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ARTÍCULO ORIGINAL

FIRST ASSAY OF PHOTO-IDENTIFICATION IN MARINE TURTLES' NESTING POPULATION

Primer ensayo de la foto-identificación en una población anidadora de tortugas marinas

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ABSTRACT

Guanahacabibes, the westernmost part of Cuba, contains important habitat for many terrestrial and marine species, particularly marine turtles. Previous studies of turtles in this area have focused on biological characteristics such as re-migration patterns, nesting patterns, and different abiotic factors such as temperature and meteorological phenomena, which determine the fate of individuals in this population. Traditional techniques for individual identification can cause stress to the animals, however, and have a limited duration. For this reason, a study was conducted to establish the efficacy of photo-identification of morphological characters as less stressful and longer-lasting means o individual turtle identification. Of 1032 tagged individuals only 140 have been photo-identified, 135 were green turtle (*Chelonia mydas*) and five were loggerhead turtle (*Caretta caretta*). Normality and homogeneity of variance was determined for all quantitative variables. Bifactorial ANOVA were determined if there was a difference in the number of vertices of post-orbital scales over years, beaches and the interaction of the two factors. Student-Newman-Keuls was used for multiple comparison test. Errors made up only 0.71% of the photos analyzed (200 in total). The number of vertices of post-orbital scales showed no difference among years or beaches with a predominance of pentagonal scales. The coding used here for animal identification using a numerical sequence proved feasible, with some failures, to help recognize and distinguish remigrating animals. Although photo-identification is not free of errors, the information it provides is very useful in conservation studies.

KEYWORDS: Cuba; green Turtle; nesting ecology; photo-identification

RESUMEN

Guanahacabibes, ubicada al extremo más occidental de la provincia de Pinar del Río, hábitat importantes para muchas especies marinas y terrestres, entre ellas las tortugas marinas. Se han desarrollado investigaciones previas para conocer las características biológicas de la población que anida en el área tales como los

patrones de remigración y de anidación, y diferentes factores abióticos como la temperatura y eventos meteorológicos, que determinan el futuro de los individuos en esta pobblación. Las técnicas tradicionales de identificación suelen estresar a los animales y tienen una duración limitada. Por este motivo, en el presente estudio se pretende determinar la eficacia del método de foto-identificación para discriminar individuos. Se determinó el cumplimiento de la normalidad y homogeneidad de varianza de las diferentes variables. El número de vértices de las escamas post-orbitales fueron analizados mediante un ANOVA bifactorial para determinar si existían diferencias por año, por playa y en la interacción entre estos dos factores. También se realizó un ANOVA de clasificación simple para comparar el número de vértices por escama post-orbital, el número de escamas post-orbitales de las tortugas en diferentes playas. Para las comparaciones múltiples se empleó la prueba de Student Newman-Keuls (SNK). Aunque la foto-identificación no está libre de errores la información aportada es muy útil en los trabajos de conservación. En este sentido, los errores encontrados fueron del 0,71 % del total de las muestras analizadas. De 1 032 individuos marcados solamente 140 han sido foto-identificados, de ellos 135 de la especie tortuga verde (Chelonia mydas) y cinco de caguama (Caretta caretta). Se comparó el número de vértices de las escamas post-orbitales por año, por playa y en la interacción entre estos dos factores. El número de vértices por escama post-orbital, la relación entre el número de la cantidad de escamas entre las tortugas de diferentes playas sin dar diferencias significativas estos análisis La codificación utilizada para identificar mediante una secuencia numérica a un individuo demostró que es un método viable, que en algunos casos puede presentar fallas, pero también aportó información significativa que ayudo a discriminar y reconocer a individuos remigrantes.

PALABRAS CLAVE: *anidación; Cuba; ecología; foto-identificación; tortuga verde*

INTRODUCTION

The measurement of population size and the historical parameters of life history are crucial for effective wildlife management of. However, on occasion it is difficult to measure this characteristics in some animals such as those with long life spans or those that are highly migratory (Lebreton *et al*., 1992; Wang & Whitlock, 2003). Many studies of animals with high mobility rely on their physical capture and the attachment of a tag or other object that allows for identification of the animals movement (McMahon *et al*., 2007). This approach can be successful but may present problems such as stress to the animal during the capture, handling and the tagging process. In addition, tag loss is a serious issue because it interrupts the continuity of long-term studies (Bellini *et al*., 2001; Jiménez Quiróz and Márquez Millán, 2002; Reisser *et al*., 2008).

