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REPRODUCTIVE PLASTICITY IN THE NEMATODE *GYRINICOLA BATRACHIENSIS*: DOES AN INTERMEDIATE REPRODUCTIVE STRATEGY EXIST IN SEXUALLY REPRODUCING, DIDELPHIC PINWORMS?

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ABSTRACT: Phenotypic plasticity is a process in which multiple phenotypes arise from 1 genotype because of environmental selection pressures. *Gyrinicola batrachiensis* has a heterogeneous reproductive strategy such that females reproduce either via parthogenesis with thick-shelled eggs in a single uterus or sexual reproduction with thick- and thin-shelled eggs in separate uterine horns. No evidence exists that strains of *G. batrachiensis* are able to switch between parthenogenetic and sexual reproduction. Thin-shelled eggs are autoinfective, and thick-shelled eggs act as transmission agents once shed into the aquatic environment. Our primary goal was to determine whether dioecious, didelphic pinworms that infect *Rana sphenocephala*, a slow-developing tadpole, and *Osteopilus septentrionalis*, a quick-developing tadpole, display reproductive plasticity with concern to thick- and thin-egg development. We performed experimental cross-infections in aquatic mesocosms to determine if dioecious, didelphic worms vary (based on tadpole host) in their ability to produce thin-shelled eggs: *O. septentrionalis* (hylid) egg masses were exposed to infected *R. sphenocephala* (ranid) tadpoles, ranid egg masses to infected hylid tadpoles, and a fully crossed infection that exposed ranid and hylid egg masses to infected tadpoles of both anuran families. Results indicated that worms reproduced via didelphic haplodiploidy in experimental ranid and hylid hosts, but that worms from hylids produced only thick-shelled eggs, which supports an intermediate reproductive strategy in *O. septentrionalis*. There was a significant difference in the mean intensities of ranid and hylid hosts, supporting our assertion that females infecting ranids are capable of producing autoinfective, thin-shelled eggs, and females infecting hylids do not produce such thin-shelled, autoinfective eggs because of the host microenvironment.

Phenotypic plasticity is a mechanism in which abiotic or biotic environmental pressures generate multiple phenotypes from 1 genotype (Bradshaw, 1965; Via and Lande, 1985). In the past few decades, it has become clear that phenotypic plasticity is common in nature and can be expressed within an individual's lifetime or across generations (Miner et al., 2005), and that plastic responses are known to impact many aspects of an organism's phenotype, such as morphology, development, physiology, behavior, and life history traits (Agrawal et al., 1999). Interest in phenotypic plasticity is increasing because such studies bridge a gap between our understanding of an organism's genotype and evolutionary fitness in nature. Evolutionary fitness is determined by an individual's ability to survive and reproduce in its environment, which is maximized when its phenotype is matched to its environment. Indeed, phenotypically plastic traits allow for a better match of phenotypes to an environment and thus an increase in reproductive fitness (Viney and Diaz, 2012).

In nematodes, phenotypic plasticity in developmental traits has been studied most extensively in the model worm *Caenorhabditis elegans*, outlining evolutionary and ecological significance of the dauer/non-dauer life-cycle choices (Sommer and Ogawa, 2011; Viney and Diaz, 2012). Similarly, the dauer polyphenism has been studied in the model organism *Pristionchus pacificus*, where this worm has plastic feeding structures that vary depending on its diet (Kiontke and Fitch, 2010). Another study used *Plectus acuminatus* to observe the critical effect levels of contaminant-induced plasticity in sensitive nematode life-cycle traits (Kammenga et al., 1997). In parasitic nematodes, plastic development is probably best known from the numerous worms that undergo arrested development (hypobiosis), which was reviewed by Gibbs (1986). Another example of developmental plasticity is found in members of Strongyloididae that are capable of switching between free-living and parasitic forms in their life cycle (Viney and Diaz, 2012). An unusual form of plasticity is poecilogony, where an organism has intraspecific variation in developmental mode that is often dependent upon the organism's environment. A hallmark example of this reproductive strategy occurs in *Gyrinicola batrachiensis*, an oxyurid nematode that infects the gastrointestinal tract of tadpoles, wherein sexually reproducing female worms produce 2 types of eggs: 1 for autoinfections and the other for transmission to other tadpoles (Adamson, 1981a). This reproductive system is complex and is known to be controlled to some extent by the worm's environment (Rhoden and Bolek, 2011).

