You can go your own way: No evidence for social behavior based on kinship or familiarity in captive juvenile box turtles

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Abstract

Behavioral interactions between conspecific animals can be influenced by relatedness and familiarity. Compared to other vertebrate taxa, considering such aspects of social behavior when housing captive reptiles has received less attention, despite the implications this could have for informing husbandry practices, enhancing welfare, and influencing outcomes of conservation translocations. To test how kinship and familiarity influenced social behavior in juvenile Eastern Box Turtles (*Terrapene carolina*), we reared 16 captive-born individuals under semi-natural conditions in four equally sized groups, where each group comprised pairs of siblings and non-siblings. Using separation distance between pairs of turtles in rearing enclosures as a measure of gregariousness, we found no evidence suggesting siblings more frequently interacted with one another compared to non-relatives over the first five months of life (β = 0.016, 95% CI: 0.117 to 0.084). Average pair separation distance decreased during this time (β = 0.146, 95% CI: 0.228 to 0.063) but may have been due to turtles aggregating around concentrated resources like heat and moist retreat areas as cold winter temperatures approached. When subjects were eight months old, we measured repeated separation distances between unique pair combinations in an experimental environment and similarly found no support for gregariousness (associations) being influenced by kinship or familiarity (β = 1.554, 95% CI: 9.956 to 6.848). Additionally, neither differences in body size between pairs of turtles (β = −22.289, 95% CI: −68.448 to 23.870) nor the five-minute time interval during the 90-minute trial (P ≥ 0.18) had any apparent effect on associations. Agonistic interactions between individuals were never observed. Encouragingly, based on our results, group housing and rearing of juvenile box turtles did not appear to negatively impact their welfare. Unlike findings for other taxa, including some reptiles, our results suggest strategically housing groups of juvenile *T. carolina* to maintain social stability may not be an important husbandry consideration or necessary when planning releases of captive-reared individuals for conservation purposes.

1. Introduction

Kinship and familiarity can be highly influential drivers of social behavior for conspecific animals (Hamilton, 1964; Kurvers et al., 2013). These factors need not be mutually exclusive in facilitating social interactions for numerous vertebrate taxa. For example, it has been found that relatedness enhances preferences based on familiarity, and for unfamiliar conspecifics, individuals prefer a related social partner (Freed Dickson and Sackett, 1984; Clark, 2004). Individuals may also prefer associating with familiar conspecifics in which previous interactions are protracted (Kristina et al., 2015; Keller et al., 2017). Additionally, aggregating conspecifics are often similar in body size, suggesting such physical traits further mediate social interactions (Gregory, 2004). Many studies investigating how social behavior is affected by the above-mentioned factors have been conducted in the context of inclusive fitness theory (i.e., kin selection; Hamilton, 1964), reproduction (e.g., inbreeding avoidance; Lehmann and Perrin, 2003), or parental care (Hoss et al., 2015). However, kinship and familiarity have been under-studied with regard to their potential effects on more applied aspects of conservation efforts for captive animals, such as husbandry and welfare (Burghardt, 2013; Doody, 2021).

Social interaction can be an important husbandry consideration for...
optimizing health and welfare of captive animals, including taxa historically (and incorrectly) considered asocial, such as reptiles (reviewed in Burghardt, 2013; Schuett et al., 2016; Doody, 2021). Captive-breeding programs and release of captive-reared (i.e., head-started) reptiles have become common management tactics for imperiled species, but whether refinements to rearing practices may be necessary, such as strategically housing individuals to facilitate preferred social interactions, is not well understood for many species. Although considering the potential importance of social structure when planning or conducting group releases of translocated reptiles was noted decades ago (Dodd and Seigel, 1991; Reintert, 1991), this has largely been neglected (but see Berry, 1986; Burke, 1989; Elangovan et al., 2021) compared to the numerous reptile translocations that have been conducted (Germano and Bishop, 2009).

Chelonians (turtles, tortoises, and terrapins; hereafter “turtles”) are among the most threatened vertebrates globally (Lovich et al., 2018), and release of head-started juveniles has become a widely used approach to bolster wild populations (Burke, 2015). Although turtles are perhaps generally less social than other reptiles such as lizards and crocodilians, they undoubtedly benefit from socialization (Doody, 2021). Turtles provide little parental care compared to other reptilian taxa (Doody et al., 2013), so juveniles of these precocial species may benefit from social interaction early in life. For instance, communication between sibling hatchlings of some species may facilitate emergence and subsequent dispersal from nests; this in turn can reduce post-emergence predation risk, which is typically high for young turtles (Santos et al., 2016). Following dispersal from nests, temperate species may benefit from interactions with conspecifics to locate suitable overwintering locations (Laarman et al., 2018). These considerations could similarly be relevant for planning releases of head-started individuals, which are naïve to novel post-release environments.

