

Amphibian predation on larval mosquitoes

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Abstract: Mosquitoes (*Culex pipiens* L., 1758) are important vectors for diseases of both wildlife and humans. Understanding how natural factors influence mosquito abundance may provide insights into the ecology of various diseases, as well as solutions to controlling disease vectors. One of the natural factors regulating mosquito distributions and population sizes is predation. A poorly understood source of natural mosquito predation is amphibians. We determined the mosquito consumption capability of two amphibians, adult Red-spotted Newts (*Notophthalmus viridescens viridescens* (Rafinesque, 1820)) and Larval Mole Salamanders (*Ambystoma talpoideum* (Holbrook, 1838)). We also compared mosquito consumption of eastern mosquitofish (*Gambusia holbrooki* Girard, 1859), a known predator of mosquitoes, and *A. talpoideum*. Both salamander species were capable of consuming large numbers of mosquito larvae per day (least-square means \pm 1 SE = 439 ± 20 and 316 ± 35 mosquitoes/day consumed by *A. talpoideum* and *N. v. viridescens*, respectively). In *A. talpoideum*, mosquito consumption scaled with body size, with the largest individual (4.4 g) ingesting 902 mosquitoes in 1 day. *Gambusia holbrooki* consumed 3.5 \times more mosquitoes during a 24 h feeding trial than similar-sized *A. talpoideum*. Our findings suggest that amphibians could have a substantial impact on mosquito larvae abundance, especially considering that amphibians can reach densities of up to 500 000 individuals/ha. Furthermore, we hypothesize that introduction of *G. holbrooki* could reduce abundances of native mosquito predators (e.g., salamanders) indirectly, through competition for invertebrate prey.

Résumé : Les moustiques (*Culex pipiens* L., 1758) sont d'importants vecteurs de maladies pour les humains et la faune. Comprendre comment les facteurs naturels influencent l'abondance des moustiques pourrait ouvrir des perspectives nouvelles sur l'écologie de diverses maladies et fournir des solutions pour le contrôle des vecteurs pathogènes. Un des facteurs naturels qui contrôlent la répartition et la taille des populations de moustiques est la prédation. Les amphibiens constituent des agents mal connus de prédation naturelle des moustiques. Nous avons déterminé la capacité de consommation de moustiques de deux amphibiens, les tritons verts à points rouges adultes (*Notophthalmus viridescens viridescens* (Rafinesque, 1820)) et les larves de l'ambystome taupe (*Ambystoma talpoideum* (Holbrook, 1838)). Nous avons aussi comparé la consommation de moustiques de la gambusie (*Gambusia holbrooki* Girard, 1859), un prédateur connu de moustiques, à celle d'*A. talpoideum*. Les deux espèces de salamandres sont capables de consommer de grands nombres de larves de moustiques par jour (moyennes des moindres carrés \pm 1 ET = 439 ± 20 et 316 ± 35 moustiques/jour consommés respectivement par *A. talpoideum* et *N. v. viridescens*). Chez *A. talpoideum*, la consommation de moustiques est fonction de la taille, l'individu le plus grand (4,4 g) pouvant ingérer 902 moustiques en une journée. Les *Gambusia holbrooki* consomment 3,5 fois plus de moustiques dans un essai alimentaire de 24 h que des *A. talpoideum* de même taille. Nos observations indiquent que les amphibiens pourraient avoir un effet important sur l'abondance des larves de moustiques, particulièrement parce que les amphibiens peuvent atteindre des densités allant jusqu'à 500 000 individus/ha. De plus, nous formulons l'hypothèse selon laquelle l'introduction de *G. holbrooki* pourrait réduire indirectement l'abondance des prédateurs naturels de moustiques (par ex., les salamandres) par compétition pour les proies invertébrées.

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Introduction

Mosquitoes are among the most important vectors of diseases that affect wildlife and humans worldwide (Gubler 1998). In recent years, concerns over mosquito-borne diseases with important health and economic implications have increased the demand for safe and cost-effective means to control mosquitoes (Miller 2001; Rose 2001; Thier 2001). Understanding how natural factors influence mosquito abundance may provide insights into the ecology of various dis-

eases, as well as natural and engineered solutions to controlling disease vectors.

