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The effectiveness of pond-breeding salamanders as agents of larval mosquito control.

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ABSTRACT

Frogs and salamanders are well known predators on insects; however the benefits of pest control attributed to them are usually based on anecdotes. Tiger salamander, *Ambystoma tigrinum* (Green) larvae were collected from eight sites in northern Indiana and analyzed for stomach contents. The larval and pupal stages of mosquitoes made up the third largest component of the diet and were found in the stomachs of 26% of the individual tiger salamander larvae. During controlled feeding experiments in the lab, mosquito larvae were observed to be a preferred prey and tiger salamander larvae were able to eat a mean of 144 mosquito larvae per day. An extrapolation of this figure suggests that a tiger salamander population can eat a large number of mosquito larvae and could be effective natural agents in the biological control of larval mosquito populations.

INTRODUCTION

Wetlands within residential areas can produce mosquito (Culicidae) populations that are capable of spreading human diseases (Russell 1999, Sanford et al. 2003, Walton and Workman 1998). Recent instances of West Nile virus have raised concerns about mosquito control, but there is also cause for concern about people over-reacting to this threat by filling and draining wetlands that do not harbor significant mosquito populations or the overuse of pesticides. Residents generally prefer biological control programs for insect pests (Jetter and Paine 2004). Natural means of pest-control often save money and are environmentally friendly alternative that need to be explored.

The benefits of pest control attributed to amphibians are usually based on anecdotes rather than on experimental field tests (Matheson and Hinman 1929, Minton 1972). One study testing the effect of adult European frogs on mosquito density established that the frogs ate mosquitoes, but had no regulatory effect (Blum et al. 1997). In a North American study adult eastern newts (*Notophthalmus viridescens*) (Rafinesque) were efficient at reducing mosquito populations (Matheson and Hinman 1929). However, the conclusions were based on qualitative observations at just three wetlands and upon the predatory behavior of three individual newts.

Pond-breeding salamander larvae of the genus *Ambystoma* are opportunistic carnivores that often occur at high densities in temporary ponds (Brodman 1995, Peterson et al. 1992, Van Buskirk and Smith 1991). Pest dipteran species including mosquito larvae are often components of the diet of pond-breeding salamander larvae (Hutcherson et al. 1989, Sever and Dineen 1978, Smith and Petranka 1987). However several studies have had mixed results, indicating that some but not all invertebrate prey taxa are reduced in population density or biomass because of predation by certain species of salamander larvae (Harris 1995, Holomuzki 1989, Holomuzki et al. 1994, Petranka 1989). A recent study in northwest Indiana found that mosquito densities were 10-100 times less abundant in wetlands and experimental mesocosms that harbored pond-breeding salamander (*Ambystoma*) larvae compared to wetlands and mesocosms that lacked salamanders (Brodman et al. 2003). However the hypothesis that amphibians reduce mosquito larvae density or biomass due to predation has not been tested quantitatively on aquatic salamanders with adequate replication.

The objectives of this study are to analyze stomach contents of tiger salamander larvae (*Ambystoma tigrinum*) (Green) collected from natural ponds and to observe the feeding behavior to estimate predation rates of salamander larvae on mosquitoes. Prey preference experiments will be performed to test the hypothesis that mosquito larvae are a preferred prey with the null hypothesis that tiger salamander larvae eat prey taxa in proportion to availability. The results of this study will be used to suggest whether or not pond-breeding salamanders are effective natural predators that can be used to control mosquito populations.

METHODS

We used 42 tiger salamander larvae for stomach content analysis that were collected from nine sites within the Upper Wabash Drainage Basin in northern Indiana and preserved in formalin during the summers of 2001 and 2002. Stomach contents were observed under a dissecting scope and prey taxa were identified to order or family (using Pennak 2001). The total length (TL) of each salamander larva was measured to the nearest 0.1 mm. The mean and standard deviation of TL was 89.3 ± 31.6 mm and ranged from 27.2 to 127.9 mm.

