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SHORT COMMUNICATIONS

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Observations on Myiasis by the Calliphorid, *Bufolucilia silvarum,* in the Eastern American Toad (*Bufo americanus americanus*) from Southeastern Wisconsin

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ABSTRACT: Larvae of certain species of blowflies (Calliphoridae) cause myiasis in amphibians which may result in significant mortality, yet there are few reports from North America. In this study, we observed primary myiasis in a population of juvenile eastern American toads (*Bufo americanus americanus*) collected during May–July 1998 from southeastern Wisconsin (USA). Nine (6%) of 140 toads were infected by the green blow fly (*Bufolucilia silvarum*) with a mean intensity of 10.5±7.2 (range=1–24). Weekly parasite prevalence and mean intensity remained low, ranging from 0–20% and 2±1.4 to 14±6, respectively. We found: 1) flies lay eggs on healthy toads, 2) eggs hatch with first instar maggots penetrating under the skin, 3) maggots develop to mature third instars within 5–7 days, 4) maggots leave the host and form pupa within 8–11 days of hatching, and 5) maggots pupate within 7–9 days at room temperature. All infected toads died within 1–2 wk as a result of the infection. The low prevalence observed in this study and other reports of this species from mammalian and bird carcasses indicated that *B. silvarum* is probably a facultative parasite of toads and other amphibians in the United States. This is the first report of *B. silvarum* causing myiasis in Wisconsin amphibians and the first report in eastern American toads in the United States.

Key words: American toad, amphibian mortality, *Bufo americanus americanus, Bufolucilia silvarum,* myiasis, Wisconsin.

Larvae of certain species of blowflies (Calliphoridae) cause myiasis in amphibians, and can cause substantial mortality (Dasgupta, 1962; Reichenback-Klinke and Elkan, 1965). During 1998, the blowfly (*Bufolucilia silvarum*) was found infesting juvenile eastern American toads (*Bufo americanus americanus*) in southeastern Wisconsin (USA). Members of this genus have been reported as obligate parasites of amphibians, particularly toads in the genus *Bufo,* but the natural history and ecology of species causing myiasis in North American amphibians are poorly known.

In Europe *Bufolucilia bufonivora* deposits eggs on the back and flanks of the amphibian host. The larvae hatch and migrate to the head reaching the nasal cavities and eyes, where development takes place (Brumpt, 1934; Sandner, 1955). There are few reports on the two species of *Bufolucilia* known to cause myiasis in North America amphibians. *Bufolucilia elongata* caused myiasis in one boreal toad (*Bufo boreas boreas*) and six American toads from Colorado (USA) and Wisconsin, respectively, while *B. silvarum* was reported from the orbits of 48 bullfrogs (*Rana catesbeiana*) in California (USA) and one American toad each from Nova Scotia and Ontario (Canada; James and Maslin, 1947; Hall, 1948; Bleakney, 1963; Anderson and Bennett, 1963; Briggs, 1975). There are reports of *B. silvarum* being reared from recently deposited eggs on a healthy rat that was shot in Wisconsin and from a freshly collected carcass of a young duck from California (Dodge, 1952; Brothers, 1970). Because reports of myiasis in North American anurans are of second or third instar larvae, it is not known if these flies lay eggs on healthy hosts with first instar larvae penetrating the skin causing primary myiasis or if the larvae penetrate previous wounds causing secondary myiasis. Here we report observations on the life history of *B. silvarum* on American toads.

A total of 140 juvenile eastern American toads was collected weekly by hand during the day from May–July, 1998, in a 0.4 ha yard of an agricultural area of Muskego, Wisconsin (42°14.22′N, 88°2.1′W). Toads were brought to the laboratory and examined under a dissecting microscope for external lesions, eggs, or maggots. All toads suspected of being infected were placed in individual 8.45 l tanks lined with moist paper towels for observation and fed commercially raised crickets twice daily. All other toads were released within 24 hr of collection. Third stage maggots from each toad were placed in individual 70 ml plastic jars containing moist soil or sand and allowed to pupate. Some were fixed in a glycerin/formalin mixture and cleared in 10% KOH. The cephalopharingeal skeleton and posterior spiracles of third instars were dissected, dehydrated through ethanol, cleared in xylene, and mounted in Canada balsam. Adult flies were fed granulated sugar and banana peels for at least 24 hr before being killed by freezing, and pinned or preserved in 70% ethanol. Adult flies were keyed to genera (Shewell, 1987) and to species by keys in Hall (1948) and Hall and Townsend (1977). Adult and larval flies were deposited in the Harold W. Manter Laboratory collection (University of Nebraska State Museum, Lincoln, Nebraska, USA; HWML 16611, larvae; 16612, adult flies).

