

# MALE REACTION TO FEMALE STIMULI IN *RHINOCEMYS AREOLATA*: AN EXPERIMENTAL ANALYSIS TO UNDERSTAND MATE RECOGNITION BY MALES

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**Abstract.**—Animals use signals to communicate effectively with each other during various stages of their life. In turtles, the production and recognition of signals are of decisive importance during the breeding season, influencing successful mate selection. We studied behavioral responses used by male Furrowed Wood Turtles (*Rhinoclemmys areolata*) to recognize different stimuli related to conspecific females. Across four experiments, we exposed 17 males to four stimuli: (1) a concave cup; (2) a female-scented plate; (3) an empty carapace; and (4) a live female. We divided the observation area into quadrants to analyze displacements and temporal proximity of turtles to stimuli. Comprehensive documentation of male behaviors enabled classification into categories of searching or interaction. Our findings revealed that males exhibited minimal interest in the concave cup stimulus, with no discernible increase in time spent or frequency of entries in the corresponding quadrant. Conversely, males spent more time in the quadrant with scent, carapace, or a female. Specifically, searching behaviors were more frequent in the presence of a live female. In contrast, interaction behaviors were pronounced with the carapace and live female stimuli, minimally occurring with the cup or scent. Our work suggests that male turtles are multisensory organisms able to use sight or odors to detect females at short distances. Furthermore, interaction behaviors appear to occur specifically with live females, demonstrating the ability of males of this species to recognize them through different types of stimuli. These findings contribute to comprehend sensory dynamics of reproductive behaviors in turtles.

**Key Words.**—chelonian; chemical cues; communication; mating signals; turtle interactions; visual cues, Furrowed Wood Turtle

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## INTRODUCTION

Effective communication is pivotal for animals, necessitating the development of specific signaling mechanisms that can evolve to enhance signal transmission and detection (Ord and Stamps 2009). In nature, signals span diverse modalities like visual, chemical, tactile, and acoustic elements, manifesting either individually or blending through multimodal communication (Partan and Marler 2005). Turtles, known for their elaborate communication process, rely extensively on signals, particularly in courtship and mating (Galeotti et al. 2011). These signals serve a multitude of purposes, enabling the recognition of conspecifics for sex discrimination, age determination (Brejcha and Kleisner 2015), assessment of health status (Okuyama et al. 2014), and understanding of

social structure (Martín and López 2011).

Prior research has emphasized the significance of visual cues in turtle communication, such as body shape and markings, which facilitate recognition of conspecifics, sex discrimination, and assessment of reproductive conditions (Rovero et al. 1999; Belzer and Seibert 2009; Ye et al. 2020). It is crucial to acknowledge the limitations of relying solely on visual signals, however, especially at a distance or in challenging environments. For instance, Hidalgo (1982) reported that male Painted Wood Turtle (*Rhinoclemmys pulcherrima incisa*) move and drag themselves towards objects that resemble a turtle, even if those objects are in motion. Similarly, Baker and Gillingham (1983) observed, on three occasions, a male Blanding's Turtle (*Emydoidea blandingii*) mounting a rock the size of a turtle.

In contrast, chemical signals, notably pheromones, persist over time and distance, providing essential information for discriminating sex and reproductive conditions (Ibáñez et al. 2012; Bernheim et al. 2019). Given the limitations of visual cues alone, turtles may employ olfactory signals as a complementary and sometimes necessary method of communication. This is particularly evident when visibility is compromised, and visual cues may not work optimally. Within this intricate communication tapestry, tactile signals play a vital role. To secure the attention of the female or stimulate her, males commonly use tactile signals, such as rubbing, biting, and nudging that is directed at the chin, cloaca, carapace, or plastron (Liu et al. 2013; Smolowitz et al. 2015; Brejcha and Kleisner 2016; Cassola et al. 2020). These tactile interactions provide an additional layer to communication, facilitating more nuanced exchanges between individuals.

Researchers have highlighted the importance of unraveling pre-copulatory behavior and mating location in solitary organisms such as turtles (Muñoz 2004; Liu et al. 2013; Bernheim et al. 2020). Investigating these behaviors across species poses challenges, mainly due to limited observation opportunities in natural habitats and the complexity of ex-situ observations. In these settings, acquiring and maintaining adequate numbers of animals under controlled conditions is particularly challenging for solitary organisms like turtles. Despite recognizing these obstacles, comprehensive information on turtle reproductive behavioral signals is lacking, impeding our understanding of their natural behaviors and limiting the applicability of findings across species.

To address this knowledge gap, our study focused on the Furrowed Wood Turtle (*Rhinoclemmys areolata*), a terrestrial species prevalent in the Yucatán Peninsula, encompassing the Cozumel island, northern Guatemala, Belize, and north-western Honduras. This species thrives in various habitats, including savannahs, thorny scrub, deciduous forests, swampy areas, and ponds, known locally as *aguadas* (Vogt et al. 2009). Recognized as Near Threatened on the Red List of the International Union for Conservation of Nature (van Dijk et al. 2007), the Furrowed Wood Turtle boasts distinctive features like one or two red, orange, or yellow stripes on the upper head and a yellow-colored plastron adorned with a dark spot of varying sizes. Using a series of case-controlled experiments, our study aimed to determine the ability of male *R. areolata* to recognize conspecific stimuli, especially those of females, at

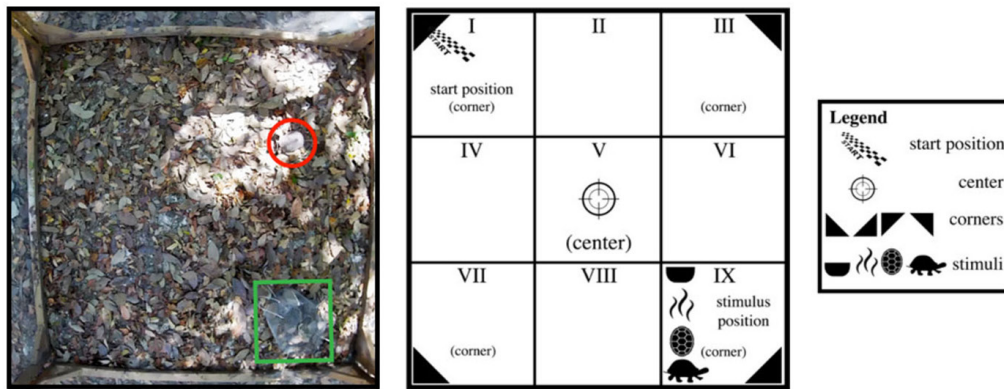
different distances, and to investigate whether males display distinct behaviors in response to different stimuli.