Oxyurid nematodes rarely infect aquatic hosts; however, *G. batrachiensis* is constrained to the larval stages of anurans (tadpoles), where female *G. batrachiensis* produce eggs within 9–19 days after establishing in the tadpole host (Adamson, 1981b; Rhoden and Bolek, 2011). As tadpoles metamorphose to the adult anuran stages, they shed all *G. batrachiensis*, and adult anurans are resistant to infections (Adamson, 1981b). These pinworms have complex reproductive strategies; females reproduce either via parthenogenesis or sexual reproduction, such that there is only 1 uterine horn in parthenogenetic females or 2 uterine horns in dioecious females. No evidence exists that strains of *G. batrachiensis* are able to switch between parthenogenetic and sexual reproduction. In this reproductive system, the gastrointestinal microenvironment of the tadpole host appears to be the environmental selection pressure influencing female nematode

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reproductive mode, with previous studies suggesting that worms recovered from relatively short-lived tadpoles are parthenogenetic and those from long-lived tadpoles are dioecious and didelphic (Adamson, 1981a, 1981b, 1981c, 1981d; Pryor and Greiner, 2004; Rhoden and Bolek, 2011). In parthenogenetic females, the single uterine horn produces thick-shelled eggs, which act as transmission agents once shed into the aquatic environment with anuran feces (Adamson, 1981a, 1981b, 1981c, 1981d, 1983; Pryor and Greiner, 2004). This reproductive strategy has been observed in the lab (Adamson, 1981c) or suggested from field collections (Pryor and Greiner, 2004; Rhoden and Bolek, 2011) to occur in quick-developing tadpoles, such as *Bufo* spp. and *Pseudacris* spp. Adamson (1981c) also found parthenogenetic worms in 3 species of *Rana* spp., but subsequent studies have not found evidence of *Rana* spp. hosting a parthenogenetic strain of pinworm, even where *Rana* sp. occupy the same water body as *Bufo* sp. and/or *Pseudacris* sp. (Pryor and Greiner, 2004; Rhoden and Bolek, 2011). Alternatively, sexually reproducing didelphic females produce thick-shelled eggs in 1 uterine horn and thin-shelled eggs in the second uterine horn. Thin-shelled eggs are autoinfective when deposited into the host gut tract and cannot survive outside the tadpole host for more than 1 hr (Adamson, 1981a, 1981b, 1981c, 1981d; Pryor and Greiner, 2004). This reproductive strategy was identified in tadpoles with longer developmental times compared to bufonids, such as *Rana* spp. (Adamson, 1981c, 1981d; Pryor and Greiner, 2004). However, Rhoden and Bolek (2011) discovered that when *Bufo* (*Anaxyrus*) *woodhousii* tadpoles co-occurred with *Rana* sp. tadpoles, female pinworms recovered from *B. woodhousii* appeared to be didelphic and produced only thick-shelled eggs. They also recovered male worms, which suggested the worms could have reproduced sexually.

