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Good vibrations: a novel method for sexing turtles

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Abstract. The ability to accurately determine the sex of individuals is important for research and conservation efforts. While most species of turtle exhibit secondary sexual dimorphisms that can be used to reliably infer sex, there are some species that are very difficult to sex, and even within many dimorphic species, it is not uncommon to encounter individuals that appear to exhibit both male and female secondary sex characteristics. Therefore, we tested the novel method of using a vibrator to sex turtles by stimulating male turtles to evert their penises. We tested this method on males of four species (three families) with known sexual dimorphisms: spiny softshell turtles (*Apalone spinifera*; n = 14), western chicken turtles (*Deirochelys reticularia miaria*; n = 17), Mississippi mud turtles (*Kinosternon subrubrum hippocrepis*; n = 10), and common musk turtles (*Sternotherus odoratus*; n = 9). The method accurately sexed 100% of *A. spinifera,* 64.7% of *D. r. miaria*, 80.0% of *K. s. hippocrepis*, and 55.6% of *S. odoratus.* Despite the low success rates in some species, there are situations in which this method will be useful for researchers working with species that are difficult to sex using external morphological characteristics.

Keywords. *Apalone*, chelonia, *Deirochelys*, *Kinosternon*, penis, *Sternotherus*, vibrator.

The ability to accurately differentiate males and females is important for ecological studies, and for many turtle species this is a relatively simple task. Turtles often exhibit a variety of secondary sexual dimorphisms in traits such as size, color, claw length, plastron shape, and pre-cloacal tail length (Gibbons and Lovich, 1990; Readel et al., 2008). Nevertheless, some species lack obvious dimorphisms, and dimorphisms may vary among populations (Iverson, 1985; Rowe, 1997). Further, even for species that are strongly dimorphic, it is not uncommon to encounter individuals that appear to have some characteristics of males and some characteristics of females, thus making them difficult to sex (McKnight, pers. obs.).

Several methods to overcome these problems are available, such as measuring testosterone levels (Owens et al., 1978; Rostal et al., 1994), laparoscopy (Wibbels et al., 1989; Ligon et al., 2009), and cloacoscopy (Ligon et al., 2013); however, these methods are often invasive, time-consuming, and difficult to implement in the field. Recently, two methods have been published for inducing penile erections in male turtles, thus allowing males and females to be differentiated. While penile eversion is a common method for sexing squamates, it has received little attention in turtles. Although this is a promising technique, the methods that have been proposed so far appear to be species-specific and have only been applied to common snapping turtles (*Chelydra serpentina*), whose penis can be everted by gently bouncing a turtle up and down (De Solla et al., 2001; Dustman, 2013), and Cotinga River toadhead turtles (*Phrynops tuberosus*), whose penis can be everted by immobilizing the neck and limbs (Rodrigues et al., 2014).

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Vibrators may provide a more broadly applicable method of penile eversion. Lefebvre et al. (2013) found that a vibrator could be used to induce ejaculation in male turtles, and ejaculation was preceded by a visible erection. Therefore, this method may be valuable as a means of sexing turtles. We examined its utility on four species of freshwater turtle representing three different families.

To test our method of using a vibrator to induce erections in males, we used species that can be sexed using external sexual dimorphisms such as size, color, and tail morphometrics. Thus, we could test the efficiency of the method by seeing how frequently it induced an erection in individuals that were known to be males. The four species that we used were: western chicken turtles (*Deirochelys reticularia miaria*; family: Emydidae), Mississippi mud turtles (*Kinosternon subrubrum hippocrepis*; family: Kinosternidae), common musk turtles (*Sternotherus odoratus*; family: Kinosternidae), and spiny softshell turtles (*Apalone spinifera*; family: Trionychidae). We captured them using hoop nets placed in ponds in southeastern Oklahoma (detailed trapping methods in McKnight et al., 2015).

Once a male turtle was captured, we attempted to induce an erection by applying an 18 cm, variable-speed, silver bullet vibrator to its shell and tail. We vibrated turtles for 10 min or until an erection was achieved, and we recorded the amount of time that it took to induce an erection. Trials were scored as "unsuccessful" if an erection had not been induced by the end of the 10-minute trial period. Our preliminary trials indicated that turtles needed to be fairly relaxed and willing to extend their limbs and tails before the method would be effective. Therefore, for the sake of time, we limited our trials to turtles that were already active at the time of capture. Although this is a drawback of our method, most turtles quickly acclimate to being handled, and a few moments of holding a turtle is generally sufficient for it to extend its limbs (McKnight, pers. obs.).