An alternative to physical tagging is the visual identification of the individual. The researcher's ability to identify an animal by its natural features has some advantages over conventional identification through tagging. Animals do not need to be captured, the characteristics are constant most of the time and there is less chance to affect animal behavior by the identification system (Reisser *et al*., 2008). In particular, photo-identification is a method by which researchers capture by photography the natural marks of individuals for identification and subsequent re-identification purposes. It has proven a handy tool in the long-term tracking of animal populations (Bradshaw *et al*., 2007; Thompson *et al*., 2000). In marine turtles, this photo-identification started as a qualitative classification of facial scales patterns (Schofield *et al*., 2008; Reisser *et al*., 2008) but evolved later to a quantita-

tive method considering scales number and position (Jean *et al*., 2010).

Tagging is one of the most common methods utilized in Cuba for studies of marine turtles. Small metal tags are inserted in the front right flipper (Moncada *et al*., 2010). However, researchers may want to try new strategies to identify individuals within a population or between different species, which allows them to avoid or minimize animal manipulation and physical damage, thereby not compromising animal health. The photo-identification method is one of the newest techniques that is providing useful results in this field (Jean *et al*., 2010). Several studies have been made to assess the usefulness of this methodology but all were underwater. Therefore it is not known how this method will actually work in the study of a nesting population.

The objective of this work was to determine the efficacy of photo-identification method identifying marine turtles' individuals at nesting sites.

MATERIAL AND METHODS

Study area

Photo-identification (Photo-ID) was conducted on four beaches at Guanahacabibes Peninsula (21°59', 22°00' N - 84°44', 84°50' W), Pinar del Río, Cuba, site of a University of Havana project for the study and conservation of sea turtles (Azanza *et al*., 2013).

Sampling desing

Data were collected as part of the University Project for the Study and Conservation of Sea Turtles in Cuba (Ibarra, *et al*., 2000), carried out by the Center for Marine Research of the University of Havana. Monitoring took place during the night, following the protocol of the Marine Turtle Conservation Program of Cuba (Moncada *et al*., 2013).

Sampling period is from 2004 to 2013. As a program, Photo-ID was initiated in 2010 following the methodology of Jean *et al.* (2010) although photographs from previous years (since 2004) also were analyzed if they fulfilled all the conditions of the followed methodology. A summary of the sample size per season is presented in Annex 1. Photos were taken from the right side of the animal's head after the females completed the nesting process to avoid any disturbance or interruption of oviposition. Since the work with turtles was made during the night the use of flash was required. Photos for analysis were chosen following two criteria: first, all photos of the same animal must be from different days or years and second, photographs must clearly demonstrate the entire facial scale. In those cases where photograph of only the left side of the head were available the photos were processed and stored in a database.

Identification of individuals: First we determined if there were any distinguishing features, such as a projecting lower jaw, atypical head shape, or any physical deformity such as an amputated flipper. Next, we described the shape, presence of spots, and different characteristics of the post-orbital scales. Finally, we counted the number of vertices of each scale of the three first columns that appear in the lateral side of the head and then followed the system proposed by Reisser *et al.* (2008) in which the first number represented the location of the analyzed column, the second number corresponded to the position of the scale in that column, from

bottom to top and the third number corresponded to the vertex scale amount.

Statistical analysis

Normality and homogeneity of variance was determined for all the quantitative variables using Kormogorov-Smirnov and Levene tests respectively. Bifactorial ANOVA was used for differences in the number of vertices of post-orbital scales among years, beaches and the interaction of the two factors. The Student-Newman-Keuls was used as a multiple comparison test.

The significance of the observed differences was evaluated with STATISTICA, version 7.0 (Statsoft, 2004, Tulsa, IL, USA) for Windows. For all the statistical test, a level of significance of 0.05 was used.

RESULTS

In the present study a total of 200 pictures of nesting females were taken but only 163 were used in the photo-identification process. Of this, 157 belong to the species *Chelonia mydas* and six to *Caretta caretta*. From this total, 140 corresponded to different individuals: 135 *C. mydas* and five *C. caretta* and the others corresponded to recaptures of some of these individuals in different years.

Prominent characteristics such as differences in the jaw, presence of fibropapilloma and damaged or amputated limbs appeared at a very low frequency and thus were not particularly helpful in individual identification. However, other features contributed to identification. For example, the presence of a lump on the head (turtle HV0194) and peculiar patterns of the face scales (HV0871) (Fig. 1).

A total of eight turtles were re-identified by means of Photo-ID. Four of them were re-migrants with re-migration intervals of two, three and six years. Among these re-migrants was a loggerhead with the larger remigration period (six years) which was photographed in 2004 and 2010, nevertheless the characteristics of the scales remained intact (Fig. 2). The others individuals were re-sighted within the same nesting season in different days with an average of 11 days between recaptures.