One of the natural factors regulating mosquito distributions and population sizes is predation. For example, bats can consume up to 500–1000 flying insects, including mosquitoes, per hour (Griffin et al. 1960; Rydell 1990) and can significantly reduce insect density in some ecosystems (Kalka et al. 2008; Williams-Guillen et al. 2008). An interesting but poorly understood source of natural mosquito predation is amphibians. All adult amphibians and larval salamanders are predatory, and accounts of amphibians preying upon larval and adult mosquitoes exist in the literature (Matheson and Hinman 1929; Minton 1972; Blum et al. 1997; Petranka 1998; Lannoo 2005; Brodman and Dorton 2006). Correlative evidence suggested that wetlands containing larval salamanders supported 98% fewer mosquitoes than wetlands without these aquatic predators (Brodman et al. 2003), but many other important ecological variables

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such as the density of salamanders and other mosquito predators were not quantified in that study. Nevertheless, these findings, coupled with numerous natural history notes (Matheson and Hinman 1929; Minton 1972; Blum et al. 1997; Petranka 1998; Lannoo 2005), indicate that the importance of amphibians as mosquito predators warrants attention.

The goal of this study was to quantify the potential role of amphibians as predators of larval mosquitoes through a series of laboratory feeding trials. Specifically, we determined mosquito consumption capability of two common aquatic amphibians, adult Red-spotted Newts (*Notophthalmus viridescens viridescens* (Rafinesque, 1820)) and larval Mole Salamanders (*Ambystoma talpoideum* (Holbrook, 1838)), that are known to consume mosquito larvae (Taylor et al. 1988; Petranka 1998). The objectives of this study were to (1) quantify the maximum number of mosquito larvae an individual could consume over a 24 h period, (2) quantify the average rate of mosquito consumption sustained by individuals of these two species over a 7 day period, and (3) compare mosquito larvae consumption by eastern mosquitofish (*Gambusia holbrooki* Girard, 1859), a known predator of mosquitoes, and *A. talpoideum*.

Materials and methods

Animal collection and husbandry

Wild larval *A. talpoideum*, aquatic adult *N. v. viridescens*, and *G. holbrooki* were collected between May and July 2006. *Ambystoma talpoideum* were collected from El-lenton Bay, an uncontaminated and fishless wetland located on the Department of Energy's Savannah River Site, Aiken County, South Carolina. *Notophthalmus viridescens viridescens* were collected from a small permanent pond located ~31 km north of Roanoke, Botetourt County, Virginia. *Gambusia holbrooki* were collected from a small pond located on the Virginia Tech campus, Montgomery County, Virginia. Both of the salamander species were transported to an outdoor mesocosm facility at Virginia Tech where they were held communally in polyethylene cattle tanks (1.85 m diameter, 1480 L volume) that were periodically infused with pond water and stocked with *Daphnia magna* Straus, 1820. *Gambusia holbrooki* were held in the laboratory in 33 L plastic bins containing aerated, dechlorinated tap water and were fed commercial fish food. During experimental trials, animals were housed in the laboratory at 24.5 ± 0.01 °C in individual 6.1 L plastic bins containing dechlorinated tap water. Experimental procedures were approved by the Virginia Polytechnic Institute and State University Institutional Animal Care and Use Committee (06-114-FIW).

Collection and rearing of mosquito larvae

Egg rafts of mosquitoes (*Culex pipiens* L., 1758) were collected in mosquito oviposition traps. Traps were constructed of 53 L plastic bins containing 3.8 L of hay infusion. Hay infusion was made by mixing 114 g of hay, 1 g of lactalbumen, 1.3 g of brewer's yeast, and 26.5 L of water (adapted from Reiter 1986 and Jackson et al. 2005) in a plastic bin and steeping the mixture in the sun for several days. Egg rafts were collected every morning from traps

and were hatched and reared to third instar in the laboratory in 33 L plastic bins containing ~25 L of aerated, dechlorinated tap water. Dry mass of third-instar larval mosquitoes was determined by drying six composite samples of 1000 larvae for 24 h at 30 °C in a drying oven.

