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Commentary

Studying mate choice in the wild using 3D printed decoys and action cameras: a case of study of male choice in the northern map turtle

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Mate choice experiments are essential to further our understanding of sexual selection, but can be challenging to design and conduct with most wild animals. 3D printing technology is creating opportunities to conduct mate choice experiments in the field by facilitating the production of biologically accurate decoys. We used pairs of 3D printed female decoys differing only in size to test whether free-ranging male northern map turtles, *Graptemys geographica*, prefer larger females. Males interacted and attempted to mate significantly more with the larger decoys. By selecting larger females, males should increase their fitness because of the correlation between female size and hatchling size. Our experiment demonstrated that 3D printing technology can be a valuable tool to study animal behaviour in the field.

Sexual selection via mate choice plays an important role in the evolution of phenotypes and has resulted in some of the most elaborate traits and behaviours found in animals (Andersson, 1994). A fundamental step in determining the evolutionary consequences of mate preferences is to identify the traits used by animals to select their mates. Mate choice experiments are particularly important in taking this step because they can directly identify the traits used in mate discrimination (Kirkpatrick, 1987). Mate choice experiments can, however, be difficult to design or perform with certain animals. For instance, factors known to influence mate choice such as mating status (King, Saporito, Ellison, & Bratzke, 2005; McNamara, Jones, & Elgar, 2004) are difficult or impossible to control for animals that can only be collected from the wild. Moreover, the social and ecological contexts in which mate choice occurs can affect mate preference (Dougherty & Shuker, 2015; Miller & Svensson, 2014), but can be poorly documented or difficult to recreate in captivity. Finally, many animals, such as large aquatic animals, are not well suited to captivity and laboratory-based mate choice experiments are thus simply impractical.

These experimental challenges can be overcome by measuring the preference of free-ranging animals for decoys of potential mates (e.g. Ellers & Boggs, 2003). The use of decoys alleviates the concern of potential confounding variables by allowing manipulation of only the trait (or traits) of interest, while performing the experiment in the field retains the natural social and ecological contexts, and eliminates any potential ethical and logistical issues associated with capture and husbandry. 3D scanning and printing systems are greatly facilitating the production of biologically accurate decoys as well as the manipulation of their 'phenotype'. This technology, in combination with the development of compact action cameras that can withstand field conditions, is providing new opportunities to use decoys in field experiments.

Here we used 3D printed female decoys and submersible action cameras to test whether free-ranging male northern map turtles, *Graptemys geographica*, prefer larger females. The northern map turtle is a species that does not fair well in captivity (Ernst & Lovich, 2009) and its courtship and mating behaviours are difficult to observe directly in nature (G. Bulté, personal observation). Moreover, this species mates while aggregated at communal hibernation sites (Vogt, 1980), a context difficult to recreate in captivity. Finally, it is impossible to determine whether males collected in the wild have previously mated and whether they have sufficient sperm to mate again, which would be a confound if these males were then to be used in mate choice trials in captivity.

Male choice can be expected to occur in the northern map turtle because many females are simultaneously present at communal hibernation sites, and females vary in size (Fig. 1; Bulté & Blouin-Demers, 2009), which is correlated with their reproductive

