

Using fluorescent paint as an effective short-term marker in a high-density rookery of Green Turtles, *Chelonia mydas* (Linnaeus, 1758), on Poilão Island, Guinea-Bissau

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Abstract. We report on a short-term marking protocol that uses weather-resistant high-contrast fluorescent dyes and non-disruptive ultraviolet lights to effectively mark and distinguish between individual green turtles (*Chelonia mydas*). We field-tested our method in the largest green turtle nesting location in Africa, on Poilão Island in Guinea-Bissau, where it proved to be quick to apply and easy to detect, and non-disruptive for the turtles. We effectively marked 2719 individuals, with some marks lasting up to 13 days. We propose that this protocol can be adapted for other animal groups and in other environmental settings.

Keywords. Tagging methods, marking, Bijagós Archipelago

Introduction

The ability to identify and single out individuals, groups, or entire populations is a key part of wildlife research and the basis for many population dynamics and behavioural studies (Krebs, 1999; Lapointe et al., 2013; Schofield et al., 2020). Mark and recapture surveys, for example, where the ability to distinguish between known and new entries is relevant, greatly benefit from it (Lindberg, 2012; Ashe and Hammond, 2022). The simplest and least invasive way of differentiating subjects is to rely on their unique individual features (Hiby et al., 2009; Moskvyyak et al., 2021). These can be assessed by either direct observation or by consulting recorded images and comparing them with previous data (Schofield et al., 2008; Marshall and Pierce, 2012; Keen et al., 2021). Unfortunately, most species lack enough and consistent intraspecific variability to allow rapid and

easy *in situ* identification (Vincent et al., 2001; Meek et al., 2013). Moreover, in the case of large populations, an extensive library of characteristics is needed for cross-reference, making direct observation an impractical way of rapid identification in the field (Lahiri et al., 2011; Andreotti et al., 2014; Nguyen et al., 2017).

The use of artificial markings, such as tags engraved with a code, is a better method for quick on-site identification. However, deployment of these tags is usually stress-inducing and/or invasive, given that it often requires interacting with the animals through capture and handling (Norman et al., 2004; McMahon et al., 2007; Rigby et al., 2012). In marine turtle studies, the most common marking methods employed are flipper and passive integrated transponder (PIT) tags (Balazs, 1999; Eckert and Beggs, 2006). These methods are useful for long-term individual tracking and have provided crucial data regarding marine turtle life history traits and connectivity between populations and critical areas (Godley et al., 2003; Moncada et al., 2010; Omeyer et al., 2019; Santos et al., 2019). However, flipper tags are somewhat prone to loss (Limpus, 1992; Pfaller et al., 2019) and have been linked to turtle mortality by entanglement (Nichols et al., 1997; Suggett and Houghton, 1998). Most adult female turtles are opportunistically tagged at nesting beaches, during the late stages of egg-laying, the occasion when they are most accessible and immobile without extreme manipulation (Eckert and Beggs, 2006). Still, even then, applying tags requires a high degree of interaction between the deployer and the turtle, known

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to be impactful and sometimes disruptive to nesting behaviour (Broderick and Godley, 1999). In addition, to get the ID code the researcher needs to approach the turtle and search for the tag, which is another potential source of disturbance for the animal.

For short-term behavioural surveys, like studies of beach emergence patterns and the tracking of individual turtle movements during nesting events, there is no need for long-lasting identification and so, temporary marks suffice. Paints and resins have previously been employed to draw patterns or characters on the carapace of turtles, both marine and freshwater species, either for individual identification or to assign them to a given group (Booth and Peters, 1972; Jones and Hartfield, 1995; Kramer, 1995; Balazs, 1999; Hamann et al., 2002; Selman and Qualls, 2007; Stokes et al., 2008; Kornilev et al., 2010, 2012). Apart from paints with long curing/drying times, or that require a clean surface to be applied, most are quick to apply, have a minimal physical impact on the turtle, and, if large and contrasting enough, can be read without approaching the animals, even from remote aerial vehicles (Dunstan et al., 2020). However, these instantaneous marks are susceptible to natural abrasion, especially in the case of nesting sea turtles where, due to their larger size and strength, marks can fade faster or be erased altogether by other turtles and sand (Balazs, 1999). This is particularly true for tropical nesting sites with heavy rainfall and storms, where wet sand pairs with the action of rain and wind, confounding markings. At high-density nesting beaches, faded marks and/or lack of contrast pose a substantial problem since reduced mark visibility thwarts or outright prevents identification. Adding to that, marks drawn with low-contrast paints may pass unnoticed, especially since most beach surveys occur at night when light usage other than red is avoided to not disturb the nesting turtles (Robertson et al., 2016). Thus, the ideal instant tag for marine turtle behavioural studies at high-density nesting beaches would be one that can be applied quickly and discretely in every weather, is resistant to immediate abrasion, and is highly visible at a distance in low light conditions, ideally by using a non-disruptive light source.