Feeding experiments

In July–August of 2006, we conducted three separate feeding experiments to examine mosquito consumption across predator species. Each individual was only used in a single experiment. Prior to each experiment, all animals were fasted and acclimated to experimental conditions for 24 h (experiment 2) or 48 h (experiments 1 and 3). All animals were weighed (nearest 1.0 mg on an electronic balance) and measured (snout–vent length (SVL), nearest 0.01 mm using digital calipers) at the beginning and end of each experimental trial. The objective of each experiment was to feed animals to satiation and not limit their ability to capture prey. Thus, we used data from a series of pilot feeding trials to determine the appropriate number of mosquito larvae to offer to ensure that animals would not consume more than ~80% of the mosquito larvae provided. Our preliminary studies were similar to the feeding trials described here, and were only conducted to give us insight into the number of mosquito larvae we should offer individuals so that not all the mosquito larvae were consumed.

In addition to number of mosquito larvae consumed, we calculated the percentage of each individual's body mass consumed in mosquito larvae. To do this we adjusted dry mass of mosquitoes to wet mass assuming 80% moisture.

We conducted a series of 24 h feeding trials to determine the number of mosquito larvae the two salamander species were capable of consuming over a short time period (experiment 1). To examine the relationship between body size and mosquito consumption, feeding rates were measured over as broad a mass range as possible for both species; 0.63–4.42 g ($n = 20$) in *A. talpoideum* and 1.87–3.39 g ($n = 10$) in adult *N. v. viridescens*. For each trial, salamanders were offered mosquito larvae in excess in the afternoon at 1600 (*A. talpoideum*: $n = 400$ –1200 larvae depending on salamander size; *N. v. viridescens*: $n = 500$ larvae). After 24 h, all remaining mosquito larvae were removed and counted.

To determine the mean number of mosquito larvae the two salamander species would consistently consume each day over an extended time period, we conducted a series of 7 day feeding trials using two size classes (~2.0 and 3.0 g) of *A. talpoideum* and *N. v. viridescens* (experiment 2). Salamanders were offered mosquito larvae in excess ($n = 450$ –600 depending on size class and species) every afternoon (1600) for 7 days. Remaining mosquito larvae were removed and counted after 18 h (in the morning at 1000). This gave animals a 6 h period each day during which food was not present. Mass ranges of 2.0 g individuals were 1.98 ± 0.07 g (mean \pm SE) for *A. talpoideum* and 2.09 ± 0.10 g for *N. v. viridescens*. Mass ranges of 3.0 g individuals were 2.80 ± 0.13 g for *A. talpoideum* and 2.92 ± 0.11 g for *N. v. viridescens*. We tested $n = 7$ individuals per species per size class in this experiment.

To compare mosquito consumption of *A. talpoideum* (1.26 ± 0.05 g, $n = 8$) and similar-sized *G. holbrooki* (1.33 ± 0.11 g, $n = 8$), we conducted a series of 24 h feeding

trials (experiment 3). After fasting, animals were offered mosquito larvae in excess (*A. talpoideum*: $n = 500$ larvae; *G. holbrooki*: $n = 1200$ larvae). Mosquitofish were offered more mosquitoes than salamanders because initial pilot feeding trials clearly revealed that more mosquitoes were required to satiate mosquitofish. After 24 h, remaining mosquito larvae were removed and counted.

Statistical analyses

All statistical models were performed using SAS (version 9.1; SAS Institute Inc., Cary, North Carolina). Statistical significance was assessed at $\alpha = 0.05$. Prior to statistical comparisons, Ryan Joiners and Bartlett's tests were used to determine whether data met assumptions of parametric models (i.e., normal distribution and heteroscedasticity, respectively).

To examine differences in mosquito consumption between *A. talpoideum* and *N. viridescens* during the 24 h feeding trial, we conducted an ANOVA (Proc Mixed procedure in SAS) and used least-square means to evaluate differences between species. The mixed model approach was used because data transformation failed to improve heteroscedasticity. We used linear regression analysis to examine relationships between body size and mosquito consumption in the two salamander species.

