UNIVERSITAT DE BARCELONA

Ecología trófica y análisis del grado de omnivoría de las tortugas verdes en la costa atlántica occidental

Trophic ecology and analysis of the degree of omnivory of the green turtles on the Western Atlantic Coast

Patricia Campos Pena

ECOLOGÍA TRÓFICA Y ANÁLISIS DEL GRADO DE OMNIVORÍA DE LAS TORTUGAS VERDES EN LA COSTA ATLÁNTICA OCCIDENTAL

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TROPHIC ECOLOGY AND ANALYSIS OF THE DEGREE OF OMNIVORY OF THE GREEN TURTLES ON THE WESTERN ATLANTIC COAST

Patrícia Campos Pena

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ECOLOGÍA TRÓFICA Y ANÁLISIS DEL GRADO DE OMNIVORÍA DE LAS TORTUGAS VERDES EN LA COSTA ATLÁNTICA OCCIDENTAL

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O mundo além das palavras –

Dentro deste mundo há outro mundo
impermeável às palavras.
Nele, nem a vida teme a morte,
nem a primavera dá lugar ao outono.

Histórias e lendas surgem dos tetos e paredes,
até mesmo as rochas e árvores exalam poesia.
Aqui, a coruja transforma-se em pavão,
o lobo, em belo pastor.

Para mudar a paisagem,
basta mudar o que sentes;
E se queres passear por esses lugares,
basta expressar o desejo.....

Rumi

Un día hablado con mi prima bióloga, Débora Campos, me dijo: “porque no intentas seguir tú carrera en Biología estudiando el medio marino, estás muy conectada con el mar”. En esta época disfrutaba de mis vacaciones en la playa, encantada de estar cerca del mar y disfrutar de la energía de su entorno. Con sus palabras pude reflexionar acerca de mi continuidad en el mundo de la Biología, sin embargo, sabía que el camino podría ser muy largo ya que en la universidad donde estudiaba sería imposible seguir los estudios en Biología Marina. Muchas gracias Débora por tú sabia intuición. Al terminar el grado, tenía la intención de irme fuera del país, quería conocer otra cultura otros lugares, pensé dónde podría ir a vivir por un tiempo. Había escuchado una vez a alguien hablando sobre Barcelona, pero no tenía ni idea. Estuve planeando el viaje con una amiga, Roberta Moura, que me dijo: “me gustaría pasar un tiempo en Barcelona”; y fue entonces cuando el gran viaje empezó. Gracias Roberta.

Yo tenía la intención de hacer un máster y pensé que quizás Barcelona podría ser el sitio ideal ya que encontraría grupos de investigaciones trabajando en el medio marino. ¡Era el comienzo! Tras un tiempo viviendo en Barcelona, empecé un máster y con ello empezó la búsqueda de alguien con quien pudiese hacer mi tesina de máster, fue entonces cuando encontré a Luis Cardona. La persona que tanto me inspiró con su sabiduría, creatividad y su gran interés por la investigación, así como su pasión y energía motivadora. Gracias por el conocimiento transmitido en muchos momentos, ha sido fundamental para seguir adelante. Terminé el máster, volví a Brasil y años después conseguí una beca para volver a Barcelona y hacer la tesis con Luis. Increíbles las coincidencias de la vida. Es por esto que quisiera dar las muchas gracias a ti Luis, por la oportunidad de desarrollar este trabajo bajo tú supervisión. No tengo palabras para agradecerle tú voto de confianza y por acompañarme en este largo viaje. En todos los momentos incluso en los más difíciles pude sentirme respaldada y acompañada.

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CONTENIDOS

Ecología trófica y análisis del grado de omnivoría de las tortugas verdes en la costa Atlántica Occidental

Resumen

Los hábitats utilizados por las tortugas marinas durante su desarrollo varían notablemente en cuanto a características ambientales y disponibilidad de alimento. Comprender los factores que determinan la selección del hábitat y de la dieta resulta esencial para comprender la interacción que existe entre dichas especies y sus hábitats. Dicho conocimiento también proporciona datos para adoptar medidas de gestión y conservación destinadas a restaurar y mantener las poblaciones de tortugas marinas y así, su función ecológica en el ecosistema. Las zonas costeras de Brasil, Uruguay y Argentina albergan diversos hábitats de importancia para la alimentación de los juveniles de la tortuga verde, pero todavía existe un gran desconocimiento sobre la ecología trófica de esta especie durante la fase juvenil de transición desde hábitats pelágicos a bentónicos. En esta tesis se ha estudiado cómo se produce el asentamiento de las tortugas verdes en la costa de Brasil, incluyendo la selección del hábitat y la adquisición de un microbioma adecuado para la digestión de material vegetal. Los resultados demuestran que el asentamiento de las tortugas verdes a lo largo de la costa oriental de Sudamérica está fuertemente influido por la corriente del Brasil y que el cambio ontogenético es rápido en las zonas tropicales y más lento en las subtropicales, a pesar de la rápida adquisición de una microbiota capaz de degradar polisacáridos complejos en ambas zonas. Dicha microbiota resulta en una gran eficiencia en la digestión de algas, especialmente de las Rodophyta, pero no impide la digestión de presas animales. Por último, las tortugas juveniles seleccionan de forma preferente para su alimentación zonas de arrecife de baja rugosidad, someras y con poco coral vivo.

Palabras claves: Tortugas marinas, Atlántico Sur, tortuga verde, asentamiento, uso del hábitat; microbiana; ecología alimentar; digestibilidad, arrecifes tropicales

Abstract

The habitats used by marine turtles during their development vary largely in environmental conditions and food availability. Understanting habitat selection is critical to the implementation of succesful management and conservation actions aiming to restore and maintain sea turtle populations and, thus, their ecological function in the ecosystem. The coastal areas of Brazil, Uruguay and Argentina have a diversity of coastal habitats supporting feeding grounds for the green turtle, but there is still a lack of knowledge about the feeding ecology of this species during the juvenile transition phase from pelagic habitats to neritic habitats. This thesis has studied the settlement of green turtles in the coastal habitats of Barzil, including the ontogenetic diet shift. Results show that the settlement of green turtles along the eastern coast of South America is strongly influenced by the Brazilian current and that the ontogenetic change is fast in the tropics and slower in the subtropics, despite a fast acquisition of a microbiota capable of fermenting complex polysaccharides shortly after settlement. Such microbiome is highly efficient for the digestion of macroalgae, particularly Rodophyta, but does not impede the digestion of animal matter. Finally, preferred foraging grounds are are shallow, have a low rugosity and little live coral.

Keywords: Sea turtles, South Atlantic, green turtle, settlement, habitat use; microbiome; feed ecology; digestibility, tropical reefs

Informe del Director

El Dr. Lluís Cardona, director de la tesis doctoral titulada " **Ecología trófica y análisis del grado de omnivoría de las tortugas verdes en la costa Atlántica Occidental**", certifica que la presente tesis ha sido realizada íntegramente por la doctoranda Patricia Campos Pena**.** Esta ha participado activamente en la planificación y preparación de cada uno de los artículos presentados en esta tesis doctoral. En concreto, la contribución de la doctoranda para cada artículo ha incluido: la planificación de los objetivos, el análisis de laboratorio de las muestras, el análisis de los resultados, la redacción del artículo y la revisión final. Cabe decir que ninguna información de estas publicaciones ha sido ni será utilizada en otras Tesis Doctorales exceptuando el artículo titulado *Contribution of green turtles to herbivore biomass in shallow tropical reefs.* Este artículo también forma parte de la tesis doctoral de la última autora, Adriana Velasquez, de la Universidad de Barcelona. La contribución de la doctoranda Patricia Campos Pena a dicho artículo ha sido la recogida de muestras en Fernando de Noronha, el análisis del conjunto de las muestras y la redacción científica.

Listado de artículos (en orden cronológico), con su estado de publicación y el factor de impacto de las revistas correspondientes:

Campos, P., Guivernau, M., Prenafeta-Boldú, F. X., & Cardona, L. (2018) Fast acquisition of a polysaccharide fermenting gut microbiome by juvenile green turtles *Chelonia mydas* after settlement in coastal habitats. *Microbiome*, 6(1), 69. doi:10.1186/s40168-018-0454-z. **Factor de impacto: 9.13.**

Campos, P., & Cardona, L. (2019) Individual variability in the settlement of juvenile green turtles in the western South Atlantic Ocean: relevance of currents and somatic growth rate. *Marine Ecology Progress Series*, 614, 173-182. doi: 10.3354/meps12909. **Factor de impacto: 2.72.**

Campos, P., & Cardona, L. (2019) Trade-offs between nutritional quality and abundance determine diet selection in juvenile benthic green turtles (en revision).

Cardona, L., **Campos, P**., Velasquez, A. (2019) Contribution of green turtles of herbivore biomass in shallow tropical reefs (en prep).

Barcelona, 19 de junio de 2015

Dr. Lluís Cardona Pascual

Introducción General

1. El ciclo de vida de las tortugas marinas

Las tortugas marinas son reptiles con un ciclo de vida largo y complejo, caracterizados por presentar un crecimiento lento y una maduración sexual tardía (Miller 1997; Bjorndal et al. 2000; Chaloupka y Limpus 2002). De las siete especies de tortugas marinas existentes, todas salvo la tortuga laud (*Dermochelys coriacea*), tienen un ciclo de vida similar, aunque la duración de cada fase puede variar en las diferentes poblaciones y especies (Parmenter 1983; Miller 1997; Hawkes et al. 2006; Da Silva et al. 2011). Durante su ciclo biológico explotan diferentes hábitats en diferentes fases de su ciclo de vida, entre las que se incluyen el ambiente terrestre, donde desovan y realizan todo el desarrollo embrionario. De todas formas, los machos son totalmente marinos y no emergen a las playas de nidificación en toda su vida (Pritchard 2017).

La vida de las tortugas marinas viene determinada en gran parte por su amplia dispersión por hábitats oceánicos como neonatos y juveniles (Putman y Naro-Maciel 2013; Mansfield et al. 2014; Briscoe et al. 2016), así como de la fidelidad de los adultos a las áreas de alimentación y a las playas de anidación (Avens et al. 2003; Bowen y Karl 2007; Broderick et al. 2007). Una vez alcanzan la madurez sexual, las tortugas adultas alternan migraciones regulares entre las zonas de alimentación y las de reproducción, en un ciclo de acumulación de energía que apoya el esfuerzo reproductivo. Tras un periodo de alimentación de varios años, las hembras migran desde las áreas de reproducción hacia las proximidades de las áreas donde nidifican (Limpus 1992; Bowen et al. 2005). El periodo de remigración de los machos es mucho más corto y a menudo se aparean anualmente (Limpus 1993; Hays et al. 2010). El periodo de apareamiento precede al periodo anidación, puede durar varias semanas e implica múltiples cópulas con diversas parejas, tanto en el caso de los machos como de las hembras. Tras varias semanas de apareamiento, los machos

vuelven a sus zonas de alimentación, y las hembras continúan en las cercanías de las zonas de nidificación (Limpus y Reed 1985; Limpus y Miller 1993; Miller 1997). Al cabo de unas semanas, salen a las playas a poner los huevos en nidos excavados en la arena(Schofield et al. 2010; Arendt et al. 2012). Durante una misma temporada, salen a la playa entre 1 y 5 veces para excavar sus nidos, Una vez concluida la última puesta, la hembra regresa a su zona de alimentación para recuperarse durante varios años, antes de llegar a las siguientes temporadas reproductivas.

Tanto las hembras como los machos presentan filopatría, comportamiento por el cual las hembras tienden a nidificar en su lugar de nacimiento y los machos retornan a las aguas próximas a las playas donde nacieron para aparearse, (Bjorndal et al. 2005; Bjorndal et al. 2006; Clusa et al. 2013; Grossman et al. 2019). De todos modos, el grado de fidelidad puede presentar algunas variantes entre especies y poblaciones. La filopatría es una estrategia altamente favorable para las hembras, ya que aseguran la viabilidad de la puesta al emplear playas de eficacia conocida, también lo es para los machos, ya que incrementa la probabilidad de encontrar una hembra con la que emparejarse (Schofield et al. 2009). De todos modos, la filopatría es relativa y la fidelidad ha de entenderse en un radio de varios cientos de kilómetros en lugar de la playa exacta donde nació (Bowen y Avise 1996; Lohmann et al. 2008).

El tiempo de incubación depende de la temperatura y la eclosión se produce tras unos 55 días de incubación (Limpus y Reed 1985; Miller 1985). El lapso entre la eclosión y la emergencia varía según la compactación de la arena, la temperatura y la profundidad del nido (Miller et al. 2003). En los primeros estadios, los recién nacidos antes de adentrarse en el mar podrían identificar la posición geográfica de las playas de nacimiento a través de los mecanismos de impresión geomagnética y de los olores y sustancias químicas disueltas en el agua (Lohmann y Lohmann 2003). Gracias a la impresión geomagnética, las tortugas son capaces de

detectar su posición en el campo magnético terrestre y a medida que crecen pueden discernir la posición que se encuentran de su zona de nacimiento (Lohmann y Lohmann 2003). Además, gracias a la impresión química las tortugas reconocen características distintivas del área específica de anidación a escala local, que podrían estar asociado con una filopatria precisa (Lohmann y Lohmann 2003; Lohmann et al. 2008). Una vez en la fase adulta, serán estos mecanismos de orientación y navegación los que permitirán el reconocimiento de la playa de nidificación y posibilitará a los adultos reproductores rastrear la posición original de sus áreas de origen, migrando desde las áreas de alimentación muy distantes hasta las áreas específicas de ovoposición (Carr 1967; Lohmann y Lohmann 1996; Lohmann et al. 2004; Lohmann et al. 2013). La próxima fase implica el transporte pasivo de los neonatos por las corrientes principales hacia las zonas de alimentación oceánicas, seguida de una etapa nerítica más o menos estricta dependiendo de la especie y población (Luschi et al. 2003).

La tortuga verde (*Chelonia mydas*) está ampliamente distribuida en las aguas tropicales y subtropicales en todos los océanos (Broderick et al. 2006; Chaloupka et al. 2008; Lemons et al. 2011; Scott et al. 2012). Las principales áreas de puesta de la tortuga verde se concentran en playas de latitudes tropicales y ocasionalmente en las subtropicales de todos los océanos, tanto en playas remotas continentales como en playas aisladas en islas oceánicas. En el Atlántico Sur, las playas de desove se ubican se en las islas oceánicas: Ascensión, Fernando de Noronha, Trindade, Atol das Rocas, Bioko, Santo Tomé y Bijagos (Bjorndal et al. 2006; Formia et al. 2006; Formia et al. 2007; de Padua Almeida et al. 2011). De todas ellas, la más importante es la isla de Ascensión (Weber et al. 2014).

Las tortugas verdes presentan uno de los ciclos más complejos de todas las tortugas marinas, pues no sólo implica cambios de hábitat, sino también cambios radicales de dieta (Reich y Arnould 2007; Arthur et al. 2008; Cardona et al. 2009; Cardona et al. 2010; Parker et al. 2011; Vélez-

Rubio et al. 2016). El asentamiento de los juveniles en los hábitats neríticos es un proceso crítico y el lugar exacto de asentamiento es fruto tanto de la selección activa como del efecto de las corrientes oceánicas, pues determinan en gran medida las trayectorias de los recién nacidos y de los juveniles antes del asentamiento (Naro-Maciel et al. 2017; Monzón-Argüello et al. 2018).

2. Las corrientes oceánicas en la biología de la tortuga verde

Las diferentes etapas de la vida de las tortugas marinas transcurren en diferentes hábitats que frecuentemente se encuentran separados por centenares o miles de kilómetros y que están conectados por migraciones complejas (Proietti et al. 2012). Dichos hábitats se clasifican como áreas juveniles de alimentación pelágica, áreas de asentamiento, hábitats de desarrollo de los juveniles neríticos y, finalmente, aéreas de alimentación de los adultos (Cowen y Sponaugle 2009).

En general, se asume que, tras el nacimiento, los neonatos de las tortugas marinas viajan largas distancias, iniciando el recorrido una vez salen del nido. Una vez en el mar, los recién nacidos comienzan a nadar de forma hiperactiva durante 24-48 horas para alcanzar las principales corrientes marinas (Luschi et al. 2003; Naro-Maciel et al. 2007; Scott et al. 2014a). Durante esta fase, pasan la mayor parte del tiempo en la superficie y nadan sin cesar. Una vez alcanzan las zonas oceánicas, los pequeños juveniles presentan un comportamiento y alimentación epipelágicas, pasando la mayor parte del tiempo en los primeros metros de la columna de agua (Bjorndal 1997; Musick y Limpus 1997; Meylan y Meylan 1999). Ello se debe a su flotabilidad positiva y a su limitada capacidad de natación (Milsom 1975; Minamikawa et al. 1997). No obstante, los neonatos parecen capaces de seleccionar hábitats oceánicos que les proporcionan

mayor beneficio térmico, lo que resulta favorable para su crecimiento, alimentación y supervivencia (Mansfield et al. 2014) .

A medida que crecen, los juveniles pueden reorientarse gracias a la natación direccional a fin de evitar áreas desfavorables para la supervivencia y permanecer en las corrientes preferidas (Prange 1976; Reich et al. 2007; Putman y Mansfield 2015). De esta manera, el movimiento de las tortugas marinas tendrá siempre la contribución de las corrientes oceánicas, aunque a medida que crecen mejora la capacidad de natación de los juveniles y disminuye la influencia de las mismas, hasta volverse irrelevantes en los adultos (Luschi et al. 2003; Hughes et al. 2006; Bentivegna et al. 2007; Hays et al. 2010; Hawkes et al. 2011).

El conocimiento sobre los primeros años de vida de las tortugas verdes ha sido inferido a través de modelos oceanográficos de dispersión de partículas virtuales y marcadores genéticos (Monzón-Argüello et al. 2010; Putman y Naro-Maciel 2013; Scott et al. 2014a; Cardona y Hays 2018). En Brasil, la presencia no reproductiva de tortugas verdes se da en toda la costa, aunque generalmente se trata de juveniles (Marcovaldi y Dei Marcovaldi 1999). Dichos ejemplares proceden principalmente de dos grandes zonas de nidificación; la primera es la Isla de Ascensión, considerada la principal fuente de agregaciones de los juveniles en áreas de alimentación en el Océano Atlántico Sur; la segunda es la Isla de Trindade (Figura 1), con corrientes oceánicas favorables para que los juveniles lleguen a las zonas de alimentación del sur de Brasil, Uruguay y Argentina (Proietti et al. 2012). Sin embargo, la contribución de Trinidade es difícil de estimar y algunos factores, como la baja temperatura, podrían contribuir negativamente a la llegada de tortugas verdes que llegan a la costa en el Atlántico Sur Occidental. Además, las tortugas verdes nacidas en Trinidade pueden llegar a la costa brasileña con un tamaño demasiado pequeño para el reclutamiento y desviarse con la Corriente del Atlántico Sur a la costa africana, o incluso ser transportadas directamente desde

Trindade a África Occidental, donde podrían reclutarse (Marcovaldi y Dei Marcovaldi 1999; Proietti et al. 2012).

En cambio, los experimentos de simulación de partículas sugieren que los neonatos nacidos en Ascensión se desplazaran directamente hacia la costa nordeste del Brasil (Putman y Naro-Maciel 2013) (Figura 1). En este contexto, la pregunta relevante es si todos los juveniles de tortugas verdes que habitan la costa oriental de América del Sur siguen una migración de desarrollo similar a lo largo de la costa brasileña o si la mayoría se asienta inmediatamente después de llegar al noreste de Brasil. Se sabe que la gran mayoría de los individuos no son lo suficientemente grande al llegar en hábitats costeros en el nordeste de Brasil como para ser capaces de asentarse en el medio bentónico (Lenz et al. 2017). Por lo tanto, cabe esperar que la mayor parte de los individuos continúen su desplazamiento hacia el sur con la corriente de Brasil, asentándose en hábitats de desarrollo subtropicales del centro y sur de Brasil (Gallo et al. 2006; Poli et al. 2014; Jardim et al. 2015; Santos et al. 2015), Uruguay (Vélez-Rubio et al. 2018) y en el norte de Argentina (González Carman et al. 2012)

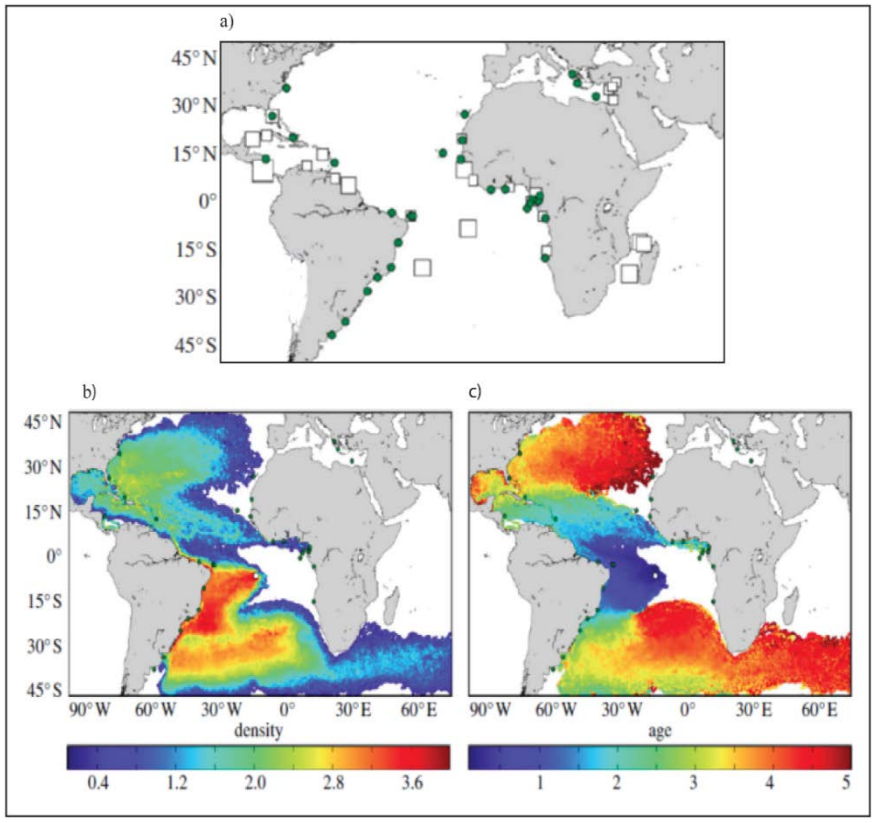

Figura 2: Panel a. Zonas de nidificación (cuadrados blancos; el tamaño de los cuadrado indica el tamaño relativo de las zonas de nidificación) y de alimentación (círculos verdes; la abundancia en las zonas de alimentación se desconoce) de la tortuga verde en el Atlántico; Paneles b y c. Distribución predicha de 13 500 partículas virtuales liberadas en las zona de nidificación de la Isla de la Ascensión; El panel de la izquierda indican la densidad de partículas por celda de la cuadrícula en todas las simulaciones (contabilizadas diariamente, escala logarítmica, no ponderada por el tamaño de la población). El panel de la derecha indica la edad media (en años) de partículas. Figura extraída de Putuman & Naro-Maciel (2013).

3. El cambio ontogenético de la dietan en la tortuga verde

Una vez en áreas costeras, los juveniles de la tortuga verde sufren un importante cambio de dieta. Los juveniles oceánicos tienen una dieta omnívora con una fuerte componente animal. En cambio, tras sus asentamientos adoptan una dieta omnívora con predominio de presas vegetales (Reich y Arnould 2007; Arthur et al. 2008; Cardona et al. 2009; Cardona et al. 2010; Carrión-Cortez et al. 2010; Parker et al. 2011; González Carman et al. 2012; Santos et al. 2015; Howell et al. 2016; Vélez-Rubio et al. 2016). El patrón de este cambió ontogenético de la dieta varía entre regiones, con fuertes diferencias entre zonas tropicales y subtropicales (Reich et al. 2007; Arthur et al. 2008; Cardona et al. 2009; Cardona et al. 2010; Howell et al. 2016; Vélez-Rubio et al. 2016). En general, en zonas subtropicales el cambio ontogénico es más lento y reversible, mientras que es rápido y representa una transición abrupta e irreversible sólo en algunas regiones tropicales caracterizada por extensas praderas de fanerógamas marinas (Bjorndal 1997; Reich et al. 2007; Arthur et al. 2008).

El cambio ontogenético de dieta de las tortugas verdes resulta notable, pues los alimentos vegetales resultan difíciles de digerir para la mayor parte de los vertebrados en ausencia de una microbiota intestinal específica. El valor nutritivo de un alimento deriva no sólo de su contenido en nutrientes y energía, sino también de su disponibilidad (Van Soest 1982). Un alimento puede contener altos niveles de nutrientes y energía y aun así ser de bajo o nulo en valor nutritivo o energético, principalmente si no puede ser digerido y asimilado adecuadamente. Por ello, los animales herbívoros maximizan la adquisición de energía y de los nutrientes esenciales mediante la ingestión de grandes cuantidades de alimento (Mattson 1980; Fleming 1995; Danell et al. 2006). En cambio, los carnívoros intentan maximizar el consumo de energía maximizando la tasa

de captura y minimizando el costo de captura (White 1978; Stephens y Krebs 1986; Kohl et al. 2015).

En el medio marino, la dieta de los mamíferos herbívoros (dugongos y manatís) está dominada por fanerógamas, mientras las iguanas marinas se alimentan solo de macroalgas (Murray et al. 1977; Aketa et al. 2003; Lanyon y Sanson 2006; Hong et al. 2011; Worthy y Worthy 2013). Las tortugas verdes exhiben dietas más flexibles que incluyen pastos marinos, macroalgas e invertebrados (Hays-Brown y Brown 1982; Bugoni et al. 2003; Amorocho y Reina 2007; Carrión-Cortez et al. 2010; González Carman et al. 2014). Los pastos marinos constituyen la base de la dieta de las tortugas verde en todo el Atlántico norte occidental y mar del Caribe (Bjorndal 1980; Mortimer 1982; Bjorndal et al. 2005; Moran y Bjorndal 2005; Williams et al. 2014; Howell et al. 2016), en el Océano Índico (Ballorain et al. 2010; Lal et al. 2010; Burkholder et al. 2011) e incluso a zonas subtropicales del Mediterráneo (Cardona et al. 2010) y el Atlántico africano (Cardona et al. 2009; Monzón-Argüello et al. 2018). La dieta de las tortugas verde en el Atlántico sudoccidental se conoce bastante bien y está basada en algas, pero también invertebrados principalmente en zonas zonas subtopicales y templadas donde el consumo de aumenta (Bugoni et al. 2003; Reisser et al. 2013; Santos et al. 2015; Vélez-Rubio et al. 2016). La gran mayoría de las especies de algas encontradas en la dieta de las tortugas verdes pertenece al grupo de algas rojas (Ferreira 1968; Seminoff et al. 2002; Arthur y Balazs 2008; Reisser et al. 2013; Vélez-Rubio et al. 2016). Aparentemente las tortugas parecen seleccionan especies de macroalgas con alto contenido de nitrógeno y carbohidratos solubles, y bajo contenido de fibra (Bjorndal 1980; Brand-Gardner y Limpus 1999).