Nine (6%) of 140 toads were infected by the green blow fly with a mean intensity of 10.5±7.2 (range=1–24). Weekly parasite prevalence and mean intensity remained low, ranging from 0–20% and 2±1.4 to 14±6, respectively (Table 1).

Third instar larvae had conically tapering bodies, narrow in front and broad and truncated behind without prominent tubercles or processes on any but the terminal segment. All third instar maggots had a well-developed cephalopharingeal skeleton. The posterior spiracles were flush with the posterior face of the anal segment and were characteristic of calliphorids. All adult flies were greenish-blue in color and were identified as *B. silvarum.*

TABLE 1. Weekly prevalence and mean intensity of *Bufolucilia silvarum* in juvenile eastern American toads (*Bufo americanus americanus*) from southeastern Wisconsin.

Date collected (1998)	Number infected/ number examined	Prevalence	Mean intensity ±1 SD (range)
31 May	2/36	5.5	2±1.4 (1–3)
7 June	0/17	0	—
14 June	3/28	10.7	11.3±6 (5–17)
20–21 June	0/30	0	—
1–2 July	4/20	20	14.3±6 (9–24)
6–12 July	0/9	0	—
Total	9/140	6.4	10.5±7.2 (1–24)

Two toads were collected with recently deposited eggs. These were white in color and glued to the amphibians back (Fig. 1). Neither individual appeared to show any discomfort and no wounds or lesions were observed on the skin of these hosts. Within 4–9 hr of collection the toads began to vigorously rub their hind legs over their back in a behavior that appeared as though they were trying to dislodge the eggs. Examination under a dissecting microscope revealed empty egg cases with first instar larvae migrating under the skin. Migrating larvae and their tracks were clearly visible under the skin for up to 12 hr after which time they disappeared presumably into the tissue. Five first instar maggots were removed from a toad as they were hatching; these had distinct heavily pigmented spines, and a well-developed cephalopharingeal skeleton (Fig. 2). After 2 days of infection some swelling was apparent on one toad (Fig. 3), and within 2–3 days of infection an open wound appeared on the animals with the posterior spiracles of maggots being visible. All toads possessed a single lesion and in these wounds, maggots congregated and fed as a group (Fig. 4). Maggots developed to mature third instars within 5–7 days of hatching. A postmortem examination of a single infected toad indicated that maggots burrowed

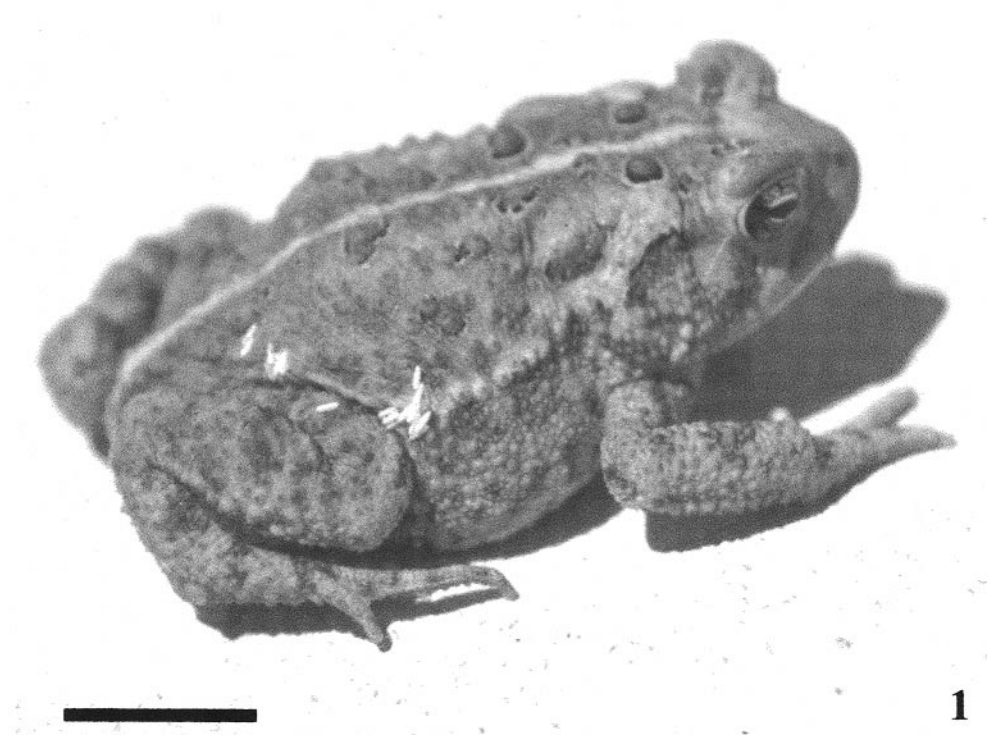

FIGURE 1. Juvenile eastern American toad with recently deposited *Bufolucilia silvarum* eggs glued to the back. Scale bar=1 cm.

