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Exotic Invasive Pomacea maculata (Giant Apple Snail) Will Depredate Eggs of Frog and Toad Species of the Southeastern US

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Exotic Invasive *Pomacea maculata* (Giant Apple Snail) Will Depredate Eggs of Frog and Toad Species of the Southeastern US

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Abstract- *Pomacea maculata* (Giant Apple Snail) is a freshwater snail from South America that is an invasive species on the Gulf of Mexico coastal plain. A sister species has been shown to prey on amphibian eggs in Asia. To test whether the Giant Apple Snail will prey on amphibian eggs, we presented eggs of *Lithobates palustris* (Pickerel Frog), *Lithobates pipiens* (Northern Leopard Frog), and *Anaxyrus americanus* (American Toad) to Giant Apple Snails in a laboratory experiment. Giant Apple Snails ate the eggs of all 3 species.

Introduction

Pomacea maculata (Perry) (Giant Apple Snail) is a freshwater snail native to South America (Hayes et al. 2015) that is an invasive species in the freshwater wetlands and waterways of the northern Gulf of Mexico, peninsular Florida (Benson 2017, Burks 2017) and globally (Hayes et al. 2015). Karraker and Dudgeon (2014) found that *Pomacea canaliculata* (Lamarck) (Channeled Apple Snail) opportunistically ate frog eggs. The Giant Apple Snail is a sister species to the Channeled Apple Snail and shares similar life-history attributes (Hayes et al. 2015). However, the literature indicates that Giant Apple Snail is presumed to be an herbivore (e.g., Burke et al. 2017, Burlakova et al. 2009). Will Giant Apple Snail eat amphibian eggs? If they do, they could have a negative impact on anuran populations throughout their introduced range. In this study, we presented Giant Apple Snails with frog and toad eggs to determine if they would eat them.

Methods

We purchased the eggs used in this experiment from Carolina Biological Supply (Burlington, NC). We tested 3 species and 4 egg masses: 1 egg mass of *Lithobates pipiens* (Schreber) (Northern Leopard Frog), 2 egg masses of *Lithobates palustris* (LeConte) (Pickerel Frog), and 1 egg mass of *Anaxyrus americanus* (Holbrook) (American Toad). Availability determined the species, age, and number of egg masses used. All the species tested are native to the US, and 2 are native to Louisiana.

The snails used for this experiment were from a population maintained in the US Geological Survey's Wetland and Aquatic Research Center in Lafayette, LA. A detailed description of the source and husbandry of this population can be found

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in Sutton et al. (2017). For this experiment, we used Giant Apple Snails that were born in captivity and are normally fed a diet of mostly green leafy produce including: apples, cilantro, cob corn, collard greens, cucumbers, iceberg and romaine lettuce, kale, mustard greens, parsley, spinach, turnip greens, yellow squash, zucchini, and other items as available. Observation of their eating behavior indicated that lettuce was a preferred food item. The snails were also given "dog chews" as a protein supplement. None of the experimental snails had been exposed to frog eggs before this feeding trial. We selected at random female Giant Apple Snails between 40 g and 60 g in weight. No snails died during or shortly after the experiments. We weighed all snails again after the experiments, but because several snails laid egg clutches during the experiments, we did not analyze weight change.

We divided each egg mass into 16 sets with approximately the same number of eggs for each of 4 treatments and 4 replicates. We randomly assigned the egg sets to 1 of 4 treatments: (C) water alone, (CL) water and 15–16g of lettuce, (S) water with 1 snail but no lettuce, and (SL) water with a snail and ~15–16 g of lettuce (as an alternative snail-food source). The C and CL were controls and the S and SL were treatments. There were 4 replicates from the single egg masses of Northern Leopard Frogs and American Toads and 8 replicates from 2 Pickerel Frog egg masses. We placed the eggs in a covered 5.7-L plastic container with 3 L of water from the snail-culture tank and we employed aquarium pumps to aerate the water. The water in the snail-culture tank came from a well on site. The experimental containers were housed in the same greenhouse as the snail tank. After 2 or 3 days, we changed the water and replaced the lettuce in the containers. We initially tried to weigh the lettuce, but it degraded too much for accurate weighing. We placed the eggs in the container with the snails for either 6 d, or until most of the eggs developed into tadpoles, i.e., 2–6 d. In most cases, the experiment was terminated because the eggs that hadn't already hatched into tadpoles were clearly dead, as indicated by discoloration. At the end of the experiment, we counted the number of remaining live eggs and the number of tadpoles; failed eggs were not used in calculating egg loss or conversion to tadpoles.

