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LEOPARD FROG POPULATIONS AND MORTALITY IN WISCONSIN

ABSTRACT

In response to reported declines in numbers and physical condition of leopard frogs *(Rana pipiens)* in Wisconsin, a study was initiated to acquire basic knowledge of leopard frog ecology through statewide and intrastate regional surveys of breeding populations and mortality from 1974-76. The statewide breeding surveys generally confirmed reports of a severe depression in the populations of leopard frogs. Of 301 sites in Wisconsin that appeared to provide good habitat, only 15 (5%) were occupied by leopard frogs in both 1974 and 1975. In a 7-county study area in East Central Wisconsin, only 30% of 83 ponds with suitable leopard frog habitat showed any leopard frog activity during 1975 and 1976.

Only 6% (in 1975) and 23% (in 1976) of the suitable ponds showed evidence of breeding activity. However, because of a severe summer drought in 1976, only 21% of ponds with breeding activity produced young in that year, while 60% of those with breeding activity produced young in 1975.

Breeding phenology showed consistent patterns in 1975 and 1976, although time of onset of mating varied, apparently requiring air and water temperatures of 10 C. Metamorphosis date varied by only about 5 days in 5 ponds despite much greater differences than this in the 2 years in breeding onset dates. Population estimates for breeding ponds in which leopard frogs were captured (4 ponds in 1975; 6 in 1976) ranged from 8 to 20 in 1975 and from 2 to 76 in 1976.

A call index was devised to estimate leopard frog populations. A comparison of its use with mark/recapture estimates showed that the call index was a valid measure of the presence of breeding frogs, but served as only a rough estimate of population size.

Sex ratio (believed to be 50:50 based on fall data), size and condition, sexual maturity (indicated by a length of 60 mm in females), egg development and movement were recorded for frogs captured in spring. Movements tended to be short and confined right after breeding to the immediate vicinity of the breeding pond. Postbreeding activity gave evidence of movement from the breeding pond for foraging and subsequent return to the home pond. The maximum movement was 401 m in 5 days.

Survival rates of newly metamorphosed young ranged from 1% to 6% in 5 breeding ponds. Sexual maturity data and postemergence movements were recorded for young frogs. No spring mortality was observed in the breeding ponds.

Statewide surveys in the fall along standard 0.5-mile transects revealed mortality ranging from 4% to 23% of the live frogs observed for the 3 years of the survey, although it was as high as 100% on some transects. The east central study area had a high proportion of Wisconsin's live frogs and a high mortality rate in both 1974 and 1975.

Mortality began at a low level soon after hibernating congregations formed in the fall, and increased to a peak just before freeze-up when frogs were entering the water for hibernation. Sick or dead frogs showed ventral skin discoloration, dry skin, and hard muscles. No blood parasites were found, nor was evidence of virus. Bacteriological examination revealed pathological symptoms in 39% (1975) to 86% (1974) of the frogs autopsied. Degenerative liver changes were discovered, some of them suggestive of ingestion or absorption of a toxic substance. Toxicity tests showed that the herbicide Atrazine retarded growth and lowered survival of tadpoles.

Monitoring of spring breeding populations and fall mortality should be continued on a more concentrated and intensive level to more accurately pinpoint and characterize both breeding areas and location and extent of mortality.

The decline in the abundance and health of the leopard frog in Wisconsin can be an indicator of stress in the ecological system that should not be ignored.

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INTRODUCTION

A decline of northern leopard frogs *(Rana pipiens)* has been documented in several states throughout the country. During the 1960's, the United States population was estimated to have dropped 50% (Gibbs et al. 1971). There are many accounts of marked decreases in frog populations, large numbers of dead frogs and eggs observed in the wild, declines in physical condition, and failures in efforts to keep frogs over winter in holding tanks of biological supply houses (Modern Medicine 1973). Declines in *Rana temporaria* in England have also been reported (Cooke and Ferguson 1976).

During the 1960's in Wisconsin, biological supply houses first noted a decreased number of frogs collected, and a reduction in the length of time frogs could be held alive. Scattered outbreaks of frog mortality were noticed in the field by frog collectors and in the early 1970's hundreds of dead and dying frogs were reported in several areas of the state. The Amphibian Facility in Ann Arbor, Michigan, reported that between 150 and 200 adult female frogs collected throughout Wisconsin in the fall of 1973 did not have pigmented eggs, the first time this situation had occurred (Richards 1973 pers. comm.). In preliminary investigations of the scattered outbreaks of mortality, many frogs had symptoms of advanced stages of redleg, a disease caused by several agents, among them the bacterium, *Aeromonas hydrophila,* which attacks other aquatic species such as the northern pike *(Esox lucius)*.

The leopard frog plays a valuable role in the natural food chain of countless mammals, birds, reptiles, and fish, in helping to control insect pests and aquatic detritus, and as an experimental animal in the biological and medical laboratory. The ability of tadpoles to reduce populations of several species of blue-green algae which are often considered unavailable as food for other vertebrates has been demonstrated by Seale (1980). Tadpoles are also significant eutrophic indicators.

Although the values of leopard frogs are recognized, and the species has been widely used as a laboratory animal, information on life history and ecology has been scanty and some of it only recently published. In the 1960's, Dole (1965*a* and *b,* 1967, 1968, 1971) carried out movement studies of *Rana pipiens* in northern Michigan; Merrell (1965, 1968, 1970) studied reproductive behavior and gene dispersal in Minnesota and in 1977 published a synthesis of his life history and ecological observations (Merrell 1977); Pace (1972) made systematic and biological studies of the *Rana pipiens* complex; and Rittschof (1975) completed an investigation of the natural history and ecology of the leopard frog in several ponds in northern Michigan.

In view of the general decline together with the mortality observed in Wisconsin, and the lack of adequate basic knowledge on the ecology of leopard frogs, an exploratory research project was set up in the spring of 1974 to obtain preliminary data on Wisconsin leopard frog populations and mortality. It consisted of a spring survey in selected areas to find ponds for intensive study in which the breeding populations 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 to collect specimens for analysis. A preliminary report on the 1974 work was released the following year (Hine et al. 1975).

While the statewide breeding survey begun in 1974 was repeated in 1975, the main focus of spring and summer studies in 1975 and 1976 was on a 7-county study area in east central Wisconsin. Specific objectives were to refine our knowledge of the types of habitats most likely to be selected as breeding areas; establish the population density of leopard frogs in sample ponds and record various characteristics of the adult population; determine reproductive success; and monitor the emergence and dispersal of the newly metamorphosed young. In the spring of 1978, a survey was run along the established transect in the east central study area to obtain a follow-up index to the leopard frog population. However, no further work was done in 1978.

The fall survey was continued statewide in 1975 and 1976 to determine where mortality was occurring and to identify factors causing it or associated with it.

STATEWIDE POPULATION

"Spot checks" of frog activity were made in selected areas throughout Wisconsin during the spring and summer of 1974-75. This was accomplished by selecting from county maps areas that contained wetlands along major highways. These were located primarily in the south central, west central, and northwestern parts of the state. Stops were made at wetland habitats while driving through these areas. Searches for leopard frogs were then conducted by walking transects through various vegetative communities and disturbing the vegetation. Areas checked in the spring were primarily breeding ponds, and in summer also included feeding areas such as wet pastures and meadows, lakes, slow-moving rivers, and streams. All locations were examined at least once during similar times of the year, both in 1974 and 1975.

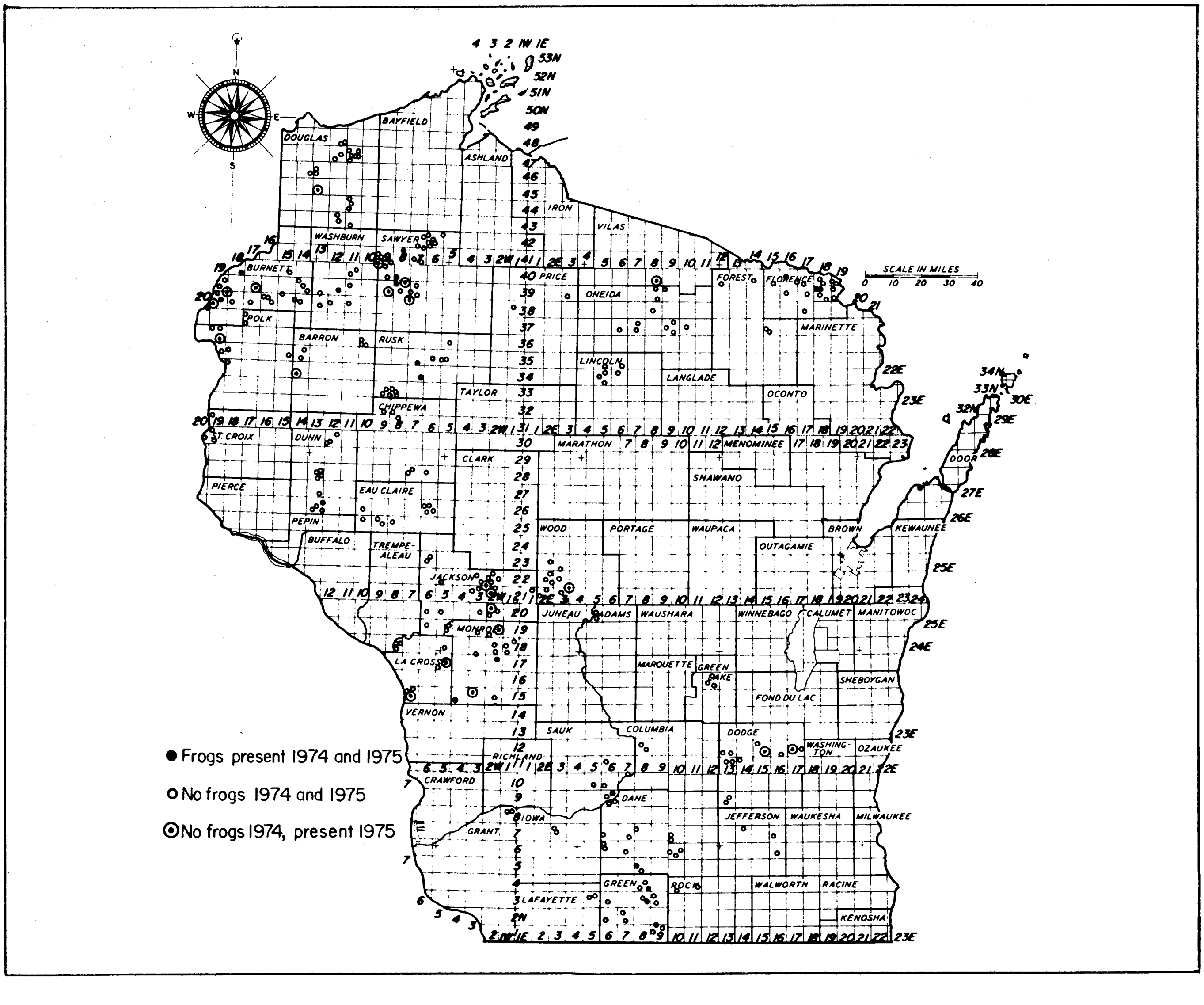
Most searches were made during the day and lasted from 10 minutes to almost 2 hours, depending on the size of the area. Night searches were limited to rather open areas and shorelines.

During the breeding season, wetlands were also checked by listening for calling males. Areas found to contain leopard frogs by hearing calls were listed as having frogs and were not visually searched.

While the whole state was not systematically surveyed, the data collected afford a clue to statewide leopard frog populations. Only 15 (5%) sites with leopard frogs were found both in 1974 and 1975 among 301 sites examined that represented what appeared to be good habitat (Fig. 1). Comparing these observations with evidence that many of the areas without frogs in 1974 and 1975 had breeding populations in prior years (Richard C. Vogt pers. comm.), we conclude that the leopard frog population was severely depressed. We conclude this in spite of the evidence that an increase in the number of areas used by leopard frogs occurred in 1975 (7% more than were recorded used in 1974).

To substantiate these observations and lend substance to the conclusion, a detailed study followed, based in the east central part of the state where leopard frogs had been reported by Vogt and local frog collectors (pers. comm.).

FIGURE 1. *Usage of ponds by leopard frogs in spring and summer, 1974-75, in selected areas of the state.*



EAST CENTRAL STUDY AREA

DESCRIPTION OF AREA

An area was chosen for intensive study in 1975-76 in east central Wisconsin that included Door, Kewaunee, Brown, Manitowoc, Calumet, Fond du Lac, and Sheboygan counties (Fig. 2). That this portion of the state apparently supported the highest leopard frog populations was further substantiated by the relative number of frogs collected during the 1974 fall mortality survey.

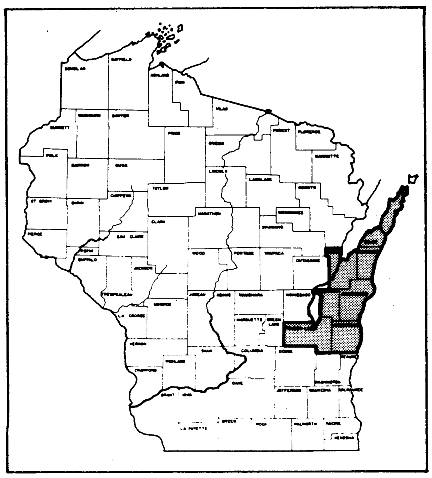


FIGURE 2. *Location of East Central Study Area.*

The topography of these counties is composed of distinct but low rolling hills interspersed with winding meadow-like valleys. The entire area is underlain by Niagara dolomite forming the Niagara Escarpment which gradually slopes eastward. Glacial drift composed of red pebbly clays, gravel, and boulders cover the area in varying thicknesses.

Most lakes and wetlands in the northern section are produced between low knolls separated by winding ravines and shallow depressions left by glacial activity. Precipitation and runoff are the sources to maintain their levels. Farther south, kettle lakes (lake-filled pits produced by melting ice blocks during the retreat of the glacier) exist in greater numbers with 75% of the lakes being of that type. The major drainage systems are divided by the Niagara Escarpment, those on the eastern slope flowing into Lake Michigan and those west, flowing into the Fox and Rock rivers and eventually into the Mississippi River. The systems include the Ahnapee, East and West Twin, Manitowoc, and Rock rivers, their tributaries, and associated springs and wetlands.

Lake Michigan, Green Bay, and Lake Winnebago have a moderating influence on the climate in the area causing less severe temperature transitions than is otherwise normal to central Wisconsin. The effect from the lakes produces a shorter spring due to the slow warming of Lake Michigan and a longer autumn due to its slow cooling. In winter, west and northwest winds limit the lake's influence allowing a normal cold season.

The warmest month is July with an average temperature between 20.5 and 22.7 C (69-73 F). The coldest month is January with an average temperature between -9.4 and -8.3 C (15-17 F). Over half of the annual precipitation falls during the growing season, May through September. Annual snowfall averages from 1 to 1.1 m (39-44 in.) with the greatest fall occurring in January.

The time of ice-out (during April) and freeze-up (about mid-December) varies depending on the location of the pond. Ponds located in Point Beach State Forest (Manitowoc County) thawed much later than ponds located in the open. Shallow ponds and those lowered in level by lack of summer rains froze up much more rapidly than deeper ponds.

The primary use of the land today is for agricultural crops (principally hay, corn, cherries, and oats) and dairying (80-85%); 7-10% is classified as commercial forests; and 5-10% consists of wetlands, rights-of-way, industrial, residential, recreation lands, and water areas.

Wetlands in the east central region are of 4 types. Classification is in accordance with the U.S. Fish and Wildlife Service's classification (Shaw and Fredine 1956): Type 2—inland fresh meadows: soil usually without standing water, but waterlogged; Type 3—inland shallow fresh marshes: soil waterlogged and often covered with as much as 6 in. of water; Type 4—inland deep fresh marshes: soil covered with 6 in. to 3 ft or more of water; and Type 5—inland open fresh water: with water usually less than 10 ft deep and fringed by a border of emergent vegetation.

METHODS

Breeding Pond Selection

Preliminary studies indicated the frog populations were at a very low level, and we decided that random selection of ponds would not yield a large enough sample of ponds with leopard frogs to be of significant value. Thus, the selection of ponds to be used as a sample for intensive study was based on predetermined criteria. Note throughout the following description that only areas of "current" leopard frog activity were selected. Unselected sites might well have been areas of leopard frog activity in the past.

Possible choices of study areas were narrowed to only a few counties in the east central section of the state from information gathered during the 1974 spring and fall surveys, coupled with reports of frog presence from Department of Natural Resources personnel. Topographic maps were used to locate bodies of water and drainage systems that might be suitable as overwintering locations or hibernacula. The factors required for consideration as a hibernaculum were permanence and size, primarily depth. Ponds of a temporary nature or shallow enough to freeze solid were not considered for study.

Ponds within 1.6 km (1 mile) of a possible hibernaculum were initially identified as possible breeding sites. This distance was selected on the basis of the experience of Vogt (pers. comm.) and professional frog collectors (Hacker pers. comm.). Merrell (1977) also noted that the distance between summering and overwintering sites is often 1 or 2 km. Areas located within towns, forests, or industrial zones were eliminated, leaving ponds near streams, wetlands, grasslands including agricultural areas, and forest

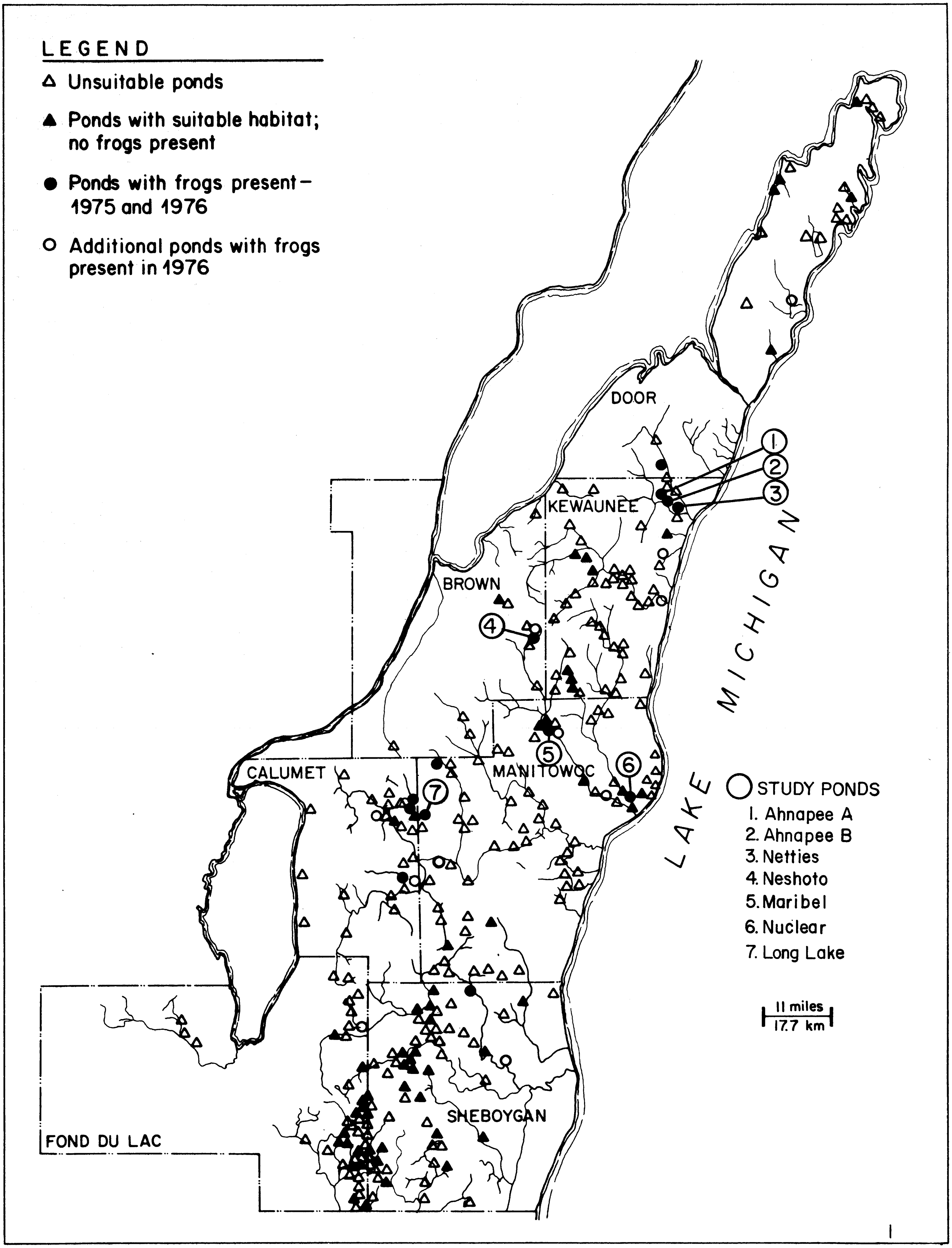


FIGURE 3. *Potential and actual breeding ponds checked in 1975-76 in the East Central Study Area.*

edges as sites for potential investigation.

