pseudonomas* and *Flavobacter*. The livers in these frogs were also a muddy, dirty gray color.

Parasites. Microscopic examination of the upper one-third of the large intestine of the sample of 23 frogs submitted for bacteriological cultures revealed the presence of the following organisms: Opalinids (Lata form), *Trichomonas*, *Proteromonas*, *Nyctotherus*, *Hexamastix*, *Blastocystis*, *Bodo*; nematodes; colonial green/blue-green algae; *Selenodesmus*.

Other Tests. Tests are being run on samples of live, sick and dead frogs collected throughout the state during the fall mortality survey: pesticides (DDT, dieldrin, PCB's), herbicides (2, 4-D, atrizene), and mercury. The results of these tests will be available at a later date.

SUMMARY

An exploratory research project was begun in 1974 to obtain preliminary data on Wisconsin leopard frog populations and mortality. Atempts to intensively study breeding ponds were limited by the absence of breeding populations in the areas selected for study. A late spring effort was finally made at a 4.5-acre area on the Ahnapee River where a population of 51.2 individuals was estimated using a mark and recapture technique.

During the fall, a statewide survey was initiated consisting of 378 transects run in 70 counties. Four thousand live and 958 sick or dead leopard frogs were observed. The heaviest mortality occurred in the east central counties.

The sex ratio of specimens collected during the fall mortality survey was 51 percent male and 49 percent female. A minimum field estimate of the proportion of young-of-the-year frogs in the fall population was 47 percent. The threshold of egg production in females appeared to be at 6 cm (2-1/2 inches) in length, with all frogs examined above 6.5 cm being sexually mature and those below this length generally being sexually immature.

Sick frogs were characterized by a variety of abnormalities including skin discoloration, hemorrhages, rigid muscles and erosion of toes and feet. Positive cultures of the organism causing redleg were obtained, and abnormalities of the liver and spleen were observed.

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TABLE 9. Survey results by county

County	No. Transects	Tot. No. Transects Run*	No. Frogs Observed		No. Frogs Collected	
			Live	Sick/Dead	Live	Sick/Dead
Adams	5	5	7	0	3	0
Ashland	5	11	15	1	11	0
Barron	5	10	31	0	0	0
Bayfield	5	15	102	5	10	5
Brown	5	10	289	0	0	0
Buffalo	5	16	9	2	0	0
Burnett	5	11	350	8	40	8
Calumet	6	11	719	258	38	153
Chippewa	7	15	34	1	4	0
Clark	5	5	0	0	0	0
Columbia	6	6	3	6	0	5
Crawford	5	10	25	1	0	1
Dane	5	8	1	3	0	3
Dodge	5	5	0	318	0	50
Door	5	10	115	3	5	0
Douglas	5	11	53	3	10	1
Dunn	5	7	31	9	0	9
Eau Claire	7	12	18	1	8	0
Florence	5	10	2	1	1	1
Fond du Lac	8	13	117	42	44	32
Forest	5	5	1	0	1	0
Grant	7	7	12	2	8	2
Green	6	10	48	16	5	7
Green Lake	7	9	95	64	34	50
Iowa	3	6	19	0	19	0
Iron	5	10	51	1	25	0
Jackson	5	10	0	0	0	0
Jefferson	3	3	0	2	0	1
Juneau	5	5	15	0	6	0
Kenosha	7	7	0	0	0	0
Kewaunee	6	12	968	19	21	15
La Crosse	5	12	74	1	1	1
Lafayette	3	3	0	0	0	0
Langlade	6	13	89	0	21	0
Lincoln	6	10	6	2	6	2
Manitowoc	6	12	33	37	15	37
Marathon	6	7	42	3	8	2
Marinette	5	13	45	1	25	1
Marquette	3	4	11	1	2	1
Milwaukee	6	6	0	0	0	0
Oconto	6	13	48	0	30	0
Oneida	5	9	12	0	0	0
Outagamie	5	5	3	0	1	0
Ozaukee	7	7	25	45	6	5
Pepin	5	5	2	0	0	0
Pierce	5	5	9	0	0	0
Polk	5	10	52	19	14	13
Portage	6	6	28	11	9	1
Price	5	11	16	1	4	1
Racine	7	7	2	0	1	0

TABLE 9. (continued)