deeply into the host tissue reaching the musculature. The location of the wounds varied among toads, but maggots were never found in the nasal cavities or eyes (Table 2). All toads died within 1 day to 2 wk of collection, although the single toad that survived for 2 wk had a single third instar larva that emerged from the animal within a day of collection. All other third

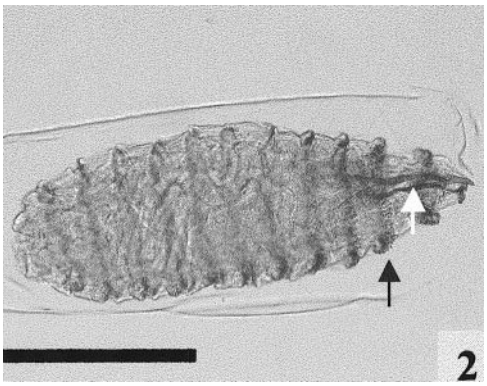

FIGURE 2. First stage larva *Bufolucilia silvarum,* removed from the skin of a juvenile eastern American toad. Note the well developed pigmented spines (black arrow) and well developed cephalopharingial skeleton (white arrow). Scale bar=0.3 mm.

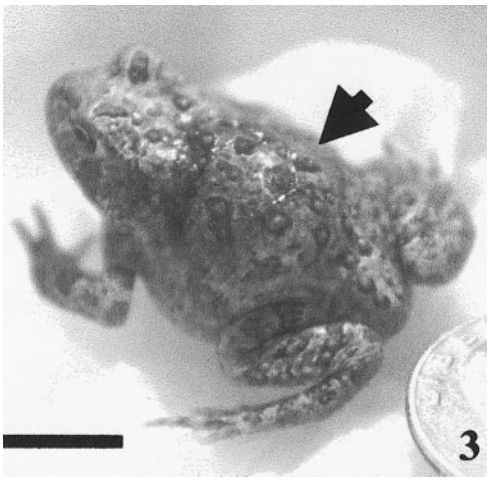

FIGURE 3. Juvenile eastern American toad within 2 days post infection by *Bufolucilia silvarum.* Note the area of swelling (black arrow). Scale bar=1 cm.

