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# The use of fluorescent powdered pigments as a tracking technique for hatchling turtles in Belize

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**Abstract.** Hatchling freshwater turtles face significant challenges during early life stages, including predation, desiccation, and habitat loss. Despite their ecological importance, little is known about their movements and habitat preferences due to their cryptic behaviour and small size. In this study, we utilized fluorescent powder tracking, a non-invasive method, to investigate the spatial ecology of hatchling turtles in southern Belize. Forty-seven hatchlings from five species (*Kinosternon acutum*, *K. leucostomum*, *Trachemys venusta*, *Claudius angustatus*, and *Chelydra rossignonii*) were tracked across old-growth rainforest and pine savanna habitats. Hatchlings exhibited movement distances ranging from 3–1987 cm, with most activity concentrated in high-elevation rainforest, particularly in ephemeral aquatic habitats such as puddles and ditches. Species-specific preferences were observed, aligning with adult habitat use. Notably, the longest movement was recorded for a *C. rossignonii* individual, which travelled over 19 m in two days. Significant differences in habitat use were found between ephemeral, permanent, and upland microhabitats, highlighting the importance of temporary aquatic environments during the rainy season. The study confirmed the effectiveness of fluorescent powder tracking as a low-cost, low-impact tool for monitoring hatchling turtles. Despite potential concerns about increased visibility to predators, only one potential predation event occurred during tracking. These findings emphasize the critical role of ephemeral habitats in supporting hatchling survival and provide valuable insights for conservation planning. By addressing a key knowledge gap, this research supports efforts to protect threatened freshwater turtle species in Belize's rapidly changing ecosystems.

**Keywords.** Dispersal, microhabitat selection, non-invasive tracking, conservation strategies, ephemeral habitats, behavioural ecology, survival vulnerability

## Introduction

The dispersal and movement of hatchling turtles during their first days post-emergence are critical to their survival. After emerging from nests, hatchlings must quickly navigate to suitable habitats that provide protection from predators and access to resources, such as food and shelter (Skibsted et al., 2023; Ultsch et al., 2007). Hatchlings are particularly vulnerable to both aquatic and terrestrial predators and environmental stressors (e.g., desiccation) during this period, making it essential to understand their dispersal strategies and habitat preferences (Mortimer, 1999; Kolbe and Janzen, 2002; Paterson et al., 2014). However, despite their ecological importance, little is known about hatchling movements, habitat use, and resting locations (Skibsted et al., 2023).

Hatchling turtles are small, cryptic, and secretive, and therefore are rarely encountered (Spangler et al., 2021). Traditional tracking methods, such as radiotelemetry, are often impractical for hatchlings due to their small size and the invasive nature of many tagging techniques (Blomquist and Hunter, 2009). Fluorescent-powder tracking offers a non-invasive, low-cost, and effective alternative, enabling researchers to track short-term movements and habitat selection without the need for bulky or invasive equipment (Roe and Georges, 2007).

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Fluorescent-powder tracking has emerged as a promising technique for studying fine-scale movements in small vertebrates (Stark and Fox, 2000; Furman et al., 2011). The technique has been used successfully to study the movement ecology of various amphibians (larval salamanders – Ireland, 1973), reptiles (lizards – Fellers and Drost, 1989; tortoises – Blankenship et al., 1990) and small mammals (Lemen and Freeman, 1985; Jike et al., 1988; Barnum et al., 1992; McShea and Gilles, 1992; Hesed, 2012). It is particularly well-suited for hatchling turtles as it does not interfere with their behaviour and can be applied to animals too small for conventional telemetry devices (Butler and Graham, 1993, 1995; Standing, 1997; Tuttle and Carroll, 2005; Buhlmann et al. 2009; Roe et al., 2009). This method involves dusting hatchlings with a non-toxic, UV-reactive powder that allows researchers to track their movements by observing the powder trails left behind on the substrate (Roe and Georges, 2007; Furman et al., 2011). Knowledge gained by tracking hatchlings can inform conservation strategies aimed at improving hatchling survival, especially in regions like Belize, where habitat loss and human activities pose significant threats to freshwater turtle populations (Platt et al., 1999; Rainwater et al., 2012).