Post-orbital scales varied in number, from two (1 individual) to five (8 individuals), but animals with four scales predominated (80 individuals). Regardless of their position, post-orbital scales had a general

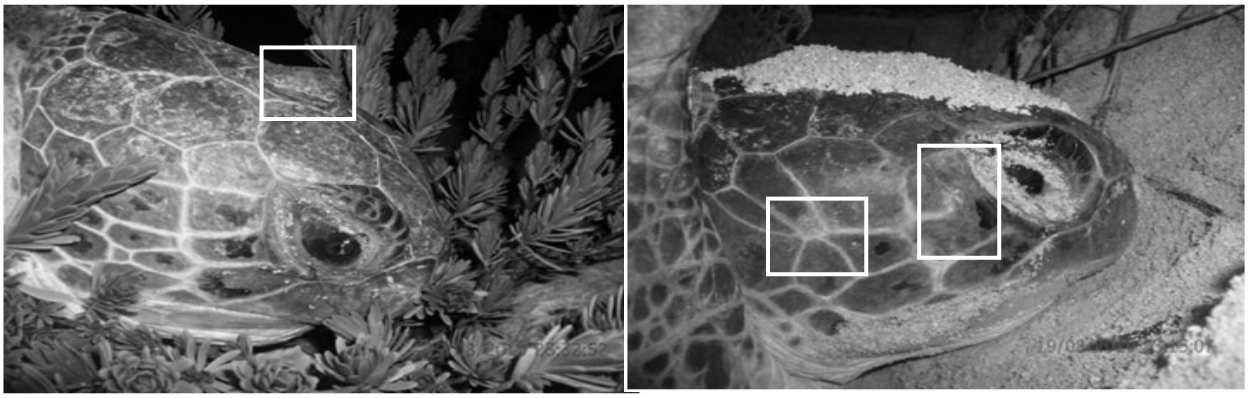

Figure 1. Distinctive prominent features used in the individual photo identification.

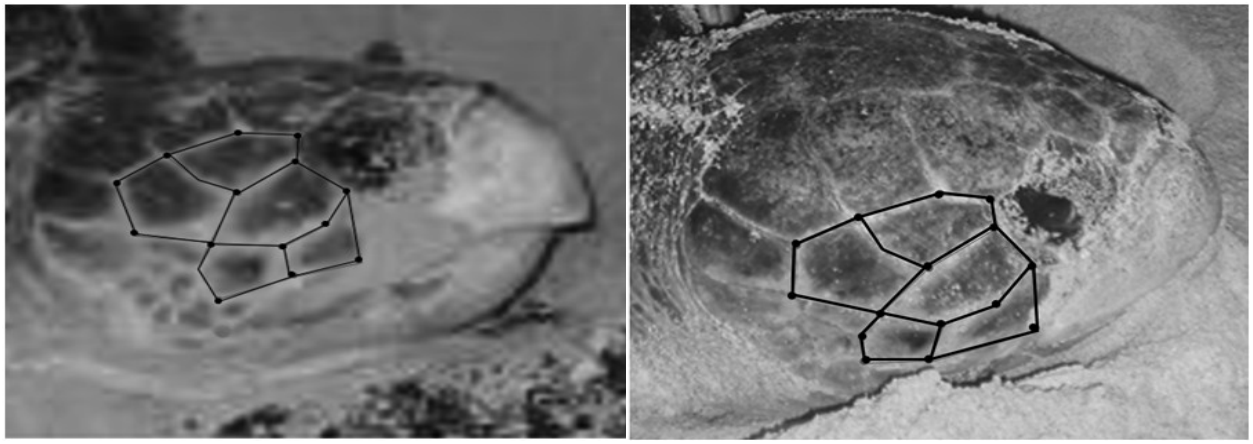

Figure 2. Scales characteristics of the loggerhead identified through photo-identification at Guanahacabibes Peninsula. First photograph in 2004 (left) and recapture in 2010 (right). The number of vertices of the lateral scales did not differ among beaches ($F_{(3,383)} = 1,89$, $p = 0,13$).

pentagonal shape. As a consequence, scales with five vertexes were the most abundant (Fig. 3). There were no significant differences in the number of vertices between scales ($F_{(3,383)} = 1,27$, $p = 0,28$).

