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Preference for Heated Substrate in Captive River Cooters (*Pseudemys concinna*): A Potential Use for the Control of Invasive Populations

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ABSTRACT

Invasive species threaten global biodiversity as well as human livelihood and much of the global lands are vulnerable to these threats. Numerous freshwater turtles from the northern hemisphere have been introduced in East Asian countries, including the Republic of Korea. Knowing turtle's behavioral ecology is valuable to manage introduced populations and a distinctive behavior is basking for behavioral thermoregulation. To understand the possibility of using basking to enhance trapping, we tested thermotaxis in the river cooter (*Pseudemys concinna*). Turtles were placed in an aquarium containing heated and non-heated mats under controlled water and air temperature, air humidity and light. We found that *P. concinna* stayed significantly longer on heated mats than on unheated control mats in 11 out of 18 trials, demonstrating that heat source is a potential attractant for *P. concinna*. We recommend the use of heat source to bait traps used for population control of invasive freshwater turtles.

Keywords: behavioral ecology, invasive species, turtle, heat attractant, population management

INTRODUCTION

Invasion by alien species has become a serious ecological issue threatening global biodiversity (Doherty et al., 2016) and leading to severe economic losses (Pimentel et al., 2000). Invasive species are likely to spread from the point of introduction and shift the ecological balance of the newly invaded ecosystems (Kolar and Lodge, 2001). Management is therefore important for the conservation of ecosystems.

Invasive species are generally removed from the habitat at a high monetary costs and in most cases only resulting in a slower dispersion rather than eradication. It is however possible to increase removal efficiency by baiting traps. For instance, attractants such as chemicals, light, and sound are used to increase trap efficiency (Schwarzkopf and Alford, 2007; Royer, 2015). Specific target species, however, require adequate cues, and light sources that work for American bullfrogs (*Lithobates catesbeianus*; reviewed by Groffen et al., 2019) would not necessarily work for invasive turtles.

Turtles are invasive globally, and especially in countries such as Taiwan, Singapore, and Vietnam due to pet trade and religious mercy release (Ramsay et al., 2007). Invasive turtles outcompete native species for food exploitation, egg-laying sites and basking places (Bury and Wolfheim, 1973; Bury, 1979). This is the case of *Trachemys scripta elegans*, which has a high ecological tolerance and is more competitive than local species (Ma and Shi, 2017). For instance, the presence of the species causes weight losses and high mortality in the native European pond turtle, *Emys orbicularis* (Cadi and Joly, 2003, 2004). Furthermore, invasive turtles can be a vector for disease and parasite transmission to local species (Iglesias et al., 2015). Thus, it is important to control the populations of invasive turtles to avert further loss of biodiversity.

One solution to manage populations of invasive turtles is to take advantage of their natural behavior to attract and capture them (Vogt, 1980). Ectotherms behaviorally regulate their body temperature by selecting specific thermal conditions (Chen and Lue, 2008). For instance, wood turtles, *Glyptemys*

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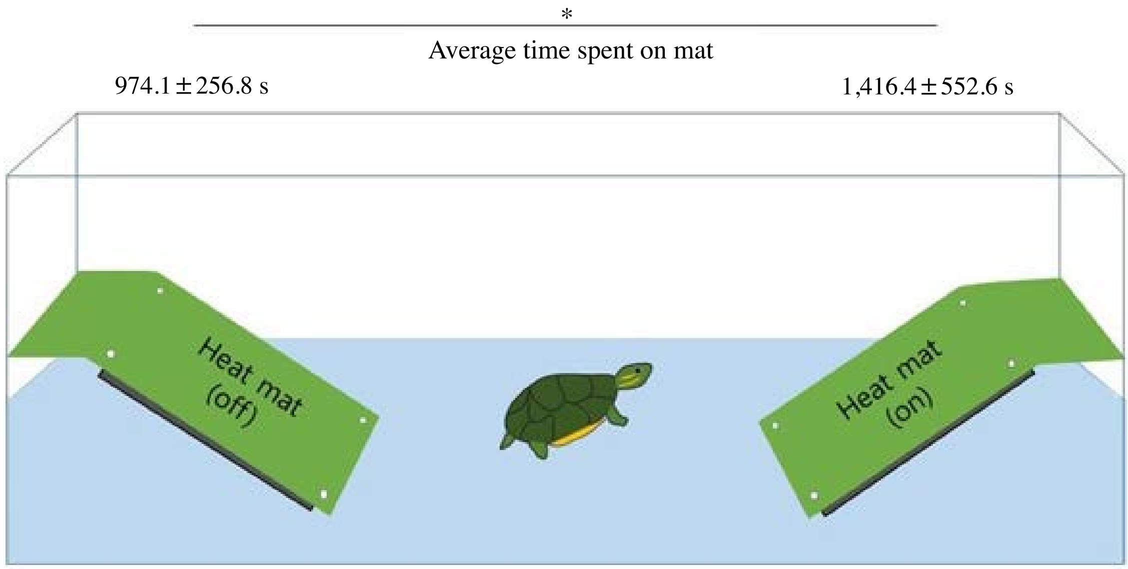

