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Amphibian infestation and host size preference by the leech *Placobdella picta* (Verrill, 1872) (Hirudinida: Rhynchobdellida: Glossiphoniidae) from the Eastern Ozarks, USA

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Abstract. Our study investigates the amphibian infestation rates of the parasitic leech *Placobdella picta* in a number of ponds from Arkansas. We also note differences in the sucker attachment on the host from what was previously published. The infestation rates reflected large populations of *P. picta* at some ponds; whereas, we could find no evidence of it in other locations. Diagnosis of picking injuries was easy and occurred on as many as 50% of some species. It also appears that this leech demonstrates host and size preference in its tadpole prey. This study adds interesting details to the paucid database of life history information on *P. picta.*

Keywords. Amphibians, Arkansas, *Desserobdella picta*, leech, behaviour, parasitism.

Introduction

Understanding the details of an organism's life history is important for understanding its role in the ecosystem (Bury, 2006; McCallum and McCallum, 2006). Despite their pivotal role in ecosystem processes, we know little about the life histories of most leeches, including *Placobdella picta* (Verrill, 1872). This aquatic species specializes in parasitizing amphibians (Barta and Sawyer, 1990), which as a group are on a global extinction trend (McCallum, 2007) and susceptible to climate change (Johnson and McKenzie, 2009; McCallum, 2010). *Placobdella picta* attacks at least 12 host species including the Wood Frog (*Lithobates sylvaticus*, McAllister et al., 1995), Bullfrog (*Lithobates catesbeianus*; Tse, Barta and Desser, 1986; Barta and Sawyer, 1990), Spotted Salamander (*Ambystoma maculatum*), Blue-spotted Salamander (*Ambystoma laterale*), Jefferson Salamander (*Ambystoma jeffersonianum*), Triploid Hybrids (*A. laterale* x *A. jeffersonianum*; Tse, Barta and Desser, 1986), American Toad (*Anaxyrus americanus*; Martin and Desser, 1991), Red-spotted Newt (*Notophthalmus viridescens*; Mock and Gill, 1984; Brigler, Lohraff and Adams, 2001; McAllister, Jamieson and Sidall, 2008), and the Green Frog (*Lithobates clamitans*; Barta and Desser, 1984), and other species (Barta and Desser, 1989; Watermolen, 1996; Bolek and Janovy, 2005). *Placobdella picta* is a vector of several amphibian blood-parasites (Woo and Bogart, 1986; Martin and Desser, 1991) and can affect tadpole fitness traits (Bervin and Boltz, 2001). Their role as amphibian specialists combined with their service as

Figure 1. Brachiate adult female *Notophthalmus viridescens* louisianensis (ASUMZ 26491) with the leech *Desserobdella picta* attached by both the anterior and posterior sucker.

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Table 1. Frequencies of picking injuries and parasitism in three afflicted ponds during 2002.

Pond	Species	Size Class	Frequency	Attached
Pond 1	*Notophthalmus viridescens*	Adult	4/19 (21.1%)	1/19 (5%)
Randolph Co., Arkansas	*Acris crepitans*	Adult	0/1 (0.0%)	0/1 (0%)
	Lithobates catesbeianus	Large Tadpoles	50/243 (20.5%)	2/243 (1%)
	Lithobates catesbeianus	Small Tadpoles	5/87 (5.7%)	0/87 (0%)
	Lithobates clamitans	Large Tadpoles	0/1 (0.0%)	0/1 (0%)
	Lithobates clamitans	Small Tadpoles	5/5 (100%)	0/5 (0%)
Pond 2	*Ambystoma maculatum*	Adult	0/5 (0.0%)	0/5 (0%)
Randolph Co., Arkansas	*Notophthalmus viridescens*	Adult	1/2 (50%)	1/2 (50%)
	Lithobates catesbeianus	Large Tadpoles	1/29 (3.4%)	0/29 (0%)
	Lithobates clamitans	Large Tadpoles	7/9 (7.8%)	0/9 (0%)
Pond 3	*Notophthalmus viridescens*	Adult	1/1 (100%)	0/1 (0%)
Sharp Co., AR			1/13 (7.7%)	0/13 (0%)
All Afflicted Ponds	All species	All tadpoles	60/350 (19.4%)	3/350 (>1%)
	Notophthalmus viridescens	All adults	6/61 (9.8%)	1/61 (1.6%)

a vector of amphibian pathogens, and the tendency for many amphibians to return to their oviposition sites (Whitford, 1966; Trauth, McCallum and Jordan, 2006) stokes speculation that this species is an important regulator of tadpole populations (Berven and Boltz, 2001). As a vector, *P. picta* could play an important role in amphibian declines.

