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Rana sphenocephala (Southern Leopard Frog) Malformities Found in Illinois with Behavioral Notes

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ABSTRACT

Several malformed southern leopard frogs (*Rana sphenocephala* [*R. utricularia*]) were discovered in a small pond in Southern Illinois. Deformities included missing limbs, split limbs, complete but abnormal limbs, missing eyes, and partial limbs. This study appears to report the first Illinois case of multiple malformities at one site and is apparently the second reported case of malformed southern leopard frogs in the country. The source pond of these frogs needs further study to resolve the many questions about these malformities at this site.

INTRODUCTION

Isolated cases of amphibian malformities have been news since 1920 (Adler, 1958; Anonymous, 1944, 1945. 1954, 1962, 1964; Banta. 1966: Bishop, 1947, 1949: Bishop and Hamilton, 1947; Bjount, 1935; Charles, 1944; Colton, 1922; Cooper, 1958; Cunningham. 1955; DuBois, 1979; Hauver, 1958; Hebard and Brunson, 1963; Hovelacque, 1920; Martinez, et al.,1992; Meyer-Rochow and Koebke, 1986; Mizgireuv, et al., 1984; Reynolds and Stevens, 1984: Rostand, 1958; and Vershinin, 1989). Although reports of malformed Amphibia are not new, the frequency of these reports has increased dramatically in recent years, especially since 1996 (Minnesota Pollution Control Agency [MPCA],1997). Early reports were very rare. By 1995, however, 91 of 95 frogs collected at one site in Meeker Co., Minnesota were abnormal and 54 of Minnesota's 87 counties had reports of deformed frogs (MPCA, 1997).

In Vermont, Ferguson and Levey (In: Allen, 1996) collected more malformed frogs in one day than had been recorded in the state in the past 75 years. Today reports are coming from all around the country as can be seen on the web site of the North American Reporting Center for Amphibian Malformities: http://www.npsc.nbs.gov/narcam/reports/reports.htm. Despite the high levels of industry and agricultural use in the Midwest, few deformities have been reported in this region. Illinois had but one isolated case of amphibian malformities (Assoc. Press, 1996). The present paper describes the anatomy and some related behaviors of a series of malformed southern leopard frogs, *Rana sphenocephala* (*R. utricularia*) discovered in Southern Illinois.

METHODS

On 20 and 25 July 1997 migrant juvenile southern leopard frogs (*R*, *sphenocephala*) were collected over 2 intensive hand search periods from a small, 1/4 acre, wooded pond on the Southern Illinois University at Edwardsville campus, Edwardsville (Madison County), IL. The 20 July search took place between 1100 and 1200 hours. The 25 July collecting period was performed between 1200 and 1400 hours. The temperature on these days was 35 – 38°C.

RESULTS

All frogs were collected at random from under coarse woody debris on the banks of the pond. Of the 31 *R. sphenocephala* collected on 20 July, one was found on open mud, 25 were found under fallen bark and small (under 5 cm diameter) logs, and four were found under large logs. One normal juvenile bullfrog (*R. catesbeiana*), one normal migrant juvenile gray treefrog (*Hyla versicolor*) identified during spring breeding chorus, and 45 normal migrant juvenile small-mouthed salamanders (*Ambystoma texanum*) were recovered from under bark and logs as well. *Rana sphenocephala* were only found under loose debris. On 25 July, I collected 35 post migrant juvenile *R. sphenocephala*, 32 from under fallen bark and three from under small logs. Thirty-seven post migrant *Ambystoma texanum* were also found. All *A. texanum* and normal *R. sphenocephala* were released.

Upon examination, one *R. sphenocephala* was discovered to possess a split-leg malformity, giving the appearance of five legs. Four other frogs in the sample also showed morphological aberrations of questionable causation. These cases appear to represent the second documented report of malformed frogs in IIIinois (Assoc. Press, 1996), and the first series of such individuals from one location. In addition, there has been only one other report of malformed *R. sphenocephala* in North America (North American Reporting Center for Amphibian Malformations, pers. comm., 1998).

