Population Decline of the Mink frog, *Rana septentrionalis* (Anura: Ranidae) in Northwest Minnesota

Running head: TENNESON AND WELLENSTEIN-MINK FROG POPULATION DECLINE

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Abstract. Mink frog populations at all sites investigated showed marked declines in population levels. At two sites in Itasca State Park, Schoolcraft Island and West Twin Lake, mink frog populations declined an order of magnitude over a period of 25 and 15 y, respectively. In addition, the overall sex ratio was female skewed, perhaps due to differential predation of males. Of a variety of potential causes of the decline investigated, none were thought to have singly caused the decline. The decline may have been related to redleg disease, caused by the bacterium *Aeromonas hydrophila*. Redleg symptoms were found in 19% of all frogs in 1982, whereas only two dead frogs with redleg symptoms were reported in in the late 1960´s. Juvenile mink frogs

showed signs of the disease more frequently than adults. Possibly redleg, combined with some other factor such as severe winters, was an important cause of mink frog mortality.

The mink frog (Rana septentrionalis), a small- to medium-sized member of the anuran family Ranidae (46 genera, 560 species worldwide; one genus, 21 species in North America), ranges from Labrador to northern New Hampshire and westward to northeastern Minnesota and southeastern Manitoba. The northern limits of its range are unknown (Hedeen, 1977).

The mink frog is one of the most nocturnal and aquatic North American ranids (Marshall and Buell, 1955; Schmid 1965). This species inhabits areas of slow moving water with dense vegetative cover (Breckenridge, 1944; Hedeen, 1970), and is found away from water only during rainstorms when individuals sometimes move between bodies of water (Hedeen, 1970).

The mink frog oviposits during mid-June to early August (Breckenridge, 1944). The tadpole stage lasts 1-2 y, and tadpoles metamorphose from 24 June to 30 August (Wright and Wright, 1949; Hedeen, 1971) at 29 to 42 mm snout-vent length (SVL) (Hedeen, 1977). Females reach maturity at a larger size (54-59 mm) than males (45-50 mm) (Hedeen, 1972a).

Hedeen, (1972a) used a "sex-index" (Martof, 1956) based on SVL/tympanum diameter to sex mink frogs in the field. This technique was verified by internal examination of the gonads and was found to be accurate for frogs longer than 48 mm (SVL) 98% of the time (Hedeen, 1972a). Mink frogs were sexed as: 1) males if the sex-index was less than 10 and the SVL was at least 48 mm, 2) females if the sex-index was at least 10 and the SVL was at least 48 mm, and 3) juveniles if the SVL was less than 48 mm.

At Itasca State Park, mink frogs are first observed on warm days during spring (April-May). On cool days the frogs remain underwater. Reproductive activity occurs from late June to early August (Hedeen, 1972a). With the onset of cool weather in fall, adults and juveniles move to overwintering sites in deep water and spend only warm days at the water's surface (Hedeen, 1970). The mink frog may not hibernate, as it has been captured under the ice mid-winter in minnow traps (J.C. Underhill pers comm).

Due to the difficulty of finding dense groups of calling males for behavioral ecology studies, an investigation of the distribution and abundance of the mink frog in Itasca State Park was undertaken. Several unpublished reports by students at the University of Minnesota Forestry and Biological Station (Lemmerman and White, 1958; MacDonald and Engebretson, 1959; McKenzie, 1962; Wunderle and Wenstrom, 1970; Peacock and Drake, 1971; Priemer et al., 1973; Caponi, 1976) and Hedeen's (1970) PhD Dissertation were used to determine past population trends and locate appropriate study areas. Observations prior to 1981 suggested a marked decline of mink frogs in the Park.

A decline since the early 1970's was reported in Wisconsin (Hine et al., 1981) and Minnesota (McKinnell et al., 1979) for the leopard frog, Rana pipiens.

