

Can an Invasive Prey Species Induce Morphological and Behavioral Changes in an Endemic Predator? Evidence from a South Korean Snake (*Oocatochus rufodorsatus*)

Jun-Haeng HEO^{1#}, Heon-Joo LEE^{1#}, Il-Hun KIM¹, Jonathan J. FONG², Ja-Kyeong KIM¹, Sumin JEONG¹ and Daesik PARK^{1*}

¹ Department of Biology, Kangwon National University, Chuncheon, Kangwon 200-701, South Korea

² Division of Science Education, Kangwon National University, Chuncheon, Kangwon 200-701, South Korea

Abstract Introduction of an invasive prey species into an ecosystem may affect an endemic predator's fitness by altering the prey-predator system. Successful adaptation may allow the endemic predator to eat and control the invasive species, while unsuccessful adaptation may result in extinction of the predator. We examine the possible effects of the invasive North American bullfrog (*Rana [Lithobates] catesbeiana*) on the endemic Red-backed rat snake (*Oocatochus rufodorsatus*) in South Korea. We do so by comparing the morphology and behavior of adult and hatchling snakes from bullfrog-exposed (Taeon) and bullfrog-unexposed (Hongcheon) populations. Among the seven morphological characteristics investigated, relative tail length (tail length/snout-vent length) of both adults and hatchlings from Taeon was significantly greater than that of adults and hatchlings from Hongcheon. Also, adult snakes from Taeon had a significantly shorter latency of first tongue flick in response to prey compared to adults from Hongcheon. This difference was not observed in hatchlings. In other snake species, a longer relative tail length and shorter latency of first tongue flick are known to improve foraging efficiency, and these characters may be adaptations of *O. rufodorsatus* to prey on bullfrogs. This study provides preliminary evidence that the presence of an invasive prey species may cause morphological and behavioral changes in an endemic predator.

Keywords invasive prey, bullfrog, *Rana catesbeiana*, *Oocatochus rufodorsatus*, predator response

1. Introduction

Invasive species can disrupt an ecosystem (Gurevitch and Padilla, 2004; Strauss *et al.*, 2006; Vellend *et al.*, 2007; Strayer, 2012) by altering the prey-predator system (Carlsson *et al.*, 2009; Anson *et al.*, 2013). Often, endemic prey are more affected by an altered prey-predator system than endemic predators (Lima, 2002; Johnson and Agrawal, 2003; Nuismer and Thompson, 2006). However, invasive prey can also affect endemic predators (Phillips and Shine, 2004, 2006; De Rivera *et al.*, 2005; King *et al.*, 2006; Langkilde, 2009; Wanger *et al.*, 2011; Harley *et al.*, 2013; Llewelyn *et al.*, 2013).

Invasive prey species may directly or indirectly decrease the fitness of endemic predators, such as by altering prey composition (Suarez and Case, 2002; Pothoven and Madenjian, 2008; Heinonen and Auster, 2012). As a response, morphological and behavioral changes due to developmental phenotypic plasticity and/or evolutionary adaptation can appear in the endemic predators (Mittelbach *et al.*, 1999; Aubret *et al.*, 2004; Phillips and Shine, 2004; Langkilde, 2009; Li *et al.*, 2011a; Wanger *et al.*, 2011). Populations of successfully adapted predators often decrease in the early stages of a prey's invasion, but soon recover to stable levels and play a key role in the long-term control of the invasive prey species (Brodie and Brodie, 1999; Phillips and Shine, 2004; Kishida *et al.*, 2006). On the contrary, populations of unsuccessful endemic predators continuously decline and are often extirpated (Case and Bolger, 1991).

Bullfrogs (*Rana [Lithobates] catesbeiana*), native

[#] These authors contributed equally to this work.

* Corresponding author: Dr. Daesik PARK, from Kangwon National University, Chuncheon, South Korea, with his research focusing on the basic and conservation ecology of Korean amphibians and reptiles.
E-mail: parkda@kangwon.ac.kr

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to North America, are now found in more than 45 countries worldwide. Due to its negative impacts in introduced areas, *R. catesbeiana* was designated one of the 100 worst invasive species by IUCN (Lowe *et al.*, 2000). *R. catesbeiana* often causes the decline of endemic insects and frogs directly through predation or indirectly through foraging competition (Kang and Youn, 1994; Kiesecker *et al.*, 2001; Hirai, 2004; Wu *et al.*, 2005; Wang *et al.*, 2007; Da Silva and Filho, 2009; Ra *et al.*, 2010; Li *et al.*, 2011a, 2011b; Jancowski and Orchard, 2013). Such effects result in altered prey-predator systems.