## MATERIALS AND METHODS

**Study site.**—We conducted our study at El Colegio de la Frontera Sur (ECOSUR) Unidad Chetumal, México (18°32'37.4"N, 88°15'48"W). We obtained the animals through loans and donations of pets from citizens of Chetumal, Quintana Roo, Mexico. Detailed explanations were provided to the donors regarding how the turtles would be used and for what purpose. The study adhered to institutional ethical standards, current regulations, and the needs for animal welfare. Turtles were returned to their owners after data collection. We housed the specimens inside a 16 × 5 m enclosure divided into two pens of the same size (5 × 8 m) containing males and females separately. This division was necessary to discourage females from collecting the semen of the males, which might make them less reactive to the presence of males (Pearse and Avise 2001), and to prevent any reproductive activity without the observer being present. The environment inside the enclosures mimicked the natural habitat of *R. areolata* (i.e., the presence of trees, foliage, and natural soil) and we limited disturbance by only adding water to the pools occasionally.

**Study design.**—We used 17 males and four sexually mature female *R. areolata*, which were able to roam, find refuge, and bathe within the enclosure. We fed the turtles with fruit, vegetables, and meat on alternate days. They were exposed to natural temperatures and rainfall during the entire time of confinement to emulate the prevailing environmental conditions.

We ran the experiments from June to July 2019, within the mating season months of the species (Vogt et al. 2009). Each simulation occurred outdoors, on sunny days, during hours of maximum activity of turtles (0830–1130 and 1730–1830). We used a mobile enclosure (2 × 2 m) as an observation area (Fig. 1). After every experiment, we moved the enclosure to an adjacent area to reduce waiting time between experiments and avoid interaction with traces of conspecific odors that could influence subsequent observations. We returned the turtles to the confinement area after the experiments.



**FIGURE 1.** (Left) A top-down view of the experimental area during the experiment with a female Furrowed Wood Turtle (*Rhynoclemmys areolata*) highlighted by a green rectangle and a male turtle by a red circle. (Right) Schematic representation of the experimental area divided into nine cells for extrapolation data. Each cell, labeled with Roman numerals from I (top left) to IX (bottom right), corresponds to a specific section of the experimental space. The introduced symbols provide a visual representation of the stimuli used and the results.

**Experiments.**—To assess the ability of males to discriminate different types of signals related to females, we observed 17 sexually mature males separately in various experimental situations within the mobile experimental area. We conducted four experiments, one for control and three for female presence discrimination: (1) Control: We placed a concave plastic cup imitating the curved shape of the carapace of a turtle in a corner of the experimental area; (2) Scent discrimination experiments: We used scents placed in a corner of the experimental area. We placed a transparent plastic dish containing the scent of a conspecific female turtle. The scent was obtained by allowing a conspecific female to move inside a plastic box with a 1 cm depth of water, which was then recollected for simulation purposes. This experiment aimed to determine if males were attracted to the scent of the female; (3) Sight discrimination experiments: In a corner of the experimental area, we positioned an empty and clean carapace (free from turtle odors) of a conspecific female in an upright position. This experiment helped us understand if there was visual recognition of a conspecific; (4) Scent and sight discrimination experiments: We placed a box-net containing a female in a corner of the experimental area and a conspecific male in the opposite direction. The box around the female enabled visual and olfactory recognition by the male but avoided physical contact between the specimens. This experiment aimed to determine if mixing both kinds of signals changed reactions of males toward the conspecific female.

For each experiment, a box-net containing a male was positioned at the diametrically opposite corner

of the arena. After a 1-min acclimation period, we released the male to move freely within the perimeter for 15 min. We observed the behavior of males throughout the experiments and recorded videos using two cameras (YiLiteCam, Xiaomi Inc, Beijing, China, and Motorola Camera G4, Motorola Mobility LLC, Libertyville, USA). The first video camera was placed above the observation area, while the second one was moved manually by the observer to document specific details of the events.

**Statistical analysis.**—We categorized behaviors exhibited by males near objects and the female into two groups: searching, encompassing the efforts of males to find a mate and gather information, and interactions, defined as actions and behaviors males display when coming into contact or engaging with objects or females. To conduct statistical analysis, these behaviors were transformed into variables, including quantified frequency (number of times a male crossed a quadrant), total time spent on the quadrant (in seconds), and an index defining the average time a male spent during each crossing (time/frequency).

We divided the experimental area into nine cells during video editing using Final Cut Pro for Mac (Apple Inc., Cupertino, California, USA). This facilitated the calculation of space use by turtles to measure search behavior, focusing on time spent and the number of times a male occupied each quadrant. To assess significant differences between group means, we employed the Friedman Test, chosen for its suitability in analyzing repeated measures data, especially when using small sample sizes and non-

independent samples (Friedman 1937, Pereira et al 2015). Subsequently, to identify specific group differences, we used the Conover post hoc test, a pairwise comparison method that assesses each pair of related groups to pinpoint where differences occur (Conover 1999). We applied Bonferroni corrections to  $P$  values to account for multiple tests of the same variable.

These analyses aimed to identify differences in behavior during various situations and assess the efficacy of different signals in recognizing a potential mate. The independent variable was the type of stimulus, while dependent variables included frequency, total time spent on the quarters, and the index (time/frequency). Additionally, we used  $G$  tests to compare individuals reaching the quadrant with the stimulus, considering both those who interacted and those who did not. We used R software (R Core Team 2024) for all analyses with an  $\alpha$  of 0.05.

