

## Research Article

**Microhabitat preference in American bullfrog tadpoles (*Lithobates catesbeianus*) in relation to predation pressure**Ming-Feng Chuang<sup>1,#</sup>, Minjee Choe<sup>1,2,#</sup>, Hakyung Kang<sup>1,2</sup>, Amaël Borzée<sup>1</sup>, Ayoung Kim<sup>1,2</sup>, Sera Kwon<sup>1,2</sup>, Musung Sung<sup>3</sup> and Yikweon Jang<sup>1,2,\*</sup><sup>1</sup>Division of EcoScience and Department of Life Science, Ewha Womans University, Seoul, Korea<sup>2</sup>Interdisciplinary Program of EcoCreative, Ewha Womans University, Seoul, Korea<sup>3</sup>College of Biological Sciences and Biotechnology, Chungnam National University, Daejeon, Korea

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**Co-Editors' Note:** This paper is a contribution to the **Behaviour in Aquatic Invasions** Special Issue of *Aquatic Invasions*. Papers in this Special Issue explore how behaviour contributes to invasion success; native species' behavioural strategies that reduce the impacts of invasions; how knowledge of behaviour can enhance management of invasive species; and potential effects of climate change on the behavioural impacts of aquatic invasive species.

**Citation:** Chuang M-F, Choe M, Kang H, Borzée A, Kim A, Kwon S, Sung M, Jang Y (2019) Microhabitat preference in American bullfrog tadpoles (*Lithobates catesbeianus*) in relation to predation pressure. *Aquatic Invasions* 14(3): 444–457, <https://doi.org/10.3391/ai.2019.14.3.04>

**Received:** 30 September 2018**Accepted:** 23 May 2019**Published:** 19 July 2019**Handling editor:** Marian Wong**Thematic editor:** Kit Magellan**Copyright:** © Chuang et al.This is an open access article distributed under terms of the Creative Commons Attribution License ([Attribution 4.0 International - CC BY 4.0](https://creativecommons.org/licenses/by/4.0/)).

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**Abstract**

Predation risk is among the key forces driving the evolution of escape behaviours and related phenotypes. Successful avoidance of predators results in increased survival rate and fitness. The behavioural response of individuals under predation pressure has been comparatively well studied, however, most studies focus on specific escape behaviours and not on behavioural plasticity. The American bullfrog (*Lithobates catesbeianus*) has been an invasive species in the Republic of Korea since the 1970s and has a negative impact on local species. Here, we evaluated the functions of microhabitat preference and behavioural plasticity in *L. catesbeianus* tadpoles through manipulative experiments. In a first experiment, we placed wild-caught tadpoles with a local predatory fish (*Channa argus*) into one of three microhabitat treatments: non-vegetated, submerged vegetation, or floating vegetation. The survival rate of tadpoles with submerged vegetation was significantly higher than that in the non-vegetated microhabitat. In contrast, the survival rates of tadpoles with floating vegetation was not significantly different from the two other treatments. In the habitat preference experiment, “unhurt” and “wounded” wild-caught tadpoles were given a choice between the three microhabitat treatments. Both types of tadpoles preferentially selected the submerged vegetation microhabitat compared to the non-vegetated microhabitat. In contrast, only wounded tadpoles significantly preferred floating vegetation over non-vegetated microhabitats. We conclude that *L. catesbeianus* tadpoles prefer vegetated microhabitats, and especially submerged vegetation. Furthermore, *L. catesbeianus* tadpoles can adjust their anti-predatory behaviour according to previous experience, and thus demonstrate behavioural plasticity by using microhabitats that they do not use in the absence of predators.

**Key words:** behavioural ecology, conservation biology, invasive species, prey-predator interaction

**Introduction**

Invasion by alien species is a serious ecological issues that threatens global biodiversity (Doherty et al. 2016) and leads to severe economic losses (Pimentel et al. 2000). Not all introduced species necessarily become

invasive, the potential for which is limited by a variety of environmental and biological factors (Allendorf and Lundquist 2003). For example, arrival in a new environment with novel predators means an introduced population needs to adapt (Blumstein 2006), and develop anti-predator strategies against local predators (Garcia et al. 2012; Liu et al. 2016). To do so, it is necessary for individuals to learn to recognize their predators in the absence of innate behavioural response (Garcia et al. 2012, 2017) and to transfer this knowledge (Ferrari et al. 2007). These limitations to the success of introduced species should be considered as a potential factor in relative management policies.