The replicates had unequal numbers of eggs at the start of the experiment due to differences in the number of eggs in different masses and the difficulty in separating eggs without damaging the embryos. We exposed the eggs to the snails for different lengths of incubation time because of differences in the age of the egg masses at the beginning of the experiment (Table 1). To account for these variables, we

Table 1. Treatment summary-statistics. Each egg mass was divided into 16 sets with approximately the same number of eggs. The 16 sets of eggs were then randomly assigned to 1 of 4 treatments, with 4 replicates per treatment. We used 1 egg mass each of American Toad and Northern Leopard Frog and 2 Pickerel Frog egg masses.

		Eggs per replicate					Days
Species	Replicates	Average	Median	SD	Min	Max	incubated
American Toad	4	25.75	25.5	4.54	19	32	2
Northern Leopard Frog	4	50.81	51.0	6.32	44	58	3
Pickerel Frog	8	64.00	64.0	17.36	25	65	3, 6

calculated the percent of eggs missing. To determine the percent of eggs missing, we subtracted the number of eggs that died from the number placed in the containers at the start of the experiment to obtain the "starting" number of eggs, added the number of eggs and tadpoles at the end of the experiment, divided by the starting number of eggs, and subtracted that percentage from 1 (Equation 1):

$$\% \text{ egg loss} = 1 - ([\text{number of eggs} + \text{number of tadpoles}]_{t1}) / [\text{number of eggs}]_{t0}),$$

where $_{t0}$ and $_{t1}$ are the first and last days of the experiment.

We then divided the percent of eggs missing by the number of days of the experimental run (t_l) to calculate percent egg loss per day (Equation 2):

$$\% \text{ egg loss per day} = (\% \text{ egg loss}) / (t_1)$$

We used the percent egg loss per day as the response variable; treatment, species, and treatment x species interaction were independent variables. We were interested in 12 specific a priori comparisons from the interaction. We performed a Bonferroni correction (alpha = 0.05/12 = 0.00417) when comparing the means of the species by treatment and the treatments by species. Our analyses employed a general linear mixed-model that adjusted for the unequal variances between the controls and snail-treatment categories. We included unequal variances between the controls and the treatments because it was biologically obvious that the control would have less variability. The variances between control and snail treatments were significantly different ($\chi_1^2 = 98.29$, $P < 0.0001$). Hence, we adjusted the mean comparisons for the unequal variance. We conducted all analyses using PROC MIXED in SAS 9.4.

Results

Giant Apple Snails consumed the eggs of all 3 anuran species tested (Carter 2018). Percent egg-loss per day for each treatment and species are presented in Table 2. At the end of the trials, there was an overall average of 12 missing eggs in the snail treatments (Table 2) with the percentage of eggs lost as high as 28.8% for 1 of the replicates.

Table 2. Mean percentage of eggs lost per day. The treatments are C = water only, CL = water and lettuce, S = snail and water, and SL = snail, water, and lettuce.

			Average % egg loss per replicate per day by treatment and species			
Species	Replicates (# masses)	Mean initial # of eggs	C	CL	S	SL
American Toad	4 (1)	25.75 ± 4.50	0.00 ± 0.00	0.00 ± 0.00	10.85 ± 5.62	9.13 ± 6.52
N. Leopard Frog	4 (1)	50.81 ± 6.32	0.43 ± 0.86	0.44 ± 0.57	21.67 ± 10.47	18.76 ± 8.43
Pickerel Frog	8 (2)	64.00 ± 17.36	2.72 ± 5.69	0.19 ± 0.38	7.95 ± 4.62	10.52 ± 3.48
All Species	16 (4)		1.30 ± 0.04	0.26 ± 0.00	12.20 ± 0.08	12.10 ± 0.07
			All controls 0.78 ± 0.03		All snail treatments 12.14 ± 0.07	