Descriptions exist of the blood-feeding behaviour and resulting feeding wounds of *P. picta*, but those authors were uncertain if their observations were isolated, host-specific, or locality specific behaviours (Barta and Sawyer, 1990). Below is an abbreviated description of their (Barta and Sawyer, 1990) observations. *Placobdella picta* finds its host by detecting vibrations from swimming amphibians. In response, *P. picta* reaches its posterior sucker from the substrate, ready to attach to its host upon contact. As the leech anchors its posterior sucker on the host tadpoles begin to thrash. Next it uses the anterior sucker to tear a portion of skin from the host (termed picking). Then the leech uses its proboscis to probe the newly formed wound for blood. Once feeding starts, it releases the posterior sucker from the host and the leech dangles free by its attached anterior sucker.

Northeast Arkansas is at the intersection of the Ozark Plateau, Crowley's Ridge, and the Mississippi Delta. The Ozark habitats comprise upland hardwood forests interspersed with cattle grazing, cold-water streams, and upland ponds. Crowley's Ridge has similar habitats, although urbanization of this geographic province is rapid. The Mississippi Delta is lowland floodplain habitat comprised of lowland hardwood forests and is dominated by agronomic pursuits including rice and cotton farming. Further, this region of Arkansas is known for instances of amphibian die-offs (Trauth, McCallum, Cartwright, 2000) and other population problems (Wheeler et al., 2002; McCallum and Trauth, 2003, McCallum and Trauth, 2002).

Our study refines previous descriptions of *P. picta* feeding behaviour, and documents an apparent prey size preference by this parasite. Additionally, we provide important information on the affliction rates of amphibians in selected ponds from the Ozarks (Randolph, Sharp and Stone Counties) and Crowleys Ridge (Craighead and Greene Counties) in Arkansas.

Materials and Methods

We collected amphibians by hand and dip-net from fishless ponds in the Ozark National Forest (n = 5; Stone County, Arkansas), Crowley's Ridge (n = 4; Greene and Craighead County, Arkansas) and on private lands in the eastern Ozarks (n = 3; Randolph and Sharp Counties, Arkansas). After collection, we examined amphibians for leeches and signs of leech attack. We relaxed leeches in a weak 1% ethanol solution, fixed them in 10% buffered formalin, and then deposited them in the Invertebrate Zoology collections of the National Museum of Natural History, Smithsonian Institution.

We identified picking marks (signs of leech attack), tabulated and compared incidences among ponds to determine the proportion of amphibians attacked by leaches in each setting. Amphibians having fresh picking marks were housed in filtered aquaria to determine the time required for picking marks to heal and to infer how long these injuries remained evident.

Results

Three ponds harboured amphibian populations infested by leeches, whereas the other ponds showed no evidence of parasitism. Ponds with leeches were located near the Randolph Co. – Sharp Co. line in Arkansas. Although no attempt was made to directly estimate the population levels of *P. picta*, this leech appeared to be

Figure 2. Adult male *Notophthalmus viridescens louisianensis* (Top, ASUMZ 26492) and *Lithobates catesbeianus* (bottom) with injuries sustained from parasitism by *Placobdella picta*. Arrows show picking injuries (newest on left to oldest on the right).

very common in the ponds and we recovered it by dip-netting nearly as frequently as amphibians.

