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Pre and post-settlement movements of juvenile green turtles in the Southwestern Atlantic Ocean

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A B S T R A C T

Detailed knowledge on migratory routes connecting distant breeding, developmental and foraging areas is a key prerequisite for the successful management of marine vertebrates. The present study combines stable isotopes analysis of carapace scutes and satellite tracking of juvenile green turtles as an experimental approach to understand the pre and post settlement (recruit to neritic habitats) movements in the Southwestern Atlantic Ocean. To this end, carapace scute biopsies were collected from 20 turtles foraging on coastal rocky outcrops in East Uruguay and sliced in successive 30-µm layers using a cryostat. The $\delta^{15}N$ and $\delta^{13}C$ values of the newest layer increased significantly with turtle size and they also increased from the oldest to the newest layer in most of the individuals. According to the regional isoscape, such a pattern was consisted with the shift from tropical, oceanic habitats to neritic habitats in northern Brazil and the subsequent southward movement along the coast. Using the $\delta^{15}N$ values of the scute layers, seven turtles were considered new-settlers and 13 residents, as only the latter had $\delta^{15}N$ and $\delta^{13}C$ values consistent with those of local potential prey. According to satellite tracking, some resident turtles perform short seasonal migrations to northern Brazilian waters during the austral winter. This behaviour is also recorded in scute layers as a small drop of $\delta^{15}N$ values. The present study thus provides empirical evidence supporting the hypothesis that turtles reaching 40–45 cm of curved carapace length arrive to Uruguayan waters following a coastal migratory route along the Brazilian coast and confirms the existence of seasonal movements between Uruguayan and South-Brazilian waters and a high fidelity for feeding grounds in the Southwestern Atlantic Ocean.

1. Introduction

Many marine vertebrates perform long migrations connecting distant breeding, developmental and adult foraging areas (Webster et al., 2002). Hence, the detailed knowledge of these regular movements is required for the successful management of those species (e.g. González-Solís et al., 2007; Scott et al., 2012; Graham et al., 2012; López-Castro et al., 2014). Recently, advances in biologging have increased greatly our knowledge about those processes (Rutz and Hays, 2009; Bograd et al., 2010; Payne et al., 2014), but the migratory routes of some species still remain largely unknown, in part because of the impossibility of instrumenting too small juvenile individuals and the incapacity of retrieving archival tags from juveniles engaged in multi-year dispersal.

Stable isotope ratios in tissues such as feathers, whiskers, carapace scutes, dentine and bones provide an alternative approach to reconstruct ontogenetic changes in diet and habitat, although resolution is often coarse (e.g. Hobson and Sease, 1998; Hobson and Schell, 1998; Reich et al., 2007; Newland et al., 2011; Borrell et al., 2013; Tomaszewicz et al., 2017). The basic assumptions of this method are (1) that stable isotope ratios in animal tissues integrate those in diet, plus a trophic discrimination factor that is tissue, diet and taxa specific (Caut

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et al., 2008); (2) that metabolically inert tissues represent a consumer's diet at the time of deposition and hence these tissues can be used as a timeline of the consumer's isotopic history; and (3) that variations of stable isotope ratios across habitats (isoscapes) are known (Bowen, 2010; Graham et al., 2010; Somes et al., 2010; Maggozi et al., 2017). The applications of stable isotope analysis (SIA) in marine ecosystems have increased substantially in recent decades as analytical cost has decreased, the capabilities of laboratories and the statistical methodologies for interpretation of isotopic data have improved and become more accessible for non-specialized researchers (Jackson et al., 2011; Layman et al., 2012; Navarro et al., 2013).

Most cheloniid turtles have complex life cycles encompassing huge areas across entire oceans and involving both neritic and oceanic habitats (Bolten, 2003; Meylan et al., 2011). Early juveniles are typically oceanic and usually recruit to neritic habitats after a few years, which results into the spatial separation between juveniles and adults, something rather unusual in animals with direct development (Congdon et al., 1992; Meylan et al., 2011). Available evidence indicates that juvenile cheloniid turtles have some control of their movements (Lohmann et al., 2001; Mansfield et al., 2014; Putman and Mansfield, 2015; Briscoe et al., 2016), but their tracks largely agree with those of major currents, which in turn determine the migratory routes they will latter follow as adults (Scott et al., 2014a; Cardona and Hays, 2018).

The green turtle, *Chelonia mydas,* is a circumglobal species occurring throughout tropical and to subtropical waters throughout the world's oceans (Seminoff et al., 2003). The migratory movements of adult females are relatively well known worldwide thanks to satellite telemetry and passive tagging (Luschi et al., 1998; Broderick et al., 2007; Troëng et al., 2005; Seminoff et al., 2008; Stokes et al., 2015; Christiansen et al., 2017). Conversely, most of our knowledge about the early years of life of green turtles has been inferred through oceanographic modeling (Monzón-Argüello et al., 2010; Putman and Naro-Maciel, 2013; Scott et al., 2014a) and changes in the stable isotope ratios of C, N and S across the layers of carapace scute (Reich et al., 2007; Cardona et al., 2009, 2010; Vander Zanden et al., 2013) although juveniles have also been satellite-tracked (Gonzalez Carman et al., 2012; Putman and Mansfield, 2015; Williard et al., 2017). The keratinous tissue of carapace scutes is inert, grows continuously and scute layers offer a record of several intrinsic biomarkers such as metal levels and stable isotope ratios of C and N (Alibardi, 2005; Reich et al., 2007, 2008; Hobson, 2008; López-Castro et al., 2013, 2014). Moreover, scutes can be sampled from live turtles more easily than other tissues.