Rhoden and Bolek (2011) examined field-collected tadpoles, thus the exact strategy of apparent didelphic reproduction in pinworms from the quick-developing *B. woodhousii* is unresolved; a few potential scenarios could explain their observations. First, toad tadpoles may have been infected with parthenogenetic worms that only produced thick-shelled eggs, but simultaneously hosted sexually reproducing males that never mated with the parthenogenetic worms. Yet, such development would require parthenogenetic worms to develop a 2-horn uterus, which seems unlikely. Alternatively, toad tadpoles could have been infected with male and female dioecious worms, with didelphic females producing only thick-shelled eggs in a single uterine horn with a second uterine horn that does not produce eggs. These explanations are not exclusive, as some combination of parthenogenetic and dioecious females and males could have infected the toad tadpoles, which would generate the appearance of a dioecious strain of worms that did not produce thin-shelled eggs. Given the complex nature of this system, experimental cross infections are needed to resolve whether an intermediate reproductive strategy exists in dioecious, didelphic pinworms as suggested by Rhoden and Bolek (2011). Ideally, 2 anuran species with different developmental times (quick vs. slow) that host sexually reproducing, didelphic pinworms would be cross-infected. The slow-developing species should host a standard pinworm that produces both thick- and thin-shelled eggs, whereas the quick-developing tadpole would host the apparently developmentally plastic pinworm that only produces thick-shelled eggs for transmission, yet minimize the potential for hosting parthenogenetic worms (primarily found in *Bufo* spp.).

To this end, the goal of this study was to determine whether dioecious, didelphic pinworms that infect *Rana* (*Lithobates*) *sphenocephala* (slow-developing) and *Osteopilus septentrionalis* (quick-developing) tadpoles show reproductive plasticity with concern to thick and thin-shelled egg development with the use of experimental cross-infections. We hypothesize that the reproductive strategy in a sexually reproducing didelphic strain of *G. batrachiensis* is plastic, and thus we expect to find didelphic females consistently producing only thick-shelled eggs in a single uterus from *O. septentrionalis*, whereas standard production of thick- and thin-shelled eggs in separate uteri is expected in *R. sphenocephala*. Further, we do not expect to find evidence of monodelphic or parthenogenetic reproduction in this study. Not only does this study add to the expanding reproductive plasticity literature of parasitic nematodes, but it also serves as a baseline for exploring a model laboratory system on the unconventional reproductive strategies found in nematodes. Most importantly, it strongly suggests that some aspect of host ecology and/or physiology is dictating uterine reproductive plasticity in a pinworm.

MATERIALS AND METHODS

The southern leopard frog, *R. sphenocephala*, has been known to exist in larval form for 270 days before undergoing metamorphosis (Dodd, 2013), and other members of the family Ranidae can exist in the tadpole stage for up to 3 yr (Lannoo, 2005). For this purpose, *R. sphenocephala* (referred to as ranids) were classified as slow-developing tadpoles. Alternatively, the Cuban tree frog, *O. septentrionalis*, metamorphoses into a frog in less than 30 days (Lannoo, 2005). Because of this distinct difference in developmental time, we chose *O. septentrionalis* (referred to as hylids) as the quick-developing tadpole species. In addition, we chose *O. septentrionalis* over a bufonid toad, such as those used in Rhoden and Bolek (2011), because hylids are not known to host parthenogenetic forms of *G. batrachiensis*, which reduces a potential confounding factor when testing for the type of egg production in sexually reproducing pinworms.

During May and June of 2014, ranid and hylid tadpoles were wild-caught from Pride Elementary School in Tampa, Florida (PE) and Common Ground Park in Lakeland, Florida (CGP), respectively. Both sets of tadpoles were removed from retention pools, and the wild-caught anurans were the only tadpole present at their respective ponds. Tadpoles were transported to the laboratory in 19-L buckets filled with pond water and identified in accordance with Altig et al. (2008). Thirty *O. septentrionalis* and 48 *R. sphenocephala* tadpoles were necropsied to establish wild *G. batrachiensis* prevalence (P), mean abundance (MA), and mean intensity (MI) according to Bush et al. (1997). The snout–vent length (SVL) and total length (TL) were recorded, and the developmental Gosner stage (GS) was noted for each individual according to Gosner (1960). All organs and body cavities were searched within 24 hr of collection with special attention given to gastrointestinal tracts. *Gyrinicola batrachiensis* were counted and examined via light microscopy to identify egg types produced by females.

