

---

# Increased Prevalence of Mature Females Bearing Pigmented Oocytes from Populations of Minnesota *Rana pipiens*<sup>1,2</sup>

---

Robert G McKinnell, Eville Gorham, Frank B Martin and John W Schaad

**Summary** | Mature female leopard frogs, *Rana pipiens*, collected from several sites in Minnesota from 1967 through 1977, were examined for the presence of pigmented oocytes. An increase in the percent of mature females with pigmented oocytes was observed in the frogs captured in 1977 compared to those captured earlier. Coincident with the increase in prevalence of females with pigmented oocytes, there was a decline in abundance of the frogs.

**Key Words** | Oocytes — Pigment — *Rana pipiens*

The Institute for Laboratory Animal Resources reported that 2 million amphibians, principally *Rana pipiens*, were used each year for research. Only rodents were used in greater numbers. Researchers are becoming increasingly concerned with the health of the frogs they use (1).

Minnesota was a primary source of *R. pipiens* for biomedical research and instruction until 1972. The state was closed for sale of mature frogs in 1975 because of a precipitous decline in frog populations.

Prevalence of the Lucké renal adenocarcinoma in natural populations of *R. pipiens* from Minnesota had been monitored since 1963 (2-6). Necropsies for renal adenocarcinomas revealed an increase in the prevalence of pigmented oocytes among mature female frogs observed in the autumn of 1977. Therefore, the 1977 data were compared with necropsy data of previous years.

## Materials and Methods

**Frogs:** During the period 1967 through 1977, 700 female, wild-caught, non-

conditioned *R. pipiens* (1) were collected by net or by hand. Of this number, 306 were obtained from Diamond Lake, Harrison Township, Kandiyohi County, Minnesota. The remainder (394) were collected from Douglas, Otter Tail, Pope, Scott and Todd counties in Minnesota. Frogs collected from the time of spawning (mid- to late April) through July were not included in this study because pigmented oocytes are not expected during this period. In order to assure that only adult frogs were examined, only those with snout-vent (head plus body) lengths of 65 mm or longer were analyzed.

**Necropsy:** The frogs were killed by pithing the brain and spine (1). Sex and snout-vent length were recorded, and the animals were necropsied under a magnifier (3X) with fluorescent illuminator. The presence or absence of pigmented oocytes was recorded. The degree of maturation of the oviduct was observed if no oocytes were pigmented.

**Statistical methods:** The Chi square test with two degrees of freedom was used to test 2 X 3 tables of the prevalence of oocyte pigmentation.

## Results

Some females had ovaries without any darkly colored oocytes. Other females had ovaries that were comprised of great masses of darkly colored oocytes and, of course, im-

---

<sup>1</sup>From the Department of Genetics and Cell Biology (McKinnell and Schaad), the Department of Ecology and Behavioral Biology (Gorham), and the Statistical Center (Martin), University of Minnesota, Saint Paul, MN 55108.

<sup>2</sup>This investigation was supported by grant RR01072 from the Division of Research Resources, National Institutes of Health.

**Table 1**Prevalence of female *Rana pipiens* collected in Kandiyohi county, Minnesota, without pigmented oocytes

	1967	1971	1977 <sup>a</sup>
Females without pigmented oocytes	7	31	1
Females with pigmented oocytes	42	159	66
Females without pigmented oocytes (%)	14.3	16.3	1.5

<sup>a</sup> Significantly different from earlier observations  
Chi square = 9.91,  $p < 0.01$

mature unpigmented oocytes that would have grown in subsequent years.

The prevalence of female frogs without pigmented oocytes collected in Kandiyohi County in 1977 was about one-tenth that of 1967 and 1971 (Table 1). There was a significant difference between the frogs collected in 1977 and those collected earlier ( $p < 0.01$ ).

