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# [Copulation Phenology of Ornate Box Turtles \(Terrapene ornata\)](https://www.researchgate.net/publication/372761463_Copulation_Phenology_of_Ornate_Box_Turtles_Terrapene_ornata?enrichId=rgreq-251d679dda0f97a4b1120d4067f76cc6-XXX&enrichSource=Y292ZXJQYWdlOzM3Mjc2MTQ2MztBUzoxMTQzMTI4MTE4MDI1NzE4M0AxNjkxNTM5MjM1Nzkz&el=1_x_3&_esc=publicationCoverPdf)

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## Copulation Phenology of Ornate Box Turtles (Terrapene ornata)

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ABSTRACT.—Many organisms respond to environmental change by altering the timing of various life-cycle events. Understanding an organism's phenology, therefore, is crucial for predicting the effects of anthropogenic impacts such as climate change. Nevertheless, collecting adequate data to test hypotheses in secretive species is difficult, especially for rare behaviors. We integrated data from online citizen science platforms, published literature, and unpublished studies to clarify the seasonal timing of copulation in Terrapene ornata, a behavior that is rarely observed. We identified 132 copulation events involving an estimated 256 individual turtles: 19 instances from 5,465 photo-vouchered observations, 78 from 11 publications, and 35 from radiotracking 267 turtles for 8 yr across five sites. Copulations in T. ornata occurred in every month from April to October, with the fewest records in April and October. We found no copulation records in two months of purported aboveground activity (March and November), suggesting that mating in the wild may not occur at any time during the active season. The frequency of reproductive events exhibited multimodality with evident peaks in May and September. Records from the northern portion of the species' range were most frequent in the spring, whereas southern records were most frequent in the fall. Our approach generated a large number of empirical records for a rare behavior in a secretive species across vast spatial scales, which would not have been possible using any of the individual data sources alone.

Novel patterns in reptile biology can be inferred from studies of behavior in individual species (O'Connell and Crews, 2022). For example, serendipitous behavioral observations of a parthenogenetic lizard led to theoretical advances in behavioral neuroendocrinology (Crews et al., 1996) and reproductive biology (Crews and Fitzgerald, 1980). Moreover, empirical experiments with a single species (Janzen, 1995) have extended our knowledge of the role that behaviors, such as nest-site selection, play in the evolution of environmental sex determination (Janzen and Phillips, 2006). Data on geographic variation in behavior have important implications for predicting how species will respond to environmental change (Bodensteiner et al., 2019) and for documenting differential selection pressures within species (Horváthová et al., 2013). Information gathered from behavioral studies has even become increasingly useful for designing effective conservation strategies (Cockrem, 2005; Buchholz, 2007; Berger-Tal et al., 2011; Caro and Sherman, 2011; Tobias and Pigot, 2019). Ethology has implications for many different fields, yet we still lack basic behavioral data for several North American reptiles (Doody et al., 2021). Rare behaviors are particularly data deficient, in part, because researchers are seldom afforded the opportunity to study animals in the wild long enough to witness them, let alone quantify such uncommon events to test hypotheses adequately (Bull et al., 2017).

In general, reptiles exhibit cryptic habits, making it inherently difficult to study their behavior in the wild (Steen, 2010; Rodda et al., 2015), and, as a result, they are underrepresented in the ethology literature (Doody et al., 2021). Techniques such as radiotelemetry have helped to circumvent some of the issues with studying secretive animals in the wild (Újvári and Korsós, 2000), which has led to novel insights about reptiles that would not have been discovered using other methods (e.g., Plummer, 1990; Ligon and Stone, 2003). Data collection for radiotelemetry,

however, has logistical drawbacks because resources (e.g., time, money, and personnel) not only place limits on sample sizes and study durations (e.g., Shine and Lambeck [1985] tracked 15 snakes for  $<$  6 mo), but they also influence our ability to observe behaviors continuously. Thus, it can take many years to generate sufficient observations to test hypotheses (e.g., Petersen et al. [2019] tracked 54 snakes for  $>17$  yr). Furthermore, infrequent behaviors, such as courtship and breeding, are often difficult to observe in situ because individuals may only do them a few times per year (or not at all in some years) (e.g., Fernández-Gil et al., 2006), which has led to gaps in our understanding of reptiles (Doody et al., 2013; Burghardt, 2021). Given the constraints of traditional techniques and the inherent difficulty of quantifying infrequent habits (e.g., Mann, 1999), there is a need for alternative ways of collecting basic ethological data for wild reptiles at broad spatial and temporal scales (Maritz and Maritz, 2020).

Terrapene ornata (Agassiz, 1857) (Ornate Box Turtle; Testudines: Emydidae) is a terrestrial emydid with a highly domed and distinctively marked carapace depicting light radiating lines on each scute set against a black or dark-brown background (Dodd, 2001). The distribution of T. ornata extends latitudinally from southern South Dakota to the northern states of Mexico, and longitudinally from western Indiana to central Arizona (Ernst and Lovich, 2009). Terrapene ornata is considered an open-habitat generalist because it primarily inhabits mixed grasslands and prairies, but it also uses forests, woodlands, and deserts (van Dijk and Hammerson, 2011). As a temperate species, T. ornata exhibits a strong seasonal pattern of activity where half of the year is spent brumating underground, which usually lasts from October-November to March-April throughout its range (Ernst and Lovich, 2009). Sexual maturity is reached after about 7 to 8 yr with no obvious adult sexual dimorphism in size or color, but males generally have greater relative tail length and larger inner claws than do females (Legler, 1960). Clutch size estimates range from one to eight eggs and mean clutch size increases with increasing latitude,

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TABLE 1. Hypotheses of peak copulation seasons in the Ornate Box Turtle (Terrapene ornata).