The behaviour of individuals is plastic in response to surrounding threats (Wechsler 1995) and this reaction has been coined anti-predatory behaviour, a result of predation acting as selective pressure (Lima and Dill 1990). One the behaviours expressed by prey individuals is to escape to shelter (Cooper and Blumstein 2015), however, those with limited moving abilities have to adopt alternative strategies to lower predation risks. For instance, microhabitat use is one such anti-predation behaviour (Schlosser 1987; Longland and Price 1991; Koivisto et al. 2017; Vijayan et al. 2018). For example, Goldfish (*Carassius auratus*) spent less time foraging in open habitats compared to covered ones in the presence of predators (Vijayan et al. 2018), and tiger salamander larvae (*Ambystoma trigrinum*) displayed differences in microhabitat use in the presence of predatory diving beetles (Holomuzki 1986). Therefore, prey animals exhibit microhabitat preferences to decrease predation risks, although microhabitat preference should be plastic enough to change according to predation pressure, microhabitat type, and other environmental variables.

Amphibian larvae (i.e. tadpoles) are highly plastic in terms of morphology and behaviour (Altig and McDiarmid 1999; Wells 2010), and are therefore ideal models to test plasticity in microhabitat use in relation to predation pressure (Morin 1986; Sazima and Eterovick 2000). Most amphibians live in aquatic environments as tadpoles until metamorphosis, and display morphological variations and specific behavioural syndromes when facing predation pressure (Lardner 2000; Relyea 2001; Wilson et al. 2005; Shaffery and Relyea 2015). Accordingly, tadpoles exploit specific microhabitats or modify their behaviour so that predation risk decreases in the presence of threats (Lawler 1989). For instance, Lawler (1989) demonstrated that *Pseudacris crucifer*, *Dryophytes versicolor*, *D. andersonii* and *Anaxyrus woodhousii* decreased activity in the presence of predatory red-spotted newt, *Notophthalmus viridescens*, and black-banded sunfish, *Enneacanthus obesus*. These responses to predation risk not only promote survivorship for local species, but also for invasive ones. For example, tadpoles of the now widespread American bullfrog (*Lithobates* [*Rana*] *catesbeianus*) (Hammond et al. 2017) can modify their phenotype in response to predatory cues (Peacor 2006).

The American bullfrog *Lithobates catesbeianus* is an invasive predatory species (Lowe et al. 2000; Lever 2003) whose invasion has resulted in numerous negative impacts on amphibians (Blaustein and Kiesecker 2002; Kats and Ferrer 2003; Li et al. 2011b), including behavioural alterations (Kiesecker et al. 2001; D'Amore et al. 2009). The species arrived in the Republic of Korea to be farmed for meat production and was later released into the environment (Park et al. 2014; Groffen et al. 2019). Because of the presence of this species, other species modified their behaviour (Heo et al. 2014), or otherwise suffered from its presence (Kim et al. 2008; Borzée et al. 2017; Kwon et al. 2017). However, *L. catesbeianus* is preyed upon by a few local species (Li et al. 2011a; Heo et al. 2014). For instance, tadpoles are eaten by native fish, including Northern snakeheads (*Channa argus*), Yellowhead catfish (*Pseudobagrus fulvidraco*) and Amur catfish (*Silurus asotus*). They are, however, ignored by all birds tested (No et al. 2017).

We conducted two sets of experiments to assess microhabitat selection in invasive *L. catesbeianus* tadpoles in relation to predation risk. First, we tested the anti-predatory function of microhabitat use, then we compared vegetation microhabitat preference for “unhurt” and “wounded” individuals. Because *L. catesbeianus* tadpoles are principally benthic feeders (Smith and Doupnik 2005), we predicted that survivorship of tadpoles presented with submerged vegetation would be higher than that of those presented with floating vegetation. We also predicted that survivorships in both floating and submerged vegetation would be higher than that in non-vegetated microhabitats. Finally, we expected tadpoles subjected to a proxy for high predation pressure to preferentially use both of the vegetated microhabitats, in contrast with submerged vegetation preference when under low predation pressure.