All *P. picta* held on to their amphibian hosts with both suckers (Fig. 1) before and during blood-feeding. Hematoma (Fig. 2) resulted from repeated attachment and release of the anterior sucker. Leech blood-feeding or "picking" injuries faded in a stepwise pattern and disappeared after 2-3 days at 22°C. Fresh marks appeared as bright-red hematoma. After one day these began to fade to brown and then gradually transformed into a white mark slightly smaller than the original injury (Fig. 2). We determined the prevalence of *P. picta* infestation and frequency of leech "picking" injuries throughout the study (Table 1). Both anuran larvae (60/340, 17.65%) and adult caudates (7/63, 11.11%) showed signs of leech attacks. Picking marks occurred on tadpoles of *L. catesbeianus* (18.3%, 62/339) and *L. clamitans* (54.5%, 5/10). Adult *A. maculatum* (1/2, 50%) and *N. viridescens* (6/61, 9.8%) had signs of leech-picking. Picking injuries occurred in 100% (5/5) of 2yr *L. clamitans* tadpoles; whereas, none (0/5) of 1yr *L. clamitans* had these signs and no *L. clamitans* had leeches attached. Only 0.5% (2/340) of *L. catesbeianus* tadpoles had leeches attached (these leeches released before they could be photographed). Still, 18.2% (62/340) had obvious signs of "picking injuries". Frogs had higher infestation prevalence than tadpoles, and hosts had a higher number of leech feeding injuries than would be indicated by the number of leeches we found attached to them. Although we collected several hundred *Acris crepitans*, *Hyla cinerea*, and *L. catesbeianus* tadpoles at the Mammoth Spring National Fish Hatchery (Randolph Co., Arkansas), no leeches were associated with specimens from that location.

Discussion

Although reports exist for *P. picta* attacks on *N. v. louisianensis* (Briggler, Lohraff and Adams, 2001; McAllister, Jamieson and Sidall, 2008), this study represents the first known account of widespread *P. picta* blood-feeding across an amphibian community. Except for observations of *Placobdella cryptobranchii* on the Ozark Hellbender (*Cryptobranchus alleganiensis*; Benjamin Wheeler, pers. comm.; Moser et al. 2008), this is the largest known frequency of leech infestation in Ozark amphibian populations.

Barta and Sawyer (1990) suggested that *P. picta* released its posterior sucker during feeding. We did not observe this. All leeches used both suckers both prior to and during blood feeding. This minor difference in observed behaviour may reflect the prey involved or geographical variation.

The difference between the infestation rates of *L. clamitans* and *L. catesbeianus* suggests that anti-predator defences by *L. catesbeianus* may also resist leeches. *Lithobates catesbeianus* tadpoles secrete compounds that are distasteful to fish (Evenson and Kruse, 1982) and their nutritional qualities cause fish to lose weight (Evenson and Kruse, 1982), suggesting that these tadpoles have potent chemical defences. Alternatively, *L. catesbeianus* may have more evidence of leech attacks because they are better able to deal with the stress associated with this insult than does *L. clamitans*. In fact, *L. catesbeianus* has a well-developed antioxidant stress response, suggesting it can better survive stressors such as injuries associated with leech attack than can other species (Jones et al., 2010). Little is known about the stress response in *L. clamitans*. The role of the chemical defences of tadpoles in repelling leeches such as *P. picta* and the ability of amphibians to deal with this stressor are interesting ecological questions in need of further inquiry.

Host size preference has implications for the evolution of parasitism in this leech. Generally, parasites are on an evolutionary pathway to mutualism (Weeks et al., 2007 and citations within). Natural selection should benefit leeches that cause the least harm to their host. Otherwise, hosts capable of resisting parasites should proliferate in the population due to selective pressure exerted by harmful parasites. Leech picking injuries appear similar in size regardless of tadpole size (anecdotal observation). Also, a leech should remove up to the same volume of blood whether the tadpole is small or large. Small tadpoles should have smaller blood volumes than large tadpoles. Therefore, smaller tadpoles should experience more distress from tissue damage and blood loss from leech parasitism than would a larger tadpole. Many questions remain about how these parasites discern hosts and host size.

As a side note, Barta and Sawyer (1990) suggested that *P. picta* released its posterior sucker during feeding. The leeches we observed attached to their host by both suckers, suggesting that either they recently attacked the tadpoles/newts, or there is variation in this behaviour. The variation in leech attachment requires more investigation to pinpoint its relevance.

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