Como ya se ha dicho, los vertebrados herbívoros nos expresan hidrolasas que permitan degradar polisacáridos estructurales complejos (Barboza et al. 2010) y, por lo tanto, requieren de una microbiota intestinal

especial para su digestión. La composición de la microbiota intestinal de los vertebrados no sólo refleja sus relaciones evolutivas, sino que también sus hábitos de alimentación, así como de la ubicación de la cavidad de fermentación en el intestino (Tabla 1) (Edwards y Ullrey 1999; Clauss et al. 2003; Miyake et al. 2015). Entre los herbívoros, los manatís y dugongos presentan una flora intestinal similar dominada por los *Firmicutes* y *Barcteriodetes* (Eigeland et al. 2012; Merson et al. 2014). Dentro de los *Firmicutes* predominan las familias *Clostridiaceae*, *Ruminococcaceae* y dentro de los *Bacteroidetes* las *Bacteroidaceae*. Adicionalmente los dugongos presentaran *Lachnospiraceae* y *Peptostreptococcaceae*, comúnmente asociados a un papel importante en la degradación de las fibra de las fanerógamas marinas en lo intestino posterior de estos animales (Eigeland et al. 2012; Merson et al. 2014). Los peces cirujanos (Acanthuridae), por otra parte, presentan una flora intestinal dominada por *Firmicutes* especialmente la clase *Clostridia*; además, las *Proteobacteria* aparecen en gran cantidad en las especies omnívoras (Miyake et al. 2015). En los reptiles, la microbiota digestiva de las iguanas terrestres (*Iguana iguana* y *Conolophus spp.*) y marinas (*Amblyrynchus cristatus*) está domina por *Firmicutes;* la microbiota de los intestinos de las iguanas terrestres presenta una elevada prevalencia de *Ruminococcaceae*, en contraste con la baja presencia encontrada en las iguanas marinas, donde en cambio abundan las *Lachnospiraceae y Clostridiaceae* (Hong et al. 2011). Por otro lado, la microbiota del intestino las tortugas terrestres *Gopherus polyphemus* y *Geochelone nigra* es muy diferente. Las tortugas gopher presentan una flora intestinal con relativa abundancia de *Firmicutes* pero en menores cantidades en comparación con otros reptiles herbívoros, incluyendo las iguanas marinas y terrestres y la tortuga de las Galápagos (Hong et al. 2011; Yuan et al. 2015). La microbiota de las tortugas gopher está constituida principalmente por *Firmicutes-Clostridia* (*Ruminococcaceae*, *Lachnospiraceae y Clostridiaceae)* y *Barcterioidetes-*

Filo	Familia	Características
Firmicutes		Alta capacidad para descomponer y utilizar energía y los nutrientes de los hidratos de carbono complejos, como celulosa, hemicelulosa y xilano presentes en las fibras vegetales.
	Clostridiaceae *Ruminococcaceae* *Lachnospiraceae*	Anaerobios obligados que fermentan diversos polisacáridos complejos y proteínas para producir alcoholes y ácidos grasos de cadena corta.
Bacteroidetes		Contribuir significativamente al ataque inicial en hidratos de carbono simples y complejos.
	Prophyromonas *Bacteroidaceae*	Capaces de descomponer polisacáridos tales como agar, carragenina, laminarina y porfirano.
Spiroquetas		Grupo de bacterias no celulolíticas, que se asocian con los sustratos vegetales específicos durante la digestión, facilitando la descomposición de la celulosa por las bacterias coexistentes.
Proteocbacterias		Incapaces de degradar polisacáridos estructurales complejos. Son conocidas por establecer relaciones patógenas y simbióticas con sus anfitriones.
Verrumicrobiaceae		Inducen inflamación intestinal y está asociado a enfermedades colon en mamíferos, pero nada se sabe sobre su patogenicidad en reptiles.

Tabla 1: Filos y familias bacterianas más importantes en la microbiota intestinal de vertebrados omnívoros y herbívoros.

Bacteroidaceae (Yuan et al. 2015). Sin embargo, el microbiana de las tortugas gigante de Galápagos está dominado básicamente por *Firmicutes* con cuantidades relativas de *Ruminococcaceae* y alta abundancia de *Clostridiaceae* (Hong et al. 2011).

El cambio ontogenético de la dieta en vertebrados influye en la composición de la microbiota digestiva (Stevens y Hume 1998) y a su vez se ve facilitado por la adquisición de una flora intestinal capaz de digerir los carbohidratos complejos presentes en la pared celular de las plantas (Bjorndal 1980). Desafortunadamente se sabe muy poco sobre cómo cambia la composición del microbioma de las tortugas verdes durante la transición carnivoría-omnivoría asociada a su asentamiento en hábitats neríticos. Se ha especulado sobre la posibilidad de que el cambio ontogenético más lento en las regiones subtropicales se deba a una adquisición más lenta de un microbioma capaz de degradar polisacáridos estructurales o bien a su menor eficacia a bajas temperaturas. Se ha especulado sobre la posibilidad de que el cambio ontogenético más lento en las regiones subtropicales se deba a una adquisición más lenta de un microbioma capaz de degradar polisacáridos estructurales o bien a su menor eficacia a bajas temperaturas.

4. La selección del hábitat de asentamiento en la tortuga verde

Los hábitats utilizados por las tortugas marinas durante el desarrollo son heterogéneos y presentan una gran variabilidad en las condiciones ambientales y en la disponibilidad de alimentos (López-Mendilaharsu et al. 2005). Como se comentó, las tortugas verdes se asientan en hábitats costeros una vez alcanzan un control eficiente de su natación y flotabilidad y en dicho proceso cambian de dieta. Se desconocen cuáles son los factores que determinan las características del hábitat de asentamiento.

Las praderas marinas extensas escasean en el Atlántico occidental occidental tropical y en la mayor parte del Océano Pacífico tropical (Green et al. 2003), donde los arrecifes rocosos y coralinos constituyen el hábitat principal de las tortugas verdes (Goatley et al. 2012; Santos et al. 2015; Balazs et al. 2017; Becker et al. 2019). Por ello, las macroalgas y los céspedes algales, conocidos también como turf, constituyen la mayor parte de la dieta de tortugas verdes en estas regiones (Arthur y Balazs 2008; Russell y Balazs 2009; Santos et al. 2015). De todos modos, se sabe muy poco sobre el papel de las tortugas verdes en la dinámica de estos ecosistemas, donde los erizos y los peces se consideran los herbívoros más relevantes (Goatley et al. 2012).

Las zonas costeras de Brasil, Uruguay y Argentina albergan diversos hábitats de importancia para la alimentación de la tortuga verde (Ferreira 1968; Bugoni et al. 2003; González Carman et al. 2011; Reisser et al. 2013; Santos et al. 2015), pero todavía existe un gran desconocimiento sobre la ecología alimentaría de esta especie, principalmente de la fase juvenil de transición desde hábitats pelágicos hasta hábitats neríticos. Entender el papel ecológico de la tortuga verde es importante para entender lo que se ha perdido en términos de estructura y función en los ecosistemas costeros fruto del histórico declive de las poblaciones de tortuga verde. Estos datos son importantes para la elaboración de acciones de manejo y conservación para mantener las populaciones de tortugas verdes de modo que éstas sean capaces de seguir desempeñando sus funciones ecológicas.

Objetivos

OBJETIVOS

La presente tesis doctoral tiene como objetivo principal proporcionar un mayor conocimiento sobre la ecología alimentaria de la tortuga verde en la costa Atlántica de América del Sur. La tesis se organiza en torno a cuatro temas principales: dispersión juvenil y cambio ontogenético de la dieta (Capítulo 1), cambios ontogenéticos del microbioma intestinal durante el asentamiento (Capítulo 2), digestibilidad de rodófitos y feófitos por los juveniles de las tortugas verdes (Capítulo 3) y selección del hábitat de alimentación por los juveniles neríticos y sy contribución a la biomasa total de herbívoros en arrecifes tropicales (Capítulo 4).

Para poder hacerlo, ha sido necesario determinar en primer lugar las rutas migratorias de los individuos juveniles desde los hábitats oceánicos hasta la plataforma continental de Sudamérica, asociadas a las corrientes oceánicas. La hipótesis inicial era que la mayoría de las tortugas verdes que habitan las áreas costeras del Océano Atlántico sudoccidental proceden principalmente de las zonas de nidificación de la Isla Ascensión y se asientan en una gran área comprendida entre el norte de Brasil y en norte de Argentina, toda ella bajo la influencia de la Corriente del Brasil. Se esperaba que los juveniles de tortugas verdes que asentaban inmediatamente tras alcanzar el nordeste de Brasil presenten pocos cambios de dieta y zona de alimentación tras el asentamiento, al hacerlo directamente en zonas favoragles. En cambio, los ejemplares asentados más al sur presentarían cambios de dieta y hábita con mayor frecuencia, debido a la necesitad de migrar hacia el norte y también a la mayor inestabilidad ambiental. En el primer capítulo se han evaluado ambas cuestiones mediante las relaciones de isotopos estables de carbono y nitrógeno en las capas de los escudos del caparazón de juveniles de tortugas verdes capturada en hábitats neríticos del nordeste y sudeste de Brasil-

Gracias a ello, ha sido posible reconstruir retrospectivamente sus dietas individuales y los patrones de uso del hábitat.

La segunda cuestión abordad ha sido la caracterización de la composición de la comunidad microbiana de los juveniles de la tortuga verde en función de la dieta y de los diferentes tipos de hábitat, aspectos muy poco documentado en la especie. La hipótesis inicial era que debía producirse un cambio ontogenético de la flora microbiana de las tortugas después del asentamiento en las áreas costeras y en asociación con el aumento del consumo de material vegetal, aunque dicho cambio sea más lento que en las subtropicales que en las tropicales y debería estar influido por el consumo de presas animales. En el según capítulo se ha evaluado ambas hipótesis mediante la caracterización del microbioma digestivo mediante la extracción de ADN microbianao de las heces e identificación de los taxones presentes en base al rRNA 16S. Se han estudiado tanto tortugas salvajes de zonas tropicales y subtropicales como tortugas cautivas con una dieta mixta de pescado y macroalgas. De este modo se ha podido evaluar si: 1) si la composición y la diversidad de la flora microbiana cambiaba gradualmente después del asentamiento y del cambio ontogénico de la dieta de las tortugas; 2) si existían diferencias en la composición y diversidad de la microbiota intestinal de las tortugas verdes de las regiones tropicales y subtropicales; 3) y se el consumo de dietas omnívoras modifica la microbiota intestinal.

Una vez aclarada las cuestiones anteriores se ha procedido a evaluar las diferencias en los coeficientes de digestibilidad y la capacidad para digerir los carbohidratos estructurales de macroalgas de dos grupos diferentes, así como el posible papel de la digestibilidad en la selección de la dieta. Para realizamos un experimento en cautividad, con dietas controladas destinadas a conocer el tiempo de tránsito intestinal y la digestibidilidad.

Por último, investigamos los determinantes del uso del hábitat de los juveniles de tortuga verde en arrecifes coralinos de Fernando de Noronha (03° 50’S, 32° 24’O), situado a 380 km desde la costa noreste de Brasil) y Oahu y Hawái (21° 18′41″N, 157°47′O), situados en la región oceánica del Pacifico Norte Central. En ambas zonas, realizamos censos de tortugas verdes, peces herbívoros (escáridos, acanturidos y kifósido), erizos y macroalgas para documentar el patrón del uso de los hábitats en relación a la disponibilidad de alimento, la cobertura de coral, la rugosidad y la profundidad.

Discusión

La presente tesis doctoral demuestra que el asentamiento de las tortugas verdes a lo largo de las costa oriental de Sudamérica está fuertemente influido por la corriente del Brasil, que el cambio ontogenético es rápido en las zonas tropicales y más lento en las subtropicales a pesar de que la adquisición de una microbiota capaz de degradar polisacáridos complejos es rápida en ambas, y que las tortugas seleccionan de forma preferente para su alimentación zonas de arrecife de baja rugosidad, someras y con poco coral vivo y que dicho patrón de selección se refuerza con la talla.

El conocimiento de la composición de la dieta de la tortuga verde es fundamental para identificar las zonas de alimentación, favoreciendo así su conservación (Seminoff et al. 2002). Comprender los factores que influyen en la composición y variabilidad de la dieta mejora nuestra compresión de las interacciones entre la especie y el hábitat, que a su vez está directamente relacionada con las tasas de la supervivencia, crecimiento y reproducción (Bjorndal 1985; Balazs et al. 1995).

Los resultados aquí presentados sugieren que las tortugas verdes presentes en la costa oriental de América del Sur consumen de forma preferente algas rojas (Reisser et al. 2013; Santos et al. 2015; Vélez-Rubio et al. 2016) debido a su mayor digestibilidad en comparación con las algas pardas. Si esta interpretación es correcta, las algas rojas constituyen un recurso crítico para la conservación de las tortugas verdes en el Atlántico sur. Además, esto explica por qué en zonas como el Mediterráneo, donde las comunidades algales están dominadas por algas pardas, la dieta se basa en la (*Cymodocea nodosa*) una fanerógama marina relativamente escasa (Cardona et al. 2010).

Otro resultado importante de esta tesis es la selección de áreas someras para alimentarse. Este patrón es consistente lo que ya se sabía sobre el uso del hábitat por parte de las tortugas verdes (Godley et al. 2002; Hays et al. 2002; Lipkin et al. 2003; Broderick et al. 2007; Reisser et al.

2013; Santos et al. 2015) y pone de manifiesto la vulnerabilidad de la tortuga verde a actividades pesqueras que ocurren cerca de la costa, especialmente en las redes de enmalle, que son comúnmente utilizadas en la pesca artesanal en la costa de Brasil. Evaluar el impacto de la pesca artesanal puede ser logísticamente difícil debido a la naturaleza difusa de esta actividad, motivo por el cual por esta razón el impacto de la pesca artesanal rara vez se cuantifica (Humber et al. 2011).

La preservación de los hábitats de alimentación es crítica para la conservación de las tortugas verdes, visto que su degradación limita la disponibilidad de alimento (Godley et al. 2002; Hays et al. 2002; Broderick et al. 2007; Santos et al. 2015). La limitación de alimento puede generar la disminución de la tasa de crecimiento corporal y consecuentemente reducir la velocidad de recuperación de la población adulta, una vez cesada la explotación comercial (Bjorndal 1985). De esta manera, el aumento del impacto humano sobre las zonas costeras, incluso sin provocar mortalidades observables, puede amenazar a las poblaciones de tortugas verdes de manera sostenida en el tiempo.

Adicionalmente, la conservación de las especies migratorias requiere el conocimiento de la distribución de los juveniles y la conservación de sus áreas de alimentación. Los individuos de una misma población se mueven de manera independiente y en algunas poblaciones emprenden migraciones que abarcan enormes áreas, con zonas de reproducción y alimentación separadas por centenares o miles de kilómetros. En las últimas décadas, la telemetría por satélite ha mejorado nuestra comprensión de la conectividad entre los diferentes hábitats empleados por las tortugas marinas en diferentes fases de su ciclo vital (Godley et al. 2008; Costa et al. 2012), pero aún así, nuestro conocimiento sobre las rutas seguidas durante los primeros estadios juvenil y el proceso de transición de los hábitats pelágicos a los hábitats neríticos resulta poco conocido.

Aunque, la miniaturización de los emisores satelitales ha permitido realizar importantes avances (González Carman et al. 2012; Putman y Mansfield 2015; Williard et al. 2017), sigue siendo imposible instrumentar y hacer el seguimiento por satélites de individuos de talla muy pequeña; además, resulta imposible recuperar los pequeños transmisores implantados en los juveniles durante la etapa oceánica. De igual forma, el seguimiento por satélite presenta limitaciones, pues los emisores permanecen unidos a los juveniles durante sólo unos pocos meses (Godley et al. 2003; González Carman et al. 2012; Putman y Mansfield 2015; Williard et al. 2017) y no ofrecen una alternativa viable para el seguimiento a largo plazo. Por todo ello, gran parte del conocimiento sobre los primeros años de vida de las tortugas verdes ha sido inferido a partir de modelos oceanográficos.

La composición isotópica de los tejidos de una animal deriva de la composición isotópica de su dieta a lo largo del tiempo más el factor de discriminación del depredador a la presa (DeNiro y Epstein 1981; Michener y Schell 1994; Hobson et al. 1996; Roth y Hobson 2000; Phillips y Gregg 2003; Revelles et al. 2007; Caut et al. 2009). La razón isotópica del nitrógeno nos informa del nivel trófico, mientras la razón isotópica del carbono permite discriminar entre el consumo de organismos planctónicos y diferentes tipos de productores primarios bentónicos (Minagawa y Wada 1984; Hobson y Clark 1992; Godley et al. 1998; McCutchan Jr et al. 2003; Vizzini y Mazzola 2003; Seminoff et al. 2006; Reich et al. 2008). Los isótopos estables de diferentes tejidos proporcionan información sobre la dieta de las tortugas verdes en una ventana temporal variable, pero en el caso de las capas de queratina del corazón representan la dieta del consumidor en el momento de la deposición (Reich y Arnould 2007; Cardona et al. 2009; Cardona et al. 2010; Vander Zanden et al. 2013; Vélez-Rubio et al.

2018). Por lo tanto, el método permite reconstruir los cambios en el uso del hábitat y la dieta de la especie en aquellos momentos de la fase juvenil difíciles de estudiar de otro modo. Además, el método permite detectar el consumo de organismos que se digieren rápidamente, evitando así el sesgo provocado por las diferencias en la tasa de digestión, inherente al análisis de los contenidos digestivos (Hobson 1999; Dalerum y Angerbjörn 2005).

Analizar las relaciones de isótopos estables en las capas de escudos de caparazón es un enfoque alternativo para reconstruir el historial de vida y los recursos utilizados, así como los cambios ontogenéticos en las dietas y hábitats de los juveniles de tortugas verdes(Reich et al. 2007; Cardona et al. 2009; Cardona et al. 2010; Vander Zanden et al. 2013). De todos modos, la interpretación de los resultados de los isotopos estables no siempre resulta sencilla debido a las variaciones complejas en el paisaje isotópico de cada región. Existe un fuerte gradiente latitudinal en la línea de base del $\delta^{15}N$ de las especies pelágicas y bentónicas a lo largo de la costa brasileña que aumentan hacia el sur desde el norte de Brasil hasta Uruguay (Somes et al. 2010; Navarro et al. 2013; Vélez-Rubio et al. 2018).

Sabemos que los juveniles de las tortugas verdes se encuentran en gran cantidad por toda la costa de Brasil, Uruguay y el norte de Argentina (González Carman et al. 2012; Vélez-Rubio et al. 2013; Santos et al. 2015). Las tortugas verdes en el Atlántico Sur Occidental utilizan una amplia diversidad de hábitats tropicales y subtropicales a lo largo de la costa del Sur de América, donde existe un claro gradiente climático latitudinal. La región tropical, representada por Praia do Forte en esta tesis, tiene una flora relativamente rica, y se caracteriza por la abundancia de sustratos consolidados (De Araújo y De Jesus Machado 2008). En cambio, la región subtropical se caracteriza por costas rocosas con una complejidad de sustrato relativamente menor

comparada con el sustrato consolidado de la región tropical. La región templada cálida, que corresponde a las zonas del Sudeste y Sur de Brasil, también posee una flora relativamente rica en comparación con la región tropical, pero hay menor riqueza. En el sudeste, representado en esta tesis por Ubatuba, se encuentra en el límite sur de la zona tropical de Brasil (Gallo et al. 2006). La región recibe la influencia de tres masas de agua responsables de un cambio estacional en salinidad, concentración de nutrientes y la temperatura varía estacionalmente desde 17,6 ºC en invierno a 24,6 ºC en verano, (de Castro Filho et al. 1987; Silva et al. 2017). Por otro lado, la costa de la región sur de Brasil está influenciada por la zona oceánica caracterizada por una alta productividad biológica, debido a la confluencia entre las corrientes oligotrófica de Brasil y la de Malvinas (Seeliger y Odebrecht 1998). Tras el asentamiento en los hábitats costeros, las características y las variaciones del hábitat local juegan un papel importante en la estrategia de alimentación y en el uso del hábitat por los juveniles de las tortugas verdes.

Se sabe que la longitud del caparazón de los juveniles de tortuga verde en el momento del reclutamiento varía entre diferentes regiones del planeta (Zug y Glor 1998; Zug et al. 2002; Reich y Arnould 2007; Arthur et al. 2008) y que las corrientes oceánicas determinan las trayectorias de los recién nacidos (Hays et al. 2010; Casale y Mariani 2014) y en consecuencia, las áreas de alimentación donde se asientan los juveniles (Schofield et al. 2013; Cardona et al. 2014; Scott et al. 2014b). Los valores del $\delta^{13}C$ analizados en los escudos del caparazón de tortugas verdes durante la presente tesis doctoral fueran complejos de interpretar. Las macroalgas analizadas difirieron en sus valores medios de $\delta^{13}C$ y revelaron un elevado nivel de variabilidad regional en la línea de base, sin un patrón latitudinal claro. Como consecuencia, los valores de $\delta^{13}C$ a través de las capas en el escudo del caparazón de los

juveniles de tortugas verdes analizados no sirvieron para la reconstrucción de los movimientos entre hábitats, pero sí han sido útiles para reconstruir la dieta utilizando modelos de mezcla.

Por lo que a los valores de δ^{15} N en las capas de los escudos de caparazón se refiere, sí reflejaron cambios en el nivel trófico y en los hábitats de alimentación, debido al fuerte gradiente en la variación latitudinal existe en la línea de base del δ^{15}N entre el norte de Brasil y el sur, Uruguay y Argentina (Vélez-Rubio et al. 2018). Las relaciones de los isotopos estables de nitrógeno permitieron diferenciar claramente los patrones del uso del hábitat de los juveniles de tortugas verdes que usaban los hábitats costeros en el nordeste de Brasil (Praia do Forte), y en el sudeste de Brasil (Ubatuba). Los resultados de isotopos estables de nitrógeno obtenidos durante la realización de la tesis doctoral demostraron diferentes trayectorias ontogenéticas para las tortugas verdes antes y después del asentamiento en los hábitats neríticos del Atlántico Sur Occidental.

Así, las tortugas verdes del nordeste de Brasil no registraron cambios relevantes de hábitat o dieta tras el asentamiento, tal como indican los valores isotópicos de las capas de los escudos del caparazón. En cambio, los individuos del sudeste de Brasil presentaban altos niveles de variabilidad individual y temporal en los valores de δ^{15}N, indicativos de cambios frecuentes de hábitat o dieta, así como una elevada diversidad de trayectorias ontogenéticas individuales al largo del isoespacio. Estos resultados se explican por la interacción entre tasa de crecimiento y deriva con la corriente desde la Isla de Ascensión, principal zona de origen de las tortugas verdes presentes en las zonas de alimentación del litoral sudamericano (Caraccio Noriega 2008; Monzón-Argüello et al. 2010; Naro-Maciel et al. 2012; Proietti et al. 2012; Prosdocimi et al. 2012).

Los recién nacidos tras abandonar Ascensión emprenden una migración de miles de kilómetros con la corriente principal con una alta probabilidad de alcanzar la costa norte de Brasil entre los primeros dos años de vida (Naro-Maciel et al. 2012). Sin embargo, el asentamiento en las áreas de alimentación del norte de Brasil se produce únicamente en aquellos individuos de cada cohorte que hayan crecido lo suficiente y que tengan el estricto control de la flotabilidad necesario para establecerse en hábitats neríticos (Hochscheid et al. 2003; Putman y Naro-Maciel 2013). La gran mayoría de los juveniles que no crecerán lo suficiente para alcanzar este estadio durante sus dos primeros años de vida y se ven obligados a continuar a la deriva hacia el sur con la corriente de Brasil, antes de asentarse en hábitats costeros en las áreas subtropicales de Brasil y Uruguay. Esta hipótesis se ve apoyada por los experimentos de simulación de partículas que sugieren que los recién nacidos procedentes de la isla de Ascensión llegarían al nordeste de Brasil con menos de dos años (Putman y Naro-Maciel 2013) y con menos de 30 cm CCL de largo de acuerdo con los datos disponibles de la tasa de crecimiento somático la tortuga verde en el Atlántico Sudoeste (Lenz et al. 2017).

Se sabe que las tasas de crecimiento de las tortugas verdes varían entre individuos, poblaciones y ecosistemas (Tomaszewicz et al. 2018). Así mismo los individuos con el mismo tamaño o edad pueden crecer a diferentes tasas, incluso compartiendo una misma área de alimentación (Bjorndal et al. 2000). En el océano Atlántico occidental las tasas de crecimiento registradas en juveniles de tortugas verdes varían de 3,1 cm.año^{-1} (Barreto 2017) a 3,7 cm.año^{-1} (Lenz et al. 2017), mientras que las poblaciones del Atlántico norte varían entre 3,0 y 5,0 cm año^{-1} (Zug y Glor 1998). Otro aspecto variable entre las poblaciones de tortugas verdes es la edad y el tamaño en que los juveniles se reclutan a los hábitats neríticos. Los juveniles de tortugas verde presentes en el

Océano Pacifico noroeste y enAustralia se asientan a tallas superiores a los 44 cm SCL (Arthur et al. 2008; Shimada et al. 2014; Fukuoka et al. 2015), mientras en el Caribe y el Atlántico Norte lo hacen entre 25-35 cm (Bjorndal 1985; Bjorndal 1997; Reich y Arnould 2007).

El asentamiento puede tener lugar en las mismas áreas de alimentación utilizados por los adultos (Chaloupka et al. 2004; Arthur et al. 2008) o, alternativamente, en hábitats de desarrollo distintos de las zonas de alimentación usadas por los adultos, especialmente en regiones subtropicales y templadas cálidas (Cardona et al. 2009; González Carman et al. 2012; Williams et al. 2014; Jardim et al. 2015; Santos et al. 2015; Howell et al. 2016). Los datos disponibles indican que sólo aparecen adultos en las áreas de alimentación mixta de las regiones nordeste y norte de Brasil, mientras que al sur de la latitud 12ºS sólo existen zonas de alimentación de juveniles (Jardim et al. 2015).

En este escenario la amplia área de asentamiento de tortugas verdes a lo largo de la costa oriental de América del Sur resulta de la interacción entre las grandes diferencias de la tasa de crecimiento individual de los juveniles y la existencia de meandros en la corriente de Brasil al sur de la latitud 20º S. Debido a ello, la mayoría de los individuos juveniles que alcanzan dicha latitud se ven obligados a un prolongado período de residencia oceánica, asentándose en Uruguay a una talla algo superior a la registrada en el nordeste de Brasil. Esto implica que las tortugas asentadas al norte de la latitud 12º S pasan la mayor parte de su vida en las mismas áreas de alimentación, mientras que los individuos asentados más al sur de esta latitud tengan que realizar una migración desde las zonas de asentamiento y desarrollo de juveniles hasta las zonas de alimentación de adultos. De todo lo anterior se desprende que las tortugas verdes no eligen exactamente la región donde se asientan en el medio bentónico, sino que el asentamiento se

produce allí donde alcanzan una talla suficiente como para poder hacerlo.

Tal como han demostrado otros trabajos previos, el asentamiento en zonas tropicales va seguido de un rápido cambio ontogenético y la adopción de una dieta básicamente herbívora (Reich y Arnould 2007; Arthur et al. 2008), mientas que en zonas subtropicales la dieta continúa siendo omnívora tras el asentamiento (Cardona et al. 2009; Cardona et al. 2010; Carrión-Cortez et al. 2010; Lemons et al. 2011; Santos et al. 2015; Vélez-Rubio et al. 2016). Sin embargo, incluso en algunas regiones tropicales la dieta omnívora persiste tras el reclutamiento (Amorocho y Reina 2007; Russell et al. 2011).

En general, las tortugas verdes en hábitats neríticos en las zonas subtropicales presentan altos niveles de omnivoría con cambios más lentos y reversibles. En la dieta omnívora ocurre un aumento del consumo de material vegetal con la longitud del caparazón (Cardona et al. 2009; Cardona et al. 2010; Burkholder et al. 2011; Lemons et al. 2011; Russell et al. 2011; González Carman et al. 2012; Howell et al. 2016) y sólo los adultos son principalmente herbívoros (Arthur et al. 2008; Cardona et al. 2009; Cardona et al. 2010; Lemons et al. 2011; Williams et al. 2014; Santos et al. 2015; Howell et al. 2016; Vélez-Rubio et al. 2016). Sin embargo, e incluso los adultos pueden revelar estilos de vida no homogéneos con la persistencia de una dieta carnívora alimentándose en el océano abierto a lo largo de su vida (Hatase et al. 2006; Kelez 2011; Parker et al. 2011).

La evidencia general indica que las tortugas verdes en los hábitats neríticos en el sur de Brasil y Uruguay comienzan a alimentarse de marcroalgas tan pronto ocurre el reclutamiento. No obstante, la transición carnívora-herbívora es gradual, y la proporción de material vegetal en la dieta aumenta a medida que los juveniles de tortugas verdes crecen, como reportado para las tortugas verdes que habitan en

otras regiones templadas cálidas. En ésta etapa, además intercalan movimientos estacionales entre las aguas del sur de Brasil y Uruguay (Vélez-Rubio et al. 2018), asimismo, la proporción del material animal se vuelve insignificante solamente en los individuos mayores de 50 cm CCL (Lenz et al. 2017; Vélez-Rubio et al. 2018).

Las tortugas verdes son fermentadores del intestino posterior y dependen de la flora intestinal para digerir los carbohidratos complejos presentes en la pared celular de las plantas (Bjorndal 1980) cuya actividad se ve claramente determinada por la temperatura del agua. Tras el asentamiento en el hábitat nerítico los juveniles deben cambiar la composición de la flora microbiana que va asociada al consumo de material vegetal. Se ha sugerido que la alta presencia de animales en la dieta de los juveniles recién asentados en las zonas subtropicales se debe a la baja digestibilidad del material vegetal (Hadjichristophorou y Grove 1983; Amorocho y Reina 2007), principalmente en los juveniles (Bjorndal 1980). Se ha sugerido que esto podría ser debido a la lenta adquisición de una microbiota fermentadora en zonas subtropicales.

