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Vulnerability and behavioral responses of South Chinese anuran tadpoles to native dragonfly (*Pantala flavescens*) naiads and introduced western mosquitofish (*Gambusia affinis*)

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ABSTRACT

Anti-predator behavior is an important fitness component in most animals. Alien predator species are a serious threat to amphibian populations. We studied the vulnerability and behavioral defenses of tadpoles of six Chinese anurans in response to the introduced, active foraging predator *Gambusia affinis* (western mosquitofish) and compared them with the responses to the native ambush predator *Pantala flavescens* (dragonfly and naiad stage) in laboratory experiments. The tadpoles were *Bufo gargarizans*, *Duttaphrynus* (formerly *Bufo*) *melanostictus*, *Rana zhenhaiensis*, *Fejervarya multistriata*, *Microhyla onata*, and *Hoplobatrachus chinensis* from Lishui, Zhejiang, South China. Our data showed that both native *P. flavescens* and introduced *G. affinis* could prey on the six tadpole species to different degrees and that the two toad tadpoles (*B. gargarizans* and *D. melanostictus*) were significantly less vulnerable to predation than the frog ones. The reduced vulnerability of toad tadpoles to predation may be attributed to their unpalatability and their continuous swimming ability, traits that are adaptive in more permanent habitats. Compared with the ambush predator *P. flavescens*, tadpoles reduced their activity level and used spatial avoidance measures when encountering *G. affinis*. Overall, our results suggest that some of the tadpoles in the study area are likely to recognize and respond to the predation threats of *G. affinis*.

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Anuran; tadpole; predation risk; activity level; spatial avoidance

Introduction

Animals face varying degrees of predation risk in their natural habitats and predation is an important selective force acting on the behavior of prey species; therefore, behavior plays a decisive role in shaping the outcome of predator–prey interactions (Lima 1998; Ferrari et al. 2010; Nunes et al. 2013). Previous studies demonstrate that amphibian larvae have evolved a variety of behavioral defenses against predators, including reduction in activity level (Laurila et al. 1997; Smith et al. 2009, 2011; Carlson and Langkilde 2014), increased aggregation behavior (Sih et al. 2010; Casillas-Barragán et al. 2016), changes in diel activity patterns (Casillas-Barragán et al. 2016), and shifts in microhabitat use to minimize predation risk (Semlitsch and Gavasso 1992; Niecieza 2000; Pearl et al. 2003; Smith and Awan 2009). A decrease in activity level is one of the most common and effective behavioral anti-predator responses (Ferrari et al. 2010; Zhang et al. 2015). Smith et al. (2010) found that there was variation in behavioral responses (activity level) of *Rana clamitans* tadpoles from different sibships to a non-native predator *Gambusia affinis*. Spatial avoidance of predators also acts as

an anti-predator defense mechanism by reducing the encounter rate with predators (Laurila et al. 1997; Nicieza 2000). However, the benefits in survival gained from these behavioral shifts often come at a cost to other components of fitness, which can reduce resource acquisition and alter growth, development, and life-history patterns (McCollum and Van Buskirk 1996). Therefore, there should be strong selection on prey to recognize dangerous predators, accurately determine predation risk, adjust anti-predator responses accordingly, and balance levels of defense and predation risk (McCoy 2007).