## Materials and methods

### *Species introduction*

American bullfrogs (*Lithobates catesbeianus*) were imported to the Republic of Korea and introduced in the environment in the 1970s (Park et al. 2014). *Lithobates catesbeianus* is widespread on the west coast of the Korean Peninsula, where it has a negative impact on local amphibians (Borzée et al. 2017), but it is not common on the eastern side of the peninsula (Andersen and Jang 2018). The predatory fish used in this experiment is *Channa argus* (Northern snakehead), which is commonly distributed in the eastern part of Asia, including Korea. *Channa argus* reaches up to 50 cm in length at around four years of age and breeds between May and June (Jang 1991).

### *Tadpole collection and maintenance*

We collected *L. catesbeianus* tadpoles at Gosner stage 35 (Gosner 1960) with funnel traps set in water between May and August 2018. All tadpoles

were caught within four localities in the western lowlands of the Republic of Korea (37.7674°N; 126.4108°E, 37.2805°N; 126.8045°E, 36.724876°N; 126.346469°E, 35.926833°N; 126.724556°E).

Prior to the experiment, tadpoles were kept in glass aquaria (30 × 30 × 30 cm, L × W × D) filled with aged tap water for at least 24 h for acclimation with around 20–30 tadpoles per aquarium. Aquaria were filled with 18 litres of water filtered through a 24-hour working sponge filter (Sponge filter SP-L4, Aquatech; Seoul, Republic of Korea) connected to an air pump (DK-3000 Air Pump; Dae-Kwang Electronics; Seoul, Republic of Korea). Thirty percent of the water was changed every three days. The tadpoles were kept at room temperature and fed *ad libitum* daily with flakes for herbivorous aquatic animals (Formula Two Flakes, Ocean Nutrition; Essen, Belgium).

#### *Predatory fish collection, maintenance and measurement*

When we collected tadpoles from the field, we occasionally captured *C. argus* juveniles that co-existed with bullfrog tadpoles. Hence, we purchased ten juvenile *C. argus* (20 to 30 cm in length) and used them as predatory fish for the experiment. Each individual was kept separately in the same setting as the one used for tadpoles and given a number for trial randomization. To ensure palatability of *L. catesbeianus* tadpoles, all fish were fed with tadpoles prior to the experiment, complemented by blocks of frozen blood worms (Bio-Pure Frozen Blood Worms, Hikari; Imeji, Japan) every other day. To ensure adequate hunger levels, each fish was starved for 48 hours prior to each trial. If a fish did not eat any tadpole during the experiment, it was fed after the experiment. Before each trial, we took pictures of each tadpole in a petri dish with a scale bar placed underneath using a digital microscope (Dimis V7; Anyang, Republic of Korea) and measured the total length with ImageJ (Schneider et al. 2012), down to the second decimal. All fish were set in the experimental aquaria at least two hours prior to the experiment for acclimation to the environment.

#### *Preparation of “wounded” tadpoles*

Anti-predator behaviours are usually triggered by the detection of predators via visual or chemical cues (Feminella and Hawkins 1994; Skelly 1994; Kiesecker et al. 1996; Griffiths et al. 1998; Chivers and Mirza 2001; Relyea 2001, 2002, 2003). Interestingly, *L. catesbeianus* tadpoles generally fail to detect the presence of potential predators (Smith et al. 2008a, b), resulting in the absence of response. To simulate a predation event, we increased predation pressure and stress by non-lethally “wounding” tadpoles through the removal of a fin section, following the protocol presented by Van Buskirk and McCollum (2000). One fifth of the tadpole’s tail was cut, right before the muscle to avoid bleeding and unnecessary

pain. Individuals recovered immediately after release in the aquaria, and the missing portions of the fin regenerated within approximately 10 days.

### *Experimental design*

We conducted two sets of experiments related to microhabitat preference in relation to a local predatory fish: (1) predation experiment, and (2) habitat preference under different predatory pressures. For all experiments, the glass aquaria used were 60 × 35 × 39 cm in size and filled with 46.2 litres of water. All aquaria were washed after each trial to avoid chemical cues being carried over. Prior to the experiment, the water temperature was measured to the nearest 0.01 °C using an electrical thermometer (Waterproof PCSTestr 35 pH/ Conductivity/ TDS/ Salinity Tester, Oakton; Vernon Hills, USA). Temperature ranged from 18 to 28 °C.