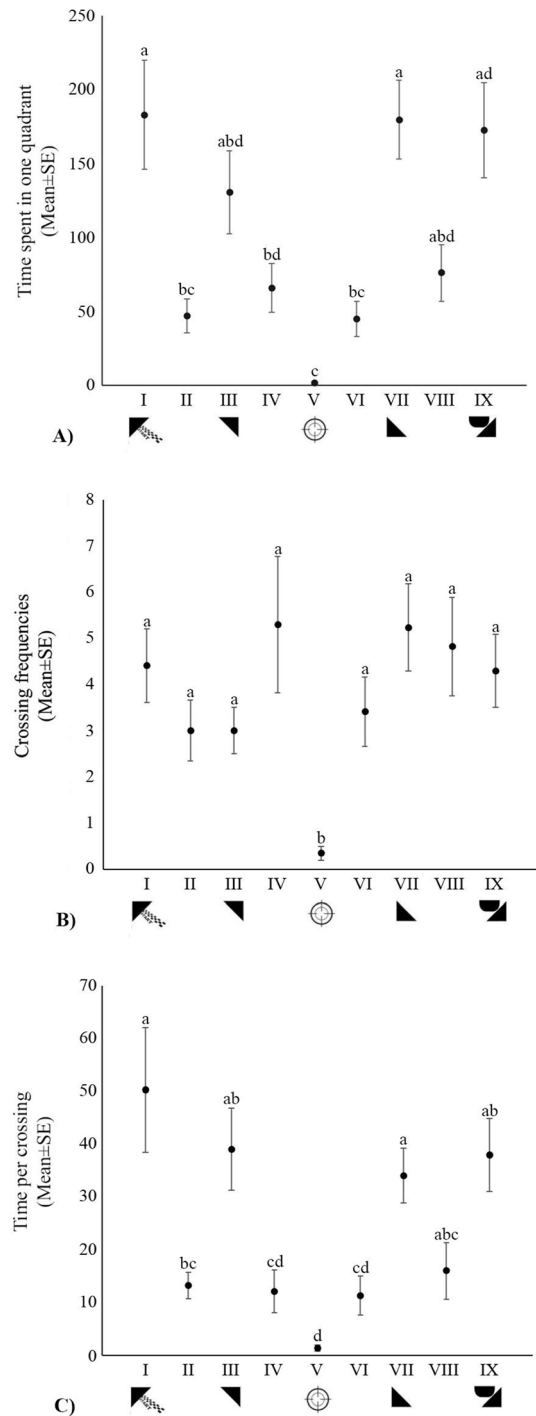
## RESULTS

### *Experiment 1: male reaction to a cup (control).—*

Significantly more males approached quadrant IX containing the cup during the experiment than quadrants without the cup (15 of 17,  $G = 11.25$ ,  $df = 1$ ,  $P < 0.001$ ; Fig. 2). Seven males exhibited search behavior (sniffing), and two interacted with the cup. There were significant differences in time spent (TS) per quadrant ( $\chi^2 = 58.02$ ,  $df = 8$ ,  $P < 0.001$ ), crossing frequencies (CF;  $\chi^2 = 38.54$ ,  $df = 8$ ,  $P < 0.001$ ), and the time per crossing index (T/C;  $\chi^2 = 63.87$ ,  $df = 8$ ,  $P < 0.001$ ). Males spent significantly less time in the central quadrant V and more time in quadrants I, III, VII and IX (Supplemental Information Table S2A). The crossing frequency of males was significantly low in quadrant V (Supplemental Information Table S2B), and the T/C index reflected a similar trend to that of the TS, indicating that males spent less time per crossing in the central quadrant V compared to the other quadrants (Supplemental Information Table S2C). The T/C was highest in corners I, III and VII, including the stimulus-containing quadrant IX (Fig. 2).

### *Experiment 2: male reaction to female scent.—*

All males reached quadrant IX using female odor. Turtles differ significantly in time spent per quadrant ( $\chi^2 = 70.62$ ,  $df = 8$ ,  $P < 0.001$ ), crossing frequencies ( $\chi^2 = 44.72$ ,  $df = 8$ ,  $P < 0.001$ ), and time per crossing ( $\chi^2 = 76.21$ ,  $df = 8$ ,  $P < 0.001$ ). TS showed that males spent significantly less time in central quadrant

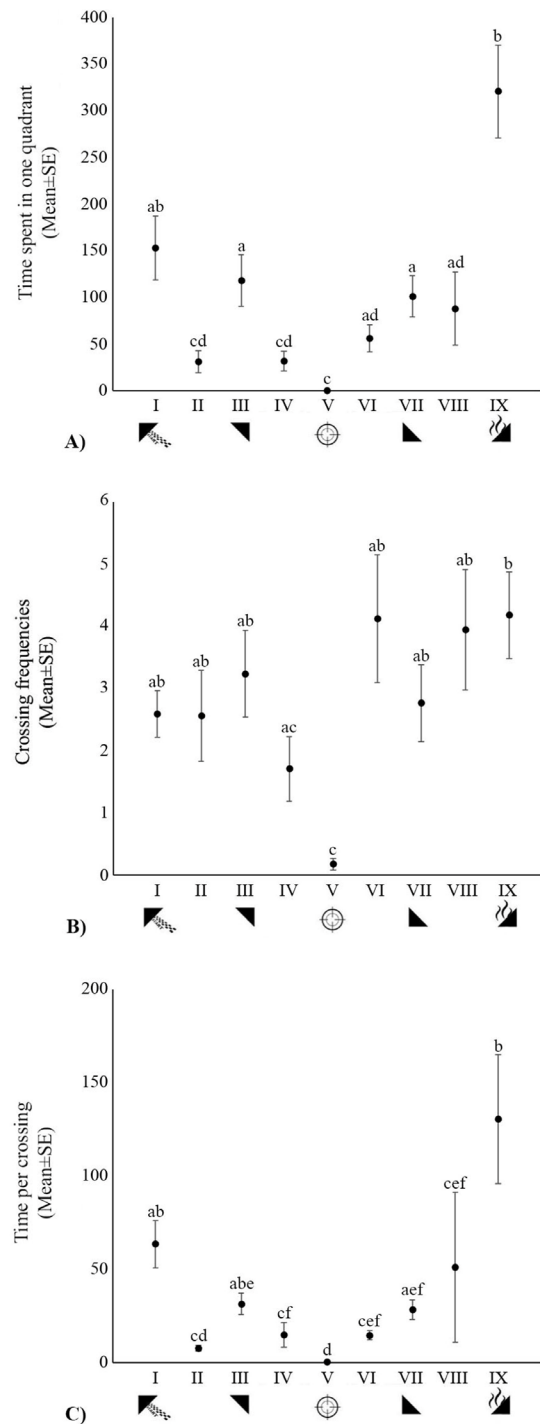


**FIGURE 2.** Means and standard errors of (A) time spent in seconds, (B) crossing frequencies, and (C) time per crossing index of male Furrowed Wood Turtles (*Rhinoclemmys areolata*) in each quadrant during the control experiment (cup). Pairwise results from Conover tests are denoted by letters atop each column. Different letters signify significant differences between quadrants, while identical letters indicate no significant difference.