County	No. Transects	Tot. No. Transects Run*	No. Frogs Observed		No. Frogs Collected	
			Live	Sick/Dead	Live	Sick/Dead
Richland	8	8	7	1	4	0
Rock	6	7	4	2	1	2
Rusk	5	5	7	0	4	0
St. Croix	5	6	3	0	0	0
Sauk	3	6	0	0	0	0
Sawyer	5	5	1	0	1	0
Shawano	7	14	47	0	28	0
Sheboygan	5	14	120	26	6	25
Taylor	5	8	29	0	14	0
Trempealeau	5	16	50	0	5	0
Vernon	5	10	9	1	0	1
Vilas	5	20	22	0	0	0
Walworth	7	7	8	0	7	0
Washburn	5	10	1	0	0	0
Washington	5	5	1	12	0	8
Waukesha	6	6	1	0	0	0
Waupaca	5	10	1	0	0	0
Waushara	5	11	40	0	10	0
Winnebago	5	6	88	26	40	17
Wood	6	8	36	3	26	2
TOTAL	378	625	4,086	958	582	462

* Does not include trial runs.

TABLE 10. Survey stations evidencing mortality of five or more leopard frogs.

County	Station Name	Station Location	No. Observed Sick/Dead	Predominant Location Observed
Burnett	Grettum Flowage	T37R19S11	28	Ice, Lakebed
Calumet	Daul Farm Pond	T19R19S33	25	Shore
	Killsnake River	T19R19S21	7	Streambed
	Round Lake	T19R20S1	107	Shore
	Spring Creek	T20R20S15	11	Streambed
	S. Br. Manitowoc	T18R20S16	103	Streambed
	River	T18R20S13	58	---
Columbia	Crawfish River	T11R12S9	5	Streambed
Dodge	Burnett Ditch	T12R15S14	38	Streambed
	Mieske Ditch	T12R15S23	270	Streambed
	Crawfish River	T10R13S22, 23	8	Streambed
Door	Ahnapee River	T26R25S29	29	Streambed
Dunn	Muddy Creek	T28R12S13	9	Streambed
Fond du Lac	E. Br. Milwaukee			
	River	T13R19S35	47	Streambed
	Inlet Rush Lake	T16R14S3	5	Streambed
Green	Sugar River	T4R8S23	10	Streambed
Green Lake	Grand River	T14R12S13, 14	38	Streambed
	Town Line Ditch	T17R12S5	26	Streambed
Kewaunee	Ahnapee River	T25R25S9, 16	11	Streambed
Manitowoc	Branch River	T20R22S21	12	Streambed
	Manitowoc River	T19R21S33, 34	12	Streambed
Ozaukee	Milwaukee River	T12R21S27	45	Streambed
Polk	Prose Pond	T35R17S8	18	Pondbed
Portage	Wisconsin River	T25R7S10	10	Ice
Sheboygan	Sheboygan River	T16R22S7	26	Streambed
Washington	Rock River	T12R18S32	11	Shore
Winnebago	Waukau Creek	T17R18S13	25	Streambed

TABLE 11. Survey stations evidencing low level mortality (1-4 frogs/station).

County	Station	No. Frogs Observed Live	Sick/Dead
Marinette	Little River Coho Pond		1
Florence	Fisher Lake		1
Lincoln	Echo Lake		2
Marathon	McGinnis Creek		3
Polk	Icehouse Lake		3
Bayfield	Pike Lake Chain		4
Bayfield	Fish Creek		1
Douglas	Brule River		2
Douglas	St. Croix River		1
Ashland	Bad River		1
Iron	Gile Flowage		1
Price	N. Fk. Flambeau River		1
Washburn	Long Lake		1
Buffalo	Delta Fish and Fur Farm (Miss. R.)		2
Chippewa	Chapman Lake		1
Eau Claire	Rodells Pond		1
Wood	Yellow River		3
Marquette	Ennis Lake		1
Kewaunee	East Alaska Lake		2
Door	Strawberry Creek		1
Winnebago	Fountain Pond		1
Washington	Cedar Creek		1
Rock	Gibbs Lake		2
Green	Beckman Lake		1
Green	Tipperary Creek		2
Green	Little Sugar River		3
La Crosse	Black River		1
Vernon	Trib. Coon Creek		1
Crawford	Rush Creek Spring Pond		1
Columbia	Tarrant Lake		1
Jefferson	Rome Pond		2
Dane	Dunlap Creek		1
Dane	Mt. Vernon Creek		2
Grant	Fennimore Fk.		1
Grant	Blue River		1
Fond du Lac	Private Pond		2
Fond du Lac	Long Lake		1
Fond du Lac	Private Pond		2
Calumet	Spring Pond		2
TOTAL COUNTIES 31	TOTAL SITES 39	TOTAL:	61