In Korea, *R. catesbeiana* was first introduced from Japan for commercial purposes in the early 1970s and dispersed to most parts of South Korea in the mid-1990s (Kim, 1972; Kang and Youn, 1994; Choi *et al.*, 1998; Kim and Ko, 1998). *R. catesbeiana* has been observed feeding on insects, amphibians, fish, and juvenile snakes in freshwater habitats (Kim and Ko, 1998; Chung, 2005; Oh and Hong, 2007). The distribution of *R. catesbeiana* in South Korea was frequently monitored (Kim and Ko, 1998; Jang and Suh, 2010; Figure 1). The Red-backed rat snake, *Oocatochus rufodorsatus*, is a predator of *R. catesbeiana* and provides a good opportunity to study the effects of prey introduction on an endemic predator because they share the same habitat (Sung *et al.*, 2006). A negative correlation between the presence of *Pelophylax nigromaculatus*, a prey item of *O. rufodorsatus*, and *R. catesbeiana* (Ra *et al.*, 2010) provides evidence that *R. catesbeiana* is altering the prey-predator system. In this study, we compared morphological characteristics and prey response behaviors of adult and hatchling *O. rufodorsatus* from bullfrog-exposed and unexposed populations in South Korea to study the effect of an altered prey-predator system.

2. Materials and Methods

2.1 Collection and housing Animal handling and experimental procedures were conducted in accordance with guidelines established by the Kangwon National University Institutional Animal Care and Use Committee. For the bullfrog-exposed populations, we selected two sites in Taean County, Chungcheongnam Province, South Korea (populations hereafter referred to as “Taean”), where *R. catesbeiana* were introduced before 1994 and are currently found (Kim and Ko, 1998; Jang and Suh, 2010; Jang *et al.*, 2011; Figure 1). The two sites in Taean are approximately 40 km apart in Wonbuk (Taean 1) (36°49'17.15" N, 126°14'40.44" E) and Anmyeon (Taean 2)

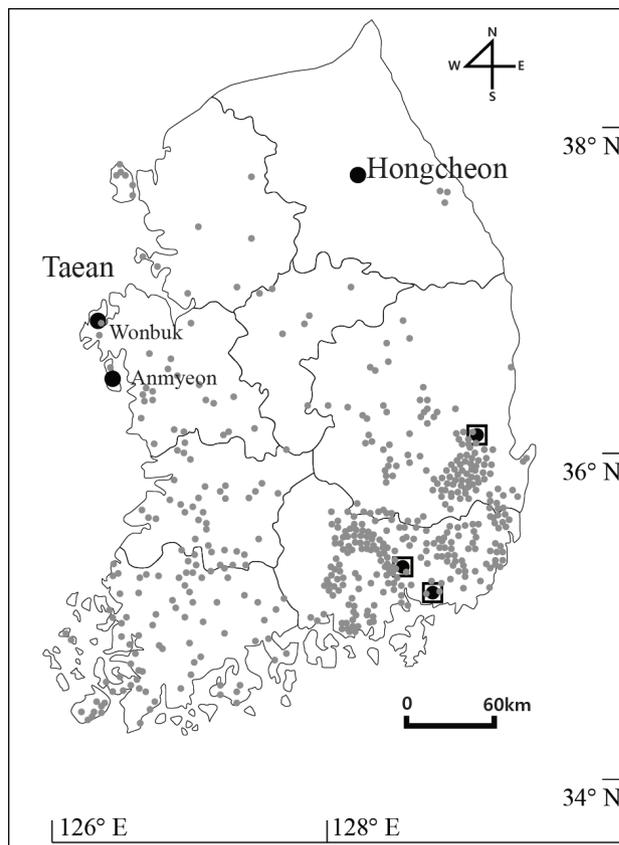


Figure 1 Locations (black filled circles) of sites where adult *Oocatochus rufodorsatus* were collected. The frogs used as prey items, *Rana (Lithobates) catesbeiana* and *Pelophylax nigromaculatus*, were collected from the same sites. Distribution of *R. catesbeiana* in South Korea reported in 2010 (Jang and Suh, 2010), represented by gray dots. Three locations where *R. catesbeiana* was first introduced are indicated by black filled circles surrounded by a square.

(36°28'53.37" N, 126°20'0.42" E) (Figure 1).

For the bullfrog-unexposed population, one site was selected in Hongcheon County, Gangwon Province, South Korea (population hereafter referred to as “Hongcheon”) (37°28'53.37" N, 128°04'33.67" E), where there are neither historical nor modern records of *R. catesbeiana* (Kim and Ko, 1998; Suh, 2005; Jang and Suh, 2010; Jang *et al.*, 2011; Figure 1).

All sampling was conducted in July of 2013. At Taean 1, we collected 9 adult snakes (9 females) from an unused rice paddy with an agricultural pond. This rice paddy had other rice paddies on one side and low mountains on the other three sides. The size of the paddy was 2188 m² and the nearest rural town (> 5 houses) was 800 m away. During sampling, we observed one *O. rufodorsatus* foraging for *R. catesbeiana*. At Taean 2, we collected 15 adult snakes (10 females, 5 males). The habitat consisted of an agricultural pond adjoined with rice paddies on one

side and pine tree windbreaks on the other three sides. The size of the pond was 1732 m² and approximately half of the perimeter was occupied by fully-grown Oriental cattails (*Typha orientalis*). The nearest rural town was 630 m away. Although we did not observe *R. catesbeiana* during our sampling they have been found in neighboring areas (< 3 km) (Jang *et al.*, 2011). At Hongcheon, we collected 22 adult snakes (15 females, 7 males). This site consisted of an agricultural pond adjoined to rice paddies on two sides and low mountains on the other two sides. The size of the pond was 1675 m² and most of the perimeter was occupied by *T. orientalis*. The nearest rural town was 170 m away. During the sampling, we did not observe any *R. catesbeiana*.