Ranid and hylid egg masses were retrieved from a permanent water body at Lakeland Highland Scrub Preserve in Lakeland, Florida. Ten 1,900-L mesocosm cattle tanks were set up outside on the Florida Southern College campus. The cattle tanks were

TABLE I. Prevalence (P), mean abundance (MA), and mean intensity (MI) of *Gyrinicola batrachiensis* in wild and experimental tadpoles from central Florida.

Host species	Measure of parasitism	Tadpole group		Statistic	*P*
		Wild	Experimental		
Osteopilus septentrionalis	P (no. infected/no. examined)	63.3 (19/30)	33.1 (41/124)	$\chi^2 = 6.21$	< 0.05
	MA ± 1 SD	1.7 ± 1.7	0.5 ± 0.9	$t = 5.2$	< 0.001
	MI ± 1 SD (range)	2.7 ± 1.5 (1–6)	1.6 ± 1.0 (1–5)	$t = 3.8$	< 0.001
Rana sphenocephala	P (no. infected/no. examined)	81.6 (40/49)	76.5 (26/34)	$\chi^2 = 0.4$	> 0.05
	MA ± 1 SD	1.3 ± 1.0	16.2 ± 19.9	$t = 5.2$	< 0.001
	MI ± 1 SD (range)	1.6 ± 0.9 (1–4)	21.2 ± 20.3 (1–71)	$t = 6.06$	< 0.001

initially filled with 1,700 L of tap water on May 1, 2014 and maintained at this level by almost daily rainfall throughout the study. The tanks were stocked 2 days later with phytoplankton, periphyton, duckweed (*Lemna valdiviana*), and other small organisms acquired from plankton net tows from nearby Lake Hollingsworth, which provided a natural, self-sustaining food source for the tadpoles. Tanks were exposed to full sunshine for >7 hr/day and maintained temperatures of 28–30.4 C during the study. Mesocosm tanks were covered and secured with a heavy mesh that prevented the intrusion of organisms or material greater than 1.6 mm, but permitted plenty of sunlight for photosynthesis. Experiments began 45 days after filling and stocking the tanks; qualitatively, all tanks were similar in appearance, with high abundance of stocked organisms. Tanks were assigned 1 of 3 treatments. Tanks 1–3 were each seeded with 200 hylid eggs (experimental hosts), 50 wild ranid tadpoles (PE) as a source of nematode eggs, and 50 ranid eggs for a time-t control. Tanks 4–6 were each seeded with 200 ranid eggs (experimental hosts), 50 wild hylid tadpoles (CGP) as a source of nematode eggs, and 50 hylid eggs for a time-t control. Tanks 7–9 were each seeded with 200 ranid eggs and 200 hylid eggs (experimental hosts), with 50 wild ranids (PE) and 50 wild hylids (CGP) as a nematode egg source. Tank 10 was designated as the supplemental pool used to harbor all excess, uninfected egg masses and tadpoles. Fifty clean ranid tadpoles were supplemented 6 days into the experiment in tanks 4–6 as a result of carnivorous *O. septentrionalis* behavior. Supplemented tadpoles were kept in submerged 11-L critter keepers, which prevented predation but allowed nematode eggs, phytoplankton, and water to move between the critter keepers and the larger mesocosm.