Belize hosts a diverse freshwater turtle fauna, including species of conservation concern, such as the Central American River Turtle, *Dermatemys mawii* Gray, 1847, the Mexican Giant Musk Turtle, *Staurotypus triporcatus* (Wiegmann, 1828), and the Narrow-bridged Musk Turtle, *Claudius angustatus* Cope, 1865 (Munscher et al., 2022; 2023). These species inhabit a range of aquatic environments, from rivers and lagoons to wetlands, many of which are increasingly threatened by deforestation, agriculture, and pollution (Platt et al., 1999; Rainwater et al., 2012; Munscher et al., 2023). Hatchlings, in particular, face significant challenges as they navigate fragmented landscapes in search of suitable habitats. Despite their importance, the early life stages of these species remain understudied, leaving critical gaps in our knowledge of their spatial ecology and behaviour.

The application of fluorescent-powder tracking to hatchling freshwater turtles in Belize offers a unique opportunity to gain insights into their movement patterns and habitat use. By tracking movement from ephemeral habitats and identifying key resting locations, this study aims to provide valuable data to inform conservation strategies aimed at improving hatchling survival and protecting critical habitats in Belize's freshwater ecosystems. This study had three main objectives: (1) assess the efficacy of fluorescent-powder tracking as a non-invasive, cost-effective method for studying small vertebrate movements; (2) document the total distance travelled and dispersal distances of hatchling freshwater turtles in Belize; and (3) identify key resting locations and habitat preferences. By addressing these objectives, this research evaluates the use of fluorescent powder in tracking hatchling turtles, contributes to a deeper understanding of hatchling turtle ecology in Belize, and provides critical information for the conservation of these threatened species (Rhodin et al. 2021).

## Materials and Methods

**Study location.** The study area is located on a private protected area that borders four public protected areas: the Bladen Nature Reserve, Cockscomb Basin Wildlife Sanctuary, Deep River Forest Reserve, and Maya Mountain Forest Reserve. These protected areas are all part of the larger Maya Mountain Massif, which includes 16 protected areas and covers almost 809,000 hectares of continuous tropical forest, making it one of the largest contiguous tracts of rainforest in the western hemisphere (Munscher et al., 2023). The Belize Foundation for Research and Environmental Education (BFREE) is a conservation organization that owns and manages a 466-ha privately protected area (centred on 16.5558°N, 88.7077°W), which consists of and is adjacent to multiple habitat types, including savannas, pine savannas, and old-growth rainforest. The location is a biodiversity hotspot and supports numerous animal and plant species (Sanville, 2022).

**Specimen collection.** Hatchling turtles were captured during hikes along the BFREE boundary road after intense rainfall. The BFREE boundary road, a two-track dirt road wide enough for an all-terrain vehicle, has frequent vernal pools and ephemeral drainages. BFREE has many dirt roads that cross and surround the property. During heavy rainfall events, refugia pools fill up along and within these dirt roads. Some of the pools reach depths of over 15 cm. Many species utilize these ephemeral refugia pools as sources of water, habitats for mating, and potential food sources (Skibsted et al., 2023). On many occasions, hatchling turtles were observed using these ephemeral pools to forage and actively feed (Skibsted et al., 2023).

We placed captured hatchling turtles plastron-first into a 7.5-cm diameter flat plastic lid with a 0.85-cm lip. The lid was coated with either orange or green fluorescent powder (approx. USD 22 per kg, Radiant Color Series T1, DayGlo Color Corporation, Cleveland, Ohio, USA). The turtle was then allowed to walk around in the lid, where it obtained a thorough coating with fluorescent powder on its limbs and belly. Once the turtle's limbs and underside were suitably coated, the turtle was placed back on the ground at its point of capture and left alone. The research team returned to each point of capture after sunset to begin tracking the turtles. We used high-powered tactical fluorescent flashlights (Streamlight ProTac HL-X Dual Fuel High Lumen Tactical Flashlight, BassPro corporation, Springfield, Missouri, USA), fluorescent string, and a measuring tape. The area around the point of capture was canvassed using these flashlights. Once the fluorescent trail of the hatchling turtle was found, the tracks were traced with the fluorescent string by one team member holding the string at the track origin while the second team member would measure the movement with the unrolled string. The total unrolled string was then measured with the measuring tape to determine the total distance travelled (Fig. 1A, B).