After capture, adult snakes were immediately transported to the laboratory at Kangwon University (37°52'06.06" N, 127°44'33.32" E; 180 km away from Taean and 30 km away from Hongcheon). Individuals were marked by inserting a passive integrated transponder tag (TX1411L, Biomark, Boise, Idaho, USA) under the skin. All snakes were individually kept in plastic boxes (39 cm × 26 cm × 17 cm) and fed one mouse (1.4 g – 2.3 g) every two weeks. A bowl with 200 ml of water was placed inside the box and changed every two days. For shelter and effective skin shedding, paper towels and a plastic T-shape pipe were put inside the box. Temperature and light–dark cycles were kept at ambient levels throughout the study. After completing behavioral experiments, adult and hatchling snakes were all released at their respective collection sites after health examination based on skin color, body condition, and locomotion.

2.2 Morphological characteristics of adult snakes

Before conducting experiments, we measured seven morphological characteristics of 22 adult snakes (18 females and 4 males) from Taean and 21 (15 females and 6 males) from Hongcheon; three snakes with partially damaged tails were excluded (1 female/1 male from Taean, 1 male from Hongcheon). We measured the snout-vent length (SVL; tip of the snout to the posterior of the vent), tail length (TAL; posterior of the vent to the tip of the tail) to the nearest 0.1 cm using a measuring tape and the body weight (BW) to the nearest 0.1 g using a digital balance (SI-132, Excel Precision Co., New Taipei City, the People's Republic of China). From these data, we calculated the relative tail length (RTL) by dividing the TAL by SVL. In addition, we measured the head length (HL; tip of the snout to the quadrate-articular jaw joint), head width (HW; at the widest point of the head), chin length (CL; tip of low jaw to the line which connects two points of the most posterior end of the mouth) and

chin width (CW; width between the two points of the most posterior end of the mouth) to the nearest 0.01 mm using digital vernier calipers (IP66, Mitutoyo, Kawasaki, Japan). Using the data of CL and CW, we calculated the gape index (GI) of each snake following $GI = \pi(CL)/(CW)/4$ (King, 2002).

2.3 Adult snake response to frog prey

2.3.1 Preparing prey Three different frog prey were used for trials: 1) metamorphosed *R. catesbeiana* from Taean 1, 2) *P. nigromaculatus* from Taean 1, and 3) *P. nigromaculatus* from Hongcheon. All prey items were caught in rice paddies near where the adult snakes were collected. The frogs were transported to the laboratory and kept at a density of 20 individuals in aquaria (53 cm × 40 cm × 28 cm) that had both aquatic and terrestrial parts. For food, we provided three crickets per frog once every three days. To facilitate successful prey foraging of adult snakes, only recently metamorphosed frogs (~2 weeks) were used.

2.3.2 Experimental procedures Experiments were conducted with adult *O. rufodorsatus*, 16 (12 females, 4 males) from Taean and 16 (11 females, 5 males) from Hongcheon between 10:00 and 19:00 from August 6 to 11, 2013. We presented pithed, unconscious frogs to the snakes. None of the frogs were recovered during the experiment and the uneaten frogs were finally sacrificed by guillotine.

To acclimate the snakes, each individual was placed in an opaque experimental box (30 cm × 30 cm × 30 cm) 1 hour prior to starting the trial. During the experiments, light and temperature followed local, ambient conditions. All behaviors were recorded using a digital video camcorder (DCR-SR65, Sony, Tokyo, Japan) positioned 30 cm above the arena. We presented a frog in the center of the box using forceps (30.5 cm long), and following previous studies (Cooper and Burghardt, 1990; Labra *et al.*, 2001), the trial was complete when the snake ate the frog or after 10 min. The time taken to eat the prey was measured from the snake's first touch to completely swallowing the frog. The snake and prey type for each trial were randomly assigned using an online random number generator (<http://www.random.org/>). Each snake was exposed to each type of prey once, and was given two days rest between trials. After each experiment, the experimental boxes were washed with hot water and dried before using again.

2.4 Hatchling snake response to frog odors

2.4.1 Obtaining hatchlings and preparing frog odors After the prey response experiments, female adult snakes

were individually kept in plastic boxes (39 cm × 26 cm × 17 cm) and fed one mouse once a week. Most females gave birth between August 8 and 20. The clutch size of the 13 pregnant females (7 in Taean, 6 in Hongcheon) was 9.6 ± 3.3 SD (range: 5–14). We kept each clutch in separate aquaria (45 cm × 32 cm × 35 cm), placing paper towels and a T-shaped plastic pipe for shelter. We marked each hatchling with a number on the dorsal plate. The birth day and the day of skin shedding were recorded for each individual. After the prey odor experiments, we measured the SVL, BW, TAL, and RTL of the hatchlings using the same method for adult snakes. Since the hatchlings were immature (7–10 days old), we could not determine their sex.