Before ice-out on lakes and ponds, the potential sites identified on the topographic maps were visited on a field survey. Sites currently lacking ponds or containing only marginally suitable ponds, and ponds which were too inaccessible or too close to sources of pollution, such as barnyards or heavily salted highways, were eliminated as possible study areas.

Shortly after ice-out and before frogs came out of hibernation, ponds were revisited and evaluated on the basis of the following characteristics (those not meeting 4 out of the 5 were eliminated):

(1) Free standing water was at least 1 m in depth, which under normal conditions should yield water available for tadpoles until mid-August. Pond vegetation was used as an indicator of water permanence. Most important was a successional gradient from dry to wet associations, commonly indicated by: alder, willow, cattail, sedge, arrowhead, pond lily, and coontail. Information on water conditions could also be obtained by consulting local persons knowledgeable of the pond's conditions in previous years.
(2) Vegetation surrounding pond consisted of 50% or more of meadow-like grasses, sedges, or emergent aquatic vegetation.
(3) At least 25% of pond surface area was free of emergent vegetation and could be expected to be open through May.
(4) There was less than 50% forest cover on land area extending 300 m or more out from the pond.
(5) The pond was free from any direct source of pollution.

These requirements were formulated on the basis of information gained through contacts with persons experienced in amphibian requirements, and from observations made on breeding ponds during the initial stages of this study.

The presence of leopard frogs was the final determinant in selecting ponds for intensive study. Direct visual and aural searches for frogs in the potential study site areas and at other sites where others reported leopard frog activity were begun after ice-out and continued until the onset of breeding activity. If leopard frogs or breeding activity were not observed by that time, the site was no longer visited frequently.

Searching for leopard frogs consisted of walking through the pond and along its perimeter following overlapping transects which systematically covered the entire area out to 50 m from the pond's edge. Although arbitrary this distance was chosen to apply a standard to all searches. A dip net was used to disturb the vegetation and to probe into crevices to displace frogs which might otherwise have gone unnoticed. These searches were made during late morning and early afternoon in early spring before leafing out of vegetation had occurred. Frogs were identified to species and adult leopard frogs were marked whenever captured. All ponds were searched in this way twice and some 3 times until the onset of breeding.

In addition to visual searches, calling frogs were located and recorded. Male leopard frogs, whose calls can be easily distinguished from those of other species in the area, often begin calling 1-2 weeks in advance of mating, making it a simple matter to locate breeding ponds before mating begins. Merely listening for the call of a male leopard frog during a 3- to 5-minute stop at a pond at a time when water and air temperatures are 10 C or above can identify a breeding pond. Because the period required to locate and identify these calls is so short, numerous ponds over a wide area could be and were checked in 24-hour search excursions. Ponds were visited an average of 4 times before they were abandoned as having no calling males.

Visits to locate frogs either visually or aurally were made under various weather conditions and light intensities; visual searches at night were made while wearing a head lamp.

In an initial screening in 1975, 83 ponds out of 279 examined were selected as meeting 4 out of the 5 criteria listed above. These 83 ponds constituted the reference study sites, and were surveyed for the presence of breeding frogs in 1975, 1976, and 1978. Of these, 7 were studied intensively throughout the breeding, summering, and fall migration seasons in the first 2 years: Ahnapee A and B, Netties, Maribel, Long Lake, Neshoto, and Nuclear. None of these ponds, with the exception of Long Lake, are officially mapped or named but are designated on Figure 3.

Pond Mapping

The ponds selected for intensive study were mapped to record their individual habitat characteristics. Their general shapes and relations to surrounding features were obtained from topographic maps and aerial photographs. Details were filled in on site from compass bearings and measurements. Ponds too small to appear on aerial photos were mapped from compass bearings and measurements taken from 2 or more positions along the pond.

Environmental Conditions

Environmental conditions (temperature, wind speed, relative humidity, and percent cloud cover) were recorded each time observations were made. Air temperature was taken at 1 m above the ground and water temperature at 5-cm depths. Additional water temperatures were taken wherever a frog was captured or at 5 cm below the water surface.

Population Size and Density

Mark and Recapture Estimates

The mark/recapture technique was used throughout this study on all age classes of frogs to obtain data on population levels. Adults were captured in a 5- to 8-day period during the peak of calling and the beginning of egg laying. Young were caught within the first 2 weeks after metamorphosis. Population size was calculated using the Schnabel method (Smith 1966:653), a method which allows for recaptures to be counted through time rather than on a one-time basis:

$$\frac{\left[\text{Number trapped} \times \text{Marked animals in area}\right]}{\text{Sum of recaptures}} = \text{Estimated Population}$$

The population estimate was adjusted according to the formula described by Merrell (1968) to compensate for fewer females caught in proportion to those present:

$$\frac{N_m + N_f}{2N_m} = \frac{E}{N_a}$$

where N_m = number of males in samples, N_f = number of females in samples, E = Lincoln-Peterson (here Schnabel) estimate, and N_a = adjusted estimate. The total number marked and recaptured for 1975 and 1976 is shown in Table 1.

Searches for frogs were performed at various times during the day and night. Captures in drier habitats were made during the daytime, either by hand, or with a 30 mm mesh dip net 51 cm in diameter. Dole (1965*b*) was

TABLE 1. *Number of leopard frogs marked and recaptured during 1975-76.*

	1975				1976			
	Male		Female		Male		Female	
Ponds	Marked	Recaptured	Marked	Recaptured	Marked	Recaptured	Marked	Recaptured
ADULTS								
Ahnapee A & B	4	14	1	2	6	26	1	1
Netties	4	11	1	0	8	20	1	0
Maribel	0	0	0	0	0	0	0	0
Long Lake	14	17	3	1	21	13	5	2
Neshoto	6	12	0	0	10	17	2	0
Nuclear	0	0	0	0	13	5	4	0
Total	28	54	5	3	58	81	13	3
YOUNG	Marked		Recaptured		Marked		Recaptured	
Ahnapee A & B	230		48		40		59	
Netties	17		15		24		23	
Long Lake	98		44		188		68	
Total	345		107		252		150	

4 times more successful in obtaining frogs in upland habitats during the daylight than at night.

Surveys of ponds and other water bodies, however, were more successful at night. Night forays involved the use of a head lamp, with captures made by hand. In darkness frogs are blinded in a beam of light allowing the investigator enough time to move through the water and deep muck to capture the frog.

All ponds were systematically searched until the entire area was covered. Captured frogs were placed in individual plastic mesh bags to prevent escape and were examined for external abnormalities, weighed on a spring balance, measured snout to vent using a plastic ruler with the frog flat in the palm of the hand, and marked by toe-clipping. Mature males were identified by the presence of a swollen and pigmented thumb pad. Frogs without a developed thumb pad were listed as females or immatures.

Toe-clipping was performed in a manner similar to that described by Martof (1953), with no more than 2 toes clipped on any 1 foot. Toes were clipped in the center of a digit to help distinguish clipped frogs from those accidentally losing toes. Little bleeding resulted from clipped toes and no regeneration was evident in the course of this study.

Dole (1965*b*) also used toe-clipping and observed that the procedure did not seem to influence either the activities or survival of the marked frogs.

Call Index

Data on frog calling were gathered to determine the feasibility of using a call index as a measure of adult male population density. Several approaches were tested: different lengths of listening periods, a variety of listening positions around the pond, and different listening times during a 24-hour period. All estimates of numbers of males calling were verified by mark/recapture estimates.

Tests of these approaches resulted in the selection of the following procedure which was applied at any time during a 24-hour period. The call index was calculated from data collected in six steps as described here:

(1) After a position was taken at the pond edge, 5 minutes were allowed for the frogs to resume calling.
(2) The calling rate for the pond was then determined. This was done by counting all the calls made by a single frog during a 5-minute period, and dividing by 5 to obtain the number of calls per minute characteristic of the frogs in that pond under the prevailing conditions.
(3) All the calls heard during a subsequent 3-minute period were counted.
(4) Steps (1) and (3) were repeated from a different position at least 10 m from the 1st position.
(5) The results from steps (3) and (4) were added, and the sum divided by 6 to obtain the number of calls heard per minute from all calling males.
(6) The quotient obtained in step (5) was divided by the number of calls per minute made by the single frog, step (2), to obtain the number of calling males in the pond.

Egg Mass Counts

Egg masses were counted to obtain additional information on population size and also as a measure of breeding success. It was possible to count all of them because of the small size of the test ponds. Searches were made in the warmest part of the pond on calm, sunny days, if possible. On a 1st pass the area was scanned for egg masses around emergent vegetation 5-10 cm below the water surface. The globular, glistening egg masses looked like slightly crinkled plastic wrap against the dark pond bottom. A second pass, after the sediment settled, was made farther along the edge or farther out into the pond.

The dimensions of each egg mass were measured, and the vegetation to which it was attached and its position in the pond were recorded. To prevent recounting all masses were marked with 15-cm lengths of red marking tape tied to nearby vegetation or to a stake.

Weighing and measuring leopard frogs.

Note enlarged thumb pad of male on right (female on left).

BREEDING POPULATION DYNAMICS

Breeding Ponds

Location and Distribution. Within the east central section of the state, 279 ponds or still water situations were identified as possible breeding ponds before field examination in 1975 (Fig. 3). These possible breeding sites were all within 1.6 km of major bodies of water which could be utilized by the frogs as hibernacula. The 279 ponds were investigated after the spring thaw and before the frogs arrived, and 83 were judged suitable as leopard frog breeding ponds, meeting at least 4 of the 5 criteria against which all ponds were analyzed for suitability (Table 2).

TABLE 2. *Spring transect results on 279 potential leopard frog ponds.*

County	Ponds With Unsuitable Habitat*	Ponds With Suitable Habitat*	Ponds With Frogs Present		
			1975	1976	1978**
Door	14	7	1	2	3
Brown	8	3	1	2	–
Kewaunee	38	12	3	4	3
Calumet	19	7	3	5	4
Manitowoc	55	15	4	7	6
Fond du Lac	30	17	0	1	4
Sheboygan	32	22	1	2	3
Total	196	83	13 (16%)	23 (28%)	23 (32%)

*Suitability based on passing 4 of 5 criteria outlined in the text.
**11 ponds were not checked thus making 72 ponds the base for 1978.

All 83 ponds were checked for frog activity during the normal breeding season (April-May) and again during the emergence of the young (July-August). The second check during July also insured that no ponds had been missed during the breeding period. Ponds with frogs present ranged from 16% in 1975 to 32% in 1978. At least 1 pond in which breeding activity had not been observed was found to produce young. Such ponds were subsequently classed as successfully producing young of the year.

Of the 83 ponds judged suitable for breeding use by leopard frogs, only 5 (6%) in 1975 and 19 (23%) in 1976 had signs of breeding activity.

Size and Characteristics. The 5 ponds in which leopard frogs were breeding in the spring of 1975 were chosen as intensive study areas for 1975 and 1976. Two other ponds, Ahnapee B and the Nuclear Pond, were added in the summer of 1975 because of Pond B's close proximity to Ahnapee A and its use by young frogs, and the Nuclear Pond's large hatch, which provided information on the young of the year. The major characteristics of these areas are summarized in Table 3, and cover types are shown in Figure 4. Generally the ponds were in Type 4 wetlands, inland deep fresh marshes, and Type 5, inland open fresh water (Shaw and Fredine 1956).

The ponds provided a variety of conditions of water availability as the summer progressed. Maribel and Neshoto were plagued both years by early dry-up before any tadpoles could metamorphose, even though reportedly they "always had water" in the past and met the criteria used to judge ponds for suitability in the spring. Netties, Long Lake, and the Ahnapee ponds had relatively stable water levels throughout both summers. At Ahnapee the opportunity to observe the effects of man-made disturbances presented itself when a farm pond was dredged nearby and the dredged material was dumped along one side of Pond A. Vegetation along the perimeter of the pond was destroyed by this action and siltation became a problem as rain eroded material from the dredged spill into the pond, degrading it severely.

The Nuclear Pond was located immediately adjacent to Lake Michigan, and during storms cold lake water spilled into the pond cooling it by as much as several degrees. Because of the filling by Lake Michigan, it remained very stable in depth.





Habitat characteristics of leopard frog breeding ponds (clockwise): Ahnapee A, Ahnapee B, Long Lake, Netties, and Maribel.

Sympatric Anuran Species. Other frog species collected in and near the study ponds and their relative abundance were: *Hyla crucifer crucifer,* northern spring peeper (abundant); *Hyla versicolor* and *Hyla chrysoscelis,* eastern gray and Cope's gray treefrog (common); *Pseudacris triseriata triseriata,* western chorus frog (abundant); *Rana clamitans melanota,* green frog (common in 1974; abundant in 1975-76); *Rana palustris,* pickerel frog (rare); and *Rana sylvatica,* wood frog (uncommon).

Portrait of an "Ideal" Breeding Pond. Merrell (1968) described a typical breeding pond for leopard frogs in Minnesota as a temporary pond that does not support any fish population, is not connected with any other body of water, dries up periodically in time of

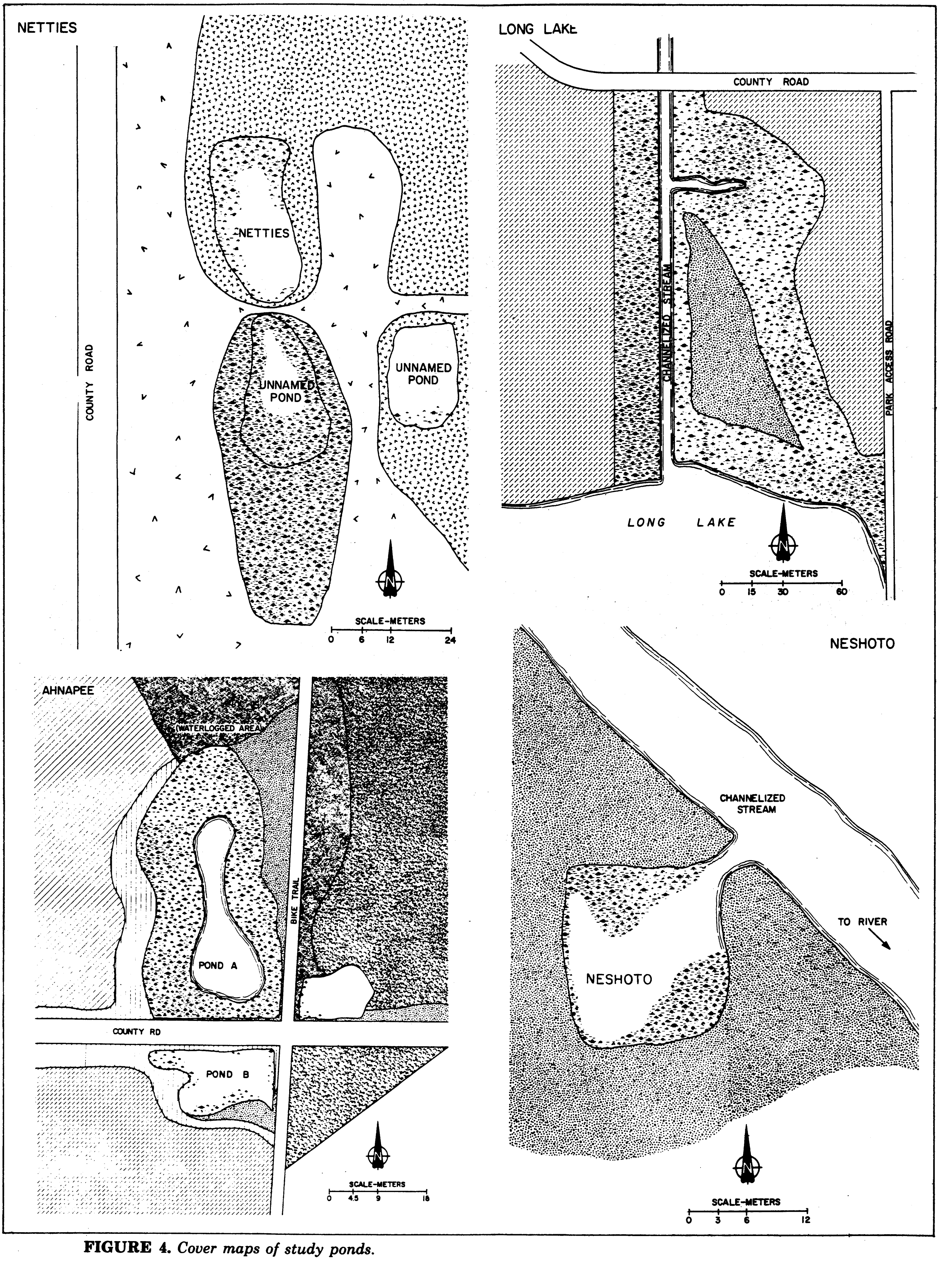


FIGURE 4. *Cover maps of study ponds.*

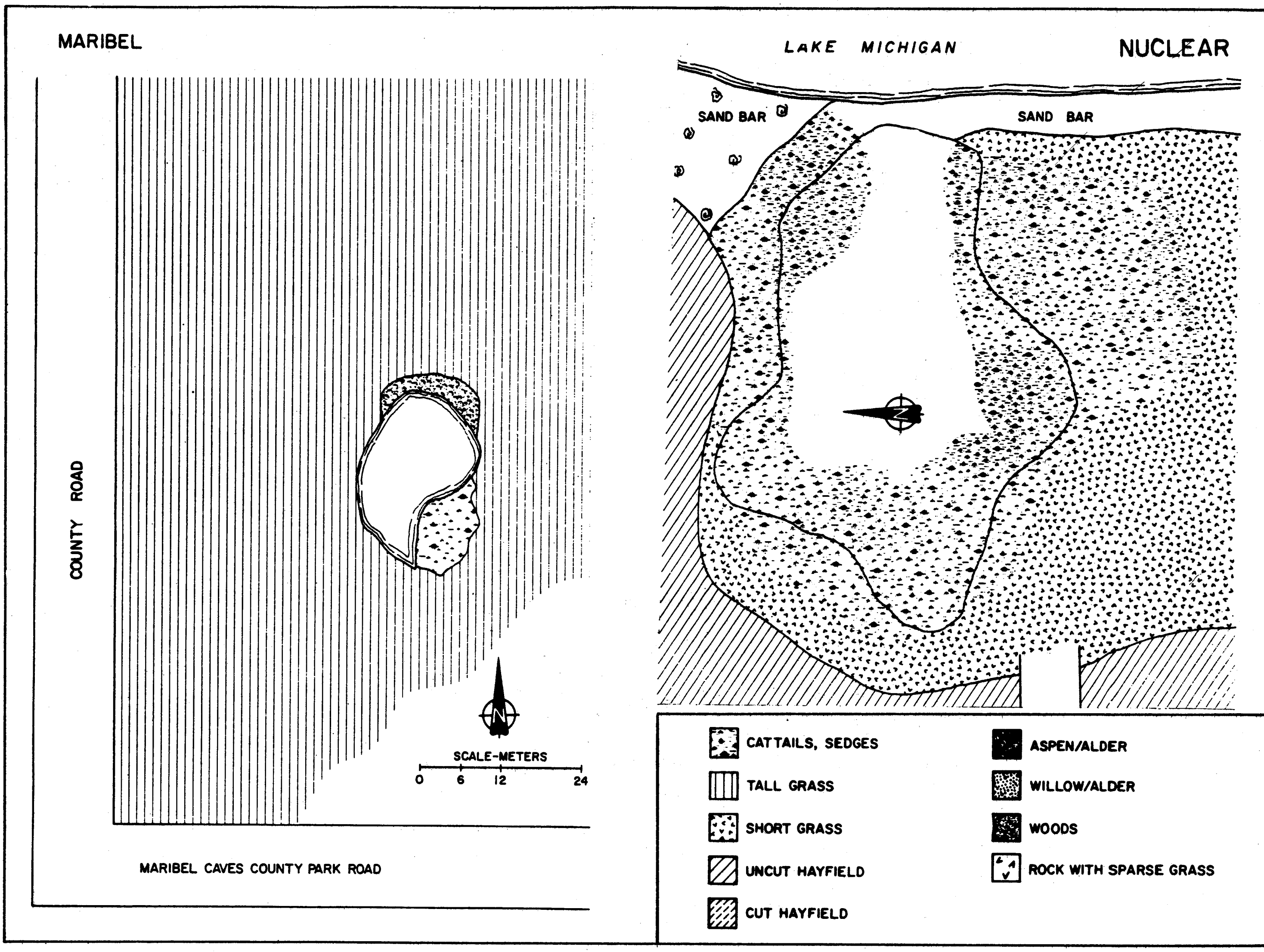


TABLE 3. *Characteristics of ponds selected as intensive study areas.**

	Type water	Surface Area (ha)	Depth (m)	Flow	Bottom substrate	Percent Surface Area Covered by Submergent Vegetation	Percent Surface Area Covered by Emergent Vegetation	Perimeter Vegetation	Drainage Area
Ahnapee Pond A Kewaunee Co.	Pond	0.32	Max. 2.4 Min. 1.5	ground water and runoff	silt and muck	60	50	30% willow 20% cattails 50% grass sp.	50% Hay 30% Cedar 10% Orchard 10% roadway
Ahnapee Pond B Kewaunee Co.	Pond	0.16	Max. 2.4 Min. 0.5	ground water and runoff	silt and muck	80	80	40% willow 60% grass sp.	70% Hay 20% Cedar 10% roadway
Netties Kewaunee Co.	abandoned gravel pit	0.14	1.2	ground water	gravel overlain with decayed veg.	60	80	90% cattails 10% gravel	50% grass 40% gravel 10% roadway
Maribel Manitowoc Co.	Pond	0.04	Max. 1.2 Min. dry	runoff	Muck	10	30	25% willow 30% cattails 45% grass sp.	90% hay 10% roadway
Long Lake Manitowoc Co.	Dead end channel off lake	0.02	Max. 3 Min. 2	runoff	silt and decaying veg.	30	80	90% cattails 10% willow	90% hay 10% road
Neshoto Brown Co.	Shale pit	0.04	Max. 1 Min. dry	runoff	shale overlain with decayed veg.	None	75	40% alder 40% willow 20% dogwood	90% shale 10% grass
Nuclear Manitowoc Co.	Pond	1.52	Max. 3 Min. 2	runoff including Lake Michigan overflow	silt and muck	70	80	80% cattails 15% willow 5% sand	90% oatfield 5% grass 5% roadway

*In spring at the beginning of the calling period.