Studies examining whether juvenile turtles choose to associate with conspecifics based on kinship and/or familiarity have met with mixed results, even for species with similar natural histories. For example, captive hatchlings of the diamondback terrapin (Malaclemys terrapin) that were familiar kin basked in larger groups than turtles that were unfamiliar or not related (Rife, 2007), suggesting releasing cohorts of such groups could enhance post-release survival by conferring group-level benefits (e.g., enhanced predator awareness). Hatchlings of other semi-aquatic species (Emydoidea blandingii and Graptemys geographica) showed no preference for water with chemosensory cues from conspecifics over unscented water (Whitear et al., 2017). Nonetheless, hatchlings of a predominantly aquatic species (Apalone spinifera) preferred water scented by conspecific hatchlings to unscented water; A. spinifera also preferred water scented by distantly related conspecifics to water scented by close kin, suggesting there may be post-hatching benefits from interacting with less familiar individuals (Whitear et al., 2017). Although, hatchling tortoises (Testudo marginata and Testudo graeca) avoided unfamiliar conspecifics more than familiar individuals (Versace et al., 2018).

Here, we investigated how kinship and familiarity influenced social interactions of captive-born juveniles of the Eastern Box Turtle (Terrapene carolina) as part of a captive-rearing and release program. This species is listed as Vulnerable by the International Union for Conservation of Nature (IUCN) (van Dijk, 2011) and is included in the Convention on International Trade in Endangered Species (CITES) Appendix II (https://cites.org/eng/app/appendices.php). Head-starting has been proposed as a potential conservation measure for the species (Dodd, 2001; Tetzlaff et al., 2019a). To further understand potential drivers of social behavior for juvenile turtles, we tested several hypotheses using individuals from captive-hatched clutches of Eastern Box Turtles (Terrapene carolina, Emydidae); If hatchlings prefer associating with siblings, then these individuals should be in closer proximity (aggregate) more often compared to non-siblings. Furthermore, this preference may be enhanced by familiarity when turtles are housed together for longer periods; thus, an interactive effect of kinship and time could influence aggregations as individuals had time to build social relationships. We also tested the following two hypotheses after captive-reared turtles had been housed in groups for several months: 1) When pairs of turtles are placed in novel conditions, we expected social interactions to decrease according to the following pattern of relatedness and familiarity: familiar siblings > familiar non-siblings > non-familiar siblings > nonfamiliar non-siblings. And 2), if body size influences sociality regardless of kinship or familiarity, then we expect non-aggressive interactions to decrease (i.e., an increase in affiliative behavior) as differences in body sizes increase.

2. Materials and methods

2.1. Ethical note

This research was conducted under an approved protocol by the University of Illinois Institutional Animal Care and Use Committee (#16017) and Scientific Collector’s Permits granted by the States of Michigan and Illinois (#NH17.5980).

2.2. Study species and husbandry

Eastern Box Turtles are long-lived (regularly >50 years) reptiles that inhabit temperate and subtropical regions of the eastern United States (Dodd, 2001). Due to population declines resulting from habitat loss, road mortality, intense predation (particularly of nests and juveniles), and collection for the pet trade, the species is listed as Vulnerable by the IUCN (van Dijk, 2011) and is included in CITES Appendix II (https://cites.org/eng/app/appendices.php).

The subjects for this study (n = 16) were collected as eggs from six insitu nests at Fort Custer Training Center in Augusta, Michigan, USA during June of 2016. Although Eastern Box Turtles can lay more than one clutch per year in some populations (Dodd, 2001), our short duration of egg collection excluded the possibility of collecting more than one clutch from a given female. Turtles from the same clutch were at least maternal siblings; yet, owing to the fact that some emydyl turtles exhibit multiple paternity (Pearse et al., 2002; Refsnider, 2009), and because we did not perform genetic analyses on our study population, we cannot exclude the possibility that individuals from separate clutches were related. Clutches were artificially incubated indoors at a constant temperature of 26.7 °C inside the incubator (Hova-Bator, Model 1602 N; GQF Manufacturing Company Inc., Savannah, Georgia, USA). We were unable to sex hatchlings without invasive procedures, but the incubation temperature we selected should have produced a relatively even number of males and females (Dodd, 2001). Once eggs began hatching, we allowed neonates to remain in the incubator until they fully emerged from their egg (~48 h).