In these experiments, we used prey odor instead of frogs, as hatchling *O. rufodorsatus* have a smaller gape than the body size of metamorphosed *R. catesbeiana*. To prepare prey odors, individuals of the three prey types (*R. catesbeiana* Taean, *P. nigromaculatus* Taean, *P. nigromaculatus* Hongcheon) were weighed, pithed and sacrificed using a guillotine, and cut into small pieces using scissors. To prevent possible contamination of odor sources, we did not use euthanizing chemicals. The pieces were ground using a mortar and pestle, adding 100 ml distilled water per 10 g BW of the frog. The solution was centrifuged for 5 min at 3500 rpm (NF-80, Hanil Science Co., Seoul, South Korea). The supernatant was decanted into 50 ml tubes and preserved at -20°C until use. As a control odor, we used distilled water.

2.4.2 Experimental procedures To know if exposure to invasive *R. catesbeiana* affects the response of newborn *O. rufodorsatus*, prey odors were sequentially presented to the hatchlings. The experiment was conducted with each of the 25 *O. rufodorsatus* hatchlings from each five adult females from both Taean and Hongcheon, between 10:00 and 19:00 from August 17 to 28, 2013. As five individuals was the smallest clutch size, we arbitrarily selected five hatchlings from each female for experimentation. At approximately 7 days old, *O. rufodorsatus* hatchlings show tongue-flicking behavior towards prey odors (personal observation), so we used only 7–10 day old hatchlings for this experiment.

During experiments, light and temperature followed ambient conditions. We recorded all behaviors with a digital video camcorder. For acclimation, each hatchling was placed in an opaque experimental box (10 cm × 10 cm × 10 cm) 1 hour prior to starting each trial. We presented gauze (2 cm × 2 cm) soaked with 1.5 ml frog odor solution in the center of the box using forceps and the trial was considered complete after 10 min. The frog

odor type and individual hatchling used were randomly assigned for each trial. As in previous olfactory tests of reptiles (Cooper and Burghardt, 1990; Labra and Niemeyer, 1999), only one odor type was presented per day. After each experiment, the boxes were washed with hot water and dried before using again.

2.5 Data analyses

2.5.1 Comparison of the morphological characteristics of adult snakes SVL, BW, TAL, RTL, HL, HW, and GI of adult *O. rufodorsatus* were compared using a General Linear Model (GLM). In the analysis, we used the locality as the independent variable, sex as a co-variable, and the seven morphological characteristics as dependent variables. In each analysis, we assessed the interaction of locality * sex. In addition, we compared the morphological characteristics of the adult female snakes caught from the Taean 1 (8 females) and Taean 2 (10 females) using an independent *t*-test or Mann-Whitney *U* test based on the normal distribution of the data (Shapiro-Wilk test). Since males were only collected from Taean 2, we could not perform any comparison between males.

2.5.2 Adult snake response to frog prey The number of adult *O. rufodorsatus* that responded by tongue flicking, touching prey, and foraging prey were compared between Taean and Hongcheon using a Chi-square test (Preacher, 2001) and between each pair of prey types within each population using the Fisher exact test (Preacher and Briggs, 2001). The significance of the Fisher exact test was set at $P = 0.017$ after Bonferroni correction (Zar, 1999). The prey response of adult *O. rufodorsatus* was analyzed using a GLM. We used locality, sex, and prey type as independent variables, SVL and BW as covariables, and the latency of the first tongue flick, time to the first touch of prey, the number of tongue flicks within the first 1 min after the first tongue flick, number of tongue flicks during the 10 min experiment, and the time taken to eat a prey item from the first touch of prey as dependent variables (Cowles and Phelan, 1958; Cooper and Burghardt, 1990; Labra *et al.*, 2001; Saviola *et al.*, 2011). In each analysis, we also assessed the interactions of locality * sex, locality * prey type, sex * prey type, and locality * sex * prey type.

When the prey type significantly affected the dependent variables, a Turkey post-hoc test in the GLM was applied to compare the differences between each pair of prey type. Relationships between the SVL and BW of frog prey and the snake's response variables were analyzed using a Spearman correlation. We did not compare the responses of the two Taean populations because the sample sizes were too small.

2.5.3 Hatchling snake response to frog odors SVL, BW, TAL, RTL, and age of hatchlings were compared between Taean and Hongcheon using an independent *t*-test or Mann-Whitney *U* test based on the normal distribution of the data (Shapiro-Wilk test). Due to the small sample size (*n* = 5), the characteristics of hatchlings' mothers were only compared between Taean and Hongcheon using the Mann-Whitney *U* test.