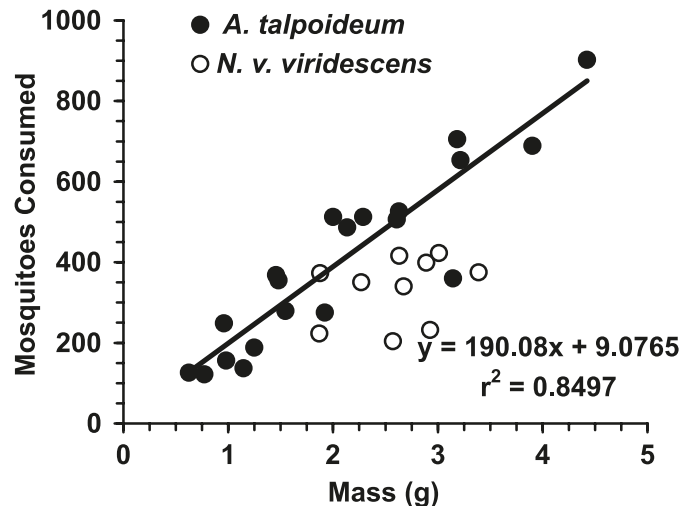
To determine mean daily mosquito consumption rates, we averaged daily mosquito consumption for each individual over the 7 day period and then compared mean daily consumption rates for each species \times size class combination. To determine the effects of species, size class, and their interaction on sustained mosquito consumption for the 7 day trial, we conducted a two-way ANOVA (Proc GLM in SAS). All data met assumptions of normality and heteroscedasticity.

To determine differences in mosquito consumption between *A. talpoideum* and *G. holbrooki*, we conducted a Wilcoxon test because data were not normally distributed. One-way ANOVAs revealed no difference in mass between the two species ($p = 0.66$), therefore mass was not included in the nonparametric model.

Results

In experiment 1, both species of salamanders consumed large numbers of mosquito larvae during the 24 h feeding trial (*A. talpoideum*: 122–902 mosquito larvae; *N. v. viridescens*: 204–423 mosquito larvae). *Ambystoma talpoideum* ate $65\% \pm 0.05\%$ of mosquito larvae offered to them, which is equivalent to $3.0\% \pm 0.15\%$ of their body mass. *Notophthalmus viridescens viridescens* ate $67\% \pm 0.05\%$ of mosquito larvae offered to them, which is equivalent to $2.0\% \pm 0.18\%$ of their body mass. We found that mosquito consumption was positively correlated with body mass in *A. talpoideum*, with the largest individual (4.4 g) capable of ingesting 902 mosquitoes in 1 day ($r^2 = 0.85$, $p > 0.001$; Fig. 1). *Notophthalmus viridescens viridescens* consumed, on average, 333.5 ± 26 mosquito larvae over a 24 h period, but mosquito consumption did not scale with mass in this species ($r^2 = 0.09$, $p = 0.40$), most likely because of their smaller mass range. There was a significant difference in mosquito consumption between the two species (least-square

Fig. 1. Consumption of mosquito (*Culex pipiens*) larvae by *Ambystoma talpoideum* ($N = 20$) and *Notophthalmus viridescens viridescens* ($N = 10$) over a 24 h period. Mosquito consumption scaled with body mass in *A. talpoideum* (solid line), but not in *N. v. viridescens* (no line).



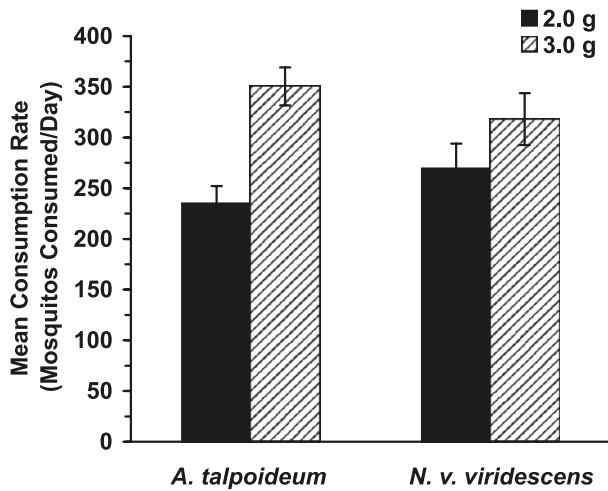
means, $p = 0.005$), with *A. talpoideum* consuming 39% more mosquitoes than similar-sized *N. v. viridescens* (*A. talpoideum*: least-square means = 439, SE = 20; *N. viridescens*: least-square means = 316, SE = 35).