V (mean TS =  $0.46 \pm$  standard error  $0.28$  sec) and significantly more time in corners and the stimulus quadrant (Fig. 3, Supplemental Information Table S3A). CF showed a significantly lower frequency in V (Supplemental Information Table S3B), and the T/C index demonstrated subdued activity in V compared to other quadrants (Fig. 3, Supplemental Information Table S3C). Notably, the time spent during each passage in a quadrant was significantly higher in the corners.

**Experiment 3: male reaction to empty female shell (sight).**—Significantly more males (16 of 17;  $G = 15.96$ ,  $df = 1$ ,  $P < 0.001$ ) entered quadrant IX than any other quadrant. Of these, a significantly higher proportion interacted with the stimulus (12 of 16;  $G = 4.18$ ,  $df = 1$ ,  $P = 0.041$ ) compared to those that entered the quadrant but did not interact with it. There were significant variations across all three variables: time spent per quadrant ( $\chi^2 = 66.41$ ,  $df = 8$ ,  $P < 0.001$ ), crossing frequencies ( $\chi^2 = 34.84$ ,  $df = 8$ ,  $P < 0.001$ ), and time per crossing ( $\chi^2 = 70.66$ ,  $df = 8$ ,  $P < 0.001$ ). In line with previous observations, males spent significantly less time in the central quadrant (V) than in all corners of the arena (Fig. 4, Supplementary Information Table S4A). In terms of crossing frequencies (CF), individuals tended to cross quadrants 2–4 times, except for the central quadrant V (mean CF =  $0.41 \pm 0.14$ ; Fig 4, Supplementary Information Table S4B). The time per crossing (T/C) was significantly higher in the quadrant containing the stimulus IX (mean T/C =  $110.50 \pm 26.77$ ) and in the corners when compared to the other quadrants (Fig. 4, Supplementary Information Table S4C).

**Experiment 4: male reaction to female (scent and sight).**—Significantly more males (15 of 17) were registered in quadrant IX ( $G = 11.25$ ,  $df = 1$ ,  $P < 0.001$ ), than other quadrants. There were significant variations in turtles in time spent per quadrant ( $\chi^2 = 52.19$ ,  $df = 8$ ,  $P < 0.001$ ), crossing frequencies ( $\chi^2 = 26.92$ ,  $df = 8$ ,  $P < 0.001$ ), and time per crossing ( $\chi^2 = 59.58$ ,  $P < 0.001$ ). Males spent significantly more time in quadrants I-start (mean TS =  $248.48 \pm 59.29$ ) and IX-stimulus (mean TS =  $337.95 \pm 63.33$ ) than in all other quadrants, but there was no significant difference between quadrants I-start and IX-stimulus (Fig. 5, Supplementary Information Table S5A). TS values were significantly lower in quadrant V compared to the corners and quadrant VI (Supplementary Information Table S5A).



**FIGURE 3.** Means and standard errors of (A) time spent in seconds, (B) crossing frequencies, and (C) time per crossing index of male Furrowed Wood Turtles (*Rhinoclemmys areolata*) in each quadrant during the scent experiment. Pairwise results from Conover tests are denoted by letters atop each column. Different letters signify significant differences between quadrants, while identical letters indicate no significant difference.

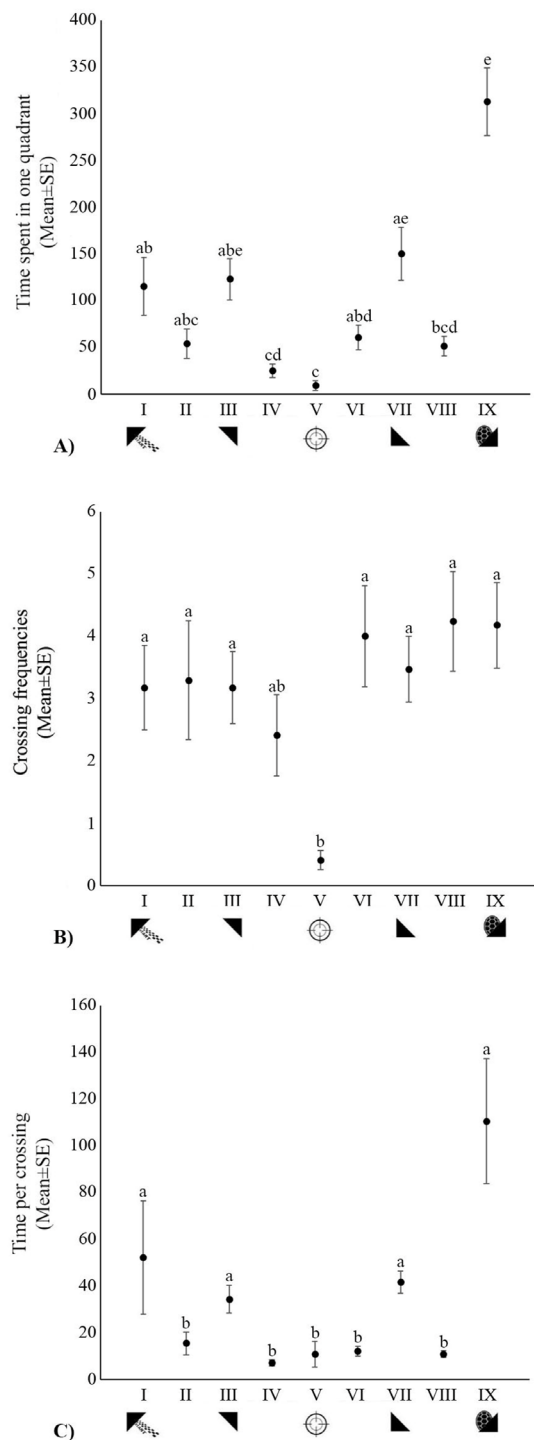


Consistent with observations in other experiments, males tended to cross the central quadrant V significantly less frequently (mean CF =  $0.47 \pm 0.17$ ) compared to the remaining eight quadrants (Fig. 5, Supplementary Information Table S5B). Lastly, turtles tended to spend significantly more time per crossing in the corners compared to quadrant V. The highest values were observed in quadrants I and IX, which were significantly higher than those in all other quadrants (Fig. 5, Supplementary Information Table S5C).