Tadpoles of Gosner stages 30–37 (average age of 45 days) for *R. sphenocephala* and 36–40 (average age of 41.2 days) for *O. septentrionalis* were removed from mesocosms, euthanized, and frozen immediately. The experiment was terminated at 45 days, as most hylid tadpoles had been removed from the mesocosms due to rapid development, and ranid tadpoles should have developed autoinfections according to Adamson (1981b). For data collection, tadpoles were thawed, identified as *R. sphenocephala* or *O. septentrionalis*, and dissected. The SVL, TL, GS, number of worms, sex of the worm, and egg types within each individual female *G. batrachiensis* were recorded. The uterine anatomy was dissected out for 25 randomly selected worms from each group: wild ranid, experimental ranid, wild hylid, and experimental hylid. Prevalence, mean abundance (MA), and mean intensity (MI) were established for wild tadpoles and experimental tadpoles, and all means are reported ±1 SD. A chi-square test for independence was used to compare the prevalence of wild and experimental groups. A *t*-test was used to compare MA and MI between groups, in which data for experimental tadpoles were combined by treatment to increase sample size for MA and MI analysis (no significant differences were found in MA and MI among treatments).

Voucher specimens of adult male, adult female, and juvenile pinworms from wild and experimental amphibian species have been deposited in the H. W. Manter Parasitology Collection, University of Nebraska, Lincoln, Nebraska (accession numbers HWML 110093–110096).

RESULTS

In total, 154 hylids and 83 ranids were used to determine ecological parameters or used for experimental or control purposes. Prevalence for wild hylids was approximately double compared to experimental hylids (Table I; $\chi^2 = 6.21$, $P < 0.05$), and prevalence for wild ranids was also greater than experimental ranids, but not significantly ($\chi^2 = 0.4$, $P > 0.05$). We observed a significantly greater mean abundance and intensity of experimental ranids when compared to wild ranids ($t = 5.2$, $P < 0.001$ for MA; $t = 6.06$, $P < 0.001$ for MI), whereas we observed a significant decrease in mean abundance for experimental hylids ($t = 5.2$, $P < 0.001$ for MA; $t = 3.8$, $P < 0.001$ for MI). In wild collections, we found significantly higher prevalence for ranid infections over hylids ($\chi^2 = 3.76$, $P < 0.05$), but no significant relationship for mean intensity and abundance ($t = 0.3$, $P > 0.05$ for MA; $t = 1.14$, $P > 0.05$ for MI). In experimental treatments, we found significantly greater infection parameters for ranids over hylids ($\chi^2 = 6.89$, $P < 0.001$ for P; $t = 5.5$, $P < 0.001$ for MA; $t = 6.11$, $P < 0.001$ for MI).

We recovered primarily adult female nematodes, which were larger in size compared to males. Fourteen of 87 (16.1%) adult nematodes were male in experimental hylid tadpoles, which was similar to wild hylids 8 of 52 (15.3%); however, males represented 11.4% (n = 61) in wild ranids and 9.1% (n = 552) from experimental ranids. Worms were recovered from tadpoles between Gosner stages 25 and 46, with 46 indicating complete metamorphosis. Metacercariae, identified as *Apharyngostrigea pipientis* (Trematoda: Strigeatida: Strigeidae), were recovered near the heart of a few tadpoles used in establishing prevalence of wild ranids and hylids. No trematodes or other nontarget metazoan parasites were recovered from experimental tadpoles in the mesocosms.

Dissection of the uterus suggests that no parthenogenesis occurred in female nematodes found in this experiment, as all observed worms (n = 100) had 2 developed uteri, although the nonfunctioning uteri were not fully developed in hylid infections. Upon examination of females collected from wild hylids, no uteri contained thin-shelled eggs; instead a single uterus functioned to produce thick-shelled eggs while the other remained empty. All females collected from experimental hylids produced thick-shelled eggs in 1 uterus, whereas the other uterus was empty for 24 of 25 (96%) examined experimental hylids. One female worm from an experimental hylid contained 2 thin-shelled eggs with apparently viable, developing autoinfective worms. Examination of female uteri from ranids yielded 1 uterus with at least 1 thick-shelled egg and the other with at least 1 thin-shelled egg in all 50 experimental and wild worms.