FROG MORTALITY
A Brief Summary of Evidence

DECLINE

General

Dr. George W. Nace (Director of Amphibian Facility, University of Michigan, Ann Arbor) -- Estimated that during past 10 years, U.S. population of frogs has dropped 50%. (Modern Medicine; Gibbs et al. 1971)

Dr. Erich Gibbs (Director, Research and Development, Ultrascience, Inc., Evanston, Ill.) -- Not uncommon for 50-90% of frogs to die within few days or weeks of delivery. (Modern Medicine)

Medical investigators: Some having a hard time getting enough frogs (especially gravid ones; many dying before they can be used; "sickly" -- from a septicemia from overwhelming infection with *Aeromonas hydrophila*. (Modern Medicine)

Supply houses and research labs:

NASCO (Wis.-based) -- Until 5 years ago collected and shipped 30 plus tons (ca. one million animals) in average year. In 1973, collected only 5 tons. Frogs from Minnesota and S.D. have appeared to be in "ill-health" and have not been hibernating properly. Less resistant to bacterial infection. (Modern Medicine)

Dahl Scientific (Calif.) -- definite decrease in frog catch in last 3 years. (Belcher 1974)

Amphibian Research Facility, Univ. of Michigan -- Dr. Christina Richard: Feels there is a chemical imbalance. Quantity of hormones required to induce ovulation has been steadily increasing over past 10 years. (Pers. Comm. with Vogt)

Three major suppliers, according to Dr. Nace, lost nearly 90% of their stock; many others lost ca. one-half of supply. (Modern Medicine)

California

General agreement that there is a distinct decline in frog populations of Calif. Fish and Game Commission has banned indefinitely all kinds of frog hunting in 4 cos. (Belcher 1974).

Minnesota

Dr. John Moyle, (Minn. DNR) -- Populations of frogs are down in Minn., as much as 90% below the past years. (Modern Medicine)

Dr. Robert McKinnell, (Prof. Zoology, Univ. of Minn.) -- observed a "marked decline in the quality of life among frogs" in past 2 years. (Modern Medicine) One frog pond visited for past 7 years: saw large number of dead frog eggs (first time he observed this in nature).

Gib Hedstrom (veteran (55 years) frog collector from Alexandria, Minn.) -- Finding fewer leopard frogs in past 3-4 years; "sickly" also. 5000-6000 lb. 4 years ago for every 1000 lb. last year. Many had leathery skin and rigid belly. (Modern Medicine)

Otter Tail Co. -- Found masses of frogs, in areas where none found for 20 years, last spring; collected 500; 3 weeks later, collected only 15 frogs; all others had died. (McKinnell, Modern Medicine)

Michigan

Daniel Rittschof (Zoology Grad. Student, Univ. of Mich.) -- conducted population study of Rana pipiens. Observed 8000 animals in one year; the next, only 50 frogs returned to the lake (possible die-off in summer since they didn't return to hibernate). (Pers. Comm. with Vogt)

Jim Collins (Grad. Student, Univ. of Mich.) -- Observed drastic reduction in Rana pipiens; appears to be replaced by Rana clamitans. No mass die-offs in fall noticed.

Wisconsin

Amphibian Research Facility (Ann Arbor, Mich.) -- 150-200 adult female frogs collected from different areas in Wisconsin did not have eggs this fall, for the first time. (Pers. Comm. with Vogt)

Collectors:

Robert Robel (Hoffman Biological Supply House, Oshkosh) -- Die-offs noticeable in 1958; length of time frogs could be held in tanks steadily decreased. (Vogt 1974)

Currently -- frog mortality occurring in many areas in late fall. (Field inspection by Robel, Vern Hacker and Vogt). (Hacker, 1973; Vogt 1974)

Fish Management Survey (1973) -- 35 replies received to questionnaire; over half reported a decrease or observed mortality of frogs.