The three types of microhabitats to which tadpoles were exposed in all experiments were non-vegetated habitat, floating vegetation, and submerged vegetation. For the non-vegetated habitat, no vegetation was used, but the bottom of the aquarium was covered with small pebbles. For floating vegetation, the aquarium was filled with floating Common water hyacinths (*Eichhornia crassipes*). Finally, for submerged vegetation, the bottom of the aquarium was covered with erect plastic vegetation (ca. 15 cm high). Each microhabitat covered the entire area of the experimental aquarium. We argue that using plastic vegetation over live vegetation does not impede the results of this study as the plastic vegetation was purposely selected to stand erect and provide a microhabitat similar to those present at the bottom of water bodies. Furthermore, previous experiments have not been impaired by the type of hybrid design implemented here (e.g. Smith and Doupnik 2005). For each experiment, in addition to the ID number and measurements of each tadpole, date, time, water temperature, fish ID number and treatment type were recorded. Each tadpole was used once only for a single experiment.

### Experiment #1: Predation experiment

Prior to the experiment, an aquarium was covered by a dark sheet on all vertical sides to reduce the influence of investigator. A single *C. argus* was added to the aquarium; each fish being starved for at least 48 hr before the test to ensure fish were motivated to forage. Then, a tadpole was placed in the aquarium and the behaviours of the tadpoles and fish were observed for 30 min. Recorded variables were: the time until the predator bit the tadpole (in min from the beginning of experiment); the number of attacks in a 30-min trial; and the time until the tadpole was fully eaten or survival until the end of each experiment. After the observation period, the surviving tadpoles were removed and placed in their aquarium of origin for re-acclimation. Each fish was only tested once per day and tested in each treatment twice with different tadpoles to obtain 20 replicates for each treatment.

### Experiment #2: Habitat preference experiment

Two types of tadpole, “unhurt” and “wounded”, were used as a proxy for predation pressure. We tested for microhabitat preference for two types of vegetation (floating and submerged). For the trials, the aquaria were divided such that one half was filled with either floating or submerged vegetation and the other half was left empty. A total of 80 tadpoles was used, divided into 20 trials for each type of microhabitat and tadpole, in a standard two by two experimental design. Trials were carried under similar light condition to eliminate bias. Each tadpole was released in the middle of the testing aquarium, and was observed for 60 min, while noting the location of the tadpole every five min. After the 60-min observation period, the tadpoles were placed back into their tank of origin for re-acclimation. We noted the horizontal (i.e. non-vegetated or vegetated side) and the vertical location (bottom or on the surface) for each observation. For each trial, the date, time, temperature of water, tadpole ID number, tadpole developmental stage and vegetation type were recorded.

### *Statistical analysis*

For statistical analysis of the predation experiment, we encoded the survival rate as the proportion of tadpoles alive at the end of each trial. We calculated vegetation preference as the frequency at which tadpoles selected the vegetation side in the habitat preference experiment. We used a Shapiro-Wilk *W* test to determine data normality of water temperature, tadpole length, and frequency of vegetation preference. To test for the factors affecting survival rates in the predation experiment, we used a Generalized linear mixed model (GLMM). We assumed a binomial distribution and a logit link function for the dependent variable, survival rate. Water temperature and tadpole length were set as random explanatory variables, and vegetation treatment was set as a fixed explanatory variable. This analysis was selected after testing a range of models and comparing the AIC value to select the most appropriate model. To test the vegetation preference by tadpoles, we used a *t*-test to compare the frequency of vegetation preference with random frequency (assumed to be 0.5). To test for factors related to vegetation preference by tadpoles, we used a Generalized Linear Model (GLM) with the dependent variable of the frequency of vegetation preference having a normal distribution assumed and log link function. Vegetation type and predation pressure (i.e. unhurt vs wounded tadpoles) were set as fixed factors, while water temperature, tadpole length, and the interaction between vegetation type and predation pressure were random factors. All statistical analyses were performed in Statistica 10 (StatSoft 2011; Palo Alto, USA). Values of each variable were presented as means  $\pm$  SD or presented as median (range: min–max) when the data were not normally distributed. Significant criterion was set as  $\alpha = 0.05$ .