**Habitat categorization and analyses.** We categorized the habitat where each turtle was tracked into two broad ecotypes: (1) old-growth rainforest, characterized by high canopied trees such as ceiba (*Ceiba pentandra*) and Honduran mahogany (*Swietenia humilis*) with a dense understory consisting of many palm species, including cohune palm (*Attalea cohune*); and (2) pine savanna, characterized by semi-open grassland dominated by Caribbean pine trees (*Pinus caribaea*). We also classified the microhabitats within these ecotypes as ephemeral aquatic habitats, permanent aquatic habitats, and upland. We further split ephemeral aquatic habitats into puddles and ditches, and permanent aquatic habitats into ponds and rivers. Next, the number of turtles per species tracked in each of the habitat categories were tallied and tests for significant differences between the observed counts across all species and within each species were conducted using a Chi-square ($\chi^2$) test with the $p$-value simulated using 2000 bootstraps. We also repeated this procedure within ecotypes and the ephemeral and permanent aquatic microhabitat classes. Distinguishing between turtle species was straightforward as the hatchlings for the five species included in this study are highly distinctive from one another and not easy to misidentify.

## Results

We caught and tracked 47 hatchling turtles, representing five of the nine freshwater turtle species native to Belize – 24 *Kinosternon acutum* Gray, 1831, 12 *K. leucostomum* (Duméril et al., 1851), seven *Trachemys venusta* (Gray, 1856), three *Claudius angustatus*, and one *Chelydra rossignonii* (Bocourt, 1868). All five species of hatchlings are highly distinctive and do not resemble one another. Movement varied by species, with many of the mud turtles exhibiting small movements < 50 cm. The longest movement observed was by a *Chelydra rossignonii*, which moved 1987 cm (Fig. 2). Most turtles were tracked in old-growth rainforest habitats ($n = 42$), while only five were tracked in pine savannah (Table 1). However, there were no significant differences across turtles ($\chi^2 = 4.05$, $p = 0.365$). There was a significant difference across turtles in the old-growth rainforest (Table 2). Ephemeral habitats ($n = 37$), ditches ($n = 24$), and puddles ($n = 13$) had the most tracked turtles, with a significant difference across turtles in ephemeral microhabitats (Table 2). Only four turtles were tracked in permanent pond habitats, and three of those were *K. acutum* (Table 2).

**Table 1.** Number of turtles by species tracked in old-growth rainforest and pine savanna ecotypes at the Belize Foundation for Research and Environmental Education. The $\chi^2$ statistic and simulated $p$-value are provided for comparisons within the ecotypes across turtles and across ecotypes by species. Significant tests are denoted in bold.

| Species | High Rainforest | Pine Savannah | $\chi^2$ Tests |
|---|---|---|---|
| *Chelydra rossignonii* | 1 | 0 | $\chi^2 = 1, p = 1$ |
| *Claudius angustatus* | 3 | 0 | $\chi^2 = 3, p = 0.243$ |
| *Kinosternon acutum* | 22 | 2 | $\boldsymbol{\chi^2 = 16.7, p < 0.001}$ |
| *Kinosternon leucostomum* | 9 | 3 | $\chi^2 = 3, p = 0.135$ |
| *Trachemys venusta* | 7 | 0 | $\boldsymbol{\chi^2 = 7, p = 0.015}$ |
| Total | 42 | 5 | |
| $\chi^2$ test | $\chi^2 = 50.0, p = 0.025$ | $\chi^2 = 5, p = 0.19$ | |