Manitoba

"Although Manitoba has had severe winter kills in previous years which involved large numbers of frogs, 1974 was the first time we had reports of unexplainable deaths on a large scale. I did not personally see any of the animals but reports received in Winnipeg spoke of dead frogs piled several inches deep along a lake shore, sometimes a reddish colouration on the ventral surface of the body and hind legs and, in living specimens, edema. Examination of several dead specimens by the provincial veterinary laboratory revealed no pathogenic bacteria or abnormal tissues. Although the frogs were not tested for any pesticide residues, samples taken from this area two years ago revealed no significant levels. The area supports little agriculture and no industry; it appears unlikely that direct chemical contamination could be involved in the present die-off." -- Carol Scott, Dept. Mines, Resources and Environ. Mgt. Winnipeg.

REASONS FOR DECLINE

Gibbs, Nace and Emmons (1971) postulate the following reasons for the 50% decline in frog populations in the U.S. during the past 10 years:

Insecticides, fertilizers, chemical or biological wastes (disastrous effects of these shown by Saunders, 1970)
Man's impacts -- causing loss of habitat
Overcollection
Weather

Dr. Erich Gibbs speculates that Aeromonas hydrophila is a "shipping disease" (Gibbs 1973).

Why is "redleg" infection (since the organism is ubiquitous in nature) gaining such an ascendency? (Modern Medicine)

Dr. John Moyle (Minnesota) -- Blames trouble on habitat loss, "redleg" -- not chemicals. (Modern Medicine)

Dr. McKinnell (Minnesota) -- Believes environmental insults are lowering frog's resistance to bacteria. Reasonable first guess: insecticides and herbicides (loss of habitat alone doesn't cause a frog to get sick). (Modern Medicine) Also, if mortality due to "shipping disease" and stress of capture, etc., why did frogs thrive in the past after shipping to labs?

Robert Treanor (Calif.) -- Preliminary report (Feb. 1973) -- habitat of bullfrogs diminished substantially; also becoming more scarce in remaining habitat. Suspicion exists that pesticide use in adjacent croplands may be involved. $30,000 study authorized on bullfrog. (Belcher 1974)

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About the Authors

Ruth L. Hine, Bureau of Research, coordinated the study and compiled the progress report from parts prepared by all investigators; Betty L. Les, Bureau of Fish and Wildlife Management, initiated and supervised the fall mortality survey; Bruce F. Hellmich, Bureau of Research, carried out the field investigations and collected and examined the specimens taken during the fall mortality survey; Richard C. Vogt, Bureau of Research, gave technical advice and assistance throughout, and supervised the field studies and the specimen collections.

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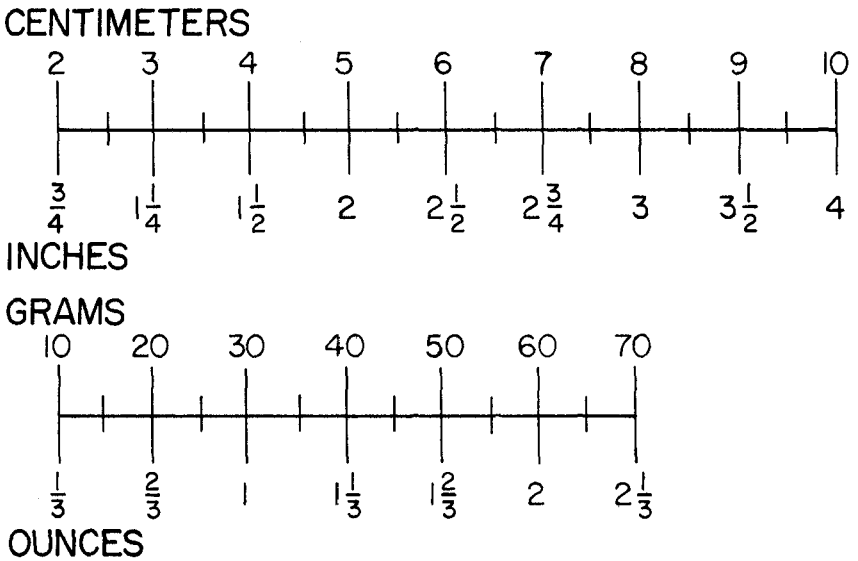
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