Specific objectives of this study are to 1) determine the current abundance of mink frogs at sites previously studied, and 2) compare current estimates with those of previous workers to estimate mink frog population trends.

METHODS AND MATERIALS

The study was initiated on 18 June 1982 and was terminated on 27 August 1982 in Itasca State Park in northwestern Minnesota. This area was chosen for study because it consisted of the same area as an ongoing behavioral ecology project. Mark-recapture data were gathered on 24 nights. Few samples were taken in July due to a conflict with a concurrent behavioral ecology project (Tenneson, in prep.). Twelve initial sites were chosen to compare with previous population estimates in the literature . Of these, five were eliminated because of low mink frog population densities or extreme vegetation density precluding the use of a canoe. The six mark-recapture study sites chosen were Bog D, Deming Lake, Schoolcraft Island, South French Creek Bay, West Arm Bay, and West Twin Lake (Fig. 1). In addition to these six, the population size of

South Deming Pond was estimated by total removal; the captured frogs were used to stock an enclosure on Lake Itasca for behavioral studies.

Each area was sampled in random order once every three to four nights. Investigators used headlamps and hand-lanterns to help locate frogs, which were captured by hand. Frogs were toe-clipped (Martof 1956) and SVL (with the animal pressed flat) was measured to the nearest 1 mm. Tympanum diameter was measured to the nearest 0.1 mm. Frogs were weighed using a Pesola 50 g scale and plastic bag to the nearest 0.25 g. Handling time was 1-2 min per frog and animals were released immediately after being weighed. Individuals were sexed in the manner of Hedeen (1972a). The methods of Seber (1973) were used to determine population densities based on multiple recapture data (Schnabel Index). The SAS (Statistical Analysis System) computer package was used for much of the statistical analysis (SAS Institute, 1982a,b). Nonparametric methods (Siegel, 1956) were used, and a probability of correctly rejecting the null hypothesis of $\underline{P}<0.05$ was considered statistically significant. Comparative population data were obtained from several class reports from the University of Minnesota Forestry and Biological Station (Lemmerman and White, 1958; MacDonald and Engebretson, 1959; McKenzie, 1962; Peacock and Drake, 1971; Priemer et al., 1973; Caponi, 1976).

RESULTS

A total of 314 mink frogs were captured. Of these, 47 (15%) were adult males, 64 (21%) were adult females, and the rest (193 or 63%) were juveniles. Ten males were captured as the result of non-random sampling and were eliminated from the following considerations.

The sex ratio of all captured adults was 1.0:1.4 (males:females). The sex ratio varied from a low of no males at Bog D, Schoolcraft Island, West Arm Bay, and West Twin Lake to a high of 5.0:1.0 at Deming Lake (Table 1). Also, the frequency of each sex-age class (males, females, juveniles) varied between capture sites significantly (Table 1).

Table 2 shows population estimates and confidence intervals for those study sites (Deming Lake, South French Creek Bay, West Arm Bay, West Twin Lake) where recapture numbers were high enough to calculate Schnabel Indices (Seber, 1973). Deming Lake had the largest population (178) and West Arm Bay had the smallest (16).

Mean weight (Table 3) and mean snout-vent length (SVL) (Table 4) varied significantly between study sites for males and juveniles, but not females.

Redleg symptoms (reddish discoloration of the ventral portion of the limbs and body) (Emerson and Norris, 1905), attributed to the bacterium *Aeromonas hydrophila*, was found in 19% of all captured frogs. Juveniles showed signs of redleg more often than either adult sex ($P<0.025$, $G=7.97$, $df=2$, G-test). Small frogs (based on snout-vent length) were not affected significantly more often than large frogs ($P>0.05$, Mann-Whitney U-test). Of the three sex-age classes, only females showed a significant difference in the proportion of affected individuals between study sites ($P<0.05$, $G=14.22$, $df=6$, G-test).