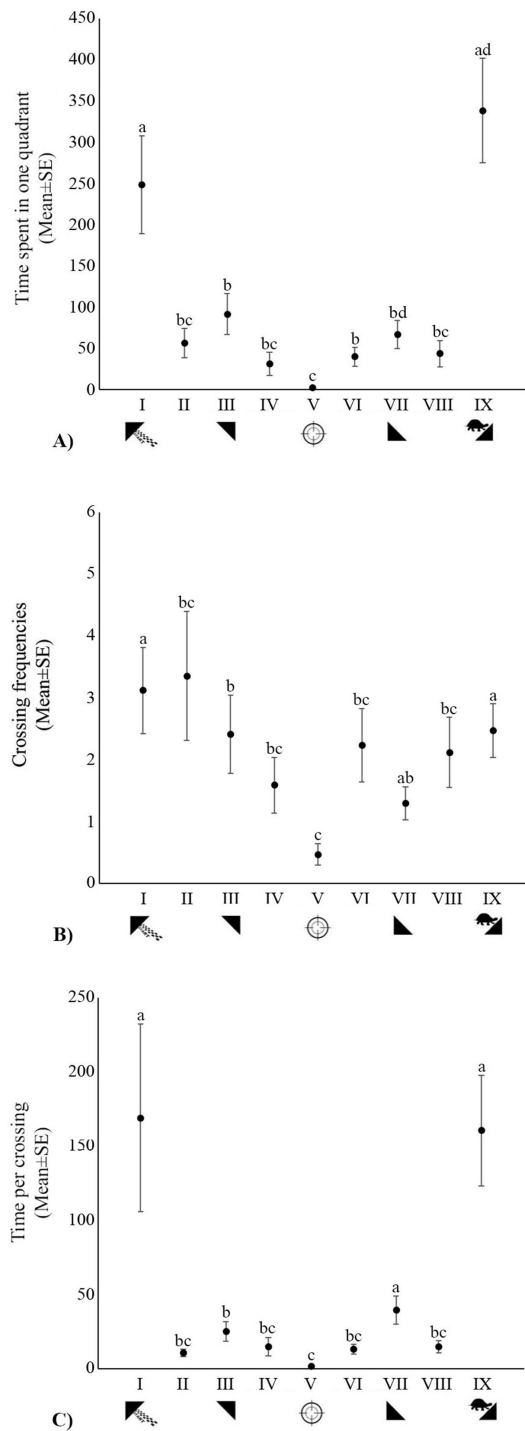
**Comparison of searching and interaction behaviors between experiments.**—We observed 32 behaviors (Supplemental Information Table S1) that we classified into two categories: Searching (seeking information) and Interaction (action oriented towards object/female). We found a significant difference between these behaviors ( $\chi^2 = 11.60$ ,  $df = 3$ ,  $P = 0.009$ ). The main significant difference was between the frequency of searching around the cup and the female (Supplemental Information Table S6A), with a lower frequency for the cup (Cup:  $0.411 \pm 0.12$ ; Female  $0.88 \pm 0.60$ ;  $P = 0.01$ ; Fig. 6). Interactions were significantly more frequent with the female and the carapace compared to the cup and the scent, separately (Supplemental Information Table S6B); however, no significant differences were found between the cup and the scent, nor between the female and the carapace (Fig. 6).

## DISCUSSION

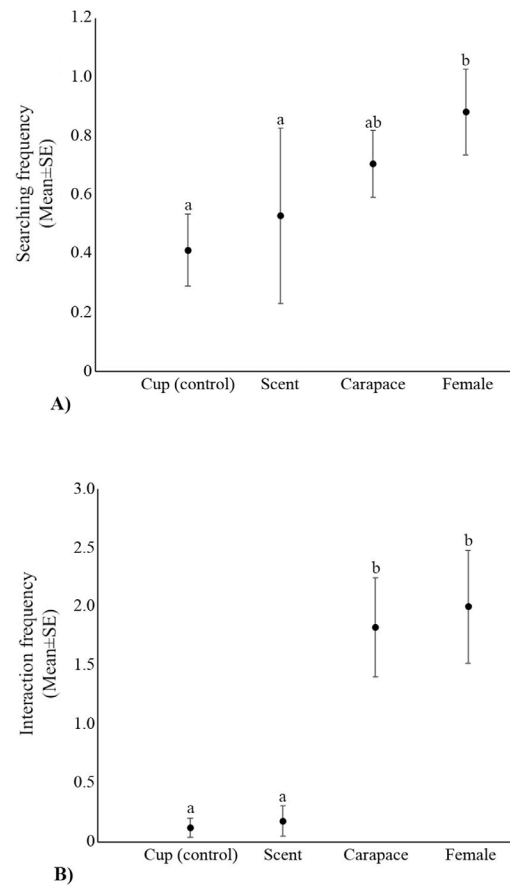
This is the first work on *Rhinoclemmys areolata* that links the receptivity of males to stimuli associated with conspecifics and elucidates their corresponding behavioral responses. Additionally, our study contributes novel insights into communication dynamics among turtles and the intricacies of partner-seeking behaviors within terrestrial species, particularly in semi-captive conditions where research remains notably scarce. Our findings reveal a nuanced male response, demonstrating a capacity to distinguish stimuli feigning female presence. Interestingly, males showed greater engagement with authentic female stimuli, including the scent and empty carapace treatments, and displayed interactive behaviors when encountering a live female. In contrast, the artificial stimuli, exemplified by a control cup, failed to elicit comparable interest despite its potential visual resemblance to a female form.