During May and June 2003 we collected 117 tiger salamander larvae from two wetlands in Jasper County, IN. We used these larvae for behavioral studies and maintained them in the lab by placing salamander larvae into plastic containers that were 9 cm high x 17 cm wide x 32 cm long and filled to a depth of 8 cm with unchlorinated tap water. Each container housed one larva. The TL of each salamander larva was measured to the nearest 0.1 mm at the beginning of the experiment. The mean and standard deviation of TL was 66.9 ± 16.4 mm and ranged from 37.0-97.4 mm.

In the lab we fed prey taxa to salamander larvae that we collected each week from one of the wetlands and additional mosquito larvae from the genera *Aedes*, *Culex*, and *Culiseta* that we collected from 80 L tubs that we set outside to collect rainwater. Prey taxa were identified to family, sorted, and then maintained in the lab so that we could feed each salamander larva a controlled diet. Observations of tiger salamander larvae were made to record predatory behavior and to estimate predation efficiencies.

Experiment 1

In the first experiment, thirteen randomly selected salamander larvae were fed 150 mosquito larvae to determine the predation rates over a range of time periods. The mean and standard deviation of TL for these larvae were 64.9 ± 16.6 mm. The number of mosquito larvae eaten was recorded after 20 min, 100 min, 5 hr, 6 hr and 24 hr. Data was analyzed using regression to test for a significant effect of salamander larva TL on the number of mosquitoes eaten.

Experiment 2

We randomly selected seven groups of thirteen salamander larvae to test whether tiger salamander larva prefer mosquito larvae to other commonly encountered prey taxa. The mean and standard deviation of TL for these larvae were 68.7 ± 16.9 mm. Every day for one week each tiger salamander larva was fed 10 mosquito larvae along with 10 individuals from another prey taxon in a series of pairwise trials to test the hypothesis that salamander larvae would eat equal numbers of each prey. Group 1 were fed were fed mosquitoes and spire snails (Gastropoda: Lymnaeidae). Group 2 were fed mosquitoes and waterboatmen (Hemiptera: Corixidae). Group 3 were fed mosquitoes and crawling water beetles (Coleoptera: Halipidae). Group 4 were fed mosquitoes and chironomid midges (Diptera: Chironomidae). Group 5 were fed mosquitoes and backswimmers (Hemiptera: Notonectidae). Group 6 were fed mosquitoes and zooplankton (mostly Crustacea: Ostracoda and Cladocera). The taxa fed to groups 1-6 were selected because they were the most abundant in the natural wetlands. Group 7 were fed 10 mosquito larvae, three isopods and one of each of the following seven taxa: frog tadpoles (*Pseudacris sp.* < 3 cm TL and *Rana sp.* = 4 cm TL), predaceous diving beetle larvae (Coleoptera: Dytiscidae < 1 cm TL), water bugs (Hemiptera: Belestomidae < 1 cm TL), water scorpions (Hemiptera: Nepidae), damselflies (Odonta: Zygoptera), and small crayfish (Crustacea: Decapoda < 2.5 cm TL). These prey taxa were chosen because they were relatively large and uncommonly found in the natural wetlands.

We observed each tiger salamander larva for 20 minutes after prey taxa were added to the container and recorded the number eaten of each prey taxon eaten and the number that were captured but then spat out. Data was analyzed by MANOVA to test for significant differences between the number of mosquitoes eaten vs. other taxa and by regression to test for the affect of salamander TL on the number of each prey taxon eaten.