DISCUSSION

There are several possible explanations for the observed sex ratio of 1.0:1.4. First, the observed sex ratio may be biased due to sampling error. Males were found more often in deeper water (resting or calling) than females or juveniles which were found most frequently in dense vegetation near shore. The variability of vegetative cover between study sites may have resulted in one or the other sex having a greater probability of capture. This, however, did not seem to be the case as careful searches were made at all the sites. If anything, males should have been easiest to capture, thus artificially biasing the sex-ratio toward males.

An explanation for the female-skewed sex ratio proposed by Hedeen (1970) assumes that territorial males are more susceptible to predation than females. Hedeen (1972b) observed great blue herons (Ardea herodias) feeding on mink frogs, but had no data indicating that males were taken preferentially over females. Male mink frogs are sometimes active throughout the day during the breeding season (late June through early August). Active males are likely to attract the attention of diurnal/nocturnal predators while establishing or maintaining territories (fighting and vocalizing). Active, territorial male bullfrogs (Rana catesbiana) were the most common category of bullfrog predated by snapping turtles (Chelydra serpentina) (Howard, 1978) at night. A similar situation may be in effect for the mink frog. Large fish are potential predators of mink frogs, but fish appear to find them distasteful (Oring pers. comm.,1981).

Hedeen (1970) found that the sex-age classes (juveniles, females, males) exhibited different movement patterns during the post-breeding season (mid-August until first ice). Most males remained at the breeding sites, although a few dispersed to other aquatic habitats. Females usually occupied shallow non-breeding habitats, and juveniles were much less predictable in habitat preference. Juveniles were observed to emigrate in large numbers from Deming Lake in 1967, although no corresponding emigration occurred at West Twin Lake. Based on these observations, Hedeen proposed several possible stimuli for differential juvenile migration between lakes, indicating that juveniles prefer areas of low population density, abundant food, and little open (non-vegetated) water. These potential differences in habitat preferences, that relate to sex-age classes, may contribute to the inequality in observed sex ratios between study sites.

Comparisons of current population estimates with those of previous workers is found in Table 5. Mink frog population declines (of up to an order of magnitude: Schoolcraft Island and West Twin Lake) were noted at all sites investigated. A portion of the observed decline at Schoolcraft Island may have been due to the removal of mink frogs from that site. A total of 100 adults were removed from the island in 1959 (MacDonald and Engebretson, 1959), and in 1962 71 adults were taken (McKenzie, 1962). However, the Schoolcraft Island population appeared to recover as evidenced by relatively high population levels in the late 1960's. Other sites investigated, to the best of our knowledge, were not subjected to removal of adults from the area.

A 50% decline in leopard frog numbers occurred in the United States during the 1960's (Hine et al., 1981; Hird et al., 1981). Only 16% (1975) and 28% (1976) of suitable habitat sites were occupied by leopard frogs in Wisconsin (Hine et al., 1981). Many formerly good breeding areas in Wisconsin were not used by leopard frogs, and one area (East Central Study Area) studied by Hine et al. (1981) yielded spring population estimates from 2 to 76 leopard frogs in 1975 and 1976. This contrasted with populations of 124 to 1568 during the 1950´s and early 1960´s in similar areas of Minnesota (Merrell, 1968).