Los resultados del microbioma obtenidos durante la realización de la presente tesis doctoral sugiere que el cambio ontogenético progresivo en la dieta con frecuencia reportado para las tortugas verdes en las áreas de alimentación templado cálido, no se debe a una adquisición lenta de una microbiota especializada para degradar los polisacáridos, sino que está probablemente relacionado con la baja eficiencia de la fermentación microbiana y la baja temperatura experimentada por los juveniles de tortugas verde en regiones subtropicales. Esta conclusión de deriva de la presencia de una microbiota rica en fermentadores en tortugas verdes juveniles tanto del norte como del centro de Brasil, a pesar de la existencia de importantes diferencias en el consumo de presas animales en ambas zonas.

Las tortugas verdes son ectodérmicas, y la temperatura corporal de los juveniles que habitan las regiones subtropicales se ve afectada y cerca de la temperatura del agua circundante (Read et al. 1996). A la inversa, la temperatura corporal de las tortugas verdes adultas puede estar a 2 ºC por encima de la temperatura del agua circundante debido a la gigantotermia (Standora et al. 1982). Por lo tanto, una temperatura corporal más alta en las tortugas verdes más grandes surge como la explicación más probable para el aumento de la digestibilidad del material vegetal con la talla (Bjorndal 1980), lo que sería especialmente relevante en zonas subtopicales.

Los resultados obtenidos de la caracterización de la flora intestinal en la presente tesis doctoral tanto para los juveniles de tortugas verdes salvajes capturados en hábitats costeros como para los juveniles cautivos, confirman la adquisición rápida de una microbiota capaz de fermentar polisacáridos estructurales independientes del tamaño (31.1 a 64.7 cm CCL), del origen de los juveniles (tropical – Praia do Forte; subtropical – Ubatuba), y de la dieta mista sobre macroalgas y/o pescado para los juveniles en cautividad. Las bacterias dominantes en el microbioma de los juveniles de tortugas verdes fueran los filos de los *Bacteroidetes* (*Bacteroidaceae; Porphyromonadaceae*) y *Firmicutes* (*Clostridiaceae; Ruminococcaceae; Lachnospiraceae*), los primeros implicados en la degradación de polisacáridos estructurales complejos propios de las algas y los segundos en la degradación de la celulosa. En cambio, en la cloaca de juveniles pelágicos se ha encontrado un predominio de *Proteocbacteria,* (Ahasan et al. 2017; Price et al. 2017), grupo que domina la microbiota de los vertebrados carnívoros pero de baja importancia en herbívoros salvajes, incluyendo las iguanas marinas y dugongos (Tsukinowa et al. 2008; Hong et al. 2011).

Los *Firmicutes*, juegan un papel crítico en la hidrólisis de la fermentación de polisacáridos complejos en los vertebrados herbívoros. La gran abundancia de *Firmicutes* encontrada en los intestinos de los juveniles de tortugas verdes (Ahasan et al. 2017) es consistente con los resultados encontrados en mamíferos (Tsukinowa et al. 2008; Nelson et al. 2013; Merson et al. 2014). Por otra parte, resulta interesante los resultados presentados aquí en lo cual detectamos la prevalencia de *Lachnispiraceae* sobre *Ruminococcaceae* en los intestinos de las tortugas verdes y de las iguanas marinas (Hong et al. 2011), cuando lo contrario sucede en otros reptiles herbívoros (Hong et al. 2011). Esta diferencia podría estar relacionada con el consumo de algas en las tortugas verdes de Brasil y en las iguanas marinas.

Los *Bacteroidetes* fueron el segundo filo más abundante en los resultados de la presente tesis doctoral, la importancia de estas bacterias puede ser atribuidas a la salud intestinal del microbioma. Los *Bacteroidetes* pueden contribuir. significativamente al ataque inicial de los carbohidratos simples y complejos (Shah y Gharbia 1993). En las tortugas gopher la alta prevalencia de los *Bacteroidetes* ha sido atribuida a las bajas temperaturas de las regiones donde habitan (Yuan et al. 2015). Sin embargo, los resultados presentados aquí tuvieron una prevalencia similar tanto para las tortugas verdes de los hábitats tropical de Praia do Forte como para las tortugas de los hábitats subtropical de Ubatuba, por lo que su presencia no parece estar relacionada con la temperatura del agua.

En definitiva, las tortugas verdes adquieren una microbiota que les capacita para digerir carbohidratos complejos poco después de su asentamiento en hábitats bentónicos. No obstante, la digestión efectiva de una dieta herbívora por las tortugas verdes depende de otros factores, además del microbioma. La eficiencia de la digestión del material vegetal en vertebrados depende en gran medida de la composición de la

pared celular de la planta y de la presencia de los metabolitos secundarios que puede inhibir la actividad de las enzimas hidrolíticas. La abundancia de polisacáridos estructurales hace de las plantas un alimento desequilibrado y pobre en nitrógeno. Para contrarrestar esto, las tortugas verdes tienen intestinos muy largos (Bjorndal 1979), pero podrían existir diferencias entre el tipo de plantas seleccionadas.

Los modelos teóricos de optimización del alimento y del hábitat sugieren que los animales seleccionan los hábitats con recursos de alta calidad sobre aquellos de baja calidad y que la composición de la dieta de un animal depende de una variedad de factores interrelacionados que incluyen la selección de presas que maximizan el rendimiento nutricional. Para las tortugas verdes, este comportamiento es importante, ya que se necesitan cantidades suficientes de alimentos nutricionalmente adecuados para el crecimiento y la reproducción.

La densidad energética y el contenido en proteína y en fibras insolubles son parámetros clave que influyen en la elección de la dieta y en la cantidad de ingesta de un herbívoro. Los resultados del experimento de digestibilidad obtenidos en la presente tesis doctoral sugieren la preferencia alimentar de las tortugas verdes por las algas rojas (*Pterocladiela capilacea)* sobre las algas pardas (*Sargassum cf vulgare)*, pues las primeras son de más fácil digestión. Este resultado explica porque las tortugas verdes en Brasil se alimentan principalmente de algas rojas, principalmente *Pterocladiela capilacea*, aunque también consumen algas verdes y pardas (Reisser et al. 2013; Santos et al. 2015). Los datos publicados sobre la dieta en otras regiones confirman también la prevalencia de algas rojas y la escasez de algas pardas en la dieta de tortugas verdes allí donde no existen praderas de fanerógamas marinas o estas son pequeñas (Ferreira 1968; Sazima y Sazima 1983; Arthur y Balazs 2008; Russell y Balazs 2009; Vélez-Rubio et al. 2016). Un factor adicional que explicaría el bajo consumo

de algas pardas es, junto a una menor digestibilidad, las altas concentraciones de compuestos fenólicos (Wong y Cheung 2001).

Otro resultado relevante de este estudio fue observar una digestibilidad aparente similar para la materia seca del alga roja y el filete de pescado. Esto indicaría que una dieta rica en fermentadores anaeróbicos no impide utilizar presas animales de forma eficiente cuando están disponibles. Ahora bien, la mayor biomasa y el menor coste de captura de las algas hace que en cuanto las tortugas verdes adquieran la capacidad para bucear y controlar su flotabilidad de forma eficiente adquieran una dieta herbívora. Sólo cuando las presas animales son muy abundantes o la baja temperatura reduce la digestibilidad del material vegetal, es rentable afrontar el mayor coste de captura que implica su consumo.

Por otro lado, se sabe que los juveniles de tortugas verdes se alimentan principalmente de pastos marinos (*Thalassia testudinum*) en el Gran Caribe (Bjorndal 1980; Mortimer 1981; Moran y Bjorndal 2005; Williams et al. 2014; Howell et al. 2016), a pesar de un coeficiente de digestibilidad relativamente bajo (Bjorndal 1980) en comparación con los aquí indicados para algas rojas y pardas. Las fanerógamas marinas constituyen el grueso de la dieta de las tortugas verdes también en el Océano Índico (Ballorain et al. 2010; Lal et al. 2010; Burkholder et al. 2011) e incluso en zonas subtropicales del Mediterráneo (Cardona et al. 2010) y el Atlántico africano (Cardona et al. 2009; Monzón-Argüello et al. 2018). Esta diferencia se explica por la abundancia y extensión de las praderas de fanerógamas marinas en el Gran Caribe, el Océano Índico y el Mediterráneo, así como su escasez en el Atlántico occidental. En este contexto, la preferencia general de la dieta por un tipo de alimento puede variar según el conjunto de la composición de las especies, posiblemente las tortugas verdes modifiquen su dieta cuando los ítems de la dieta preferidas son escasos

o ausente. A este respecto cabe remarcar que la selección de la dieta por las tortugas verdes no puede explicarse únicamente por los coeficientes aparentes de digestibilidad y que la disponibilidad juega un papel importante.

El predominio de las macroalgas en la dieta de las tortugas verde no es exclusivo del Atlántico sudoccidental, sino que ocurre en todas aquellas regiones donde las praderas de fanerógamas escasean (Ferreira 1968; Sazima y Sazima 1983; Arthur y Balazs 2008; Russell y Balazs 2009; Reisser et al. 2013; Santos et al. 2015; Vélez-Rubio et al. 2016). En estas regiones, las tortugas verdes se alimentan en hábitats arrecifales, tanto en Brasil (Santos et al. 2011; Santos et al. 2015), como en Hawaii (Russell y Balazs 2009; Russell y Balazs 2015) y Australia (Goatley et al. 2012). Sin embargo, se sabe sobre el papel de las tortugas verdes en la dinámica de la vegetación de los arrecifes tropicales.

La biomasa de macroalgas puede ser elevada a los arrecifes rocosos (Oke (Okey et al. 2004; Reisser et al. 2013) y por lo tanto la existencia de un mega herbívoro en estos ecosistemas no debería sorprender. No obstante, no ocurre lo mismo en los arrecifes coralinos, donde la biomasa de macroalgas es baja como consecuencia de la acción de los erizos de mar y de los peces herbívoros de las familias Scaridae y Acanthuridae (Hughes et al. 2003; Bellwood et al. 2004; Mumby et al. 2006; Cheal et al. 2010; Estes et al. 2011).

Los resultados de los de censos submarinos en arrecifes tropicales de Fernando de Noronha y Hawaii realizados en el trascurso de la presente tesis doctoral demuestran que las tortugas verdes contribuyen en muy poco a la biomasa total de herbívoros de los arrecifes tropicales. En Fernando de Noronha, fueron los peces los principales herbívoros, mientras que ese papel correspondió a los erizos en Hawaii. Esto se debe a que las tortugas verdes prefieren los habitas de poca complejidad y rugosidad, situados en zonas poco profundas y

protegidas del oleaje. En cambio, los peces prefieren las zonas de elevada complejidad y rugosidad, mientras que en Hawaii los erizos abundan en todos los hábitats, salvo los resguardados del oleaje (Russo 1977; Johansson et al. 2013). Cabe remarcar la práctica ausencia de erizos de Fernando de Noronha, sin que se sepa el motivo. Dicha escasez data al menos de mediados de la década de 1980 (Eston et al. 1986) y se ignora si está relacionada con la epidemia que diezmó las poblaciones de *Diadema antillarum* en el Caribe (Mumby et al. 2007; Mumby y Steneck 2008), pero se sabe poco sobre la dinámica de las poblaciones de *Diadema antillarum* en el resto del Atlántico tropical occidental.

A pesar de estas diferencias, la biomasa de las tortugas verdes fue similar en ambas regiones, si bien se alimentaban de forma diferente. Las tortugas en el Hawaii se alimentaban en áreas intermareales, ya que los hábitats submareales presentaban baja cobertura de macroalgas. Los bajíos de Heeia fue el único sitio donde las tortugas se alimentaban en áreas submareal debido a una alta cobertura vegetal posible gracias a la ausencia de erizos debido a su protección del oleaje. En consecuencia, fue el sitio en Hawaii con mayor biomasa de tortugas verdes. En cambio, en Fernando de Noronha las tortugas se alimentaban en áreas submareales debido a una mayor disponibilidad de alimento, fruto de la ausencia de erizos. No obstante, la disponibilidad de alimento no explico la preferencia por áreas planas y protegidas de W.Boldro, E.Boldro y Porto.

En conjunto, los resultados obtenidos sugieres que las tortugas verdes probablemente desempeñan un papel insignificante en la dinámica de la vegetación en los arrecifes tropicales, salvo en zonas resguardadas de fondo plano.

Conclusiones

- ✓ Las tortugas verdes se asientan en las zonas de alimentación juveniles a medida que crecen y alcanzan un control de su flotabilidad. La existencia de una notable variabilidad individual en la tasa de crecimiento corporal hace que el asentamiento se produzca a lo largo de toda la costa atlántica de Sudamérica, aunque los juveniles que se asientan más al sur se verán obligados a desplazarse hacia las zonas de alimentación de adultos del norte a medida que crezcan.
- ✓ Las tortugas verdes neríticas de zonas tropicales adoptan rápidamente una dieta herbívora y muestran pocos cambios de hábitat y dieta tras el asentamiento. Por el contrario, las tortugas verdes neríticas de zonas subtropicales adoptan una dieta omnívora tras el asentamiento y muestran frecuentes cambios de dieta o zona de alimentación.
- ✓ Los juveniles de tortugas verdes adquieren rápidamente tras el asentamiento una microbiota intestinal rica en fermentadores de polisacáridos complejos, sin que existn diferencias entre zonas tropicales y subtropicales. Por lo tanto, el cambio tardío a una dieta completamente herbívora tras el asentamiento en hábitats subtropicales se debe a las bajas temperaturas invernales y no a un retraso en la adquisición de un microbioma rico en fermentadores.
- ✓ La adquisición de una microbiota intestinal rica en fermentadores de carbohidratos complejos no impide la digestibilidad de presas de origen animal y por lo tanto el cambio de dieta es fácilmente reversible.
- ✓ El coeficiente de digestibilidad aparente del material vegetal para las tortugas verdes varía según las características de los carbohidratos estructurales presentes en las paredes celulares de las plantas consumido, siendo mayor para las algas rojas que para las algas pardas. En consecuencia, las algas rojas predominan sobre las algas pardas en la dieta de las tortugas verdes que habitan arrecifes tropicales y subtropicales.

- ✓ El predominio de las fanerógamas marinas sobre las macroalgas en la dieta de las tortugas verdes de ciertas regiones se explica por su mayor abundancia, no por una mejor digestibilidad.
- ✓ Las tortugas verdes están presentes de forma habitual en la mayor parte de los arrecifes tropicales someros, pero su contribución a la biomasa total de herbívoros es generalmente baja. Los erizos y los peces son los herbívoros más abundantes.
- ✓ La competencia con los erizos hace que allí donde su biomasa es muy elevada, las tortugas verdes se vean obligadas a alimentarse principalmente del turf intermareal.
- ✓ Las tortugas verdes que habitan arrecifes tropicales prefieren zonas de poca complejidad estructural y con baja rugosidad, situadas en áreas poco profundas y protegidas del oleaje. Dicha selección estaría motivada por una menor presencia de depredadores (tiburones) y competidores (peces herbívoros y

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1ª Capitulo: Dispersión juvenil y cambio ontogenético de la dieta

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Individual variability in the settlement of juvenile green turtles in the western South Atlantic Ocean: Relevance of currents and somatic growth rate

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Abstract

The settlement of demersal animals is influenced both by physical processes ruling the distribution of pelagic juveniles in the open ocean and by their active selection of suitable benthic habitats by those same pelagic juveniles. Green turtles inhabiting the coastal areas of the western South Atlantic Ocean derive primarily from the rookery at Ascension Island and settle over a huge area spanning from northern Brazil to Uruguay. Here, we have analysed the stable isotope ratios of C and N in 30 μm of carapace layers from juvenile green turtles collected from two distinct areas of Brazil (Praia do Forte, 12º 38′S 38º 05′W, and Ubatuba, 23º 26′S, 45º 05′W), with the goal of retrospectively reconstructing their individual diets and habitat use patterns. Juvenile neritic green turtles from Praia do Forte usually have herbivorous diets, with limited individual variability and few temporal changes in diet or habitat. Conversely, most juvenile green turtles from Ubatuba had omnivorous diets, although they exhibited high levels of individual and temporal variability. These contrasting patterns could be linked to a less abundant and predictable fodder availability in subtropical

Ubatuba compared to tropical Praia do Forte. It is unknown why large numbers of juvenile green turtles skip past foraging grounds in north-eastern Brazil to settle in subtropical or warm temperate areas, although it may be related to individual differences in growth rate and their size being too small when reaching Brazil from Ascension Island.

Key words
Juvenile turtles, *Chelonia mydas*, settlement, developmental habitat, stable isotopes

Introduction

Demersal marine animals often use a diversity of habitats throughout their lives, with juveniles typically inhabiting the water column and adults residing near the sea bed (Rodriguez and Bastida 1993; Harmelin-Vivien et al. 1995; Juanes 2007). Settlement into benthic habitats is a critical process for all these demersal animals, and this usually involves the juveniles actively selecting suitable habitats (Harmelin-Vivien et al. 1995; Montgomery et al. 2001; Jenkins 2005). Nonetheless, currents may profoundly impact the dispersal of juvenile, pelagic stages because of their small body size and limited swimming skills. This, in turn, may result in long-distance dispersal, thus connecting pelagic foraging grounds, settlement areas, the developmental habitats of neritic juveniles and, finally, the foraging grounds for adults (Cowen and Sponaugle 2009).

Marine turtles offer a good example of these complex life cycles, which result largely in part from the broad dispersal of post-hatchlings and oceanic juveniles (Putman and Naro-Maciel 2013; Mansfield et al. 2014; Briscoe et al. 2016), and also from the fidelity of adults to foraging grounds and nesting beaches (Bowen and Karl 2007). Green turtles *Chelonia mydas*

inhabit all the tropical regions of the planet (Wallace et al. 2010), and the dispersal of post-hatchlings and oceanic juveniles is strongly influenced by oceanic currents (Monzón-Argüello et al. 2010; Naro-Maciel et al. 2017). Juvenile green turtles settle into neritic habitats when their curved carapace length (CCL hereafter) reaches 25-50 cm (Bjorndal 1985; Bjorndal 1997; Seminoff et al. 2002; Reich et al. 2007; Arthur et al. 2008; Cardona et al. 2009; Williams et al. 2014; Howell et al. 2016; Williard et al. 2017). At this point, they shift from a carnivorous diet based on gelatinous zooplankton to an herbivorous or omnivorous plant-based diet (Reich et al. 2007; Arthur et al. 2008; Cardona et al. 2009; Cardona et al. 2010; Parker et al. 2011; Vélez-Rubio et al. 2016). Settlement may take place at the same feeding grounds used by adults (Chaloupka et al. 2004; Arthur et al. 2008) or, alternatively, at developmental habitats that are different from those used by adults, particularly in subtropical and warm temperate regions (Cardona et al. 2009; González Carman et al. 2012; Williams et al. 2014; Jardim et al. 2015; Santos, Martins, et al. 2015; Howell et al. 2016). In the latter situation, green turtles will eventually move to the adult foraging grounds as they grow older. The benefits of settling in developmental habitats remain unknown(Meylan et al. 2011) , but using neritic foraging grounds that are distinct from those of adults might simply be a result of oceanic juveniles drifting with the currents until they grow large enough to control their buoyancy (Scott et al. 2014).

According to tagging and genetic markers, the majority of the green turtles inhabiting the western South Atlantic derive from the rookery at Ascension Island (Carr et al. 1978; Caraccio 2008; Proietti et al. 2012; Prosdocimi et al. 2012; Putman and Naro-Maciel 2013; Scott et al. 2014). Virtual particle modelling indicates that most hatchlings leave Ascension Island and drift westward along the Atlantic South Equatorial Current (Fig. 1) before reaching the neritic habitats off the coast of north-eastern Brazil in less than two years (Putman and Naro-Maciel 2013; Scott et al. 2014).

Mixed adult/juvenile foraging grounds exist only at latitude 12ºS and farther north (Gallo et al. 2006; Poli et al. 2014a; Jardim et al. 2015; Santos, Martins, et al. 2015) and, hence, most juvenile green turtles are expected to settle immediately after reaching north-western Brazil. However, large numbers of juvenile green turtles continue drifting southward along the Brazil Current before settling at developmental habitats off central and

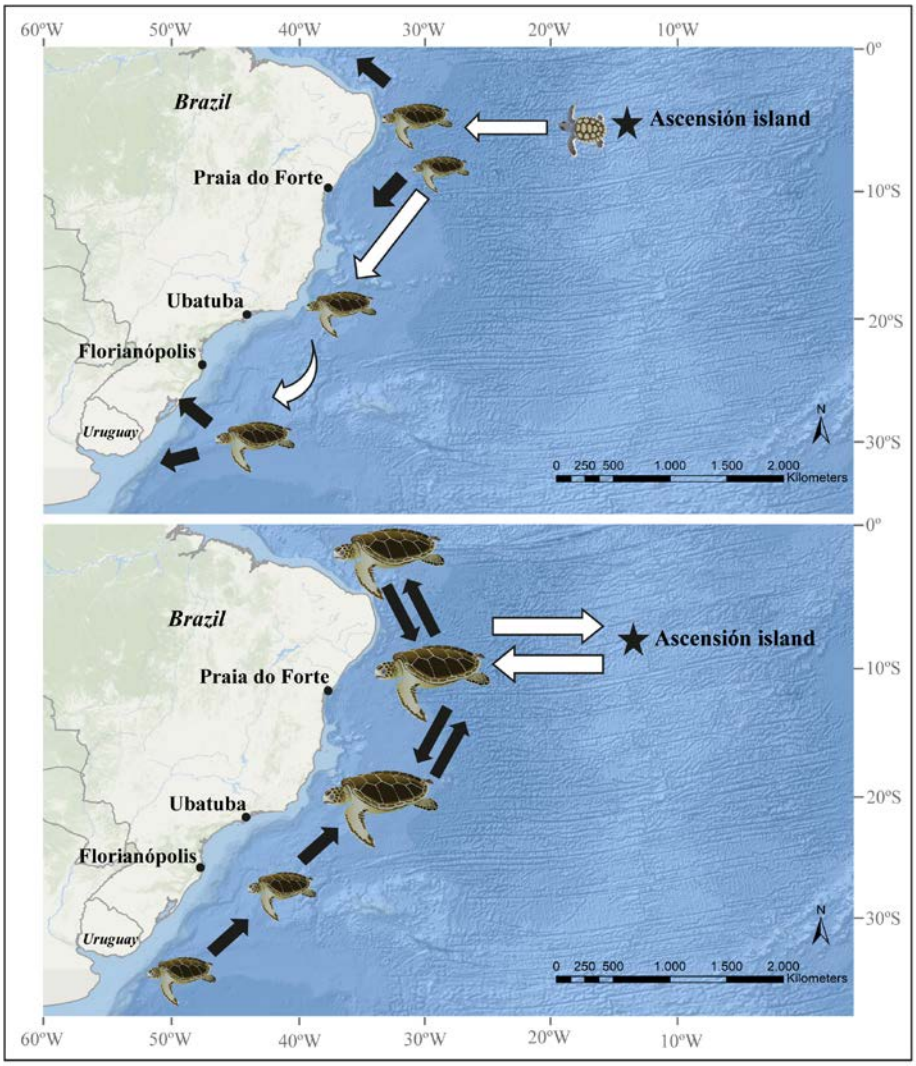

Fig. 1: Movements of green turtles from Ascension Islands across the western South Atlantic. Top panel: Developmental dispersal from Ascension Island to neritic foraging grounds along South America. Bottom panel: Movements from developmental habitats in central Brazil, Uruguay and northern Argentina to adult foraging grounds in north-eastern Brazil and reproductive migration. Empty arrows denote oceanic (deeper than 200 m) pathways and solid arrows denote neritic (shallower than 200 m) pathways. Turtle drawings (source: IAN image library) denote hatchlings, small and larger juveniles, and adults.

southern Brazil (Gallo et al. 2006; Poli et al. 2014a; Jardim et al. 2015; Santos, Martins, et al. 2015), Uruguay (Vélez-Rubio et al. 2018) and northern Argentina .

The climate is tropical in north-eastern Brazil, with increasing seasonality southwards and a warm temperate climate in Uruguay and northern Argentina. In this scenario, green turtles inhabiting the western South Atlantic are expected to exhibit a diversity of life histories. Those settling in tropical mixed adult/ juvenile foraging grounds will inhabit a rather constant and predictable environment; hence, they will experience little variability in diet and habitat throughout their lifetimes, with the exception of their periodical breeding migration to Ascension after adulthood. In contrast, individuals settling in subtropical and warm temperate developmental habitats will shift habitats frequently as a result of not only increasing seasonality but also the northward displacement towards the tropical adult foraging grounds off north-eastern Brazil.

Satellite tagging has offered some evidence of seasonal migration in juvenile green turtles from northern Argentina (González Carman et al. 2011; González Carman et al. 2012) and Uruguay (Vélez-Rubio et al. 2018), but the tags remain attached to small green turtles for only a few months (Godley et al. 2003; González Carman et al. 2012; Putman and Mansfield 2015; Williard et al. 2017); thus, they do not serve as a viable alternative for long-term tracking. Furthermore, satellite tagging offers no information about diet. Analysing stable isotope ratios in the layers of carapace scutes is an alternative approach to reconstructing ontogenetic changes in the diets and habitats of juvenile green turtles, because this metabolically inert tissue records a timeline of the consumer's isotopic history spanning several years, even if the resolution is often coarse (Reich et al. 2007; Cardona et al. 2009; Cardona et al. 2010; Vander Zanden et al. 2013; Vélez-Rubio et al. 2018).

The basic assumptions of the stable isotope are: (1) that stable isotope ratios in animal tissues integrate those in their diet and the trophic discrimination factor is tissue specific; (2) that the stable isotope ratios of metabolically inert tissues do not change after deposition and hence integrate the diet during short periods (days to weeks); and (3) that variations of stable isotope ratios across habitats and prey are known. The first two assumptions are not redundant, because the stable isotope ratios of metabolically active tissues, such as skin or muscle, change over time and hence offer no timeline, whereas the opposite is true for layers of metabolically inert tissues.

This paper uses the stable isotope ratios of N and C in the carapace scute layers of juvenile green turtles captured in neritic habitats from north-eastern and central Brazil to track their individual ontogenetic trajectories in diet and habitat use. Turtles from north-eastern Brazil are expected to have settled immediately after arriving from Ascension and hence exhibit rather constant stable isotope ratios across their carapace scutes after the drop associated with the settlement (Reich et al. 2007; Vander Zanden et al. 2013). Conversely, as a result of frequent shifts in both diet and habitat in a more seasonal environment, juvenile green turtles from central Brazil are expected to exhibit more variable stable isotope profiles across carapace scutes and higher individual variability.

Material and Methods

Study Area

We collected samples from February to March 2016 in two different regions of the Brazilian coast: 16 were collected from tropical Praia do Forte (12º 38′ S 38º 05′ W), located 70 km from Salvador do Bahia, and 14 were collected from subtropical Ubatuba (23º 26′S, 45º 05′ W), off the northern coast of the state of Sao Paulo (Fig. 1).

Sampling

At both sites, most turtles were captured alive through free diving or with a monofilament nylon net (30 cm mesh size) by members of Projeto Tamar (www.tamar.org.br), and this formed a part of their long-term study on the abundance and habitat use of green turtles along the Brazilian coast. Some of the juvenile green turtles from Ubatuba were captured alive in pound nets (Gallo et al. 2006). The mortality in pound nets is low because turtles are free to breathe, especially when the gear is open-roofed (Silva et al. 2017). Additional samples were collected at Praia do Forte during the necropsy of five recently dead turtles that had been caught incidentally by local fishermen.

Curved carapace length (CCL) was measured with flexible tape (CCL, notch to tip) and carapace scute samples 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 Miltex biopsy punch (Reich et al. 2007).

Previous research has shown that the macroalgae *Ulva* spp., *Chondracanthus* spp. and *Pterocladiella capillacea* are the staple food of green turtles along the coast of Brazil (Jardim et al. 2015; Santos, Silva Martins, et al. 2015) and Uruguay (Vélez-Rubio et al. 2018), and that a steep latitudinal gradient exists for their $\delta^{15}N$ values but not for $\delta^{13}C$. Furthermore, green turtles regularly consume gelatinous zooplankton in southern Brazil and Uruguay (Santos et al. 2015; Vélez-Rubio et al. 2018). According to this information, we collected *Ulva* sp., *Chondracanthus* sp., *Pterocladiella capillacea* and other macroalgae for stable isotope analysis at Praia do Forte and Ubatuba (five replicates each). The jellyfish *Velella velella* were also collected at Ubatuba. Prey samples were kept frozen (-20ºC) prior to analysis.

