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First Record and Notes on the Ecology of the Boreal Chorus Frog (*Pseudacris maculata*) in Arkansas

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Pseudacris maculata, boreal chorus frog, is a small hylid frog found throughout the midwestern United States. In northwestern Arkansas, all *Pseudacris* were previously referred to as *P. triseriata* (Trauth *et al*. 2004). However, the majority of populations of *P. triseriata* in Arkansas were redescribed as *P. fouquettei* (Lemmon *et al*. 2008) based on genetic data from Lemmon *et al*. (2007). Based on these genetic data, Lemmon *et al*. (2007) suggested *P. maculata* occurs in extreme northwestern Arkansas; however, no specimens of *P. maculata* from Arkansas were included in the study. Thus, our study was conducted to confirm occurrence in the state and to examine the ecology of this species in Arkansas, specifically regarding habitat, diet, reproduction, and parasites.

Populations of chorus frogs were sampled from select areas of the northwestern portion of the state (Benton and Madison counties) to determine if *Pseudacris maculata* occurs in Arkansas as suggested by Lemmon *et al*. (2007). Between March 2008 and March 2016, opportunistic data were collected during the spring breeding season by listening for breeding choruses of male *Pseudacris* frogs in roadside ditches, fishless ponds, and ephemeral wetlands (Fig. 1). When a *Pseudacris* population was located, a sample of individuals was collected and identified using mitochondrial DNA analysis. Methods for the mitochondrial DNA analysis followed Moriarty and Cannatella (2004). DNA was extracted from tissue using the Qiagen DNeasy kit. Two primers (16sc/16sd) were used to amplify the region of the 16S rRNA mitochondrial genes via polymerase chain reaction. When *P. maculata* were positively identified, a subsample was necropsied for parasite infections, diet, and reproductive notes. Specimens were placed in individual bags on ice and within 48 hrs frogs were overdosed with a 10% v/v ethanol solution (HACC 2004). A mid-ventral incision from mouth to cloaca was made to expose the gastrointestinal tract. Specimens were examined for select protists, including the gall bladder for myxozoans and the rectum for opalinids and ciliates (McAllister 1987; 1991). For helminths, the entire gastrointestinal tract was examined. Trematodes were stained with acetocarmine and mounted in Canada balsam for identification. Reproductive status of females was noted by the presence of ovarian eggs. When females were gravid, clutch size was determined by counting yolked ovarian follicles. Additionally, food items were identified to the lowest taxon possible.

Voucher specimens of parasites that were new host records were deposited in the Harold W. Manter Parasitology Lab (HWML), Lincoln Nebraska. Voucher specimens of *Pseudacris maculata* were deposited in the Sternberg Museum of Natural History (MHP), Fort Hayes, Kansas, Henderson State University Herpetological Collection (HSU), Arkadelphia, Arkansas, and Arkansas State University Herpetological Collection (ASUMZ), State University, Arkansas.

The only confirmed site for *Pseudacris maculata* was in Benton Co. near Pea Ridge (N 36°27’26; W 94°03’36). On 2 March 2008, a male *Pseudacris* frog was the first specimen from Arkansas to be genetically identified as *P. maculata* (MHP 14025). All other populations that we sampled were genetically confirmed to be *Pseudacris fouquettei.* Ten *P. maculata* were collected in 2015 and 2016, respectively. We collected limited data on food habits of *P. maculata* as only a few frogs that were necropsied contained food in their stomachs. However, 3 of 20 frogs had a single food item each: terrestrial isopod, gastropod (Hydrobidae), and Hirudinae (only contained half of the mid body). Most breeding activity that we observed occurred during February and March at this site with calling choruses and both males and females present. Three female *P. maculata* collected were gravid and had the following clutch sizes: SVL 28 mm- 480 eggs; SVL 29 mm- 185 eggs; SVL 30 mm-371 eggs. Three species of

endoparasites were found in *Pseudacris maculata*: *Opalina* sp., *Myxidium melleni,* and *Langeronia microcirra* (HWML 98399). *Opalina* and *Myxidium* were collected in 2015 with 2 of 10 frogs infected with *Opalina* and 4 of 10 frogs infected with *Myxidium. Langeronia* were collected in 2016 with 6 of 10 frogs infected with an average of 2.5 trematodes per host (range 1—5).

Figure 1. Typical breeding habitat of *Pseudacris maculata*.

This study is the first to report a genetically confirmed population of *Pseudacris maculata* in Arkansas. The breeding season we observed in Arkansas is similar as previously reported elsewhere (Dodd 2013). Our egg count range of 185—371 falls within the reported range of 137—793 (Pettus and Angleton 1967). Our limited data over food habits do not elucidate much regarding diet. However, chorus frogs eat mainly small invertebrates (Dodd 2013) as we found in our study.

Opalina sp. and *Myxidium* have been reported from every hylid host that inhabits Arkansas (Muzzall and Sonntag 2012; McAllister *et al*. 2013), including the newly documented *Hyla squirella* from Arkansas (Connior *et al*. 2014). Both of these parasites are ubiquitous in amphibians. The trematode *L. microcirra* is a new host record and distributional record for the state. Although we were only able to confirm one population of *P. maculata,* we suspect further systematic distributional surveys will produce additional breeding populations within the extreme northwestern portion of Arkansas. In fact, during March 2020, some small populations of chorus frogs were heard in the vicinity of the known locale but were not collected or analyzed for species identification.

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