In our sustained feeding studies (experiment 2), each day all individuals consumed between 39% and 83% of the mosquito larvae offered to them, which was equivalent to 1.4%–3.1% of their body mass. On average, individuals consumed a total of 1764 ± 109 and 2340 ± 109 mosquitoes per week (2.0 and 3.0 g individuals, respectively), or $13.3\% \pm 0.01\%$ and $12.7\% \pm 0.01\%$ of their body mass (2.0 and 3.0 g individuals, respectively). Results of the two-way ANOVA revealed that size class had a significant effect on mean mosquito consumption rate ($F_{[1,24]} = 14.2$, $p = 0.001$), in which 2.0 and 3.0 g individuals (both species combined) consumed 252 ± 10 and 334 ± 10 mosquitoes per day, respectively (Fig. 2). However, there was no significant species effect ($p = 0.96$) or species \times size class interaction ($p = 0.14$) on mean mosquito consumption rate in this experiment.

In experiment 3, *G. holbrooki* consumed 992 ± 39 mosquito larvae during the 24 h feeding trial, which was $3.5\times$ more mosquitoes than consumed by similar-sized *A. talpoideum* (284 ± 31 mosquito larvae, $p < 0.001$; Fig. 3). *Gambusia holbrooki* consumed $83\% \pm 0.03\%$ of mosquito larvae offered, which is equivalent to $12\% \pm 0.9\%$ of their body mass, whereas *A. talpoideum* consumed $57\% \pm 0.06\%$ of mosquitoes offered, which is equivalent to $3.5\% \pm 0.4\%$ of their body mass.

Similar discrepancies in mosquito consumption were evident when comparing *G. holbrooki* to *N. v. viridescens* across experiments. Despite the fact that *G. holbrooki* used in this experiment were half the size of *N. v. viridescens* used in experiment 1 (*N. v. viridescens*: 2.61 ± 0.15 g; *G. holbrooki*: 1.33 ± 0.11 g), mosquito consumption by

Fig. 2. Mean daily consumption of mosquito (*Culex pipiens*) larvae over a 7 day period by ~2.0 g and ~3.0 g *Ambystoma talpoideum* and *Notophthalmus viridescens viridescens*. Error bars are ± 1 SE and $N = 7$ individuals per species per size class.



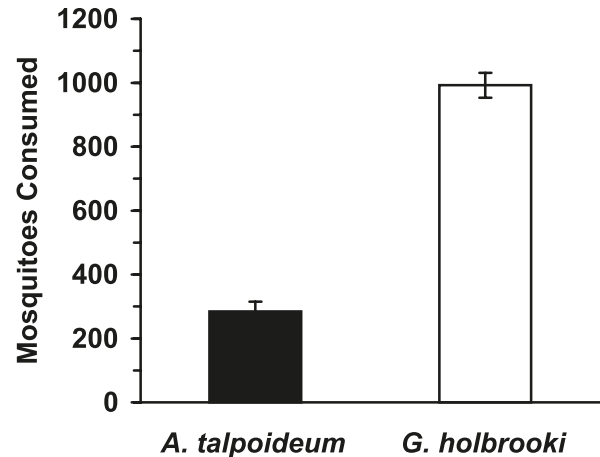
G. holbrooki was 3 \times higher compared with *N. v. viridescens*.

Discussion

Under laboratory conditions, amphibians consumed large numbers of mosquito larvae. *Ambystoma talpoideum* ranging from 2.0 to 3.0 g were capable of maximally consuming 390–580 third-instar mosquito larvae in a single day, and both salamander species (*A. talpoideum* and *N. v. viridescens*) ranging from 2.0 to 3.0 g were capable of consistently consuming 200–400 third-instar mosquito larvae per day. These results suggest that aquatic salamanders could play a key role in mosquito control. This may be particularly true in ephemeral water bodies where fish are typically absent. Clearly, the results of our laboratory studies are only a first step towards understanding the role of amphibian predation on regulation of mosquito populations. Next, mesocosm and field studies are needed to elucidate the impact amphibians have on mosquito populations in a natural setting. For example, given the opportunity salamanders may select alternative prey (e.g., cladocerans) instead of feeding on mosquito larvae. In addition, mosquitoes may be able to avoid amphibian predation in certain situations because mosquitoes can oviposit within the dry wetland (Sota and Mogi 1992), enabling mosquitoes to hatch and reach the adult stage before predators are present. Mosquitoes may also avoid predation by using chemosensory cues to choose oviposition sites with fewer predators (Angelon and Petranka 2002). However, our results in conjunction with the findings of Brodman et al. (2003) indicate that the impact of amphibian predation on mosquito populations deserves further investigation.