**Table 1.** Results of microhabitat preference experiments with *Lithobates catesbeianus* tadpoles. The three microhabitats tested were non-vegetated habitat, floating vegetation, and submerged vegetation. Values are presented as mean  $\pm$  SD.

	Non-vegetated habitat	Floating vegetation	Submerged vegetation
Sample size	20	20	20
Temperature ( $^{\circ}$ C)	24.57 $\pm$ 2.25	25.47 $\pm$ 2.00	25.39 $\pm$ 2.13
Tadpole length (cm)	8.05 $\pm$ 1.22	8.40 $\pm$ 0.84	8.63 $\pm$ 1.00
Survival rate	35%	50%	75%

**Table 2.** Results of the GLMMs for the survivorship of tadpoles in the predation experiment ( $n = 60$ ). The optimal model, as highlighted by the lowest AIC value, is underlined.  $P$ -values in bold denote significance.

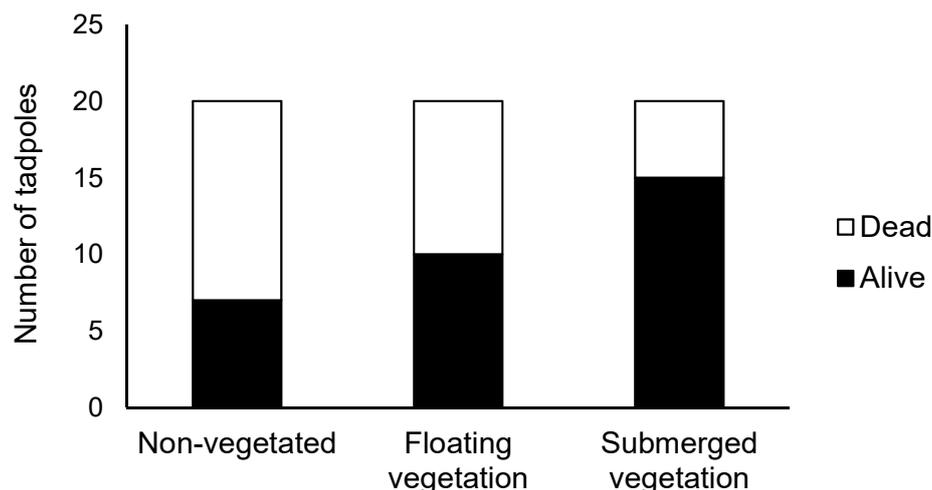
Variables	Estimate	Wald Stat	Confidence Interval	$P$
<b>Null model (AIC = 84.9)</b>				
<b>Full model (AIC = 74.9)</b>				
Water temperature (WT)	-0.34	5.17	-0.64 – -0.05	<b>0.023</b>
Tadpole length (TL)	-0.42	1.95	-1.01 – 0.17	0.163
Treatment	NaN	4.03	NaN	0.133
<b>Partial models (73.9 &lt; AIC &lt; 83.6)</b>				
<u>WT + TL (AIC = 73.9)</u>				
Water temperature	-0.35	5.59	-0.64 – -0.06	<b>0.018</b>
Tadpole length	-0.52	3.09	-1.11 – 0.06	0.078
WT + Treatment (AIC = 75.7)				
Water temperature	-0.33	4.82	-0.63 – -0.04	<b>0.028</b>
Treatment	NaN	5.12	NaN	0.077
TL + Treatment (AIC = 82.3)				
Tadpole length	-0.37	1.76	-0.91 – 0.18	0.184
Treatment	NaN	4.89	NaN	0.087
Water temperature (AIC = 80.4)				
Water temperature	-0.33	5.82	-0.61 – -0.06	<b>0.016</b>
Tadpole length (AIC = 83.6)				
Tadpole length	-0.47	3.12	-1.00 – 0.05	0.078
Treatment (AIC = 82.1)				
Treatment	NaN	6.14	NaN	<b>0.046</b>
Treatment (non-vegetated)	0.78	4.06	0.02 – 1.54	<b>0.044</b>
Treatment (floating vegetation)	0.16	0.18	-0.58 – 0.90	0.672

All the experiments conducted in this study followed the laws of the Republic of Korea (permit 2018-1; from the Han River riverine environment, Ministry of Environment) and all animal experiments were approved by the Institutional Animal Care and Use Committees of Ewha Womans University (permit 18-051).