<sup>a</sup> Source indicates that copulations may take place at any time during the activity period.

while clutch frequency exhibits the inverse relationship with latitude (Edmonds et al., 2020). Mating in T. ornata is thought by some to occur at any time during the active season (Dodd, 2001); however, the seasonal timing of copulations is poorly understood, and many accounts report contradictory conclusions (e.g., Vogt, 1981; Minton, 2001; Ballinger et al., 2010). Copulatory behavior in many turtles is controlled by the secretion of gonadal sex hormones, which typically follows a seasonal pattern for species at temperate latitudes (Woolley et al., 2004). While the literature on physiology of T. ornata is sparse, a study on circulating testosterone in wild individuals of T. carolina found a bimodal pattern with peaks in the spring and fall (Currylow et al., 2013). The apparent confusion in the literature surrounding the copulation phenology of T. ornata stems from either small sample sizes that yielded narrow inferences or from an unfamiliarity with prior research (i.e., Legler, 1960; Blair, 1976). There seems to be a common pattern among sources referencing the timing of copulations in T. ornata (Table 1), where, evidently, the rarity of observing mating in the wild has contributed to a general misunderstanding surrounding the phenology of this behavior.

Available information on reproductive timing in T. ornata comes from a handful of historical studies that often contained just a limited number of copulation events observed in wild individuals from a single site (e.g., Brumwell, 1940; Legler, 1960; Smith et al., 1965; Blair, 1976). As a result, subsequent reports lend varying support to one of three hypotheses concerning mating phenology in T. ornata: 1) copulations peak in the spring, 2) copulations peak in the spring and fall, or 3) copulations occur at any time during the active season (Table 1). Because copulation in T. ornata is a rarely observed behavior that has not been subjected to robust quantification or analysis, we set out to clarify hypotheses on seasonal mating in this species by combining diverse sources of data. Our approach to pool data across sources not only demonstrated that T. ornata copulate most frequently in the spring and fall, but also that citizen science can be integrated into the study of rare behaviors that are otherwise inherently difficult to quantify from directed study.

#### MATERIALS AND METHODS

To collect as much mating phenology data as possible for our study, we: 1) scraped data from citizen science platforms for copulation events, 2) used unpublished data from ongoing longterm field projects, and 3) conducted an extensive literature review of published data. Below we detail our methods for each of these approaches.

To search for photo-vouchered observations on citizen science platforms, we generally followed the procedures outlined in Urquidi and Putman (2021) and Putman et al. (2021) with slight modifications. We have elected to use the term ''citizen science'' over "community science" because we agree with Cooper et al.

(2021) that the latter phrase should be reserved for projects that focus on local priorities and led by local communities. We searched for Ornate Box Turtle (Terrapene ornata, T. ornata ornata, and T. ornata luteola) observations on iNaturalist (www. inaturalist.org) and HerpMapper (www.herpmapper.org). We chose iNaturalist because it is the most widely used program for citizen science (87.9 million observations as of 3 January 2022) and HerpMapper because it is the largest reptile and amphibian repository for curated photo-vouchered observations (359,511 observations as of 3 January 2022). We searched all records on both platforms that had photo-vouchered observations. For iNaturalist, we used only Research Grade observations (i.e., verified by at least one other observer). We visually inspected all photographs associated with each record to look for two turtles in the act of copulation, and we also searched the metadata of each observation if there may have been notes on whether they were found copulating before the pictures were taken. We did this by visually searching the metadata for recorded notes and using global searches with various breeding-related terms, such as "reproducing," "copulating," and "mating." To ensure that we did not count copulations as two events if they were posted on both platforms, we closely examined the dates, locations, and photographs of each event for possible overlap, of which we found none. In our final citizen science dataset, we note that only one user submitted more than a single observation of copulating turtles, with the top observer submitting four, all of which were of different turtles mating at different times and at different sites.

We compiled copulation records from unpublished radiotelemetry studies on T. ornata over 8 yr from five sites across three midwestern states (Iowa, Kansas, and Nebraska). We have opted to not disclose specific locality information for these studies as they are ongoing and revealing such data often hurts wild populations because they can be co-opted for the illegal turtle trade (Lindenmayer and Scheele, 2017; Litzgus, 2017; Quinn, 2021). At the first site in eastern Iowa, 17 T. ornata were tracked at least two times per week from April to October in 2021 (Table 2). At the second site in eastern Iowa, 13 total T. ornata were tracked over 3 yr: 2018, 2019, and 2021. Turtles were tracked at the second Iowa site from April to October three times per week in 2018; once per week in 2019; and in 2021, turtles were tracked daily from April until the end of July and then once per week through October. At a site in western Nebraska, a total of 161 turtles were tracked during 2014–2016, 2018, and 2020–2021. At this site in 2014 and 2016, turtles were tracked every day from 14 May to 4 August; in 2015, turtles were tracked at least two times per week from May to August; in 2018, turtles were tracked every day from 20 May to 16 June; and during 2020–2021, turtles were tracked every day from 8 June to 17 July. In northeastern Kansas, 52 total turtles were tracked at one site for two seasons (2019 and 2020), and 24 turtles were tracked at an additional site in 2021. At the first northeastern Kansas site, turtles were tracked at least three

TABLE 2. Summary of radiotracked Ornate Box Turtles (Terrapene ornata) from the unpublished radiotelemetry dataset.

Site	Males	Females	Relocations
lowa 1		10	764
Iowa 2	5	8	2,178
Nebraska	65	96	6,904
Kansas 1	26	26	3,726
Kansas 2	10	14	1,656
Total	113	154	15,228

times per week from May to October in 2019, and from April to September in 2020. At the second northeastern Kansas site, turtles were tracked at least three times per week from May to September in 2021. We note that none of our radiotelemetry studies in the Midwest tracked turtles in March or November, but turtles at these sites have not been observed active on the surface until April and they consistently began overwintering before November (Bernstein and Black, 2005; Bernstein and Richtsmeier, 2007; Reed, 2018). The data underpinning these analyses are available from the authors upon request.