Experiment 3

For the final experiment we randomly selected 13 salamander larvae to test whether tiger salamander larvae eat prey taxa in proportion to availability. We fed prey taxa to each salamander larva in the following proportions: 10 mosquito larvae, 10 backswimmers, 10 zooplankton (mostly ostracods and cladocerans), 10 spire snails, 10 chironomids, 5 crawling water beetles, 5 water boatmen, and 5 other prey taxa randomly selected from among frog tadpoles, isopods, small predaceous diving beetle larvae, small water bugs, damselflies, and small crayfish. These proportions represent what was available in the natural pond at the time of the experiment. Each salamander larva was observed for 60 min after prey taxa were added to the container. In each trial the number eaten and the number captured but spat out was recorded for each prey taxa. Data was analyzed using chi-square goodness of fit test to test if there is a significant difference in the proportion of each prey taxa eaten compared to availability.

RESULTS

A total of 2334 prey items from 22 taxa were identified from the stomach contents of 42 tiger salamander larvae (table 1). While crustacean zooplankton (cladacerans and ostracods) made up most (92%) of the prey items and were found in 38% of the salamander larvae, mosquitoes were third most abundant prey taxon and were found in 27% of the tiger salamander larvae ranking second behind cladocerans.

We observed that mosquitoes were readily eaten by tiger salamander larvae in the lab. The salamander larvae usually remained still on the bottom or floating in the water column, but would also slowly stalk prey by crawling or slowly swimming, especially when prey items were at low density. The salamander larvae typically responded to movements of the mosquitoes by orienting and then lunging towards the mosquitoes. Of the 2,379 observations that we made of tiger salamander larvae eating mosquito larvae, we only observed four instances when the captured mosquito was spit out. In each of these cases the mosquito was recaptured and eaten. A small number of mosquito larvae developed into pupae during the experiment and all were eaten by tiger salamander larvae before they could metamorphose to the adult. The mean rate of consumption was approximately 20 mosquito larvae per hour for 6 hours until reaching a mean saturation of 144 mosquitoes per day (Fig. 1). There was a weak negative effect of TL on the number of mosquitoes eaten ($F_{1,57} = 3.986$, $r = -0.252$, $p = 0.05$).

Tiger salamander larvae were observed to eat significantly more mosquitoes ($F_{6,6} = 14.720$, $p < 0.001$) than spire snails, zooplankton, crawling water beetles and backswimmers, but there was no significant difference in the number of mosquitoes eaten compared to water boatmen, chironomids, or large prey (Fig 2). The TL of salamander larvae was significantly associated with the number of *Rana* tadpoles ($F_{1,12} = 24.786$, $r = 0.832$, $p < 0.001$), small crayfish ($F_{1,12} = 18.041$, $r = 0.802$, $p = 0.003$), crawling water beetles ($F_{1,12} = 11.747$ $r = 0.719$, $p = 0.006$), and backswimmers ($F_{1,12} = 24.927$, $r = 0.833$, $p < 0.001$) eaten. Tiger salamander larvae generally attempted to consume any prey item that moved and was in striking distance. However while some prey taxa were almost always swallowed, some prey taxa were usually released after capture. The majority (74) of the 91 prey items that were observed to be captured by tiger salamander larvae but then released were backswimmers and crawling water beetles. Water scorpions were the only potential prey taxa that were never observed to be eaten by tiger salamander larvae and also were not present in the stomach contents of preserved salamander larvae. Only two water scorpions were observed to be captured but both were quickly released.

The abundance of each prey taxon eaten by tiger salamander larvae (Fig 3) was significantly different from the proportion of taxa available ($chi^2 = 115$, $df = 6$, $p < 0.001$). Mosquito larvae, chironomid larvae, and some of the larger prey taxa (tadpoles, isopods, damselflies, small dytiscid larvae, and small crayfish) were eaten relatively more often than expected, whereas water boatmen were eaten in numbers similar to expected. Backswimmers, spire snails, crawling water beetles, and zooplankton were eaten in numbers less than expected based on availability.

DISCUSSION

Stomach content analysis supports the conclusion that tiger salamander larvae are carnivore generalists eating a variety of aquatic invertebrate and vertebrate prey (Black 1969, Brophy 1980, Brunkow and Collins 1996, Collins and Holomuzki 1984, Dodson and Dodson 1971, Lannoo and Bachman 1984, Rose and Armentrout 1976, Seaver and Dineen 1978, Wilbur 1972). Water scorpions were the only invertebrates in the size class of other prey that we found in natural habitats but were not observed in stomach contents.