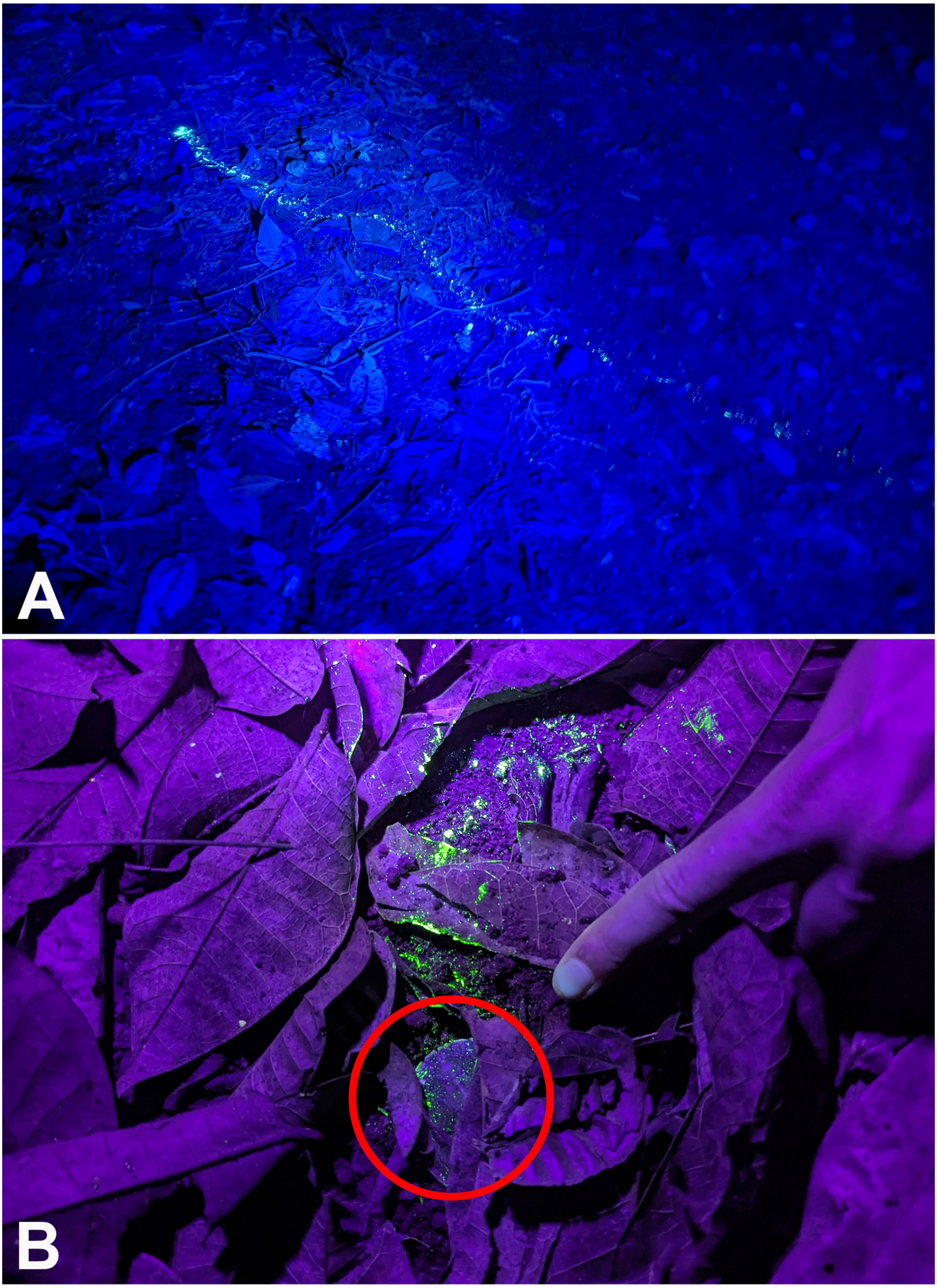


**Figure 1.** *Trachemys venusta* tracked at the Belize Foundation for Research and Environmental Education. (A) Trail of a tracked hatchling. (B) Powdered hatchling located within leaf litter (red circle).