We synthesized records of copulation in T. ornata observed by researchers in the wild by conducting a search of the published literature using Google Scholar. We used the species name and search terms in various combinations to find studies that included empirical data on copulations in the wild. Examples of search phrases included "Terrapene ornata reproduction" and ''Ornate Box Turtle copulation.'' We conducted searches using the custom date range tool in Google Scholar to collect results on a decadal basis from when the species was first described to the present (Agassiz, 1857). Our temporally guided approach enhanced the detection of papers published in otherwise obscure sources (Cacciatore et al., 2012). We also obtained all papers cited as containing reproductive data in the most authoritative books published on the species to date: Dodd (2001) and Ernst and Lovich (2009). Lastly, we searched every state field guide where T. ornata occurs for additional records and sources that may have records (e.g., Phillips et al., 1999; Trauth et al., 2004; Lewis, 2011; Legler and Vogt, 2013; Powell et al., 2016; Boundy and Carr, 2017). For every source, we examined the literature cited sections for additional studies that may have contained records. We scrutinized all potential records to determine if the copulation event was observed under captive settings, and if found, we excluded these from the final data set (e.g., Norris and Zwiefel, 1950). Our searches ultimately resulted in 11 sources that contained records with dates of copulation events (Brumwell, 1940; Legler, 1960; Smith et al., 1965; Blair, 1976; Doroff and Keith, 1990; Hammerson, 1999; Minton, 2001; Grant, 2010; Refsnider et al., 2012; Quinn et al., 2014; Forrester et al., 2020). When needed, we emailed the corresponding author of these studies to supplement their published data (e.g., Refsnider et al., 2012). We collected data on all copulation events described in each source, including the date (month and year) and location (county and state). To the best of our abilities, we screened all observations to remove incidences of hybridization between T. ornata and other Terrapene species (Cureton et al., 2011) and same-sex mounting (Reed et al., 2022).

The North America base map was obtained from dmaps.com, the range map for T. ornata was adopted from iNaturalist, and both were redrawn in Adobe Illustrator. We used Microsoft Excel 2016 and R version 4.0.5 (R Core Team, 2021) to organize data, conduct analyses, and generate graphics.

We constructed frequency histograms for copulations by grouping events into monthly bins for data from citizen science platforms, published papers, and unpublished studies. By pooling data across time periods, we assumed that historical climate changes have had little effect on copulatory behavior, but we note that 91% of our copulation records were observed after 1978. We used the R package ''diptest'' (Maechler, 2021) to compute Hartigan's dip-test statistic to test for unimodality of frequency histograms (Hartigan, 1985; Hartigan and Hartigan, 1985) using a Monte Carlo simulation of a uniform distribution set for 10,000 replicates. The null hypothesis for Hartigan's dip test is that the data display a unimodal distribution, and the alternative hypothesis is that the data exhibit multimodality. To explore whether the timing of these events exhibited any geographic variation, we also analyzed frequency histograms as described above by grouping copulations into 4-degree latitudinal bins that roughly corresponded to major temperature gradients in the United States (Daly et al., 2008), while encompassing sufficient records for spatial comparisons. We recognized statistical significance at  $P < 0.05$ .

#### **RESULTS**

From 5,465 observations of T. ornata posted on two citizen science platforms (3,933 from iNaturalist and 1,532 from HerpMapper), we detected 19 copulation events with at least one record in every month from April to October and records ranged spatially from Wisconsin, United States to Chihuahua, Mexico. Most copulations in the citizen science records occurred during May to August (89.5% of citizen records) with no obvious peak. From radiotracking 267 turtles (113 males and 154 females) across five sites in the Midwest with a total of 15,228 relocations, we detected 35 copulation events with at least one record in every month from April to October. Most copulation records from these unpublished studies occurred in May (48.6% of unpublished records), June (17.1% of unpublished records), and September (14.3% of unpublished records), with sporadic records in the other months. From 11 literature sources, we found 78 copulation events with at least one record in every month from April to October and records ranged spatially in the United States from South Dakota to Texas. Most copulations in published accounts fell within two seasonal peaks that constituted 50% of these records: May (22% of published records) and September (28% of published records).

By pooling data from all sources, we amassed 132 copulation events with records in every month during April to October from southern South Dakota to northern Mexico (Fig. 1). The monthly frequency distribution for all copulation events exhibited significant multimodality (bandwidth =  $0.601$ , D = 0.106,  $P < 0.001$ ) and appeared bimodal in structure with the highest percentage of records during May (28.8%) to June (17.4%), and another peak during August (14.8%) to September (21.6%). The lowest frequency of events occurred in the months of July (8.3%), April (6.1%), and October (3%).

The monthly frequency of copulations from locations across the species' range showed generally overlapping distributions but seemingly different peaks (Fig. 2). The shape of seasonal copulation frequencies was multimodal for records in the northern (bandwidth = 0.610, D = 0.091, P < 0.001), central (bandwidth = 0.972, D = 0.125,  $P = 0.022$ ), and southern (bandwidth =  $0.878$ , D =  $0.096$ , P =  $0.007$ ) portions of the range. Copulations in the northern portion of the range exhibited a distinct peak in May (37% of northern records), whereas



FIG. 1. Monthly frequency of pooled copulation events in Ornate Box Turtles (T. ornata). Frequency is presented as the percentage of copulations in each month divided by the total. Density represents the curve of the frequency distribution with tick marks indicating individual observations.

copulations in the southern portion of the range exhibited a distinct peak in September (34.3% of southern records). No clear peak was apparent in the central portion of the range with most copulation events occurring in April (25% of central records), June (25% of central records), and September (18.8% of central records), but we note that only 16 total copulations were observed from this region.