Tiger salamander larvae forage for food by floating in the water column, resting motionless on the bottom and then lunging at prey that approach, or by actively crawling on the bottom in search of prey (Anderson and Graham 1967, Hassinger et al. 1970, Leff and Bachmann 1986). We observed each of these foraging behaviors. The most common foraging strategy was to respond to moving mosquitoes by orienting and lunging towards the mosquitoes.

Tiger salamander larvae shift their diet as they grow (Petranka 1998, Lannoo and Phillips 2005). Smaller larvae primarily eat cladocerans and diptera larvae while larger larvae also eat snails, tadpoles and a wider variety of aquatic insects (Holomuzki and Collins 1987, Werner and McPeek 1994). We observed that the TL of tiger salamander larvae was positively associated with the number of tadpoles, beetles, backswimmers, and crayfish eaten and negatively associated with the number of mosquitoes eaten. However, mosquitoes were regularly eaten by the largest of our study animals.

Tiger salamander larvae often eat prey in proportion to their abundance, although larger dipterans and zooplankton are often preferred while adult beetles, backswimmers and caddisfles larvae are rarely eaten (Dodson 1970, Dodson and Dodson 1971). None of the prey tested in pairwise comparison or within an experimental community were preferred prey by tiger salamander larvae over mosquito larvae. However salamander larvae preferred mosquito larvae as prey over spire snails, crawling water beetles, backswimmers, water scorpions and zooplankton and were eaten in greater numbers than expected by relative density.

Wetlands with populations of salamander larvae typically have low densities of mosquito larvae (Brodman et al. 2003). We observed that tiger salamander larvae can eat a large number of mosquito larvae in short periods of time. Let us assume that a moderately sized population of tiger salamanders has 40 adult females breeding per year and each lays an average of 300-400 eggs (Anderson et al. 1971, Wilbur 1977). If half of these eggs survive and develop into larvae of the size and age that we studied, then a predation rate of 144 mosquitoes per day in such a population would extrapolate to over 1,000,000 mosquitoes eaten per day. However it is unlikely that mosquito populations could attain such densities in water bodies with populations of predatory salamander larvae. These observations and experimental results suggest that salamander larvae, such as the tiger salamander, could be capable of naturally reducing larval mosquito populations in ponds and wetlands. Future research needs to determine if the reduction of mosquito larvae by predatory salamanders in breeding habitats is sufficient to control adult mosquito densities.

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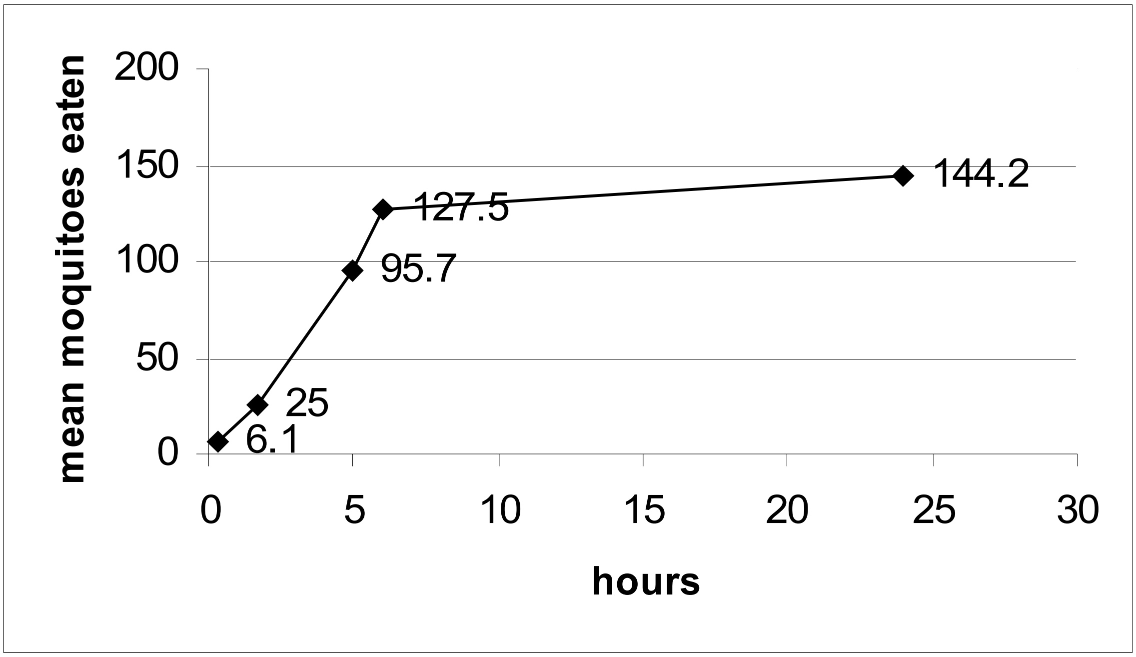