The number of the *O. rufodorsatus* hatchlings that responded by tongue flicking and touching prey odors were compared between Taean and Hongcheon using a Chi-square test (Preacher, 2001) and between each pair of odor type within each population using the Fisher exact test (Preacher and Briggs, 2001). The significance of the Fisher exact test was determined at *P* = 0.008 after Bonferroni correction (Zar, 1999).

The responses of the *O. rufodorsatus* hatchlings to prey odors were analyzed using a GLM. We used the locality and the prey odor type as independent variables, the SVL and BW of the hatchlings and their ages as co-variables, and the latency to the first tongue flick, time to the first touch of prey and the number of tongue flicks during 10 min as dependent variables. In each analysis, we also assessed the interaction of locality * prey odor type.

We performed the Chi-square test and Fisher exact test online (<http://www.quantpsy.org>; Preacher, 2001; Preacher and Briggs, 2001). For the remaining analyses,

we used software SPSS v. 20.0 (SPSS Inc., Chicago, IL, USA). All data are presented as mean ± SE.

3. Results

3.1 Comparison of the morphological characteristics of adult snakes

RTL of adult snakes was significantly greater in Taean than Hongcheon ($F_{1,43}=14.97, P < 0.01$; Table 1; Figure 2A). The remaining six morphological characteristics were not statistically different between Taean and Hongcheon (*P* > 0.05 for all cases; Table 1). Comparing males and females, all morphological characteristics except TAL (*P* = 0.97) were significantly different between the sexes (*P* < 0.01 for all cases; Table 1). RTL was greater in males than that of females, but the remaining five characteristics were greater in females than in males (Table 1). None of the characteristics were significantly different between the two Taean populations (*P* > 0.05 for all cases; data not shown).

3.2 Adult snake response to frog prey

The number of adults that responded by tongue flicking, touching prey, and eating prey was not significantly different between Taean and Hongcheon (Chi-square test, *P* > 0.05 for all cases; Table 2) and between each pair of prey type within each population (Fisher exact test, *P* > 0.017 for all cases; Table 2).

Adults from Taean had a significantly shorter latency to the first tongue flick than adults from Hongcheon ($F_{1,69}$

Table 1 Morphological characteristics of adult *Oocatochus rufodorsatus* caught from bullfrog-exposed populations at Taean and bullfrog-unexposed population at Hongcheon. Data are presented as mean ± SE based on the populations and the sex. Gape index (GI) = π (chin length) (chin width) /4. SVL, snout-vent length; BW, body weight; TAL, tail length; RTL, relative tail length (TAL/ SVL); HL, head length; HW, head width.

Population (Pop) or Sex		Characteristics						
		SVL (cm)	BW (g)	TAL (cm)	RTL (ratio)	HL (mm)	HW (mm)	GI
Taeon (<i>n</i> = 22)	Female (<i>n</i> = 18)	58.68 ± 1.88	109.17 ± 10.45	10.99 ± 0.39	0.188 ± 0.005	19.82 ± 0.58	13.22 ± 0.46	165.14 ± 9.05
	Male (<i>n</i> = 4)	40.78 ± 3.70	33.4 ± 4.26	10.38 ± 1.00	0.255 ± 0.007	16.97 ± 0.48	10.25 ± 0.33	160.58 ± 9.40
	Total	55.43 ± 2.23	95.4 ± 10.65	10.88 ± 0.36	0.201 ± 0.007	19.31 ± 0.53	12.68 ± 0.45	154.62 ± 8.93
Pop Hongcheon (<i>n</i> = 21)	Female (<i>n</i> = 15)	61.35 ± 2.08	112.39 ± 10.93	10.17 ± 0.48	0.170 ± 0.006	20.21 ± 0.57	12.77 ± 0.51	160.58 ± 9.40
	Male (<i>n</i> = 6)	46.17 ± 1.62	40.02 ± 3.65	10.6 ± 0.55	0.232 ± 0.007	17.07 ± 0.43	10.65 ± 0.38	112.81 ± 6.98
	Total	57.01 ± 2.17	91.71 ± 10.68	10.3 ± 0.37	0.185 ± 0.008	19.31 ± 0.53	12.17 ± 0.43	146.93 ± 8.43
Statistical results (<i>F</i> / <i>P</i> value)		2.04/ 0.16	0.11/ 0.74	1.20/ 0.28	14.97/ <0.01	0.24/ 0.63	0.22/ 0.64	0.05/ 0.83
Sex	Female (<i>n</i> = 33)	59.89 ± 1.39	110.63 ± 7.45	10.62 ± 0.31	0.178 ± 0.004	20.00 ± 0.4	13.02 ± 0.34	163.07 ± 6.44
	Male (<i>n</i> = 10)	44.01 ± 1.86	37.37 ± 2.84	10.51 ± 0.49	0.241 ± 0.006	17.03 ± 0.31	10.49 ± 0.26	110.59 ± 5.07
	Statistical results (<i>F</i> / <i>P</i> value)	35.99/ < 0.01	27.88/ < 0.01	0.00/ 0.97	91.76/ < 0.01	15.40/ < 0.01	15.00/ < 0.01	17.85/ < 0.01

Table 2 The number of adult *Oocatochus rufodorsatus* from bullfrog-exposed populations at Taeon and bullfrog-unexposed population at Hongcheon and their hatchlings that responded in each response measurements to frog prey and to frog prey odors, respectively. CatTA: *Rana (Lithobates) catesbeiana* or their odors from Taeon; NigTA and NigHC: *Pelophylax nigromaculatus* or their odors from Taeon and Hongcheon; DH2O: distilled water-used as control odor.