Alien predators are a threat to biodiversity worldwide (Webb and Joss 1997; Hamer et al. 2002; Gurevitch and Padilla 2004; Reynolds 2009; Hussain and Pandit 2012; Shulse et al. 2013). Amphibians are especially vulnerable to the introduction of new predators because they often have complex life cycles with aquatic eggs and larvae (Kats and Ferrer 2003; Vonesh 2005; Segev et al. 2009; Smith et al. 2013), which are consumed in large amounts by aquatic alien predators (*G. affinis*, Goodsell and Kats 1999; *Gambusia holbrooki*, Stanback 2010; *Pomacea canaliculata*, Karraker and Dudgeon 2014; *P. clarkii*, Polo-Cavia and Gomez-Mestre 2014). The western mosquitofish *G. affinis* (Poeciliidae), one of the 100 worst invasive species listed by the World Conservation Union, is native to the fresh waters of the eastern and southern United States (Lowe et al. 2000). Its widespread introduction can be attributed to its purported effectiveness in consuming larval mosquitoes (Pyke 2008). Previous studies have shown that *G. affinis* readily preys on amphibian eggs and larvae, both within (Walls et al. 2002; Baber and Babbitt 2003; Zeiber et al. 2008) and outside of its native range (Goodsell and Kats 1999; Komak and Crossland 2000; Segev et al. 2009; Shulse and Semlitsch 2014; Fan et al. 2016). *G. affinis* was introduced to China for mosquito control in 1927, and it now occurs throughout the southern water bodies of the Yangtze River (Li and Jie 2002). The introduction of *G. affinis* to China caused a serious threat to the diversity and population dynamics of amphibians, but unfortunately, empirical studies to examine the impacts of the mosquitofish on diversity of native anuran species in China have been scarce.

Naiads of the dragonfly *Pantala flavescens* (Fabricius) have been recognized as voracious native predators of anuran larvae and are widely abundant in lentic water bodies (Caldwell et al. 1980; Touchon and Wojdak 2014). The two predators have qualitatively different foraging styles. *P. flavescens* naiads are typical ambush predator invertebrates, resting at the bottom of the body of water or perching on the grass nearby, and directly ambushing the head and abdomen of prey with their piercing-sucking mouthpart after slowly approaching prey through the water column (Touchon and Wojdak 2014). Mosquitofish are usually actively swimming and foraging predators (Komak and Crossland 2000; Touchon and Wojdak 2014). Because of limited gape, mosquitofish often maim individual tadpoles by biting the tail fin; therefore, this species is considered a sublethal predator, but it can kill prey through multiple injuries (Walls et al. 2002; Shulse and Semlitsch 2014; Touchon and Wojdak 2014).

Lishui University (28°27'N, 119°53'E) is in Zhejiang, South China. There is an abundance of permanent ponds and temporary water bodies providing favorable breeding sites for a large number of amphibians, including toads *Bufo gargarizans* and *Duttaphrynus* (formerly *Bufo*) *melanostictus*, and frogs *Rana zhenhaiensis*, *Fejervarya multistriata*, *Microhyla onata*, and *Hoplobatrachus chinensis* (Fei et al. 2009). Tadpoles of these anurans are at a risk of predation from both native *P. flavescens* and alien *G. affinis*. Unfortunately, the ability of these tadpoles to detect and respond to the two predators is largely unknown; this information is important for understanding the extent to which native amphibian larvae are able to cope with this alien predator. Therefore, in this study, we performed a series of laboratory experiments to assess vulnerability, activity level, and spatial predator avoidance of six species of tadpoles under direct predation risk by free-ranging alien mosquitofish *G. affinis* and native dragonfly *P. flavescens* naiads. This study provides the simultaneous assessment of anti-predator behavior of six anuran larvae species in response to the native dragonfly *P. flavescens* naiads and the introduced mosquitofish *G. affinis*.

Table 1. The time of collection, morphological characteristics, and experimental temperature of six tadpole species including *Bufo gargarizans*, *Duttaphrynus melanostictus*, *Rana zhenhaiensis*, *Microhyla ornate*, *Fejervaya multistriata*, and *Hoplobatrachus chinensis* in our experiments.