Stable isotope analysis

All carapace scute samples were rinsed with deionized water in the laboratory prior to analysis. Each sample was embedded in an O.C.T. (Optimal Cutting Temperature) compound manufactured by Tissue-Teck®, with the dorsal side (oldest tissue) down and frozen. Then, scute samples were subsampled in successive 30-µm layers using a cryostat (Leica Cryostat CM 3050S). Each layer was rinsed with deionized water for 24 hours and kept separately. Previous tests confirmed that this procedure removed O.C.T. traces and did not modify the stable isotope ratios of C or N (Monzón-Argüello et al. 2018; Vélez-Rubio et al. 2018). Samples were dried in a stove at 55°C for one day.

The number of layers obtained was proportional to the scute thickness, which varied individually. As scute grows outward, the oldest tissue remains in the outermost part of the scute and the most recent tissue forms the innermost section of the scute (Alibardi 2005). According to previous studies (Reich et al. 2007; Cardona et al. 2010; Vander Zanden et al. 2013), each 30-µm-thick layer integrates approximately 54 days, although the associated variance is unknown and hence should be considered as a coarse estimate only. Each layer was analysed independently for the stable isotope ratios of carbon and nitrogen. Subsamples were weighed in tin cups with a microbalance (approximately 0.3 mg of sample), combusted at 1,000°C, and analysed by continuous flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA; Thermo Finnigan) at the Centres Científics i Tecnològics de la Universitat de Barcelona (Spain).

Stable isotope abundances were expressed in δ notation according to the following expression:

$$\delta X = \left[\left(R_{\text{sample}}/R_{\text{standard}}\right) - 1\right] \times 10^3$$

where X is ^{13}C or ^{15}N, Rsample is the heavy to light isotope ratio of the sample ($^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively), and Rstandard is the heavy to light isotope ratio of the reference standards, which were VPDB (Vienna Pee Dee Belemnite) calcium carbonate for ^{13}C and atmospheric nitrogen (air) for ^{15}N. For calibration at a precision of 0.2‰, we used international isotope secondary standards of known $^{13}C/^{12}C$ ratios, as given by the IAEA (International Atomic Energy Agency IAEA), and these were namely polyethylene (IAEA CH_7, $\delta^{13}C$ = –31.8‰), graphite (IAEA USGS24, $\delta^{13}C$ = –16.1‰) and sucrose (IAEA CH_6, $\delta^{13}C$ = –10.4‰). For nitrogen, we obtained a precision of 0.3 ‰ using international isotope secondary standards of known $^{15}N/^{14}N$ ratios, namely $(NH_4)_2SO_4$ (IAEA N1, $\delta^{15}N$ = + 0.4 ‰ and IAEA N_2, $\delta^{15}N$ = + 20.3 ‰) and KNO_3 (IAEA NO_3, $\delta^{15}N$ = + 4.7 ‰).

Data analysis

The coefficient of variation was used as a measure of variability across carapace layers (Table 1). The Fisher test was used to compare the variability across individuals in the coefficient of variation for the two populations.

The prey-to-consumer trophic discrimination factor for the carapace scute of green turtles has been assessed empirically by Shimada et al. (2014) as -1.4‰ for $\delta^{13}C$ and 2.5‰ for $\delta^{15}N$. The stable isotope ratios of local macroalgae from Praia do Forte and Ubatuba were corrected accordingly to define the polygon within the $\delta^{13}C$-$\delta^{15}N$ isospace that is expected for enclosing the stable isotope ratios of carapace scute layers in a way that is compatible with herbivorous diets at each locality.

We used the Bayesian stable isotope mixing model in the Stable Isotope Analysis in R (SIAR) package (Parnell et al. 2010) to assess the feasible contribution of the jellyfish *Velella velella* and several macroalgae to the diet of those green turtles, with stable isotope ratios lying outside the

mixing polygon. SIAR assumes that the variability associated with food sources and trophic enrichment is normally distributed (Parnell et al. 2010). To better restrict our model, we used elemental concentrations (%C and %N) in each prey (Claudino et al. 2013). Only the innermost layer from each turtle was included in SIAR, because older samples may correspond to foraging somewhere else and do not reveal local diet.

Data are always reported as mean ± SD, unless stated otherwise.

Study area	ID	CCL (cm)	$\delta^{13}C$ range (‰)	$\delta^{13}C$ CV (%)	$\delta^{15}N$ range(‰)	$\delta^{15}N$ CV (%)	Time (days)
Praia do Forte - BA	PF1	45.6	-17.6/-21.7	7.0	7.7/7.9	1.0	378
Praia do Forte - BA	PF2	85.9	-18.0/-18.7	1.7	8.7/ 9.0	1.4	270
Praia do Forte - BA	PF3	104	-15.2/-16.6	3.7	8.0/8.6	2.6	270
Praia do Forte - BA	PF4	70.2	-15.4/-15.7	6.7	8.0/8.1	1.7	486
Praia do Forte - BA	PF5	53.7	-14.9/-16.2	3.8	8.1/ 9.1	4.9	216
Praia do Forte - BA	PF6	56.4	-14.1/-14.2	0.3	7.7/4.8	0.7	162
Praia do Forte - BA	PF7	60.5	-16.3/-18.0	4.1	9.4/10.7	4.6	270
Praia do Forte - BA	PF8	66.0	18.6/-19.6	3.9	6.7/10.4	2.5	216
Praia do Forte - BA	PF9	49.3	-15.4/-15.7	0.7	8.0/8.1	0.7	378
Praia do Forte - BA	PF10	57.8	-15.4/-17.4	4.7	8.1/8.5	1.6	432
Praia do Forte - BA	PF11	49.6	-15.5/-15.9	1.0	8.2/8.5	1.3	216
Praia do Forte - BA	PF12	38.8	-13.9/-14.5	4.7	11.3/11.6	1.1	270
Praia do Forte - BA	PF13	44.0	-15.0/-21.1	11.1	6.6/10.0	6.2	162
Praia do Forte - BA	PF14	31.1	-15.7/-17.6	4.2	5.7/7.5	12.5	216
Praia do Forte - BA	PF15	35.0	-17.6/-18.8	2.5	8.9/10.1	4.8	270
Praia do Forte - BA	PF16	40.0	-14.5/-16.2	3.4	8.4/9.0	2.7	486
Ubatuba - SP	UB1	41.3	-14.9/-17.4	6.0	10.5/11.2	2.5	270
Ubatuba - SP	UB2	45.0	-16.2/-16.6	1.0	9.8/10.0	1.1	162
Ubatuba - SP	UB3	58.3	-15.8/-19.5	7.0	10.8/12.3	5.3	378
Ubatuba - SP	UB4	53.3	-15.0/-17.3	5.2	10.1/11.7	5.0	324
Ubatuba - SP	UB5	54.2	-19.8/-20.5	1.4	12.2/12.4	0.5	378
Ubatuba - SP	UB6	61.4	-17.4/-18.3	5.7	10.4/13.4	21.0	324
Ubatuba - SP	UB7	45.7	-18.6/-19.6	1.6	6.7/10.4	17.0	378
Ubatuba - SP	UB8	39.7	-18.5/-20.1	3.7	5.3/6.6	10.3	216
Ubatuba - SP	UB9	40.0	-18.1/-20.1	3.7	8.1/11.4	14.4	270
Ubatuba - SP	UB10	44.7	-18.9/-19.8	44.3	6.9/11.8	48.1	378
Ubatuba – SP	UB11	47.0	-19.6/-20.2	1.0	10.9/11.8	2.5	432
Ubatuba – SP	UB12	37.0	-17.3/-19.3	3.6	5.6/6.0	2.0	324
Ubatuba – SP	UB13	70.7	-17.2/-18.0	2.0	13.5/14.0	1.6	270
Ubatuba – SP	UB14	34.0	-16.7/-19.7	4.6	8.4/9.8	4.7	486

Table 1: Summary of stable isotope descriptors for the 16 green turtles (*Chelonia mydas*) sampled from Praia do Forte, and the 14 from Ubatuba, São Paulo, Brazil. ID: identification number; CCL: curved carapace length; CV: coefficient of variability.

Results

Turtles ranged 31.1 to 104.0 cm CCL in Praia do Forte, and 34.9 to 70.2 cm CCL in Ubatuba (Table 1).

Variability in $\delta^{13}C$ values across carapace layers was similar in the juvenile green turtles from Praia do Forte and Ubatuba (Table 1; F= 2.255, p= 0.144). Conversely, variability in $\delta^{15}N$ values across carapace layers was much greater in the green turtles from Ubatuba than in those from Praia do Forte for (Table 1; F= 8,703, p=0.006).

The stable isotope ratios of most of the turtles from Praia do Forte (14 out of 16) were highly consistent across carapace layers (Table 1) and were also compatible with a local herbivorous diet (Fig. 1). Only two of the smallest turtles (measuring 31.1 and 38.1 cm CCL) had stable isotope ratios incompatible with a local herbivorous diet in at least some carapace layers (Fig. 2; panels P6 and P16). The layers outside the mixing polygon were more enriched than expected in ^{13}C, but not in ^{15}N. This suggests that unusual stable isotope ratios resulted from foraging somewhere else and not because of local mixed diets that included animal prey.

Conversely, only two turtles from Ubatuba (measuring 34.0 and 45.0 cm CCL) had stable isotope ratios in their carapace scutes compatible with a local herbivorous diet (Fig. 3; panels P2 and P14). The remaining 12 turtles (ranging from 37.0 to 70.7 cm CCL (Table 1) had stable isotope ratios lying outside the local mixing polygon and they varied largely across layers in $\delta^{13}C$, $\delta^{15}N$, or both (Fig. 3). Two of them (Fig. 3; panels P8 and P12) had $\delta^{15}N$ values lower than those expected for a local herbivorous diet; seven had $\delta^{15}N$ values higher than those expected for a local herbivorous diet (Fig. 3; panels P1 to P6); and three had values both above and below those expected for a green turtle with a local herbivorous diet (Fig. 3; panels P9, P10 and P7). SIAR revealed that the stable isotope ratios above the mixing polygon were consistent with a mixed diet deriving approximately 20% of the nutrients from *Velella velella* and 80% from local macroalgae

(Fig. 4). However, turtles from southern Brazil eating a plant-based diet were expected to have similar stable isotope ratios; thus, a recent arrival from more southern foraging grounds cannot be ruled out. On the other hand, $\delta^{15}N$ values lower than those in the mixing polygon certainly revealed foraging somewhere else, and these were observed in two of the smallest turtles from Ubatuba (measuring 37.0 and 39.7 cm CCL) (Fig. 3; panels UB8 and UB12).

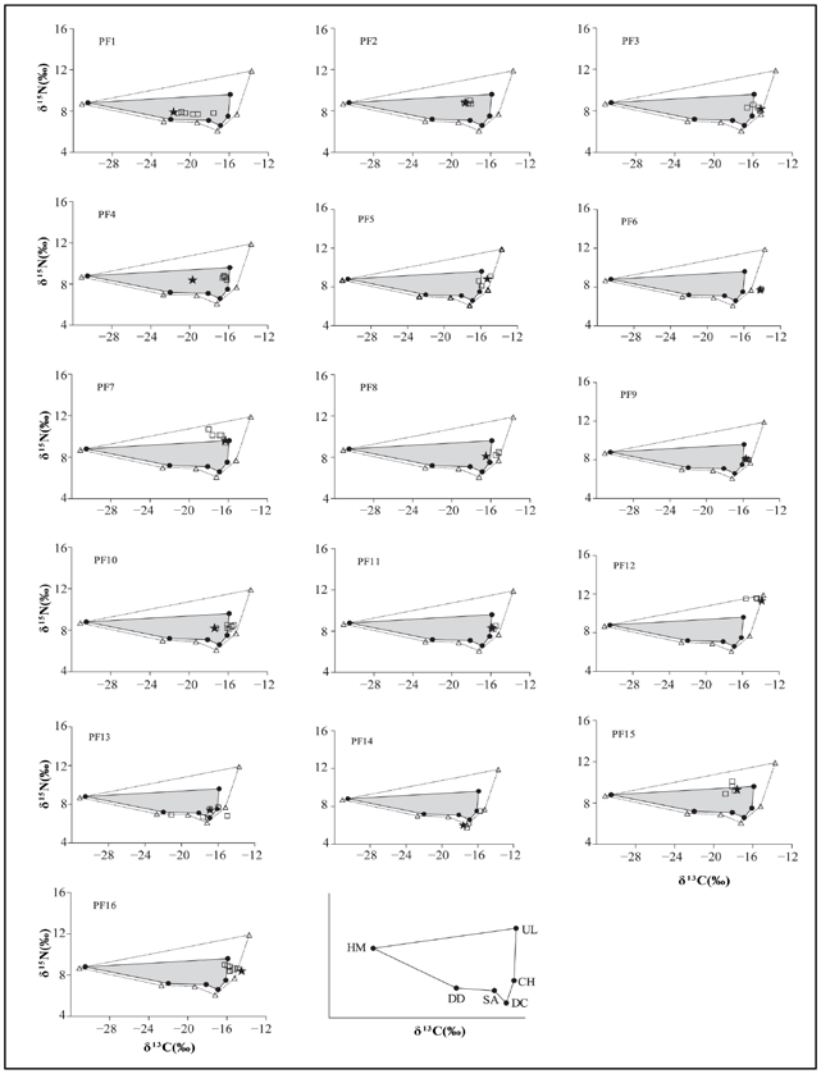

Fig. 2: $\delta^{15}N$-$\delta^{13}C$ biplot showing the position of 16 green turtles (PF1-PF16; see Table 1) in relation to the mixing polygon delimited by the macroalgae from Praia do Forte. The solid star denotes the innermost carapace layer and the open squares the older layers. The solid line connecting the solid circles shows the mixing polygon delimited by the average values of macroalgae, and the dashed line connecting the triangles shows the 95% confidence interval contour. The bottom right panel shows the position of each macroalgae in the mixing

polygon (CH: *Chondracanthus* spp.; DD: *Dictyopteris delicatula*; DY: *Dictyota dichotoma;* HM: *Hypnea musciformis;* SA: Sargassum sp.; UL: *Ulva* spp.).

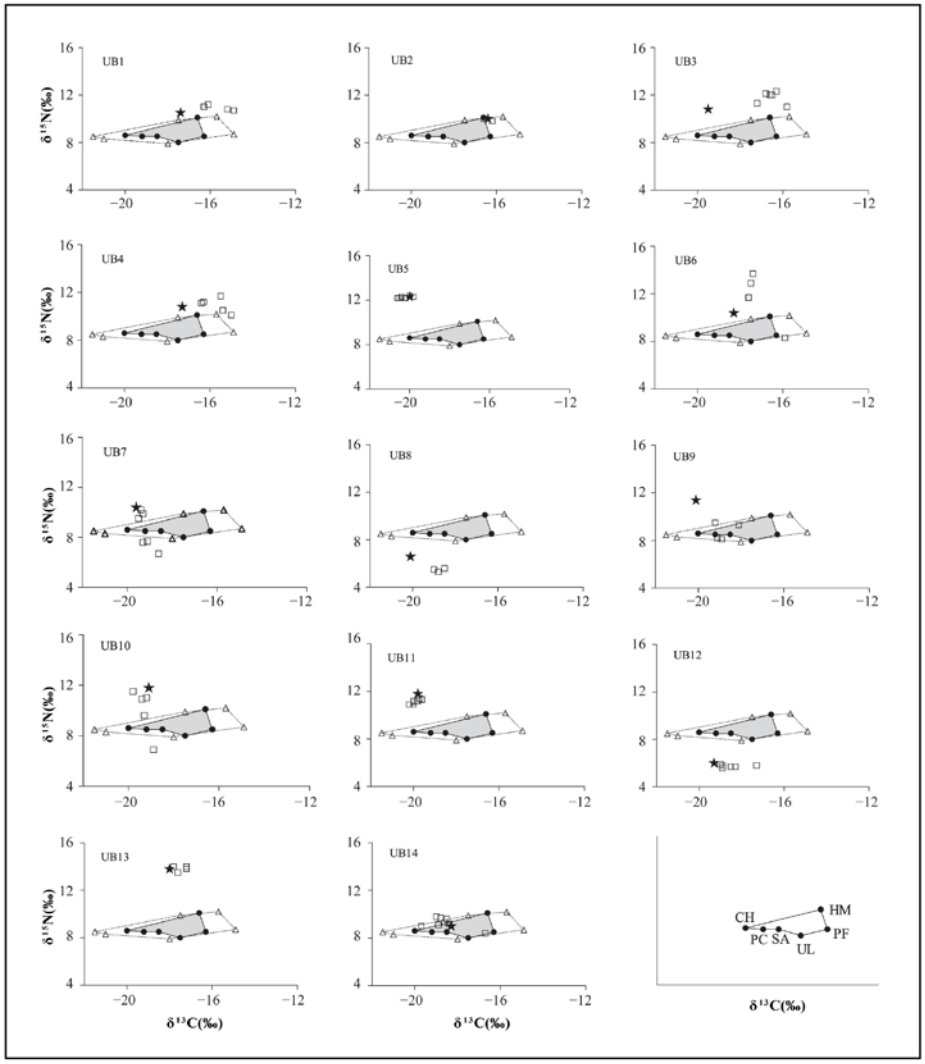

Fig. 3: $\delta^{15}N$-$\delta^{13}C$ biplot showing the position of 13 green turtles (UB1-UB14; see Table1) in relation to the mixing polygon delimited by the macroalgae from Ubatuba. The bottom right panel shows the position of each macroalgae in the mixing polygon (CH: *Chondracanthus* spp.; HM: *Hypnea musciformis;* PC: *Pterocladiella capillacea;* PF: *Palisada*

flagelifera; SA: *Sargassum* sp.; UL: *Ulva* spp.). Al other details as in Fig.2.

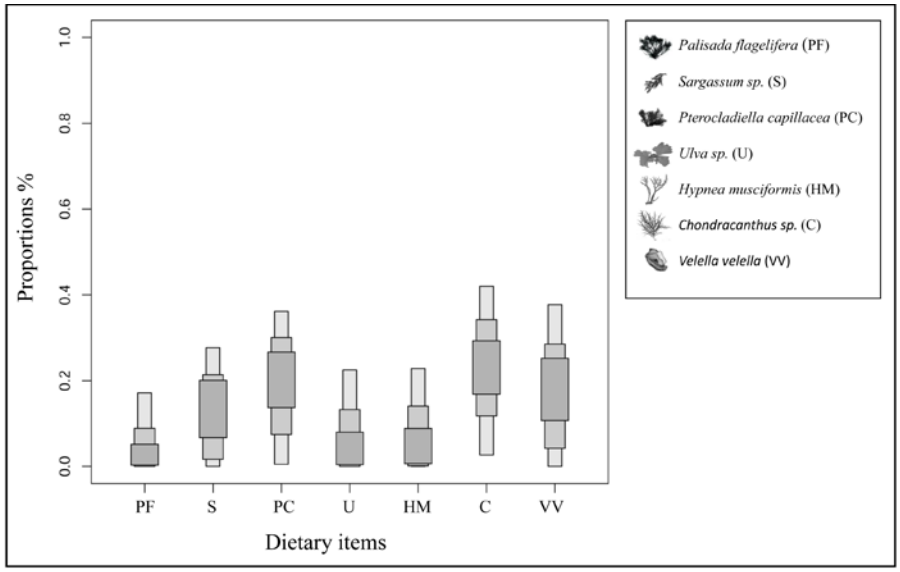

Fig.4: Feasible contribution of prey to the diet of juvenile green turtles from Ubatuba (n=12), according to the Stable Isotope Analysis in R (SIAR); including 95, 75 and 50% credibility intervals (light, medium and dark grey shading, respectively)

Discussion

It is no straightforward task to interpret whether an isotope ratio within a given portion of a turtle's scute is due to local trophic status or to prior foraging location, particularly if residency times are unknown and individual diet preferences exist. Turtles from southern Brazil were particularly challenging, as the stable isotope ratios observed in their more recent carapace scute layers were consistent with either a local omnivorous diet or a plant based diet from Uruguay (Vélez-Rubio et al. 2018) that would thus represent a recent arrival to the sampling area.

Despite those challenges, juvenile neritic green turtles from Praia do Forte and Ubatuba certainly differed in their diets and patterns of habitat use, as revealed by stable isotope ratios across carapace layers. Those from Praia do Forte had relatively consistent isotope ratios, which were usually consistent with an herbivorous diet based on local seaweeds. This evidence suggests that most of them settled in the area and shifted to an herbivorous diet more than one year prior to sampling. They also exhibited limited individual variability and few temporal changes in diet or habitat. Conversely, most juvenile green turtles from Ubatuba had omnivorous diets, with high levels of individual and temporal variability in stable isotope ratios, particularly in $\delta^{15}N$. This evidence suggests a more complex life history, with frequent changes in diet and habitat.

Previous research had already reported not only a rapid dietary shift from a carnivorous to an herbivorous diet after the settlement of juvenile green turtles in tropical neritic habitats (Reich et al. 2007; Guebert-Bartholo et al. 2011; Santos et al. 2011; Nagaoka et al. 2012; Bezerra et al. 2015; Jardim et al. 2015; Gama et al. 2016), but also a remarkable temporal consistency in diet and habitat use thereafter (Vander Zanden et al. 2013). In contrast, juvenile green turtles settling in warm temperate or subtropical habitats usually consume plant-based omnivorous diets and exhibit frequent habitat and diet shifts (Cardona et al. 2009; Cardona et al. 2010; González

Carman et al. 2012; González Carman et al. 2014; Morais et al. 2014; Vélez-Rubio et al. 2016; Williard et al. 2017).

Campos et al. (2018) revealed the fast acquisition of a carbohydrate-fermenting gut microbiota by neritic green turtles after settlement both in Praia do Forte and Ubatuba, without major differences in the composition of the gut microbiota at the two localities. Accordingly, the hypothesis should be ruled out that green turtles in subtropical and warm temperate regions have more carnivorous diets because of a delayed acquisition of a carbohydrate-fermenting gut microbiota (Cardona et al. 2010). Alternatively, higher levels of omnivory and frequent diet and habitat shifts in subtropical and warm temperate regions might result from a lower and highly seasonal availability of fodder. If so, subtropical and warm temperate developmental habitats are indeed suboptimal, and juvenile green turtles settle there only because they drift there with the currents.

This is particularly dramatic in the western South Atlantic, where most oceanic juveniles from Ascension Island drift to north-eastern Brazil (Putman and Naro-Maciel 2013) while skipping past the mixed juvenile/adult foraging grounds stretching north to latitude 12ºS, and they instead settle in southern developmental habitats. As adults occur only north to latitude 12ºS, they must move north as they grow older, thus returning to areas they skipped past as juveniles (Fig. 1).

Laboratory experiments, satellite telemetry and genetic markers have revealed that juvenile green turtles ranging from 14 to 30 cm are able to sustain short periods of directional swimming to avoid areas and conditions unfavourable for survival (Prange 1976; Reich et al. 2007; Putman and Mansfield 2015). However, they probably lack the necessarily tight control of their buoyancy for settling in neritic habitats (Hochscheid et al. 2003). Indeed, even larger juveniles (38-48 cm CCL) may follow prevailing currents (González Carman et al. 2011).

Most post-hatchlings from Ascension reach north-western Brazil in less than two years (Putman and Naro-Maciel 2013). Juvenile green turtles that age in the western South Atlantic are certainly less than 35 cm CCL (Andrade et al. 2016; Lenz et al. 2017; Tomaszewicz et al. 2018)), although young green turtles from other populations can grow much faster (Tomaszewicz et al. 2018). This indicates that only the fastest growing members of each cohort from Ascension Island are probably large enough to settle in the neritic habitats of north-eastern Brazil upon reaching them for the first time. The majority of each cohort will likely continue drifting southwards along the Brazil Current until they grow large enough to control buoyancy and settle in neritic habitats.

The meandering of the Brazil Current far away from the continental shelf south to Cape Sao Tomé (Longhurst 1998; Da Silveira et al. 2008) may delay settlement even more, because most oceanic juveniles reaching latitude 20ºS are probably averted off-shore and reside in the ocean for an extended period before once again approaching the continental shelf off southern Brazil and Uruguay (Putman and Naro-Maciel 2013). They will then be 3-4 years old (Putman and Naro-Maciel 2013) and 40 cm CCL (Andrade et al. 2016; Lenz et al. 2017), which corresponds to the size at settlement reported for Uruguay (Vélez-Rubio et al. 2018). The high level of individual variability observed in the $\delta^{15}N$ values of juvenile green turtles from Ubatuba likely reveals the diversity of drifting trajectories during the pre-settlement and settlement phases of the life cycle.

In conclusion, this study demonstrates a diversity of ontogenetic trajectories for green turtles before and after settlement in the neritic habitats of the western South Atlantic, which likely results from broad variability in the rate of somatic growth of juvenile green turtles. This suggests that juvenile green turtles settle in developmental habitats south to latitude 12ºS not because they are optimal, but merely because they serve

as convenient end points in the oceanic dispersal phase once they grow large enough to become neritic.

Declarations

Ethics approval and consent to participate

The fieldwork in natural reserves and handling of wild animals was carried out under the authority of the Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio (license reference ICMBio/SISBIO 52128-1), and of the Convention on International Trade in Endangered Species of Wild Fauna and Flora – CITES (license reference CITES 16BR020234/DF).

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Fast acquisition of a polysaccharide fermenting gut microbiome by juvenile green turtles *Chelonia mydas* after settlement in coastal habitats

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Abstract

Tetrapods do not express hydrolases for cellulose and hemicellulose assimilation and, hence, the independent acquisition of herbivory required the establishment of new endosymbiotic relationships between tetrapods and microbes. Green turtles (*Chelonia mydas*) are one of the three groups of marine tetrapods with an herbivorous diet and acquire it after several years consuming pelagic animals. We characterized the microbiota present in the feces and rectum of 24 young wild and captive green turtles from the coastal waters of from Brazil, ranging in curved carapace length from 31.1 to 64.7 cm, to test the hypotheses that (1) the ontogenetic dietary shift after settlement is followed by a gradual change in the composition and diversity of the gut microbiome, (2) that differences exist between the composition and diversity of the gut microbiome of green turtles from tropical and subtropical regions and (3) that the consumption of omnivorous diets modifies the gut microbiota of green turtles.A genomic library of 2,186,596

valid bacterial 16S rRNA reads was obtained and these sequences were grouped into 6,321 different OTUs (at 97% sequence homology cutoff). The results indicated that most of the juvenile green turtles less than 45 cm of curved carapace length exhibited a fecal microbiota co-dominated by representatives of the phyla *Bacteroidetes* and *Firmicutes* and high levels of *Clostridiaceae, Prophyromonas, Ruminococaceae* and *Lachnospiraceae* within the latter phylum. Furthermore, this was the only microbiota profile found in wild green turtles >45 cm CCL and in most of the captive green turtles of any size feeding on a macroalgae/fish mixed diet. Nevertheless, microbial diversity increased with turtle size and was higher in turtles from tropical than from subtropical regions.These results indicate that juvenile green turtles from the coastal waters of Brazil had the same general microbiota, regardless body size and origin, and suggest a fast acquisition of a polysaccharide fermenting gut microbiota by juvenile green turtles after settlement into coastal habitats.

Keywords: Tetrapods, herbivorous, microbial communities, *Chelonia mydas*, 16S rRNA, fermentation.

Background

Herbivory has evolved independently in several groups of tetrapods belonging to diverse evolutionary lineages(Sues and Reisz 1998). Unlike some invertebrates, tetrapods do not express hydrolases for cellulose and hemicellulose (Barboza et al. 2010) and hence the independent acquisition of herbivory required the establishment of new endosymbiotic relationships between tetrapods and microbes (Sues and Reisz 1998; Mackie et al. 2008; Hong et al. 2011; Keenan et al. 2013). As a consequence, the composition, abundance and diversity of the gut microbiota of herbivorous tetrapods varies widely across groups, reflecting not only their evolutionary relationships but also their foraging habits and the location of the cavity of

fermentation into the gut - hindgut vs. foregut fermenters (Edwards and Ullrey 1999; Clauss et al. 2003; Miyake et al. 2015).