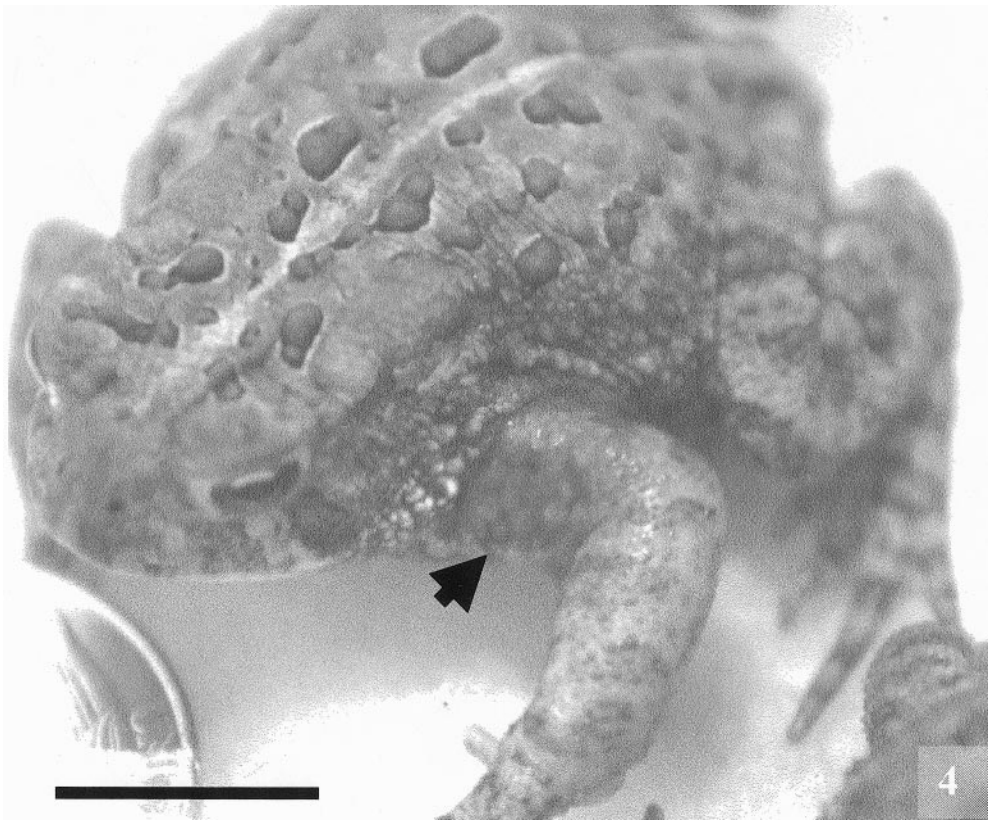

FIGURE 4. Juvenile eastern American toad infected with 17 third instar larvae *Bufolucilia silvarum* infecting the left front leg (black arrow). Note that all maggots aggregated in a single lesion and fed as a group. Scale bar=1 cm.

instar maggots continued to feed on the carcasses for 1–2 days following host death, eventually migrating out and actively seeking a suitable area to pupate. When placed in moist soil, maggots turned into pupae within 12–24 hr, and pupated within 7–9 days at room temperature.

Hall (1948) stated that although there

TABLE 2. Record of wet weight (WW), snout vent length (SVL), location of infection, and fate of juvenile eastern American toads (*Bufo americanus americanus*) infected by *Bufolucilia silvarum* from southeastern Wisconsin.

Date collected (1998)	WW (g)	SVL (cm)	Location of fly larvae on toad	Fate of toad
31 May	5.6	4	Paratoid gland	Died 31 May
31 May	4.8	3.8	Back	Died 13 June
14 June	11.4	4.5	Neck	Died 18 June
14 June	10.2	3.9	Left front leg	Died 16 June
14 June	9.8	4	Right hind leg	Died 24 June
1 July	11.4	4.3	Left hind leg	Died 2 July
1 July*	5.8	4	Right hind leg	Died 5 July
2 July	11.4	4.4	Back	Died 5 July
2 July*	8.18	4	Back	Died 8 July

* Collected with recently deposited visible *B. silvarum* eggs.

are reports of *B. silvarum* infecting toads and other amphibians in Europe, it cannot be ascertained whether records of toad and frog parasitism reported from Palaearctic region were *B. silvarum* or *B. bufonivora* because some authors have not distinguished between these two species (Stadler, 1930). Our observations are the first to report an estimate of mortality of American toads from one population caused by *B. silvarum.* The only other study of *Bufolucilia* mortality in a single toad population is of *Bufo bufo* from the Netherlands (Strijbosch, 1980). Strijbosch (1980) captured 2,216 amphibians of nine species from an area of about 300 ha over a period of 2 yr. Nineteen (8%) of 244 subadult and adult *B. bufo* were infected by *B. bufonivora.* In this study, *B. bufonivora* apparently selected *B. bufo* according to their size with larger toads having a higher frequency of infection.

Similar to the observations of Strijbosch (1980), our data suggests that myiasis caused by *B. silvarum* in Wisconsin amphibians is rare. Over the last 6 yr, our laboratory has examined over 1,000 amphibians of 12 species, including 256 eastern American toads, for parasite and life history studies with only nine juvenile American toads being infected by *B. silvarum* (Bolek, 1998; Bolek and Coggins, 1998, 2000, 2001; Yoder, 1998; Yoder et al., 2001). At our study site only juvenile American toads were present during the day, while night searches also revealed 10 uninfected adult toads. Therefore, our data differs from that of Strijbosch (1980) in that only juvenile American toads were infected at our study site in Wisconsin. These observations may be important because Hall (1948) indicated that *B. silvarum* is a diurnal species that occurs upon foliage in early morning and late afternoon and is found in woods and meadows, being active from May–October, and in Wisconsin, is most abundant during June and July (Hall, 1948; Dicke and Eastwood, 1952). Importantly, following metamorphosis American toads are diurnal and form dense, heliothermic aggregations on the margins of ponds from which they emerge. After a period of growth and development, the juveniles disperse from ponds during early to mid summer and assume the solitary nocturnal habits of the adults (Taigen and Pough, 1981; Vogt, 1981). To our knowledge, no studies exist on juvenile American toads following metamorphosis but hundreds of juvenile toads are usually seen during May–August in marshes, agricultural fields, gardens, and woods in southeastern Wisconsin (Vogt, 1981; Bolek, unpubl. data). Because of their diurnal habits, we hypothesize that this age group of toads overlaps ecologically more commonly with *B. silvarum* and may be more prone to infection by this fly than is currently known.

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