Anatomical Descriptions and Possible Causes

The five-legged frog possessed a "split limb" malformity creating the appearance of a fifth limb (fig. 1). The forelimbs and left, rear limb were normal. The extra limb was representative of a typical left leg, and was complete from the tibiotarsus distally. The extra limb extended cranio-dorsally from the joint of the right tibiotarsus and femur, but the normally-ventraI surface faced cranially. The primary right leg was positioned normally, but possessed an incomplete, shortened femoral section shared by both extensions of the split-limb. A swollen area containing the femur appeared on the ventrurn of the pelvic region. This frog died 3 August 1997.

A second frog had a minor, almost unnoticeable malformity in the right rear limb. This leg was not held in the normal sifting posture usually observed in frogs. Typically, the tibiotarsus of the rear limb can be folded flush against the femur portion of the leg in preparation for jumping. Instead, the angle between the femur and tibiotarsus was never held closer than 20 to 30 degrees. Since every other frog in the sample held their rear legs normally, it suggests this was also a developmental abnormality. An alternative explana-

tion is that this frog suffered an injury such as a sprained joint causing it to be unable to fold its leg properly.

The other three frogs could actually represent survivors of predation events, but they are included here because of their association with the split-limbed frog. The third frog was missing its right eye (fig.1). The tibiotarsus of its left leg was visibly shortened, and was missing any remnants of a foot. The right leg was normal through to the distal joint of the tibiotarsus. The right foot was reduced in size and lacking digits. The missing parts of the hind legs may have been caused by crayfish which prey on young froglets as they metamorphose (Tucker, Illinois Natural History Survey, pers. comm., 1997). The fourth frog appeared normal except for the complete absence of a right rear leg.

The fifth frog was missing its front right limb from the elbow distally. The distal end of this limb was swollen and red as if infected or recently amputated. Still, it was similar to pictured malformities of *Ambystoma* induced by Pietsch (1991). This individual was released at the pond.

The cause of these malformities remains unknown, but several alternatives have been suggested at other sites. Harkey and Semlitsch (1988) provided evidence that ornate chorus frog (*Pseudacris ornata*) larvae developing at temperatures between 20°C and 25°C tended to result in relatively fewer malformities than those raised at extremes of 15°C and 30°C. Frogs raised at extreme temperatures often developed partially and totally nonfunctional rear legs. Muto (1969) found that improper temperatures could induce skeletal malformities in unidentified toad larvae (*Bufo*) as well. Air temperature trends during April 1997 were not typical for this region (pers. obs.). Whether acute exposure to freezing temperatures can induce malformities such as those seen at this site is unknown. If *R. sphenocephala* are similarly affected by temperature, the unusually cool temperatures of spring and summer of 1997 may have contributed to malformities at this site.

This pond is located near grass fields that are harvested for hay. Although I have not observed fertilizer or pesticide application on these fields, I have observed personnel of Southern Illinois Unviersity, Edwardsville applying pesticides or herbicides along buildings and lamp posts with "high volume sprayers" with garden hose attachments. This procedure was very sloppy. Chemicals were captured by the wind and the volume of water created runoff. Such spraying could be a partial causal agent of these malformities. Hind limb amphibian malformities have been connected to agricultural use (Bonin. et al., 1997; Hecnar, 1995; Marchal-Segault and Ramade, 1981; Oellet. et al.,1997; Pawar, et al., 1983: Pawar and Katdare,1984; Rzehak, et al., 1977; Schuytema, et al., 1991). If fertilizers or pesticides were applied to these grasslands, this may explain the Edwardsville occurrence.

Methoprene is a commonly applied mosquito control that has been connected to amphibian malformities (Conlon,1996). This compound breaks down into a compound structurally and functionally similar to retinoic acid. Retinoic acid stimulates limb regeneration and has been shown to induce malformities in Amphibia when present at improper levels (Madden, 1993; Pietsch,1991). Mosquitoes are a Public health concern in the St. Louis area due to occurrences of meningitis and encephalitis, consequently, mosquito spraying

is widespread. If Methoprene was applied, it might have mimicked the effects of retinoic acid causing malformities like I have observed.