**FIGURE 4.** Means and standard errors of (A) time spent in seconds, (B) crossing frequencies, and (C) time per crossing index of male Furrowed Wood Turtles (*Rhinoclemmys areolata*) in each quadrant during the sight experiment. Pairwise results from Conover tests are denoted by letters atop each column. Different letters signify significant differences between quadrants, while identical letters indicate no significant difference.



**FIGURE 5.** Means and standard errors of (A) time spent in seconds, (B) crossing frequencies, and (C) time per crossing index of male Furrowed Wood Turtles (*Rhinoclemmys areolata*) in each quadrant during the female presence experiment. Pairwise results from Conover tests are denoted by letters atop each column. Different letters signify significant differences between quadrants, while identical letters indicate no significant difference.



**FIGURE 6.** Means and standard errors of (A) Searching and (B) Interaction behaviors frequencies for control and stimuli experiments. Pairwise results from Conover tests are denoted by letters atop each column. Different letters signify significant differences between quadrants, while identical letters indicate no significant difference.

**Long distance recognition.**—Our results revealed a noteworthy pattern: all stimuli successfully captured the attention of male *R. areolata*. To establish a recognizable conspecific shape at a distance, we selected a cup with a circular diameter, influenced by the demonstrated ability of turtles to discriminate known shapes visually (Wilkinson et al. 2013; Glavaschi and Beaumont 2014). Although visually recognized by males in quadrant IX, the cup failed to elicit distinctive interactions related to reproductive behavior, contrary to observations in other turtle species (Hidalgo 1982; Baker and Gillingham 1983).

In investigating male reactions to natural stimuli, we noted their capacity to discern a female-associated cue at a distance, prompting their approach and sustained proximity. While olfactory stimuli are commonly

associated with short-distance recognition (Liu et al. 2013), our experimental findings underscore their efficacy across longer distances. The scent contained in the plate not only attracted males to quadrant IX but also prolonged their stay, surpassing the duration observed in other quadrants.

Males were also observed approaching the quadrant with an empty carapace. This initial, distant recognition, similar to that with the cup, is consistent with previous observations showing that turtles can respond to objects resembling conspecifics (Hidalgo 1982; Baker and Gillingham 1983), highlighting the potential role of visual recognition in their response to the conspecific-shaped object. Moreover, males engaged in a range of interactions upon closer approach.

Similarly, when encountering a live female, males swiftly recognized her, likely prompted by the simultaneous influence of various stimuli emanating from the female specimen. The combined effect of the scent secreted by the female and the familiar shape seemed to be what attracted males, initiating immediate interaction behaviors upon recognition. Additionally, the limited but unrestricted movement of females within the metal box may have proved enticing to males, as observed in *R. pulcherrima*, where males follow anything resembling a moving turtle (Hidalgo 1983). This multifaceted interaction highlights the nuanced and responsive nature of male behavior to a range of stimuli, both artificial and natural, in their quest for conspecifics.

**Short distance recognition and behavior.**—Upon approaching the quadrant with the cup, males neither avoided nor exhibited specific behaviors toward the object. During the searching phase, there were no notable differences in male actions between the cup, the scent, and the carapace experiments; however, a significant distinction emerged when compared to the presence of a live female. Searching behaviors towards the cup were minimal, with a few observed with the carapace but numerous searching behaviors were evident in response to the scent and, as expected, the live female. The limited searching behaviors directed at the cup can be rationalized by considering that these actions are geared towards recognizing a familiar shape, a cup resembling a carapace. This is evident as some individuals attempted to insert their heads under it, a behavior commonly observed in many turtle species when males search for the cloaca of a conspecific female (Liu et al. 2013). Intriguingly, males did not exhibit attempts to mate

with the object, a behavior observed in other turtle species (Hidalgo 1982; Baker and Gillingham 1983). This could be attributed to the absence of odors or the capability of males, at a short distance, to discern that the object was not a female. Unfortunately, a comprehensive comparison with existing literature is not possible, as previous research has mainly focused on courtship behaviors in aquatic species (Liu et al. 2013), particularly marine and freshwater turtles, often overlooking terrestrial species and providing limited detail on communication and signaling between specimens (Mason and Parker 2010). Another possible explanation for this lack of interaction can be attributed to individual behavioral traits, such as temperament (Cassola et al. 2020), where bolder animals are known for exhibiting greater exploratory tendencies. In our experiment, once males recognized the artificial object, their searching behaviors ceased, and interest waned.

For olfactory stimulus, males were initially attracted to the scent, but their behavior shifted upon reaching the stimuli quadrant. Engaging in searching behaviors, such as moving their heads in the plate, suggested active efforts to locate the scent source in the absence of the female at the point of maximum odor intensity. Searching behaviors were consistently less diverse but more frequent for both the carapace and the live female, suggesting potential specific recognition. When approaching a carapace, males visually identified a conspecific structure at a short distance despite its immobility. For live females, the influence might stem from a combination of sensory inputs, including smell and the movements of the live female; however, male searching activities diminished upon encountering the conspecific, changing to more varied interaction behaviors.

Interactions with the cup and scent stimuli were similar: both showed low frequencies and limited variety in behaviors, contrasting with the carapace and female stimuli that triggered a broader range of behaviors, indicating specific recognition at short distances. Despite the infrequent interactive actions with the cup and scented dish, the observed movements were reminiscent of behaviors associated with conspecific recognition, such as lowering their heads beneath the cup as they were looking for the cloaca and using limbs for interaction. Conversely, the odorless empty carapace prompted a higher rate of interactions, including behaviors akin to those observed with live females. Males were engaged in activities such as searching for the cloaca, circling the empty shell to search for the cloacal area, or



attempting to insert the head under the carapace. This specific interaction behavior activates another action, the olfactory recognition of the cloaca, allowing them to distinguish the individual in front of them. Notably, even after acknowledging the carapace as empty, males consistently interacted with it upon returning to the IX quadrant, resulting in similar frequencies between the empty carapace and the live female.