Fig. 1. A male spiny softshell turtle (*Apalone spinifera*) being vibrated on the tail.

During our trials, we held turtles vertically, with their plastrons facing the researcher that was operating the vibrator. Then, we gently applied the tip of the vibrator to the plastron, carapace, and tail (Fig. 1). We moved it among those regions based on the turtles' responses (i.e., if a turtle responded by tightly pulling its limbs and tail against its body, we moved to a different area). For each species, erections generally occurred when the vibrator was placed on the tail itself, but it was often necessary to first vibrate areas other than the tail, because starting with the tail generally resulted in the turtles pulling the tail tightly against the body, rather than extending it. Therefore, we started with the plastron or carapace, and moved to the tail once a turtle had fully extended its tail.

In general, turtles appeared to respond best when only the tip of the vibrator was touching them and when the vibrator had fresh batteries and was set on the fastest setting. Also, they seemed to respond best when the tip was held firmly against them (rather than allowing it to bounce), but not be pressed hard against them. Both allowing it to bounce and pressing it too hard generally resulted in turtles holding their limbs and tail tightly against the body, rather than relaxing. Additionally, it was often useful to move the vibrator around in small, slow, steady circles. As a general rule, we tried to hold the vibrator against the tail whenever possible (including following the tail if the turtle is waving it from side to side), but if this caused the turtle to retract its tail, then we moved the vibrator to a different position until the tail was extended again. Finally, sometimes males only protracted their penises briefly and quickly retracted them, rather than maintaining an erection. Therefore, it was necessary to watch the cloaca closely.

Although this was the general pattern, each species responded differently, so we had to adapt our protocol based both on the species and individual responses, and it will be necessary to test different positions and techniques when trying this method on a new species. For *A. spinifera* it was generally not necessary to spend time on parts of the body other than the tail. They usually extended their tails immediately and would allow us to hold the vibrator against their tails. They did frequently wave their tails from side to side, forcing us to move the vibrator with the tail, but they generally did not hold the tail against the body in a stressed position.

In contrast, *K. s. hippocrepis, S. odoratus*, and *D. r. miaria* usually held their tails against their bodies initial-

ly and required stimulation to other parts of their body before they would relax and extend their tails. For *K. s. hippocrepis* and *S. odoratus*, this generally involved moving the vibrator in slow, small circles on the abdominal and pectoral scutes (the diameter of the circle was only 1-2 cm). *Deirochelys r. miaria* was similar, but it was usually necessary to vibrate slightly higher (more on the pectoral scutes than abdominal scutes). Also, sometimes they responded to being vibrated on the carapace (usually on the first vertebral scute). Finally, Lefebvre et al. (2013) reported that, when inducing male turtles to ejaculate, vibrating turtles on their heads was often effective, however, that method generally did not work in our study. This further illustrates the differences among species and highlights the need for testing several different locations and methods when vibrating a species for the first time.

In addition to the differences in the techniques necessary for stimulating the different species, our success rates also varied among species (Table 1). The method was the most successful for *A. spinifera* (100%), followed by *K. s. hippocrepis* (80.0%). It was less successful for *D. r. miaria* (64.7%) and *S. odoratus* (55.6%). We compared the success rates among species using a Fisher's exact test, and this showed that there was a statistically significant difference (P = 0.026). The median time required to induce an erection also varied among the species, but it was lowest in *A. spinifera* and *K. s. hippocrepis*. Because the utility of this method varied among species, it will need to be validated on a species by species basis.

Despite the low success rate in some species, we think that this method has potential to be useful in several situations. First, based on our success employing this technique on *A. spinifera* and *K. s. hippocrepis*, it should be useful for some species or populations that are difficult to sex. However, it will first need to validated using individuals of a known sex (such as individuals in a captive population that have been sexed by other methods). If it is successful on those known individuals, then it will provide a cheap and non-invasive way of sexing that species in the field.

Second, even for species that can usually be sexed via secondary sexual dimorphisms, it is not uncommon to find individuals that possess some characteristics of both males and females, thus making them difficult to sex. This method can be applied to those individuals even if it has not been validated for that species. In other words, if the method has been validated, then the outcome of vibrating the turtle can be used to assign the sex as either male or female; however, if it has not been validated for that species, inducing an erection would allow the turtle to be sexed as a male, and failure to induce an erection would simply leave the turtle with an unassigned sex code. Using a vibrator in this manner had already been helpful for sexing problematic individuals in our own research (McKnight, pers. obs.).