Adult experiment		Presented frog prey			
Population	Responses	CatTA	NigTA	NigHC	
Taeon (n = 16)	Tongue flicking	13	11	15	
	Frog touch	8	4	11	
	Frog eating	3	3	9	
Hongcheon (n = 16)	Tongue flicking	9	11	10	
	Frog touch	5	4	5	
	Frog eating	1	2	2	
Hatchling experiment		Presented frog prey odor			
Population	Responses	CatTA	NigTA	NigHC	DH2O
Taeon (n = 25)	Tongue flicking	22	14	17	20
	Odor touch	5	3	1	1
Hongcheon (n = 25)	Tongue flicking	25	22	18	19
	Odor touch	9	8	4	2

= 8.88, $P = 0.004$; Figure 3), but the latency time was not different between sexes ($F_{1,69} = 0.32$, $P = 0.58$) and among different prey types ($F_{2,69} = 0.29$, $P = 0.75$). None of the interactions between locality-sex, locality-prey type, sex-prey type, and locality-sex-prey type were significant ($P > 0.05$ for all cases). The number of tongue flicks within the first 1 min, during the first 10 min, and the time to the first touch of the prey were not significantly different between Taeon and Hongcheon ($P = 0.78$, $P = 0.13$, and $P = 0.76$, respectively), between the sexes ($P = 0.41$, $P = 0.14$, and $P = 0.37$, respectively), and among different prey types ($P = 0.63$, $P = 0.31$, and $P = 0.13$, respectively). None of the interactions between variables were significant ($P > 0.05$ for all cases). The time taken to eat prey was not different between Taeon and Hongcheon ($F_{1,45} = 0.04$, $P = 0.84$) and between the sexes ($F_{1,45} = 0.53$, $P = 0.47$), but was different among different prey types ($F_{2,45} = 6.66$, $P = 0.004$). In particular, snakes took more time to eat *R. catesbeiana* than *P. nigromaculatus* from both Taeon ($P < 0.01$) and Hongcheon ($P = 0.008$), but the time to eat *P. nigromaculatus* from Taeon and Hongcheon ($P = 0.32$). The SVL and BW of adult snakes were not significantly

correlated with any of the response measurements ($P > 0.05$ for all cases).

The SVL and BW of the prey were positively correlated with the number of tongue flicks during the first 10 min ($r = 0.263$, $P = 0.029$ for the SVL; $r = 0.265$, $P = 0.028$ for the BW) and the time taken to eat prey ($r = 0.609$, $P = 0.004$ for the SVL; $r = 0.642$, $P = 0.002$ for the BW), but were not correlated with the time to the first tongue flick and the number of tongue flicks within the first 1 min ($P > 0.05$ for the cases).

3.3 Hatchling snake response to frog odors TAL and RTL of hatchlings from Taeon were greater than that from Hongcheon (TAL: $t = 2.58$, $df = 48$, $P = 0.013$; RTL: $U = 153.5$, $P = 0.002$; Table 3, Figure 2B), but the SVL, BW, and age were not different ($P > 0.05$ for the cases, Table 3). Comparing between hatchlings' mothers, RTL was greater in Taeon than Hongcheon ($U = 1.00$, $P = 0.016$), but the SVL, BW, TAL and GI were not different ($P > 0.05$ for the cases, Table 3).

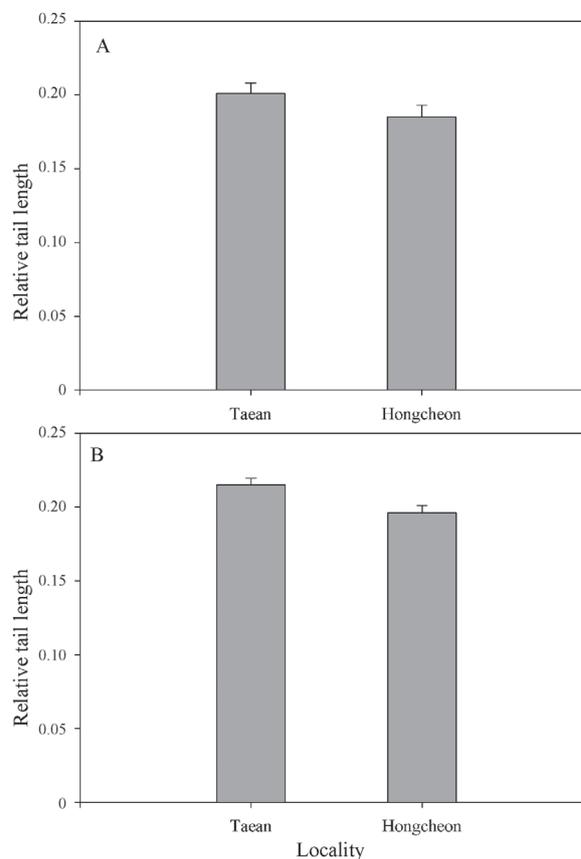


Figure 2 Relative tail length (tail length/snout-vent length) of (A) adult female *Oocatochus rufodorsatus* and (B) hatchlings used in the study. Females and hatchlings from bullfrog-exposed populations in Taeon had a greater relative tail length than those from bullfrog-unexposed population in Hongcheon ($P < 0.01$ for females; $P = 0.002$ for hatchlings).

