Comparison of Field and Lab Behavior and Mirror Responses Of Ornate Box Turtles (*Terrapene ornata*) in Nebraska

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Introduction

Behavioral types are made of five axes: boldness, aggression, sociality, activity, and exploration. Specifically, personality is significant because variation may influence the way different individuals interact within their environment in certain behaviors such as resource acquisition, ranging, philopatry, and navigation. Behavioral types (personality) have been shown to be consistent and repeatable for multiple populations of Ornate Box Turtles in a lab environment. While laboratory tests may be more controlled, they may not reflect what the animal does in the wild. Comparing lab and field results using the same testing protocol allows us to examine differences and find results that may be more applicable to the animal's ecology. In this study, we investigate field behavioral types by testing boldness and activity ("den assay" and "activity assay", respectfully) in a western Nebraska population of ornate box turtles (*Terrapene ornata*) as it compares to lab data. To our knowledge this is the first study to investigate the comparison between behavioral types in field and lab data in Ornate Box Turtles. This study also included a preliminary behavioral investigation into reflected image responses induced by a mirror.

Questions

- Are behavior types repeatable in the field and laboratory?
- Do field assay results reflect laboratory assay results?
- Does the average behavioral responses vary across different groups (male, female, juvenile) and context (laboratory vs field)
- Do turtles respond to mirrored reflections and, if so, do these responses vary across individuals?

Methodology

- Used radio telemetry to locate and collect turtles. Replaced or added transmitters to carapace of turtles throughout the season (late May-July 2023)
- Performed outdoor and indoor behavioral assays and weighed and measured each turtle after assay (n=37)
  - "Den assay": emergence time from inner space in 300 seconds
  - "Activity assay": total quadrants entered over 300 seconds in a wading pool subdivided into four sections as a perimeter walking track
- Estimated repeatability for each assay
- Compared outdoor and indoor behavioral syndrome results between groups.

Outdoor Assay Methods

1) Selected turtle groups based on general location and proximity to each other
   - 3 to 5 turtles were assayed per trial day
   - Sample sizes: F=20, M=11, J=6
2) Randomized trial order within selected turtle group each day
3) Collected turtles via radio telemetry and transported to assay location
4) Recorded ambient temperature (°C) at start of each trial using Kestrel Pocket Weather Meter
5) Assays conducted in fixed order: Den, Activity, Mirror
6) Weighed turtle and returned to bag (placed in shaded area)
7) Repeated steps 4-5 for remaining turtles
8) Returned all turtles to their collected location (marked by GPS and labeled flags)
9) Assayed all 37 turtles once in June (except 3) and once in July

Results

1)

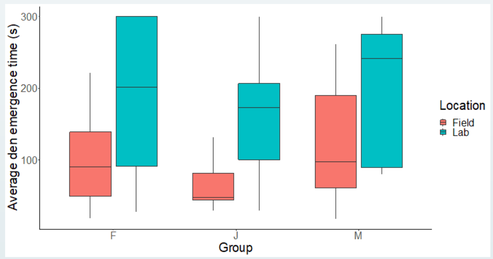

Figure 1. Average den emergence time (s) between females, juveniles, and males in field and lab studies (field: F=1.46, df=34, p=0.25; lab: F= 0.19, df=34, p=0.83). Average difference between laboratory and field behaviors was significant (F= 17.3, df=34, p<0.01).

2)

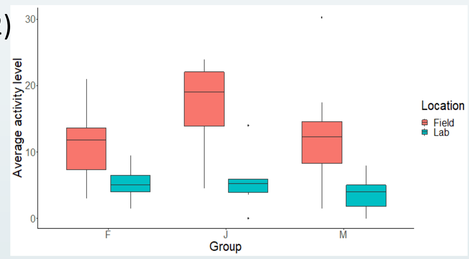

Figure 2. Average activity level (total quadrants entered) between females, juveniles, and males in field and lab studies (field: F=1.70 , df=34, p=0.20; lab: F=1.76, df=34, p=0.19). Average difference between laboratory and field behaviors (F=50, df=34, p<0.01).

3)

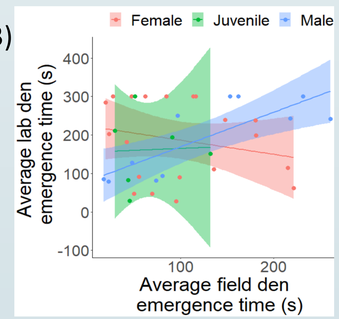

Figure 3. Interaction between field den emergence time and laboratory den emergence time by group (F=3.75, df=31, p<0.01; significant interaction of group (t=2.78, p<0.01)).

4)

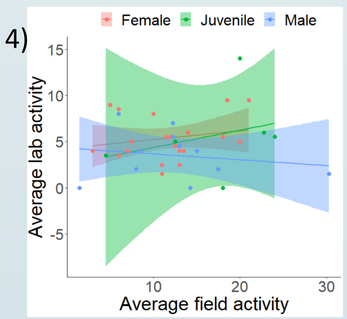

Figure 4. No interaction between field activity scores and laboratory activity scores by group (F=0.95, df=31, p=0.47)

5)

Assay	Location	R	CI
Den	Field	0.362	[0.042, 0.623]
Den	Lab	0.408	[0, 0.735]
Activity	Field	0.578	[0.264, 0.761]
Activity	Lab	0.493	[0.152, 0.703]
Den	Field and Lab	0.176	[0, 0.389]
Activity	Field and Lab	0.254	[0.051, 0.432]

Figure 5. Repeatability values (R) and confidence intervals (CI) of each assay in the field and laboratory separately and when scored together.

SCAN ME

This QR code leads to a YouTube video depicting various types of behaviors displayed in the mirror assay.

Conclusion

This field component of this study was designed to be as similar as possible to pre-existing laboratory protocols to make the results comparable. However, there were some aspects that were, understandably, impossible to recreate in the field. The laboratory protocol requires each turtle to be kept overnight and tested back-to-back mornings. The field protocol tested each turtle in the same morning they were caught in June and again in the same manner in July. First, the field results for both the den and activity assays were repeatable on the population level, indicating that both assays are capturing personality traits. Our results show a weak relationship between the field and laboratory den assays for any group of turtles; however, males appeared to react similarly across field and laboratory assays while the females and juveniles reacted less similarly. Knowing that field tests have many more unaccountable variables, it is difficult to conclude a specific reason for these results. Because the results of the field and laboratory tests are repeatable but not related, the field tests may be testing short-term boldness as opposed to long-term boldness or a different axis of personality entirely. Field boldness may be more closely described as escape behavior due to procedural differences. Also, the results suggest more plasticity in female behavior than male behavior. Previous studies have indicated sexual dimorphism in ranging behavior and possibly cognition, which also supports why sexes may respond differently across assay contexts. The preliminary mirror assay showed that turtles reacted to their reflections and had varying responses. Turtles exhibited stark contrasts in reactivity across the population, including ramming, hiding, shuffling, nose tapping, etc. Because scoring proved to be difficult, we currently have no repeatability data, but have provided examples of behavioral responses in the QR code given.

Future Directions

- Examine relative impact of other variables (such as temperature, egg-bearing status, etc.) on field assay performance
- Continue field assays on different populations
- Complete more trials with juveniles to better compare across groups
- Expand mirror assay to potentially use as a measure of sociality

Acknowledgements

Samuel Wagner, Zoe Edlund, Timothy Speer, Keetan Munsell, Mason Chanay, Aubrey Gauntt, Dr. Tracy Wagner and Dr. Paul Wagner