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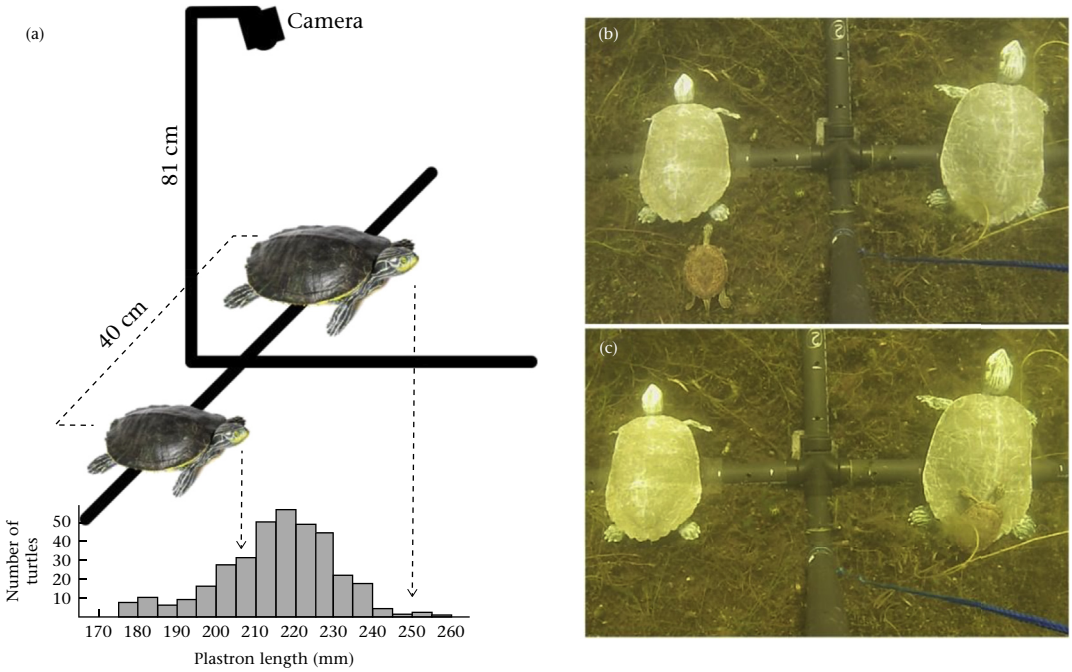

Figure 1. (a) Diagram of mount for male mate choice experiment in northern map turtles showing the female decoys used in the experiment. The size of the decoys in relation to the size distribution of mature females from Lake Opinicon, Ontario, Canada is indicated. Size data are from Bulté and Blouin-Demers (2009). (b) Example of a male approaching a female decoy without making physical contact. (c) Example of a male attempting to mate with a female decoy.

output (Bulté, Irschick, & Blouin-Demers, 2008). Based on these observations, we predicted that male northern map turtles would prefer larger females when given the choice.

METHODS

Study Site

We conducted this study in April 2016 at two northern map turtle communal hibernation sites in Lake Opinicon located 130 km south of Ottawa, Ontario, Canada.

Printing the Female Decoys

A female map turtle was created by digitally scanning of a dried carapace and appendages of a preserved specimen using a desktop 3D scanner (Ultra HD, NextEngine Inc., Santa Monica, CA, U.S.A.). Because of the poor condition of the soft parts of the preserved specimen, a head was digitally rendered using photographs via 3D Studio Max 8 (Autodesk Inc., San Rafael, CA). A complete digital decoy was assembled and scaled in preparation for 3D printing in sections (ABS plastic) using a Makerbot® Replicator 2x and accompanying software (MakerBot, New York, NY, U.S.A.). We printed four decoys, scaled to two sizes: 210 mm and 250 mm (plastron length). An artist was commissioned to paint each decoy to represent biologically accurate coloration to mimic live turtles as closely as possible (Fig. 1a).

Male Mate Choice Experiment

We mounted the decoys in pairs (one large female decoy and one small female decoy) 40 cm apart on a platform made of 3.81 cm diameter ABS pipes (Fig. 1a). Each of the two decoy stations was equipped with a GoPro Hero 3 ® camera (GoPro, San Mateo, CA) with an extended Wasabi® battery pack (Wasabi Power, Pomona, CA). The camera was installed 80 cm above the decoys facing down. Both decoy stations were deployed between 0700 and 0800 hours and recovered at 1900 hours on 9 days. The location of both mate choice stations was determined haphazardly in the hibernation areas each day. For each deployment, we recorded the number of males approaching the decoys and the number of males attempting to mate with the decoys. We defined an approach as an event in which a male approached a female decoy to less than its own body length (Fig. 1b; Supplementary Video S1). Mature males range in carapace length from 10 to 16 cm (Bulté & Blouin-Demers, 2009). We defined a mating attempt as when the male mounted the dorsoposterior end of the female decoy (Fig. 1c, Supplementary Video S1). If male map turtles do not exhibit female size preferences, then the expected number of approaches and mating attempts should not differ between large and small female decoys. We used chi-square analysis to test whether the frequency of behaviours (approaches and mating attempts) directed towards the large decoy differed significantly from the expected 50%.