We transported neonates to a common greenhouse for rearing on the University of Illinois at Urbana-Champaign campus. We initially housed hatchlings individually in 60.3 cm long × 42.2 cm wide × 27.9 cm tall transparent plastic tanks with an 11.5 cm long × 8.5 cm wide × 8 cm tall plastic hide box, reptile cage carpet (Zoo Med Eco Carpet; Zoo Med Laboratories, Inc., San Luis Obispo, California, USA), and a shallow food bowl. We kept these tanks on a slight angle to hold fresh standing water (ca. 4 cm deep) in the lower end for drinking and soaking. Once we confirmed that each turtle was healthy and regularly eating (less than two weeks after hatching), we randomly assigned each turtle to a permanent rearing environment.

We housed turtles in 132 cm long × 79 cm wide × 30 cm deep Rubbermaid® stock tanks (Fig. 1). We designed these enclosures to reasonably mimic natural habitats, consisting of structural features functionally similar to those commonly used by wild box turtles. Each enclosure had substrate of ca. 6 cm deep of coconut fiber (Zoo Med Eco Earth®) to promote digging and burrowing, shrubby and herbaceous artificial plants, sphagnum moss, two half logs (Zoo Med Habbah Hut®) for additional hiding places, and two naturalistic shallow rock water
We recorded individuals in each enclosure contained two pairs of clutch mates from separate clutches. We also noted whether turtles were engaging in aggressive behaviors, which we defined as biting, forcefully clawing or nudging, or otherwise attempting to show dominance over another. We immediately placed turtles back in their primary enclosure once a trial ended. Between each trial, we thoroughly cleaned the arena with bleach solution, dried it, and placed fresh substrate before initiating another trial.

2.4. Experimental trials

We conducted trials by observing the behavior of pairs of turtles placed together in a novel environment that were raised together and were either clutch mates or not as well as pairs of turtles not raised together that were either clutch mates or not. This resulted in pair combinations consisting of familiar siblings, familiar non-siblings, unfamiliar siblings, and unfamiliar non-siblings. Our sample of turtles was conducive to forming a maximum of eight unique pair combinations of familiar siblings and non-familiar siblings. There were 16 possible pair combinations of familiar non-siblings, and 80 possible pairs of non-familiar non-siblings. Because there were many more possible pair combinations in the latter two treatments, we randomly selected eight pairs from each to balance them to the size of the former two treatments.

We conducted trials during daylight hours from 2 to 17 March 2017. Each trial lasted for 90 min and took place in the greenhouse where the turtles were housed. We used a 60 cm long x 42 cm wide x 34 cm tall, open-topped plastic storage container to serve as a testing arena. We taped white copy paper on all sides of the arena to reduce stress to the turtles. We also covered the floor of the arena with an evenly spread, thin (~0.5 cm deep) layer of the same coconut fiber substrate the turtles were raised on. No other materials were present in the arena.

We estimated the minimum separation distance between pairs of turtles to the nearest 5 cm as a measure of social preference, where turtles that were observed closer together—but not behaving aggressively towards one another—were assumed to be engaging in social behavior since there was no common attractant (e.g., food, water, or shelter) in the arena. We pre-marked the arena at 5 cm increments to aid in our estimation of separation distance between a pair (Clark, 2004). This distance interval was approximately the carapace length of each turtle at the time of testing. We placed a pair of turtles from each treatment 20 cm apart into the center of the arena at the beginning of a trial. The observer then was out of view of the animals and only peered into the arenas to quickly record data at five-minute intervals during each trial. We visually estimated the minimum separation distance between a pair of turtles. We also noted whether turtles were engaging in aggressive behaviors, which we defined as biting, forcefully clawing or nudging, or otherwise attempting to show dominance over another.

3. Results

3.1. Observational study

We collected 510 pair separation distance measurements across the four enclosures (mean ± SD measurements per enclosure = 127 ± 25)
over 149 days. Individual growth increased steadily over this period (see Fig., 2b in Tetzlaff et al., 2019a). Agonistic interactions were never observed between individuals when collecting measurements or providing husbandry. Considering an alpha level of 0.05, we found no evidence for an interactive effect of kinship and days in captivity affecting pair separation distance ($\beta = -0.016$, 95% confidence interval [CI]: $-0.117$ to $0.084$, $p = 0.750$). Similarly, when considered as additive effects, we found no support for kinship affecting separation distance ($\beta = 2.586$, 95% CI: $-10.785$ to $15.957$, $p = 0.693$), but this was affected by days in captivity, where separation distance decreased with time ($\beta = -0.146$, 95% CI: $-0.228$ to $-0.063$, $p = 0.006$). Average pair separation distance sharply decreased from approximately 55 cm after being placed in group housing to about 35 cm after ~2.5 months in captivity, then slowly declined to and remained at ~30 cm at the conclusion of the five-month observational study (Fig. 2).