Several factors have been proposed as possible causes of frog population declines. These include loss of wetland habitat (Cooke, 1972), toxicity due to pesticides and other chemicals (Hine et al., 1981), and redleg disease (Anonymous, 1973). The Lake Itasca region is part of the Minnesota State Park system and, to the best of our knowledge, no wetlands within the park have been drained. Loss of wetland habitat is probably not a significant factor in the decline of mink frog populations in the Park. Leopard frog population declines in Wisconsin are thought to have resulted from the effects of chemicals (Hine et al., 1981). Periodic administrations of herbicides have occurred in parts of the Park. In the late 1950´s, 2-4-5-T was used to eliminate brush from turnout vistas along the East edge of Lake Itasca. Runoff from these areas would have carried the herbicide into Lake Itasca. In the early 1970´s, a pine regeneration area was sprayed with herbicides to facilitate pine growth. Herbicides may have been carried by runoff into an adjacent sewage lagoon. This same sewage lagoon was later (mid-1970's) sprayed to control cattails, Typha sp. Some herbicides may have then entered Lake Itasca via Bear Paw Creek, which runs between the sewage lagoon and the lake (supposedly isolated from each other by a clay dam). Recently (summers of 1982-3), the boat marina on the East shore of Lake Itasca was treated with an herbicide to remove aquatic vegetation. With the above exceptions, no other sites within the Park have been sprayed (B. Toma, Park Naturalist, pers. comm.). Toxic chemicals may have had an effect on populations of mink frogs on Lake Itasca, but could not have affected populations away from the lake that were not subjected to herbicide spraying. Because the mink frog decline is so widespread, even in areas where no spraying of chemicals occurred, it is unlikely that toxic chemicals played an important role in the decline of mink frog populations in the Park.

One possibility, not yet investigated, is the potential for an epidemic spread of an unidentified virus. McKinnell et al. (1979) found a reduced prevalence of a virus caused adenocarcinoma when leopard frog populations declined. They did not, however, indicate the existence of a causal relationship between viral infection and population decline. The potential effect/existence of an epidemic viral disease could only be refuted/substantiated with laboratory techniques beyond the scope of this study.

The aquatic bacterium, A. hydrophila, has been identified as an important source of mortality in anurans. Captive animals infected with A. hydrophila invariably die within several days unless isolated and maintained at cold temperatures (Emerson and Norris, 1905). It is not known what proportion of frogs infected in the wild die. In studies of leopard frogs Hird et al. (1981) found that A. hydrophila could be isolated from both healthy appearing and obviously ill frogs, indicating that the proportion of infected individuals may have been much higher than the number of animals showing symptoms of redleg. They also reported that the degree of infestation was related to overwintering, such that animals that overwintered were most susceptible to the disease. It was proposed that A. hydrophila was present in the water at all times, and that the combined stress of overwintering and carrying the bacterium may have contributed to leopard frog mortality.

Redleg symptoms were found in 19% of all mink frogs captured, and symptoms occurred more frequently in juveniles than either adult sex. The data reported here for mink frogs contrasted with those of Hird et al. (1981), where it was reported that newly metamorphosed frogs (juveniles) were least affected by A. hydrophila. The Lake Itasca region does experience severe winter conditions, and redleg may contribute to overall patterns of mink frog mortality, along with other factors such as low [O_2] resulting from long, severe winter conditions.

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Fig. 1. Map of six sites in Itasca State Park chosen for mark-recapture studies in 1982.