Fig. 1. Experimental setup for the thermotaxis experiment. We randomly selected the mat on either side to be heated and the other side was used as control. A single river cooter (*Pseudemys concinna*) was placed between the two heat mats to determine thermotaxis in freshwater turtles. Asterisk indicates a significant difference between the average time spent on the heated mat and control mat.

insculpta, select open areas with access to solar radiation for thermoregulation (Dubois et al., 2009).

Numerous species of invasive turtles have been found in the Republic of Korea, including *Chrysemys picta*, *Pseudemys concinna*, and *Trachemys scripta* (Lee et al., 2016). *Trachemys s. elegans* is listed as one of the 100 worst invasive species in the world (Lowe et al., 2000). The species was introduced to the Republic of Korea in the 1970s (Ramsay et al., 2007) from Buddhist mercy releases and through the pet trade, along with numerous and yet to be listed turtle species (Platt and Fontenot, 1992; Koo et al., 2018). Millions of turtles were imported to the Republic of Korea until import was banned in 2001 (Lee et al., 2016). Currently, it is the most widespread invasive reptiles in Korea (Koo et al., 2018), observed basking in multiple locations near rivers and reservoirs (Lee and Park, 2010). A hypothesis why North American invasive species are more competitive than native ones is that they originate from species assemblages comprising up to six species (Whitfield Gibbons, 1990), where they have to be highly competitive. This condition favors their establishment in regions such as Korea where only two species with very distinct ecological preferences were originally present.

Here, we used *P. concinna* as a proxy to determine thermotaxis in invasive turtles. We conducted manipulative experiments to test whether *P. concinna* were attracted to higher temperature. Based on the thermotaxic behavior displayed, we predict that individuals will stay longer in the warmer basking area than in that with environmental temperature.

MATERIALS AND METHODS

Animal preparation and maintenance

Pseudemys concinna are common turtles found in rivers and lakes of North America. Populations in the New River of West Virginia were observed basking from March to September on logs and rocks (Buhlmann and Vaughan, 1991). In September 2018, we bought five *P. concinna* from a local pet shop (median length = 5.83 cm; min–max, 4.84–7.60) and kept them indoors at room temperatures. Prior to the experiment, they were kept together in a glass aquarium (30×30×30 cm), filled with aged tap water and a 24 hour working sponge filter (Sponge filter SP-L4; Aquatech, Seoul, Korea) under the natural photoperiod (ca. 11.5 h of light : 12.5 h of darkness). The water temperature was set to 25°C with an aquatic heater (Aqua Heater AH-55, Zhongshan, China). The aquarium was washed and was re-filled with aged water once a week. The turtles were fed reptile pellet feed (Hikari Turtle Crest; Kyorin Co. Ltd., Himeji, Japan) and dried shrimp (Animal Bab Gammarus, China) twice a day *ad libitum*. All experiments were conducted between 5 and 21 November 2018.