## Results

### *Predation experiment*

In 28 out of 60 trials, tadpoles were swallowed entirely by the predatory fish *Channa argus*, within  $6.54 \pm 8.31$  min (mean  $\pm$  SD) of the trial beginning. Survival rates of tadpoles were significantly different among three treatments (Tables 1 and 2, Figure 1). Tadpoles in submerged vegetation had the highest survival rate, with 15 out of 20 individuals surviving. In the model containing only treatment the non-vegetated group was significantly related to the lower survivorship of tadpoles, but the lowest AIC values for the GLMM (Table 2) indicates a partial model (water



**Figure 1.** Survival rate of *Lithobates catesbeianus* tadpoles in the three different treatments: floating vegetation, submerged vegetation and non-vegetated.

**Table 3.** Results of microhabitat preference experiments for “unhurt” and “wounded” *Lithobates catesbeianus* tadpoles. The two microhabitats tested were floating vegetation and submerged vegetation. Values are presented as mean  $\pm$  SD.

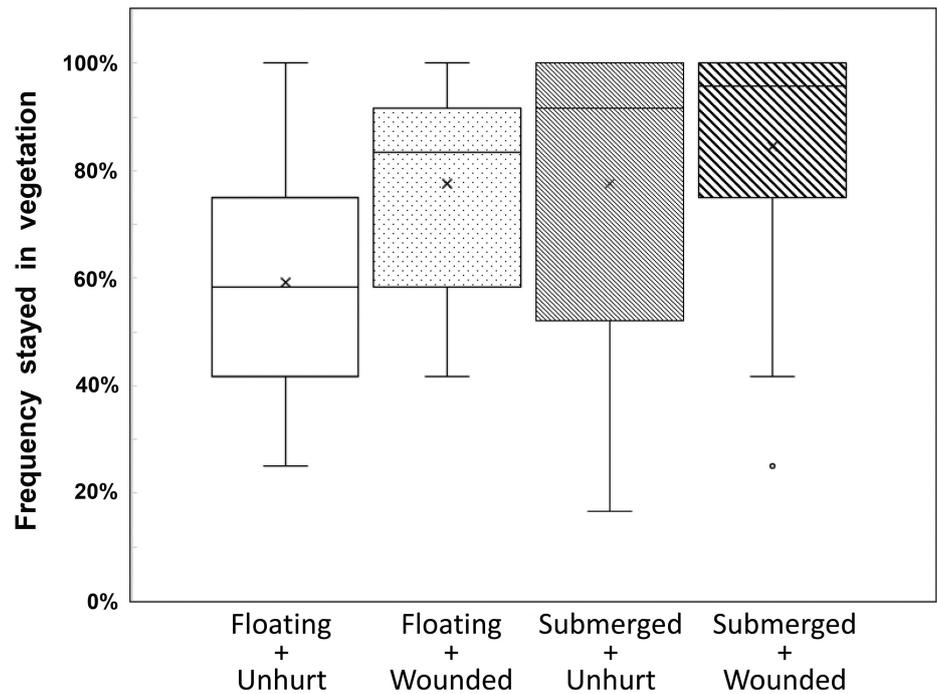
Variable	Unhurt tadpole		Wounded tadpole	
	Floating vegetation	Submerged vegetation	Floating vegetation	Submerged vegetation
Sample size ( <i>n</i> )	20	20	20	20
Water temperature (°C)	22.10 $\pm$ 2.80	22.92 $\pm$ 1.88	28.26 $\pm$ 0.68	23.90 $\pm$ 1.13
Tadpole length (cm)	9.39 $\pm$ 0.94	8.78 $\pm$ 1.51	8.46 $\pm$ 0.70	8.23 $\pm$ 1.00
Frequency on bottom (%)	89.17 $\pm$ 15.56	84.17 $\pm$ 20.39	83.75 $\pm$ 23.80	93.33 $\pm$ 13.68
Preference for vegetation (%)	59.17 $\pm$ 21.10	77.50 $\pm$ 29.26	77.50 $\pm$ 17.54	84.58 $\pm$ 22.99

temperature + tadpole length) as most explanatory for survivorship of tadpoles, where increasing water temperature was related to higher survivorship.