Poilão Island, in the Bijagós Archipelago of Guinea-Bissau, hosts one of the largest green turtle rookeries in the world, with ca. 25,000 clutches estimated to be laid annually (Patrício et al., 2018). The nesting period occurs during the height of the wet season, from mid-June to mid-December and peaking in August and September (Patrício et al., 2017). Every year since 2004, a survey effort directed toward green turtle nesting has been conducted during this time along a 1.8-km extension of

the beach divided into four sections, encompassing ~78% of the available beach coastline (Patrício et al., 2017; Madeira et al., 2020). As in most campaigns, one of the main objectives is to determine and monitor the size of this nesting population. This is mostly done by counting tracks and nightly beach attendance of nesting females, two methods typically used in sea turtle surveys (Eckert, 1999). However, Poilão is a high-density nesting location, with the yearly maximum number of nesting female emergences per night averaging 649 ± 531 between 2007 and 2020, reaching its highest in 2020 with 2065 individuals in a single night. The sheer number of turtles impacts the quality of the monitoring data since tracks overlap and are erased, and nesting turtle accumulation may interfere with nightly survey counts. To improve these metrics, we aimed to assess their accuracy by determining the actual number of nesting turtles that they should represent, which required us to quickly and discretely identify every single nesting female to assure we counted it only once. As such, we created a short-term marking protocol, reported herein, that uses high-contrast fluorescent dyes and ultraviolet light to improve turtle detection and identification with minimal disturbance.

Materials and Methods

Surveying. During parts of the green turtle breeding season in 2019 and 2020 (23 August–5 November 2019, 7–15 September 2020), we conducted 20 and six turtle surveys, respectively, on non-consecutive nights. The aim was to count all turtle emergences per night in each of the four monitored beach sections. For each survey, two observers took turns patrolling a given beach section, one from sunset to midnight and another from midnight to sunrise. Each section was traversed constantly in successive counting rounds, with only 2–5 min intervals between passes to avoid missing turtles. During each round, we counted old and new individuals and marked all new arrivals.

Marking. We used green, yellow, orange, and pink fluorescent Markal Paintstik livestock markers (LA-CO Industries, Elk Grove Village, Illinois, USA). These markers are commonly used to mark livestock and are water and weather resistant and non-toxic. Marks were applied without cleaning the turtle's carapace. To eliminate confusion between new marks and those from previous nights, we pattern-coded them with a vertical or horizontal stripe at either the left or right posterior end of the carapace (Fig. 1). Furthermore, we alternated colours cyclically to further discriminate between survey nights and beach sections. To minimize our