TABLE 4. *Summary of breeding pond activity.*

	1975		1976	
	No.	Percent	No.	Percent
Suitable breeding ponds (without frogs)	83	–	83	–
Ponds with frogs	13	16	23	28
Ponds with breeding activity	5	6	19	23
Ponds with young of the year	3	4	4	6
Percent ponds with breeding activity producing young		60		21

drought, and has a maximum depth of 1.5-2 m.

Upon combining all the data gathered on characteristics of the ponds used by leopard frogs for breeding in the east central study area, we suggest the following characteristics for an "ideal pond". These represent refinement, based on 2 years of field study, of the original criteria.

(1) Distance from hibernaculum: Ponds within 1.6 km from a hibernaculum are more likely to be located by adults seeking breeding ponds in the spring; conversely in the fall, young frogs leaving their juvenile ranges are more likely to find a suitable hibernaculum. This distance was originally selected on the basis of Merrell's (1968) observations and on the experience of Wisconsin frog collectors, and is supported by our studies.

(2) Depth in spring: Ponds 1.5 m or more in depth generally have a better balance of open water to vegetation cover. Depth appeared to be more important than area of pond.

(3) Littoral vegetation: Emergent vegetation (e.g., cattail, sedge, arrowhead) in May on approximately 2/3 of the circumference of a pond provides cover for escape from predators and for attachment of egg masses. Submergent vegetation (e.g., coontail) on 50% of the surface area also provides cover, substrate for egg mass attachment, and a source of food for the tadpoles. Clean, clear recreation ponds have little value to a frog.

(4) Bottom contour: A gradual slope provides a greater area of emergent vegetation which in turn provides more cover.

(5) Pond exposure: Open water exposed to the direct rays of the sun through most of the day aids in the warming of the pond. Ponds or areas of ponds that warm first usually have the most breeding activity.

(6) Surrounding land: Inland fresh meadows (Type 2 wetlands) or inland fresh shallow marshes (Type 3 wetland), unmowed pastures, and hayfield are preferred. Land around the pond is important as summer habitat for adults after breeding, for juvenile growth, and later on as a highway for young frogs during their fall migration. Grassy meadow areas with a high moisture content provide ideal cover and feeding habitat. A field under cultivation does not.

(7) Permanence of water: Standing water in the pond most of the time throughout the year is necessary to maintain the desired balance of vegetative cover. However, it is necessary for the pond to dry up every decade or so to eliminate any fish population that may have become established. Predation by fish can substantially reduce a frog population.

Breeding Activity

Summary of Pond Activity. Of the 83 ponds judged suitable for breeding use in 1975, 13 had leopard frogs in the spring. Five of these had calling frogs, egg masses, and tadpoles. Of the 5 active breeding ponds, 2 dried up by midsummer, leaving only 3 ponds which produced young (Table 4).

In 1976, 23 of the 83 suitable ponds had frogs present in early spring; 19 of these had breeding activity, as evidenced by calling frogs, egg masses, and tadpoles (Table 4). A drought in the summer, recorded by the National Weather Bureau as the worst since 1869 for much of Wisconsin, destroyed most of the ponds with tadpoles, allowing only 4 ponds to produce emerging young.

Almost twice as many ponds had frogs present in 1976 as in 1975, and 4 times as many showed evidence of breeding activity in 1976. The increase in breeding activity in 1976 may have been due to a greater number of frogs which had emerged in 1974 and were sexually immature in 1975. The good climatic conditions in the late summer and fall of 1975 most likely allowed a relatively high proportion of them, now sexually mature, to enter hibernation and later to emerge in 1976 to breed during the early wet spring.

The percentage of ponds with breeding activity that produced young was 60% in 1975 and 21% in 1976. The low success rate in 1976, in spite of a larger breeding population, was caused by the severe drought. In 1976, 79% of the study areas dried up before the young emerged, while in 1975, only 40% dried up.

During both years, only 30% of the 83 suitable ponds showed any frog activity. Such evidence possibly indicates an already low population of leopard frogs. Although figures are not available, the reports of professional frog collectors indicate that past densities of leopard frogs were considerably higher than those found during these study years.

Breeding Phenology. Breeding time varied by several weeks over the 2-year study, since in maintaining their own internal body temperatures, leopard frogs are completely dependent upon environmental temperatures. Thus the onset of breeding (calling by males) appeared to depend upon water temperatures reaching at least 10 C. At no time in the approximately 180 hours of observation time from 20 April to 11 May were leopard frogs heard calling regularly or observed mating at water or air temperatures below 10 C.

Temperatures of 10 C and the beginning of breeding activity occurred in the 4th week of April in 1974, the 3rd-4th week of April in 1975, and in the 1st-2nd week of April in 1976. Air temperatures were significant in depressing breeding activity when they dropped below 10 C once breeding activity had begun.

The general pattern of breeding activity was the same each year. Male

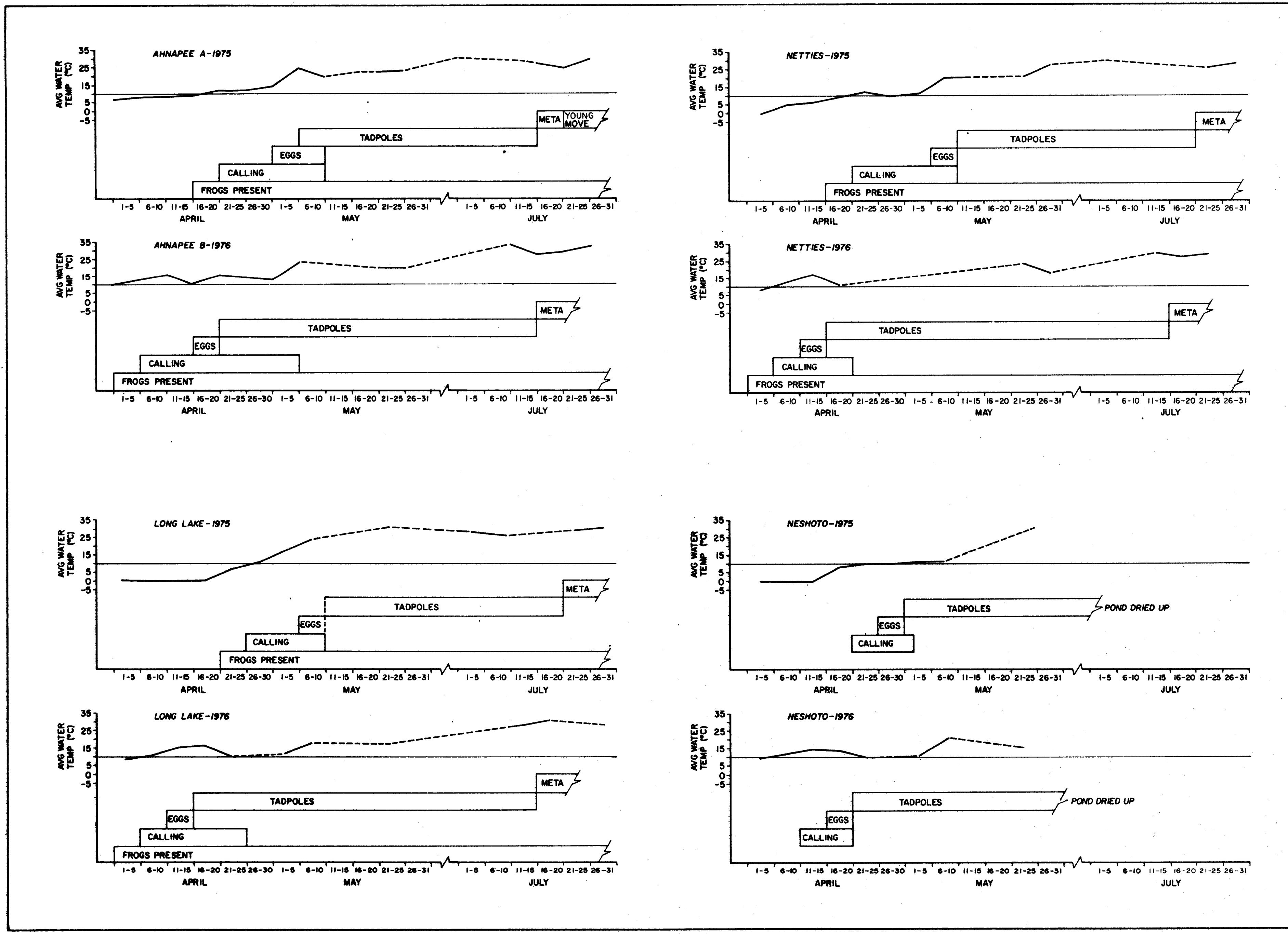


FIGURE 5. *Breeding phenology of 5 study ponds, 1975-76.*

frogs arrived first at the ponds and began to call as soon as water and air temperatures were appropriate, about 10 C. Females arrived from 3 to 14 (usually 5-7) days after calling began. Once females were evident at the ponds and temperatures continued at 10 C or more, mating would proceed. All breeding females deposited egg masses within 2-7 days of arrival at the ponds. If low temperatures halted breeding activity before all breeding females were mated, the females would resume mating once temperatures were again appropriate. Sometimes the periods of low temperatures separated warmer periods by 2 weeks, and resulted in 2 or more mating periods and several size classes of young.

Breeding activity generally took place in the warmest part of the ponds. Since water temperatures in the ponds did not reach 10 C until late morning, the leopard frogs bred mostly during the daylight hours. In the evening and on cloudy days when the air temperature dropped below 10 C, breeding activity slowed or stopped completely even though the water temperature was several degrees warmer as a result of heating during the day.

The breeding phenology of 5 ponds is shown in Figure 5 for 1975 and 1976. Breeding activity began during the 4th week of April in 1975 and about 2 weeks earlier in 1976. In spite of the difference in starting dates between the 2 years, and the variability in the length of calling and egg laying periods, there was a remarkable constancy in dates for initiation of metamorphosis, varying by only an average of 5 days. A similar conclusion was reached by Merrell (1977) in his Minnesota breeding ponds.

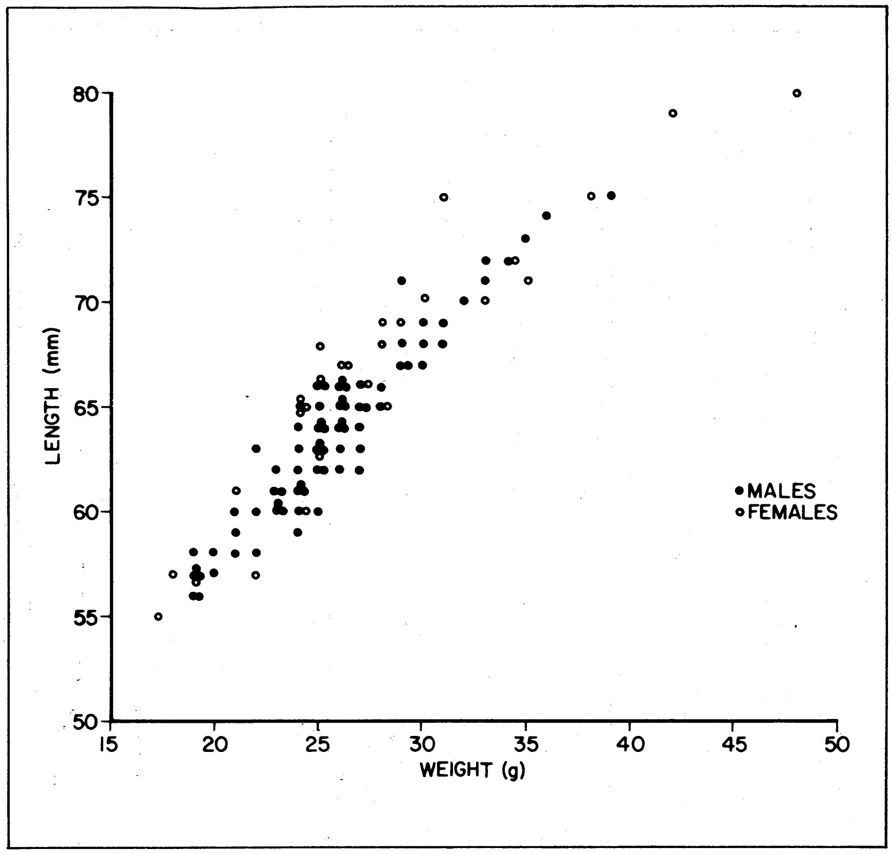


FIGURE 6. *Size of leopard frogs taken at breeding ponds during May and June, 1975 (after breeding and before appearance of young).*

Characteristics of the Breeding Population

Sex Ratio. Of 104 adult frogs captured in the springs of 1975 and 1976, 77 (86%) were males and 27 (13%) were females and possibly a small number of subadult males. The high percentage of males reflects the difference in behavior exhibited by the 2 sexes during the breeding season. Females are extremely secretive and difficult to locate. Males are just the opposite—their calling making them conspicuous and easy to locate. They were found sitting on floating plants away from dense vegetation. Merrell (1968) obtained similar data, with only 11% of the frogs captured during the breeding season being female. The actual sex ratio was believed to be 50:50 based on data collected during our fall survey (Table 18). Merrell (1968) and Rittschof (1975) also reported an equal sex ratio at other times of the year.

Size and Condition. Females captured during the spring averaged both longer and heavier than the males. The average length of females was 67 mm (55-80 mm) and the average weight was 27.8 g (17-48 g) after egg laying. The average length of males was 63.8 mm (55-75 mm) and their average weight was 25.8 g (17-39 g). These measurements were recorded in May and June after egg laying and before any young of the year had emerged.

The adult frogs examined appeared healthy during breeding except for 1 female found dead at Netties Pond. Symptoms of advanced redleg were evident on her legs and abdomen.

Sexual Maturity. According to data collected in the fall, 97% of the females with pigmented eggs (and therefore sexually mature) were 60 mm or more in length (Table 20). Females caught in the spring, during and after egg laying but before the appearance of young, ranged from 55 to 80 mm in length (Fig. 6). Only 85% of these were 60 mm or more in length, and on the basis of the fall data, could be classified as sexually mature. The remaining 15% could be either among the 3% of sexually mature females that are smaller than 60 mm, or subadults that came to the breeding ponds but did not breed.

Merrell (1977) has stated that only sexually mature frogs come to the breeding ponds, while immatures tend to remain near the larger bodies of water. Dole (1967), however, found that the immatures came to his breeding ponds about a week after the adults.

Although we were not able to determine the time at which males reached sexual maturity, it appears from the range of sizes of males in spring (55-75 mm) that both 1-year-old and 2-year-old males might be at the breeding ponds (Fig. 6). Since all had swollen thumb pads, they were thought to be sexually mature.

Movements. Movements of adults during and immediately after breeding were confined to the pond itself or the immediate perimeter. In over 30 hours of direct observation of leopard frogs, movements were observed to be short (less than 1 m), with no evidence of territoriality. Most movements were to change position into or out of the warmth of the sun, to feed, or to escape a disturbance. Frogs often sat for hours in the same location, only moving for better position to catch a passing insect.

Escape Behavior. Behavior patterns concerning escape varied with

TABLE 5. *Population estimates in spring breeding ponds, based on 104 marked adults.*

	1975				1976				1978
	Mark/ Recapture*	Adjusted Estimate**	Call Index[1]	No. Egg Masses (× 2)	Mark/ Recapture*	Adjusted Estimates**	Call Index[1]	No. Egg Masses (× 2)	Call Index[1]
Ahnapee A	10	16	14	4(8)	2[2]	–	0	–	6
Ahnapee B	0	–	6	4(8)	4[2]	–	4	2(4)	0
Netties	8	13	14	2(4)	10	18	8	3(6)	10
Maribel[3]	0	–	0	43(86)	0	–	0	–	18
Long Lake	20	33	18	11(22)	44	71	46	19(38)	50+
Neshoto	14	28	14	7(14)	18	30	18	8(16)	0[4]
Nuclear[3]	missed	–	(missed)	–	76	116	88	24(48)	

*Both males and females caught.
**Adjusted according to Merrell (1968).
[1]Number of males heard multiplied by 2.
[2]Actual number of frogs present.
[3]No adults found or calling males heard in 1975 but evidence of breeding activity later observed.
[4]Increased construction activity made this pond unsuitable for breeding.

the time of day or night, with temperature, and with location of the individual. During light periods, whether during daylight or full moon, the frogs showed an increased wariness to disturbance both on land and in the water. They were difficult to capture in bright moonlight.

Higher temperatures generally made it more difficult to capture individuals than lower temperatures. Frogs in air or water temperatures of 8-10 C were relatively easy to capture while "warm frogs", those in water with temperatures higher than 10 C would move more quickly to escape.

Frogs situated in or very near the water would remain stationary longer in mid-day or in a beam of light at night than frogs located a meter or more away from the water's edge. In response to disturbance, they moved toward the safety of water whenever possible, or moved essentially back and forth until reaching dense grass cover. At no time was any observation made of a frog moving to an area where it would be more exposed.

Population Estimates

Population estimates obtained through mark/recapture showed leopard frog populations ranging from 8 to 20 in the 4 ponds in which breeding frogs were captured in 1975, and from 2 to 76 in 6 ponds in 1976 (Table 5). In his study of adult breeding populations in 6 ponds in Minnesota from 1958 to 1966, Merrell (1968) estimated population size by the Lincoln-Petersen method. He obtained average estimates of from 124 to 1,568 adults in his study ponds, a level considerably higher than the 1975-76 east central Wisconsin populations.