We assumed normality because the residuals of the general linear model were unimodal and symmetric, and the residuals passed the test for homogeneity after fitting unequal variances (*P*-value < 0.0001; Table 3). We found that the overall model was significant ($\chi_{11}^2 = 221.4$, $P < 0.0001$) at $\alpha = 0.05$, the treatment x species interaction was significant ($F_{6,52} = 3.06$, $P < 0.0122$), and the overall model $r^2 = 74.71\%$. All replicates exposed to snails had a significant loss of eggs as compared to their matched controls, except for the American Toad C vs. S comparison (Table 3).

Discussion

The current range of the Giant Apple Snail overlaps with the ranges of the Pickerel Frog and American Toad (Benson 2017, USGS National Amphibian Atlas 2014). Although the current range of the Giant Apple Snail does not overlap the range of the Northern Leopard Frog, it does overlap the range of the closely related *Lithobates sphenocephalus* (Cope) (Southern Leopard Frog). All 4 of these frog species lay eggs in potential snail habitat—slow-moving or still bodies of water, at shallow depths on the bottom, or attached to vegetation in the water (AmphbiaWeb 2018). We divided egg masses into treatments and replicates; thus, the number of eggs encountered by the snails in this study was fewer than what they would have encountered if they had come upon an egg mass of hundreds to thousands of eggs.

In 3 out of 4 experimental runs, the eggs matured into tadpoles within 3 d, significantly reducing the time the snails could discover and eat the eggs compared to the time of exposure to the eggs snails might have in natural field settings. The incubation time from egg laying to hatching into tadpoles is variable by species—American Toads = 3–12 d, Northern Leopard Frog = 2–17 d, and Pickerel Frog = 10–24 d (AmphibiaWeb 2018). Therefore, we would expect, accounting for shipping time, the American Toad eggs should have hatched soon after arrival.

Table 3. Matched comparisons of egg treatments within species. The treatments are C = water only, CL = water and lettuce, S = snail and water, and SL = snail, water, and lettuce. An asterisk (*) indicates significance at the 0.05/12 = 0.0041 alpha level.

Comparison	Species	$t_{1,52}$	*P*
C vs. S	Northern Leopard Frog	-6.81	<0.0001*
C vs. S	Pickerel Frog	-3.52	<0.0009*
C vs. S	American Toad	-2.86	<0.0060
CL vs. SL	Northern Leopard Frog	-5.87	<0.0001*
CL vs. SL	Pickerel Frog	-4.51	<0.0001*
CL vs. SL	American Toad	-3.48	0.0010*
C vs. CL	Northern Leopard Frog	-0.03	0.9762
C vs. CL	Pickerel Frog	0.27	0.7884
C vs. CL	American Toad	0.00	1.0000
S vs. SL	Northern Leopard Frog	0.66	0.5117
S vs. SL	Pickerel Frog	0.68	0.5002
S vs. SL	American Toad	-0.43	0.6657

Even when snails were provided with an alternative preferred food item from their regular diet, they still ate frog eggs. We have demonstrated that Giant Apple Snails will eat frog eggs under laboratory conditions. It remains to be demonstrated, but it is very likely that Giant Apple Snail depredate amphibian egg in natural settings, just as Karraker and Dudgeon (2014) found in their study on Channeled Apple Snails in Hong Kong.

Our findings demonstrate that Giant Apple Snails could potentially have a significant impact on amphibian reproduction. We also suspect that the eggs of other species of amphibians not tested here may also be susceptible to Giant Apple Snail predation. More laboratory and field studies are needed to determine if Giant Apple Snails depredate amphibian eggs in the wild and if so, how might this predation impact amphibian populations. The results of this study, in concert with the continued range-expansion of Giant Apple Snails, may cause concern for those interested in amphibian conservation in the Gulf South.

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