**Table 2**Prevalence of female *Rana pipiens* collected in Douglas, Otter Tail, Pope, Scott, and Todd counties, Minnesota, without pigmented oocytes

	1967-1968	1971-1972	1977 <sup>a</sup>
Females without pigmented oocytes	29	19	2
Females with pigmented oocytes	158	65	121
Females without pigmented oocytes (%)	15.5	22.6	1.6

<sup>a</sup> Significantly different from earlier observations  
Chi square = 20.7,  $p < 0.005$

The results of the collections made in Douglas, Otter Tail, Pope, Scott and Todd counties are shown in Table 2. Fifty female frogs did not have pigmented oocytes among the 394 frogs with snout-vent length 65 mm or greater. A decline in prevalence of mature female frogs lacking pigmented oocytes was observed in 1977 ( $p < 0.005$ ). Mature oviducts were present in all frogs that were studied.

## Discussion

All frogs studied were considered to be sexually mature because of the presence of mature oviducts. Additionally, it should be noted that wild-caught females with snout-vent lengths as short as 49 mm and

laboratory-reared females as short as 40 mm may have pigmented oocytes (George W Nace, The Amphibian Facility, University of Michigan, Ann Arbor, MI, 9 May 1978, personal communication).

Coincident with the increased prevalence of pigmented oocytes reported here, there has been a decline in abundance of *R. pipiens* in Minnesota. There was evidence of decreasing abundance over the past 7 years dating from the summer of 1971 (Philip Economon, Department of Natural Resources, State of Minnesota, Saint Paul, MN, 12 January 1978, personal communication). An average of 7.5 mature frogs were sighted per 1,000 yards walked by Department of Natural Resources personnel in 12 Minnesota counties in 1975. The Department judged from these data that frog populations were at a very low level during that year. The summer of 1976 census revealed that frogs were reduced by another 50% in the 12 counties surveyed in 1975. The census of summer 1977 showed continued decline statewide except for isolated populations. Field observations by the senior author since 1965 also indicated a population decrease in Minnesota frogs.

It is suggested that there may be a correlation of increased prevalence of oocyte pigmentation with a reduction in frog abundance. It is postulated that during the late 1960's, the environment of Minnesota was so favorable that not only healthy frogs but those failing to produce mature oocytes survived. Many of the frogs were afflicted with renal tumors during the years of abundant frogs (2-4). Frogs became scarce during the 1970's, suggesting intense selection pressure. Renal tumors virtually disappeared (5). Presumably, genotypes tending to produce poor oocytes, as evidenced by lack of pigmentation, were selected against during the period of frog scarcity of the 1970's. Alternatively, it could be postulated that crowded conditions in the 1960's created an environmental stress resulting in poor oocyte quality.

## References

1. Nace G W, Culley D D, Emmons M B, et al: *Amphibians: Guidelines for the Breeding, Care, and Management of Laboratory Animals*, National Academy of Sciences, Washington, 1974
2. McKinnell R G: Incidence and histology of renal tumors of leopard frogs from the

north central states. *Ann NY Acad Sci* 126:85-98, 1965

3. McKinnell R G: Lucké renal adenocarcinoma: Epidemiological aspects. In *Biology of Amphibian Tumors*, p 254-260, Mizell M, Ed, Springer-Verlag, New York, 1969

4. McKinnell R G, Ellis V L: Epidemiology of the frog renal tumour and the significance of tumour nuclear transplantation studies to a viral aetiology of the tumour - A review. In *Oncogenesis and Herpesviruses*, p 183-197, Biggs P M, de-The G, Payne L N, Ed, In-

ternational Agency for Research on Cancer, Lyon, 1972

5. McKinnell R G, Gorham E, Martin F B, et al: The benefits of catastrophe: A major reduction in tumor prevalence associated with greatly diminished frog populations. *Proc Am Assoc Cancer Res* 19:212, 1978

6. McKinnell R G, McKinnell B K: Seasonal fluctuation of frog renal adenocarcinoma prevalence in natural populations. *Cancer Res* 28:440-444, 1968