Several groups of tetrapods have recolonised the marine environment after independent evolution in land, but only three of them are herbivores: sirenians (manatees and the dugong), the marine iguana (*Amblyrhynchus cristatus)* and the green turtle (*Chelonia mydas*). Sirenian diet is dominated by seagrasses (Marsh et al. 1982; Mignucci-Giannoni and Beck 1998; André et al. 2005; Castelblanco-Martinez et al. 2009) which are vascular plants rich in cellulose (Bjorndal 1980; Yamamuro and Chirapart 2005). Consequently, sirenians host microorganisms producing the enzymes needed for the fermentative digestion of cellulose (Eigeland et al. 2012; Merson et al. 2014). On the other hand, marine iguanas feed only on macroalgae (Wikelski and Trillmich 1993). The cell wall of macroalgae differs from that of seagrasses and other vascular plants in the abundance of sulfated polysaccharides and alginic acid and low levels of cellulose (Graham and Wilcox 2000). As a consequence, the microbiota of marine iguanas is characterized by the presence of some specific groups of methanogens and differs largely from that of terrestrial iguanas, despite a close evolutionary relationship (Hong et al. 2011). Green turtles exhibit a much larger dietary flexibility than sirenians and marine iguanas, as they undergo a major ontogenetic dietary shift from animal-based to plant-based diets following settlement in coastal areas (Reich et al. 2007; Arthur et al. 2008; Cardona et al. 2009; Cardona et al. 2010; Parker et al. 2011; González Carman et al. 2012; Howell et al. 2016). Nevertheless, they also exhibit a high level of regional variability in the degree of omnivory after settlement and the relative importance of seagrasses and seaweeds in their diets (Ferreira 1968; Mortimer 1982; Bjorndal 1995; Cardona et al. 2009; Russell and Balazs 2009; Cardona et al. 2010; Carrión-Cortez et al. 2010; Burkholder et al. 2011; Santos et al. 2015; Howell et al. 2016; Vélez-Rubio et al. 2016; Reisser et al. 2013).

The acquisition of a specialized microbiota is facilitated by lactation and intimate calve/mother relationships in mammals (Rey et al. 2013) and the consumption of conspecific excrements in marine iguanas (Wikelski and Trillmich 1993). On the contrary, the solitary lives of green turtles may delay the acquisition of a specialized gut microbiota, which in combination with the higher body temperature of larger turtles in winter may explain the improved digestibility and assimilation of plant material as green turtles grow (Bjorndal 1980; Cardona et al. 2010). This is because green turtles are ectothermic and the body temperature of inactive adult green turtles can be 2 ºC above water temperature thanks to gigantothermy (Standora et al. 1982), whereas that of juveniles matches that of the environment [83]. It has also been suggested that mixed seagrass/macroalgae diets are uncommon in green turtles because the entirely different structure of polysaccharides in their cell walls would require different compositions of the gut microbiota (Bjorndal 1985). In such case, frequent and short term shifts in diet may reduce the efficiency of plant digestion (Bjorndal et al. 1991).

Unfortunately, very little is known about the gut microbiota of green turtles, how it changes after settlement in coastal areas in association to the increase in the consumption of plant material and the influence of turtle diet on microbiota composition. The only information available to our knowledge is about the microbiota present in the cloaca of pelagic and recently settled green turtles, which reveals a high prevalence of *Proteobacteria* and a low occurrence of bacteria associated to the fermentation of structural polysaccharides (Price et al. 2017). In this study, we characterize the microbiota present in the feces and rectum of young wild and captive green turtles from Brazil to test the hypotheses that [1] the ontogenetic dietary shift after settlement is followed by a gradual change in the composition and diversity of the gut microbiome, [2] differences exist in the composition and diversity of the gut microbiome of green turtles from

tropical and subtropical regions and [3] the consumption of omnivorous diets modifies the gut microbiota of green turtles.

Methods

Study Area

Two different areas of Brazil were sampled in February to March 2016. Most samples (n=20) were collected from subtropical Ubatuba (23º 26′S, 45º 05′ W), in the northern coast of the state of Sao Paulo. Rocky reefs and sandy beaches dominate the coastline of Ubatuba (Gallo et al. 2006). A few additional samples (n = 5) were collected from tropical Praia do Forte (12º 38′ S 38º 05′ W), located 70 km from Salvador do Bahia. The coastline is characterized by the presence of shallow coral reefs with substantial air exposition during low tide (Moraes and Machado 2003).

Sampling

Fecal samples were collected from 8 turtles held in captive at the facilities of Projeto Tamar at Ubatuba and 11 wild turtles from Ubatuba. Some wild green turtles were captured alive in weirs ("*Cercos flutuantes*") used by local fishermen and consisting on fixed nets attached to the seafloor (Silva et al. 2017), and others were captured alive through free diving by members of Projeto Tamar (www.tamar.org.br), as part of the long-term study on the abundance and habitat use of green turtles along the Brazilian coast. After capture, curved carapace length (CCL) was measured with a flexible tape (CCL, notch to tip) and turtles were moved to the facilities of Projeto Tamar in Ubatuba. These turtles were confined in individual PVC tanks until the moment they defecated, between 24 and 36 hours after capture, and then released back to the sea at the same place of capture. Tanks had been previously disinfected with regular bleach. The core of each fecal pellet was accessed using sterilized forceps and sampled with a swab, to reduce as much as possible contamination from water. Additionally,

rectal samples (n=5) were collected with a swab during the necropsy of recently dead turtles at Praia do Forte.

Fecal and rectal samples were stored at 4ºC immediately after collection and then at -20ºC until DNA extraction. No buffers were used. All procedures were non-invasive and conducted in accordance with guidelines from the Projeto TAMAR and ICMBio.

DNA extraction and Next Generation Sequencing

DNA was extracted from a subsample of 0.25 g from each fecal or rectal sample using the PowerSoil DNA kit (MO BIO Laboratories, Carlsbad, CA) following the manufacturer's instructions. All DNA extracts were kept frozen at -20ºC until further analysis. Massive bar-coded 16S rRNA gene-based libraries in the *Eubacteria* domain were sequenced by using the MiSeq Illumina platform (Molecular Research DNA LP, Shallowater, USA). These gene libraries were constructed by targeting the V1-V3 hypervariable regions with the primer set 27F (5'-AGRGTTTGATCMTGGCTCAG–3') / 519R (5'-GTNTTACNGCGGCKGCTG-3') as previously described in (Dowd et al. 2008) . The obtained DNA reads were compiled in FASTq files for further bioinformatic processing. Trimming of the 16S rRNA barcoded sequences into libraries was carried out using QIIME software version 1.8.0 (Caporaso et al. 2010). Quality filtering of the reads was performed at Q25, the default set in QIIME, the default set in QIIME, prior to the grouping into Operational Taxonomic Units (OTU) at a 97% sequence homology cutoff. The following steps were performed using QIIME: Denoising of sequence data using Denoiser (Reeder and Knight 2010), picking up of OTU reference sequences via the first method of the UCLUST algorithm (Edgar 2010) and, for sequence alignment and chimera detection, processing by PyNAST (Caporaso et al. 2010b) and ChimeraSlayer (Haas et al. 2011). OTUs were then taxonomically classified using BLASTn against

GreenGenes and RDP (Bayesian Classifier) databases and compiled into each taxonomic level (DeSantis et al. 2006).

Biostatistical methods

A general lineal model (GLM) using locality (Ubatuba vs. Praia do Forte) as a fixed factor and turtle curved carapace length as a covariable was used to test the hypothesis that the microbial diversity of wild green turtles increases with turtle size and varies across localities. A general lineal model using origin (captive vs. wild) as a fixed factor and turtles curved carapace length as a covariable was used to test the hypothesis that the microbial diversity of green turtles increases with turtle size and differs between captive and wild green turtles from subtropical Ubatuba. GLMs were run in IBM SPSS Statistics 23. Multivariate Principal Coordinate Analysis (PCoA) based on Bray-Curtis similarity distances was carried out on the OTUs incidence matrix using the CANOCO software package, version 5 (Microcomputer Power, Ithaca, NY, USA), to identify clusters of green turtles differing in the community structure of their microbiomes

Results

The gut microbiome of 24 green turtles ranging in curved carapace length (CCL) from 31.1 to 64.7 cm, was studied. A genomic library of 2,187,066 valid eubacterial 16S rRNA reads was obtained from their faeces (Supplementary Material). These sequences were grouped into 6,321 different OTUs (at 97% sequence homology cutoff), ranging from 473 to 1,952 in individual turtles (Table 1). The Good's coverage estimator on the percentage of the total species (as OTUs) represented in any given sample

Study area	Origin	Turtle	CCL (cm)	Total reads	OTUs	Coverage	Shannon (ave ± SD)*	Chao1 (ave ± SD)*
Praia do Forte -BA	wild	PF1	31.1	70792	1589	99%	4.69 ± 0.006	2015 ± 78
Praia do Forte -BA	wild	PF2	35.0	111405	1794	99%	4.17 ± 0.008	1790 ± 88
Praia do Forte -BA	wild	PF3	38.8	70850	1997	98%	5.14 ± 0.006	2523 ± 85
Praia do Forte -BA	wild	PF4	40.0	90045	1911	99%	4.22 ± 0.008	2148 ± 87
Praia do Forte -BA	wild	PF5	44.0	89351	2211	98%	5.05 ± 0.007	2466 ± 90
Ubatuba - SP	wild	UB6	37.0	127862	1217	99%	2.16 ± 0.009	1071 ± 69
Ubatuba - SP	wild	UB7	39.7	68389	601	99%	2.59 ± 0.006	956 ± 80
Ubatuba - SP	wild	UB8	40.0	98513	1954	99%	4.70 ± 0.007	2053 ± 77
Ubatuba - SP	wild	UB9	41.3	76055	1947	99%	4.82 ± 0.006	2389 ± 90
Ubatuba - SP	wild	UB10	44.7	61852	598	99%	3.08 ± 0.005	953 ± 67
Ubatuba - SP	wild	UB11	45.0	119273	2150	99%	4.37 ± 0.008	2036 ± 93
Ubatuba - SP	wild	UB12	47.0	119764	2206	99%	4.47 ± 0.008	2050 ± 81
Ubatuba - SP	wild	UB13	53.3	84889	2006	99%	4.60 ± 0.008	2264 ± 79
Ubatuba - SP	wild	UB14	54.2	107097	1670	99%	3.24 ± 0.009	1657 ± 71
Ubatuba - SP	wild	UB15	58.3	90582	1951	99%	4.53 ± 0.007	2187 ± 90
Ubatuba - SP	wild	UB16	61.4	79361	2179	98%	5.15 ± 0.006	2540 ± 83
Ubatuba - SP	captivity	UB17	32.5	103168	2284	99%	4.55 ± 0.008	2355 ± 88

Ubatuba - SP	captivity	UB18	34.9	121100	1481	99%	2.88 ± 0.009	1374 ± 75
Ubatuba - SP	captivity	UB19	38.6	56987	1723	99%	4.85 ± 0.005	2447 ± 77
Ubatuba - SP	captivity	UB20	40.0	123937	1442	99%	2.79 ± 0.009	1302 ± 71
Ubatuba - SP	captivity	UB21	41.3	101478	2436	98%	4.68 ± 0.008	2549 ± 93
Ubatuba - SP	captivity	UB22	53.5	99346	2079	99%	4.17 ± 0.009	2118 ± 75
Ubatuba - SP	captivity	UB23	58.6	70520	2330	98%	5.38 ± 0.006	2802 ± 77
Ubatuba - SP	captivity	UB24	64.7	43980	1036	99%	4.70 ± 0.001	1875 ± 34
Range			31.1-64.7	70792-127862	1589-2436	98-99%	2.16-5.38	953-2549

** Calculated upon sample rarefaction at 43000 reads*

Table 1: Descriptors of bacterial diversity in fecal and rectal samples of juvenile green turtles *Chelonia mydas* from Brazil. CCL= curved carapace length, BA= State of Bahia, SP= State of Sao Paulo; ave =average. Fecal samples were collected at Ubatuba and rectal samples at Praia do Forte.

was above 98%, indicating that the observed species encompassed a very significant proportion of the entire sample populations. With this respect, the number of expected OTUs (Chao 1) ranged from 959 to 2,818 and the Shannon index from 2.17 to 5.38 (Table 1). The number of recovered and expected OTUs in wild turtles form Praia del Forte was larger than in wild turtles from Ubatuba and increased significantly with curved carapace length in both areas according to GLM (Table 2). However, the indices of microbial diversity did not differ between wild and captive turtles from Ubatuba (GLM; OTUs: $F_{2,18}$=1.750, p=0.205; Chao1: $F_{2,18}$=1.922, p=0.179; Shannon: $F_{2,18}$=2.445, p=0.118).

Microbial diversity		**F**	**df**	**p**	**r^2**
OTUs	Model	4.155	2,15	0.040	0.296
	CCL	6.016	2,16	0.023	
	Area	6.205	2,16	0.028	
Chao 1	Model	4.517	2,16	0.032	0.319
	CCL	6.177	2,15	0.027	
	Area	7.671	2,15	0.016	
Shannon	Model	3.180	2,16	0.075	NA
	CCL	3.939	2,15	0.069	
	Area	2.708	2,15	0.033	

Table 2: Summary statistics of general lineal models describing the relationship between indices of microbial diversity in fecal and rectal samples of wild juvenile green turtles *Chelonia mydas* from Brazil and carapace length (CCL

The dominant phyla in the majority of wild and captive turtles were *Bacteroidetes*, ranging 20-70% of relative abundance (RA), and *Firmicutes* with a 24-56% of RA (Figure 1). In most of the studied turtles (Figure 2), the predominant families within *Bacteroidetes* phylum were *Bacteroidaceae* and *Porphyromonadaceae*, while within *Firmicutes* phylum the predominant families were *Clostridiaceae*, *Lachnospiraceae* and *Ruminococcaceae*, with the exception of two wild individuals and one captive individual from Ubatuba . The bacterial community structure of these two anomalous wild turtles (UB7 and UB10) was characterized by a high RA of representatives from (60% RA) and *Actinobacteria*, which in this latter phylum belonged to the *Mycobacterium* genus (1.2% and 4.7% RA in UB7 and UB10, respectively). The main OTUs of the former *Proteobacteria* phylum were related to *Burkholderia* spp. (*Betaproteobacteria*), *Sphingopyxis* spp. (*Alphaproteobacteria*) and *Pseudomonas* spp. (*Gammaproteobacteria*), which combined represented 49.5% and 38.3% RA for UB7 and UB10, respectively. Except for *Sphingopyxis*, these genera have been associated to the presence of *Staphylococcus* spp., in the phylum *Firmicutes* (5.0% and 3.5% of RA in UB7 and UB10, respectively). Regarding the bacterial community of the anomalous captive turtle (UB18), it was characterized by a high abundance of the phylum *Fusobacteria* (27% RA). Such dominance was primary caused by OTU7, affiliated with the microaerotolerant fermentative *Cetobacterium* sp. (96% of similarity to *Cetobacterium ceti*), also found in whale, dolphin and porpoise gut flora (Bik *et al* 2016).

When those three anomalous turtles (UB7, UB10, UB18) were removed from the analysis, the abundance of *Proteobacteria* was consistently higher in captive (range: 0.7-7.7% RA) than in wild (range: 0.2-1.9% RA) turtles from Ubatuba (Mann-Whitney test; U=57.00, p=0.046). On the other hand, *Akkermansia* spp., belonging to the phylum *Verrumicrobia*, was found with a RA of 8-15% in captive turtles UB18, UB20 and UB21. It is noteworthy that in one of the wild individuals

(UB14), *Akkermansia* was enriched up to a 30% of RA and, curiously, the microbiome of this individual was rather different from that of other wild turtles.

The family *Clostridiaceae* comprised a ribotype (OTU1) that was predominant in almost all samples (from 1% to 8% RA). OTU1 belongs to the unclassified *Clostridiaceae* 1 subfamily. Interestingly, the RA of OTU1 in the wild turtles with the most dissimilar microbiome (UB7 and UB10) was < 0.1% RA (Figure 1 and 2). Moreover, predominant OTUs of *Bacteriaceae* in those anomalous turtles were present at a comparatively low RA. On the other hand, representatives of the genus *Spirochaetes* were detected in all samples, but only in turtles from Praia do Forte this phylum appeared in significant amounts, especially in PF3, PF4 and PF5, where OTU6 was predominant. This OTU was distantly related (88% in sequence homology) to *Treponema brennaborense* and might therefore correspond to an undescribed species. Furthermore, samples from Praia do Forte had a lower abundance of representatives in the *Actinobacteria* and *Verrumicrobia*, when compared to the Ubatuba individuals.

Multivariate analysis (PCoA of samples'- Bray-Curtis distances based on OTUs incidence) (Figure 3), showed three major clusters in relation to the microbial community structure of the gut microbiome from the studied turtles (Figure 3). The smallest and more specific group confirmed the uniqueness of the bacterial community in the two anomalous wild turtles described above, UB7 and UB10. No significant segregation was observed between wild and captive turtles, but individuals from Ubatuba displayed a significant variability and two major groups were apparent. The minor cluster encompassed the previously described individuals that were characterized by a relatively high abundance of *Akkermansia* spp., while a second larger one also contained the samples from Praia do Forte forming a very compact subcluster.

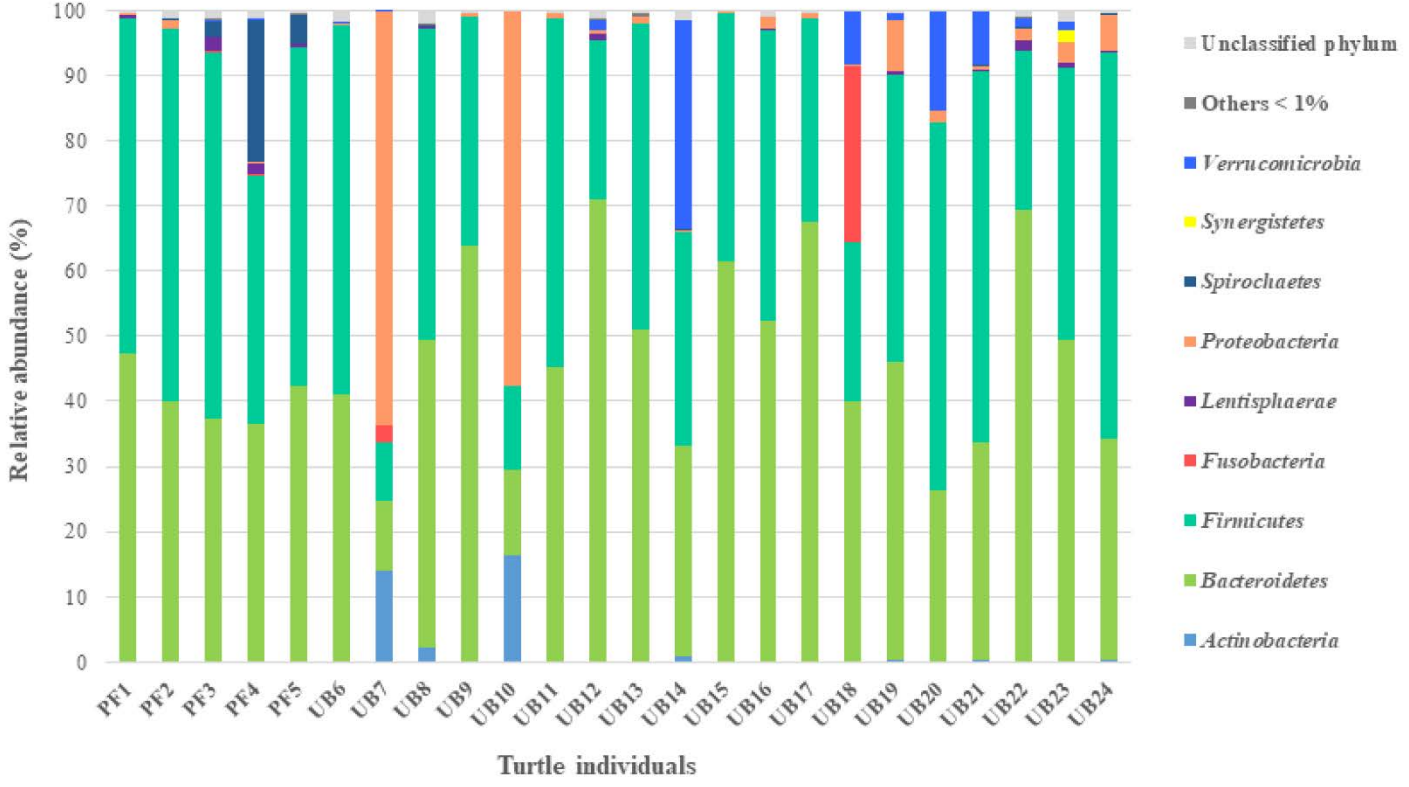

Figure 1: Percentages of sequences from each individual turtle, fecal or rectal sample assigned at the phylogenetic level of phylum, according to the RDP Bayesian Classifier database with a bootstrap confidence above 80%. PF1 to PF5= wild turtles from Praia do Forte; UB6 to UB16= wild turtles from Ubatuba; UB12 to UB24= captive turtles from Ubatuba. Taxa with a RA lower than 1% is grouped as “others”.

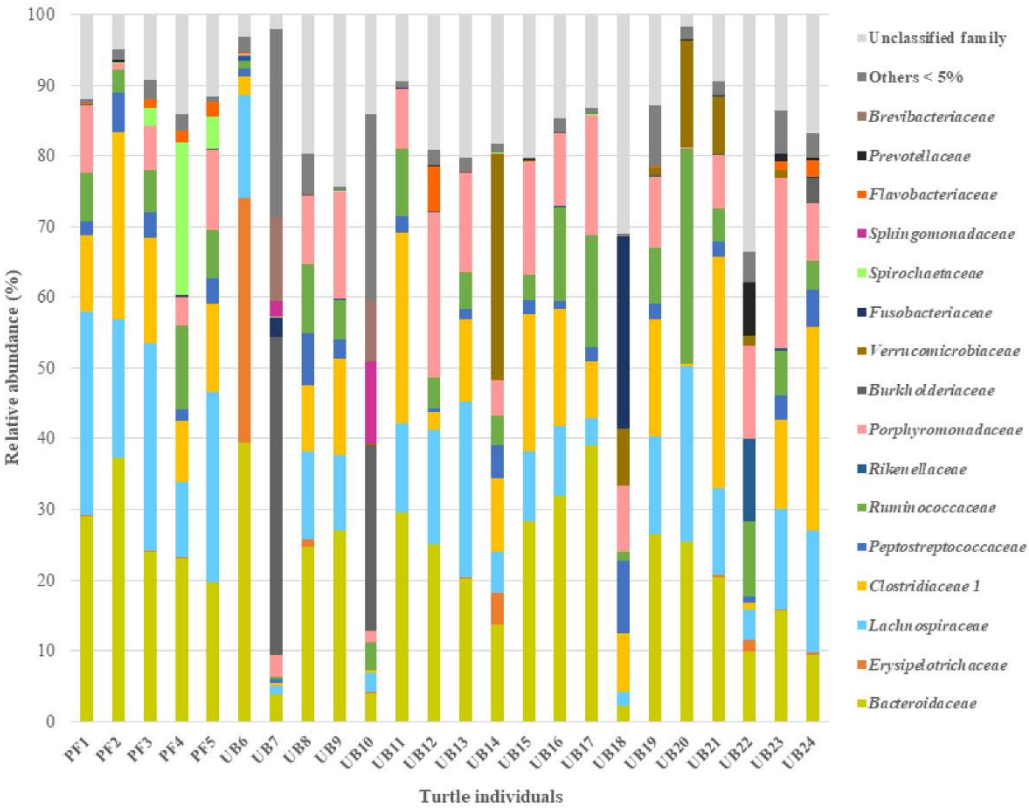

Figure 2: Percentages of sequences from each individual turtle, fecal or rectal sample assigned at the phylogenetic level of family, according to the RDP Bayesian Classifier database with a bootstrap confidence above 80%. PF1 to PF5= wild turtles from Praia do Forte; UB6 to UB16= wild turtles from Ubatuba; UB12 to UB24= captive turtles from Ubatuba. Taxa with a RA lower than 5% is grouped as “others”.

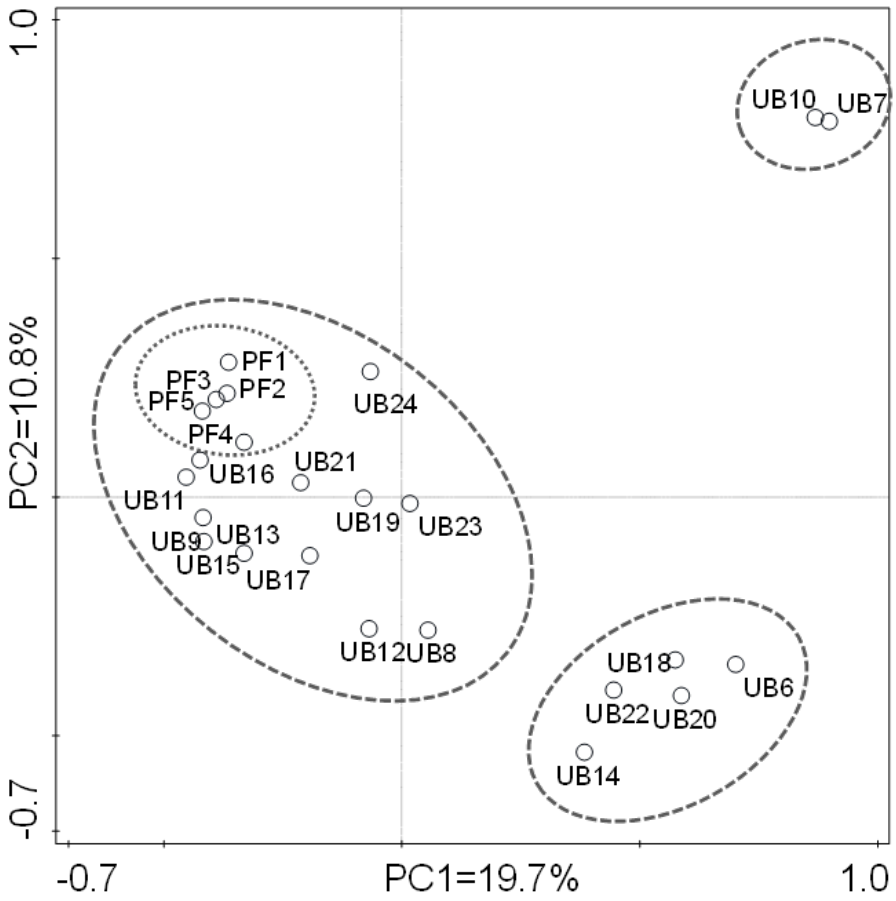

Figure 3: PCoA biplot of the gut microbiome in Brazilian green turtles based on the Bray-Curtis distance matrix. Wild turtles came from Praia do Forte (PF1 to PF5) and Ubatuba (UB6 to UB16). Captive turtle came only from Ubatuba (UB17 to UB24). The percentage of explain variation encompassed by the two main axis has been indicated. The main sample score clusters (dashed contours) and the more specific subcluster from Praia do Forte (dotted contour) have been highlighted.

Discussion

Green turtles settle in the coastal habitats of the south-western South Atlantic when they are 30-45 cm in CCL (Gallo et al. 2006; González Carman et al. 2014; Vélez-Rubio et al. 2016). The results reported here indicated that most of the green turtles less than 45 cm CCL from Brazil

exhibited a fecal microbiota co-dominated by phyla *Bacteroidetes* and *Firmicutes* and high levels of *Clostridiaceae*, *Porphyromonas*, *Ruminococcaceae* and *Lachnospiraceae* within the latter phylum. Furthermore, this was the only microbiota profile found in wild green turtles >45 cm CCL and in most of the captive green turtles of any size feeding on a macroalgae/fish mixed diet. These results suggest a fast acquisition of a polysaccharide fermenting gut microbiota by juvenile green turtles after settlement into coastal habitats.

A high abundance of *Proteobacteria* had been previously reported from the cloaca of pelagic (range: 17.1-21.7 cm CCL) and recently settled (29.4-34.6 cm CCL) juvenile green turtles from Florida and from the gut of omnivorous marine fishes, but not from other groups of herbivorous vertebrates (Table 3). A high abundance of *Proteobacteria* has been observed also in two wild and one captive green turtles from Brazil less than 45 cm CCL (this study), but this is probably because they were immunodepressed and not because of recent settlement. We hypothesize that the prevalence of the *Proteobacteria* phylum in those three individuals was because of lesions from anthropogenic impacts (Orós et al. 2005). The same is true for *Mycobacterium*, from the *Actinobacteria* phylum, a genus very uncommon in turtles but which includes several well-known pathogens for reptiles and amphibians (Rhodin and Anver 1977; Donnelly et al. 2016). Furthermore, three captive and one wild turtle shared OTUs affiliated to the *Akkermansia* genus (*Verrumicrobiaceae* family). *Akkermansia* is a mucin-degrading bacterium commonly found in the human gut and recently isolated in reptiles (Rawski et al. 2016; Ouwerkerk et al. 2017). Several studies showed that the enrichment of *Akkermansia* induces gut inflammation and is associated with colonic diseases in mammals, but nothing is known about its pathogenicity in reptiles. It is also worth noting a small captive turtle (34.9 cm CCL) with a microbiota dominated by *Bacteroidetes* and *Firmicutes* but with a high relative

abundance of *Fusobacteria*, a group occurring sporadically in carnivorous marine mammals (Keenan et al. 2013).