**Table 2.** Number of turtles by species tracked in microhabitats at the Belize Foundation for Research and Environmental Education. The $\chi^2$ statistics and simulated *p*-value are provided for comparisons within the microhabitats across turtles. Two $\chi^2$ statistics for comparisons by species are provided: (1) across the broad microhabitat classes ($\chi^2_B$) and (2) across all microhabitat classes ($\chi^2$). Significant tests are denoted in bold.

| **Species** | **Ephemeral** | | **Permanent** | | **Upland** | **$\chi^2$ Tests** |
|---|---|---|---|---|---|---|
| | **Ditch** | **Puddle** | **Pond** | **River** | | |
| *Chelydra rossignonii* | 0 | 0 | 0 | 0 | 1 | $\chi^2_B = 2$; $\boldsymbol{\chi^2 = 4}$ |
| *Claudius angustatus* | 3 | 0 | 0 | 0 | 0 | $\chi^2_B = 6$; $\boldsymbol{\chi^2 = 12}$ |
| *Kinosternon acutum* | 16 | 5 | 3 | 0 | 0 | $\boldsymbol{\chi^2_B = 32.3}$; $\boldsymbol{\chi^2 = 36.4}$ |
| *Kinosternon leucostomum* | 4 | 8 | 0 | 0 | 0 | $\boldsymbol{\chi^2_B = 24}$; $\boldsymbol{\chi^2 = 21.3}$ |
| *Trachemys venusta* | 1 | 0 | 0 | 1 | 5 | $\chi^2_B = 4.6$; $\boldsymbol{\chi^2 = 12.3}$ |
| Subtotal | 24 | 13 | 3 | 1 | 6 | |
| Total | 37 | | 4 | | 6 | |
| $\chi^2$ test | $\chi^2 = 8.58$, $p = 0.025$ | | $\chi^2 = 4$, $p = 0.252$ | | | |

*Chelydra rossignonii* and *T. venusta* were only tracked in upland habitats (Table 2). There were significant differences across turtles between the broad microhabitats – ephemeral, permanent, and upland ($\chi^2 = 37.47$, $p < 0.001$) – as well as within the specific microhabitats ($\chi^2 = 54.10$, $p = 0.018$).

There were clear differences among the species in terms of the ecotype or microhabitat where they were most commonly found and tracked (Tables 1, 2). Across ecotypes, *K. acutum* and *T. venusta* were tracked significantly more frequently in old-growth rainforest than pine savanna (Table 1). Across broad microhabitats, *K. acutum* and *K. leucostomum* had significantly more tracks in ephemeral microhabitats than permanent aquatic habitats (Table 2). All turtles, except for *C. rossignonii*, had significant differences in track counts across the specific microhabitat classes. Both *C. angustatus* and *K. acutum* had more tracks in ditches, while *K. leucostomum* had more tracks in puddles, and *T. venusta* had the most tracks in upland habitats (Table 2).

## Discussion

The use of fluorescent powder to track hatchling semi-aquatic turtles in Belize has proven to be an effective and economical method for studying short-term movement patterns and use of temporary habitats. Recorded track lengths ranged from 3–1987 cm (Fig. 2), with average movement distances comparable to those documented in similar fluorescent-powder tracking studies of amphibians and reptiles. While most movements aligned with typical short-range activity, several individuals from three different turtle species displayed longer movement patterns. For example, prior research on amphibian and reptile species reported movements of 2–350 m for amphibians, including Marbled Salamanders, *Ambystoma opacum* (Gravenhorst, 1807), Southern Toads, *Anaxyrus terrestris* (Bonnaterre, 1789), and Leopard Frogs, *Lithobates sphenocephalus* (Cope, 1886) (Graeter and Rothermal, 2007); 1–71 m for Six-Lined Racerunners, *Aspidoscelis sexlineatus* (Linnaeus, 1766) (Dodd, 1992); 5–225 m for Texas Horned Lizards, *Phrynosoma cornutum* (Harlan, 1825) (Stark and Fox, 2000); and 3–203 m for gartersnakes, genus *Thamnophis* (Furman et al., 2011). Notably, a hatchling *C. rossignonii* in our study showed extensive movement, traveling distances of 1107 cm and 1987 cm over two days, and was found resting under cohune palm leaf litter.