| Species | Date of collection | Development stage (Gosner) | Total length (mm) | Experimental temperature (°C) |
|-----------------------------------|--------------------|-------------------------------|-------------------------------|-------------------------------|
| <i>Bufo gargarizans</i> | 23 December 2013 | 26.20 ± 0.03 (26.00–27.00) | 13.20 ± 0.21 (11.86–14.55) | 18 |
| <i>Rana zhenhaiensis</i> | 14 January 2014 | 26.70 ± 0.09 (26.00–27.00) | 16.04 ± 0.14 (14.30–17.59) | 18 |
| <i>Duttaphrynus melanostictus</i> | 14 March 2014 | 26.68 ± 0.07 (26.00–27.00) | 16.23 ± 0.09 (15.21–18.54) | 28 |
| <i>Fejervaya multistriata</i> | 8 June 2014 | 26.08 ± 0.12 (26.00–27.00) | 15.83 ± 0.07 (15.22–16.35) | 28 |
| <i>Microhyla ornate</i> | 8 June 2014 | 26.56 ± 0.03 (26.00–27.00) | 16.11 ± 0.12 (15.54–17.37) | 28 |
| <i>Hoplobatrachus chinensis</i> | 21 May 2014 | 26.72 ± 0.05 (26.00–27.00) | 16.88 ± 0.64 (16.42–18.95) | 28 |

Note: Gosner stage and total length of the tadpoles are mean ± SE, range given in parentheses.

Methods

Collection and maintenance of animals

The twining egg strings of *B. gargarizans* (simply called Bg) were collected from a permanent pond at Lishui University on 23 December 2013. The spherical egg masses of *R. zhenhaiensis* (Rz) were collected from a ditch on 14 January 2014. We also collected *D. melanostictus* (Dm) egg strings from the same pond on 14 March 2014, and obtained *H. chinensis* (Hc) egg masses from our Laboratory of Herpetology on 21 May 2014. The floating egg films of *F. multistriata* (Fm) and *M. onata* (Mo) were collected from several short-lived puddles on 8 June 2014. We collected three clutches of the six anurans. The extended experimental period is due to differences in breeding phenology of the six anurans. Clutches were brought back to our herpetological laboratory and kept individually in species-specific plastic containers (700 × 500 × 400 mm³) filled with aged tap water to a 200 mm depth, until tadpoles reached Gosner stage 26–27 (Gosner 1960). The development stages of tadpoles were determined using an anatomical microscope (Nikon XTS30). The tadpoles were fed with commercial bullfrog powder (Tianbang Special Aquatic Mixed Feed Co.) *ad libitum* every two days during the experiment. As the breeding date of our six anurans differed among species, total length, Gosner developmental stage, the dates of predation trials, and behavioral observations also varied (see Table 1). The temperature in the room was approximately 18 or 28 °C, according to the species-specific breeding season temperature, under a 12:12 light:dark cycle.

We dip-netted about 70 similar *P. flavescens* naiads from the same pond within the campus to be used as native predators. Meanwhile, female *G. affinis* with black embryo spots on both ventral sides (Deaton and Cureton 2011) were collected as introduced predators. All anuran species included in this study co-occur with both predators. Predators were housed in identical plastic containers (700 × 500 × 400 mm³) in a climatic chamber. Several small stones provided a perching site for dragonflies, while floating aquatic plants provided a hiding site for mosquitofish. The predators were separated from tadpoles to avoid chemical or visual contact prior to the experiments. All predators were assigned randomly to test groups and starved for 24 h before the experiment.

Experimental design

Predation of dragonflies and mosquitofish on the tadpoles: Our experimental arrangement was a 2 × 6 factorial randomized block design with predators (levels: dragonfly and mosquitofish) and six kinds of anuran tadpoles (levels: *B. gargarizans*, *D. melanostictus*, *R. zhenhaiensis*, *F. multistriata*, *M. onata*, and *H. chinensis*) as the main factors. Every predator combination was replicated 11 times,

for a total of 132 experimental units. Each unit consisted of an opaque plastic container ($180 \times 135 \times 65 \text{ mm}^3$) filled with aged tap water to a 50 mm depth. We placed a dragonfly naiad or mosquitofish in a container with 10 tadpoles at 20:00, and counted the number of tadpoles remaining in the bowls of all treatments at 24 h to estimate survivorship. All docked-tail and dead tadpoles were regarded as predation events. Throughout the experiment, all containers were arranged on the floor to avoid slight temperature variation in the laboratory room. Throughout the experiment, laboratory conditions were maintained as in the pre-experiment phase.