Finally, males arriving next to the live female started some typical male-female interaction behaviors. Although these were found to be limited (likely due to the presence of the protection around the female), this did not stop the male from activating courtship behaviors towards the latter. Nevertheless, these behaviors persisted for longer durations and exhibited higher frequencies compared to responses to other stimuli. This intriguing interaction likely can be attributed to increased male receptivity during the breeding season, which coincided with the experiments. Alternatively, it may be influenced by potential behavioral cues from the female, a facet we did not explore. It is essential to emphasize the need for the participation of both sexes for successful mating. Consequently, we cannot exclude the possibility that a specific female response, distinct from the scent or shape stimuli, triggered the high frequency of certain responses by males. Future research focusing on female responses and active participation, especially in courtship behaviors, is recommended to better understand the reproductive behavior in *R. areolata*.

**Use of the area in the presence of a stimulus.**—A peculiarity that we observed in all four experiments, regardless of the type of stimulus used, was that males showed a lower interest in crossing the fifth quadrant. Notably, in this region, both time and frequency values were markedly low, almost nearing zero. Conversely, the four corners exhibited considerably higher values, particularly in terms of time spent and crossing frequency.

It is interesting to note that the turtles did not cross the arena directly toward the stimuli; instead, they closely followed the perimeter. This behavioral tendency aligns with thigmotaxis, a phenomenon where certain animals exhibit towards vertical walls or surfaces in general and, in more specific cases, toward angles. While thigmotaxis has been observed in laboratory settings with other species, including rodents (Rozen-Rechels et al. 2018) and some reptiles (e.g., snakes and lizards; Green et al. 2001; LeMaster et al. 2001), its exploration in turtles, particularly

*R. areolata*, has been limited. Thigmotaxis is often linked to a state of anxiety, where specimens feel safer in proximity to vertical surfaces (Walz et al. 2016; Zhang et al. 2020). Consequently, the greater presence of males in the corners may indicate anxiety. Therefore, we propose further investigation of this phenomenon in a round arena to gain deeper insights.

Our research successfully delineated two distinct categories of behaviors, namely searching and interaction, in male *R. areolata* based on the type of stimulus encountered. Despite the inherent challenges in studying olfactory stimuli and responses in a terrestrial environment, our findings reveal that males can recognize olfactory stimuli associated with a conspecific female from a distance. Their subsequent attraction and search for the source of the scent highlight the significance of olfactory recognition in these turtles.

Moreover, in the absence of obstacles, males demonstrated the recognition of shapes resembling the carapace of a turtle. This recognition prompted the turtles to approach the object and engage in tactile interaction behaviors. Interestingly, upon recognizing a conspecific, males initiated courtship actions, contributing valuable insights into the behavioral repertoire of *R. areolata*.

In analyzing the observed interaction behaviors, it is crucial to contextualize them within the well-documented and species-specific courtship rituals that has been observed in *R. areolata* (Pérez-Higareda and Smith 1988; Artner 2009; Cassola et al. 2020). We observed these behaviors during the mating season, a period characterized by heightened male receptivity and courtship activities. The persistent alignment of our findings with established courtship patterns strongly suggests a direct association between the observed behaviors and the courtship process. Importantly, these courtship behaviors, encompassing interactions and displays, are inherently social. Notably, distinctive courtship actions like circling and cloacal inspection exhibited by males conform to well-established courtship patterns integral to the broader spectrum of social behaviors (Mason and Parker 2010; Brejcha and Kleisner 2016).

Our study represents the first effort to unravel the complexities of stimulus recognition in terrestrial environments for turtles, particularly in olfactory stimuli, which are notoriously challenging to observe in the wild. The diversity of male responses to different stimuli underscores the intricate interplay of sensory cues required for partner selection. Beyond short-distance olfactory recognition for cloacal

identification, our findings emphasize the importance of visual stimulus recognition within a critical distance, where the conjunction of olfactory and tactile stimuli becomes crucial for discriminating conspecific objects. Our study also underscores the multisensory nature of turtles, challenging conventional notions and necessitating a broader perspective. The insights gained from this research have practical applications in understanding inter-individual interactions and environmental relationships within wild populations. Additionally, in captive settings, applying diverse sensory stimuli could enhance cognitive abilities and searching behaviors of these turtles.

The methodology we employed in this research expands the scope for experimental studies on the communication of terrestrial turtles, paving the way for cross-species behavioral comparisons. This knowledge not only contributes to the conservation and management of *R. areolata* but also provides a foundation for broader studies exploring the communication patterns of terrestrial turtles. Future research avenues could delve into the nuanced role of female behaviors in male-female interactions, shedding further light on the intricacies of their social dynamics and reproductive strategies.

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#### LITERATURE CITED

- Artner, H. 2009. Field observations and captive breeding of the Furrowed Wood Turtle *Rhinoclemmys areolata* (Duméril and Bibron, 1851). *Emys* 16:4–19.
- Baker, R.E., and J.C. Gillingham. 1983. An analysis of courtship behavior in Blanding's Turtle, *Emydoidea blandingi*. *Herpetologica* 39:166–173.
- Belzer, W.R., and S. Seibert. 2009. How do male box turtles find mates. *Turtle and Tortoise Newsletter* 13:11–21.
- Bernheim, M., S. Livne, and U. Shanas. 2020. Mediterranean Spur-thighed Tortoises (*Testudo graeca*) exhibit pre-copulatory behavior particularly under specific experimental setups. *Journal of Ethology* 38:355–364.
- Brejcha, J., and K. Kleisner. 2016. Turtles are not just walking stones: conspicuous coloration and sexual selection in freshwater turtles. *Biosemiotics* 9:247–266.
- Cassola, F.M., Y. Henaut, J.R. Cedeño-Vázquez, F.R. Méndez-de la Cruz, and B. Morales-Vela. 2020. Temperament and sexual behaviour in the Furrowed Wood Turtle *Rhinoclemmys areolata*. *PLoS ONE* 15:e0244561 <https://doi.org/10.1371/journal.pone.0244561>.
- Conover, W.J. 1999. *Practical Nonparametric Statistics*. 3rd Edition. Wiley, Hoboken, New Jersey, USA.
- Friedman, M. 1937. The use of ranks to avoid the assumption of normality implicit in the analysis of variance. *American Statistical Association* 32:675–701.
- Galeotti, P., R. Sacchi, D. Pellitteri-Rosa, and M. Fasola. 2011. The yellow cheek-patches of the Hermann's Tortoise (Reptilia, Chelonia): sexual dimorphism and relationship with body condition. *Italian Journal of Zoology* 78:464–470.
- Glavaschi, A., and E.S. Beaumont. 2014. The escape behaviour of wild Greek Tortoises *Testudo graeca* with an emphasis on geometrical shape. *Basic and Applied Herpetology* 28:21–33.
- Greene, M.J., S.L. Stark, and R.T. Mason. 2001. Pheromone trailing behavior of the Brown Tree Snake, *Boiga irregularis*. *Journal of Chemical Ecology* 27: 2193–2201.
- Hidalgo, H. 1982. Courtship and mating behavior in *Rhinoclemmys pulcherrima incisa* (Testudines: Emydidae: Batagurinae). *Transactions of the Kansas Academy of Science* 85:82–95.
- Ibáñez, A., P. López, and J. Martín. 2012. Discrimination of conspecifics' chemicals may allow Spanish terrapins to find better partners and avoid competitors. *Animal Behaviour* 83:1107–1113.
- LeMaster, M.P., and R.T. Mason. 2001. Evidence for a female sex pheromone mediating male