High levels of *Firmicutes* are characteristic of the gut and fecal microbiota of herbivorous vertebrates (Table 3), as this phylum plays a critical role in the fermentation of complex polysaccharides (Xu et al. 2003; Hong et al. 2011). The families *Ruminococcaceae* and *Lachnospiracea* are particularly relevant, as both are obligate anaerobes with capacity to

Species	**Diet**	***Firmicutes***	***Bacteroidetes***	***Verrucomicrobia***	***Spirochaetes***	***Proteobacteria***	***Actinobacteria***	**Other**
Teleosteans								
Acanthurus gahhm[1]	Omn/Alg	**29.5**	0.6	0.0	1.3	**49.4**	7.7	9.1
Naso elegans[1]	Herb/Alg	**97.4**	0.0	0.0	0.0	0.0	0.0	2.6
Naso unicornis[1]	Herb/Alg	**83.3**	9.0	**2.6**	0.0	2.6	1.3	1.2
Siganus stellatus[1]	Omn/Alg	**42.3**	11.5	0.0	2.6	**37.2**	0.0	6.4
Turtles								
Chelonia mydas[a,2]	Omn/Alg	6.5	27.1	0.6	0.0	**60.5**	0.1	5.2
Chelonia mydas[b, 2]	Herb/Seg	8.3	15.4	0.2	0.2	**66.6**	1.7	7.6
Chelonia mydas[c*,3]	Herb/Alg	10.8	11.8	0.1	0.1	**60.7**	15.2	1.3
Chelonia mydas [d,3]	Herb/Alg	**44.8**	**46.6**	**3.8**	1.3	1.1	0.3	2.1
Geochelone nigra[3]	Herb/Ter	**81.1**	4.4	0.1	0.0	2.0	0.8	11.6
Gopherus polyphemus[3]	Herb/Ter	**38.4**	**36.9**	**3.0**	4.4	<3.0	<3.0	7.4
Lizards								
Amblyrynchus cristatus[3]	Herb/Alg	**75.1**	8.2	1.0	0.0	0.6	0.6	14.5
Conolophus spp. [3]	Herb/Ter	**63.9**	4.2	0.2	0.0	1.4	1.3	29.0
Iguana iguana[3]	Herb/Ter	**74.0**	10.1	1.0	0.6	3.1	0.1	11.1
Mammals								
Antidorcas marsupialis[3]	Herb/Ter	**75.6**	24.4	0.0	0.0	0.0	0.0	0.0

Dugong dugong[3]	Herb/Seg	**57.5**	**42.5**	0.0	0.0	0.0	0.0	0.0
Gorilla gorilla[3]	Herb/Ter	**67.4**	3.5	**10.5**	2.3	0.0	11.6	4.7
Loxodonta africana[3]	Herb/Ter	**80.5**	2.5	**1.8**	0.2	10.1	4.7	0.2
Ovis canadensis[3]	Herb/Ter	**64.0**	3.0	**2.7**	0.0	2.1	25.8	2.4
Trichechus manatus[3]	Herb/Seg	**77.3**	19.5	0.0	0.1	0.3	2.0	0.8

Table 3: Relative abundance of bacterial phyla to the gut microbiota of omnivorous and herbivorous vertebrates. Bold type denote accumulated RA higher that 60%. Superscript numbers denote sample source as follows, 1: whole intestinal tract, 2: cloaca; 3: rectum or feces. Diet: ormnivores (Omn) or herbivores (Herb). Major group of plants in diet: algae (Alg), seagrasses (Seg) and terrestrial plants (Ter). Length of green turtles *Chelonia mydas*: a= 17.1-21.7 cm CCL, b=29.4-34.6 cm CCL, c=39.7-44.7, d=31.1-64.7. *= potentially immunodepressed individuals

polysaccharides into short chain volatile fatty acids (Mountfort et al. 2002; Flint et al. 2008; Pope et al. 2010; Hong et al. 2011; Biddle et al. 2013; Yuan et al. 2015) and occur in large numbers only in the gut and feces of herbivorous tetrapodes (Hong et al. 2011; Meehan and Beiko 2014; Miyake et al. 2015; Yuan et al. 2015). Short chain volatile fatty acids are indeed the main product of fermentation of plant material in the large intestine of green turtles (Bjorndal 1979; Bjorndal et al. 1991) and the analysis of the green turtle microbiota reported here revealed that *Ruminococcaceae* and *Lachnospiraceae* represented 3-30% of the OTUs recovered from the rectal and fecal samples of most juvenile green turtles, thus confirming their capacity to ferment structural polysaccharides. This suggests that juvenile green turtles with a *Firmicutes-Bacteroidetes* dominated fecal microbiota were plant-based omnivores or herbivores, which agrees with available dietary information (Santos et al. 2011; Nagaoka et al. 2012; Morais et al. 2014; Santos et al. 2015; Gama et al. 2016; Vélez-Rubio et al. 2016; Reisser et al. 2013).

Interestingly, *Ruminococcaceae* prevail over *Lachnospiraceae* in terrestrial herbivorous reptiles (Hong et al. 2011) but the opposite appears to be true in marine iguanas (Hong et al. 2011) and in green turtles. Macroalgae are the staple food of both groups and differ from seagrasses and terrestrial plants in high levels of sulfated polysaccharides and alginic acid and low levels of cellulose (Graham and Wilcox 2000). This suggests that the prevalence of *Lachnospiraceae* over *Ruminococcaceea* in marine iguanas and green turtles is related to the similar composition of the polysaccharides in their diets. Nothing is known about the microbiota of green turtles feeding on seagrasses, but the profiles of the short chain volatile fatty acids produced in the large intestine of green turtles feeding on seagrasses and those feeding on macroalgae differ (Bjorndal 1979; Bjorndal et al. 1991), thus suggesting potential differences in their microbiota worth exploring in further research.

Another major difference between the rectal and fecal microbiota of green turtles and those of other herbivorous vertebrates is the high abundance of *Bacteroidetes* in the former, a pattern reported previously only from dugongs (*Dugong dugong*) and gopher tortoises (*Gopherus polyphemus*) (Table 3). *Bacteroidetes* may contribute significantly to the initial attack on both simple and complex carbohydrates (Shah and Gharbia 1993) and Yuan et al (2015) speculated that the high prevalence of *Bacteroidetes* in gopher tortoises might be related to the seasonally low temperatures experienced in subtropical environments. However, *Bacteroidetes* had a similar prevalence in green turtles from tropical Praia do Forte and from subtropical Ubatuba (this study), thus suggesting that seasonal differences in temperature are unlikely to not induce major changes in the relative abundance of *Bacteoidetes* and *Firmicutes*, although samples were collected in summer in both areas. A high abundance of *Bacteroidetes* is neither characteristic of the gut microbiota of herbivorous chelonians, as they represent only 4% of the relative abundance of bacteria in the microbiota of Galapagos giant tortoises (*Geochelone nigra*) (Hong et al. 2011). It is suggested that the high presence of this phylum in all the samples of green turtles from Brazil, except those of the three anomalous individuals, could be related to the presence of high levels of organic matter in coastal waters, which allow copiotrophs (such as *Bacteroidetes*) to thrive and dominate the microbial community structure (Troussellier et al. 2017). Moreover, a recent study of gut microbiota of the loggerhead sea turtle *Caretta caretta (Abdelrhman et al. 2016)*, found that *Firmicutes*, *Proteobacteria* and *Bacteroidetes* were the most predominant microbial population in turtle feces.

Spirochaetes is another group of non-cellulolytic bacteria associate with specific plant substrates during digestion (Bekele et al. 2011), facilitating the breakdown of cellulose by co-occurring bacteria (Kudo et al. 1987). Within this phylum, the *Spirochaetes* members exhibit enormous

diversity in a free living or host associated life, being pathogenic or non-pathogenic, and aerobic or anaerobic (Gupta 2016). This phylum has also been reported to be a major component of the microbiota of gopher tortoises, omnivorous fishes and gorilla, but not in other herbivorous reptiles (Table 3). OTU 6, an unidentified *Spirochaetes*, was detected in all the samples, but only in the rectal samples of three individuals from Praia do Forte (PF3, PF4 and PF5) did it represented more than 2% of the relative abundance.

The fact that *Bacteroidetes* and *Firmicutes* were the dominant bacteria in the feces and the rectal samples of most juvenile green turtles less than 45 cm CCL, including four specimens ranging 31.1-35.0 cm CCL, indicates that they acquired a microbiota adapted to digest polysaccharides shortly after settlement. How this specialized bacterial flora is acquired by settlers remains unknown, but land and marine iguanas have been observed consuming conspecific excrements (Troyer 1982; Wikelski and Trillmich 1993), which certainly facilitate acquiring a plant degrading microbiota. Juvenile green turtles are not gregarious, but may form dense aggregations (Bresette et al. 2010; Reisser et al. 2013), which might facilitate feces consumption and hence the quick acquisition of a bacterial flora adapted to digest polysaccharides. Alternatively, fermenters might be transferred through the diet, as they can be associated with algal surfaces (Ibrahim et al. 2015).

Algae and seaweeds are typically rich in sulfated polysaccharides that are absent in terrestrial plants. Hence, microbiota from the phylosphere of seaweeds are characterized by high copy numbers of sulfatases in their genomes (Wasmund et al. 2017). A recent study suggested that traditional sushi food, which is largely composed of seaweeds, significantly affected the gut microbiome of the Japanese population (Hehemann et al. 2010; Hehemann et al. 2012). It was then observed that carbohydrate-active enzymes (CAZymes) in the gut microbiome, which are absent in the human

genome, were acquired by horizontal gene transfer (HGT) from the marine bacteria associated with seaweeds. Moreover, (Thomas et al. 2011), reviewed several studies on the HGT phenomena between environmental and gut bacteria within the phyla of *Bacteroidetes* and *Firmicutes* in different organisms, including the grazer surgeonfish. Hence, it is well possible that the seaweed-based diet of turtles could similarly affect their gut microbiota by gene acquisition, considering that CAZymes and sulfatases are required for efficient seaweed degradation (Benjdia et al. 2011). This topic merits further research taking advantage of the existing programs on captive breeding of green turtles by performing gut metagenomics analysis.

In any case, the fast acquisition after settlement in coastal areas of a microbiota adapted to ferment polysaccharides should enable green turtles to adopt an herbivorous diet soon after recruitment. This is the pattern reported from tropical areas (Reich et al. 2007; Stringell et al. 2016), but in warm temperate and subtropical regions juvenile green turtles are best described as plant-based omnivory and only adults are primarily herbivores (Arthur et al. 2008; Cardona et al. 2009; Cardona et al. 2010; Lemons et al. 2011; Williams et al. 2013; Arthur et al. 2015; Santos et al. 2015; Howell et al. 2016; Vélez-Rubio et al. 2016). The results presented here indicate an increase in the taxonomic richness of the gut microbiome as turtles grow, but this is an unlikely explanation by the progressive ontogenetic dietary shift, because even small turtles had a high abundance of *Ruminococcaceae* and *Lachnospiraceae*. Consumption of animal material results into a slight and statistically significant increase in the relative abundance of *Proteobacteria*, as revealed by the differences between captive and wild healthy turtles, but the abundance of *Ruminococcaceae* and *Lachnospiraceae* remains high anyway. This suggests that omnivory is unlikely to reduce the capacity of green turtles to digest plant material.

Digestibility of plant material in green turtles increases with temperature (Bjorndal 1980) and the body temperature of juvenile green turtles inhabiting subtropical regions is close to that of water during winter months (Read et al. 1996). Conversely, the body temperatures of inactive adult green turtles can be 2 ºC above water temperature thanks to gigantothermy (Standora et al. 1982), which explains why the digestibility of plant material by green turtles increases with body size even in tropical settings (Bjorndal 1980). Interestingly, the apparent digestibility of plant material does not increase with body size in marine iguanas (Read et al. 1996), because even very small individuals can rise significantly their body temperature through basking in black lava (Wikelski and Trillmich 1993). Green turtles bask regularly in the beaches of Hawaii and Galapagos (Whitton and Balaz 1982; Snell and Fritts 1983) and this behavior has been suggested to improve digestion, but beach basking has never been reported in other areas to our knowledge. If green turtles inhabiting subtropical and warm temperate regions do not bask in winter, the digestibility of plant material by small individuals can be compromised during winter, even if they support a specialized microbiota rich in *Ruminococcaceae* and *Lachnospiraceae*, which may explain the progressive dietary shift as they grow.

Conclusions

This study revealed that juvenile green turtles from the coastal waters of Brazil had the same general microbiota profile, regardless of size and origin (wild vs. captive; subtropical Ubatuba vs. tropical Praia do Forte). This indicates a fast acquisition of a microbiota with capacity to ferment structural polysaccharides soon after settlement in the coastal waters of Brazil and that the regular consumption of animal prey does not significantly reduce the presence of *Ruminococcaceae* and

Lachnospiraceae and, hence, does not impair the capacity to ferment structural polysaccharides. However, subtropical specimens displayed a larger variability in the gut microbial community structure, which in the most extreme cases was clearly related to poor physical condition. In summary, there is no reason for a delayed ontogenetic dietary shift after settlement, unless low winter temperature reduces their capacity to digest plant material.

Abbreviations

BA: State of Bahia; CCL: Curved carapace length; NGS: Next generation sequencing; OTU: Operational taxonomic unit;, SP: State of Sao Paulo

Declarations

Ethics approval and consent to participate

Field work in natural reserves and handling of wild animals was carried out under the authority of the Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio (license reference ICMBio/SISBIO 52128-1), and of the Convention on International Trade in Endangered Species of Wild Fauna and Flora – CITES (license reference CITES 16BR020234/DF).

Consent for publication

Not applicable.

Availability of data and materials

DNA sequence data from the MiSeq NGS assessment was submitted to the Sequence Read Archive (SRA) of the National Center for Biotechnology

Information – NCBI (https://www.ncbi.nlm.nih.gov/) under the accession number SRP114384. The bioestatistical data generated and analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LC directed the overall research project. LC and FPB designed the experiments and drafted the manuscript. PC carried out the field work and was the first author. MG performed the DNA sequencing and bioinformatics processing. All authors participated in the analysis and interpretation of the data, and contributed significantly in writing the final manuscript.

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3ª Capitulo: Digestibilidad de rodófitos y feófitos por los juveniles de las tortugas verdes

In revision

Trade-offs between nutritional quality and abundance determine diet selection in juvenile benthic green turtles.

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Abstract

Herbivores consume foods that are often low in many essential nutrients and high in structural constituents difficult to digest. Accordingly, nutritional quality in highly relevant in food selection. Benthic green turtles in the western South Atlantic rely primarily on red macroalgae, although brown macroalae are highly available. Furthermore, they consume some animal prey. We investigated the digestibility coefficient and calculated the intake passage time of the red macroalgae *Pterocladiela capilacea*, the brown macroalgae *Sargassum cf. vulgare* and the fish *Cynoscion leiarchus* for juvenile green turtles ranging 48.0-63.0 cm in curved carapace length to test the hypothesis that diet is based on national quality. Results indicated that the apparent digestibility coefficient of *Pterocladiela capilacea* and

fish fillets were similar (93.9% and 98.9%) respectively and significantly higher than that of the *Sargassum cf. vulgare* t 75.8%. Those differences arose partially because of the higher apparent digestibility of insoluble fibre of *Pterocladiela capilacea* compared to that of *Sargassum cf. vulgare* (95.2% and 84.0%) respectively. The intake passage time, at 24.5 ºC, was 20.6 ± 3.8 days for the three diets. The overall evidence indicates that foraging on *Pterocladiela capilacea* is more profitable than foraging on *Sargassum cf. vulgare*, which may explain the prevalence of red algae in the diet of green turtles off Brazil. Furthermore, evidence indicates that red algae diets are similar to animal diets as far as digestibility is considered, although a higher daily intake is necessary to acquire the same energy intake, due to a lower energy density.

Key words

Green turtle, digestibility, intake passage time, nutrition, feeding ecology

Introduction

The nutrients and energy available to meet the metabolic requirements of an animal are determined by its diet, the amount of food eaten and its digestive efficiency (Demment and Van Soest 1985; Buchsbaum et al. 1986). Carnivory and herbivory represent contrasting dietary strategies prioritizing differently the cost of foraging over the quality of the diet. Carnivores consume prey rich in protein but relatively scarce and difficult to capture and handle, so they try to maximize energy consumption by maximizing the capture rate and minimizing the cost of capture (White 1978; Stephens and Krebs 1986; Kohl et al. 2015). On the contrary, herbivores consume abundant prey easy to capture and handle but with a low ratio of nitrogen to structural carbohydrates (fiber) difficult to

digest (White 1978; Mattson 1980; Fleming 1995). Accordingly, herbivores try to maximize nitrogen acquisition by ingesting large amounts of food, although low levels of many essential nutrients and high levels of structural constituents reduce the efficiency of digestion (Boyd and Goodyear 1971; Buchsbaum et al. 1986). The assessment of the digestibility coefficient is thus essential for measuring the daily ration and the daily energy intake of herbivores. These coefficients are also useful for understanding resource partitioning by different species of herbivores that graze/browse in the same area (Clauss and Kienzle 2010).

Globally, most marine herbivorous vertebrates are fishes, with marine herbivorous tetrapods occurring only in certain regions (Ogden and Lobel 1978; Montgomery 1980). Seagrasses dominate the diet of extant herbivorous marine mammals (Marsh et al. 1982; André et al. 2005), whereas marine iguanas feed only on macroalgae (Wikelski and Trillmich 1993). Green turtles and ducks exhibit more flexible diets and consume variable proportions of seagrasses, seaweeds and invertebrates (Goudie and Ankney 1986; Seminoff et al. 2002; Amorocho and Reina 2007; Cardona et al. 2009; Takekawa et al. 2009; Carrión-Cortez et al. 2010; Carman et al. 2014; Santos et al. 2015; Vélez-Rubio et al. 2016).

Early juvenile, oceanic green turtles have animal-based omnivorous diets and shift to plant-based diets only after settlement in neritic habitats (Reich and Arnould 2007; Arthur et al. 2008; Cardona et al. 2009; Cardona et al. 2010; Parker et al. 2011; Carman et al. 2012; Howell et al. 2016). This process is facilitated by the fast acquisition of a microbiota rich in polysaccharide fermenting bacteria after settlement in neritic habitats (Ahasan et al. 2017; Price et al. 2017; Campos et al. 2018), as green turtles lack enzymes to break up structural carbohydrates (Bjorndal 1997).

In the Caribbean, green turtles rely mainly on the highly abundant seagrass *Thalassia testudinum* (Bjorndal 1980; Bjorndal 1985; Bjorndal 1987), but macroalgae are the staple food of green turtles in regions where

seagrasses are scarce: the Eastern Pacific Ocean, (Hays-Brown and Brown 1982; Seminoff et al. 2002; Amorocho and Reina 2007), the Galapagos islands (Carrión-Cortez et al. 2010), Hawaii (Russell et al. 2003; Arthur and Balazs 2008) and the western South Atlantic (Ferreira 1968; Sazima and Sazima 1983; Santos et al. 2015; Vélez-Rubio et al. 2016; Reisser et al. 2013). Nothing is known about the apparent digestibility coefficients of major groups of macroalgae for green turtles, although they differ largely in the composition of their cell walls (Percival and McDowell 1981; Graham and Wilcox 2000; Davis et al. 2003; Costa et al. 2010) and their capacity to produce secondary metabolites acting as herbivore deterrents (Van Alstyne 1988; Van Alstyne et al. 2001).

Red macroalgae dominate the diet of green turtles in Brazil (Santos et al. 2015; Campos and Cardona 2019; Reisser et al. 2013). Whether this is because of its dominance in the algal assemblages of Brazilian shallow reefs (Jardim et al. 2015), a high digestibility coefficient or both remains unknown. In this study, we conduct feeding trials to assess the apparent digestibility coefficient and intake passage time (IPT of the red macroalgae *Pterocladiela capilacea*, the brown macroalgae *Sargassum cf. vulgare* and the fish *Cynoscion leiarchus* for juvenile green turtles to better understand the relevance of the digestibility coefficient on diet selection.

Material and methods

Study Area

The digestibility experiments were carried out in March and April 2017 at the facilities of Projeto Tamar at Ubatuba (23º 26′S, 45º 05′ W), in the northern coast of the state of Sao Paulo (Brazil). The experiments were performed in accordance with the Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio (license reference ICMBio/SISBIO 52128-1). Water temperature off Ubatuba ranges seasonally from 17.6 ºC in winter to

24.6 ºC in summer (Gallo et al. 2006) and the region supports macroalgae foraging grounds used extensively by juvenile green turtles .

Experimental animals and diets

A group of 12 wild, healthy juvenile green turtles ranging in curved carapace length 48.0-63.0 cm were housed in individual 500 L PVC containers. Turtles were assigned randomly to three experimental groups, each feed one of the following diets through the experiment: fillets of the local fish *Cynoscion leiarchus*, the read macroalgae *Pterocladiella capillacea* or the brown macroalgae *Sargassum cf vulgare*. These two species of macroalgae are consumed regularly by juvenile green turtles off Brazil, although *Pterocladiella capillacea* prevails over *Sargassum spp.* (Santos et al. 2011; Morais et al. 2014; Santos et al. 2015).

Fish and macroalgae were kept frozen at -18 ºC until diet preparation. Daily, they were chopped, divided in 5 g aliquots, mixed with 4 plastic beads (diameter= 6 mm) and encased with gelatin. The plastic beads were used as markers to assess the intake passage, as they were not chewable in smaller pieces and could be easily recovered and counted (Van Soest 1994; Amorocho and Reina 2007). Daily ration was 1% of turtle body weight. Nevertheless, any plastic bead and fish/macroalge fragment uneaten was collected and weighted, to record the actual amount of plastic beads and food consumed daily by each turtle.

Sample collection

Each tank was checked daily and any excrement or plastic beads removed. After the detection of the first plastic bead by the faces of each turtle, subsequent excrements were weighted, dried in a stove at 55 ° C until a constant weight was reached and stored dry for subsequent chemical analysis Diet samples were also dried for storage.

Composition Analyses

Total lipid, total protein, soluble carbohydrates, insoluble fiber and ash contents were analyzed in five replicates of each experimental diet and replicates of feces collected from each diet. All samples were ground into a fine powder using a SPEX model 6850 freezer/mill, and then stored in hermetically labeled glass vials. Homogenized samples were split in four subsamples. One of them was weighted and burnt for 1 h in a muffle oven at 600 °C for ash contents determination. Another subsample was processed to extract lipids using a gravimetric method with a purified chloroform / methanol solution (2: 1) , evaporated to dryness under a stream of filtered and heavy N_2 gas (Folch et al. 1957; Bligh and Dyer 1959). Total proteins were extracted from a third subsample and amino acids were analyzed by HPLC using the AccQTag pre-column derivatization method. The amino acid reaction with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate yields derivatives that are detected at 254 nm (Cohen et al. 1993; Cohen and DeAntonis 1994). Soluble carbohydrates were extracted from samples by hydrolysis of carbohydrates using acid trifluoroacetic (TFA) 4 mol/L. The colorimetric method, gas / liquid chromatography (GLC) was used to quantify the monosaccharides released, with column Aminex HPX 87C. The mobile phase consisted of water saturated with $CaSO_4$, flowing at 0.8 ml min^{-1} and 75 ºC (Chaplin and Kennedy 1986; Garna et al. 2004). The percentage of insoluble fiber was calculated by subtracting the total percentage of all components (lipid, total protein, soluble carbohydrates, and ash. To calculate the energy supplied by lipids and proteins, we used an average combustion equivalent of these compounds of 23.9 kJ g^{-1} for proteins and carbohydrates and 39.5 kJ g^{-1} for lipids (Clarke et al. 1992).

All values were reported in relation to dry weight (DW), except water contents. All were analyzed at the Scientific and Technological Centers of the University of Barcelona (CCiT UB).

Statistical analysis

MANOVA was used to compare differences in the composition of the three diets and ANOVA was used as pot hoc tests to compare the contribution of each component soluble carbohydrate, total protein, crude lipid and insoluble fibre in the three diets. ANOVA was also run to compare the intake passage time of the three experimental diets and the apparent digestibility coefficients of dry matter, carbohydrate, protein, lipids and insoluble fibre. All statistical analyses were conducted with the SPSS 15 software package. Data are always shown as mean ± standard deviation (SD), unless otherwise stated.

Results

Turtles fed the brown diet ranged 42.8-63.0 cm CCL, those fed the red macroalgae diet ranged 48-58.5 cm CCL and those fed the fish diet ranged 50.7-57.5 cm CCL (Table 1). Water temperature in the experimental tanks ranged 24.5 ± 0.2 ºC throughout the experiment.

The water content of the three experimental diets was on average 88.0 ± 1.6 % in *S. cf. vulgare*, 80.0 ± 1.4 % in *P. capillacea* and 60.0 ± 2.8 % in *C. leiarchus* (Table 2). There was a significant difference in the proximate composition of the three diets on a dry weight basis (MANOVA; Pillai's Trace = 33772.546; P= 0.001) an all the components analysed contributed to that difference (ANOVA; soluble carbohydrates: $F_{2,14}$= 16.617, P=0.001; total protein: $F_{2,14}$=1438.587, P= 0.001; lipids: $F_{2,14}$=76.595, P= 0.001; ash: $F_{2,14}$=332.097, P= 0.001; insoluble fibre: $F_{2,14}$=63.286, P=0.001). The red macroalgae diet was characterized by a high contents of soluble carbohydrates (33.6 ± 16.8 % DW,) a moderate

amount of proteins (16.8 ± 1.5 % DW), a moderate contents of insoluble fiber (45.7 ± 16.8 % DW) and a low ash contents (4.0±.8 % DW). Conversely, the brown macroalgae diet had a low content of soluble carbohydrates (5.2±1.1% DW,) a low contents of proteins (10.9±2.0 % DW) and a high content of insoluble fiber (68.4 ± % DW) and ash (13.81±0.8 % DW). Compared to the red and brown macroalgae diets, the fish diet has very high amounts of protein (82.5 ± 3.2 % DW) and crude fat (15.8 ± 3.5 % DW) and negligible amounts of soluble carbohydrates and insoluble fiber.

Diet	Turtle ID	CCL (cm)	Turtle weight (Kg)
red macroalgae	T1	51.0	18.6
red macroalgae	T2	57.6	18.0
red macroalgae	T3	52.8	15.8
red macroalgae	T4	47.6	11.0
brown macroalgae	T5	62.0	28.7
brown macroalgae	T6	60.8	25.0
brown macroalgae	T7	44.0	8.3
brown macroalgae	T8	42.7	7.5
fish	T9	52.5	15.0
fish	T10	52.0	13.8
fish	T11	50.2	12.7
fish	T12	51.6	17.6

Table 1: Experimental groups of juvenile green turtles (*Chelonia mydas*). Red macroalgae: *Pterocladiella capillacea*. Brown macroalgae: *Sargassum cf. vulgare*. Fish: *Cynoscion leiarchus*. CCL: curved carapace length

Diet	Water (%)	Soluble carbohydrate (% DW)	Total protein (% DW)	Crude lipid (% DW)	Ash (% DW)	Insoluble fibres (% DW)	Gross energy density (KJ $g^{-1)}$
Red macroalgae	80.0 ± 1.4	33.6 ± 16.8	16.8 ± 1.5	2.5 ± 0.2	4.0 ± 0.8	45.7 ± 16.8	4.7 ± 0.0
Brown macroalgae	88.0 ± 1.6	5.2 ± 1.1	10.9 ± 2.0	1.7 ± 0.5	13.8 ± 0.8	68.4 ± 2.6	2.5 ± 0.0
Fish	60.0 ± 2.8	0.1 ± 0.0	82.5 ± 3.2	15.8 ± 3.5	3.6 ± 0.5	0.0 ± 0.0	9.4 ± 0.1

Table 2: Proximate composition of the three experimental diets. Red macroalgae: *Pterocladiella capillacea*. Brown macroalgae: *Sargassum cf. vulgare*. Fish: *Cynoscion leiarchus*. DW: dry weight. Values are reported as mean ± standard deviation (SD) of five replicates. Gross energy density has been calculated assuming a dry matter digestibility coefficient of 100%. See Table 3 for available energy density.