Figure 1. The mean number of mosquitoes eaten during per hour. N = 13 tiger salamander larvae.

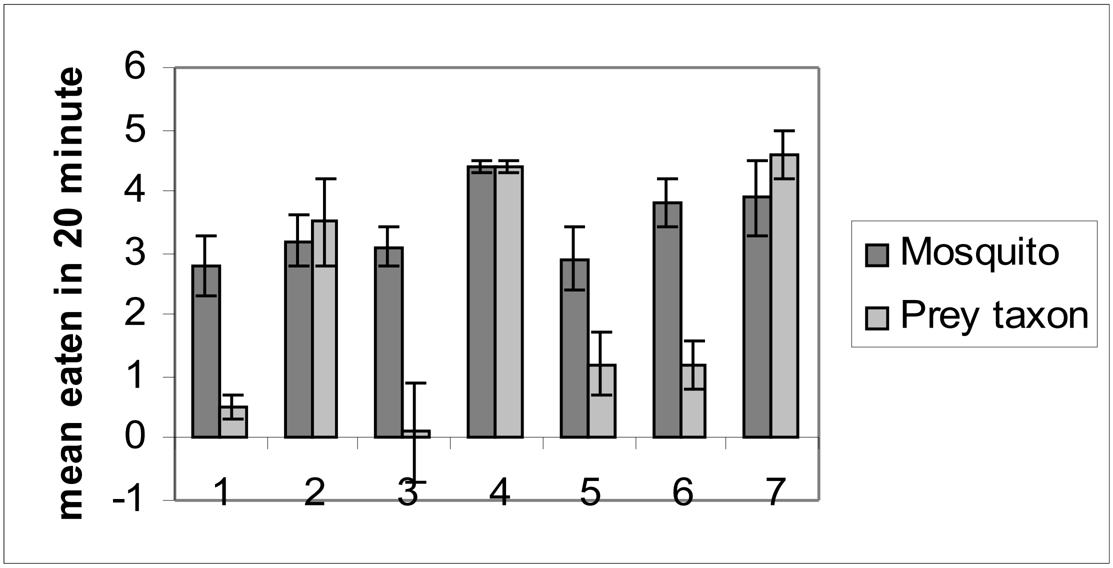

Figure 2. Pairwise comparison of mosquitoes and alternate prey taxa eaten by tiger salamander larvae. Alternate prey taxa include (1) spire snails, (2) waterboatmen, (3) crawling water beetles, (4) chironomid midges, (5) backswimmers, and (6) zooplankton (Cladocerans and Ostracods), and (7) large prey (frog tadpoles, predaceous diving beetle larvae, water bugs, water scorpions, damselflies, and small crayfish). N = 13 tiger salamander larvae for each group.