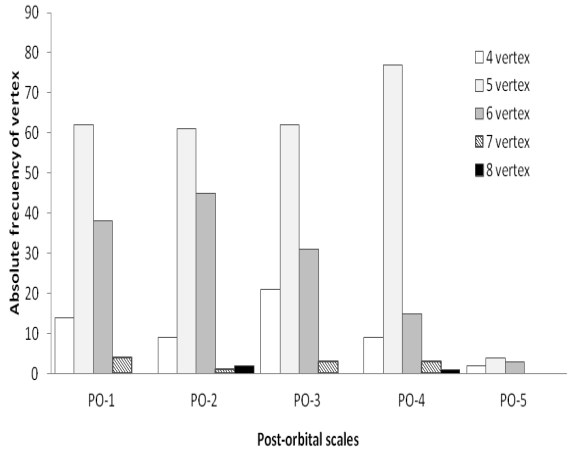

Figure 3. Number of vertices in post-orbital (PO) scale on heads of nesting females of *C. mydas* in the Guanahacabibes Peninsula.

found exclusively between 2011 and other years as a result of an increase in the number of turtles with six and seven scales in the second and the third columns.

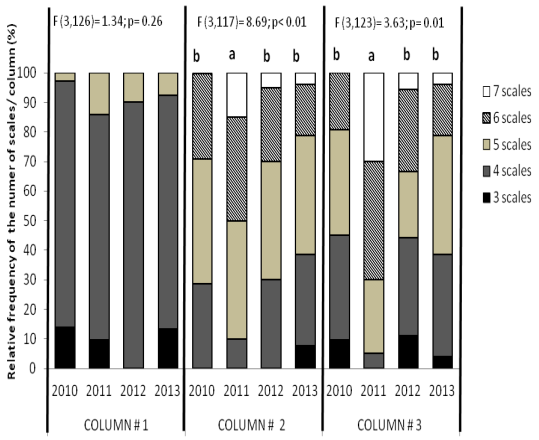

Figure 4. Relative frequency of the lateral scale's number in the first three lateral columns of the head of *C. mydas* females nesting at Guanahacabibes Peninsula.

Also, there were no significant differences among years with respect to the number of vertices of the first column of post-orbital scales but there were differences in the second and third columns of scales, which were more variable (Fig. 4). This difference was

Despite these differences, it was not possible to distinguish individuals solely by noting the number of scales. For example, combining the number of scales and vertices per scale of the post-orbital scales we found the same code repeated 11 times in different ani-

mals. The second column showed more variability in the number of scales (between three and seven) therefore there were less repeated codes in this column and the same occurred with the third one. For this reason the combination of the number of post-orbital, temporal, sub-temporal, central and tympanic scales and their vertices into a unique identification code made individual identification possible.

Although the photo-ID program has not been active as long as the physical tagging program, it is possible to make a preliminary analysis of their utility for the identification at the individual level. Thanks to the Photo-ID technique a re-migrant female was identified as the same individual since she appears in the database with two different tag codes: HV1178 and HV1354. The first tag (HV1178) corresponds to the first time that she was sighted in 2010, this tag was lost and the same turtle was re-tagged later on the same beach two years later. Despite the difference in the tagging database, photo-identification determined that was the same individual (Fig. 5).

A total of 140 separate codes were obtained from the 140 individual turtles, none of them repeated in different animals. However, in one case the same turtle had different codes representing 0,71 % of the total. Nevertheless this difference was of only one scale in the entire code of scale patterns.

DISCUSSION

Photo-ID can be one of the most efficient methods for long-term monitoring of a population, despite it not being error-free. Different individuals can present similar mark patterns that can lead us to make errors in an individual's identification (false positive), or multiple identifications of the same individual (false negative). Low quality of the photos also diminishes the efficiency of this method (Kelly, 2001; Speed *et al*., 2007). Using the numeric code of the scales proposed by Jean *et al.* (2010) improve the identification progress with regards the qualitative method used previously (Reisser *et al*., 2008; Schofield *et al*., 2008). Photo-ID is still in development and new approaches to improve