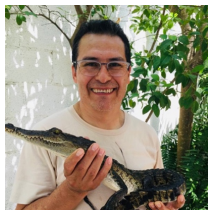
- trailing behavior in the Red-sided Garter Snake, *Thamnophis sirtalis parietalis*. *Chemoecology* 11:149–152.
- Liu, Y.X., C.M. Davy, H.T. Shi, and R.W. Murphy. 2013. Sex in the half-shell: a review of the functions and evolution of courtship behavior in freshwater turtles. *Chelonian Conservation and Biology* 12:84–100.
- Martín, J., and P. López. 2011. Pheromones and reproduction in reptiles. Pp. 141–167 *In* Hormones and Reproduction of Vertebrates. Norris, D.O., and K.H. Lopez (Eds.). Academic Press, San Diego, California, USA.
- Mason, R.T., and M.R. Parker. 2010. Social behavior and pheromonal communication in reptiles. *Journal of Comparative Physiology A* 196:729–749.
- Muñoz, A. 2004. Chemo-orientation using conspecific chemical cues in the Stripe-necked Terrapin (*Mauremys leprosa*). *Journal of Chemical Ecology* 30:519–530.
- Okuyama, J., S. Kagawa, and N. Arai. 2014. Random mate searching: male sea turtle targets juvenile for mating behavior. *Chelonian Conservation and Biology* 13:278–281.
- Ord, T.J., and J.A. Stamps. 2009. Species identity cues in animal communication. *American Naturalist* 174:585–593.
- Partan, S.R., and P. Marler. 2005. Issues in the classification of multimodal communication signals. *American Naturalist* 166:231–245.
- Pearse, D.E., and J.C. Avise. 2001. Turtle mating systems: behavior, sperm storage, and genetic paternity. *Journal of Heredity* 92:206–211.
- Pereira, D.G., A. Afonso, and F.M. Medeiros. 2015. Overview of Friedman's test and post-hoc analysis. *Communications in Statistics-Simulation and Computation* 44:2636–2653.
- Pérez-Higareda, G., and H.M. Smith. 1988. Courtship behavior in *Rhinoclemmys areolata* from western Tabasco, Mexico (Testudines: Emydidae). *Great Basin Naturalist* 48:263–266.
- R Core Team (2024). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Rovero, F., M. Lebboroni, and G. Chelazzi. 1999. Aggressive interactions and mating in wild populations of the European Pond Turtle *Emys orbicularis*. *Journal of Herpetology* 33:258–263.
- Rozen-Rechels, D., A. Dupoué, S. Meylan, B. Decenière, S. Guingand, and J.F. Le Galliard. 2018. Water restriction in viviparous lizards causes transgenerational effects on behavioral anxiety and immediate effects on exploration behavior. *Behavioral Ecology and Sociobiology* (2018) 72:23 <https://doi.org/10.1007/s00265-018-2443-4>.
- Smolowitz, R.J., S.H. Patel, H.L. Haas, and S.A. Miller. 2015. Using a remotely operated vehicle (ROV) to observe Loggerhead Sea Turtle (*Caretta caretta*) behavior on foraging grounds off the mid-Atlantic United States. *Journal of Experimental Marine Biology and Ecology* 471:84–91.
- van Dijk, P.P., J. Lee, R. Calderón Mandujano, O. Flores-Villela, and M.A. Lopez-Luna. *Rhinoclemmys areolata*. The IUCN Red List of Threatened Species 2007. International Union for Conservation of Nature. <http://www.iucnredlist.org>.
- Vogt, R.C., S.G. Platt, and T.R. Rainwater. 2009. *Rhinoclemmys areolata* (Duméril and Bibron 1851), Furrowed Wood Turtle, Black-bellied Turtle, Mojina. Pp. 022.1–022.7 *In* Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group. Chelonian Research Monographs No.5. Rhodin, A.G.J., P.C.H. Pritchard, P.P. van Dijk, R.A. Saumure, K.A. Buhlmann, and J.B. Iverson (Eds.). Chelonian Research Foundation, Arlington, Virginia, USA.
- Walz, N., A. Mühlberger, and P. Pauli. 2016. A human open field test reveals thigmotaxis related to agoraphobic fear. *Biological Psychiatry* 80:390–397.
- Wilkinson, A., J. Mueller-Paul, and L. Huber. 2013. Picture-object recognition in the tortoise *Chelonoidis carbonaria*. *Animal Cognition* 16:99–107.
- Ye, M., H. Chen, M. Li, J. Duan, and P. Li. 2020. Observations on the courtship and mating behavior of captive Green Turtles (*Chelonia mydas*). *Herpetological Conservation and Biology* 15:284–292.
- Zhang, X.Y., J. Vollert, E.S. Sena, A.S. Rice, and N. Soliman. 2021. A protocol for the systematic review and meta-analysis of thigmotactic behaviour in the open field test in rodent models associated with persistent pain. *BMJ Open Science* 5(1) <https://doi.org/10.1136/bmjos-2020-100135>.



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