Third, it is often desirable to collect or monitor several individuals of a known sex (e.g., for movement studies). This is another situation where the method can be used even for species for which vibrating has a low or undetermined success rate. For example, if a research endeavor requires ten males of a species that is difficult to sex, an investigator could simply vibrate turtles until they had ten with erect phalli.

Finally, in situations where a researcher is working with a species, subspecies, or population for which secondary sexual dimorphisms are unknown or questionable, this method can be used to help validate a secondary sexual dimorphism. It is often possible to identify some individuals as females by palpating turtles for the presence of eggs (Zuffi et al., 1999) or employing a sonogram to look for evidence of enlarged follicles, and using the method we described to vibrate turtles will allows some males to be identified. Therefore, the combination of these two methods would allow researchers to easily compare the morphometrics of known males and known females to identify secondary sexual dimorphisms.

Indeed, this final method has proved useful in our research. At the outset of this project, we were not certain if our populations of *D. r. miaria* (a subspecies that has been poorly studied) were sexually dimorphic. We had a few known females (identified by the presence of eggs or enlarged follicles), but the majority of individuals appeared to be males (with a few immature females), resulting in a strongly skewed sex ratio (8:1 M:F [adult sex ratio], 4.7:1 [including suspected immature females]). Based on our extensive trapping, the sex ratio did not appear to be a trapping artifact, but it was skewed enough that we were not confident that published sexual dimorphisms were correct for this subspecies at this location (Ernst and Lovich, 2009). However, by using the vibrator method to confirm that a subset of the suspected males were actually males, we were able to plot regressions (Fig. 2), which then allowed us to use secondary sexual dimorphisms to confidently assign sex to turtles in our populations.

Table 1. Sample sizes and results for the species that we vibrated. Both "Median time" and "Time range" represent the time for trials that were successful. Unsuccessful trials were aborted after 600 s.

	Deirochelys reticularia miaria	*Kinosternon subrubrum hippocrepis*	*Sternotherus odoratus*	*Apalone spinifera*
N	17	10	9	14
% successful	64.7	80	55.6	100
Median time (s)	145	82	112	77
Time range (s)	20–580	6–332	42–162	7–150

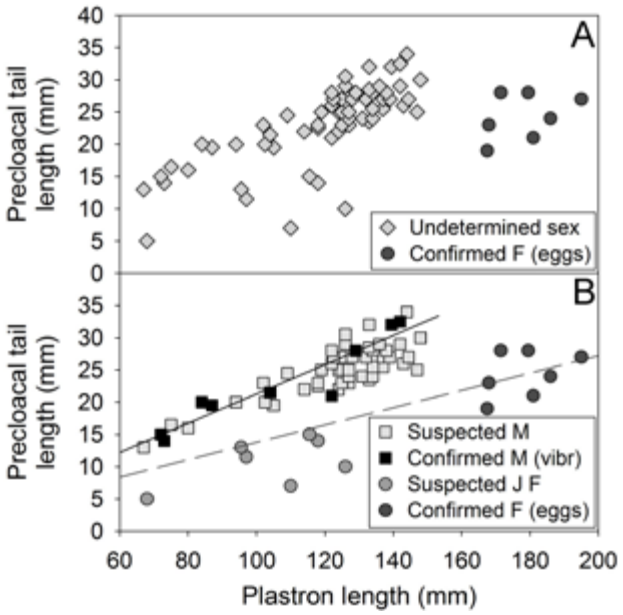

Fig. 2. Secondary sexual dimorphisms in western chicken turtles (*Deirochelys reticularia miaria*). A) Prior to vibrating several males, we had identified females by the presence of eggs, but because of the skewed sex ratio and large size of the confirmed females, we were not confident that the relative precloacal tail length was reliable for this species. B) The vibrator allowed us to confirm the sex of several males, thus establishing that this is a true sexual dimorphism (precloacal tail length was not recorded for two of the vibrated males). M = males, F = females, J = juveniles, (vibr) = confirmed via the vibrator, (eggs) = confirmed via the presence of eggs or enlarged follicles. Regression lines are only shown for confirmed males and confirmed females.

In conclusion, although this method may not be a silver bullet for sexing all problematic turtle species, it is reliable for some species, and it has value even for species with low or undetermined rates of efficiency. It is cheaper, easier to implement in the field, and less invasive than many of the alternative techniques, and it has already proved useful in our own research. Therefore, we think that it will enhance other research projects as well.

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