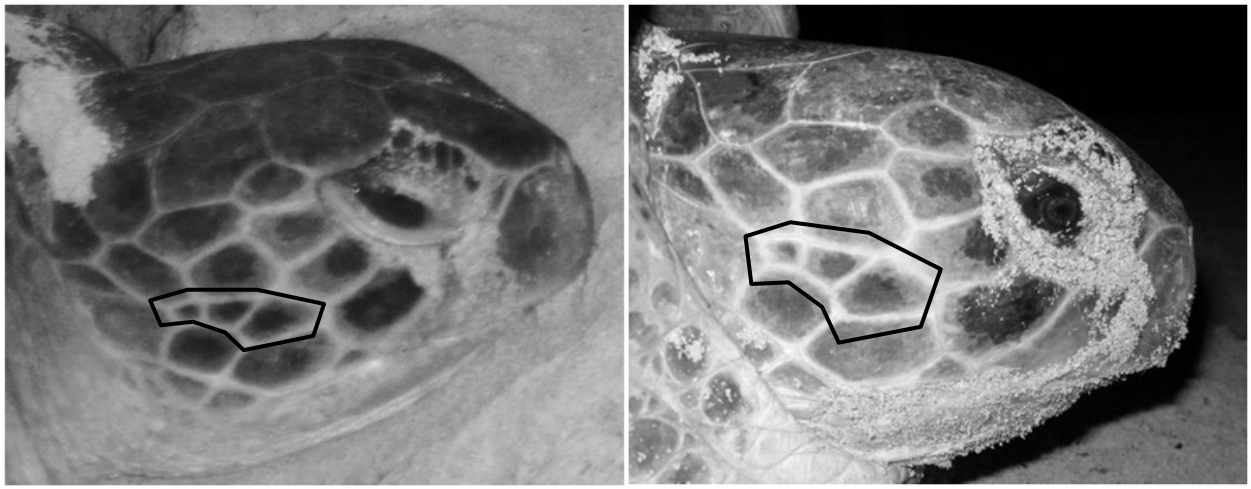

Figure 5. Photographs of the individual catalogued with different tags: In the left, photo taken in 2010, tag ID: HV1178; in the right photo taken in 2012, tag ID: HV1354. The contoured area highlights one of the distinctive features of the scale pattern in the face.

the method keeps coming out, such as a newly developed method for automated recognition of photographs (Carter *et al*., 2014).

The photo-ID system that we used has been employed previously by other authors such as Jean *et al.* (2010) and Reisser *et al.* (2008) but only for underwater identification. Photo-ID proved to be useful for ecological characterization of feeding aggregations (Reisser *et al*. 2013) and for management decisions such as the limits of a Marine Protected Area (Schofield *et al*., 2013). In all studies the authors noted the advantages of the method and were successful in identifying different individual animals. Similar results were obtained in our study with only one identification error. Nevertheless 37 pictures had to be discarded because the visibility of the scales were compromised due to sand or head retraction. In those cases, identification would be erroneous and would lead to mistakes in the database. Application of photo-ID in nocturnal studies could have also the disadvantage of stressing the animals when are flashed. Nevertheless, this process is always performed after the females finished laying the eggs and, so far, no sign of disturbance had been noticed. In any case, we concur with Reisser *et al.* (2008) with the fact that the possible disturbance that the flash may cause to nesters needs a follow up since Campbell (1994) in Costa Rica, recorded a reduction in the time spent by females covering the nest when they were photographed.

In this study, working during the night and at a nesting beach presented certain difficulties in detecting facial scale patterns. For one, photos taken during the night are often not clear enough to identify and count all scales. Secondly, in several photos the turtles have sand in their faces, which impede the clear identification with clarity of the characteristics of scale characteristics. Thirdly, when a turtle is on land it often retracts its head, covering some of the scales and making the deciphering of facial patterns tricky. To avoid at least the second impediment, Jean *et al*. (2010) suggested taking photos during the nesting event only after washing the head with seawater.

Our results indicate that the most informative pattern in turtles studied on land come from the second column of scales of the right side of the head, continued by the post-orbital ones. This is mostly due to the sand that collects on the head while the female digs as well as the retraction of the head. Marshall and Cooper (1988) pointed out that the existence of the secretions from the salt gland created a humid patch on which particles of sand can easily accumulate, further limiting the observation of the post-orbital scales to the eyes. However the only error found was not in the post-orbital scales. Another previously mentioned disadvantage during nesting is that while the female is in her nesting position, she usually takes positions that sometimes impede the full extension of the neck. In these cases the shell covers part of the scales or an entire column of them, usually, the third column and beyond. These are the reasons that determine that the second column was the easiest to observe, with limited observation very rare due to shell or sand.

Although the second column presents a smaller likelihood for loss of information, this is not enough to distinguish an individual from the rest of the population. Therefore a 49 49ll information should be supplemented with the post-orbital and the third

column of scales. Our results demonstrate that the combination of the three columns is accurate enough to identify individuals. Analysis of other columns such as the fourth is not necessary or recommended because of interference from the carapace.

Despite photo-identification in turtles has had different approaches, all authors agrees in the fact unlike many animals, marine turtles have distinctive facial characteristics, making them ideal candidates for photo-ID systems (Carter *et al*., 2014). Photo-identification method used here proved to be feasible for individual turtle identification in sandy beach conditions. Although it can sometimes fail, it can be used to give important information to help recognize and identify re-migrant animals and serve as a useful complement to tagging programs for long-term monitoring of sea turtles.

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