Our estimates obtained through mark/recapture were adjusted according to the formula devised by Merrell (1968) to compensate for the lower numbers of females caught in spring. In Table 5 these are compared with the call index. The population estimates based on the index of calling males was derived by multiplying the call index by 2, since the sex ratio was believed to be 50:50.

There is a close similarity between estimates obtained from the adjusted mark/recapture data and the call indexes in only 2 instances. In the other cases, the estimates based on call index were consistently lower than the adjusted estimates. This could be explained by the fact that call indexes are based on 1 or 2 listening periods, and there is a certain amount of chance involved in hearing all the frogs at any one time. Although this has not been reported for leopard frogs, there could be suppression of subdominant males by dominant calling males. Fellers (1979) found this to be true for *Hyla chrysoscelis*.

Comparing the 2 estimates allows an evaluation of the call index. We believe that it is valid as a measure of the presence of breeding frogs, but that it can serve only as a rough estimate of population size. Furthermore, we were working with small populations; it is difficult to apply the technique to populations higher than about 25 males since individual calls would be difficult to differentiate, as we found at Nuclear Pond in 1976.

The number of adults estimated through mark/recapture methods exceeded the size of the breeding population as indicated by the number of egg masses. This agrees with Merrell (1968) who stated that even the "effective size" of the breeding population, based on doubling the number of egg masses, is considerably lower than the total population of frogs present at the breeding ponds based on census data. This further suggests the presence of nonbreeding frogs at the breeding ponds in spring.

Egg Development

Egg masses appeared 5-14 days after males began calling; cooler conditions extended the period between calling and egg laying and warm conditions compressed the time. Egg masses in all the study ponds were grouped closely together in the shallow part of the pond that received the greatest amount of early morning and early afternoon sunlight, allowing that area to warm faster than the rest of the pond. The egg masses were attached to standing or fallen vegetation—a firm twig, sedge, or dead cattail—and floated at or near the surface of the water. Merrell (1968) noted that practically all egg masses were laid in one 1- to 4-m^2 area of the breeding pond. He also noted that egg mass concentrations coincided with the place in the pond where calling males had congregated.

Compared with masses submerged by only as much as 1 cm, a higher percentage of eggs failed to develop in egg masses at the pond surface; approximately 15-20% of these egg masses appeared to be lost to drying. Based on visual inspection, about 5% of the eggs in each egg mass were lost to parasitism, disease, or other factors.

The size of 97 egg masses measured during the springs of 1975 and 1976 averaged 87 mm long (40-150 mm) by 63 mm wide (30-100 mm) by 69 mm deep (40-110 mm). Hatching time varied depending on water tempera-

Leopard frog egg masses.

TABLE 6. *Movements of marked leopard frogs at 5 ponds over periods from 2 to 17 days in July 1975.*

Frog No.	Total Distance Moved (Minimum) (m)	Maximum Distance Caught away from Pond (m)	Longest Movement (m)	Shortest Movement (m)
Ahnapee A				
F1	220	30	84	4.6
F2	ca. 200	190	140	14
F3	46	2	18	9
F4	55	150	30	25
F5	30	16	16	11
F6	9	3	–	–
Ahnapee B				
F1	28	4	25	3
F2	7	4	–	–
Netties				
F1	28	20	21	7
F2	12	1	11	1
F3	20	20	–	–
Neshoto				
F1	3	1	–	—
F2	400	401	–	–
F3	37	40	–	–
Long Lake				
F1	10	20	–	–
F2	27	30	36	2
F3	4	2	3	1
F4	23	40	–	–
F5	11	25	–	–
F6	8	5	6	2
F7	11	1	–	–

ture. During a constant temperature period (10 C or above), tadpoles were observed in 5-9 days. Cool periods prolonged the time. The numbers of eggs per mass were not counted during this study, but Merrell (1968) estimated that in Minnesota between 2,000 and 5,000 eggs were produced per mass.

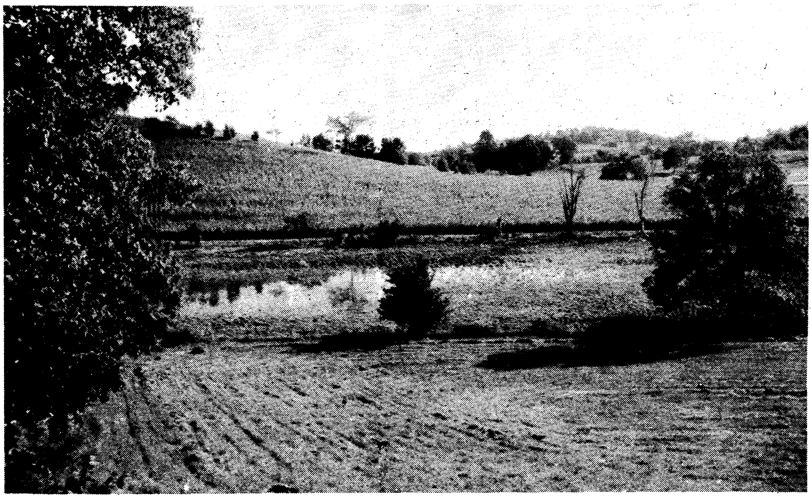
Postbreeding Activity

Adults

Movement. An intensive search and marking effort was undertaken on 5 of the study ponds in mid-July, 1975. Searches were made for 2-7 consecutive days at Ahnapee, Neshoto and Netties ponds, and over a 17-day period at Long Lake. The search was begun in a 10-m strip around each pond to locate frogs, and then was extended into adjacent habitats. Movements were recorded for 21 marked frogs. Fewer adults were found around the ponds in midsummer compared to the number earlier in the spring, for many had left the pond area for summer habitats away from the pond.

Ahnapee Ponds. The ponds at Ahnapee provided some of the best comparisons of movement and habitat utilization by leopard frogs (Fig. 7 and Table 6) since the 2 distinct ponds were surrounded by several types of vegetation.

At pond A, the largest of the 2, 6 frogs were recaptured during a 7-day period, with searches being made each day. Among these, 3 frogs moved into a waterlogged meadow with aspen and alder for distances of up to 150 m from the nearest pond edge. Two of the 3 did not return, while F1 returned to within 27 m of its starting point after moving a total distance of 220 m. F1 was the only one observed to leave a small area where it was recaptured 3 times and then to return to the same



Leopard frog summer habitat.

area. F2 was also found near the pond, but then moved out and was not recaptured again near the pond. F3 and F6 remained near the pond and only moved along the perimeter. F4 was recaptured 3 times in the meadow area and was not observed near the pond. F5 changed ponds over a 5-day period from Pond B to Pond A, crossing a blacktop road to get there.

The frogs in Pond B remained along its perimeter. Movement tended to be to other locations within the tall grass border of the pond.

The frog movements associated with Ponds A and B tended to be within areas where adequate ground moisture was available. During the 7-day period, heavy rains were not experienced though light rain and mist were. Frogs were not observed moving in the mowed or unmowed hayfields nor were any observed in the mixed or cedar woodlots. Sampling procedures in those areas were hindered by dense vegetation, and some frogs may have been missed because of numerous cavities and holes. We are reasonably certain, however, that most activity associated with these ponds was in the wetter zones which were mainly the marsh area.

Neshoto. Neshoto's vegetation cover represented good leopard frog habitat, but its ground moisture all but disappeared early in the summer. During the spring breeding period, adequate water levels provided many areas for frogs to lay eggs, and many were used; but by midsummer the ponds had dried and the frogs had moved to moister areas in the surrounding river bottom (Fig. 7 and Table 6). Rather long movements (40 and 400 m) of the 2 frogs toward the waterlogged areas of the river bottom were recorded. A third frog stayed nearer the pond, but was not captured after the pond dried up.

The captures for F3 were 2 days apart, during which it moved 40 m. F2 was recaptured twice within a 5-day interval, during which it moved 400 m from its 1st point of capture.

Netties. Dry rocky ridges surrounding the main pond on 3 sides limited the available areas with ground moisture. On the north side of the pond, a wet area fed by a seeping spring proved to be the habitat used most by the leopard frogs.

Only 3 frogs were recaptured in the area over a 7-day period (Fig. 7 and Table 6). Two of the 3 were first captured near the pond and were later found in the spring area. A third moved only a few meters to another area of the pond.

Long Lake. Long Lake is representative of the good leopard frog habitat found throughout east central Wisconsin. Leopard frog habitat requirements were met in the immediately adjacent areas. A stable water supply maintained a balance of habitats throughout the season. A low wet hayfield and numerous waterlogged meadow areas provided adequate summer feeding grounds, while nearby Long Lake provided suitable overwintering cover.

Frog movement data gathered at Long Lake consisted of recaptures of 7 individuals on dates separated by 15-17 days (Fig. 7 and Table 6). Additional captures of 30 frogs were also mapped.

Movements tended to be rather short and adult recapture sites were located in the cattail-sedge marsh not more than 60 m from the edge of the breeding site. The area was covered with tall grasses which assured the availability of ground moisture. Movements to and from the breeding area were not observed. Frogs which were captured only once were generally found along the borders of the marsh or just into the hayfield. Most of these captures were on rainy nights or after a day of rain. These frogs may have been on excursions out of their home ranges or, since they were not recaptured, they may have been only temporary residents of the area.

Movements of adult leopard frogs in early summer appeared to range from very short distances along the edges of the ponds to rather lengthy forays into adjacent habitats. Forty-three percent of the recorded July movements were within 1-5 m of a home pond's edge. Movements away from the pond were generally toward adjacent wet areas. The longest movement in a 24-hour period was 140 m from a pond edge (F2, Ahnapee A). The greatest movement from a pond edge made by any frog during this period was 401 m over 5 days (F2, Neshoto). This movement carried the frog from a pond that was drying (Neshoto) to a moist river bottom habitat. Some movement, however, may have been for foraging rather than translocating, as illustrated by the reappearance of F1 at Ahnapee A near the original site of capture, after a movement of at least 30 m away from the pond. Although the frog was not seen feeding, this suggests that some frogs move out from the pond and forage in adjacent favorable habitat, and return again to the home pond. This behavior is further suggested in our study by the numbers of frogs captured only once at Long Lake.

These findings agree with those of Dole (1965 a, b) who found that adults showed a strong attachment to a relatively small area (home range) during the summer months. However, he also observed that although some individuals embarked on more extended excursions from their home ranges (distances up to 160 m were recorded), they returned without occupying the excursion area for more than a few days.

Feeding. In order to determine the food used by adults, gut content analyses were performed on 11 frogs. They

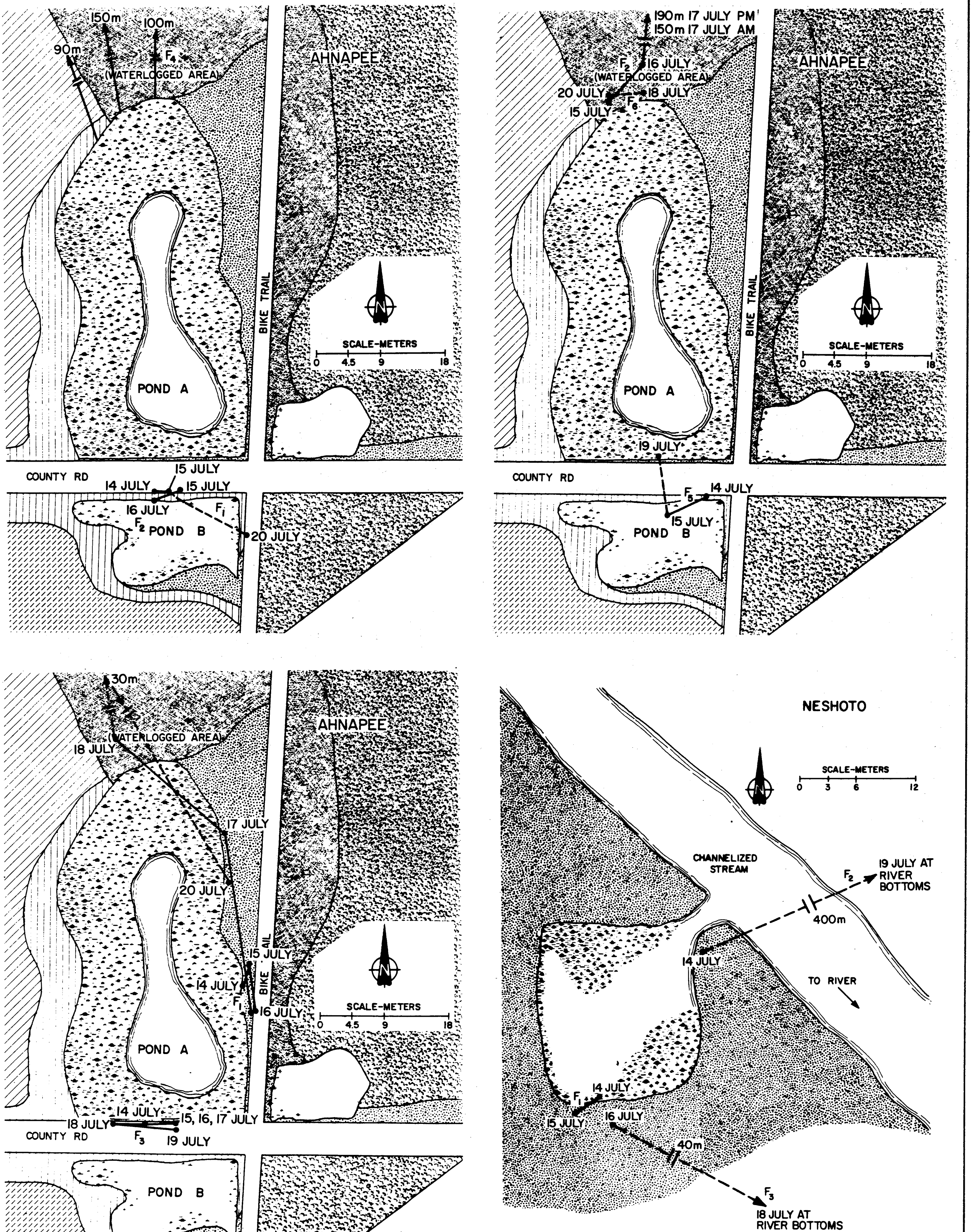
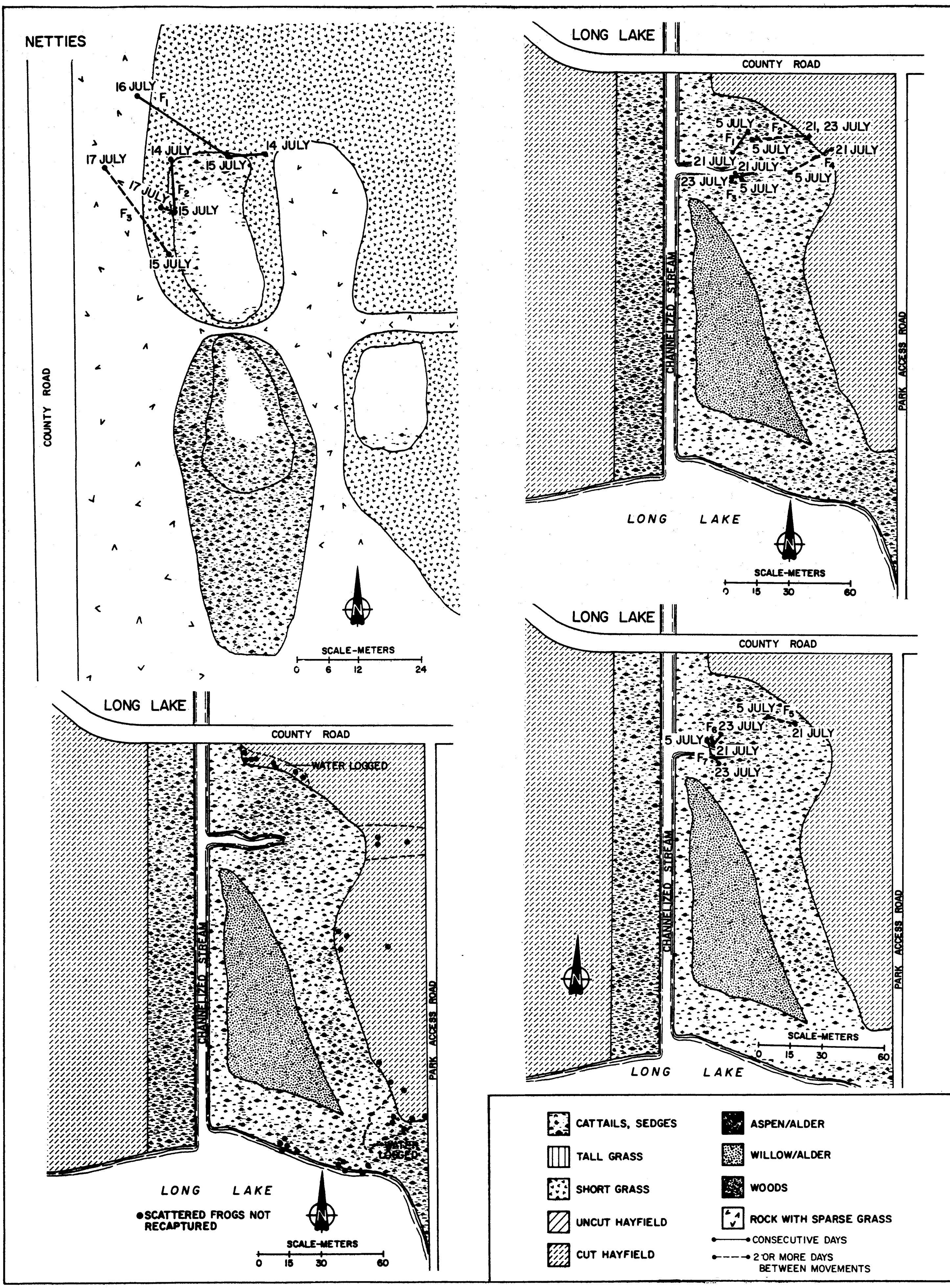


FIGURE 7. *Movement of marked adult frogs on 5 study ponds. (Note: Breeding areas are shown on Figure 9.)*


NETTIES
16 JULY
F1
14 JULY
14 JULY
15 JULY
17 JULY
17 JULY
F2
15 JULY
F3
15 JULY
COUNTY ROAD
SCALE-METERS
0 6 12 24
LONG LAKE
COUNTY ROAD
5 JULY
F1
F2
21, 23 JULY
5 JULY
21 JULY
21 JULY
21 JULY
F4
23 JULY
F3
5 JULY
5 JULY
CHANNELIZED STREAM
PARK ACCESS ROAD
LONG LAKE
SCALE-METERS
0 15 30 60
LONG LAKE
COUNTY ROAD
WATER LOGGED
CHANNELIZED STREAM
PARK ACCESS ROAD
WATER LOGGED
LONG LAKE
SCATTERED FROGS NOT RECAPTURED
SCALE-METERS
0 15 30 60
LONG LAKE
COUNTY ROAD
5 JULY
F5
F6
23 JULY
21 JULY
5 JULY
21 JULY
F7
23 JULY
CHANNELIZED STREAM
PARK ACCESS ROAD
SCALE-METERS
0 15 30 60
LONG LAKE
CATTAILS, SEDGES
TALL GRASS
SHORT GRASS
UNCUT HAYFIELD
CUT HAYFIELD
ASPEN/ALDER
WILLOW/ALDER
WOODS
ROCK WITH SPARSE GRASS
CONSECUTIVE DAYS
2 OR MORE DAYS BETWEEN MOVEMENTS

were taken in areas far from the study areas, so that the ponds under investigation would not be influenced by their removal. Animal parts were identified to phylum or in the case of insects, to orders.

The occurrence of items in order of their abundance was: (1) Isopoda, (2) Coleoptera, (3) Lepidoptera larvae, (4) Annelida, (5) Hemiptera, (6) Hymenoptera, (7) Odonata, and (8) Trichoptera. Major prey items of frogs in Michigan were Coleoptera, Orthoptera and Lepidoptera larvae (Rittschof 1975). Feeding probably took place both during the day and night since both diurnal and nocturnal prey items were represented.