3.2. Experimental trials

We recorded 576 pair separation distance measurements across 32 trials. Agonistic interactions between individuals were never observed during trials. We found no support for an interactive effect of familiarity and kinship affecting separation distance, nor as additive effects (Table 1). We also found no evidence suggesting the additive effects of time interval in the trial or size difference between a pair of turtles (mean ± SD = 3.59 ± 2.58 mm) affected separation distance (Table 1).

4. Discussion

We found little evidence suggesting the social associations or structure of captive-born juvenile Eastern Box Turtles are related to kinship, familiarity, or body size. The benefits of associating or forming groups based on these factors may be limited for this species in nature. Wild juvenile Eastern Box Turtles have numerous potential predators, and their primary anti-predator strategy entails exhibiting secretive behavior or cryptism (Dodd, 2001). Remaining in close proximity to conspecifics may therefore increase predation risk by enhancing visual and olfactory cues to predators (Shine et al., 2001; Tetzlaff et al., 2020).

Additionally, choosing not to associate with kin may indicate early signs of inbreeding avoidance, as box turtles have short natal dispersal, are not known to be territorial, and likely encounter numerous conspecifics when traversing small home ranges across their long lifespans (Dodd, 2001).

The only indication of social behavior we observed was that pair separation distance declined the longer turtles were kept in captivity. Although this finding could be interpreted as increasing sociality as turtles had time to build relationships, this pattern temporally mirrored when wild Eastern Box Turtles become more sedentary in preparation for overwintering (Dodd, 2001). Throughout their range, Eastern Box Turtles are not known to communally overwinter (Dodd, 2001); thus, alternatively, pair separation distance decreased because turtles were aggregating around concentrated resources such as heat and moist retreat areas to prevent desiccation as cold winter temperatures approached (Gregory, 2004). This could be tested by replicating rearing conditions as we did but also having another treatment with multiple areas of heat and high humidity in enclosures to provide options for turtles to aggregate or separate based on individual preferences.

Although results of the other tests performed were not significant, we suggest our study provides a foundation for refining future related experiments. First, evaluating relatedness between individuals using genetic data will give greater confidence in assessing social behavior based on kinship. Implementing longer observation periods (hours to days) once placed into novel environments may lead to more informative observations than conducting 90-minute trials (Clark, 2004; Skinner and Miller, 2020). Additionally, instead of testing study animals in pairs during experimental trials as we did and others have done (e.g., Clark, 2004), an alternative approach could incorporate social network analyses for the entire captive population, which may reveal subtleties of the often-cryptic social behavior exhibited by reptiles (Schuett et al., 2016; Skinner and Miller, 2020).

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
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<th>UCL</th>
<th>p-value</th>
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</table>

Fig. 2. Separation distance (cm) of pairs of juvenile captive-born Eastern Box Turtles (Terrapene carolina) based on kinship (kin or non-kin) and the number of days they had been in captivity, starting in August 2016. The lines represent means for each group fit by loess smoothing, and the ribbons are 95% confidence intervals.
5. Conclusion

Box turtles (Terrapene spp.) are commonly kept and bred in zoos, aquariums, nature centers, museums, and by private hobbyists. Our results suggest efforts to maintain opportunities for social relationships for captive juvenile *T. carolina* may not be required. Because we never observed behaviors such as anorexia (Tetzlaff et al., 2019a), prolonged inactivity, or agonistic interactions between individuals, juveniles can likely be mixed in new groups with little concern of social disruption or compromised welfare (McArthur et al., 2004; Doody, 2021; Warwick et al., 2013). The subjects in this study were part of a head-starting program, and our findings therefore have implications for translocations of this species. Our previous work on juvenile box turtles suggests behaviors that could influence post-release survival are innate (e.g., habitat selection; Tetzlaff et al., 2018) or enhanced through learning (e.g., foraging and predator avoidance; Tetzlaff et al., 2018a). Consequently, inherited traits and individual experience may be more important than potential benefits garnered from certain types of social interactions. Importantly, our findings also suggest that releasing cohorts of Eastern Box Turtles based on relatedness or social familiarity may not be required for translocation success, similar to findings in other species (Armstrong, 1995; Armstrong and Craig, 1995).

Author agreement

This manuscript has not been published, accepted, or is not under consideration for publication in any other journal or book. It has been read by all authors and submission has been agreed upon. All persons entitled to authorship have been included. Additionally, all relevant permits and animal use permissions were in place to ensure humane treatment of all animals involved.

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References