Table 3 Morphological characteristics of hatchling *Oocatochus rufodorsatus* used in the behavioral experiment and their mothers caught from bullfrog-exposed populations at Taean and bullfrog-unexposed population at Hongcheon. Data are presented as mean \pm SE. GI (Gape index) = π (chin length) (chin width) /4. SVL, snout-vent length; BW, body weight; TAL, tail length; RTL, relative tail length (TAL/SVL).

Population		Characteristics				
		SVL (cm)	BW (g)	TAL (cm)	RTL (ratio)	Age (days)
Hatchling (<i>n</i> = 25)	Taean	18.6 \pm 0.23	2.81 \pm 0.07	4.02 \pm 0.07	0.215 \pm 0.004	8.4 \pm 0.1
	Hongcheon	19.13 \pm 0.17	2.72 \pm 0.04	3.73 \pm 0.08	0.196 \pm 0.005	8.4 \pm 0.2
	Statistical results (<i>t</i> or <i>U</i> / <i>P</i>)	1.84/ 0.072	1.17/ 0.248	2.58/ 0.013	153.5/ 0.002	300.0/ 0.792
Population		SVL (cm)	BW (g)	TAL (cm)	RTL (ratio)	GI
Female (<i>n</i> = 5)	Taean	61.36 \pm 1.75	135.16 \pm 17.30	11.48 \pm 0.31	0.186 \pm 0.002	179.1 \pm 12.00
	Hongcheon	64.62 \pm 2.46	136.58 \pm 15.15	10.86 \pm 0.43	0.168 \pm 0.004	180.2 \pm 13.78
	Statistical results (<i>U</i> / <i>P</i>)	11.00/ 0.84	8.00/ 0.42	9.00/ 0.55	1.00/ 0.016	12.00/ 1.00

The number of hatchlings that responded by tongue flicking and touching odor gauze was not different between the two populations (Chi-square test, $P > 0.05$ for all cases) and between each pair of prey odor types (Fisher exact test, $P > 0.008$ for all cases; Table 2).

The latency time to the first tongue flick, time to the first touch of prey odor gauze, and number of tongue flicks during 10 min were not different between hatchlings from Taean and Hongcheon ($P = 0.53$, $P = 0.10$, $P = 0.99$, respectively) and among different prey odor types ($P = 0.33$, $P = 0.38$, $P = 0.17$, respectively). None of the interactions between locality and odor type were significant ($P > 0.05$ for all cases).

4. Discussion

Adult and hatchling *O. rufodorsatus* from bullfrog-exposed populations in Taean had a significantly greater relative tail length (RTL) than those from bullfrog-unexposed population in Hongcheon. In addition, adult snakes from Taean responded more rapidly to prey with tongue flicking compared to Hongcheon. These differences tentatively demonstrate that invasive *R. catesbeiana* affect the morphology and behavior of endemic predator snakes. In the case of *O. rufodorsatus*, both of these changes may be adaptations to increased foraging efficiency. Discussion of both characters follows below.

A snake's tail causes forward propulsive forces and balances the body during movement, and the loss of tail tip significantly decreased the burst speed of snakes in

aquatic habitats (Aubret *et al.*, 2005). In our study, adult snakes from Taean had a greater RTL than those from Hongcheon. RTL is probably heritable as female snakes in our study with greater RTL gave birth to hatchlings with a greater RTL. We propose three main explanations for the greater RTL in Taean: 1) an adaptation for prey capture (Clark, 1966; King, 1986)—bullfrogs are more agile and common than endemic frogs in Taean, 2) an adaptation