The gross energy density also differed between diets (ANOVA; $F_{2,14}$=10610.024; P= 0.001). The gross energy density of the red macroalgae diet was roughly half that of the fish diet and twice that of the brown macroalgae diet (Table 2). Those differences become more dramatic when digestibility coefficients were considered (see below).

The percentage of plastic beads swallowed with the diet during the first week of the experiment and recovered 33 days latter was 73.1 ± 46.2 % for the red algae diet, 81.3 ± 37.5 % for the brown algae diet and 54.3 ± 10.5 % for the fish diet. No marker from turtle T9 was recovered after 33 days. Excluding that turtle from the analysis, the mean IPT for the remaining 11 turtles was 20.6 ± 3.8 days, without significant differences between diets (ANOVA $F_{2,8}$ = 0.110, P = 0.897).

The proximate composition of green turtle faeces is reported in Table 3. The mean apparent digestibility coefficient for dry matter, soluble carbohydrate, total protein, crude lipid and insoluble fibre of the three diets are reported in Table 4. There were significant difference in the apparent digestibility of dry matter, protein and lipids of three diets (ANOVA $F_{2,10}$= 15.742, P=0.002; $F_{2,10}$=1.149, P= 0.001; $F_{2,10}$=26.634, P= 0.001; respectively). Furthermore, the apparent digestibility of the fibre from the red macroalgae diet was higher than that of the brown macroalgae diet (ANOVA; $F_{1,9}$= 8,965, P= 0.017). Differences in the apparent digestibility of soluble carbohydrates in the three diets were not statistically significant (ANOVA; $F_{2,10}$=18.064, P= 0.364). The available energy density differed largely between diets (ANOVA; $F_{2,14}$=11251.112; P= 0.001). That from the fish diet was twice that of the red macroalgae diet, which in turn was almost four times higher than that of the brown macroalgae diet (Table 4).

Diet	Soluble carbohydrate (%)	Total protein (%)	Crude lipid (%)	Ash (%)	Insoluble fibre (%)
Red macroalgae	3.5 ± 0.5	27.9 ± 8.7	24.1 ± 3.0	13.9 ± 1.7	33.0 ± 11.4
Brown macroalgae	5.7 ± 4.7	24.6 ± 0.5	7.5 ± 3.6	18.5 ± 9.6	43.7 ± 11.4
Fish	13.3 ± 12.1	38.7 ± 17.6	26.7 ± 24.0	21.8 ± 15.1	0.0 ± 0.0

Table 3: Proximate composition of the faeces of juvenile green turtles fed three different diets. Red macroalgae: *Pterocladiella capillacea*. Brown macroalgae: *Sargassum cf. vulgare*. Fish: *Cynoscion leiarchus*. DW: dry weight. Data are reported as mean ± SD of four replicates

Diet	Dry matter (%)	Soluble carbohydrate (%)	Total protein (%)	Crude lipid (%)	Insoluble fibers (%)	Available energy density (KJ g^{-1})
Red macroalgae	93.9 ± 3.2	99.4 ± 0.3	90.0 ± 7.0	48.8 ± 22.1	95.2 ± 3.2	4.4 ± 0.0
Brown macroalgae	75.8 ± 8.9	68.1 ± 35.2	45.3 ± 20.3	8.9 ± 11.8	84.0 ± 7.2	1.8 ± 0.0
Fish	98.9 ± 1.7	63.8 ± 55.3	99.8 ± 0.2	98.2 ± 3.0	-	8.6 ± 0.1

Table 4: Apparent digestibility of dry matter, soluble carbohydrates, total proteins, crude lipids, insoluble fibres and energy assimilated according to the three experimental diets. Red macroalgae: *Pterocladiella capillacea*. Brown macroalgae: *Sargassum cf. vulgare*. Fish: *Cynoscion leiarchus*. Data are (means ± SD).

Discussion

The results reported here showed that juvenile green turtles are highly efficient assimilating the macronutrients present in the red macroalgae *P. capilacea* but less efficient assimilating nutrients from the brown macroalgae *S. cf. vulgare.* Those differences are not only because of a much higher apparent digestibility of insoluble fibre from *P. capilacea*, but also because of a higher apparent digestibility of total protein and crude fat from *P. capilacea* than from *S. cf. vulgare*. On the other hand, juvenile green turtles are highly efficient digesting fish fillets, thus revealing that the acquisition of a fermenting microbiome after settlement in benthic habitats does not impede the consumption of animal prey when available.

The efficiency of plant matter digestion in vertebrates is highly dependent on three different factors: the existence of a carbohydrate fermenting microbiome, the complexity and length of the gut and the composition of the cell walls and the presence of secondary metabolism of the plan material. Green turtles are well equipped for the digestion of plant material because they acquire a gut microbiome rich in anaerobic fermenters quickly after settlement in benthic habitats (Ahasan et al. 2017; Price et al. 2017; Campos et al. 2018) and have very long intestines (Bjorndal 1979). This morphological trait is supposed to enhance the digestion of plant material high in fiber and low in nitrogen contents due to long IPT (Robbins 1993; Worthy and Worthy 2013). IPT values in green turtles range from 156 h to 672 h and usually exceed 300 h (Brand-Gardner and Limpus 1999; Amorocho and Reina 2007) (this study). ITP values are shorter in terrestrial herbivorous tortoises foraging on vascular plants and seldom exceeds 300 h: 240-384 h in Gopher tortoises (*Gopherus polyphemus* and 192-288 h in Galapagos giant tortoises *Geochelone nigra* (Bjorndal 1987; Hatt et al. 2002). IPT values are even shorter in sirenians foraging on seagrasses: 120-168 h in manatees (Lanyon and Marsh 1995; Larkin et al. 2007; Worthy and Worthy 2013), and 144–168 h in dugongs

(Kataoka 1997; Aketa et al. 2003). Those differences could be related to a higher body temperature in terrestrial tortoises and mammals comparted to green turtles, resulting from a higher body temperature because endothermy in sirenians and basking in tortoises.

Despite the possession of a fermenting microbiome and a long intestine, green turtles are less efficient digesting the structural carbohydrates from brown macroalgae and those from seagrasses at 24ºC than those of red macroalgae (Bjorndal 1980). Those differences are likely related to the differences in the composition of the cell walls of red macroalgae, brown macroalgae and seagrasses. The cell walls of red algae generally contain cellulose, xylan and several sulfated galactans (Davis et al. 2003), whereas alginate and fucans prevail in the wall cells of brown seaweeds (Graham and Wilcox 2000; Davis et al. 2003). As a result, red macroalgae have softer cell walls than brown macroalgae (Graham and Wilcox 2000), which results into a higher apparent digestibility (Foster and Hodgson 1998; Wong and Cheung 2001). On the other hand, the cell walls of vascular plants, including seagrasses, is dominated by cellulose and lignin; the latter is particularly difficult to digest and a strong negative correlation between apparent digestibility and lignin contents has been reported for herbivorous mammals, including marine sirenians grazing on seagrasses (Murray et al. 1977; Aketa et al. 2003; Lanyon and Sanson 2006; Worthy and Worthy 2013).

It is worth noting that the apparent digestibility for green turtles of protein and crude fats from brown macroalgae is also lower than those from red macroalgae. The most likely explanation is the presence of secondary metabolites that inhibit the action of trypsin and lipase respectively, as these this kind of chemical deterrents are particularly abundant in *Sargassum* spp. and other Fucales (Van Alstyne 1988; Barwell et al. 1989; Fleming 1995; Targett and Arnold 1998; Amsler et al. 2001)

In summary, the overall evidence derived from the digestibility experiments suggests that fleshy red macroalgae should be preferred to brown macroalgae and seagrasses as the staple food of green turtles. However, dietary studies confirm only partially those predictions. Red macroalgae prevail in the diet of green turtles from the western south Atlantic, Hawaii and the Galapagos Islands (Ferreira 1968; Sazima and Sazima 1983; Arthur and Balazs 2008; Russell and Balazs 2009; Santos et al. 2015; Vélez-Rubio et al. 2016; Reisser et al. 2013) and they are also major components of macroalgal communities in Galapagos (Shepherd and Hawkes 2005) and Brazil (Jardim et al. 2015), so it is hardly surprising that green turtle diet is based on them. Furthermore, seagrass meadows are poorly developed in all those regions (Green and Osteling 2003) and hence there is no real avoidance.

On the contrary, seagrasses dominate the diet of green turtles in the Caribbean, northwestern Africa, the Mediterranean and Australia (Bjorndal 1980; Moran and Bjorndal 2005; Heithaus et al. 2007; Heithaus et al. 2008; Cardona et al. 2009; Cardona et al. 2010), where extensive meadows exist (Green and Osteling 2003). Interestingly, brown macroalgae usually dominate the macroalgal community in those regions and red macroalgae seldom form dense strands in either coral or rocky reefs (Lirman and Biber 2000; Tuya et al. 2004; Shepherd and Hawkes 2005; Fuentes et al. 2007; Ramos-Esplá et al. 2007). In this scenario, the cost of searching for profitable red macroalgae could be much higher that the benefit derived from a high digestibility coefficient and green turtles forage primarily on highly abundant seagrasses.

Furthermore, sustained grazing of seagrasses improves its productivity and the nutritional quality of young leaves (Moran and Bjorndal 2005), thus adding another reason for the preference of seagrasses over red macroalgae where environmental conditions allow the development of extensive seagrass meadows. Eastern Australia offers a

good example, as green turtles clearly preferred some seagrass species, but not all of them, over red macroalgae (Fuentes et al. 2007). This evidence suggests that the high biomass typical of most seagrass meadows may balance its lower nutritional quality compared to that of red macroalgae. Finally, it is worth considering the tradeoff between quality and quantity in the context of the ontogenetic dietary shift in green turtles (Fig 1). The results reported here revealed similar apparent digestibility coefficients for the dry matter from red macroalgae and fish flesh, although the latter has a much higher energy density. It should be noted, however, that jelly-

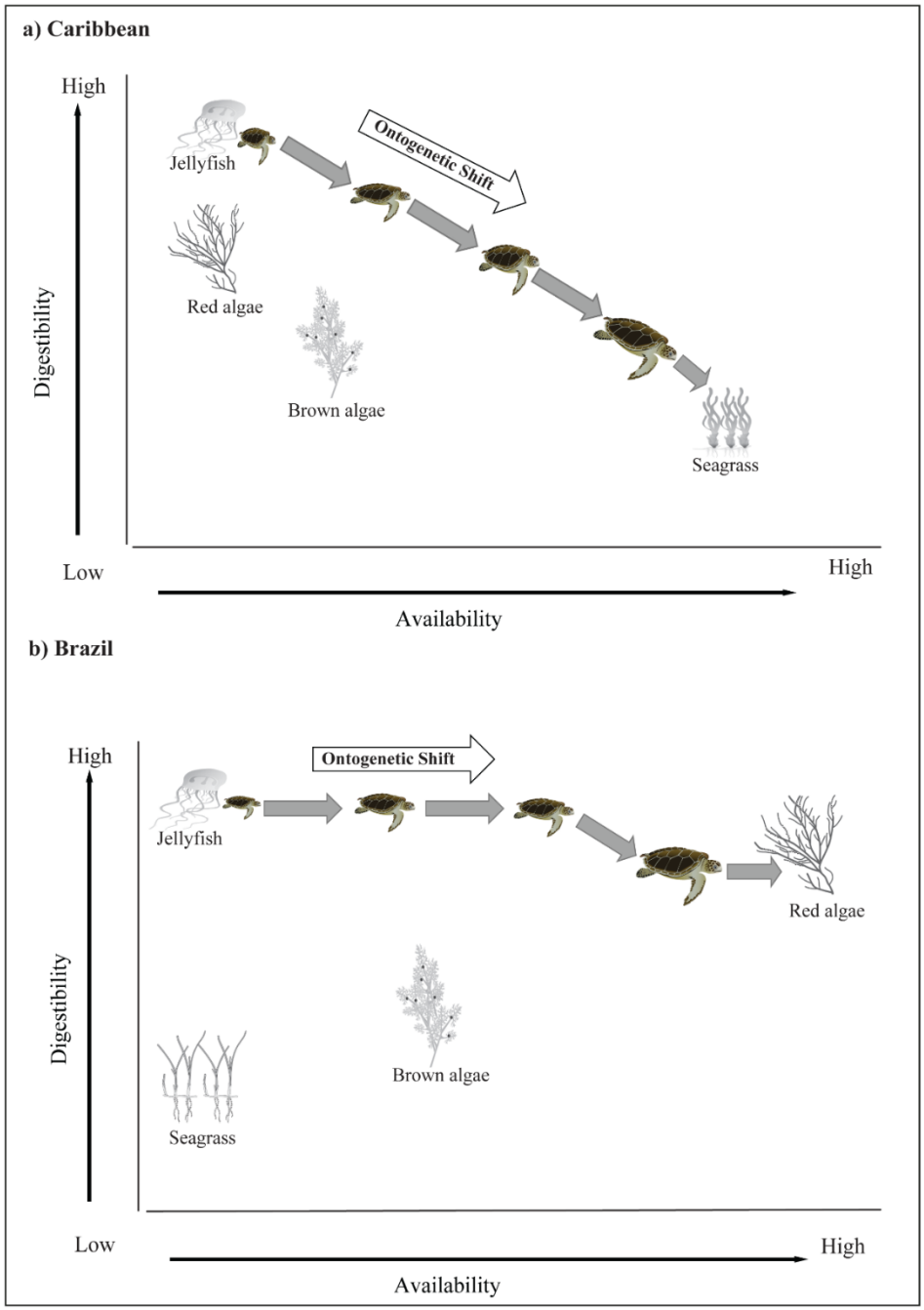

Figure 1: Trade-offs between digestibility and availability of potential prey for juvenile green turtles during the ontogenetic shift in the Caribbean (top) and Brazil (bottom).

fish and not fish is the most likely animal prey consumed by neritic juvenile green turtles (Burkholder et al. 2011; Carman et al. 2014; Vélez-Rubio et al. 2016) and that the energy density of jellyfish (Cardona et al. 2012) is much lower than that of fish or macroalge. Furthermore, jellyfish distribution in the ocean in patch and unpredictable, although jellyplankton and other neustonic species are the only prey accessible for positively buoyant post-hatchlings. This means that a plant-based diet is highly convenient for young green turtles as soon as they acquire the swimming skills necessary for benthic foraging and a carbohydrate fermenting microbiome. Certainly, green turtles consume animal prey when available, particularly in subtropical and warm temperate regions (Bugoni et al. 2003; Cardona et al. 2009; Cardona et al. 2010; González Carman et al. 2011; Fukuoka et al. 2015; Santos et al. 2015; Vélez-Rubio et al. 2016; Spier and Gerum 2017; Campos and Cardona 2019), where low water temperature may reduce seasonally the efficiency of microbial fermentation (Bjorndal 1980). However, there is little reason why green turtles should prefer animal to plant food where extensive and dense pastures exist, either seagrass meadows or algal habitats, and water temperature is above 20 ºC.

In conclusion, the apparent digestibility coefficient of plant material for green turtles vary according to the characteristics of the structural carbohydrates present in cell walls. Nevertheless, diet selection by green turtles cannot be explained solely by apparent digestibility coefficients. Seagrasses dominate the diet of benthic green turtles where extensive meadows exist, because the benefits of easy access to massive amounts of fodder overcome the limitations of a low digestibility coefficient. On the other hand, red macroalgae prevail in the diet of benthic green turtles were seagrasses are scarce, because their cell walls are easier to digest than those of brown seaweeds and they usually lack the chemical deterrents present in the latter.

Declarations

Ethics approval and consent to participate

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4ª Capitulo: La selección del hábitat de alimentación por los juveniles neríticos y contribución a la biomasa total de herbívoros

In prep.

Contribution of green turtles of herbivore biomass in shallow tropical reefs

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Abstract

Herbivory is a critical process in shallow marine ecosystems worldwide and changes in herbivore biomass may have profound effects on ecosystem structure. Tropical reefs are not an exception and the foraging activity of sea urchins, fish and, to a lesser extent, crabs keep the standing crop of macroalgae low and allows the settlement and growth of coral colonies. Green turtles are megahebivores frequently observed in tropical reef habitats, but little is known about its role in the ecosystem. Here, we have conducted underwater censuses of green turtles, herbivorous fishes and sea urchins in two distinct areas of the tropical Atlantic Ocean (Fernando de Noronha) and Pacific Ocean (Hawaii) to understand the major determinants of green turtle distribution in tropical reefs and assess their potential contribution to total herbivore biomass. Results revealed a significant decline of vegetation cover with increasing total herbivore biomass. Although juvenile green turtles ranging 40-60 cm were observed at most of

the sites surveyed, they usually represented less than 10% of the total herbivore biomass, except in sheltered areas with low rugosity. This is both because turtles favored them and were avoid by herbivorous fishes and sea urchins. The overall evidence indicates a minor role for green turtles as herbivores in shallow, tropical reef habitats.

Key words

Green turtle, habitat use, tropical reefs, underwater censuses

Introduction

Herbivory is a critical process in shallow marine ecosystems worldwide and changes in herbivore biomass may have profound effects on ecosystem structure (e.g. Steneck et al. 2002; Vinueza et al. 2006; Kelkar et al. 2013). Tropical reefs are not an exception and the foraging activity of sea urchins, fish and, to a lesser extent, crabs keep the standing crop of macroalgae low and allows the settlement and growth of coral colonies (Fox and Bellwood 2007; Mumby et al. 2007; Mumby and Steneck 2008; Lefcheck et al. 2019). Although the exact relevance of individual herbivore species in the control of macroalgae in tropical reefs is debated (Choat et al. 2002; Mantyka and Bellwood 2007; Mumby et al. 2007) (Bruno et al. 2019), the existence of abundant and diverse assemblages of herbivores is thought to increase coral reef resilience and create a buffer for natural and human induced disturbances (Burkepile and Hay 2008; Mumby and Steneck 2008; Lefcheck et al. 2019).

Green turtles are magaherbivores occurring in tropical regions worldwide (Wallace et al. 2010). Populations were decimated historically due to overharvesting but their population is currently increasing thanks to conservation actions implemented in the past decades (Chaloupka et al. 2008; Kittinger et al. 2013; Silva et al. 2017). Recent evidence demonstrates

that green turtle grazing is a major structuring force in seagrass meadows once populations are rebuilt (Bjorndal et al. 2005; Moran and Bjorndal 2005; Burkholder et al. 2013; Kelkar et al. 2013). Extended sea grass meadows, however, are scarce in the western South Atlantic and most of the tropical Pacific (Green and Osteling 2003), where tropical reefs are the main habitat of green turtles (Goatley et al. 2012; Santos et al. 2015; Balazs et al. 2017; Becker et al. 2019). Macroalgae and turf algae represent the bulk of green turtle diet in those regions (Arthur and Balazs 2008; Russell and Balazs 2009; Santos et al. 2015), but little else is known about the role of green turtles on tropical reef dynamics. Ecosystem modeling suggest that sea urchins are the major determinants of algal cover in Hawaiian reefs, with a relevant role for green turtles only in interidal rocky habitats (Wabnitz et al. 2010), whereas Goatley (2012) hypothesized a relevant role for green turtles in the Great Barrier Reef if population reached pre-exploitation levels.

This paper aims to assess the potential contribution of green turtles to the herbivore biomass and assess potential consumption of plant matter in tropical reefs of the western South Atlantic and the Central Pacific Ocean, by conducting underwater censuses of green turtles, herbivorous fishes and sea urchins and identify the major determinants of green turtle distribution in the tropical reefs.

Material and methods

Underwater surveys were conducted in September 2017 at seven sites in Fernando de Noronha (western South Atlantic Ocean) and September 2018 at 8 sites the islands of Hawaii and Oahu (Hawaii Archipelago, central Pacific Ocean). The characteristics of the sampling sites are detailed in table 1. Surveys coincided with the end of the dry season in both areas and were conducted always at high tide.

Herbivorous fishes were censed visually using 50m x 5m transects (Friedlander et al. 2003; Friedlander et al. 2007; Williams et al. 2008; Jouffray et al. 2015) parallel to the shore. Four independent and non-overlapping transects were surveyed at each sampling site. Each fish in the transect was identified to the species level, included in a 5 cm length class, and counted. Fish size was later converted to fish biomass using the equation weight=a*(lenght^b) with a and b values for that species from www.fishbase.org. When TL data was not available, we converted FL to TL. If weight-length data was not available for the species, the genus equation was used. Only the following roving herbivorous species were included in the censuses: *Kyphosus sectatrix*, *Sparisoma amplum*, Sparisoma axilare, *Sparisoma frondosum* and *Sparisoma radians* at Fernando Fernando de Noronha (Bonaldo et al. 2006) and *Acanthurus achille, Acanthurus blochii,Acanthurus guttatus, Acanthurus leucopareius, Acanthurus nigricans, Acanthurus nigrois, Acanthurus triostegus, Calotomus carolinus, Calotomus zonarchus, Kyphosus spp., Naso unicornis, Naso lituratus, Scarus dubius, Scarus perspecillatus, Scarus psittacus, Scarus rubroviolaceus, Scarus sordidus, Zebrasoma flavescens* and *Zebrasoma veliferum* at Hawaii (Jones 1968; Choat et al. 2002; Choat et al. 2004; Crossman et al. 2005). Other species of Acanthuridae rely primarily on detritus or zooplankton. Territorial herbivores (damselfish and blennies) were not considered.

Once fish were counted, depth was recorded at 0, 10, 20, 30, 40, and 50 m from the starting point of the transect, to calculate the average depth of the transect. Habitat rugosity was assessed using a relative scale ranging from 1 (flat sea bead) to 4 (seabed with large rocks or coral heads). The abundance of sea urchins was measured at flat sites along the fish transects (roughly at 10, 20, 30, 40, and 50 m from the starting point) using 0.5 x 0.5 m PVC quadrants. All sea urchins found inside each 0.5 x 0.5 m quadrant (25 quadrants per transect) were measured with a plastic caliper

(horizontal test diameter without spines), and counted. Horizontal test diameter was converted to biomass following McClanahan (1988). The cover of erect algae, turf-forming alga and live coral was assessed at the same quadrants (Friedlander (Friedlander et al. 2003; Friedlander et al. 2007; Williams et al. 2008; Jouffray et al. 2015). Finally, the abundance of green turtles was assessed in four 100 m x 10 m transects parallel to the shore (Roos et al. 2005; Ballorain et al. 2010; Gitirana and Souza 2012). Turtle transects overlapped with those used for fish censuses. Each green turtle 10 cm length class and carapace length was converted to biomass using an equation derived by the authors from juvenile green turtles from Brazil (W= -35.823 + 0.966CCL, where W is weight in kg and CCL length in cm; r^2= 0.887, p< 0.001). Furthermore, the behavior of each turtle (foraging, resting, swimming) was noted.

Statistical analysis

Normality was checked with the Lilliefors test and data were transformed ($\log_{10}(x+1)$ or asin (x)) when necessary to ensure normality. Pearson correlation was used to explore the relationship between the biomass of sea uchins, herbivorous fishes, green turtles and total herbivore biomass with habitat depth, rugosity and vegetation cover. Analyses were run in IBM SPSS 24.

Site	Hab.	MPA	Depth (m)	Rugosity	Live coral (% cover)	Turf (% cover)	Macroalgae (% cover)
Fernando de Noronha							
Porto	V	Y	1.9 ± 0.5	1.4 ± 0.3	0.0 ± 0.0	19.2 ± 3.1	5.1 ± 2.9
E Boldro	R	Y	1.8 ± 0.7	2.3 ± 0.3	0.0 ± 0.0	44.9 ± 4.4	14.5 ± 5.5
W Boldro	V	Y	1.8 ± 0.3	1.4 ± 0.3	0.0 ± 0.0	22.3 ± 1.8	2.7 ± 8.9
Morro da Fora	RC	Y	1.8 ± 0.7	2.4 ± 0.5	1.0 ± 0.1	19.2 ± 3.5	4.0 ± 2.0
Morro Pico	V	Y	1.9 ± 0.5	1.3 ± 0.3	0.0 ± 0.0	76.3 ± 1.9	5.9 ± 1.9
Porcos	RC	Y	1.8 ± 0.6	2.1 ± 0.5	0.2 ± 0.5	24.3 ± 0.7	0.5 ± 0.4
Sancho	RC	Y	2.0 ± 0.6	2.4 ± 0.5	3.2 ± 1.8	18.2 ± 3.6	3.2 ± 1.7
Hawaii/Kona coast							
Old Kona Air.	C	Y	5.0 ± 0.8	3.0 ± 0.2	22.4 ± 16.8	8.0 ± 2.8	0.0 ± 0.0
Kua	RC	N	3.0 ± 0.4	1.5 ± 0.5	6.0 ± 1.2	13.6 ± 14.4	0.0 ± 0.0
Kiholo	RC	N	2.5 ± 0.1	1.0 ± 0.1	31.6 ± 2.8	4.4 ± 5.2	0.0 ± 0.0
Waialea	RC	Y	3.0 ± 0.7	2.0 ± 0.5	1.6 ± 1.6	26.8 ± 23.6	0.0 ± 0.0
Hawaii/Oahu							
Pupukea	RC	Y	2.0 ± 0.6	2.5± 0.5	13.2 ± 1.2	36.4 ± 30.0	0.0 ± 0.0
Heeia flats	CR	N	1.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	48.0 ± 10.5	6.2 ± 4.3
Kaneohe reef 1	C	N	2.5 ± 0.2	3.0 ± 0.2	93.6 ± 3.6	0.0 ± 0.0	6.5 ± 4.5
Kaneohe reef 2	C	N	2.0 ± 0.3	3.0 ± 0.1	96.4 ± 2.0	0.0 ± 0.0	8.4 ± 4.8

Table 1. Major characteristics of sampling sites in the western South Atlantic Ocean (Fernando de Noronha) and the central Pacific Ocean (Hawaiian Islands). Hab: habitat (V: vermetid reef, R: rocky reef, RC: rocky reef with scattered coral; C: coral reef, CR: coral rubble), MPA: protection from fishing (Y: spear fishing and set nets forbidde, N: spear fishing and set nets allow allowed).

Results

Live coral was scarce covered less than 5% of the seafloor in the Fernando de Noronha sites. Hawaiian sites were more variable and live coral cover ranged 0-96 %. Turf was abundant in both areas, covering 18-76 % of the seafloor at Fernando de Noronha and 0-48 % at Hawaii. Conversely, macroalgae cover was low in both areas: 0.5-15% off Fernando de Noronha and 0-8% off Hawaii. The most common macroalgae species were *Sargassum* spp, *Dictypteris plagiogramma* and *Caulerpa racemosa* in Fernando de Noronha. *Dictiosphaeria cavernosa* was the only macroalgae observed Hawaii. Turf and macroalgae cover were added for latter analysis and total vegetation cover was negatively correlated with depth (r=-0.624, p= 0.011) (Figure1). When the two coral heads from Kaneohe bay were removed from the analysis, because off a high liver coral cover, total vegetation cover was negatively correlated with total herbivore biomass (r=-0.512, p=0.037) and depth (r=-0.624, p=0.011) (Figure 1).

Sea urchins were virtually absent from Fernando de Noronha, were only two specimens of two different species (*Diadema antillarum* and *Tripnestes ventricosus*) were observed, none of them inside any sampling quadrant (Figure 2). Sea urchins, mainly *Echinometra mathaei*, occurred at most sites in Hawaiian, at an average density of 3.7 urchins m^{-2}. Nevertheless, sea urchins were absent from the two coral heads at Kaneohe Bay and the Heeia flats (Figure 2). Sea urchin biomass ranged 0-237 t km^{-2} at Hawaii and was unrelated to the seabed rugosity index (p=0.473) or depth (p=0.285).