#### **DISCUSSION**

We used an integrative approach to generate the largest collection of copulation records for T. ornata to date, which has helped to clarify previous hypotheses on seasonal mating in this species. We found a bimodal pattern for copulation in T. ornata range wide. The empirical evidence underlying our knowledge of the copulation phenology in T. ornata was based on a handful



FIG. 2. Geographic distribution of copulation events observed in Ornate Box Turtles (T. ornata) based on records from citizen science (squares), unpublished data (triangles), and published studies (circles). Left: histograms showing the frequency of monthly copulations separated by three general regions: north (black), central (grey), and south (white). Right: map depicting copulation records superimposed on the species' range map, which was modified from iNaturalist.

of primary data sources (Brumwell, 1940; Smith et al., 1965; Legler, 1960; Blair, 1976), but subsequent literature on the subject frequently stated contradictory conclusions, especially in synoptic guides (e.g., Vogt, 1981; Degenhardt et al., 1996; Hammerson, 1999; Dodd, 2001; Minton, 2001; Ernst and Lovich, 2009; Ballinger et al., 2010; McGinnis and Stebbins, 2018). Among the historical studies, Legler (1960) found patterns in Kansas—derived from an independent source of data—that were the most congruent with our results. Based on 40 male T. ornata preserved throughout the active season, Legler (1960) found that testes were largest in spring and fall, where testis size increased from April through early June, receded from late-June to July, increased again in August to reach their largest size from September through October. Legler (1960) also found that mature sperm in the epididymis were most numerous in spring and fall between the spermatogenic cycles. Based on 68 female T. ornata preserved throughout the active season, Legler (1960) found the seasonal occurrence of females with enlarged ovarian follicles was highest in October and March through May, reduced in June and July, and lowest in August and September. The two annual mating periods—or the ability to mate throughout much of the activity season (Dodd, 2001)—in T. ornata would be advantageous for males that complete spermatogenesis prior to female ovulation.

We wish to highlight general biases unique to studies using citizen science and radiotelemetry data that should be considered when interpreting their results (Kosmala et al., 2016; Di Cecco et al., 2021). One major source of bias in citizen science data is related to the human tendency to overemphasize larger animals that are engaged in actions deemed interesting enough for the viewer to photograph (Callaghan et al., 2021; Hughes et al., 2021). We processed over 5,000 observations from citizen platforms—all of which were verified as T. ornata—and only 0.35% of these records included turtles photographed in copula. Furthermore, our unpublished radiotelemetry dataset was based on over 15,000 relocations of turtles in the wild and only 0.23% of these observations were of turtles engaged in copulation. Taken together, the independent sources corroborate the notion that copulation is an uncommonly encountered event. Another common bias in citizen data is that observations tend to be skewed towards times when humans are most active throughout the year (Courter et al., 2013). We did not find any evidence that the bimodality in copulations was because fewer people were looking for turtles during the summer. For example, when examining all iNaturalist observations for T. ornata, we found a unimodal pattern with most occurring in June, July, and August, which was the same when looking at observations from southern states in isolation, such as Texas. Lastly, we wish to point out that radiotelemetry studies are not without their own biases (Millspaugh and Marzluff, 2001), particularly with respect to the season that researchers elect to follow tagged animals.

Our results from wild individuals revealed two general peaks in the seasonal copulation frequency of T. ornata, a pattern that was geographically variable with fall copulations more common in southern locations and spring copulations more common in northern sites. Sites in the central portion of the range showed no clear pattern, with generally equal numbers from April to September. The widest breeding window from any single study observed copulations from 5 April to 13 October in Texas (Blair, 1976), but several studies at northern locales also reported wide breeding windows, including from 15 May to 7 September in Illinois (Refsnider et al., 2012) and 18 April to 20 September in

South Dakota (Quinn et al., 2014). We found instances of mating throughout April to October across much of the species' range, indicating that copulatory behavior can occur at nearly any time during the active season (Dodd, 2001; Redder et al., 2006). However, we note that none of our records occurred in March or November, suggesting that wild individuals may be constrained by the extreme cold temperatures often occurring in these months. Corroborative evidence comes from the most southern, and presumably warmer, site of Blair (1976) who observed turtles active on the surface from March through December, yet only saw copulations during April to October. There are accounts, nonetheless, of captive T. ornata copulating in every month of the year (e.g., Rodeck, 1949; Vogt, 1981).

Even though many turtle species display complex social behaviors (e.g., Kaufmann, 1992), they, in general, do not appear to form obvious bonds with other turtles, nor does either sex provide much parental care after copulation and nesting (Iverson, 1990). Consequently, turtle mating decisions are most affected by mate quality, availability of mates, timing of mating or remating, and sperm storage (Pearse and Avise, 2001). Sperm storage allows for asynchronous mating (Godley et al., 2002; Riley et al., 2021) and appears universal in the turtles studied to date (Gist and Jones, 1989; Pearse and Avise, 2001; Tuberville et al., 2011; Cutuli et al., 2013; Orr and Brennan, 2015), including the closely related species T. carolina (Hattan and Gist, 1975). Despite the apparent dissociation between fertilization and mating, we found a seasonal pattern in T. ornata copulations, which mirrored the spermatogenesis cycle (Legler, 1960) and seasonal changes in circulating testosterone levels in T. carolina (Currylow et al., 2013). Turtles at temperate latitudes exhibit seasonal cycles of gonadal sex hormones (Woolley et al., 2004), and perhaps, copulatory behavior in T. ornata is constrained to a similar pattern. Studies of space use in T. ornata demonstrate that the greatest overlap of home ranges occurs in the spring and fall (Dodd, 2001), indicating that these are the periods of the year when individuals are most likely to encounter each other, and potentially copulate. A series of mating experiments in T. carolina by Belzer (2002) and Belzer and Seibert (2009) revealed that males could not find females to copulate with if they were not close enough to be seen or heard, indicating that population density and degree of home-range overlap significantly influence the onset of coital behavior (Stickel, 1989).

Female turtles achieve multiple paternity through sperm storage or by mating with multiple males in the same season, which can increase offspring fitness (Uller and Olsson, 2008; Riley et al., 2021). In our unpublished radiotelemetry dataset, 35 mating events involved just 56 turtles, with several individuals seen copulating multiple times. Males were observed copulating multiple times in one year more frequently than females, with six males copulating more than once compared to four females. From a Kansas site, a single female and a single male were observed copulating with each other on two different occasions in 2021: 29 April and 15 September. From an Iowa site, a single female was observed copulating on three different occasions with three different males in 2018: 24 May, 25 June, and 4 September. We note that the 14 observations were included in the larger analysis because none occurred on the same day and thus were temporally independent. Hoekert et al. (2002) found clutches that exhibited multiple paternity, but there were extreme differences in fertilizations amongst competing males. Such unequal paternity could result from sperm competition, cryptic female choice, or sperm precedence due to mating order. Physical competition among males for access to females may

occur in T. ornata, which would facilitate precopulatory female choice. For example, Brumwell (1940) in Kansas observed four males pursuing a single female on 9 May 1939, eventually resulting in one male copulating with the female and the other males walking away. Future research exploring the effect of mating order on paternity in T. ornata would shed light on the relative contributions of sperm storage, multiple paternity, and cryptic female choice to the genetic diversity of offspring (Pearse et al., 2001).