Behavioral response of tadpoles to predators: At the beginning of the experiment, a single tadpole was randomly assigned to each circular plastic bowl with a 200 mm diameter and 50 mm water depth. After allowing the tadpoles to acclimate for 5 min, a predator (dragonfly naiad or mosquitofish) was placed into the container. We monitored each tadpole for 30 min using the instantaneous scan sampling method (Polo-Cavia and Gomez-Mestre 2014), recording the activity level, spatial vertical distribution, and horizontal distance from the free-roaming predator every 2 min (15 scans per tadpole in total). One observer measured activity state by counting the number of active tadpoles at the instant of observation. The tadpole was considered to be active when it was actively swimming (either slowly or with bursts of speed), feeding (even if not substantially altering position), or simply undulating its tail (without actively swimming) (Nunes et al. 2013). Tadpoles were considered to be preferring the upper part of the water column when located at 0–25 mm depth, and preferring the bottom when located below 25 mm in depth. The second observer photographed the predators from above using a digital camera (Sony DSC-T100), then the tadpoles' horizontal distance from the predators (from the base of the tadpole's tail-fin to the snout of the predator, $\pm 0.01 \text{ mm}$) was measured using ImageJ 1.44p software. A 20 mm long metal wire was placed at the bottom of every bowl to provide a size standard in photographs. All behavioral measurements were made between 08:00 and 12:00. The two observers, who sat quietly on both sides of the experiment containers from approximately 0.5 m away, did not appear to affect tadpole behavior (Nicieza 2000). Treatments were monitored in a random sequence within 45–55 min to prevent possible bias due to diel activity cycles (Nicieza 2000). At the end of each trial, we immediately replaced the tadpole, the water (with aged tap water), and the predator, then repeated the procedure. Fifteen replicates for each kind of predator, and all the tadpoles and predators were used only once. After the experiments, the surviving tadpoles were released back to the capture ponds, whereas the predators (dragonflies and mosquitofish) were frozen to death.

Data analysis

All statistical analyses were performed using Statistica 5.0 software. All variables were tested for normality and homogeneity using the Kolmogorov–Smirnov test and the F -max test, respectively. To analyze the interspecific differences in the vulnerability of tadpoles to the two predators, we performed a two-way ANOVA with treatment ('dragonfly naiads' vs. 'mosquitofish') as a between-subject factor and the six anuran species as a within-subject factor, then Tukey's *post hoc* test with different predators or tadpoles species as factor. We performed the similar above statistical methods to compare the behavioral responses of the six tadpole species to the two predators. If the tadpoles were consumed by the predator during the behavior experiments, the data of those individuals were excluded from the analysis. All results were expressed as mean \pm SE, with $\alpha = 0.05$ considered statistically significant.

Results

Vulnerability

There were no significant differences in the survival rates of tadpoles paired with dragonfly naiads versus those paired with mosquitofish either at 24 h ($F_{1,131} = 0.53$, $P = 0.468$, Figure 1). However,