#### Habitat preference experiment

We then compared the habitat preference of unhurt wild-caught and wounded *L. catesbeianus* tadpoles. Tadpoles stayed on the bottom of aquarium in most of the observations (87.60  $\pm$  18.84 % for all 80 tadpoles, Table 3). The habitat preference results were different for each treatment (Figure 2): for unhurt tadpoles with floating vegetation, the frequency of tadpoles staying within the vegetation was not significantly different from random expectations (*t*-test;  $t(19) = 1.94$ ,  $P = 0.067$ ). For the other three treatments, the frequency at which tadpoles stayed within the vegetation were all significantly higher than randomly expected: *t*-test;  $t(19) = 4.20$ ,  $P < 0.001$  for unhurt tadpoles in submerged vegetation;  $t(19) = 7.01$ ,  $P < 0.001$  for wounded tadpole in floating vegetation;  $t(19) = 6.73$ ,  $P < 0.001$  for wounded tadpole in submerged vegetation.

There was a significant interaction between vegetation and predation pressure (Table 4). The percentage of submerged vegetation use seemed similar between unhurt and wounded tadpoles. However, wounded tadpoles were more likely to go to the floating vegetation, compared to unhurt tadpoles



**Figure 2.** Boxplot representation of microhabitat use frequency for *Lithobates catesbeianus* tadpoles under two vegetation treatments and for low and high predation pressure. All tadpoles are wild-caught, and “wounded” tadpoles had a part of their fin removed. Boxplot shows 90% range, 1<sup>st</sup> and 3<sup>rd</sup> quartiles, medians (line), mean (mark), and outliers.

**Table 4.** Results of the GLM univariate tests of significance, effect sizes, and powers for vegetation preference. Alpha values are set at 0.05. *P*-values in bold denote significance.

Variables	DF	<i>F</i>	<i>P</i>	Partial $\eta^2$	Observed power
Intercept	1	13.6	< <b>0.001</b>	0.156	0.954
Water temperature	1	3.9	0.053	0.050	0.491
Tadpole length	1	2.8	0.100	0.036	0.377
Vegetation type	1	0.8	0.361	0.011	0.148
Predation pressure	1	7.9	<b>0.006</b>	0.097	0.794
Vegetation type * Predation pressure	1	4.0	<b>0.050</b>	0.051	0.501
Error	74				

(Figure 2). Other variables, including water temperature, tadpole length, and vegetation type, did not significantly affect tadpoles’ preference (Table 4).

## Discussion

Submerged vegetation may be a refuge from predation for American bullfrog tadpoles (*Lithobates catesbeianus*), and tadpoles avoid predation by preferentially selecting this microhabitat. Furthermore, *L. catesbeianus* tadpoles conditionally modified the frequency of microhabitat use when exposed to predatory cues. Preference for submerged vegetation, but not floating vegetation, increased with predation pressure. Our results support our prediction that tadpoles selectively use vegetation to increase survival rates.

Predation risks influence behavioural decisions made by animals, and consequently the fitness of individuals (Lima 1998; Chuang et al. 2019). We demonstrated that predation pressure influenced the decision of *L. catesbeianus* tadpoles to shelter in vegetation, resulting in higher survival

rates. The underlying mechanism to this microhabitat selection is the complexity of the environment (Kopp et al. 2006), exemplified by the vegetation used in this study. Sheltering in vegetation results in decreased predation because of diminished visual detection (Babbitt and Tanner 1998) and increased foraging difficulty (Folsom and Collins 1984; Warfe and Barmuta 2004). Several other species of anuran tadpoles also exhibit significant preference for coverage or complex microhabitats (e.g. Alford and Crump 1982) in connection with anti-predatory behaviour (reviewed by Lima 1998).

In agreement with previous studies (e.g. Semlitsch and Reyer 1992), we found that “wounded” tadpoles preferentially selected vegetation refuges, indicating conditional adjustment, or “fine tuning”, of their anti-predatory behaviour. “Wounded” tadpoles likely selected vegetation refuges because their swimming performance decreased with the partial removal of their fins, lessening their chance of escaping (Van Buskirk and McCollum 2000). This kind of plasticity in response to predation risks allows individuals to save energy in low-risk situations and allocate more energy to anti-predation behaviour in high-risk conditions (e.g. Quinn and Cresswell 2005; Lima 2009; Elvidge et al. 2014). Most anuran tadpoles decrease their activity upon detecting the presence of predators (Feminella and Hawkins 1994; Skelly 1994; Kiesecker et al. 1996; Griffiths et al. 1998; Relyea 2001, 2002, 2003). This reduction in activity decreases the probability of a chance encounter with predators and thus increases survivorship. However, tadpoles displaying lowered activity budgets also spend less time foraging, resulting in slower growth and development (Skelly 1992). Therefore, the ability to adjust anti-predator behaviours benefits individuals and enables them to reach an optimal balance between costs and benefits in response to predation risks.