Terrapene ornata are listed as Near Threatened by the International Union for Conservation of Nature's Red List (van Dijk and Hammerson, 2011) and considered endangered in many of the states where they occur (Redder et al., 2006). The greatest threats to T. ornata are road mortality, habitat loss, and collection for the animal trade (Dodd, 2001), none of which show any signs of diminishing for turtles in the near future (Stanford et al., 2020). Although T. ornata is apparently well studied (Dodd, 2001; Ernst and Lovich, 2009), our analysis revealed that some previous conclusions about its mating phenology were based on limited data from wild individuals. Robust data on a species' behavior can aid its conservation by allowing for a more fine-scaled analysis of how it responds to change (Cockrem, 2005). Natural history is widely regarded as a critical piece to the conservation of biodiversity on a changing planet (Tewksbury et al., 2014). However, natural history as a discipline is founded on detailed observations of organisms in nature (Arnold, 2003; Greene, 2005). Yet achieving descriptive ethology to the level that advances conservation requires arduous effort (Dayton, 2003), a task made even more challenging by a species with cryptic habits. The large amount of natural history data we gathered for T. ornata would not have been possible without dedicated researchers that tracked wild turtles with radiotelemetry and individuals willing to contribute observations to citizen science platforms (Irwin, 2018). Citizen science platforms, in fact, represent important sources of biodiversity data that can be leveraged to study organisms across unprecedented spatiotemporal scales of analysis (e.g., Hantak et al., 2022; Perry et al., 2022). Citizen science helped us elucidate the reproductive phenology T. ornata, and it will continue to be important for biodiversity in the future.