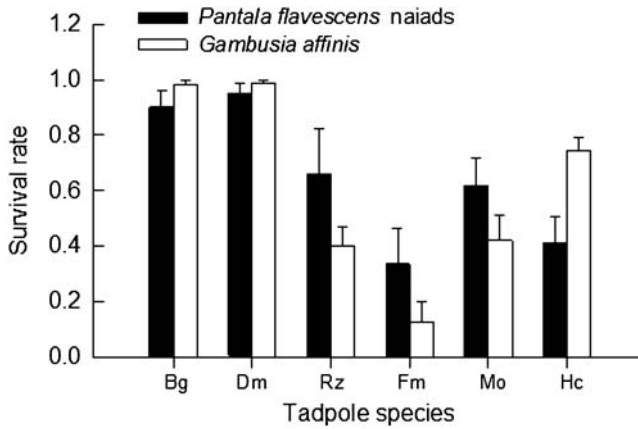


Figure 1. Survival rate (mean ± SE) of the six tadpole species at 24 h coexisting with indigenous dragonfly *Pantala flavescens* naiads or introduced mosquitofish *Gambusia affinis*.
 Note: Bg: *Bufo gargarizans*, Dm: *Duttaphrynus melanostictus*, Rz: *Rana zhenhaiensis*, Mo: *Microhyla ornate*, Fm: *Fejervaya multistriata*, Hc: *Hoplobatrachus chinensis*.

there were significant differences in the survival rates among the six anuran tadpoles at 24 h ($F_{5,131} = 20.23, P < 0.001, Bg^a, Dm^a, Rz^b, Fm^c, Mo^b, Hc^b$). Different superscripts indicate significant differences ($a > b > c$). The effect of the interaction between predators and anuran species on the survival rate of tadpoles was significant ($F_{5,131} = 4.62, P < 0.001$).

Behavioral response

Activity level: There were more active tadpoles in the presence of dragonfly naiads than in the presence of mosquitofish ($F_{1,160} = 49.92, P < 0.001, \text{Figure 2}$). The active state of the six tadpole species significantly differed ($F_{5,160} = 127.87, P < 0.001, Bg^{ab}, Dm^a, Rz^b, Fm^c, Mo^c, Hc^b$). The interaction between predators and tadpole species was significant ($F_{5,160} = 27.34, P < 0.001$).

Vertical spatial distribution: There were no significant effects of the presence of predators on the bottom distribution of tadpoles ($F_{1,160} = 1.26, P = 0.264, \text{Figure 3}$), but this distribution was significantly correlated with anuran species ($F_{5,160} = 53.89, P < 0.001, Bg^d, Dm^{bc}, Rz^a, Fm^c, Mo^a, Hc^b$).

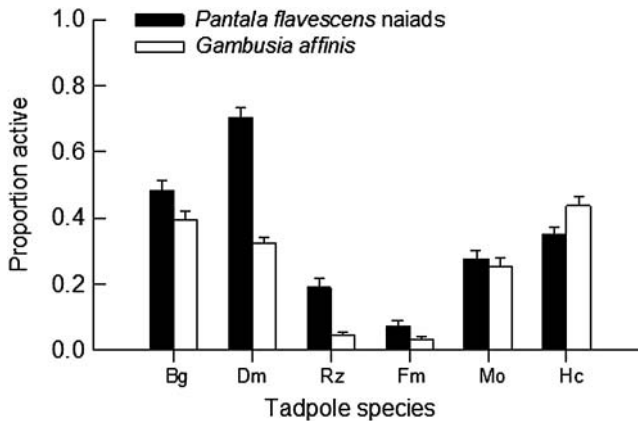


Figure 2. Percentage of six species tadpoles keeping active level in the presence of *Pantala flavescens* naiads or *Gambusia affinis* predators. Data are mean (± SE) of all observations taken at 2 min intervals over 30 min.
 Note: Bg: *Bufo gargarizans*, Dm: *Duttaphrynus melanostictus*, Rz: *Rana zhenhaiensis*, Mo: *Microhyla ornate*, Fm: *Fejervaya multistriata*, Hc: *Hoplobatrachus chinensis*.