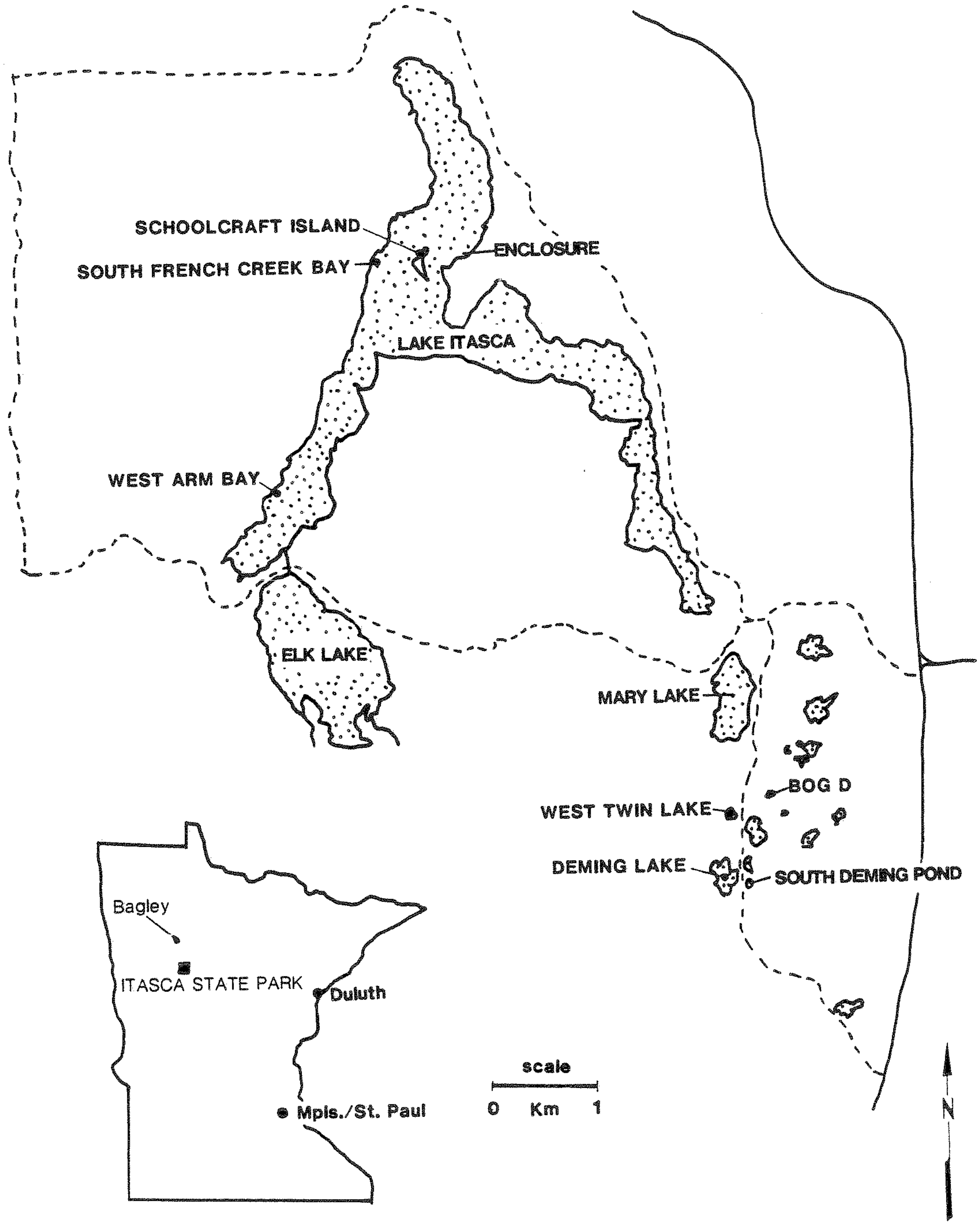
SCHOOLCRAFT ISLAND
SOUTH FRENCH CREEK BAY
ENCLOSURE
LAKE ITASCA
WEST ARM BAY
ELK LAKE
MARY LAKE
BOG D
WEST TWIN LAKE
DEMING LAKE
SOUTH DEMING POND
Bagley
ITASCA STATE PARK
Duluth
Mpls./St. Paul
scale
0 Km 1
N

TABLE 1. NUMBER OF EACH SEX-AGE CLASS CAPTURED AT EACH STUDY SITE. BD=Bog D, DL=Deming Lake, SI=Schoolcraft Island, SDP=South Deming Pond, SFCB=South French Creek Bay, WAB=West Arm Bay, WTL=West Twin Lake.

	Capture site						
Sex	BD	DL	SI	SDP	SFCB	WAB	WTL
Males	0	20	0	10	14	0	0
Females	7	4	1	22	19	2	2
Juveniles	9	97	11	0	25	8	43
Total	16	121	12	32	58	10	45

$\underline{P}<0.001$, $G=181.99$, $df=12$, G-test

TABLE 2. SCHNABEL POPULATION ESTIMATES WITH 95% CONFIDENCE INTERVALS FOR FOUR STUDY SITES.