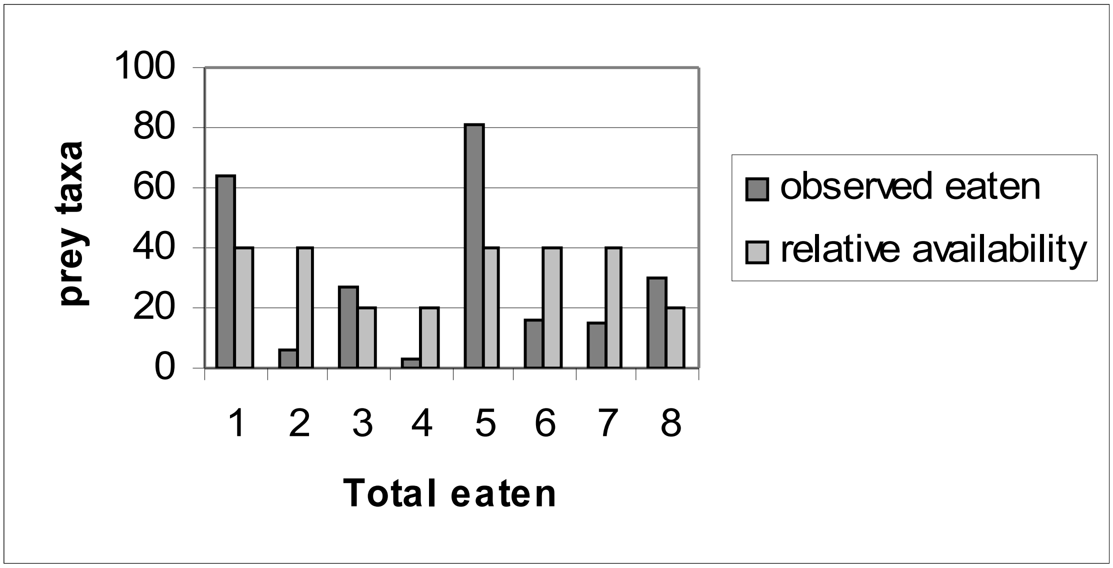

Figure 3. Relative number of each prey taxa eaten in 60 min compared to the relative number available in an experimental community. Prey taxa include (1) mosquitoes, (2) spire snails, (3) waterboatmen, (4) crawling water beetles, (5) chironomid midges, (6) backswimmers, and (7) zooplankton (Cladocerans and Ostracods), and (8) large prey (frog tadpoles, predaceous diving beetle larvae, water bugs, water scorpions, damselflies, and small crayfish). N = 13 tiger salamander larvae.

Table 1. Stomach contents of 42 tiger salamander larvae collected from 9 sites. The data are the total number (#) of each prey taxon and the percentage (%) of larvae with the prey taxon in its stomach.

Prey Taxa	#	%
Cladocera		
Ostracoda	2079	35.7
Mosquito (Culicidae)	82	19.0
Spire snail (Lymnaeidae)	39	26.2
Copepod	37	16.7
Crawling water beetle (Haliplidae)	18	16.7
Dragonfly (Anisoptera)	11	16.7
Water boatman (Corixidae)	10	16.7
Backswimmer (Notonectidae)	8	16.7
Predaceous diving beetle	8	14.3
(Dytiscidae)	7	11.9
Rana tadpoles	6	14.3
Chironomid midge	5	9.5
Fingernail clam (Sphaeriidae)	5	2.4
Phantom midge (Chaoboridae)	4	7.1
Ambystoma tigrinum salamander	3	7.1
Isopods	3	4.8
Damselfly (Zygoptera)	2	4.8
Fishfly (Corydalidae)	2	4.8
Fairy shrimp (Anostraca)	2	4.8
Bufo tadpoles	1	2.4
Amphipod	1	2.4
Leech (Hiruninae)	1	2.4
total	2334	