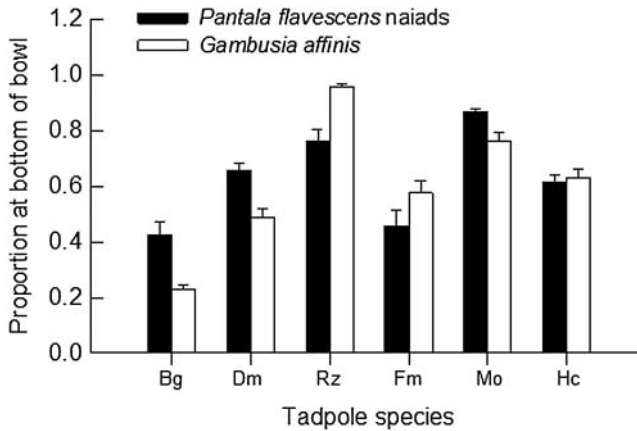


Figure 3. Proportion at the bottom of the bowls of six species tadpoles co-existing with *Pantala flavescens* or *Gambusia affinis* predators. Data are mean (\pm SE) of all observations taken at 2 min intervals over 30 min.

Note: Bg: *Bufo gargarizans*, Dm: *Duttaphrynus melanostictus*, Rz: *Rana zhenhaiensis*, Mo: *Microhyla ornate*, Fm: *Fejervaya multistriata*, Hc: *Hoplobatrachus chinensis*.

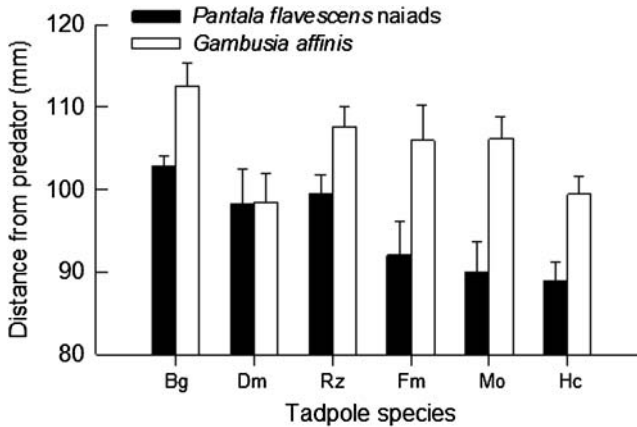


Figure 4. Mean (\pm SE) distance between the six tadpole species and the predators (*Pantala flavescens* naiads or *Gambusia affinis*).

Note: Bg: *Bufo gargarizans*, Dm: *Duttaphrynus melanostictus*, Rz: *Rana zhenhaiensis*, Mo: *Microhyla ornate*, Fm: *Fejervaya multistriata*, Hc: *Hoplobatrachus chinensis*.

The interaction between predators and tadpole species significantly affected the distribution of tadpoles ($F_{5,160} = 9.62$, $P < 0.001$).

Horizontal distance from predators: The average horizontal distance between tadpole and dragonfly was significantly shorter than that between tadpole and mosquitofish ($F_{1,161} = 25.27$, $P < 0.001$, Figure 4). There were also significant differences in the horizontal distances between tadpole and predator among anuran species ($F_{5,161} = 3.76$, $P < 0.003$, Bg^a, Dm^{ab}, Rz^b, Fm^b, Mo^b, Hc^b). The interaction of predators and tadpoles species was not significant ($F_{5,161} = 1.21$, $P = 0.308$).

Discussion

In our survival rate trials, both native ambush predator *P. flavescens* and introduced active foraging predator *G. affinis* could prey on the six species of tadpoles within 24 h, so our results confirm that there really are predator–prey relationships among them as other studies have reported (Goodsell and Kats 1999; Komak and Crossland 2000; *Litoria aurea*, Pyke and White 2000). We also observed that the two predators took different predatory strategies when attacking the tadpoles. *P. flavescens*

often ate the head and mid-ventral region along with the internal organs of tadpoles, while *G. affinis* first consumed the tail-fin of tadpoles and then ate the remainder.