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### LITERATURE CITED

- AGASSIZ, L. 1857. Contributions to the Natural History of the United States of America, Volume 1. Little, Brown and Company, USA.
- ARNOLD, S. J. 2003. Too much natural history, or too little? Animal Behaviour 65:1065–1068.
- BALLINGER, R. E., J. D. LYNCH, AND G. R. SMITH. 2010. Amphibians and Reptiles of Nebraska. Rusty Lizard Press, USA.
- BARTLETT, R. D., AND P. P. BARTLETT. 2013. New Mexico's Reptiles and Amphibians: A Field Guide. University of New Mexico Press, USA.
- BELZER, B. 2002. A nine year study of eastern box turtle courtship with implications for reproductive success and conservation in a translocated population. Turtle and Tortoise Newsletter 6:17–26.
- BELZER, W. R., AND S. SEIBERT. 2009. How do male box turtles find mates? Turtle and Tortoise Newsletter 13:11–21.
- BERGER-TAL, O., T. POLAK, A. ORON, Y. LUBIN, B. P. KOTLER, AND D. SALTZ. 2011. Integrating animal behavior and conservation biology: a conceptual framework. Behavioral Ecology 22:236–239.
- BERNSTEIN, N. P., AND R. W. BLACK. 2005. Thermal environment of overwintering ornate box turtles, Terrapene ornata ornata, in Iowa. American Midland Naturalist 153:370–377.
- BERNSTEIN, N. P., AND R. J. RICHTSMEIER. 2007. Home range and philopatry in the ornate box turtle, Terrapene ornata ornata, in Iowa. American Midland Naturalist 157:162–174.
- BLAIR, W. F. 1976. Some aspects of the biology of the ornate box turtle, Terrapene ornata. Southwestern Naturalist 21:89–104.
- BODENSTEINER, B. L., D. A. WARNER, J. B. IVERSON, C. L. MILNE-ZELMAN, T. S. MITCHELL, J. M. REFSNIDER, AND F. J. JANZEN. 2019. Geographic variation in thermal sensitivity of early life traits in a widespread reptile. Ecology and Evolution 9:2791–2802.
- BOUNDY, J., AND J. L. CARR. 2017. Amphibians and Reptiles of Louisiana: An Identification and Reference Guide. Louisiana State University Press, USA.
- BRUMWELL, M. J. 1940. Notes on the courtship of the turtle, Terrapene ornata. Transactions of the Kansas Academy of Science 43:391–392.
- BUCHHOLZ, R. 2007. Behavioural biology: an effective and relevant conservation tool. Trends in Ecology and Evolution 22:401–407.
- BULL, C. M., M. G. GARDNER, A. SIH, O. SPIEGEL, S. S. GODFREY, AND S. T. LEU. 2017. Why is social behavior rare in reptiles? Lessons from sleepy lizards. Advances in the Study of Behavior 49:1–26.
- BURGHARDT, G. M. 2021. How comparative was (is) the Journal of Comparative Psychology? A reptilian perspective. Journal of Comparative Psychology 135:286–290.
- CACCIATORE, M. A., A. A. ANDERSON, D. H. CHOI, D. BROSSARD, D. A. SCHEUFELE, X. LIANG, P. J. LADWIG, M. XENOS, AND A. DUDO. 2012. Coverage of emerging technologies: a comparison between print and online media. New Media and Society 14:1039–1059.
- CALLAGHAN, C. T., A. G. POORE, M. HOFMANN, C. J. ROBERTS, AND H. M. PEREIRA. 2021. Large-bodied birds are over-represented in unstructured citizen science data. Scientific Reports 11:19073.
- CARO, T., AND P. W. SHERMAN. 2011. Endangered species and a threatened discipline: behavioural ecology. Trends in Ecology and Evolution 26: 111–118.
- COCKREM, J. F. 2005. Conservation and behavioral neuroendocrinology. Hormones and Behavior 48:492–501.
- COLLINS, J. T., S. L. COLLINS, AND T. W. TAGGART. 2010. Amphibians, Reptiles, and Turtles in Kansas. Eagle Mountain Publishing, USA.
- COOPER, C. B., C. L. HAWN, L. R. LARSON, J. K. PARRISH, G. BOWSER, D. CAVALIER, R. R. DUNN, M. HAKLAY, K. K. GUPTA, N. T. O. JELKS, ET AL. 2021. Inclusion in citizen science: the conundrum of rebranding. Science 372:1386–1388.
- COURTER, J. R., R. J. JOHNSON, C. M. STUYCK, B. A. LANG, AND E. W. KAISER. 2013. Weekend bias in Citizen Science data reporting: implications for phenology studies. International Journal of Biometeorology 57: 715–720.
- CREWS, D., AND K. T. FITZGERALD. 1980. ''Sexual'' behavior in parthenogenetic lizards (Cnemidophorus). Proceedings of the National Academy of Sciences USA 77:499–502.
- CREWS, D., J. GODWIN, V. HARTMAN, M. GRAMMER, E. A. PREDIGER, AND R. SHEPPHERD. 1996. Intrahypothalamic implantation of progesterone in castrated male whiptail lizards (Cnemidophorus inornatus) elicits courtship and copulatory behavior and affects androgen receptorand progesterone receptor-mRNA expression in the brain. Journal of Neuroscience 16:7347–7352.
- CURETON, J. C., A. B. BUCHMAN, R. DEATON, AND W. I. LUTTERSCHMIDT. 2011. Molecular analysis of hybridization between the box turtles Terrapene carolina and T. ornata. Copeia 2011:270–277.
- CURRYLOW, A. F., M. S. TIFT, J. L. MEYER, D. E. CROCKER, AND R. N. WILLIAMS. 2013. Seasonal variations in plasma vitellogenin and sex steroids in male and female eastern box turtles, Terrapene carolina carolina. General and Comparative Endocrinology 180:48–55.
- CUTULI, G., S. CANNICCI, M. VANNINI, AND S. FRATINI. 2013. Influence of mating order on courtship displays and stored sperm utilization in Hermann's tortoises (Testudo hermanni hermanni). Behavioral Ecology and Sociobiology 67:273–281.
- DALY, C., M. HALBLEIB, J. I. SMITH, W. P. GIBSON, M. K. DOGGETT, G. H. TAYLOR, J. CURTIS, AND P. P. PASTERIS. 2008. Physiographically sensitive mapping of climatological temperature and precipitation across the coterminous United States. International Journal of Climatology 28: 2031–2064.
- DAYTON, P. K. 2003. The importance of the natural sciences to conservation. American Naturalist 162:1–13.
- DEGENHARDT, W. G., C. W. PAINTER, AND A. H. PRICE. 1996. Amphibians and Reptiles of New Mexico. University of New Mexico Press, USA.
- DI CECCO, G. J., V. BARVE, M. W. BELITZ, B. J. STUCKY, R. P. GURALNICK, AND A. H. HURLBERT. 2021. Observing the observers: how participants contribute data to iNaturalist and implications for biodiversity science. BioScience 71:1179–1188.
- DODD, C. K., JR. 2001. North American Box Turtles: A Natural History. Johns Hopkins University Press, USA.
- DOODY, J. S., G. M. BURGHARDT, AND V. DINETS. 2013. Breaking the social– non-social dichotomy: a role for reptiles in vertebrate social behavior research? Ethology 119:95–103.
- DOODY, J. S., V. DINETS, AND G. M. BURGHARDT. 2021. The Secret Social Lives of Reptiles. Johns Hopkins University Press, USA.
- DOROFF, A. M., AND L. B. KEITH. 1990. Demography and ecology of an ornate box turtle (Terrapene ornata) population in south-central Wisconsin. Copeia 1990:387–399.
- EDMONDS, D., L. ADAMOVICZ, M. C. ALLENDER, AND M. J. DRESLIK. 2020. Reproductive output of ornate box turtles (Terrapene ornata) in Illinois, USA. Herpetological Conservation and Biology 15:467–475.
- ERNST, C. H., AND J. E. LOVICH. 2009. Turtles of the United States and Canada. 2nd ed. Johns Hopkins University Press, USA.
- FERNÁNDEZ-GIL, A., J. NAVES, AND M. DELIBES. 2006. Courtship of brown bears Ursus arctos in northern Spain: phenology, weather, habitat and durable mating areas. Wildlife Biology 12:367–373.
- FORRESTER, A. J., M. L. ROHDE, M. J. HARNER, C. KRUSE, AND K. GELUSO. 2020. Ornate box turtles (Terrapene ornata) copulating in water: an incidental observation or ancestral behavior. Transactions of the Nebraska Academy of Sciences 40:19–23.