The relative abundance of light and heavy isotopes of carbon (C), reported as $\delta^{13}C$, is widely used as a habitat marker (e.g. Cardona et al., 2009, 2010) and provides information on foraging strategies and feeding locations (Newsome et al., 2010; De Niro and Epstein, 1978; Drago et al., 2016); for example, to identify oceanic or neritic feeding habits (e.g. Reich et al., 2007; Eder et al., 2012). On the other hand, the relative abundance of light and heavy isotopes of nitrogen (N), reported as $\delta^{15}N$, is widely used to asses trophic level (e.g. Cardona et al., 2009) but can also be used as a habitat marker at a basin scale (e.g. Cardona et al., 2014).

Ascension Island (UK overseas territory, Fig. 1) is the main nesting rookery for the green turtle in the South Atlantic Ocean (Weber et al., 2014 and references therein) and the source of most green turtles in foraging aggregations in the Southwestern Atlantic (SWA hereafter) (Caraccio, 2008; Monzón-Argüello et al., 2010; Naro-Maciel et al., 2012; Proietti et al., 2012; Prosdocimi et al., 2012). Virtual particle drifting experiments and studies based on Lagrangian drifters suggest that hatchlings from Ascension would drift westward directly to the coast of Brazil (close to latitude 10°S) in less than one year and then will move southward, reaching Uruguay (latitude 33°S) in < 4 years (Putman and Naro-Maciel, 2013; Scott et al., 2014a). The second largest rookery in the South Atlantic Ocean is Trindade Island (Fig. 1), closer to the Brazilian coast, with clearly favorable ocean currents for turtles to arrive to the feeding grounds of South Brazil, Uruguay and Argentina (Proietti et al., 2012), although actual tracking data are lacking.

The values of $\delta^{15}N_{plankton}$ in the SWAO are known to decrease as much as 5‰ eastward from Ascension Island (7°56′S 14°22′W) to Paraiba (Brazil, 7°09′S 36°49′W) and then increase as much as 4‰ southward from Paraiba to Uruguay (33°44′S 53°21′W) (Somes et al., 2010; Navarro et al., 2013). Hence, the $\delta^{15}N$ values of the carapace scutes of green turtles would offer a good method to validate the above-proposed migratory route for early juvenile green turtles in the SWAO (Fig. 2), as far as they are not confounded by changes in $\delta^{15}N$ associated to the ontogenetic dietary shift (Vélez-Rubio et al., 2016). The same approach, combined with satellite telemetry, should allow detecting seasonal movements and provide insights into the overwintering strategy of juvenile green turtles feeding in Uruguayan waters. This is critical, because green turtles inhabiting temperate regions may suffer hypothermic stunning in winter unless they migrate to warmer waters (Broderick et al., 2007), move to oceanic waters or perform a brumation strategy (Vélez-Rubio et al., 2016; Vélez-Rubio et al., 2017).

In this context, the objectives of the present study are 1) to test the hypothesis that most juvenile turtles disperse from Ascension Island to Uruguay moving along the coastal waters of Brazil, 2) to assess the size when juvenile greens settle in the coastal waters of Uruguay and 3) to describe the seasonal migrations between Uruguay and Brazil.

2. Material and methods

2.1. Study site

The Uruguayan coast (710 km length) is part of a complex hydrological system comprising the frontal zone of the Rio de la Plata estuary and the Atlantic Ocean (Fig. 1), with a prevalence of the Malvinas/Falkland current during the austral winter and the Brazilian current during the austral summer (Garcia, 1998; Ortega and Martínez, 2007). This causes variations of > 15 °C in sea surface temperature (range 10–27 °C) throughout the year (Acha et al., 2004). The coastline is a succession of sandy beaches separated by rocky outcrops rich in mussels and macroalgae (see e.g. Borthagaray and Carranza, 2007; Gónzalez-Etchebehere et al., 2017). Juvenile green turtles are found within the whole area, but the major foraging grounds are located in the rocky outcrops at Canelones, Maldonado and Rocha Departments (López-Mendilaharsu et al., 2006; Vélez-Rubio et al., 2013).

2.2. Turtle capture, sample collection and sample processing

Green turtles were captured by the local NGO Karumbé as part of a long-term study on the abundance and habitat use of green turtles in the east coast of Uruguay, mainly in two coastal-marine protected areas (CMPA), Cerro Verde e Islas de La Coronilla and Cabo Polonio (Fig. 1). Turtles were captured alive while feeding over rocky and sandy bottoms < 5 m deep. Set nets (nylon monofilament, 50 m length × 3 m depth, 30 cm stretched mesh size) were deployed perpendicular to wave direction and were monitored constantly to avoid turtle drowning (see Martinez Souza, 2014 for more details of the capture methodology). Curved Carapace Length (CCL notch to tip) was measured for each turtle using a flexible tape (error = 0.1 cm) and all turtles were tagged with Inconel flipper tags before release. All turtles captured were in good condition after sampling and tagging and were released at the site of capture.

Carapace samples were collected from 20 juvenile green turtles in CMPAs of Cerro Verde and Cabo Polonio and adjacent areas of Rocha Department, from January to April in 2012 and 2013. We selected this period because these are the months with higher number of green turtles in the study area (Vélez-Rubio et al., 2013; López-Mendilaharsu et al., 2016). Biopsies were collected from the posterior medial region of the third left lateral scute of each individual, close to the posterior margin using a 6-mm diameter Miltex biopsy punch (Fig. 2), following Reich et al. (2007). Only the biopsy samples going all the way through