Young-of-the-year

Tadpoles began growing legs and resorbing tails in the latter half of July, after spending 10-13 weeks feeding in the floating vegetation.

Population Estimate. Population estimates of juveniles obtained from 597 marked after metamorphosis are shown in Table 7 for both years. Changes in pond character, either through human disturbance or drought, were responsible for the lack of reproductive success at 3 ponds during 1 or both years. Netties and Long Lake showed increases in the numbers of adults, egg masses, and young between 1975 and 1976, while Nuclear Pond, although the numbers of adults and egg masses were not known for 1975, showed a decrease in production of young in 1976.

Merrell (1977) found that an estimated 600,000 eggs yielded 20,000 newly metamorphosed young (a survival rate of 3%). Our study yields information on the survival of juveniles up to 6 weeks after metamorphosis. Using an average of 3,500 eggs per egg mass, and calculating overall survival for each study pond, we found survival rates of 1-6% (Table 8). The highest survival occurred in Long Lake, the largest of the study ponds and the one least affected by agricultural land. Lowest survival occurred in Ahnapee B and Nuclear ponds, which were small and surrounded by agricultural land.

Size and Condition. The average length and weight of juveniles from the time of metamorphosis (mid-July to early September) is shown in Table 9 for both years. There is an initial weight loss ranging from 1.5 to 2.5 g over a 4-day period after metamorphosis. They then increased in size, averaging 58 mm in length and 15.4 g in weight in September.

A comparison of lengths and weights on different days from 20 July to 6 September at Ahnapee B shows that young frogs from the same pond varied considerably in length and weight at any one time (Fig. 8). Although there was remarkable similarity in the time of metamorphosis between ponds (Fig. 5), delays in breeding activity caused by temperature variations within a pond could account for an extended period of transformation and resultant size differences. While the smallest frogs were taken in July, and the largest in September, there was overlap in the intervening periods.

Our data parallel the findings of Merrell (1977) in Minnesota, who found the usual size of frogs at metamorphosis to be 35-40 mm. He attributed observed differences in size at metamorphosis in 2 ponds to drought conditions (young 25-30 mm), and to uncrowded conditions (young 48-50 mm). By the end of the summer the young in the latter pond had reached 55 mm in length.

The average length of young frogs in our Wisconsin study ponds and in Minnesota (Merrell 1970) exceeded that recorded by Rittschof (1975) in northern Michigan. He recorded an average length of 21.5 mm at the time of metamorphosis, and an increase of 15.5 mm by early October (to 37 mm).

Sexual Maturity. Young-of-the-year females generally become subadults (Dole 1965a) the following spring and producers during their third spring when they are 2 years old. However, data collected during our fall survey showed a substantial number of frogs contained eggs in which vitellogenesis had begun, but which were not yet pigmented (Table 20). Of these frogs, 77% were less than 60 mm in length, the minimum length of 97% of sexually mature females (those with pigmented eggs). These frogs may represent young-of-the-year females that were able to reach an intermediate level of maturation (visible eggs but not pigmented). The remaining 23% of frogs without pigmented eggs, those larger than 60 mm, may represent fast-growing young of the year and/or possibly slow-maturing subadults. Such subadults could not become producers until their fourth spring, when they would be 3 years old.

In Minnesota, most sexually mature individuals ranged between 60 and 80 mm in length. Most young, 35-40 mm at metamorphosis, were less than 55 mm at the end of their first summer and were sexually immature (Merrell 1977). Merrell also con-

TABLE 7. *Summary of breeding activity on study ponds, 1975-76.*

	1975			1976		
Study Pond	Est. No. Adults	No. Egg Masses	Est. No. Young	Est. No. Adults	No. Egg Masses	Est. No. Young
Ahnapee A	16	4	440	2	0	0
Ahnapee B	–	0	0	4	2	80
Netties	13	2	160	18	3	220
Maribel	–	43	0	–	0	0
Long Lake	33	11	1,720	71	19	4,000
Neshoto	28	7	0	30	8	0
Nuclear	?	?	3,240	116	24	1,120

TABLE 8. *Estimated survival of young frogs from 5 breeding ponds from mid-July (metamorphosis) to 6 September.*

Pond	Year Surveyed	No. Egg Masses	Potential Production of Young*	Population Estimate of Young	Percent Survival
Ahnapee A	1975	4	14,000	440	3
Ahnapee B	1976	4	14,000	80	1
Netties	1975	2	7,000	160	2
	1976	3	10,500	220	2
Long Lake	1975	11	38,500	1,720	4
	1976	19	66,500	4,000	6
Nuclear	1976	24	84,000	1,120	1

*Average of 3,500 eggs per egg mass.

TABLE 9. *Average length (mm) and weight (g) of 597 leopard frogs captured at 5 study ponds between mid-July and 6 September.*

	1975		1976		Total	
	Avg.	Range	Avg.	Range	Avg.	Range
Avg. length at metamorphosis (mm)	40	35-40	36	34-40	39	34-40
Avg. weight at metamorphosis (g)	8.5	4.0-9	8.0	4.0-9.5	8.0	4.0-9.5
Avg. weight loss (g)	2.5	–	1.5	–	2.1	–
Time period of weight loss	4 days		4 days		4 days	
Avg. weight after 1 month (g)	11.8	5.0-14.0	11.5	5.0-13.5	11.6	5.0-14.0
Avg. length after 1 month (mm)	52	45-54	49	40-55	50	40-55

cluded that it normally took leopard frogs 2 years in the Minnesota area to reach sexual maturity.

In northern Michigan, sexual maturity was also attained near the end of 2 growing seasons, but in southern Michigan in some years a high proportion of frogs had enlarged thumb pads or enlarged ovaries at the end of the first growing season (Rittschof 1975). Data from our fall examinations showed that frogs between 50 and 60 mm sometimes had enlarged ovaries, but only rarely were the eggs pigmented. It is possible that sexual maturity could be reached by a few frogs in our ponds during the first growing season, since some juveniles were caught in September that were 60 mm in length (Fig. 8). However, this could not be documented since internal examinations for pigmented eggs were not made.

Movement. Three areas were intensively monitored for postemergence movements for a 6-week period following metamorphosis in 1975—Ahnapee, Netties, and Long Lake. The numbers of juveniles marked and recaptured are shown in Table 1.

Ahnapee. The area of Pond A with the greatest juvenile activity was approximately 1 m in depth and ranged from areas of open water through areas covered with various types and stages of emergent vegetation. Other areas of the pond tended to be less diverse and much shallower or deeper than 1 m.

Movement of the juveniles after emergence was directed south towards Ahnapee B (Fig. 9). Only 2 marked at Ahnapee A were recaptured in Ahnapee A, while 26 moved across the road to Ahnapee B. Twenty-three were captured and marked in B. They too had apparently moved in from A, since B had neither egg masses nor tadpoles in the summer of 1975.

The movement of juveniles was not en masse, nor did it seem to be triggered by rainfall. Rather, movement tended to be gradual from Ahnapee A to B. Once in B there was little movement away from the pond during the July-August observation period, and no movement was observed from B back to A.

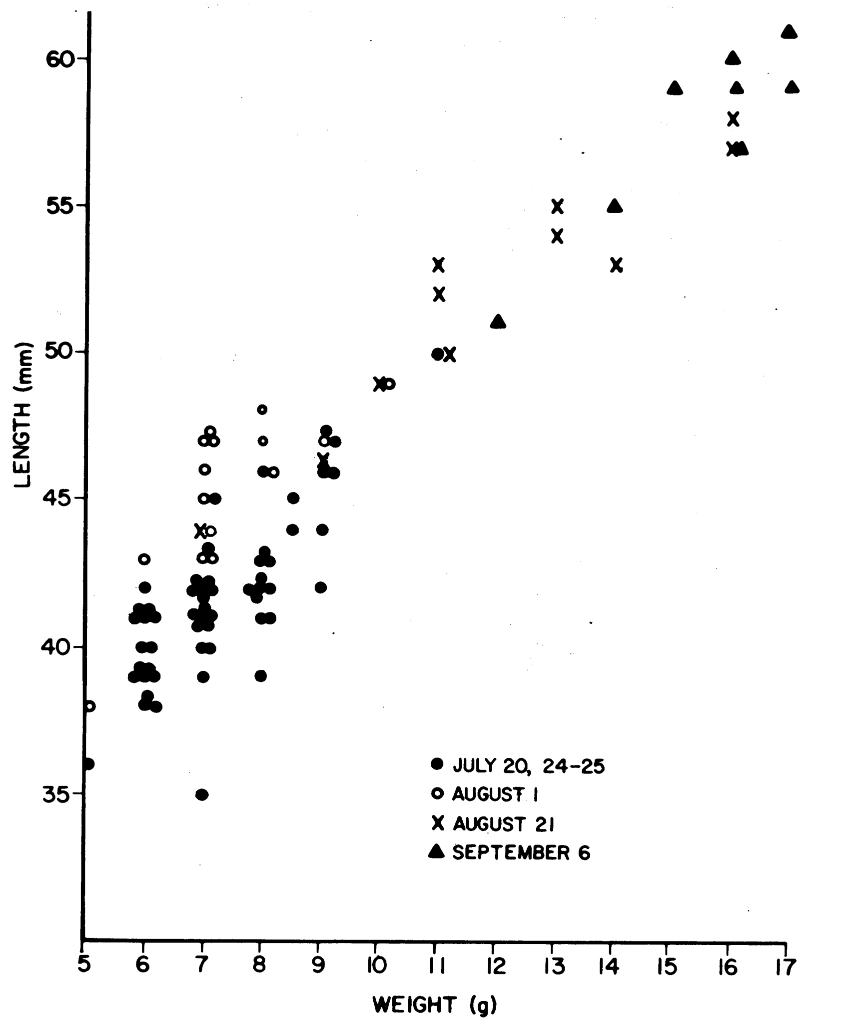


FIGURE 8. *Comparison of lengths and weights on different days from 20 July to 6 September 1975 at Ahnapee B.*

Netties Pond. Seventy percent of the young marked were captured near the location of egg masses in the pond (Fig. 9). This area of the pond was the preferred habitat. It contained a

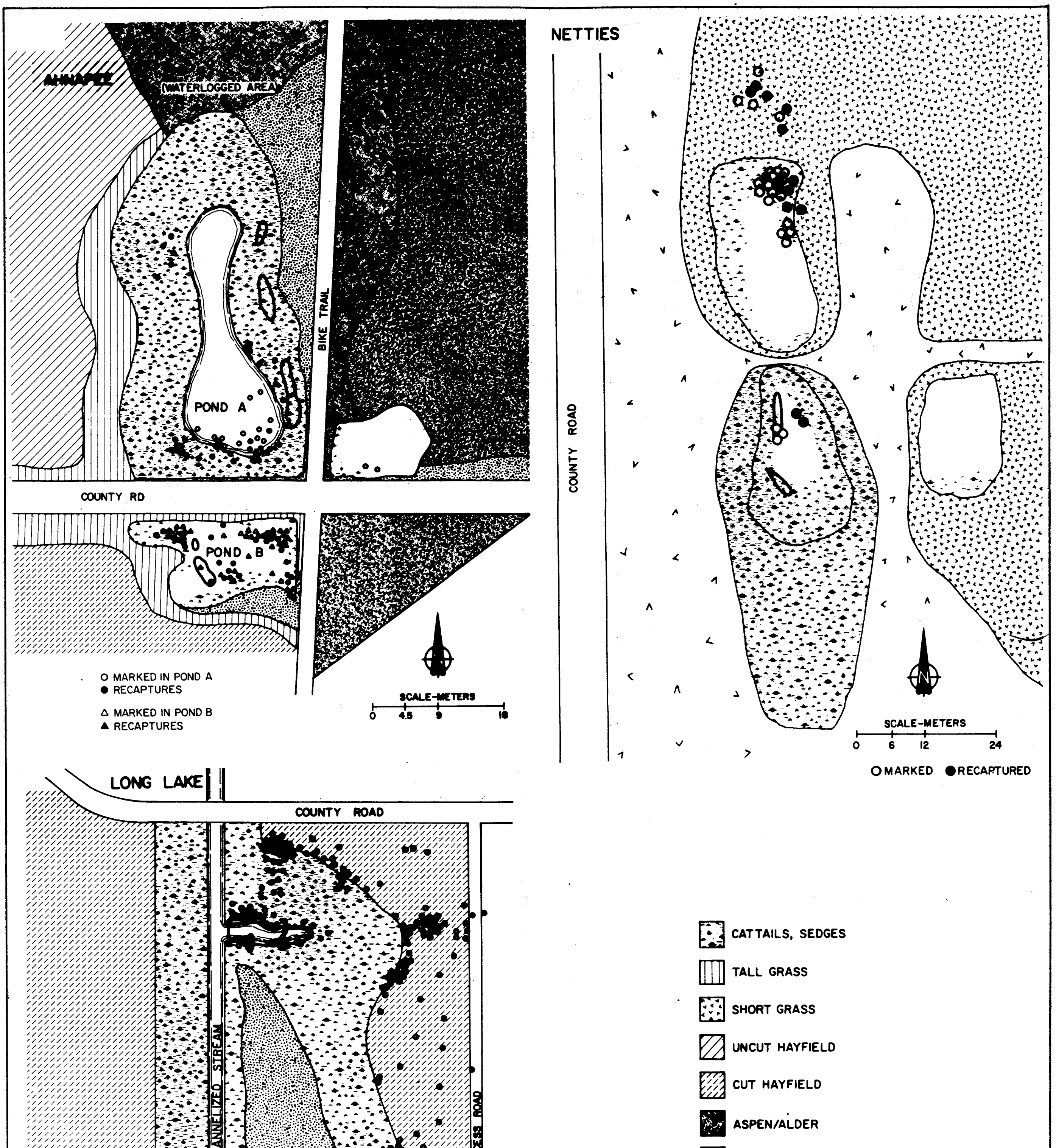


FIGURE 9. *Postemergence movements of young-of-the-year leopard frogs.*

mixture of vegetation types with a gradually sloping bank. Other areas of the pond carried either 1 type of vegetation or were next to a steep gravelly bank.

Young frogs moved singly away from the pond towards a wet, medium-light, grassy area where a seeping spring provided constant ground moisture. Young were observed in the wet area for 2-3 weeks after emergence, but then moved out of the study area, possibly toward the river, although no frogs were recaptured there.

Long Lake. Few frogs were recaptured near the site of egg laying (Fig. 9). Their distribution and recapture locations suggested that they moved first north and then south along the tall grass interface between the marsh and hayfield, a place where ground moisture remained almost constantly. This led them to a wet area of tall grass along the lake front. Young frogs were found in the hayfield or on the adjacent roadway only during rains or on wet foggy nights. Subsequent movement tended to be southward along the edge of the lake. Here there were good cover and feeding areas except for one 75-m stretch of mowed grass at a county boat landing. The presence of cottages and closely mowed lawns and boat docks characterized the north-northwest edge of the lake. Frogs were not found there which suggests such cover is unsuitable for them.

Movements of young frogs recorded from our study ponds were directed toward wet, grassy areas surrounding the ponds, or to other water areas. This is in agreement with the findings of Merrell (1970), Dole (1971), and Rittschof (1975). The latter 2 authors recorded short distance movements of 5 to several hundred meters (Dole 1971) and longer movements up to 800 m (Rittschof 1975).

Feeding. After the period of weight loss shortly after emergence, when the young apparently were not yet feeding efficiently, they fed heavily on small prey such as ants and isopods until they could take the variety of prey typical of the adult diet.

Spring Mortality

Frogs from hibernacula near the study areas did not exhibit spring mortality as judged by searches for dead or sick frogs along lake fronts and river/stream banks. Over these years, however, reports were received of spring die-offs of frogs in several other areas. These observations apparently represent overwinter mortality which was observed at hibernacula in the early spring. No mortality was observed in spring at the breeding ponds.

FALL MORTALITY SURVEY

PROCEDURES

Field Survey

A statewide survey was conducted by DNR fish managers in 1974-76 to assess the extent and characteristics of leopard frog mortality associated with fall hibernation. This is the period when sick and dead frogs had been reported in the early 1970's. Standard 0.5-mile (1.3-km) survey stations were established and examined in all Wisconsin counties except Menominee during these years, although time and budget limitations forced restriction to a smaller number of stations in 1976 (Fig. 10). For analysis purposes, the station sites were grouped into regions (Fig. 11). During the first year of the survey, DNR fish managers selected stations on the basis of habitat considerations and the known presence of frog populations. All stations were reviewed before the 1975 survey and those whose habitats had been found unsuitable were deleted or replaced by new stations. Survey stations selected for 1976 were among those where frogs had been found in either of the 2 previous years of the fall study. Survey stations totaled 378 for 1974, 292 for 1975, and 80 for 1976 and represented 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 divided into 3 phases, although weather conditions sometimes made it necessary to combine phases I and II or to delete phase III. A description of each phase follows:

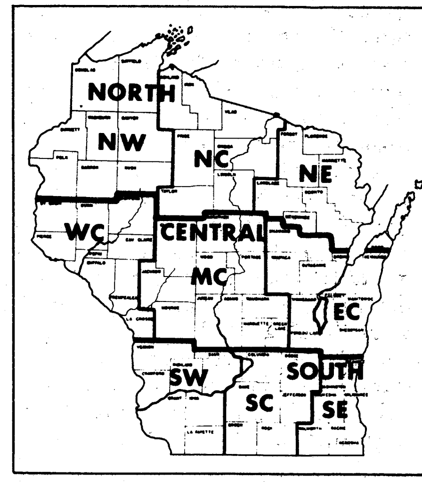


FIGURE 11. *Geographic areas used as the basis for the compilation of results in the fall mortality survey.*

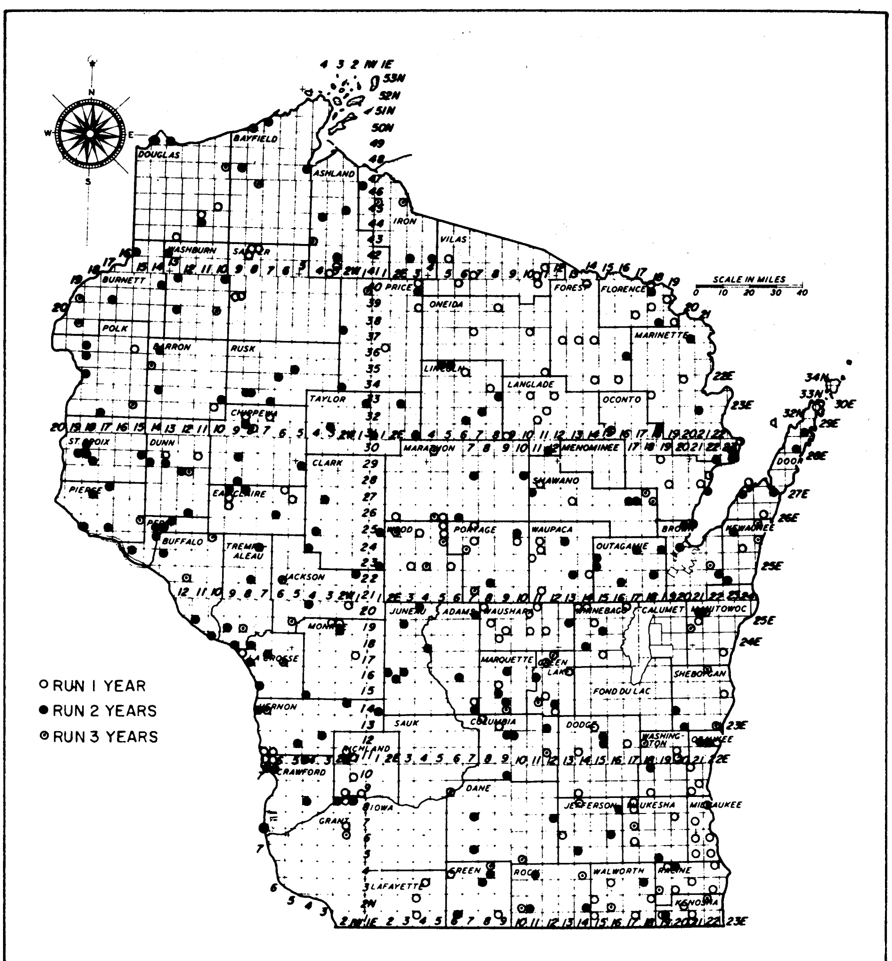


FIGURE 10. *Leopard frog stations sampled during fall mortality surveys, 1974-76.*

(1) Phase I: Initial Count. The objective was to count as many live frogs as possible, detect signs of mortality, and collect sick/dead frogs. This phase was initiated when frogs began to congregate in hibernacula and usually coincided with 4-7 days of 10-16 C temperatures coupled with freezing nighttime temperatures. Mass migrations or roadkills were observed in some locations. Survey dates varied from year to year, but in general were early October for northern counties, mid- to late October for central counties, and early November for southern counties.