- GIST, D. H., AND J. M. JONES. 1989. Sperm storage within the oviduct of turtles. Journal of Morphology 199:379–384.
- GODLEY, B. J., A. C. BRODERICK, R. FRAUENSTEIN, F. GLEN, AND G. C. HAYS. 2002. Reproductive seasonality and sexual dimorphism in green turtles. Marine Ecology Progress Series 226:125–133.
- GRANT, S. D. 2010. Spatial Ecology and Demography of the Ornate Box Turtle in a Seasonally Burned Sand Prairie Matrix. Ph.D. diss., West Texas A&M University, USA.
- GREENE, H. W. 2005. Organisms in nature as a central focus for biology. Trends in Ecology and Evolution 20:23–27.
- HAMMERSON, G. A. 1999. Amphibians and Reptiles in Colorado. University Press of Colorado, USA.
- HANTAK, M. M., R. P. GURALNICK, A. ZARE, AND B. J. STUCKY. 2022. Computer vision for assessing species color pattern variation from web-based community science images. iScience 25:104784.
- HARTIGAN, P. M. 1985. Computation of the dip statistic to test for unimodality. Applied Statistics 34:320–325.
- HARTIGAN, J. A., AND P. M. HARTIGAN. 1985. The dip test of unimodality. Annals of Statistics 13:70–84.
- HATTAN, L. R., AND D. H. GIST. 1975. Seminal receptacles in the eastern box turtle, Terrapene carolina. Copeia 1975:505–510.
- HOEKERT, W. E. J., H. NEUFEGLISE, A. D. SCHOUTEN, AND S. B. J. MENKEN. 2002. Multiple paternity and female-biased mutation at a microsatellite locus in the olive ridley sea turtle (Lepidochelys olivacea). Heredity 89:107–113.
- HORVÁTHOVÁ, T., C. R. COONEY, P. S. FITZE, T. A. OKSANEN, D. JELIĆ, I. GHIRA, T. ULLER, AND D. JANDZIK. 2013. Length of activity season drives geographic variation in body size of a widely distributed lizard. Ecology and Evolution 3:2424–2442.
- HUGHES, A. C., M. C. ORR, K. MA, M. J. COSTELLO, J. WALLER, P. PROVOOST, Q. YANG, C. ZHU, AND H. QIAO. 2021. Sampling biases shape our view of the natural world. Ecography 44:1259–1269.
- IRWIN, A. 2018. No PhDs needed: how citizen science is transforming research. Nature 562:480–482.
- IVERSON, J. B. 1990. Nesting and parental care in the mud turtle, Kinosternon flavescens. Canadian Journal of Zoology 68:230–233.
- JANZEN, F. J. 1995. Experimental evidence for the evolutionary significance of temperature-dependent sex determination. Evolution 49:864–873.
- JANZEN, F. J., AND P. C. PHILLIPS. 2006. Exploring the evolution of environmental sex determination, especially in reptiles. Journal of Evolutionary Biology 19:1775–1784.
- JOHNSON, T. R. 2000. The Amphibians and Reptiles of Missouri. 2nd ed. Missouri Department of Conservation, USA.
- KAUFMANN, J. H. 1992. The social behavior of wood turtles, Clemmys insculpta, in central Pennsylvania. Herpetological Monographs 6:1– 25.
- KIESOW, A. M. 2006. Field Guide to the Amphibians and Reptiles of South Dakota. South Dakota Department of Game, Fish and Parks, USA.
- KOSMALA, M., A. WIGGINS, A. SWANSON, AND B. SIMMONS. 2016. Assessing data quality in citizen science. Frontiers in Ecology and the Environment 14:551–560.
- LECLERE, J. B. 2013. A Field Guide to the Amphibians and Reptiles of Iowa. ECO Herpetological Publishing and Distribution, USA.
- LEGLER, J. M. 1960. Natural history of the ornate box turtle, Terrapene ornata ornata Agassiz. University of Kansas Publications, Museum of Natural History 11:527–669.
- LEGLER, J. M., AND R. C. VOGT. 2013. The Turtles of Mexico: Land and Freshwater Forms. University of California Press, USA.
- LEWIS, D. 2011. A Field Guide to the Amphibians and Reptiles of Wyoming. Wyoming Naturalist, USA.
- LIGON, D. B., AND P. A. STONE. 2003. Radiotelemetry reveals terrestrial estivation in Sonoran mud turtles (Kinosternon sonoriense). Journal of Herpetology 37:750–754.
- LINDENMAYER, D., AND B. SCHEELE. 2017. Do not publish. Science 356:800-801.
- LITZGUS, J. 2017. The illegal turtle trade: why I keep secrets. The Conversation. Available at https://theconversation.com/the-illegalturtle-trade-why-i-keep-secrets-85805.
- MAECHLER, M. 2021. Package 'diptest.' Available from: https://github. com/mmaechler/diptest.
- MANN, J. 1999. Behavioral sampling methods for cetaceans: a review and critique. Marine Mammal Science 15:102–122.
- MARITZ, R. A., AND B. MARITZ. 2020. Sharing for science: high-resolution trophic interactions revealed rapidly by social media. PeerJ 8:e9485.
- MCGINNIS, S. M., AND R. C. STEBBINS. 2018. A Field Guide to Western Reptiles and Amphibians. 4th ed. Houghton Mifflin Harcourt, USA.
- MILLSPAUGH, J., AND J. M. MARZLUFF. 2001. Radio Tracking and Animal Populations. Academic Press, USA.
- MINTON, S. A., JR. 2001. Amphibians and Reptiles of Indiana. 2nd ed. Indiana Academy of Sciences, USA.
- NORRIS, K. S., AND R. G. ZWEIFEL. 1950. Observations on the habits of the ornate box turtle, Terrapene ornata (Agassiz). Chicago Academy of Sciences 58:1–4.
- O'CONNELL, L. A., AND D. CREWS. 2022. Evolutionary insights into sexual behavior from whiptail lizards. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology 337:88–98.
- ORR, T. J., AND P. L. BRENNAN. 2015. Sperm storage: distinguishing selective processes and evaluating criteria. Trends in Ecology and Evolution 30:261–272.
- PEARSE, D. E., AND J. C. AVISE. 2001. Turtle mating systems: behavior, sperm storage, and genetic paternity. Journal of Heredity 92:206–211.
- PEARSE, D. E., F. J. JANZEN, AND J. C. AVISE. 2001. Genetic markers substantiate long-term storage and utilization of sperm by female painted turtles. Heredity 86:378–384.
- PERRY, T., A. STENHOUSE, I. WILSON, I. PERFETTO, M. W. MCKELVEY, M. COULSON, R. A. ANKENY, P. D. RISMILLER, AND F. GRÜTZNER. 2022. EchidnaCSI: engaging the public in research and conservation of the short-beaked echidna. Proceedings of the National Academy of Sciences USA 119:e2108826119.
- PETERSEN, C. E., S. M. GOETZ, M. J. DRESLIK, J. D. KLEOPFER, AND A. H. SAVITZKY. 2019. Sex, mass, and monitoring effort: keys to understanding spatial ecology of timber rattlesnakes (Crotalus horridus). Herpetologica 75:162–174.
- PHILLIPS, C. A., R. A. BRANDON, AND E. O. MOLL. 1999. Field Guide to Amphibians and Reptiles of Illinois. Illinois Natural History Survey, USA.
- PLUMMER, M. V. 1990. Nesting movements, nesting behavior, and nest sites of green snakes (Opheodrys aestivus) revealed by radiotelemetry. Herpetologica 46:190–195.
- POWELL, R., R. CONANT, AND J. T. COLLINS. 2016. Field Guide to Reptiles and Amphibians of Eastern and Central North America. 4th ed. Houghton Mifflin Harcourt, USA.
- PUTMAN, B. J., R. Williams, E. Li, and G. B. Pauly. 2021. The power of community science to quantify ecological interactions in cities. Scientific Reports 11:3069.
- QUINN, A. 2021. Transparency and secrecy in citizen science: lessons from herping. Studies in History and Philosophy of Science Part A 85:208– 217.
- QUINN, H. R., H. QUINN, AND A. HIGA. 2014. Notes on reproduction and growth of South Dakota ornate box turtles (Terrapene ornata). Chelonian Conservation and Biology 13:65–71.
- R CORE TEAM. 2021. R version 4.0.5: a language and environment for statistical computing. Available at https://www.R-project.org.
- REDDER, A. J., C. K. DODD, JR., AND D. KEINATH. 2006. Ornate box turtle (Terrapene ornata ornata): a technical conservation assessment. USDA