Although, there were no significant differences in the predation of *G. affinis* and *P. flavescens* to the six species of tadpoles. But to a certain degree, the survival rates of *B. gargarizans* and *D. melanostictus* tadpoles were significantly higher than *R. zhenhaiensis*, *F. multistriata*, *M. onata*, and *H. chinensis* tadpoles in the presence of mosquitofish and dragonflies. These results are likely attributed to multiple factors including identification capability, behavioral response of the tadpoles, and predation mode of the predators. The most likely reason may be related to the tadpoles' ability to recognize their predators. Some studies have shown that the tadpoles of many amphibian species can respond to the presence of chemical cues from local predators by reducing their activity levels or changing their space use as an adaptive anti-predator strategy (Ferrari et al. 2016; Hettyey et al. 2015). But there are actually several studies that show this is not the case and that native species do respond to non-native species. For example, Buttermore et al. (2011) found that the activity of wood frog (*Lithobates sylvaticus*) tadpoles was not affected by the presence of various cues from mosquitofish (*G. affinis*). They are unable to recognize cues from introduced predators because they may lack common evolutionary history with the introduced predator (Gomez-Mestre and Diaz-Paniagua 2011; Polo-Cavia and Gomez-Mestre 2014). Moreover, a number of studies have indicated a common lack of adaptive responses to alien predators as a major cause of decline in global amphibian populations (Gillespie 2001; Kats and Ferrer 2003; Cruz et al. 2008; Polo-Cavia and Gomez-Mestre 2014). A history of co-evolution between predator and prey is important for prey to respond adaptively to predation risk (Gomez-Mestre and Diaz-Paniagua 2011). If these tadpoles cannot effectively learn to recognize introduced predatory mosquitofish as a threat in a relatively short period of time, then the introduction of *G. affinis* poses a serious threat to our amphibian diversity. It seems that introduced mosquitofish may cause greater predation pressure on *R. zhenhaiensis*, *F. multistriata*, and *M. onata* tadpole populations, and could therefore be a greater danger to them than native dragonflies.

In addition, our results also showed that *P. flavescens* and *G. affinis* preyed on significantly fewer Bufonidae tadpoles than other tadpoles. We believe that there may be two possible reasons. First, *D. melanostictus* and *B. gargarizans* belong to the family Bufonidae. Some studies have shown that several species of Bufonidae produce noxious or toxic compounds (alarm substances) which cause unpalatability to deter native predators such as dragonflies (Laurila et al. 1997; Crossland and Alford 1998), *Procambarus clarkii* (Nunes et al. 2013), and *P. canaliculata* (Karraker and Dudgeon 2014), as well as invasive predators such as *G. affinis* (Komak and Crossland 2000). Unpalatability often is considered as an anti-predators defensive strategy for the tadpoles (Pearl et al. 2003). Gunzburger and Travis (2005) reviewed that tadpoles species that commonly breed with fish in the permanent ponds were more likely to be found unpalatable by fish predators and tend to have higher survival rates with fish predators than those amphibian species that rarely breed with fish, and species that usually breed in temporary ponds were palatable to fish. Karraker (2011) found that *D. melanostictus* larvae are known to be unpalatable to larvae of the native dragonfly *P. flavescens* and the invasive mosquitofish *G. affinis* in Hong Kong. Dragonflies consumed an average of 20% of *B. gargarizans* tadpoles, indicating that the tadpoles are somewhat palatable to the dragonfly. Second, the tadpoles of *B. gargarizans*, *D. melanostictus*, and *H. chinensis* were highly active when coexisting with the predators. We also found that the tadpoles of *B. gargarizans* and *D. melanostictus* were fond of continuous swimming in their natural persistent pond habitats (personal observation); therefore, this may be a response to having enough benefit from swimming rather than a direct response to the predator. The continuous swimming of tadpoles of the family Bufonidae aids in their escape from *G. affinis*, and may be part of social facilitation of anti-predator behavior (Crane et al. 2012). Previous research has shown that *H. chinensis* tadpoles are aggressive carnivores that often cannibalize larvae (Fan et al. 2014), which may explain why this species can keep high survivorship in the presence of predators. Other studies have shown that the interspecific differences in anti-predator behavior of tadpoles likely depend on the habitat preference of each species. Prey species that inhabit more permanent habitats tend to exhibit defensive