(2) Phase II: Count/Collect. The objective was to document any developing mortality and to collect live and sick frogs for analysis. This phase usually followed 1 week after phase I, but was postponed if warming occurred.

(3) Phase III: Final Mortality Check. The objective was to document mortality as completely as possible and to collect additional specimens for analysis. This phase was usually conducted 1-2 weeks after the animals had entered the water for hibernation. Daytime air temperatures were usually 4-6 C and nighttime temperatures -4 to -6 C. In northern and central areas of the state, this period was

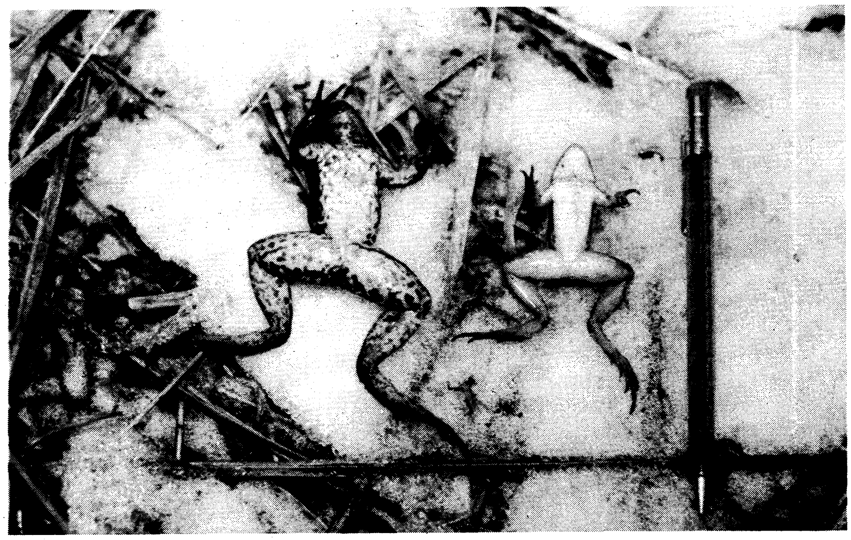
rapidly followed by freeze-up, but was often extended and interspersed with warm periods in southern areas. The phase III survey was conducted only at stations where frogs were found in phase I or II.

Whenever possible, phase I and II surveys were made during the warmer hours of the day, with calm, rainy (or humid) days preferred. Each station 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 examined by walking through them. Each leopard frog encountered was recorded as live or sick/dead. Frogs were classified as sick if they exhibited any of the following characteristics: skin lesions, ventral skin discoloration, dry skin, hard muscles, or abnormal behavior. The specimen objective of Phase II and III surveys were met by collecting 10 live frogs and 15-20 sick/dead frogs at each station. Collected frogs were grouped according to health category and placed in plastic bags. Specimens were stored in a cool place until transfer to the central processing location in Madison.

Additional survey information was gathered on the adjacent drainage area, immediate habitat, and the specific locations where frogs were observed. Other frog species encountered were counted and any mortality noted. Water samples were collected at stations where heavy mortality was found in 1974 and analyzed by the DNR water quality laboratory.

Transport to Madison for final processing and storage was done in 2 ways in 1974: some frogs, kept at central locations about the state, were picked up by private carrier and driven to Madison, while others were mailed. In 1975 and 1976, all frogs were mailed. Bags of frogs transported by private carrier were placed in ice chests. Ice was included to keep the frogs inactive thus preventing physical injury and rapid oxygen consumption. Frogs that were mailed were placed in cardboard boxes along with moisture-conserving materials such as sphagnum moss or wet paper towelling. Other types of damp vegetation were tried in a few mailings but were found to decay very quickly. Delivery in Madison was usually 2-3 days after mailing.

Most frogs mailed were received in good condition, although a few died during transport especially on warm days. In the initial days of the first survey (1974), coordination was inadequate as a few collections of frogs were lost. Dead frogs that were already in decay at the time of collection were usually unsuitable for analysis upon arrival in Madison. In general, frogs that were mailed were received in better condition than were those transported by private carrier because storage time after collection was minimized.



Dead frogs on the ice, Little Sugar River (green frog, left; leopard frog, right).

Laboratory Analysis

All frogs, except those lost to decay, were weighed and measured (snout to vent), sexed, and examined for physical abnormalities, stomach contents, fat body development, and presence of eggs. Frogs were sexed by 2 methods. In 1974, the major criterion was the enlarged thumb pad of the male, backed up by internal macroscopic observations of the gonads in a subsample of frogs to determine the size below which all frogs were immature. This size limit was determined to be 50 mm. In 1975 and 1976, all frogs, except those being preserved undissected for certain tests were sexed by internal observation. Frogs were classified as immature if sex could not be determined by either method. Physical abnormalities, fat body development, and presence of enlarged eggs were determined by visual observation. Stomach contents were recorded as either present or absent. All frogs were classified as live, sick, dead, or decayed at the time of analysis, usually within 1 day of receipt. Criteria used to classify frogs as sick were the same as those used in the field survey (e.g., presence of external or behavioral abnormality) with the addition of liver abnormality assessed through internal observation. A sample of specimens from the University of Wisconsin Zoology Museum and the Milwaukee Public Museum was examined for comparative information in 1974.

After measurements were taken, the specimens were divided among 3 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 1 or 2 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, placed in plastic bags and frozen.

Findings from the 1974-76 field surveys and 1975-76 laboratory measurements were computerized and are stored in Madison for future reference.

RESULTS

Occurrence of Mortality

Field observations for 1974-76 for each region (Fig. 11) are summarized in Table 10. Numbers in Table 10 should be interpreted with procedural limitations in mind. 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 vegetation or into the water. Once hibernation began, 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 full extent of mortality by repeated monitoring. In many cases, this would require daily monitoring. However, the numbers have value as comparative indicators

TABLE 10. *Fall mortality survey results by geographic area.**

Location	No. Survey Stations	No. Runs	Observations: Live No.	Observations: Live No./Run	Observations: Sick/Dead No.	Observations: Sick/Dead No./Run	Observations: Live: Sick/Dead	Collections: No. Live	Collections: No. Sick/Dead
1974									
Northwest	40	77	597	7.8	49	0.6	17:1	79	27
North central	36	79	151	1.9	4	0.1	30:1	60	3
Northeast	32	64	299	4.7	19	0.3	60:1	83	2
North	108	220	1,047	4.8	72	0.3	23:1	222	32
West central	49	94	230	2.5	13	0.1	16:1	18	10
Mid-central	53	70	274	3.9	83	1.2	3:1	98	56
East central	58	107	2,385	22.3	386	3.6	6:1	193	279
Central	160	271	2,889	10.7	482	1.8	6:1	309	345
Southwest	34	50	72	1.4	5	0.1	14:1	31	4
South central	31	39	41	1.1	337	8.6	1:8	6	68
Southeast	45	45	37	0.8	52	1.2	1:2	14	13
South	110	134	150	1.1	394	2.9	1:3	51	85
STATE TOTAL	378	625	4,086	6.5	948	1.5	4:1	582	462
1975									
Northwest	39	77	698	9.1	68	0.9	10:1	43	37
North central	26	63	135	2.1	5	0.1	27:1	137	5
Northeast	25	62	261	4.2	2	**	131:1	66	0
North	90	202	1,094	5.4	75	0.4	15:1	246	42
West central	48	80	759	9.5	30	0.4	25:1	54	21
Mid-central	59	102	3,390	33.2	3	**	1,130:1	105	0
East central	39	72	1,608	22.3	252	3.5	6:1	113	25
Central	146	254	5,757	22.7	285	1.1	20:1	272	46
Southwest	14	24	116	4.8	3	0.1	39:1	48	2
South central	30	63	3,326	52.8	85	1.3	39:1	45	14
Southeast	12	12	262	21.8	1	0.1	262:1	28	1
South	56	99	3,704	37.4	89	0.9	42:1	121	17
STATE TOTAL	292	555	10,555	19.0	449	0.8	24:1	639	105
1976									
Northwest	10	19	125	6.6	0	0	125:0	12	0
Northcentral	6	16	34	2.1	0	0	34:0	16	0
Northeast	7	13	11	0.9	0	0	11:0	0	0
North	23	48	170	3.5	0	0	170:0	28	0
West central	10	20	180	9.0	20	1.0	9:1	11	11
Mid-central	16	38	84	2.2	0	0	84:0	8	0
East central	12	17	9	0.5	1	0.1	9:1	3	0
Central	37	75	273	3.6	21	0.3	13:1	22	11
Southwest	6	15	28	1.9	1	0.1	28:1	9	1
South central	8	25	32	1.3	4	0.2	8:1	2	2
Southeast	6	6	2	0.3	0	0	2:0	1	0
South	20	46	62	1.3	5	0.1	12:1	12	3
STATE TOTAL	80	169	505	3.0	26	0.2	19:1	62	14

*Numbers are minimum. Live frogs were not recounted for every observation date; the highest number observed on a transect at any one time was used in the tabulation. However, all sick/dead frogs observed were counted since they are quickly removed by decay and predation.

**Less than 0.1 frogs/run.

of abundance and the occurrence of mortality. Many surveys were conducted in the 3 recommended phases and some sites were checked at least once again for mortality. Thus, at least some frogs present at a station were probably sighted at some point of the survey although numbers reported must be taken as a minimum. The number of live and sick/dead frogs observed per survey run allow general comparisons of frog numbers from year to year but cannot be used to represent an "average" number of frogs present at any given survey station.

Looking beyond the year-to-year fluctuations, the number of frogs observed is believed to be quite low, based on observations of past numbers by Department of Natural Resources personnel and professional frog collectors (Hacker pers. comm.). Mortality ranged from 4% to 23% of live frogs observed statewide for the 3 years of the survey, although it ran as high as 100% on individual transects. Degree of actual mortality is unknown.

The increase in number of live frogs observed per survey run in the central and southern areas in 1975 and the sharp decrease in 1976 may be largely attributable to prevailing weather conditions. The reduction in field effort for 1976 can be ruled out as a factor in that year's decrease because the 1976 survey stations represented most of those that had produced frogs in 1974 and 1975. Thus the results in terms of number of frogs observed can still be compared to previous years. Spring and summer, 1975, were very favorable for leopard frog breeding and foraging while spring and especially summer, 1976, were very unfavorable. The 1974 season might be described as an "average" season. Leopard frogs normally experience population fluctuations but such shifts are especially noticeable when populations are initially low.

Although Table 10 suggests reduced mortality for 1975 in the central and southern areas, phase III mortality checks were not run at many stations in these areas because of warm weather followed by sudden freeze-up. Results from stations that were sampled regularly through late November and early December indicate roughly the same level of mortality as in 1974, and it is possible that similar, though unobserved, mortality may have occurred at other stations. The number of sick/dead frogs observed in 1976 was also low, but may be related to the extreme scarcity of frogs that year. The 1976 ratio of live to sick/dead frogs is artificially high because a few stations produced most of the frogs and very little mortality was noted at these stations.

It is interesting to note that both the number of live frogs and sick/dead frogs observed per survey run remained almost equal during 1974-75 for the east central component of the central study area. This suggests that the region may be experiencing higher or more stable mortality than other regions of the state. The east central region corresponds to the intensive study area for leopard frog population dynamics covered earlier in this report. Both the number of ponds utilized for breeding and the number of breeding frogs per pond were found to be quite low.

The pattern of the locations of live and sick or dead frogs, and of those stations at which no frogs were observed, are shown in Figures 12-14, plotted by civil town. The map showing station

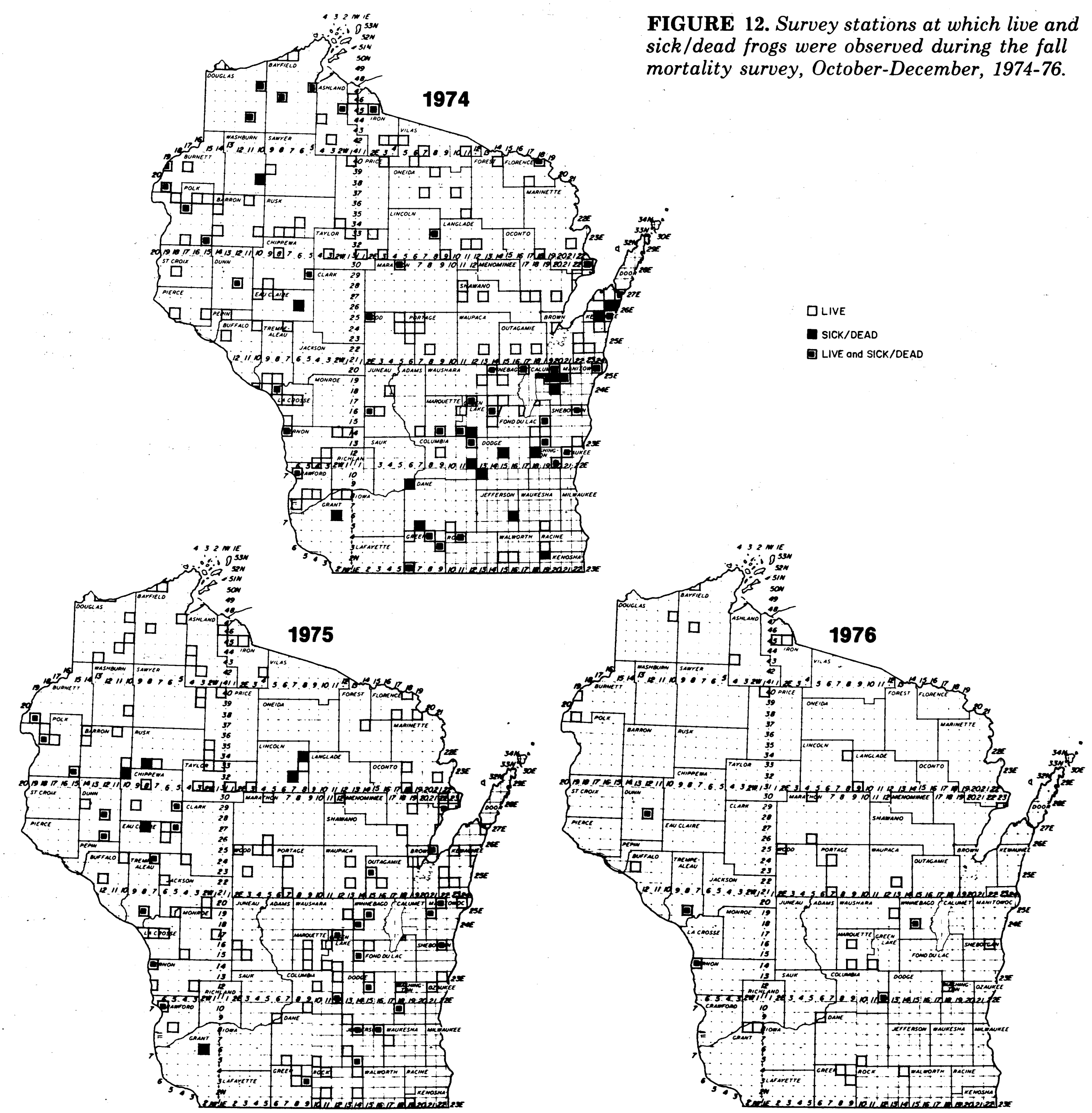
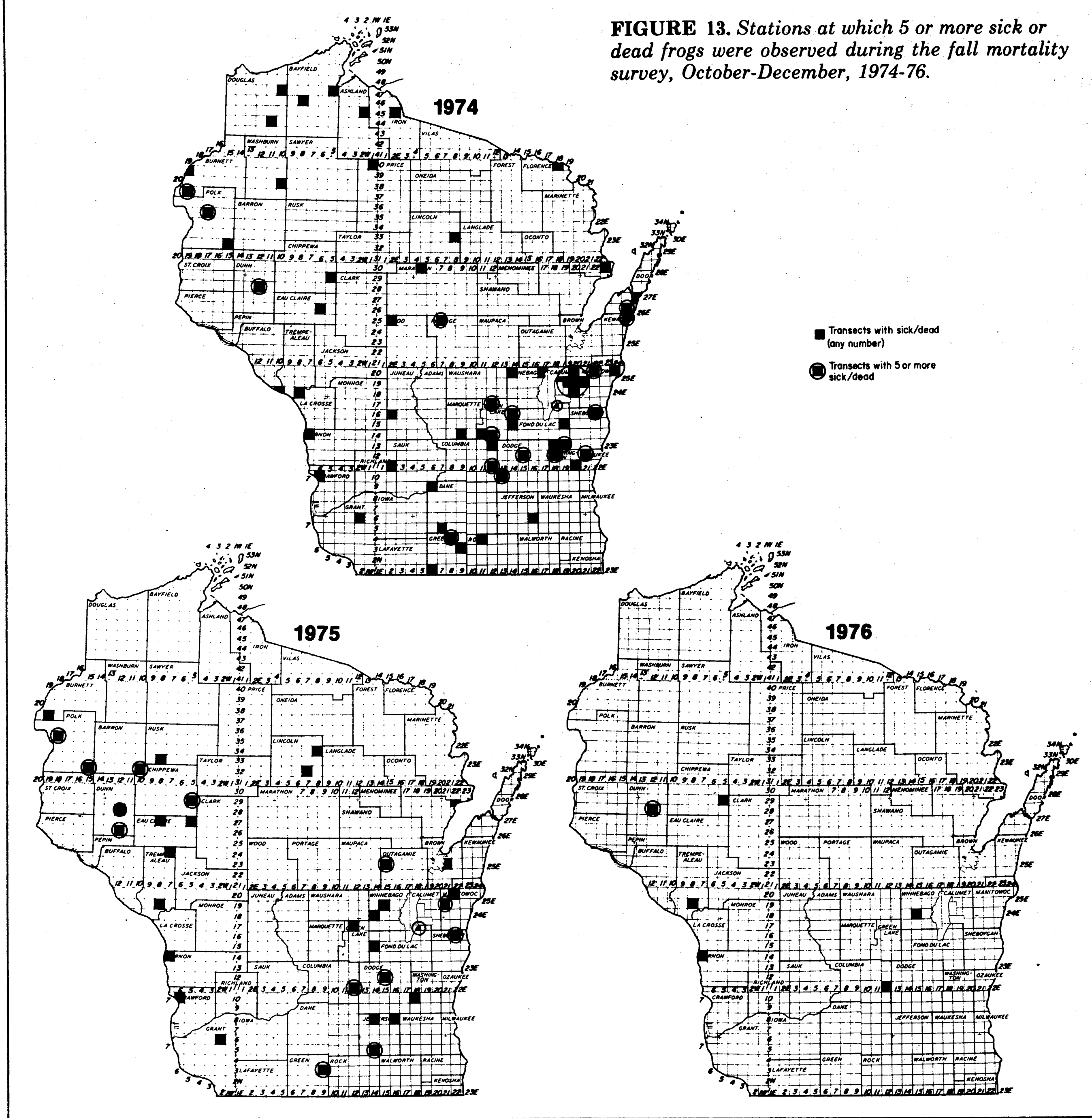


FIGURE 12. *Survey stations at which live and sick/dead frogs were observed during the fall mortality survey, October-December, 1974-76.*

FIGURE 13. *Stations at which 5 or more sick or dead frogs were observed during the fall mortality survey, October-December, 1974-76.*



locations with no frogs represents areas of good frog habitat surveyed under suitable weather conditions. In preparing this figure, information on all the 1974 stations was examined and data from those stations were eliminated where absence of frogs could be attributed to poor time of observation (e.g., early morning hours at cold temperatures) or location of the station in poor wintering habitat (e.g., edges of forested streams). Stations surveyed in 1975 and 1976 all represented suitable habitat and are therefore included.