Our species-specific results are noteworthy, as they appear to align with the habitat preferences of adult individuals of each species. In Belize, kinosternid species exhibit activity closely tied to the rainy season. As ephemeral habitats such as puddles, ditches, and wetlands become inundated, these turtles seem to emerge abruptly, likely to exploit aquatic invertebrates and amphibian eggs as food resources (Skibsted et al., 2023). The discovery of *Kinosternon acutum* hatchlings within the pine savannah ecosystem was unexpected. However, these hatchlings were located near the ecotone at the boundary of the old-growth rainforest, suggesting possible edge habitat use by the adults when looking for potential nesting habitat. The longest movement recorded in our study was by a *Chelydra rossignonii* hatchling, which mirrors findings from *Macrochelys temminckii* (Troost, 1835) hatchlings in Oklahoma, where movements ranged from 3–19 m (Spangler et al., 2021). Additionally, overwintering studies on *C. serpentina* (Linnaeus, 1758) hatchlings in New York reported movement distances ranging from 3–17 m, primarily toward aquatic environments. Notably, one instance in the New York study documented terrestrial movement ranging from 34–260 m (Ultsch et al., 2007), indicating potential for substantial dispersal in certain circumstances.

The influence of handling on this young age group remains uncertain, but a few examples do exist, Butler and Graham (1995) documented that hatchling Blanding's Turtles, *Emydoidea blandingii* (Holbrook, 1838), exhibited specific post-emergence behaviour and habitat selection patterns, potentially influenced by environmental stressors. Similarly, Tuttle and Carroll (2005) found that hatchling Wood Turtles, *Glyptemys insculpta* (Le Conte, 1830), displayed varied movement patterns and behaviour, often seeking refuge quickly, which could be a response to perceived threats or environmental conditions. These findings highlight the need for caution when interpreting movement data in juvenile turtles, as handling and environmental factors may influence behaviour.