strategies that enable them to coexist with potential predators (Gómez and Kehr 2011). In opportunistic species that breed in highly variable and/or unpredictable environments, abiotic factors are more important for the population dynamics of tadpoles, and predator-induced responses affecting their external and internal plasticity will differ from those of other species, the tadpoles of which inhabit more-predictable environments (Gómez and Kehr 2011).

Several previous studies have documented a positive correlation between predation risk and prey activity level (Crane et al. 2012; Casillas-Barragán et al. 2016), thus a decrease in activity level is one of the most common and effective behavioral anti-predator responses (DeSantis et al. 2013). For example, larvae of the ringed salamander (*Ambystoma annulatum*) responded to chemical cues from dragonfly (Family: Libellulidae) nymphs with appropriate decreased activity in a predator-recognition experiment (Crane et al. 2012). Nunes et al (2013) demonstrated that tadpoles of *Alytes cisternasii*, *Discoglossus galganoi*, *Bufo bufo*, *Hyla meridionalis*, and *Pelodytes ibericus* had strong behavioral responses to dragonflies, specifically decreased activity. Spadefoot toad (*Sphaerotherca breviceps*) tadpoles were capable of innately recognizing waterborne chemical cues from native dragonflies, and thus, adaptively reduced their activity level (Petranka and Hayes 1998; Mogali et al. 2015). Some species of tadpoles show anti-predator behaviors such as reducing their activity in response to the presence of mosquitofish or mosquitofish cues (Lawler et al. 1999; Smith et al. 2007). In general, greatly reduced activity levels in the presence of the native predator were observed.

Our results suggest that the different foraging styles of dragonfly naiads and mosquitofish have significant effects on the activity level of tadpoles. More specifically, more tadpoles were active and kept a closer distance in the presence of *P. flavescens* than in the presence of *G. affinis*. The reason may be that ambush predator *P. flavescens* slowly closed in on the tadpoles, so it scarcely attracted attention, and therefore did not induce avoidance behavior. In contrast, the tadpoles usually kept in a low activity level and used spatial avoidance measures when they encountered the active swimming predator *G. affinis*. Low activity state makes prey less noticeable to visually oriented predators, then may decrease the probability that prey will inadvertently swim near a visually cryptic ambush predator such as a dragonfly naiad. For aquatic vertebrates, decreased activity has been reported as a common response to increased predation risk (Mirza et al. 2006), and it has been shown to increase the probability of tadpoles surviving encounters with dragonfly predators (Skelly 1994). This is also consistent with previous studies demonstrating that spatial avoidance of the predator was an instinctive response for prey (Laurila et al. 1997; Nicieza et al. 2006). For instance, tadpoles (*Crinia signifera* and *Limnodynastes tasmaniensis*) showed obvious predator avoidance behavior when *G. holbrooki* was introduced into the pond (Lane and Mahony 2002).

Both native *P. flavescens* and introduced *G. affinis* can prey on the six species of tadpoles studied, and the lower vulnerability of Bufonidae tadpoles might be attributed to their unpalatability and higher level of activity as compared to Ranidae tadpoles. All tadpoles employed spatial avoidance measures when encountering active foraging predator *G. affinis* but not when encountering ambush predator *P. flavescens*. Moreover, the tadpoles were less active in the presence of *G. affinis* than in the presence of *P. flavescens*. Thus, some of the tadpoles in the study area are likely to recognize and respond to predation threats of the introduced predator *G. affinis*.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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