DISCUSSION

This study is the first to test experimentally whether sexually reproducing, didelphic pinworms that infect ecologically dissimilar tadpoles show reproductive plasticity with concern to thick- and thin-egg development in separate uterine horns. In support of our hypothesis, we found didelphic females producing only thick-shelled eggs in a single uterus from the quick-developing tadpole *O. septentrionalis* (with 1 exception discussed below), whereas reproduction with both thin- and thick-shelled eggs from separate uteri is confirmed in the slow-developing tadpole *R. sphenocephala*. Thus, our study provides empirical support for the observations of Rhoden and Bolek (2011) that suggests when haplodiploid populations of *G. batrachiensis* are shared among amphibian species that differ in developmental period; the pinworm strain is readily transmitted and hosted among ecologically dissimilar host species. As worms mature, an unknown host cue triggers an intermediate, plastic reproductive strategy, i.e., 1 functional uterus that produces only thick-shelled eggs, when the worm is hosted by *Bufo* spp. or *O. septentrionialis*. In a broader view, this suggests that *G. batrachiensis* is restricted to producing only thick-shelled eggs in at least some quick-developing tadpole species, whether via monodelphic parthenogenesis (Adamson, 1981c) or with the intermediate strategy described in this study. Thus, it may be that rapid host development has selected for "bet-hedging" in 2 different genetic strains of *G. batrachiensis* (Olofsson et al., 2009; Rhoden and Bolek, 2011). Although, future studies will need to distinguish carefully whether this intermediate strategy is caused by host development rates or if it is dependent upon host species, densities, or another unconsidered aspect of the host species. Alternative to a bet-hedging strategy, our mesocosms could have contained multiple genetic strains (or even cryptic species) of pinworm, thus permitting certain host species to host strains that produce only thick-shelled eggs. However, this alternative seems unlikely in our study given (1) the recovery of male worms, (2) presence of 2 uteri in females, (3) the cross-infection experimental design, and (4) the careful selection of wild tadpoles from small water bodies with no other tadpole species present to reduce genetic variation. Indeed, we did not find evidence of parthenogenetic reproduction, and our male recovery rate from hylid hosts was 16%, which is consistent with a previous study by Anderson (1981b) that males generally represent approximately 15% of recovered worms when females reproduce sexually. Nonetheless, our study cannot rule out the possibility of cryptic species in this pinworm system. Overall, this study has established developmental plasticity in these pinworms from ecologically diverse and distantly related tadpoles; however, additional experimental studies are needed to ascertain if host developmental time alone governs such plasticity, as our study does not eliminate the possibility of other host species triggering a plastic, intermediate developmental response regardless of their larval development time.

Although worms from *O. septentrionalis* were characterized by the intermediate developmental strategy, 1 of these tadpoles hosted a worm that contained 2 thin-shelled eggs. *Osteopilus septentrionalis* tadpoles in our mesocosms developed rapidly (as quickly as 14 days at >32 C), which may be too fast for *G. batrachiensis* to develop thin-shelled eggs, although thick-shelled eggs were common in these worms. Thus it is possible that an extended development time would have permitted more worms to produce thin-shelled eggs. It also seems possible, given the carnivorous diet and predatory nature of *O. septentrionalis* (Rodder and Weinsheimer, 2009), that the single worm producing thin-shelled eggs was acquired from consuming the gastrointestinal tract of a sympatric experimentally infected *R. sphenocephala*. Of note, the gastrointestinal tract of the latter was frequently consumed at least partially whole by carnivorous *O. septentrionalis* (G. Langford, unpubl. obs.).