Ethical Note

This research was approved by the Carleton University Animal Care Committee under the protocol no. 102679. A wildlife scientific collector's authorization was also obtained from the Ontario Ministry of Natural Resources (authorization no. 1083630).

RESULTS

Over the 9 days of trials, we recorded 112 male approaches and 12 mating attempts at both mate choice stations (Fig. 2). In 28 instances, a male made physical contact with a decoy but did not attempt to mate. In 74 of the 112 (66%) approaches, the focal male approached the large female decoy first. Ten out of 12 (83%) mating attempts were with the large female decoy. During approaches and mating attempts, male behaviours were directed towards the larger

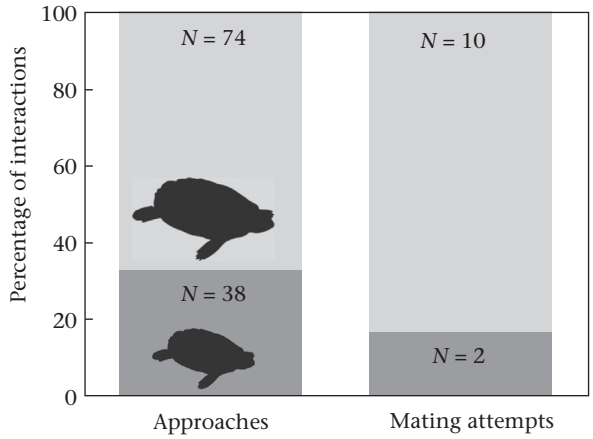

Figure 2. Percentage of approaches towards and mating attempts with large female decoys (light grey bars) and small female decoys (dark grey bars) by male northern map turtles in Lake Opinicon, Ontario, Canada. *N* indicates the number of observations in each category.

female decoy more than expected (chi-square tests: approaches: $\chi^2 = 11.78$, $P < 0.001$; mating attempts: $\chi^2 = 5.82$, $P = 0.02$).

DISCUSSION

Our objective was to test whether male northern map turtles discriminate females based on size by recording the interactions of free-ranging males with 3D printed female decoys. We found convincing evidence that males prefer larger females when given the choice. The benefits of being choosy may be important in this species due to the correlation between female size and offspring size (Bulté et al., 2008; Ryan & Lindeman, 2007). A female the size of the larger decoy would produce hatchlings that are on average 16% heavier than a female the size of the smaller decoy (Supplementary Fig. S1). Size is a determinant of early life survival in hatchling turtles (Janzen, 1993; Janzen, Tucker, & Paukstis, 2000, 2007) and a small increment in mass can lead to an important increase in the probability of surviving in the nest to water dispersal (Janzen et al., 2000). By selecting larger females, males may markedly increase the probability of their offspring surviving this critical life-history stage.

Our experimental set-up worked admirably well at attracting male map turtles (Supplementary Video S1), but we were unable to determine how many different individual males interacted with the female decoys, which could lead to pseudoreplication. Since 2003, we have captured and marked 503 males in Lake Opinicon. We are confident that the vast majority of turtles in the lake hibernate at the two hibernation sites we studied because the two sites are the only ones we found in a 3-year radiotelemetry study in which 49 individuals were tracked (Carrière et al., 2009). The high abundance of males at the hibernation sites thus limits the potential for pseudoreplication. Despite this potential caveat, we clearly demonstrated that 3D printed decoys and submersible action cameras are useful tools to study mating behaviours in the field. This approach involves minimal stress and disturbance and should be a valuable tool to document the mating and social behaviours of species that are prone to captivity stress or that are easily disturbed by observers in the field.

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Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.anbehav.2018.02.018.

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