Behavioral plasticity of microhabitat selection of invasive American bullfrog tadpoles reduces predation by local predatory fish

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Introduction

- **Research purpose:**

Predation risk is among the key forces driving the evolution of escape behaviors and related phenotypes. As invasion of introduced species is a problem worldwide, many studies focus on anti-predator behavior of native species. However, relatively less research is done on the development of anti-predatory strategies of invasive species in a new environment. We tested the anti-predator functions of microhabitat preference and behavioral plasticity of **American bullfrog (*Lithobates catesbeianus*)** tadpoles, an invasive species living in South Korea.

- **Research questions:**

1) Can micro-habitats function as anti-predator shelter?
2) Do bullfrog tadpoles prefer micro-habitats with vegetation?
3) Can bullfrog tadpoles utilize micro-habitats according to previous experience?

Material & Method

1. Tadpole collection and maintenance

- Bullfrog tadpoles under Gosner stage 35 collected from May to August 2018
- Four locations: 12 sites each for Gunsan, Ganghwado, Taean, and Soesan of the Republic of Korea

2. Predatory fish collection and maintenance

- 10 young snakeheads purchased from a local store
- Each individual kept separately in an aquarium
- Starved 48 hours prior to each trial

Northern snakehead (*Channa argus*)

3. Experimental design

- Three types of microhabitats: non-vegetated, floating vegetation, submerged vegetation
- Recorded the date, time, water temperature, treatment type, tadpole ID number, and fish ID number

- **Experiment #1: Predation experiment**

1) A starved snakehead and a tadpole placed in the aquarium
2) Observed for 30 minutes
3) Recorded variables: Time until the predator bit the tadpole, number of attacks, time until the tadpole was fully eaten or survival
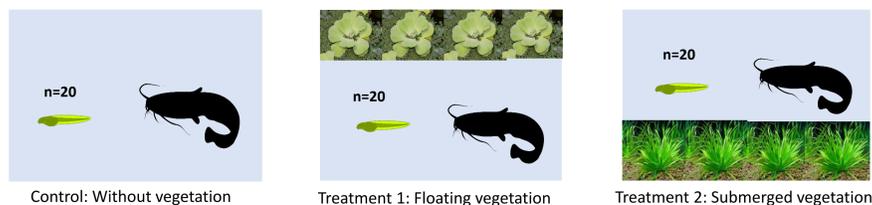4) 20 replicates for each treatment

n=20   Control: Without vegetation

n=20   Treatment 1: Floating vegetation

n=20   Treatment 2: Submerged vegetation

Figure 1. Predation experiment in the three different treatments

- **Experiment #2: Habitat preference experiment**

1) Two types of tadpoles, unhurt and wounded*
2) Aquaria divided in half with floating or submerged vegetation, other half empty
3) Observed for 60 minutes, note location of tadpole every 5 minutes
4) Recorded horizontal and vertical location of tadpole
5) 20 replicates for each type of microhabitat and tadpole

* Wounded tadpoles were prepared by removing one fifth of the tadpole's tail (Van Buskirk and McCollum 2000)

n=20   Floating vegetation
n=20   Submerged vegetation

Figure 2. Habitat preference experiment for unhurt tadpoles

n=20   Floating vegetation
n=20   Submerged vegetation

Figure 3. Habitat preference experiment for wounded tadpoles

4. Statistical analysis

- Shapiro-Wilk W test to determine data normality of water temperature, tadpole length, and frequency of vegetation preference
- T-test to test vegetation preference of tadpoles

Result

- **Experiment #1 Predation experiment**
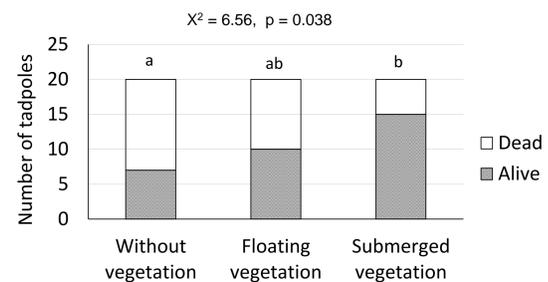
$X^2 = 6.56$, p = 0.038

Figure 4. Survival rates of *Lithobates catesbeianus* tadpoles in three treatments

- Survival rates of tadpoles were significantly different among three treatments.
- Non-vegetated habitat 35%, floating vegetation 50%, submerged vegetation 75%
- Submerged vegetation had the highest survival rate, 15 out of 20 individuals survived.

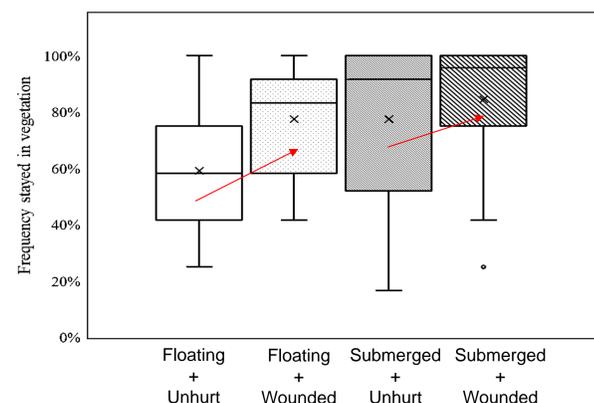- **Experiment #2 Habitat preference experiment**

Figure 5. Microhabitat use frequency for *Lithobates catesbeianus* tadpoles in two vegetation treatments

One sample t-test (vs 50%)	Floating vegetation	Submerged vegetation
Unhurt tadpole	$t = 1.94$, $p = 0.067$	$t = 4.20$, $p < 0.001$
Wounded tadpole	$t = 7.10$, $p < 0.001$	$t = 6.73$, $p < 0.001$

- Tadpoles tended to stay within the vegetation, except for unhurt tadpoles in floating vegetation.
- Wounded tadpoles stayed in vegetation longer than unhurt tadpoles, suggesting they were capable of adjusting their behavior according to predation pressure.

Discussion

- In this study, we demonstrated that (1) the vegetation increased the survival of tadpoles, thus functions as anti-predator shelter, (2) tadpoles preferred to stay in the vegetation, especially for the submerged vegetation, (3) tadpoles adjusted the utilization of microhabitats according to predation pressure.

- Tadpoles may decrease predation pressure by (1) selecting microhabitat and (2) conditionally modifying utilization frequency.

- The capacity of bullfrog tadpoles to avoid predation may be an additional factor in their rapid colonization of habitats worldwide.

Acknowledgement