Study site	N (est)	95% Confidence intervals	
Deming Lake	178	134	257
South French Creek Bay	27	19	37
West Arm Bay	16	3	46
West Twin Lake	42	23	61

TABLE 3. MEAN (AND STANDARD ERROR OF THE MEAN: SE) WEIGHTS FOR MALES, FEMALES, AND JUVENILES FOR EACH STUDY SITE IN ITASCA STATE PARK. Abbreviations the same as TABLE 1.

	Males*		Females**		Juveniles***	
Study site	Mean	SE	Mean	SE	Mean	SE
BD	-	-	11.82	3.10	4.72	1.88
DL	15.15	0.71	15.56	3.54	7.55	0.22
SI	-	-	9.00	-	8.80	0.22
SDP	14.14	1.61	19.47	0.86	-	-
SFCB	18.69	1.51	18.88	2.17	7.54	0.49
WAB	-	-	19.50	2.50	10.32	0.65
WTL	-	-	15.38	0.13	6.90	0.69

*$\underline{P}<0.001$, H=15.87, df=2, Kruskal-Wallis 1-Way ANOVA

**$\underline{P}>0.05$, Kruskal-Wallis 1-Way ANOVA

***$\underline{P}=0.008$, H=15.60, df=5, Kruskal-Wallis 1-Way ANOVA

TABLE 4. MEAN (AND STANDARD ERROR OF THE MEAN: SE) SVL´S FOR MALES, FEMALES, AND JUVENILES. Abbreviations the same as TABLE 1.

	Males*		Females**		Juveniles***	
Study site	Mean	SE	Mean	SE	Mean	SE
BD	-	-	59	2	49	1
DL	56	1	56	3	43	0
SI	-	-	54	-	46	1
SDP	52	6	63	1	-	-
SFCB	61	1	63	1	42	1
WAB	-	-	62	6	46	1
WTL	-	-	55	2	44	2

*$P=0.003$, $H=11.64$, $df=2$, Kruskal-Wallis 1-Way ANOVA

**$P>0.05$, Kruskal-Wallis 1-Way ANOVA

***$P<0.001$, $H=42.58$, $df=5$, Kruskal-Wallis 1-Way ANOVA

TABLE 5. POPULATION DATA FOR SITES ON AND AROUND LAKE ITASCA FROM THE LITERATURE AND CURRENT STUDY. N=number estimated, T=total number observed, A=number of adults of unknown sex observed, M=number of adult males observed, F=number of adult females observed, J=number of juveniles observed. Place abbreviations same as TABLE 1.

Site	Year	N	T	A	M	F	J	Method of estimate	Source
BD	1967		40	23			17	# sighted along 300' transect	Hedeen, 1970
	1982						4	# sighted along 300' transect	present study
	1982					9	7	total #	present study
SI	1955	162						Schnabel Index	MacDonald and Engebretson, 1959
	1956	176						Schnabel Index	MacDonald and Engebretson, 1959
	1958	94			56	39		Schnabel Index	Lemmerman and White, 1958
	1959		122		50	50	22	total #	MacDonald and

							observed	Engebretson, 1959
	1962		71	35	36		total # observed	McKenzie, 1962
	1969	90					Schnabel Index	Wunderle and Wenstrom, 1970
	1970	71					Schnabel Index	Wunderle and Wenstrom, 1970
	1971		21				total # observed	Peacock and Drake, 1971
	1981			2	2		total # observed	present study
	1982				1	11	total # observed	present study
SFCB	1970		50-				total #	Hedeen, 1970
	1973		4-5				total # per unit shoreline	Priemer et al., 1973
	1976		16	11	5		total # observed	Caponi, 1976
	1982	27	58	14	19	25	Schnabel Index	present study
WTL	1966		242				total # observed	Hedeen, 1970
	1967		1480				total # observed	Hedeen, 1970

1982	42	2	43	Schnabel Index	present study