Live frogs were observed throughout the state although the heavily forested areas of northern Wisconsin showed only scattered, low-level populations. The largest concentrations were usually associated with agricultural-sedge meadow complexes typical of central and southern parts of the state. Mortality likewise occurred throughout the state although it was concentrated in the east central and south central areas. The percent of total stations showing live frogs, sick/dead frogs, only live frogs, and no frogs was relatively constant for the 3 survey years (Table 11).

However, findings for individual stations varied from year to year. During the 3-year period only 4 stations showed mortality each year, 9 stations showed only live frogs each year, and no station showed a total absence of frogs all 3 years. Counting any 2 years of the survey, number of stations producing the same category of frog observations totalled 18 for sick/dead, 61 for only live frogs, and 31 for 0 frogs present. Small aggregations were easy to miss in sampling and this may partly account for the year-to-year fluctuations at stations evidencing small numbers of frogs. However, many previously large-producing stations were barren of frogs in 1976. It is unknown whether the observed differences in station results from year to year represent normal population fluctuations or manifestations of the current depressed population. It is known, how-

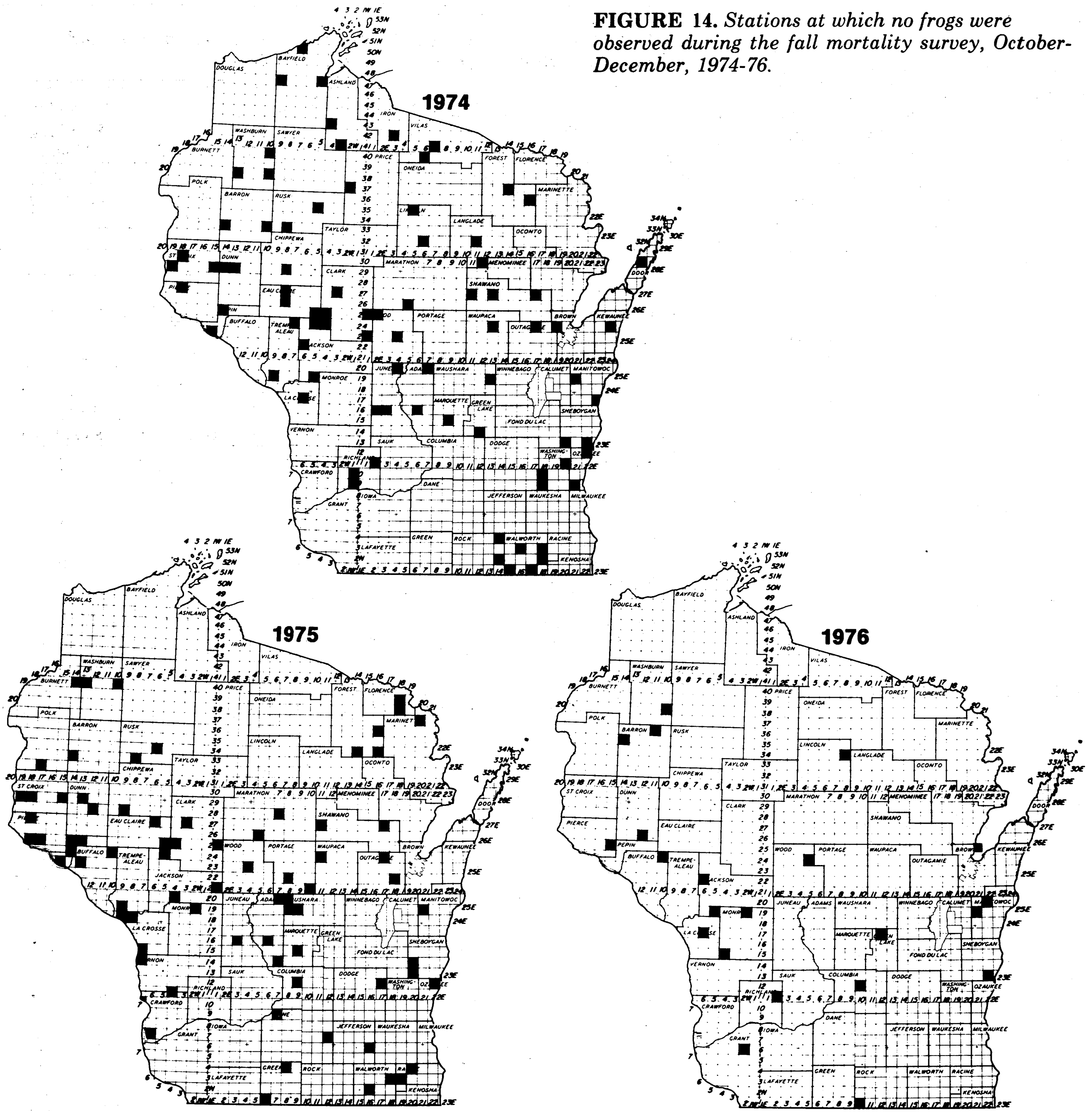


FIGURE 14. *Stations at which no frogs were observed during the fall mortality survey, October-December, 1974-76.*

ever, that in past years commercial frog collectors often relied on the same sites from year to year and only recently have had to search for hibernating congregations.

Very few sick or dead frogs of other species were noted, although observations of these species were only incidental to the leopard frog survey (Table 12). Those sick or dead frogs noted were usually observed in only a few locations in the state.

Pattern of Mortality

A chronology of observations of live and sick/dead leopard frogs is presented in Table 13. Mortality began earliest in northern areas of the state and latest in the southern areas, a pattern that coincided with the spread of cold weather. Increasing mortality was also generally associated with decreasing observations of live frogs which indicated the beginning of hibernation. At individual stations, mortality was first observed at a low level soon after hibernating congregations were formed and increased with time. At a given site, for example, 1 or 2 sick or dead frogs might initially be observed, whereas the number might increase to 50 or more within a few weeks. Limited data suggest that mortality continues throughout the winter. Large numbers of dead frogs were observed in 1975 at 2 nonwinterkill lakes by DNR fish managers shortly after ice-out in the spring and lower numbers were found on 2 others. Occasionally, dead frogs were observed through January and February in southwestern Wisconsin trout streams. An unnamed spring-fed pond in Barron County experienced a large mortality in late December, 1975, a full month and a half after freeze-up of area lakes. Dead leopard frogs have also been observed at hibernating sites in early spring, shortly after ice-out.

Most sick or dead frogs were observed in the water on the lake or stream bed although some were observed in other locations, especially prior to hibernation (Table 14). Live frogs were most often observed on the bank when cool temperatures concentrated them near water. As air temperatures became relatively colder than water temperatures, most frogs were observed in the water or at its edge.

Field observations of abnormalities were not quantified but serve to corroborate laboratory findings. Leopard frogs were observed with ventral skin discoloration, eroded digits, dry skin, and hard muscles. 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. Several healthy-looking frogs did not move when approached in warm weather and died shortly after collection.

Case Histories of Representative Stations

Although uniform procedures were established for conducting the survey, each station became an individual case, often receiving modified handling and producing unique results. Erratic weather, natural variations in habitat, leopard frog behavioral characteristics (many of which are poorly understood), and unknown factors associated with the mortality were largely responsible for the differences, which were heightened by the statewide scope of the survey and the number of individuals involved. However, broad similarities in the results were present, both within some individual stations from year to year and among stations throughout the state. The following case histories are representative of the statewide survey and illustrate the general pattern of mortality as well as some of the problems involved in timing field surveys.

Bullhead Lake. A 67-acre lake located in east central Wisconsin's Manitowoc County. Shoreline development is moderate with the surrounding drainage area composed of 50% residential, 25% wetland, and 25% agri-

TABLE 11. *Number of survey stations where frogs were present and absent during observation periods in fall 1974-76.*

Year	Total No. Stations	Stations with Frogs Present: Only Live	Live + Sick/Dead	Only Sick/Dead	Total	No. Stations With Frogs Absent
1974	378	130 (34%)	47 (12%)	21 (6%)	198 (52%)	180 (48%)
1975	292	136 (47%)	28 (10%)	5 (2%)	169 (58%)	123 (42%)
1976	80	34 (43%)	4 (5%)	1 (1%)	39 (49%)	41 (51%)

TABLE 12. *Observations on species other than* Rana pipiens *during the fall mortality survey, 1974-76.*

Species	1974 Live	1974 Sick/Dead	1975 Live	1975 Sick/Dead	1976 Live	1976 Sick/Dead
Green frog *(Rana clamitans melanota)*	928	15	693	35	318	2
Bullfrog *(Rana catesbeiana)**	128	0	65	0	25	1
Pickerel frog *(Rana palustris)*	65	1	31	0	0	0
Wood frog *(Rana sylvatica)*	28	0	16	0	2	0
Unclassified species	112	2	124	0	9	0
TOTAL	1,261	18	929	35	354	3

*Included green frogs in many instances, especially in 1974.

TABLE 13. *Number of leopard frogs observed per survey month grouped by geographic area.*

Location	Live Frogs Observed* Sep	Oct	Nov	Dec	Sick/Dead Frogs Observed Sep	Oct	Nov	Dec
1974								
North	183	997	58	0	1	50	21	0
Central	61	1,591	1,228	73	0	4	232	246
South	34	4	99	13	0	0	104	290
TOTAL	278	2,592	1,385	86	1	54	357	536
1975								
North	67	895	592	0	0	0	23	52
Central	73	3,838	3,725	7	0	4	84	197
South	0	1,182	2,639	37	0	2	72	15
TOTAL	140	5,915	6,956	44	0	6	179	264
1976								
North	121	65	0	0	0	0	0	0
Central	5	269	12	0	0	17	4	0
South	10	36	22	0	0	2	3	0
TOTAL	136	370	34	0	0	19	7	0

*All leopard frogs observed were counted, including those that may have been sighted more than once on different sampling dates.

TABLE 14. *Number of frogs observed in September-December during preparation for hibernation, summarized by location. Numbers reflect total number of frogs observed and have not been adjusted for possible multiple sightings within a given year.*

Year	Bank Live	Bank Sick/Dead	Water's Edge Live	Water's Edge Sick/Dead	In Water Live	In Water Sick/Dead	Other* Live	Other* Sick/Dead
1974	2,676	125	320	32	1,044	708	301	83
1975	7,199	50	4,183	90	1,539	305	134	4
1976	196	2	273	5	68	19	3	0
Total	10,071	177	4,776	127	2,651	1,032	438	87

*Includes frogs sighted in upland areas, roadways, and iced water surface.

Little Sugar River survey area.

TABLE 15. *Results of fall mortality survey for leopard frogs on Bullhead Lake, 1974-76.*

Date	Live Frogs Number	Live Frogs Location	Sick/Dead Number	Sick/Dead Location	Comments
1974					
4 Oct 74	4	Water edge; lake bed	0	---	Air temperature 50 F; all frogs apparently healthy.
7 Nov 74	7	Bank; water edge	0	---	Air temperature 50 F; 40 frogs of an unidentified species (probably green frogs) observed.
1975					
17 Nov 75	321	Water edge; lake bed	2	Water edge	Live frogs appeared healthy.
1 Dec 75	2	Lake bed	44	Lake bed	Skim ice present; some frogs with red skin discoloration.
5 Dec 75	5	Lake bed	146	Lake bed	Sample taken through ice; many frogs showed severe skin abnormalities; many dead.
1976					
24 Oct 76	0	---	0	---	Good survey conditions; preceded by long cold spell.
15 Nov 76	0	---	0	---	Air and water in 30's (F). Marsh observation area much drier than in past years.

cultural land. The survey station was located in a narrow band of softstem bulrush and cattail at the lake edge. Survey results are presented in Table 15. In 1974, low numbers of live frogs were observed in October and early November sampling, but no sick/dead ones. In 1975, freeze-up occurred on most area lakes by late November and was preceded by unusually warm weather. Most other area lakes were surveyed much earlier than Bullhead Lake, and were completed by mid-November. Many showed the same levels of live frogs as the 17 November Bullhead Lake survey, but subsequent surveys were not made. In 1976, no frogs at all were seen in sampling through freeze-up.

Little Sugar River. A medium-sized stream (average width at study site is 75 ft) located in south central Wisconsin's Green County. The surrounding drainage is 15% forest, 60% wetland, and 25% agricultural. The study area was located in a state-owned wildlife area with reed canary grass the dominant immediate vegetation. Survey results are presented in Table 16. Most of the streambed was not visible due to turbid water and only a limited area could be sampled by a net. Raccoon and bird tracks near the water suggested quick predation of sick or dead frogs in shallow water. Cumulatively, 1975 mortality was significant but most would have been missed if the standard sampling format had been followed. The 1974 survey of Little Sugar River was less intensive, showing moderate abundance of live frogs (both leopard and green) and low-level mortality. Green frog mortality (Table 12) was observed in several locations throughout the state in all 3 years of the survey and was the highest on the Little Sugar River.

Muddy Creek. A small, low-gradient stream (average width at study site equals 6 ft) located in west central Wisconsin's Dunn County. Surrounding drainage is 50% wetland and 50% agricultural. Survey results are presented in Table 17. Muddy Creek showed a similar pattern and level of mortality all 3 years of the survey although survey dates varied from year to year depending on weather patterns. It is likely that further mortality would have been observed if sampling under the ice had been possible. The 14 October 1976 observation of live frogs was the highest observed in the state and was unusual in that dry year.

Population Characteristics

A total of 760 leopard frogs was screened in the laboratory during the 3 years of the fall survey. Results for average length, weight, sex ratio, and egg production are tabulated in Tables 18 and 19. The sex ratio for mature frogs hovered near 1:1 throughout the study.

Immature frogs composed 24-29% of the sample in these years. These figures probably underestimate the true proportion of immature frogs present because smaller frogs may have been less susceptible to collection, and there is evidence that they migrate more erratically than the adults and might thus be more likely missed. Females were slightly longer and heavier than males all three years. Eggs, which are already ripe in the fall, may account for the heavier weight of females. Females sampled in the spring breeding pond survey were also found to be heavier and longer than males.

As expected of normal animals, females without pigmented eggs (macroscopically visible eggs or small unovulated eggs as yet unpigmented) were consistently smaller in both weight and length than females with pigmented eggs (Table 19; Fig. 15). However, 5 adult frogs (> 60 mm) with otherwise developed eggs lacked pigmentation in these eggs, suggesting a diversion from normal oogenesis patterns or pathological oogenesis.

The threshold of egg maturation was 60 mm, with 97% of the females examined above this length being sexually mature (Table 20 and Fig. 15). A large proportion of the frogs less than 50 mm long were immatures, and none had pigmented eggs. Frogs measuring 50-59 mm represented a "zone of overlap", and included immatures (21%), females without pigmented eggs (69%), and a small percentage of gravid females (3%).

TABLE 16. *Results of fall mortality survey for leopard frogs on the Little Sugar River, 1974-76.*

Date	Live Frogs Number	Live Frogs Location	Sick/Dead Number	Sick/Dead Location	Comments
1974					
11 Nov 74	27	Bank	0	---	Found in matted reed canary grass; all appeared healthy.
22 Nov 74	5	Bank	3	Stream bank	Red skin discoloration on sick/dead frogs; 4 live green frogs and 1 sick/dead observed.
4 Dec 74	0	---	0	---	Snowing.
1975					
3 Oct 75	129	Upland; bank; water edge	0	---	2 live green frogs; all frogs appear healthy.
20 Oct 75	35	Bank	0	---	Good survey conditions.
19 Nov 75	1	Bank	0	---	Freezing temperatures night before.
23 Nov 75	5	Bank, water edge	0	---	
25 Nov 75	1	Water edge	0	Streambed	Cold.
5 Dec 75	12	Bank; water	3	Water edge; streambed	4 live, 1 sick/dead green frog; warm.
7 Dec 75	3	Bank	7	Streambed	8 sick/dead green frogs; obvious reddening of skin.
12 Dec 75	0	---	4	Streambed	7 sick/dead green frogs; obvious reddening of skin.
1976					
7 Oct 76	8	Water edge; streambed	0	---	Good sunny conditions; frogs observed were adults.
12 Oct 76	0	---	0	---	1 live green frog.
2 Nov 76	0	---	0	---	Good survey conditions.
5 Nov 76	0	---	0	---	4 live green frogs.
18 Nov 76	0	---	0	---	Cold.
23 Nov 76	0	---	0	---	Cold.

Pathology

Condition of Frogs Received in Madison. Of the 578 leopard frogs received in the Madison laboratory for processing in 1975, 79% were classified as sick, dead, or decayed (Table 21). In 1976 the percentage was 43%. When broken down by sex, the percentage of females classified sick, dead, or decayed was slightly higher than males and immatures for both 1975 and 1976.

Many more frogs were classified as unhealthy (sick, dead, or decayed) in the laboratory than when they were collected in the field, 79% in the laboratory vs. 14% in the field for 1975, and 45% vs. 18% for 1976. Several differences between the 2 sets of observations must be taken into account when interpreting these data: (1) Field observations were made by many different people whose interpretations of the health category may have varied while laboratory observations were made by 1 person. (2) Frogs were held in close quarters 3-7 days between field collection and laboratory processing. (3) Observation of the liver was possible in the laboratory while it was not in the field, and abnormal liver was used in classifying frogs as sick in the laboratory. (4) Some of the frogs listed in the laboratory as dead or decayed may have died from the trauma of transport but this percentage is believed to be low (maximum of 10%).

However, it is very possible that the close confinement of shipping precipitated the emergence of sick symptoms in live frogs and accelerated death. Biological supply houses reported problems in holding leopard frogs in recent years with frogs becoming sick or dying before shipment to buyers.

Gross Abnormalities. Laboratory observations revealed a variety of internal and external abnormalities including ventral skin discoloration, hard muscles, eroded appendages, subcutaneous hemorrhages, eye hemorrhages, body emaciation, and various liver abnormalities (Table 22). Ventral skin discoloration was the most common abnormality, ranging from a slight reddish color on the hind limbs to a bright red color throughout the ventral surface, including the front limbs. Abnormal liver was also common, especially discoloration, which took 2 forms: a cream or muddy color. Normal liver color in freshly dead frogs is brownish-red.