Forest Service, Rocky Mountain Region. Available at https://www. fs.usda.gov/Internet/FSE\_DOCUMENTS/stelprdb5182076.pdf.

- REED, B. M. 2018. Interspecific and Intraspecific Variation in the Movement Ecology of Ectotherms Across Different Spatial and Temporal Scales. Ph.D. diss., University of Nebraska-Lincoln, USA.
- REED, B. M., K. HOBELMAN, AND D. F. HUGHES. 2022. Terrapene ornata (ornate box turtle): same-sex mounting. Herpetological Review 53: 126.
- REFSNIDER, J. M., J. STRICKLAND, AND F. J. JANZEN. 2012. Home range and site fidelity of imperiled ornate box turtles (Terrapene ornata) in northwestern Illinois. Chelonian Conservation and Biology 11:78–83.
- RILEY, J. L., A. STOW, P. E. BOLTON, S. DENNISON, R. W. BYRNE, AND M. J. WHITING. 2021. Sperm storage in a family-living lizard, the tree skink (Egernia striolata). Journal of Heredity 112:526–534.
- RODDA, G. H., K. DEAN-BRADLEY, E. W. CAMPBELL, T. H. FRITTS, B. LARDNER, A. A. Y. ADAMS, AND R. N. REED. 2015. Stability of detectability over 17 years at a single site and other lizard detection comparisons from Guam. Journal of Herpetology 49:513–521.
- RODECK, H. G. 1949. Notes on box turtles in Colorado. Copeia 1949:32– 34.
- SHINE, R., AND R. LAMBECK. 1985. A radiotelemetric study of movements, thermoregulation and habitat utilization of Arafura filesnakes (Serpentes: Acrochordidae). Herpetologica 41:351–361.
- SIEVERT, G., AND L. SIEVERT. 2021. A Field Guide to Oklahoma's Amphibians and Reptiles. 4th ed. Oklahoma Department of Wildlife Conservation, USA.
- SMITH, H. M., T. P. MASLIN, AND R. L. BROWN. 1965. Summary of the Distribution of the Herpetofauna of Colorado: A Supplement to an Annotated Check List of the Amphibians and Reptiles of Colorado. University of Colorado Press, USA.
- STANFORD, C. B., J. B. IVERSON, A. G. RHODIN, P. P. VAN DIJK, R. A. MITTERMEIER, G. KUCHLING, K. H. BERRY, A. BERTOLERO, K. A. BJORNDAL, T. E. BLANCK, ET AL. 2020. Turtles and tortoises are in trouble. Current Biology 30:R721–R735.
- STEEN, D. A. 2010. Snakes in the grass: secretive natural histories defy both conventional and progressive statistics. Herpetological Conservation and Biology 5:183–188.
- STICKEL, L. F. 1989. Home range behavior among box turtles (Terrapene c. carolina) of a bottomland forest in Maryland. Journal of Herpetology 23:40–44.
- STONE, L. M. 2007. Box Turtles. Lerner Publications Company, USA.
- TEWKSBURY, J. J., J. G. ANDERSON, J. D. BAKKER, T. J. BILLO, P. W. DUNWIDDIE, M. J. GROOM, S. E. HAMPTON, S. G. HERMAN, D. J. LEVEY, N. J. MACHNICKI, ET AL. 2014. Natural history's place in science and society. BioScience 64:300–310.
- TOBIAS, J. A., AND A. L. PIGOT. 2019. Integrating behaviour and ecology into global biodiversity conservation strategies. Philosophical Transactions of the Royal Society B Biological Sciences 374:20190012.
- TRAUTH, S. E., H. W. ROBISON, AND M. V. PLUMMER. 2004. The Amphibians and Reptiles of Arkansas. University of Arkansas Press, USA.
- TUBERVILLE, T. D., T. M. NORTON, B. J. WAFFA, C. HAGEN, AND T. C. GLENN. 2011. Mating system in a gopher tortoise population established through multiple translocations: apparent advantage of prior residence. Biological Conservation 144:175–183.
- ÚJVÁRI, B., AND Z. KORSÓS. 2000. Use of radiotelemetry on snakes: a review. Acta Zoologica Academiae Scientiarum Hungaricae 46:115– 146.
- ULLER, T., AND M. OLSSON. 2008. Multiple paternity in reptiles: patterns and processes. Molecular Ecology 17:2566–2580.
- URQUIDI, E. R., AND B. J. PUTMAN. 2021. Quantifying southern pacific rattlesnake (Crotalus oreganus helleri) hunting behavior through community science. Diversity 13:349.
- VAN DIJK, P. P., AND G. A. HAMMERSON. 2011. Terrapene ornata (errata version published in 2016). The IUCN Red List of Threatened Species 2011: e.T21644A97429080. Available at https://dx.doi.org/10.2305/ IUCN.UK.2011-1.RLTS.T21644A9304752.en.
- VOGT, R. C. 1981. Natural History of Amphibians and Reptiles of Wisconsin. Milwaukee Public Museum Press, USA.
- WOOLLEY, S. C., J. T. SAKATA, AND D. CREWS. 2004. Evolutionary insights into the regulation of courtship behavior in male amphibians and reptiles. Physiology and Behavior 83:347–360.

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