Cummins (1987) showed that common frog tadpoles (species unknown) developed increased frequencies of deformities when raised at a low pH. This pond received a lot of runoff directly from a neighboring parking lot. Runoff caused the vertical depth of the pond to increase about 5 m above its low point the previous summer. It is possible that products used to melt ice or compounds in the asphalt reduced the pH causing these problems. It is also possible that the asphalt itself is responsible. Furthermore, the fact that this pond is not stream fed, but obtains most of its water supply from the parking lot's runoff, reduces the opportunity for its buffering prior to entering the pond. Such an effect could reduce the pH of the pond significantly. Rain in our area is much more acidic today than in the past. In 1955 it was above 5.6 and by 1984 it was less than 4.5 (Chiras. 1991).

Another possible cause of this problem was suggested for *Hyla regilla* and *Ambystoma macrodactylum* by Sessions and Ruth (1990). They found that a parasitic trematode uses amphibians as intermediate hosts. It buries into the cloacal region of the tadpole and forms a cyst. The presence of this cyst mechanically interferes with limb development resulting in supernumery rear limbs. Since I observed many malformity types other than supernumery hind limbs, this explanation is not adequate.

Whatever the teratogenic factor, the fact that deformed frogs are now documented in Illinois makes further research in this state desirable. This pond is easily accessible for study, and easily enclosed by drift fence materials to monitor migrating juveniles. Such a study would contribute much to our understanding of this problem in Illinois. These specimens were delivered to Dr. Chris Phillips for deposition in the Collection of the Illinois Natural History Survey, Champaign, IL (accession numbers not yet available).

Behavioral Observations

The split-limb impeded the frog's ability to jump. When jumping forward**,** the extra limb created resistance that prevented much forward movement. Most movements by this frog were restricted to "walking". In addition, the small femur of the 5-legged frog reduced the leverage necessary for saltatory movement. This frog had great difficulty righting itself when turned on its back. The inability to effectively escape predators due to its lowered mobility and dexterity, would make survival of this frog in the wild unlikely.

Its reduced jumping ability also affected the feeding success of the split-limb frog, When a dewinged housefly was dropped on the water surface 1-2 cm from the frog, the frog had not successfully captured it after 6 attempts. The same fly was easily captured by a normal frog in one attempt. It seems that the normal attack behavior of *R. sphenocephala* could not be performed due to the opposing resistance of the extra limb experienced while lunging forward after prey. The only time it could catch prey was when flies were dropped directly on the frog's nose. Consequently, it is unlikely that this frog could survive very long even if it did escape predation.

All the malformed frogs appeared less active than normal individuals. These frogs were held temporarily in a 40 L aquarium. Despite constant movements of the normal frogs, the malformed frogs remained within 5 cm of their original placement in the tank 20 min,

1 hr, and 3 hrs later. This apparently low activity level may be reflective of these frog's malformities and the difficulty with which they moved. It may also represent a selective behavior on the part of these frogs. Frogs with malformities (which reduce their potential for mobilization and consequent escape) are more susceptible to predators. If these frogs continually move about in a habitat filled with snakes, bullfrogs, and other predators; they will quickly be removed from the population by these frog-eaters. Alternatively, if frogs carrying such malformities remain still and resist fleeing; they are less likely to be attacked -especially by movement dependent predators. In such cases these frogs could survive longer, increasing their potential fitness. Reduced activity due to carbaryl has been reported in tadpoles (Bridges, 1997; Semlitsch, et al., 1995).

It would be interesting to raise a number of these frogs to adulthood to determine their survivorship in the absence of predators, the heritability of their malformities, and to investigate some of the behavioral changes necessary for their persistence. It might also be interesting to look at the influence of these deformities on mate choice. Are females able to recognize malformed males visually or by their call alone? Or would these deformities affect the male's reproductive success at all? It would also be interesting to examine the actual economic costs of toxic exposure incurred by amphibians utilizing optimality models.

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Figure 1. Southern leopard frogs, *Rana sphenocephala* (*R. utricularia*) found in Edwardsville, Madison County, Illinois. One frog has its right rear leg malformed as a split limb. It showed reduced activity and had difficulty catching food. The second frog has a missing left eye, reduced and disfigured backbone, and is missing portions of its rear legs. This frog also showed reduced activity levels compared to normal frogs.