Regarding *R. sphenocephala*, thin-shelled eggs were observed in all dissected worms and the mean intensity of experimental ranids was significantly higher than the mean intensity of hylid hosts, which supports autoinfection occurring in ranid hosts but not hylid. However, this comparison may be biased because many of the hylid tadpoles developed quickly and were removed from the mesocosm before ranids. Although it is unclear if tadpole development time impacts the speed of nematode development, Adamson (1981b) found higher temperatures increased worm development. In addition, ranid behavior can contribute to increased prevalence as well. When collecting *R. sphenocephala* from mesocosms, it was imperative to sample the bottom, as most tadpoles were retrieved under a large algae mat along the bottom of the tanks. Further, we noted that experimental ranid tadpoles spent longer times hiding and feeding on the bottoms of the mesocosms when compared to hylid tadpoles that swam in the water column, which would increase contact of thick-shelled eggs and *R. sphenocephala*. Likewise, mature females from ranids reproduce via didelphic haplodiploidy, where males arise from unfertilized eggs and females arise from fertilized eggs (Adamson, 1981a, 1981b, 1981c, 1981d; Rhoden and Bolek, 2011). Males mate with females once developed to increase egg production, possibly leading to an increased number of worms within the gut tract compared to hylid-infecting nematodes (Adamson, 1981b). We also suggest that wild ranid tadpoles maintained relatively low-intensity infections when compared to experimental hosts because the wild tadpoles were relatively young (Gosner stage 26) and probably had not lived long enough to permit many autoinfections to occur, which is supported by collections we conducted later in the season from the same pond that found high-intensity infections in that population of *R. sphenocephala* at Gosner stage 39 (J. Childress, pers. obs.).

We attribute the decreases in experimental hylid infection parameters to the behavior and diet of the experimental *O. septentrionalis*. Cuban tree frog tadpoles continually preyed upon ranid tadpoles in every tank. Not only did wild hylids (larger, more mature tadpoles) feed on egg masses and newly developed tadpoles (both hylid and ranid), but *O. septentrionalis* experimental tadpoles rapidly developed while feeding on conspecifics and heterospecifics (G. Langford, unpubl. data). The carnivorous diet of experimental *O. septentrionalis* might have also reduced nematode infections because *G. batrachiensis* are thought to persist only in the guts of primarily herbivorous tadpoles (Pryor and Bjorndal, 2005; Rhoden and Bolek, 2011). Alternatively or synergistically, the behavior of the tadpoles might have affected their infection parameters in captivity. In the mesocosms *O. septentrionalis* were often found at the top of the water column and along the sides of the mesocosm tanks; however, in nature, the tadpoles are frequently collected from shallow waters that facilitate near constant substrate sampling. Thus, experimental infections were likely influenced by lower substrate sampling time for *O. septentrionalis* tadpoles, reducing the probability of encountering thick-shelled eggs.

Prior to this study, *G. batrachiensis* had not been reported from *O. septentrionalis*, and this relationship may pose a potential ecological challenge. The invasive Cuban tree frog has now expanded its territory as far north as South Carolina (Rodder and Weinsheimer, 2009). Pryor and Bjorndal (2005) provide evidence that *G. batrachiensis* increases gut fermentation and induces a faster metamorphosis time in bullfrog tadpoles (*Rana catesbeiana*). The same study noted that gastrointestinal nematodes were absent in carnivorous tadpoles, although our study found infections in *O. septentrionalis*, which we found to consume considerable animal matter. Indeed, these tree frogs are known to prey on other tadpoles and have substantial animal matter in their diets (Rodder and Weinsheimer, 2009). Furthermore, *G. batrachiensis* possibly has the same effect on this invasive anuran, i.e., more rapid development, that Pryor and Bjorndal (2005) found in bullfrogs. These invasive tree frogs already develop larger and quicker, produce more eggs per clutch, and lay more clutches per year when compared to native tree frogs (Lannoo, 2005; Rodder and Weinsheimer, 2009). Coupling this natural rapid development with pinworm infection might compound the negative effects of *O. septentrionalis* on native anurans. However, it is unclear if the carnivorous diet of the frogs prevents tadpoles from hosting high-intensity infections of *G. batrachiensis*. Future research could be directed at the ability of the invasive tree frog to host and benefit from intense pinworm infections.

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