Amphibians often represent the single most abundant vertebrate in natural systems, reaching terrestrial densities of 18 000 individuals/ha (Petranka and Murray 2001) and aquatic densities of up to 500 000 individuals/ha (Davic and Welch 2004). *Ambystoma talpoideum* densities in several wetlands in South Carolina in May were estimated at

Fig. 3. Consumption of mosquito (*Culex pipiens*) larvae by ~1.3 g *Ambystoma talpoideum* and *Gambusia holbrooki* over a 24 h period. Error bars are ± 1 SE and $N = 8$ individuals per species.



13 300 – 300 000 individuals/ha (Semlitsch 1987). Such high biomass, coupled with the typical voracious appetites of amphibian larvae (Taylor et al. 1988), further strengthens the likelihood that amphibians play important roles in mosquito control. For example, in a 10 ha wetland, if we assume that mosquito larvae constitute only 1% of a larval amphibian's diet (a conservative estimate considering that dipterans can make up to 60% of a salamander's diet; Taylor et al. 1988), simple calculations using an aquatic density of 50 000 individuals/ha, which is well within the range documented for this species, and mean mosquito consumption rates of 2.0 g salamanders suggests that amphibians could be capable of consuming ~100 000 mosquito larvae per night. Considering that *A. talpoideum* can occur at higher densities and can achieve much larger sizes (10–12 g) in the aquatic environment than what we examined, mosquito consumption capability could be even greater.

Historically, one method that has been employed to control mosquito populations is the introduction of *Gambusia* (Hoddle 2004). Our findings support previous studies (e.g., Bence 1988) demonstrating that *Gambusia* is capable of consuming large quantities of mosquito larvae. Despite the high consumption rates, however, evidence suggests that in many cases when *Gambusia* was introduced for mosquito control, they were ineffective at controlling mosquito populations (Courtenay and Meffe 1989), presumably because they often feed on alternative prey types (e.g., cladocerans; Bence 1988), including other predators of mosquito larvae (Blaustein 1992).

The introduction of *Gambusia* has led to established populations in areas that previously did not support fish but did support amphibians (Goodsell and Kats 1999). Unfortunately, studies have shown that *Gambusia* also prey on amphibian eggs and larvae (Grubb 1972; Gamradt and Kats 1996; Goodsell and Kats 1999). Given that *Gambusia* can occur at very high densities (e.g., 110 000/ha; Stewart and Miura 1985) and are capable of consuming large quantities of mosquito larvae and other aquatic invertebrates (e.g., cladocerans; Hurlbert and Mulla 1981; Bence 1988; Blaust-

tein and Karban 1990), it is possible that *Gambusia* also could impact salamander populations through competition if prey resources are limiting. However, to our knowledge, no studies have examined competition between amphibians and mosquitofish.

Because *G. holbrooki* prey on, and potentially compete with, amphibians in the wild, the introduction of these fish for mosquito control could have serious impacts on populations of native mosquito predators such as amphibians. Moreover, although *G. holbrooki* consumed 3.5× more mosquito larvae than *A. talpoideum* in our study, amphibians may serve as more effective mosquito control agents in ephemeral wetlands, where complete drying eliminates fish populations. In such situations, amphibians may serve as more effective long-term consumers of mosquito larva because terrestrial adult amphibians rapidly recolonize aquatic habitats after complete drying (Gibbons et al. 2006). Thus, because adult salamanders require terrestrial habitat (Semlitsch 1998), preservation of terrestrial habitats surrounding ephemeral water bodies may be a vital component of natural mosquito control.

In summary, the results of our laboratory experiments indicate that salamanders can consume large numbers of larval mosquitoes. Our findings lend support to the idea proposed by Brodman et al. (2003) that salamanders may play an important role in mosquito population control through predation on larval mosquitoes. Unfortunately, global amphibian population declines represent the greatest mass extinction of land vertebrates since the dinosaurs (Stuart et al. 2004). Clearly, if amphibians are efficient predators of mosquitoes in a natural setting, the loss of amphibian biodiversity may have direct implications for the health of wildlife and humans. However, additional studies are needed to fully appreciate the role of amphibians in mosquito population dynamics. Future studies should be conducted to examine mosquito predation by salamanders in a natural setting and determine feeding preferences of salamander species to examine how mosquito consumption rates are altered by invertebrate community composition.

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