Experimental design

For the experiment, we filled an acrylic tank (120×45×45 cm) with ca. 54 L of aged water. Heat mats were placed on top of slanted plastic plates, attached to the inside of the tank and adjusted so that the slanted end was at the water surface (Fig. 1). During the experiment, a heated mat was set under

Table 1. Descriptive results for a heat choice experiment in River cooters (*Pseudemys concinna*)

Variable	No.	Mean±SD	Median (min–max)	Shapiro-Wilk's W	p-value
Turtle body length (cm)	5	5.83±1.20	5.8 (4.8–7.6)	0.86	0.229
Water temperature (°C)	18	18.0±1.2	18.1 (16.3–19.7)	0.93	0.194
Date (Julian date)	18	315.1±5.4	315.0 (308–324)	0.77	**0.046**
Time in a day (%)	18	58.4±8.2	58.2 (45.3–71.5)	0.87	0.261
Temperature of control mat (°C)	18	20.6±1.1	20.55 (18.7–22.3)	0.95	0.397
Temperature of heated mat (°C)	18	30.5±0.6	30.55 (29.1–31.3)	0.94	0.293
Duration on heated mat (s)	18	1,416.4±552.6	1,253.50 (675–2,633)	0.91	0.082
Duration on control mat (s)	18	974.1±256.8	1,012.0 (408–1,308)	0.90	0.051
Thermotaxic index (heated/control)	18	1.8±1.7	1.4 (0.6–6.3)	0.61	<**0.001**

This table presents sample sizes, mean±SD, median (min–max), and the results of Shapiro-Wilk's normality test. Significant results are highlighted in bold.

both slanted plates, but only one heat mat was randomly selected and turned on, while the other one was used as a control. Before the experiment, the tank was adjusted so that both heat mats receive similar light. The tank was washed and the water was changed between each trial to remove any chemical cues.

Each of the five turtles used for the experiments was given an identification number based on the pattern of its plastron and each turtle was tested four times, with all the trials conducted in a haphazard order. For each trial, we placed the selected turtle in the middle of the experimental tank. We ran each trial for 60 min and videotaped (Sony DCR-TRV30 NTSC; Tokyo, Japan) the experiment without the presence of investigators to avoid any interferences. We then measured the total duration time (s) for which the turtle stayed on each of the heated and non-heated mat from the videotape. The individuals tested moved on and out of the heat mat several times within the trial, so we based our analyses on the total duration time spent on the heat mat. Two trials out of 20 were invalidated as the turtles had not selected a mat within the 60 min imparted. Before each trial we recorded date and time, then measured air temperature, humidity, water temperature, temperature of each heat mat and time. When averaged for the 18 trials of the experiment, the water temperature measured before each trial was 18.0±1.2°C (mean±standard deviation [SD]), the control heat mat was 20.6±1.1°C and the heat mat turned on was 30.5±0.6°C.

Statistical analyses

To compare the time spent by a single turtle on either the heated or the control mats, we created a "thermotaxic index" in the form of a ratio with the duration a turtle stayed on the heated mat divided by the duration the turtle stayed on the control mat within the same trial. For all of the measurements, we calculated mean, SD, median, minimum and maximum. We used a Shapiro-Wilk's test to determine the normality of all the measurements (Table 1). Since the date of experiments and the thermotaxic index did not follow a normal distribution, we transformed each value into their natural logarithm. We used a Generalized Linear Model (GLM) to test whether the thermotaxic index was affected by date, time of day, water temperature, air temperature, humidity and the individual identity number. Because there was no significant effect from individual on the thermotaxic index, we then used paired t-test to compare the durations stayed on the heated mat and the control mat. Also, we used a one sample t-test to test the null hypothesis where thermotaxic index is not different from the expected value (here averaged at 0.5). This test was conducted to examine whether the turtles preferentially selected the warm mat or the control mat. All analyses were performed with SPSS 21.0 (IBM Corp., Armonk, NY, USA). Values of measurements were shown as mean±SD. Significant criterion was set at alpha=0.05.