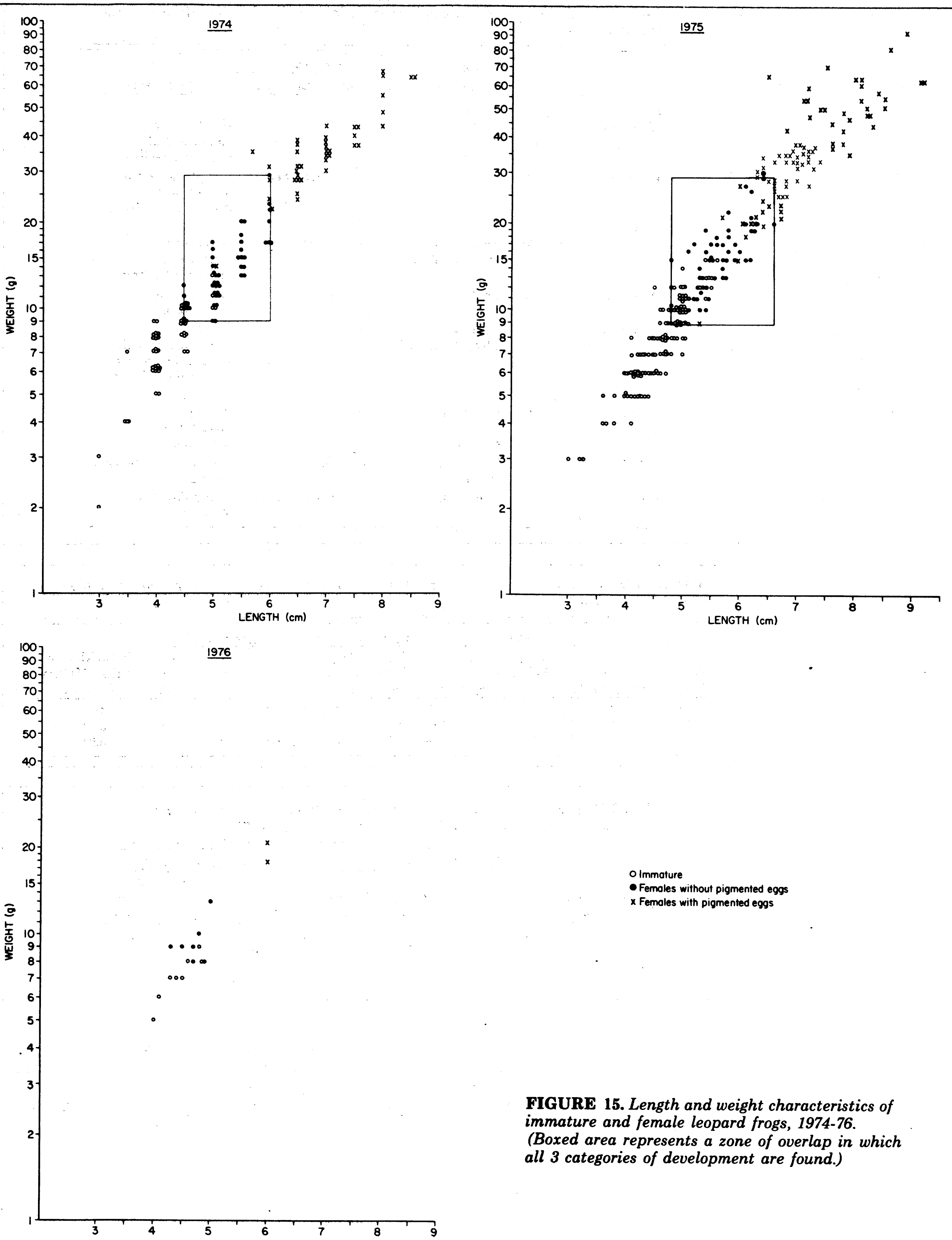


FIGURE 15. *Length and weight characteristics of immature and female leopard frogs, 1974-76. (Boxed area represents a zone of overlap in which all 3 categories of development are found.)*

TABLE 17. *Results of fall mortality survey for leopard frogs on Muddy Creek, 1974-76.*

Date	Live Frogs Number	Live Frogs Location	Sick/Dead Number	Sick/Dead Location	Comments
1974					
28 Oct 74	30	Bank	0	---	Good survey conditions; all appeared healthy.
4 Nov 74	0	---	9	Streambed	Sample taken through ice; red skin discoloration obvious.
1975					
26 Sep 75	30	Bank, water edge; streambed	0	---	Good survey conditions; all appeared healthy.
28 Oct 75	2	Bank		---	Good survey conditions.
18 Nov 75	290	Bank; water edge; streambed	15	---	Good survey conditions though water cold; red skin observed on sick frogs.
1976					
14 Oct 76	109	Bank; water edge; streambed.	0	---	All appeared healthy; good survey conditions.
25 Oct 76	2	Water edge; streambed	20	Streambed	Freezing conditions; 2 dead green frogs.
2 Nov 76	2	Water edge	2	Streambed	Warm day; 1 dead green frog.

TABLE 18. *Sex ratio and average size of leopard frogs collected from throughout Wisconsin in the fall of 1974-76.*

Survey Year	Total Sampled	Males No.	Males Avg. Weight (g)	Males Avg. Length (mm)	Females No.	Females Avg. Weight (g)	Females Avg. Length (mm)	Immatures No.	Immatures Avg. Weight (g)	Immatures Avg. Length (mm)	Ratio Mature Males : Mature Females
1974	214	77	22	60	79	26	62	58 (27%)	8	42	49:51
1975	501	186	21	62	168	28	65	147 (29%)	8	46	53:47
1976	45	18	14	52	16	15	54	11 (24%)	6	42	53:47

TABLE 19. *Presence of eggs, weight, and length of female leopard frogs collected from throughout Wisconsin in the fall of 1974-76.*

Survey Year	Total Sampled	With Pigmented Eggs No.	With Pigmented Eggs Percent	With Pigmented Eggs Avg. Weight (g)	With Pigmented Eggs Avg. Length (mm)	Without Pigmented Eggs No.	Without Pigmented Eggs Percent	Without Pigmented Eggs Avg. Weight (g)	Without Pigmented Eggs Avg. Length (mm)
1974	79	38	48	37.8	70	41	52	15.5	54
1975	135	84	62	37.3	71	51	38	15.7	57
1976	9	2	22	19.5	60	7	78	9.4	47

TABLE 20. *Size classes of immature and female leopard frogs examined during the fall survey, 1974-76.*

	< 50 mm					50-59 mm					> 60 mm					All Sizes	
	1974	1975	1976	Total	Percent	1974	1975	1976	Total	Percent	1974	1975	1976	Total	Percent	Total	Percent
Immatures	53	90	8	151	79%	5	36	0	41	21%	0	0	0	0	0	192	46%
Females without pigmented eggs	1	1	6	8	8%	31	36	1	68	69%	9	14	0	23	23%	99	24%
Females with pigmented eggs	0	0	0	0	0	1	3	0	4	3%	39	81	2	122	97%	126	30%
Total	54	91	14	159	38%	37	75	1	113	27%	48	95	2	145	35%	417	

TABLE 21. *Number of frogs classified as live, sick, dead, and decayed upon laboratory processing in Madison, 1975-76.*

Year	Condition Received				Total All	Total Sick, Dead, Decayed	
	Live	Sick	Dead	Decayed	Conditions	No.	Percent
1975							
Male	48	51	43	44	186	138	74
Female	27	63	38	40	168	141	84
Immature	41	32	16	58	147	106	72
Unreported	4	1	6	66	77	73	95
1976							
Male	11	3	2	0	16	5	31
Female	7	2	5	0	14	7	50
Immature	7	2	5	0	14	7	50
Unreported	0	0	0	0	0	0	0
TOTAL							
1975	120	147	103	208	578	458	79
1976	25	7	12	0	44	19	43

TABLE 22. *Number of leopard frogs exhibiting abnormalities, based on laboratory observation of frogs collected statewide, fall 1974-76.*

External Abnormality	1974*		1975					1976				
	Total	Percent of Frogs Exam.	Slight	Moderate	Severe	Total	Percent of Frogs Exam.	Slight	Moderate	Severe	Total	Percent of Frogs Exam.
Dry skin	11	5	12	5	0	17	3	1	0	0	1	2
Skin discoloration	27	12	87	60	32	179	31	5	4	1	10	23
Hard muscles	–	–	13	14	16	43	7	0	1	1	2	5
Subcutaneous hemorrhages	–	–	3	4	2	9	2	0	0	0	0	
Eroded appendages	–	–	4	7	1	12	2	1	0	0	1	2
Body emaciation	1	1	3	6	0	9	2	0	0	0	0	
Liver abnormality**	–	–	–	–	–	116	21	–	–	–	12	27
Other	23	11	0	0	2	2	0	0	0	0	0	
Frogs examined[1]	218					578					44	

*Hard muscles, subcutaneous hemorrhages, and eroded appendages were lumped in the "other" category for 1974.
**Includes discoloration, surface hemorrhaging, white spots, and enlargement. Primarily external examination in 1974.
[1]An individual frog often had more than 1 abnormality and was thus counted more than once.

TABLE 23. *Gross pathology of leopard frogs sacrificed for bacteriological examination.*

Year	No. Exam.	Presence of Path. Symptoms	No. with Eggs Resorbed/Resorbing		No. Positive *Aeromonas*
			With Path.	Without Path.	
1974	22	19 (86%)	–	–	5
1975	36	14 (39%)	4	5	0
1976	17	9 (53%)	0	2	3

TABLE 24. *Fat body development in frogs sampled in fall, 1975 and 1976.*

Fat Body Development	1975		1976	
	No.	Percent	No.	Percent
Lacking	32	9	8	35
Slight	103	30	9	39
Moderate	110	32	5	22
Extensive	104	30	1	4
No. Frogs	349		23	

For type of abnormality among abnormal frogs only, there is a highly significant difference ($P < 0.01$) in type of abnormality between 1974 and 1975 with or without liver included, but there is no significant difference between 1975 and 1976 (probably due to small sample size).

Blood smears did not reveal the presence of any blood parasites.

Bacteriological examination provided further evidence of pathological conditions among these leopard frogs (R. Simpson pers. comm.). From 39% to 86% of the frogs autopsied had 1 or more pathological symptoms (Table 23). The 3 years collectively differ highly ($P < 0.01$); there was also a highly significant difference between 1974 and 1975, but no difference between 1975 and 1976. An interesting incidental observation was the resorption of eggs. In 1975 eggs were resorbed or being resorbed in 9 females, only 4 of which showed any pathology.

Bacteria. Samples of leopard frogs were examined each year for the presence of bacteria, especially *Aeromonas hydrophila,* one causative agent of the well-known disease "redleg" (R. Simpson pers. comm.). Of 75 frogs examined, only 8 had positive cultures for *Aeromonas* (Table 23). One pooled sample (4-5 frogs) from Winnebago County, run separately from the above, indicated the presence primarily of *Aeromonas.* A number of species of *Aeromonas* were found in addition to *hydrophila.* Other bacterial organisms found were *Pseudomonas* and *Flavobacter.* The livers in these frogs were also a muddy gray color.

Virus. Forty pooled samples of kidneys and spleens collected from leopard frogs in several areas of the state revealed no evidence of virus in either 1975 or 1976 (J. Warren pers. comm.).

Liver pathology. Liver sections indicated degenerative changes. Intracytoplasmic inclusions were found, but their significance in frogs is not known. Inclusions are associated with certain viral diseases in mammals and birds. The presence of hemosiderin in hepatic macrophages suggests antemortem blood destruction. Necrosis of hepatocytes was also observed; such degeneration has been known to accompany ingestion or absorption of a toxic substance (N. Levin pers. comm.).

Responses to Chemicals. Toxicity tests for the herbicide, Atrazine, were run on leopard frog tadpoles in early spring of 1975 (Mauck and Olson 1976). Although there were many potential candidates for screening, Atrazine was chosen because of its wide use in Wisconsin. Tadpoles were exposed for 54 days posthatch to 6 water concentrations of Atrazine ranging from 0.31 to 12 mg/l in an effort to determine effects on survival and growth. Significant mortality did not occur in any concentration prior to 27 days exposure; however, tadpole survival decreased dramatically with continued exposure up to 54 days. Atrazine concentrations as low as 0.31 mg/l significantly ($P < 0.05$) retarded the growth of leopard frog tadpoles, but the difference in growth rates between treatments was insignificant. Tadpoles accumulated residues of Atrazine 5- to 14-fold greater than the water concentration in 54 days. Tadpole survival and growth is reduced by relatively low water concentration of Atrazine. Unexposed tadpoles contained Atrazine residues of 0.9 mg/l, which indicates the presence of the herbicide in leopard frog natural habitat.

Lethal threshold for a 96-hour exposure to Atrazine in *Rana pipiens* larvae in Minnesota was established at 8.5 mg/l by Hovey (1975). Larval leopard frogs obtained from laboratory stock were held until reaching the stage of chromatophore development (Taylor-Kollros Stage I). Groups of 20 tadpoles were distributed to 2-liter glass containers and exposed to various concentrations of Atrazine for 96 hours. Lethal threshold was defined as that level of chemical at which 1 more tadpole died in the exposed group than in the control group.

Flora and fauna. Microscopic examination of the upper 1/3 of the large intestine of the sample of 36 frogs submitted for bacteriological cultures revealed the presence of the following organisms: Opalinids (Lata form), *Trichomonas, Proteromonas, Nyctotherus, Hexamastix, Blastocystis, Bodo;* nematodes; trematode, *Megalodiscus;* colonial green/blue-green algae, *Selenodesmus.*

Fat bodies. Fat body development was slight or lacking in 39% of the frogs sampled in 1975, and 74% of those sampled in 1976 (highly significant difference, $P < 0.01$) (Table 24). Although the relationship of fat body development to overall health has not been established, it is possible that insufficient fat body development may be an abnormality since these tissues apparently are involved in sustaining the frog throughout hibernation.

DISCUSSION AND SUMMARY

Evidence has accumulated from many states and Canada that the leopard frog *(Rana pipiens)* has undergone a decline in recent years (Modern Medicine 1973, Gibbs et al. 1971, Belcher 1974). The decline in Wisconsin leopard frog populations was noticed in the early 1970's by professional frog collectors (R. Robl pers. comm.) and others associated with the use of frogs in scientific and educational work (V. Hacker, DNR, pers. comm.; M. Emmons, NASCO, pers. comm.). According to Robl (pers. comm. 1979) leopard frog populations are still depressed, although there have been 2 years of successful reproduction. Many formerly good breeding areas no longer have leopard frogs and mortality is still observed near hibernating sites in the fall.

In the study ponds located in the East Central Study Area, spring population estimates ranged from 2 to 76 leopard frogs during 1975 and 1976. These estimates are considerably lower than those cited by Merrell (1968) for his study ponds in Minnesota (from 124 to 1,568 adults) in the late 1950's and early 1960's.

Another indication of low populations was the extent of unoccupied habitat. Along transects in 7 counties in east central Wisconsin 83 ponds were identified with suitable leopard frog habitat, but only 16% (in 1975) and 28% (in 1976) were occupied by leopard frogs. Also in the fall, although many transects had leopard frogs, large concentrations were few.

Many animal populations are known to fluctuate in numbers, and amphibians, with their dependency on spring and summer water conditions, are no exception. For example, in 1976 late spring drought conditions depressed the number of ponds producing young close to the previous year's level, even though the number of ponds with breeding activity had quadrupled. It is not known, however, whether these fluctuations in the current low numbers of leopard frogs represent the downside of a natural fluctuation or a more permanent reduction. Only long-term monitoring can answer this question.

Within these low leopard frog populations, breeding phenology, sex and age ratios, size and condition of adults and young, egg production, metamorphosis, and movements generally appeared to be normal, on the basis of comparison of these population characteristics with those published by other investigators.

Large-scale sampling of leopard frogs is difficult. An attempt was made to devise an easily obtainable estimate of the number of frogs in a pond by counting the number of calls heard during a prescribed period and adjusting for the calling rate. The resulting call index was generally lower than the population estimates obtained through mark and recapture techniques, and was not believed to be a reliable estimate of the population density of a pond. However, the call index does appear to be a valid measure of the presence of breeding frogs, and could be used to obtain an index of pond use along survey stations from year to year.

The difficulty in sampling large areas was also evident in the fall mortality survey conducted in 1974-76. This survey was statewide, with 80-378 stations monitored 2-3 times over about a 2-month period each fall. Care was taken to time the sampling periods to correspond with the best chance of observing frogs and documenting mortality. However, weather anomalies and the secretive behavior of leopard frogs made it very difficult to get complete results. It is uncertain how many frogs were missed. Sick and dying frogs are the most difficult to observe, being quickly removed by predation or sinking from sight in deep or turbid water.

Conspicuous and widespread mortality associated with fall hibernation was documented throughout the 3-year study and known to occur for several years prior to the initiation of the study. Robert Robl (pers. comm. 1974) began to notice die-offs in Wisconsin as early as 1958, and by the early 1970's dead frogs were reported from many areas (Hacker 1973 pers. comm., Vogt 1974 pers. comm.). Although no quantitative data exist for past population levels of Wisconsin leopard frogs, biological supply houses reported a large decline over the past 10 years.

Similar mortality has been noted for surrounding states. McKinnell (Modern Medicine 1973) observed large numbers of dead frogs in the field in Minnesota for the first time in 1973. While there had been severe winterkills in Manitoba in the past, Carol Scott (pers. comm. 1974) reported unexplainable deaths on a large scale in 1974, with dead frogs piled several inches deep along a lakeshore and exhibiting reddish coloration on the ventral surface.

A repeated pattern was documented for the fall mortality in Wisconsin. Heaviest mortality was centered in the east central counties, with as many as several hundred sick/dead frogs reported at a single hibernaculum. At individual stations, mortality was first observed at a low level soon after hibernating congregations were formed, and increased with time. Peak mortality was observed just before freeze-up when frogs had entered the water for hibernation. However, the actual mortality peak may occur later after freeze-up when observation becomes extremely limited or impossible. Limited data suggest that mortality continues throughout the winter. Sick and dead frogs repeatedly displayed ventral skin discoloration, dry skin, and hard muscles. Other abnormalities documented were eroded digits, unusual behavior, and discolored liver.

Thorough laboratory analyses ruled out virus and bacterial infections as significant reasons for the mortality. Limited work suggests the possibility that a toxic reaction may be involved. Bioassay on larval leopard frogs exposed to the herbicide, Atrazine, showed that growth and survival were reduced by concentrations of Atrazine as low as 0.31 mg/l. Interestingly, control larvae were found to carry Atrazine residues of 0.9 mg/l. Histopathological examination of liver sections indicated necrotic tissue similar to that produced by toxic accumulations.

Reports to the Department in 1978 and 1979 continue to show the existence of leopard frog mortality in Wisconsin's lakes and streams in fall and early winter. An examination of 10 leopard frogs collected during a die-off in the Oconomowoc River, December 1978 by the U.S. Fish and Wildlife Service laboratory in Patuxent, Maryland, showed no significant amount of insecticides in the tissues. However, no herbicide analyses were made.

It is possible that mortality also occurs away from hibernating sites during late spring and summer. Leopard frogs are widely dispersed at these times, making any mortality difficult to observe and document.

RECOMMENDATIONS FOR FUTURE RESEARCH AND SURVEYS

The objectives of this preliminary study have been met: namely to provide a base of information on leopard frog populations to compare with known data from other states and provinces, and to monitor the observed mortality. But many more questions and avenues of research and survey have been opened up. An intensive study is needed to determine the cause of the observed mortality. Particularly important are histopathological studies, since gross observations suggest a possible inability of leopard frogs to enter and remain in the water at hibernation time, which in turn may be related to abnormal water metabolism beneath the skin and the change from lung to skin respiration (V. Hacker, R. Robl, M. Nickerson pers. comm. 1979). Closer pathological studies of the liver abnormalities are needed.

Preliminary toxicity tests with Atrazine showed a cumulative lethal effect. Intensive laboratory study of the single and combined effects of herbicide residues (and breakdown products) in both leopard frogs and pond water are needed to analyze the possible role of chemical contaminants in producing the mortality observed in the field.

In the meantime there should be continued vigilance. Monitoring of spring breeding populations and fall mortality should be continued. Survey stations should be run in the east central counties — Sheboygan, Fond du Lac, Manitowoc, and Kewaunee — to monitor spring breeding populations. Suitable habitat can be identified using the 7 criteria for an "ideal pond," and the percentage of suitable ponds with frogs present in spring will provide an index to spring breeding populations.

Fall mortality should also be monitored each year through examination of a representative number of areas for dead or dying frogs in late fall *immediately* prior to freeze-up. The timing is extremely critical, for the mortality is not evident before this aggregation at the hibernacula. Intensive, perhaps daily, monitoring of a small number of known hibernacula should be carried out to more thoroughly characterize mortality.

The leopard frog may likely be another subtle indicator of a greatly modified environment. The reasons for this stem primarily from the fact that the leopard frog ranges from lakes and rivers to wetlands to surrounding agricultural fields, and sometimes woodlands, a mile or more from the breeding ponds. Their environment has been and still is changing constantly, and many factors may combine to stress the frog on its breeding, summering, and wintering range. Some factors are slow and constant. Wetland drainage, for example, resulting in considerable loss of habitat, has been occurring in recent years at the rate of 1% per year (Dale E. Marsh, pers. comm.).

Other factors have led to drastically different land use. Agricultural crops planted have not greatly changed, for example, but tillage methods have, shifting from cultivation to higher pesticide use. In addition, higher costs of farming and the fast rates of price increases have intensified farming efforts to obtain higher yields. This has resulted in higher fertilizer and pesticide use, with consequent runoff into ponds and rivers. Effects of these and other environmental pollutants could be direct, or indirect, weakening the frog or increasing its susceptibility to other stresses.

The drama of the leopard frog decline that has been unfolding over the past decade may provide a vital insight into ecosystem health — it must not go on unnoticed or unattended.

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Ruth L. Hine, Bureau of Research, and Betty L. Les, formerly of the Bureau of Fish Management, coordinated and supervised the study, and compiled the final report; Bruce F. Hellmich, formerly of the Bureau of Research, carried out the field investigations and collected and examined the specimens taken during the fall mortality survey.

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