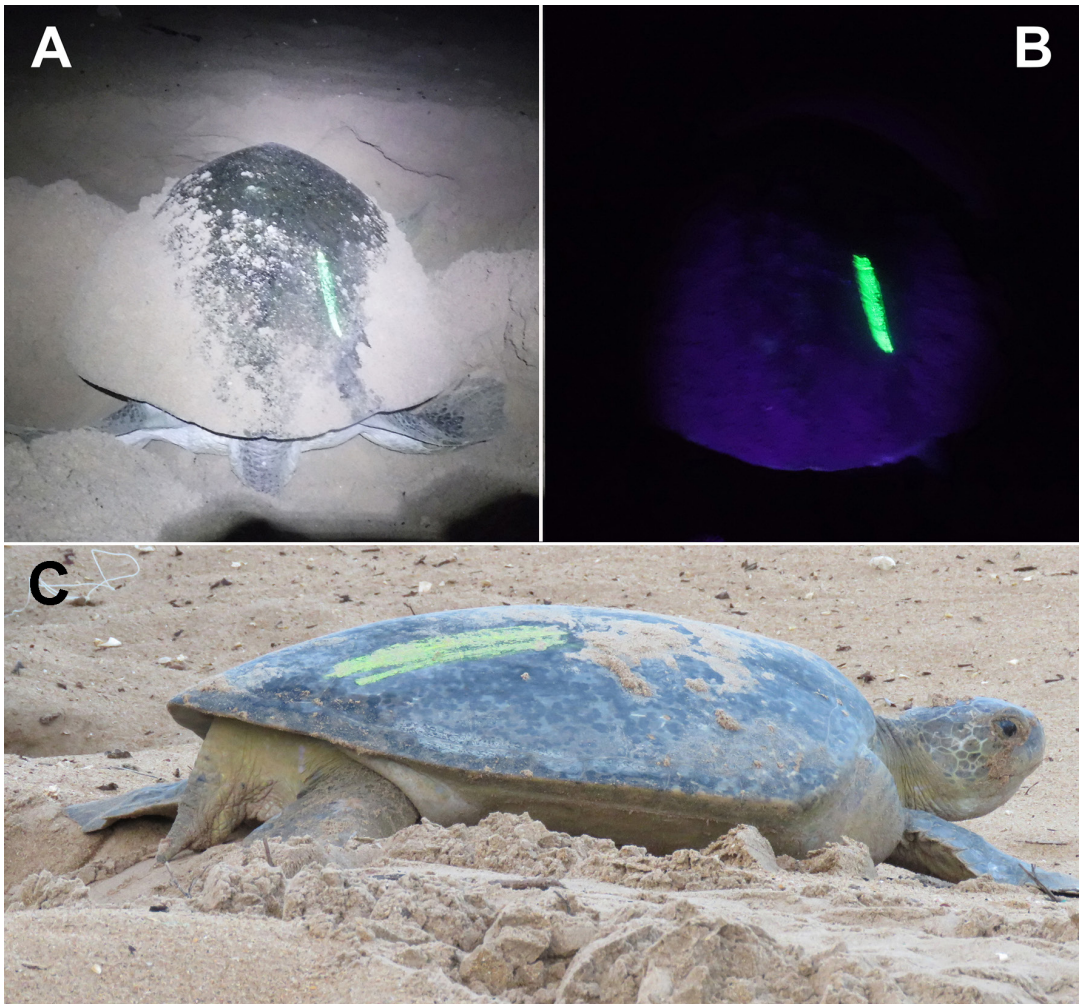


Figure 1. (A) A green turtle (*Chelonia mydas*) on a nesting beach on Poilão Island, Guinea-Bissau, marked with a green Markal Paintstik, shown under white light. (B) The same turtle under UV light, showing the highly contrasting mark. (C) A turtle marked with green paint observed during the day.

impact, we only marked females already digging nests, always approaching them discreetly and from the back.

Recording. When exposed to ultraviolet light (UV) or “blacklight”, fluorescent colours appear to glow due to the interaction of the light and the pigments. To maximize contrast and improve detection during our surveys we used a handheld UV flashlight (ahome.com). The wavelength emitted by this light (395 nm) is below the minimum threshold of the green turtle sensitivity spectrum (400 nm; Levenson et al., 2019), and should not disturb them. Still, this flashlight can be focused into a single focused beam, allowing us to only illuminate a small area of the carapace at a distance and further decrease its potential impact.

Results

In 2019, a low nesting year, we did an average of 37 ± 6 survey rounds per night, and totalled 570 marked turtles, averaging 28 ± 15 turtles per night. In contrast, in 2020, the year with the highest recorded nesting activity at Poilão, we marked 2149 turtles, averaging 358 ± 169 turtles per night.

Paints were easy to apply and quickly adhered to carapaces, even when these were covered in sand or wet. The marking process took only 5–10 s and had no apparent repercussions on turtle behaviour. No female stopped or abandoned the nesting process during the interaction. Marks remained visible all night and finding

them was fast and easy, even after being covered in sand and sometimes exposed to heavy rain (Fig. 1A). Some marks did start to fade on the same night, mainly when the weather was rougher or when the sand-covered turtles brushed against each other or the vegetation. Despite this, in 2020, with the exponential increase in abrasion caused by the higher density of nesting turtles, the marks remained detectable, even when the overall configuration of the horizontal/vertical patterns faded. In the worst cases, the paint would remain between the scutes and inside the scratches and dents of the carapace, allowing us to consistently identify marks by colour or zoning. We observed no counting discrepancies and believe that we were able to correctly identify all already marked individuals. Some colours, like green and yellow, proved to be more effective because they were brighter under UV light and consequently more visible at a distance. Due to their higher contrast, these colours were at times even visible through a significant layer of sand. All marks also remained visible during the day (Fig. 1C).

In 2019, 14 turtles still had small patches or flecks of paint at their next nesting event, sometimes retaining the overall orientation of the stripe after 11–13 days. In 2020, given the short span of the sampling period (8 days), we were unable to monitor successive nesting events for most turtles, which take place every 10–15 days (Hancock et al., 2019). Still, we found 29 turtles with markings from previous nights. This could be problematic for the long-term repetition of this protocol since colour accumulation could difficult distinction between older and newer marks. Nonetheless, as we alternated between patterns, areas of the carapace, and colours, we had no problem differentiating between relevant markings and older residual ones.

Discussion

This methodology appears to be non-invasive and reliable in the short term, even in rough conditions. Colours and zoning marks on the carapace proved to be most dependable for detection and discrimination, followed by the configuration of the painted patterns (horizontal vs. vertical stripes). It would be interesting to apply this method to a smaller sea turtle nesting population, where it is feasible to mark all turtles in a single season. This way it would be possible to test the long-term resistance of these marks in comparison with longer lasting, more technical paints (Kornilev et al., 2012). Fluorescent livestock markers could also be used to better visually discriminate turtles during in-water studies as an alternative to less contrasting paints,

as the marks are water-resistant and readable, while other alternatives, such as flipper tags and PIT tags, are hard and sometimes impossible to read underwater in moving turtles, even during the day.

In addition, given that the original intended application of Paintsticks is marking animal hair, having also been used to mark bird feathers (Romero et al., 2021), these tools could easily be applied to other integuments or surfaces besides turtle carapaces. This could be especially useful in other short-term nocturnal studies to either mark individuals or relevant locations, structures, or points of interest. Regardless of its other potential applications, the use of fluorescent markers coupled with UV flashlights was highly successful to discriminate between nesting turtles in tough conditions, with minimal disturbance, and will undoubtedly be useful for other marine turtle surveys.

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