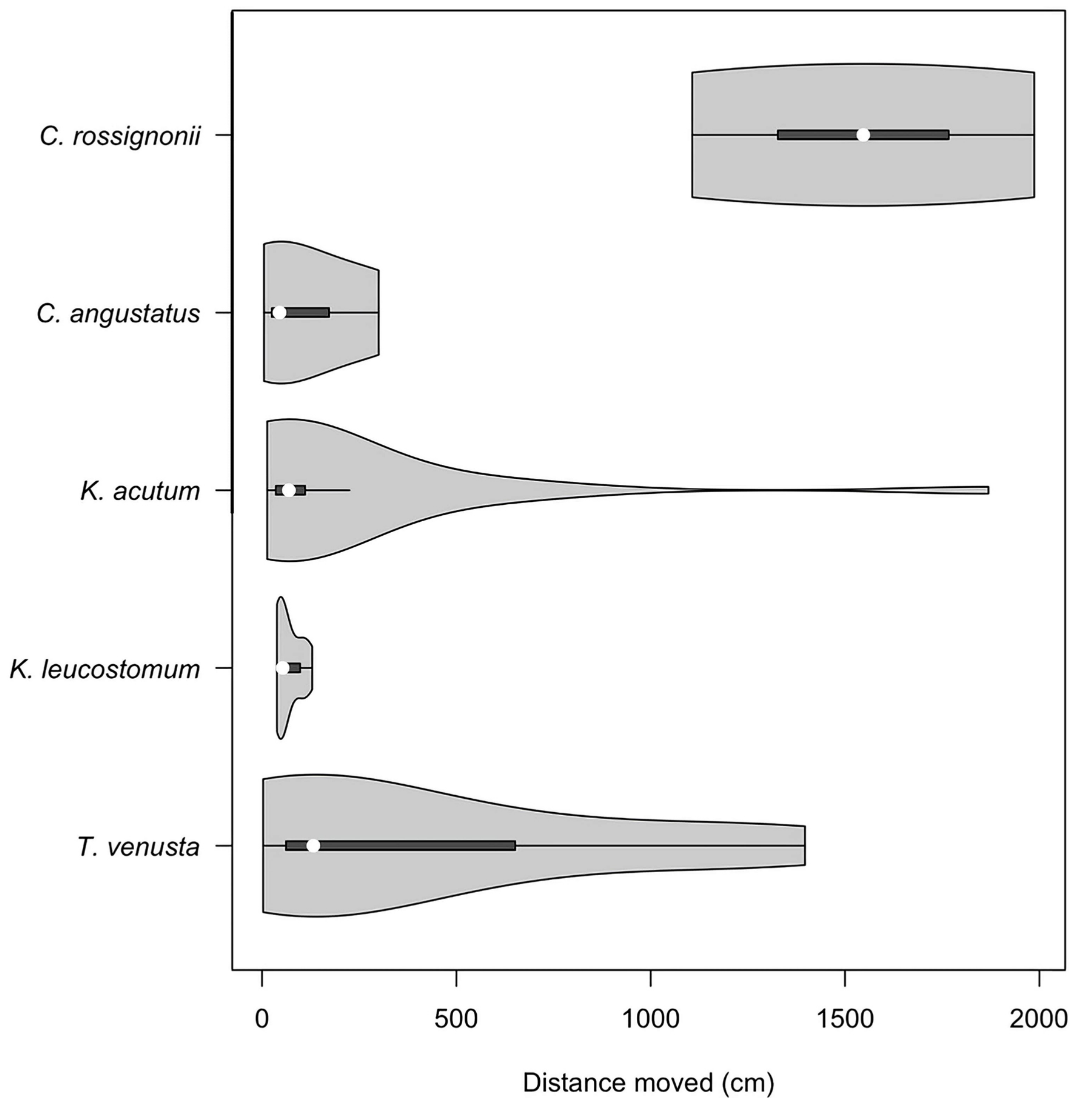


**Figure 2.** Distribution of distances moved by hatchlings during fluorescent-powder tracking at the Belize Foundation for Research and Environmental Education.

Findings from studies on snakes and lizards offer some insights. For instance, Furman et al. (2011) suggested that handling or acclimatization might influence movement patterns in gartersnakes (genus *Thamnophis*). Similarly, Dodd (1992) found that 19 of 40 tracks created with fluorescent powder in *Aspidoscelis sexlineatus* were shorter than 10 m, often leading directly to shelter, which he suggested could be a stress response to handling.

Understanding hatchling movements, habitat use, and resting locations remains challenging, as hatchling turtles are typically small, cryptic, and elusive, making encounters infrequent (Spangler et al., 2021; Skibsted et al., 2023). Developing cost-effective, efficient methods to locate and study hatchlings is crucial for advancing knowledge and conservation strategies for this life history stage. Fluorescent powder tracking has shown promise as a viable technique in field settings. We have successfully utilized this technique for two years in the southern Belize rainforest, achieving informative tracking results even under heavy rainfall conditions. This technique is less intensive and likely less stressful for hatchlings compared to radio telemetry, which is often unsuitable due to size constraints, and thread trailing, which may present risks of entanglement and additional weight burdens (Furman et al., 2011). Although fluorescent powder could theoretically increase predation risk by making hatchlings more visible, we only observed one out of the 47 tracked hatchlings being potentially lost to predators during our study. Our findings support fluorescent powder tracking as an effective method for observing hatchling semiaquatic turtles in Belize and recommend its application to other species to improve understanding of this poorly studied life stage.

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