Conversely, our result for *L. catesbeianus* tadpoles’ preference for vegetated microhabitat is not consistent with previous studies of the same species. Smith and Doupnik (2005) found that “early tadpoles of bullfrog showed a preference for non-vegetated areas, whereas late tadpoles showed no preference.” Tadpoles below Gosner stage 35 (ca. 8 cm in total length) used in the present study were more similar to the late tadpoles (ca. 7 cm in total, Gosner stage 38–40). These variations may result from several factors. First, the tadpoles used in our study were wild-caught and coexisted with predators so may not have been “naive” individuals, i.e. they may have experienced predation pressure before. However, our “wild-caught unhurt” and “wounded” *L. catesbeianus* tadpoles did show different microhabitat selection. These differences in preference likely arose from corresponding variations between “more naive” unhurt individuals than the ones experimentally “wounded”. Second, *L. catesbeianus* tadpoles may have developed adaptive behaviours since invading Korea. Similar rapid

adaptive behavioural changes have been observed in a native predatory Australian snake in response to the invasive Cane toad (*Rhinella marina*; Phillips and Shine 2006), and these types of behavioural variations are to be expected for newly co-existing species (e.g. Chuang et al. 2019; Polo-Cavia and Gomez-Mestre 2014). The reverse is also likely to occur, where a native species induces rapid behavioural changes in an invasive species.

We also determined that *L. catesbeianus* tadpoles preferred submerged over floating vegetation, corresponding to the microhabitat chosen for fitness benefits by other related species. For instance, *Hyla versicolor* tadpoles show microhabitat selection related to the presence of predatory beetle larvae in order to decrease predation risk (Formanowicz Jr and Bobka 1989). Both our results and the published literature agree in defining *L. catesbeianus* tadpoles as benthic feeders (e.g. Smith et al. 2008a), a category of species for which submerged vegetation is more readily accessible than emerged vegetation. Therefore, we found there was an interaction effect of vegetation type and the level of simulated predation pressure, in which only wounded tadpoles showed preference for floating vegetation but unhurt tadpoles did not.

The anti-predator behaviour of *L. catesbeianus* tadpoles in this study demonstrates their capacity to learn from new environments, a valuable trait when establishing new populations. Not only did they use microhabitats as refuges, they also adjusted microhabitat use frequency according to past experiences. While other species of anuran larvae do not alter their activities until detecting the presence of predators (Schoeppner and Relyea 2005, 2009; Shaffery and Relyea 2015), *L. catesbeianus* tadpoles exhibited preference for refuges both before and after experiencing predation risks. This behavioural strategy is likely to directly increase their ability to avoid predation and may be an additional factor in their rapid colonization of habitats worldwide.

The removal or control of invasive species have become severe challenges for biodiversity management in every ecosystem (Allendorf and Lundquist 2003). Invasions of the American bullfrog have resulted in numerous negative impacts on biodiversity in South Korea (Kim et al. 2008; Borzée et al. 2017; Kwon et al. 2017) and worldwide (Kats and Ferrer 2003; Li et al. 2011b). We hope the empirical evidence found in this study enhances the efficiency of biological control and contributes to the alteration of management policies for invasive species.

### Acknowledgements

We thank Juhyeon Kim and Yejin Kim for their assistance with experiments. We thank Editor Kit Magellan, Editor Vadim E. Panov, associate Editor Marian Wong, Desiree Andersen, and two anonymous reviewers for editing and helpful comments on the manuscript. All the experiments were conducted in agreement with the laws of the Republic of Korea (permit 2018-1; emitted by the Han River riverine environment, Ministry of Environment) and all animal experiments were approved by the Institutional Animal Care and Use Committees of Ewha Womans University (permit 18-051). This work was supported by Korea Environmental Industry & Technology Institute (KEITI RE201709001).

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