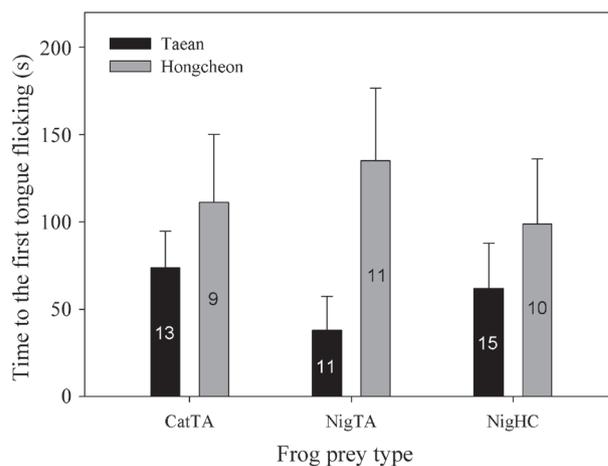


Figure 3 Comparison of time to the first tongue flick of adult *Oocatochus rufodorsatus* from bullfrog-exposed populations in Taean and bullfrog-unexposed population in Hongcheon. Experiments were run with three different prey types: *Rana (Lithobates) catesbeiana* from Taean (CatTA), *Pelophylax nigromaculatus* from Taean (NigTA), and *P. nigromaculatus* from Hongcheon (NigHC). Snakes from Taean always had faster times compared to Hongcheon ($P = 0.004$). The numbers on the bars are the number of the snakes which responded to tongue flicking of the frogs out of a total of 16 snakes investigated.

to habitat (Clark, 1966; King, 1986)—aquatic habitats are more common in Taean, or 3) pre-existing geographic variation between Taean and Hongcheon before bullfrog introductions. When comparing the two adaptation explanations, we believe RTL variation due to habitat is less likely than prey capture. Although the absolute and percentage area of marsh habitat was greater in Taean County (23 978 055 m², 4.6% of total county area; Taean, 2013) than Hongcheon County (1 597 040 m², 0.9% of total county area; Hongcheon, 2012), the time an individual *O. rufodorsatus* is exposed to aquatic habitats should be similar across sites because except hibernating on land in winter, *O. rufodorsatus* stays in or near aquatic habitats (Lee *et al.*, 2011). Our results suggest that a greater RTL in Taean improves foraging efficiency of preying upon frogs and freshwater fish (Kang and Yoon, 1975; Ji *et al.*, 1997), through better burst locomotion. To evaluate this preliminary finding and contrast it against the alternative of pre-existing intraspecific variation, a time-series comparison of historical specimens is still needed.

Rapid response to prey in adult snakes from bullfrog-exposed populations at Taean might also increase foraging efficiency. Snakes smell by flicking their tongue to deliver airborne odors to the vomeronasal organ (Halpern, 1992; Cooper, 1994). Snakes that have an earlier as opposed to late tongue flicking response can detect odor sources sooner (Cowles and Phelan, 1958) and at farther distance (Burghardt and Abeshaheen, 1971). In our study, adult snakes from Taean had a significantly shorter latency to the first tongue flick than those from Hongcheon, regardless of prey type. We propose two hypotheses for the shorter latency in Taean populations. First, a shorter latency could be due to increased motivation to capture prey. This phenomenon was observed in the lizard *Liolaemus bellii* and explained by decreased prey availability (Labra *et al.*, 2001). The same phenomenon may be possible in *O. rufodorsatus* if endemic prey (frogs and fish) are reduced by bullfrogs (Wu *et al.*, 2005; Wang *et al.*, 2007; Ra *et al.*, 2010; Da Silva *et al.*, 2011; Jancowski and Orchard 2013). Second, a changed preference for prey may influence latency. In China, the Red-banded snake (*Dinodon rufozonatum*) preferred the introduced bullfrogs over native frog species because bullfrogs showed less avoidance behaviors (Li *et al.*, 2011a). Data supporting each hypothesis should differ—if the shorter latency to first tongue flick is due to a shift in prey preference, we expect *O. rufodorsatus* response time to differ for *R. catesbeianus* and *P. nigromaculatus*.

We did not find a difference in response to the two

prey species, making the second explanation less likely. However it is still possible that a common, fast response to different prey may be learned. Our results suggest that bullfrog presence has influenced the foraging behavior of snakes from Taean. The faster reaction of individuals to prey in Taean may increase foraging success, allowing them to detect frog prey faster and at farther distances.

Unlike adult *O. rufodorsatus*, hatchlings did not show any difference in prey odor response. These results might be explained in four ways. First, frog odor sources used might be less stimulating than frog prey, resulting in less distinctive olfactory responses. It is possible that some of airborne odor stimulus might be lost during odor preparing process. Second, as shown in the study of Eastern indigo snake (*Drymarchon couperi*) (Saviola *et al.*, 2011), both olfactory and visual cues are needed. Third, hatchlings used in this study may be still undergoing olfactory developments. In general, olfactory development in hatchling snakes are modulated over time based on early odor exposure and genetically determined factors (Mushinsky and Lotz, 1980). Finally, frogs may not be a major prey of *O. rufodorsatus* hatchlings, resulting in low responses to frog odors. As far as we know, detailed diets of the hatchlings have not been studied.

In summary, our results suggest that invasive *R. catesbeiana* can affect the morphological characteristics and behaviors of the endemic predator *O. rufodorsatus*. When we compared bullfrog-exposed and unexposed populations, we found significant differences in characters that may increase foraging efficiency—relative tail length and time to first tongue flick. Studies of more populations, *O. rufodorsatus* prey items, and time series comparisons of characters during the history of bullfrog-exposed and unexposed populations are needed to elucidate the full effect of invasive prey species on endemic predators.

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