RESULTS

We found that *Pseudemys concinna* individuals demonstrated thermotaxis by staying longer on the heated mats compared to the control mats in 11 out of 18 trials. From the results of the GLM, none of the factors had an effect on the thermotaxic index (adjusted $R^2=0.01$, $F_{1,8}=1.02$, $p=0.494$) (Table 2). The duration spent on the heat mat was significantly longer than that spent on the control mat (paired t-test; $t_{1,17}=2.52$, $p=0.022$) (Fig. 2). Furthermore, the thermotaxic index was significantly larger than the expected value of 0.5 (one sample t-test; $t_{1,17}=2.35$, $p=0.031$), indicating that turtles stayed longer on the heated mat than on the control mat. Thus, *P. concinna* preferred substrates with higher temperature to normal environmental temperature.

Table 2. Results of the Generalized Linear Model testing for the impact of covariates on the thermotaxic index in river cooters (*Pseudemys concinna*)

	SS	df	MS	F	p
Date	1.19	1	1.19	2.98	0.123
Time of day	0.05	1	0.04	0.11	0.752
Water temperature	0.02	1	0.02	0.05	0.830
Air temperature	<0.01	1	<0.01	<0.01	0.952
Humidity	1.68	1	1.68	4.22	0.074
Turtle ID	0.91	3	0.30	0.76	0.546
Error	3.19	8	0.40		

For the whole model, multiple R=0.73, multiple R^2=0.53, adjusted R^2=0.01, $F_{(1,8)}$=1.0 and p=0.494. Note the absence of significant result.

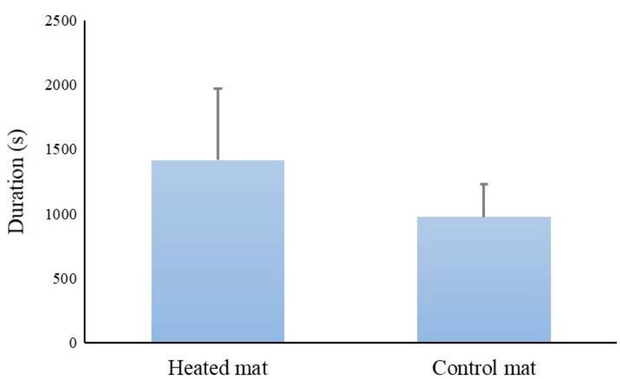

Fig. 2. Time spent on the heated mats and control mats by the river cooters (*Pseudemys concinna*). The turtles stayed longer on the heated mats than on the control mats (paired t-test; $t_{1,17}$=2.52, p=0.022).

DISCUSSION

The *Pseudemys concinna* individuals tested here stayed longer in the basking areas with a heat source. Basking is essential for turtles as their metabolism is stimulated following the increase in body temperature (Kepenis and McManus, 1974): an elevated body temperature also increases feeding and digestion rates and enhances growth (Parmenter, 1980). Our results are in agreement with that of other studies, where for instance, juvenile *Malaclemys terrapin* detect and preferentially select areas with warmer temperatures in order to boost their growth and metabolism (Tamplin et al., 2013). Similarly, *M. temminckii* selects warmer microhabitats for basking (Fitzgerald and Nelson, 2011) and juvenile *Glyptemys insculpta* select the warmest temperature available (Tamplin, 2009).

As the experiment was conducted under controlled conditions and over 17 days, we do not expect a bias in our results, and our findings highlight that thermotaxic preferences have an important potential in the control of invasive species. Attractants used for the trapping of invasive species have proven their efficiency (Witmer et al., 2010; Snow and Witmer, 2011), including in freshwater turtles where baits helped catching up to 70% of *T. scripta* individuals at a single locality (Scalera, 2007; Valdeón et al., 2010). Also, bycatch rates were higher when water temperatures was higher (Larocque et al., 2012). Therefore, using heat as an attractant is most likely to enhance trapping of freshwater turtles.

As our study shows that *P. concinna* are attracted to heat, we recommend the use of warmer microhabitats as lures when designing and developing effective traps. In addition, we recommend the use of heat as a lure before hibernation, when the turtles are still active and looking for warm microhabitats. The implementation of this recommendation should result in a higher capture rate and increased efficiency of population management. This is especially important in the Republic of Korea where native freshwater organisms are under exacerbated threats (Lee and Miller-Rushing, 2014) and conservation actions are urgently required (Borzée et al., 2019).

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CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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