Herbivorous fishes were found everywhere, except at Heeia flats (Figure 2). They were present at very low numbers in the two coral heads in Kaneohe. Biomass ranged 19-89 t km^{-2} at Fernando de Noronha and 0-132 t km^{-2} at Hawaii. Fish biomass increased significantly with the seabed rugosity index (r= 0.520; p= 0.012) (Figure 1). Juvenile green turtles

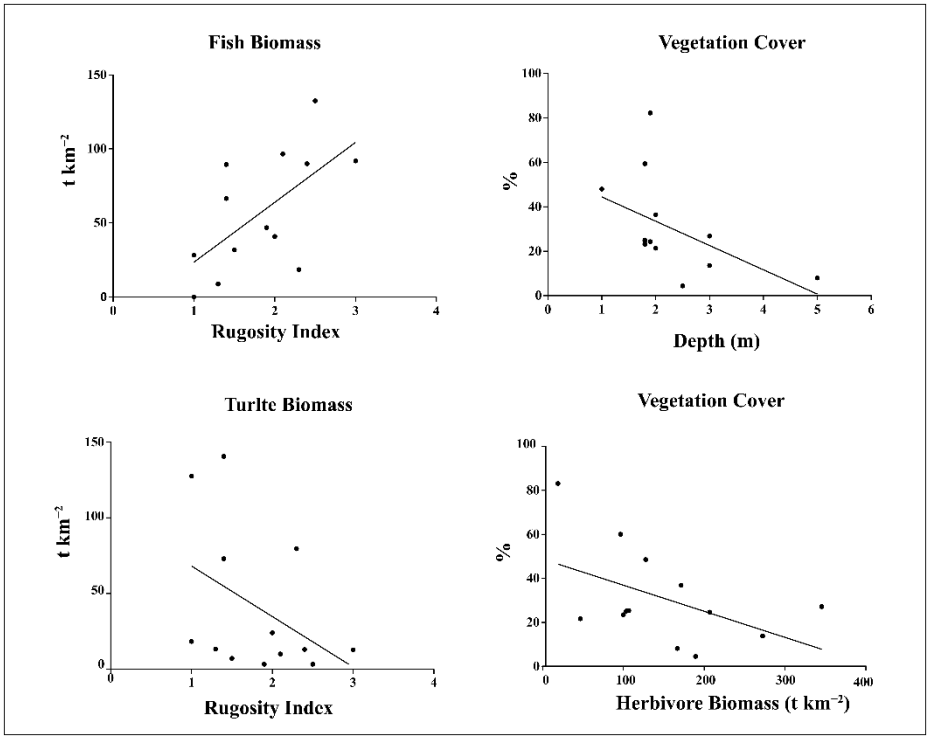

Figure 1: Determinants of fish biomass, turtle biomass and vegetation cover at shallow tropical reef habitats.

ranging 40-60 cm were observed at most sites except Sancho in Fernando de Noronha and Pupukea and the two coral heads in Kaneohe Bay in Hawaii (Figure 2). Average green turtle size was similar in both regions (Fernando de Noronha: 48.5 ± 7.8 cm; Hawaii 49.0 ± 7.4). Green turtles >50 cm were observed only at sites with a rugosity index lower than 1.5 and green turtle biomass was negatively correlated with the rugosity index (r=-0.589, p= 0.021) (Figure 1). Most of the green turtles observed while foraging were exploiting intertidal pastures in Hawaii (intertidal: 24, subtidal: 4), but the opposite was true in Fernando de Noronha (intertidal: 4, subtidal 16).

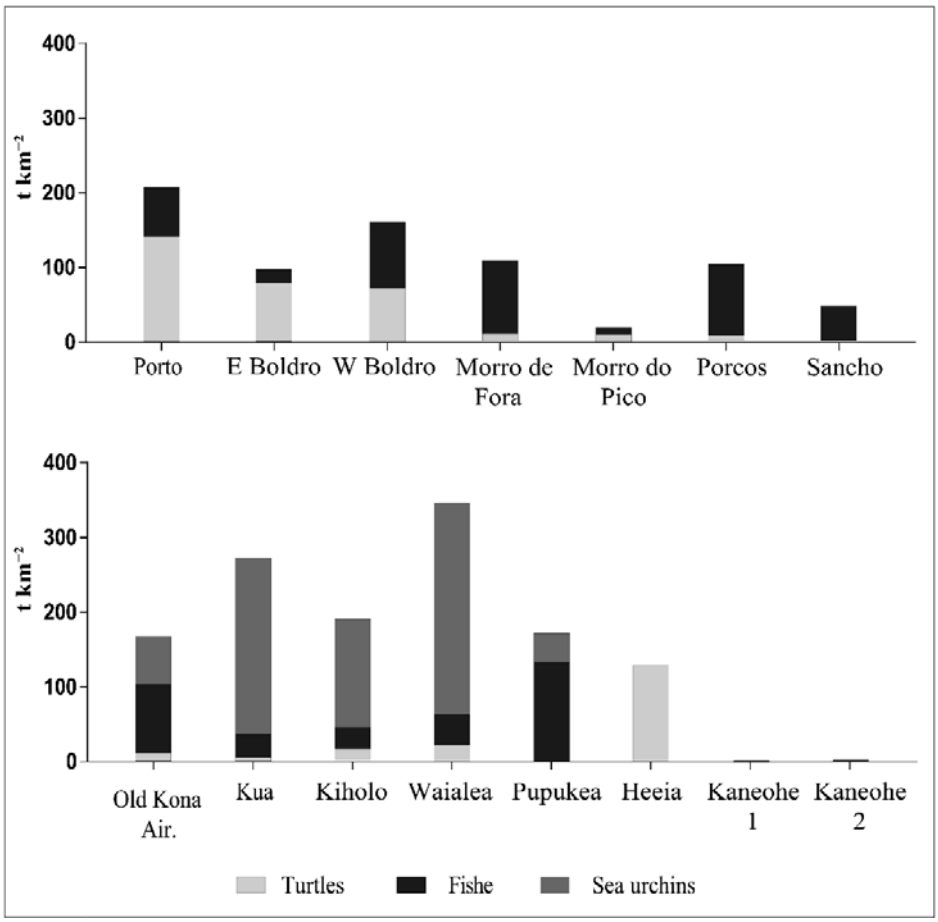

Figure 2: Biomass of herbivores at reef habitats in Fernando de Noronha (top panel) and Hawaii (bottom panel).

The range of total herbivore biomass was similar in both regions (Fernando de Noronha: 19-207 t km^{-2}; Hawaii: 3-272 t km^{-2}). Nevertheless, differences existed between the two regions in the contribution of green turtles, fishes and sea urchins to total herbivore biomass. Comparable rocky reef with scattered coral supported a much lower biomass of herbivores in Fernando de Noronha (86.7 ± 26.7 t km^{-2}) than in Hawaii (244.7 7 ± 80.4 t km^{-2}), likely because of the absence of sea urchins in the former. Indeed,

fishes were the major herbivores in most of the rocky reefs with scattered coral off Fernando de Noronha, with the exception of E Boldro; on the contrarly, sea urchins were the dominant herbivores in most Hawaiian sites (Figure 2). Green turtles represented less than 8 % of total herbivore biomass at any Hawaiia site, except in the Heeia flats, where green turtles were the only herbivores present and achieved a high biomass (128 t km^{-2}). Green turtles were also the dominant herbivores at two sites with a low rugosity index (Porto and W Boldro) in Fernando de Noronha.

Discussion

Results revealed a significant decline of vegetation cover with increasing herbivore biomass, thus supporting a role for top-down control of algae in the shallow tropical reefs of Fernando de Noronha and Hawaii. Sea urchins were the major contributors to herbivore biomass in most Hawaiian sites and fish dominated herbivore biomass at most sites in Fernando de Noronha. Green turtles usually made a small contribution to herbivore biomass in most sites, except in flat areas.

The distribution of sea urchins in tropical reefs is strongly determined by water movement and sedimentation rate (Russo 1977; Johansson et al. 2013). This explains the virtual absence of sea urchins form sheltered sites in Hawaii (Heeia flats and the two coral heads in Kaneohe bay) and a much higher abundance at exposed sites (Old Kona Airport, Kua, Kiholo, Waialea and Pupukea). Conversely, depth had no impact within the range here studied. Sea urchins were virtually absent from all the sampling sites at Fernando de Noronha. Illness decimated populations of *Diadema antillarum* in the Caribbean at the beginning of the 1980s (Mumby et al. 2007; Mumby and Steneck 2008), but little is known about the dynamics of *Diadema antillarum* populations in the rest of the tropical western Atlantic. The first comprehensive survey of benthic habitats in Fernanando de Noronha was conducted in 1985, immediately after the collapse of the

Caribbean population of *Diadema antillarum*, and sea urchins were already extremely scarce in the archipelago (Eston et al. 1986).

Habitat complexity and wave exposure are the major determinant of fish biomass in Fernando de Noronha (Krajewski and Floeter 2011) and the Hawaiian Islands, although protection from fishing is also relevant in the latter (Friedlander et al. 2003; Friedlander et al. 2007; Williams et al. 2008). The results reported here demonstrate that habitat complexity (rugosity) is also a major habitat determinant of the biomass of roving herbivorous fishes in both areas, although intense fishing may explain some anomalies observed in the Hawaiian Islands, such as the extremely scarcity of herbivorous fishes at the two coral heads in Kaneohe. High habitat complexity offer fish with protection from predators and parrotfishes are particularly dependent on the existence of adequate shelter to sleep at night (Buckman and Ogden 1973; Robertson and Sheldon 1979; Ong and Holland 2010).

The distribution pattern of green turtle biomass is similar in both areas and opposes that of herbivorous fishes; green turtle biomass peaks in sheltered areas with low habitat complexity. Morro Pico, in Fernando de Noronha, is the only sheltered area with a low biomass of green turtles, likely because of the strong currents that swept Morro Pico. As green turtles are legally protected both in Fernando de Noronha and Hawaiian Islands, the above reported patter of biomass distribution should be determined only by natural factors, although green turtle abundance is certainly lower than in historical times due to overharvesting in the past.

The reasons why green turtles, and particularly specimens >50 cm, concentrate at flat, sheltered areas are unknown but might be related to food availability and predator avoidance. Vegetation cover was usually low in subtidal habitats in the Hawaiian Islands and hence most of the turtles observed in this survey foraged intertidaly. This behavior

has been previously reported for Kaloko Honokohau, in Hawaii (Wabnitz et al. 2010), and could be rather common through the archipelago. The Heeia flats, and intertidal area covered with coral rubble and devoid of sea urchins and roving herbivorous fishes at high tide, had the highest vegetation cover of the sites surveyed in the Hawaiian island during this study. Hence, it is not surprising that large numbers of green turtles aggregated there at high tide. The existence of a *Halophila* spp. meadow at the nearby Kaneohe Sandbar, 1 km apart, may facilitate the presence of green turtles there.

Food availability, however, does not explain the preference of green turtles for the flat, sheltered areas of W Boldro and Porto. Green turtles foraged usually in subtidal habitats in Fernando de Noronha, which relates to a higher abundance of subtidal vegetation compared to Hawaii. Nevertheless, W Boldro and Porto have only a modest vegetation. Sharks (*Carcharhinus perezi* and *Negaprion bervirostris*) were spotted at all the sampling sites in Fernando de Noronha, except W Boldro, Porto and Morro Pico, and hence predatory avoidance may explain the preference of green turtles for W Boldro and Porto. Nevertheless, complex interactions may between body condition, forage quality and predation risk (Heithaus et al. 2007; Burkholder et al. 2013).

The overall evidence indicates that green turtle biomass is small in most coral reef habitats compared to that of sea urchins and roving herbivorous fishes. Only in shallow, sheltered and flat areas green turtles make most of the herbivore biomass. The biomass of sea urchins, herbivorous fishes and green turtles reported here for most sites in the Hawaiian Islands are similar to those reported by Wabnitz et al. (2010) for Kaloko Honokokau. If so, Wabnitz's et al conclusion that that green turtles had little relevance for the dynamics of algae other than the intertidal turf probably applies to most of the west coast of Hawaii. Indeed, sea urchin density is the major determinant of the dynamics of

subtidal vegetation according to ecosystem modeling. However, it should be noted that different herbivores target different algal resources, creating the potential for strong complementarity in these ecosystems and many more species might be needed to maintain ecosystem functioning across large, naturally varied reefscapes than suggested by small scale studies (Lefcheck et al. 2019). In this sense, green turtles probably play a negligible role in reef habitats, but can be critical for the dynamics of vegetation in marginal, flat areas.

Declarations

Ethics approval and consent to participate

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Apéndice

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Individual variability in the settlement of juvenile green turtles in the western South Atlantic Ocean: relevance of currents and somatic growth rate

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ABSTRACT: The settlement of demersal animals is influenced both by physical processes ruling the distribution of pelagic juveniles in the open ocean and by their active selection of suitable benthic habitats. Green turtles *Chelonia mydas* inhabiting the coastal areas of the western South Atlantic Ocean derive primarily from the rookery at Ascension Island and settle over a huge area spanning from northern Brazil to Uruguay. Here, we analysed the stable C and N isotope ratios in 30 µm of carapace layers from juvenile green turtles collected from 2 distinct areas of Brazil (Praia do Forte, 12° 38′ S, 38° 05′ W, and Ubatuba, 23° 26′ S, 45° 05′ W), with the goal of reconstructing their individual diets and habitat use patterns. Juvenile neritic green turtles from Praia do Forte usually had herbivorous diets, with limited individual variability and few temporal changes in diet or habitat. Conversely, most juvenile green turtles from Ubatuba had omnivorous diets, although they exhibited high levels of individual and temporal variability. These contrasting patterns could be linked to less abundant and predictable food availability in subtropical Ubatuba compared to tropical Praia do Forte. It is unknown why large numbers of juvenile green turtles bypass foraging grounds in north-eastern Brazil to settle in subtropical or warm temperate areas, although it may be related to individual differences in growth rate and their size being too small when reaching Brazil from Ascension Island.

KEY WORDS: Juvenile turtles · *Chelonia mydas* · Settlement · Developmental habitat · Stable isotopes

1. INTRODUCTION

Demersal marine animals often use a diversity of habitats throughout their lives, with juveniles typically inhabiting the water column and adults residing near the sea bed (Rodríguez et al. 1993, Harmelin-Vivien et al. 1995, Juanes 2007). Settlement into benthic habitats is a critical process for these demersal animals, and this usually involves juveniles actively selecting suitable habitats (Harmelin-Vivien et al. 1995, Montgomery et al. 2001, Jenkins 2005). Currents may profoundly impact the dispersal of juvenile, pelagic stages because of their small body size and limited swimming skills. This, in turn, may result in long-distance dispersal, thus connecting pelagic foraging grounds, settlement areas, developmental habitats of neritic juveniles and, finally, the foraging grounds for adults (Cowen & Sponaugle 2009).

Marine turtles offer a good example of these complex life cycles, which result largely in part from the broad dispersal of post-hatchlings and oceanic juveniles (Putman & Naro-Maciel 2013, Mansfield et al. 2014, Briscoe et al. 2016), and also from the fidelity of adults to foraging grounds and nesting beaches (Bowen & Karl 2007). Green turtles *Chelonia mydas* inhabit all the tropical regions of the planet (Wallace et al. 2010), and the dispersal of post-hatchlings and oceanic juveniles is strongly influenced by oceanic

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currents (Naro-Maciel et al. 2017, Monzón-Argüello et al. 2018; but see Shamblin et al. 2018). Juvenile green turtles settle into neritic habitats when their curved carapace length (CCL) reaches 25–50 cm (Bjorndal 1985, 1997, Seminoff et al. 2002, Reich et al. 2007, Arthur et al. 2008, Cardona et al. 2009, Williams et al. 2014, Howell et al. 2016, Williard et al. 2017). At this point, they shift from a carnivorous diet based on gelatinous zooplankton to an herbivorous or omnivorous plant-based diet (Reich et al. 2007, Arthur et al. 2008, Cardona et al. 2009, 2010, Parker et al. 2011, Vélez-Rubio et al. 2016). Settlement may take place at the same feeding grounds used by adults (Chaloupka et al. 2004, Arthur et al. 2008) or, alternatively, at developmental habitats that are different from those used by adults, particularly in subtropical and warm temperate regions (Cardona et al. 2009, González Carman et al. 2012, Williams et al. 2014, Santos et al. 2015, Howell et al. 2016, Jardim et al. 2016). In the latter situation, green turtles will eventually move to the adult foraging grounds as they grow older. The benefits of settling in developmental habitats remain unknown (Meylan et al. 2011), but using neritic foraging grounds that are distinct from those of adults might simply be a result of oceanic juveniles drifting with the currents until they grow large enough to control their buoyancy (Scott et al. 2014).

According to tagging and genetic markers, the majority of the green turtles inhabiting the western South Atlantic derive from the rookery at Ascension Island (Carr et al. 1978, Caraccio Noriega 2008, Proietti et al. 2012, Prosdocimi et al. 2012, Putman & Naro-Maciel 2013, Scott et al. 2014). Virtual particle modelling indicates that most hatchlings leave Ascension Island and drift westward along the Atlantic South Equatorial Current (Fig. 1) before reaching the neritic habitats off the coast of north-eastern Brazil in less than 2 yr (Putman & Naro-Maciel 2013, Scott et al. 2014). Mixed adult/juvenile foraging grounds exist only at latitude 12° S and farther north (Gallo et al. 2006, Poli et al. 2014, Santos et al. 2015, Jardim et al. 2016) and, hence, most juvenile green turtles are expected to settle immediately after reaching northwestern Brazil. However, large numbers of juvenile green turtles continue drifting southward along the Brazil Current before settling at developmental habitats off central and southern Brazil (Gallo et al. 2006, Poli et al. 2014, Santos et al. 2015, Jardim et al. 2016), Uruguay (Vélez-Rubio et al. 2018) and northern Argentina.

The climate is tropical in north-eastern Brazil, with increasing seasonality southwards and a warm temperate climate in Uruguay and northern Argentina. In this scenario, green turtles inhabiting the western South Atlantic are expected to exhibit a diversity of life histories. Those settling in tropical mixed adult/juvenile foraging grounds will inhabit a rather constant and predictable environment; hence, they will experience little variability in diet and habitat throughout their lifetimes, with the exception of their periodical breeding migration to Ascension after adulthood. In contrast, individuals settling in subtropical and warm temperate developmental habitats will shift habitats frequently as a result of not only increasing seasonality but also the northward displacement towards the tropical adult foraging grounds off northeastern Brazil.

Satellite tagging has offered some evidence of seasonal migration in juvenile green turtles from northern Argentina (González Carman et al. 2011, 2012) and Uruguay (Vélez-Rubio et al. 2018), but the tags remain attached to small green turtles for only a few months (Godley et al. 2003, González Carman et al.

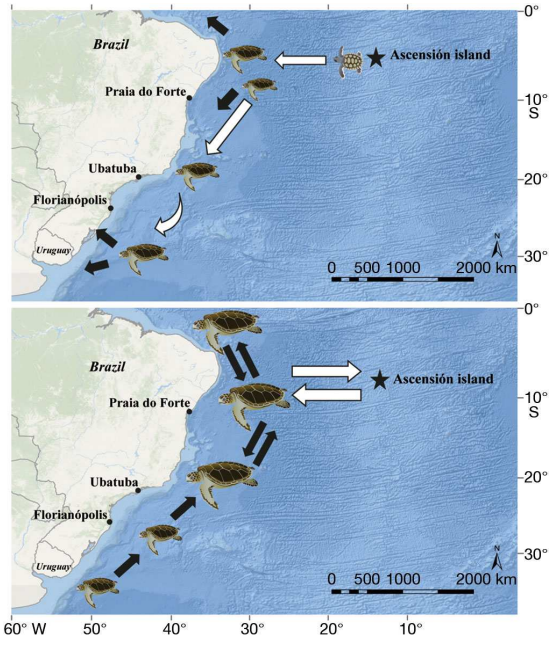

Fig. 1. Movements of green turtles from Ascension Island across the western South Atlantic. (a) Developmental dispersal from Ascension Island to neritic foraging grounds along South America. (b) Movements from developmental habitats in central Brazil, Uruguay and northern Argentina to adult foraging grounds in north-eastern Brazil and reproductive migration. White arrows denote oceanic (deeper than 200 m) pathways and black arrows denote neritic (shallower than 200 m) pathways. Turtle drawings (source: IAN image library) denote hatchlings, small and larger juveniles, and adults

2012, Putman & Mansfield 2015, Williard et al. 2017); thus, they do not serve as a viable alternative for long-term tracking. Furthermore, satellite tagging offers no information about diet. Analysing stable isotope ratios in the layers of carapace scutes is an alternative approach to reconstructing ontogenetic changes in the diets and habitats of juvenile green turtles, because this metabolically inert tissue records a timeline of the consumer's isotopic history spanning several years, even if the resolution is often coarse (Reich et al. 2007, Cardona et al. 2009, 2010, Vander Zanden et al. 2013, Vélez-Rubio et al. 2018).

The basic assumptions of the stable isotope analysis in scutes are: (1) that stable isotope ratios in animal tissues integrate those in their diet and the trophic discrimination factor is tissue specific; (2) that the stable isotope ratios of metabolically inert tissues do not change after deposition and hence integrate the diet during short periods (days to weeks); and (3) that variations of stable isotope ratios across habitats and prey are known. The first 2 assumptions are not redundant, because the stable isotope ratios of metabolically active tissues, such as skin or muscle, change over time and hence offer no timeline, whereas the opposite is true for layers of metabolically inert tissues.

We used the stable isotope ratios of N and C in the carapace scute layers of juvenile green turtles captured in neritic habitats of north-eastern and central Brazil to track their individual ontogenetic trajectories in diet and habitat use. Turtles from north-eastern Brazil are expected to have settled immediately after arriving from Ascension and hence exhibit rather constant stable isotope ratios across their carapace scutes after the drop associated with the settlement (Reich et al. 2007, Vander Zanden et al. 2013). Conversely, as a result of frequent shifts in both diet and habitat in a more seasonal environment, juvenile green turtles from central Brazil are expected to exhibit more variable stable isotope profiles across carapace scutes and higher individual variability.

2. MATERIALS AND METHODS

2.1. Study area

We collected samples from February to March 2016 in 2 different regions of the Brazilian coast: 16 were collected from tropical Praia do Forte (12° 38′ S 38° 05′ W), located 70 km from Salvador do Bahia, and 14 were collected from subtropical Ubatuba (23° 26′ S, 45° 05′ W), off the northern coast of the state of Sao Paulo (Fig. 1).

2.2. Sampling

At both sites, most turtles were captured alive through free diving or with a monofilament nylon net (30 cm mesh size) by members of Projeto Tamar (www.tamar.org.br), and this formed a part of their long-term study on the abundance and habitat use of green turtles along the Brazilian coast. Some of the juvenile green turtles from Ubatuba were captured alive in pound nets (Gallo et al. 2006). The mortality in pound nets is low because turtles are free to breathe, especially when the gear is open-roofed (Silva et al. 2017). Additional samples were collected at Praia do Forte during the necropsy of 5 recently dead turtles that had been caught incidentally by local fishermen.

Curved carapace length (CCL) was measured with flexible tape, and carapace scute samples 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 Miltex biopsy punch (Reich et al. 2007).

Previous research has shown that the macroalgae *Ulva* spp., *Chondracanthus* spp. and *Pterocladiella capillacea* are the staple food of green turtles along the coast of Brazil (Santos et al. 2015, Jardim et al. 2016) and Uruguay (Vélez-Rubio et al. 2018), and that a steep latitudinal gradient exists for their $\delta^{15}N$ values but not for $\delta^{13}C$. Furthermore, green turtles regularly consume gelatinous zooplankton in southern Brazil and Uruguay (Santos et al. 2015, Vélez-Rubio et al. 2018). According to this information, we collected *Ulva* sp., *Chondracanthus* sp., *P. capillacea* and other macroalgae for stable isotope analysis at Praia do Forte and Ubatuba (5 replicates each). The jellyfish *Velella velella* was also collected at Ubatuba. Prey samples were kept frozen (–20°C) prior to analysis.

2.3. Stable isotope analysis

All carapace scute samples were rinsed with deionized water in the laboratory prior to analysis. Each sample was embedded in an optimal cutting temperature (OCT) compound manufactured by Tissue-Teck®, with the dorsal side (oldest tissue) down and frozen. Scute samples were then subsampled in successive 30 µm layers using a cryostat (Leica Cryostat CM 3050S). Each layer was rinsed with deionized water for 24 h and kept separately. Previous tests confirmed that this procedure removed OCT traces and did not modify the stable isotope ratios of C or N (Monzón-Argüello et al. 2018, Vélez-Rubio et al. 2018). Samples were dried in an oven at 55°C for 1 d.

The number of layers obtained was proportional to the scute thickness, which varied individually. As a scute grows outward, the oldest tissue remains in the outermost part of the scute and the most recent tissue forms in the innermost section (Alibardi 2005). According to previous studies (Reich et al. 2007, Cardona et al. 2010, Vander Zanden et al. 2013), each 30 µm thick layer integrates approximately 54 d, although the associated variance is unknown and hence should be considered as a coarse estimate only. Each layer was analysed independently for the stable isotope ratios of carbon and nitrogen. Subsamples were weighed in tin cups with a microbalance (approximately 0.3 mg of sample), combusted at 1000°C and analysed with a continuous flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA; Thermo Finnigan) at the Centres Científics i Tecnològics de la Universitat de Barcelona (Spain).

Stable isotope abundances were expressed in δ notation according to the following expression:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3 \quad (1)$$

where X is ^{13}C or ^{15}N, R_{sample} is the heavy to light isotope ratio of the sample ($^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively), and $R_{standard}$ is the heavy to light isotope ratio of the reference standards, which were Vienna Pee Dee belemnite calcium carbonate for ^{13}C and atmospheric nitrogen (air) for ^{15}N. For calibration at a precision of 0.2‰, we used international isotope secondary standards of known $^{13}C/^{12}C$ ratios, as given by the International Atomic Energy Agency (IAEA), and these were namely polyethylene (IAEA CH_7, $\delta^{13}C$ = −31.8‰), graphite (IAEA USGS24, $\delta^{13}C$ = −16.1‰) and sucrose (IAEA CH_6, $\delta^{13}C$ = −10.4‰). For nitrogen, we obtained a precision of 0.3‰ using international isotope secondary standards of known $^{15}N/^{14}N$ ratios, namely $(NH_4)_2SO_4$ (IAEA N1, $\delta^{15}N$ = +0.4‰ and IAEA N_2, $\delta^{15}N$ = +20.3‰) and KNO_3 (IAEA NO_3, $\delta^{15}N$ = +4.7‰).

2.4. Data analysis

The coefficient of variation was used as a measure of variability across carapace layers (Table 1). The Fisher test was used to compare the variability across individuals in the coefficient of variation for the 2 populations.

The prey-to-consumer trophic discrimination factor for carapace scutes of green turtles was assessed empirically by Shimada et al. (2014) as −1.4‰ for $\delta^{13}C$ and 2.5‰ for $\delta^{15}N$. The stable isotope ratios of local macroalgae from Praia do Forte and Ubatuba were corrected accordingly to define the polygon within the $\delta^{13}C$–$\delta^{15}N$ isospace that is expected for enclosing the stable isotope ratios of carapace scute layers in a way that is compatible with herbivorous diets at each locality.

We used the Bayesian stable isotope mixing model in the Stable Isotope Analysis in R (SIAR) package (Parnell et al. 2010) to assess the feasible contribution

Table 1. Summary of stable isotope descriptors for 16 green turtles *Chelonia mydas* sampled from Praia do Forte, Bahia, and 14 from Ubatuba, São Paulo, Brazil. ID: identification number; CCL: curved carapace length; CV: coefficient of variability

Study area	ID	CCL (cm)	$\delta^{13}C$ range (‰)	$\delta^{13}C$ CV (%)	$\delta^{15}N$ range (‰)	$\delta^{15}N$ CV (%)	Time recorded (d)
Praia do Forte	PF1	45.6	−17.6/−21.7	7.0	7.7/7.9	1.0	378
Praia do Forte	PF2	85.9	−18.0/−18.7	1.7	8.7/9.0	1.4	270
Praia do Forte	PF3	104	−15.2/−16.6	3.7	8.0/8.6	2.6	270
Praia do Forte	PF4	70.2	−15.4/−15.7	6.7	8.0/8.1	1.7	486
Praia do Forte	PF5	53.7	−14.9/−16.2	3.8	8.1/9.1	4.9	216
Praia do Forte	PF6	56.4	−14.1/−14.2	0.3	7.7/4.8	0.7	162
Praia do Forte	PF7	60.5	−16.3/−18.0	4.1	9.4/10.7	4.6	270
Praia do Forte	PF8	66.0	−18.6/−19.6	3.9	6.7/10.4	2.5	216
Praia do Forte	PF9	49.3	−15.4/−15.7	0.7	8.0/8.1	0.7	378
Praia do Forte	PF10	57.8	−15.4/−17.4	4.7	8.1/8.5	1.6	432
Praia do Forte	PF11	49.6	−15.5/−15.9	1.0	8.2/8.5	1.3	216
Praia do Forte	PF12	38.8	−13.9/−14.5	4.7	11.3/11.6	1.1	270
Praia do Forte	PF13	44.0	−15.0/−21.1	11.1	6.6/10.0	6.2	162
Praia do Forte	PF14	31.1	−15.7/−17.6	4.2	5.7/7.5	12.5	216
Praia do Forte	PF15	35.0	−17.6/−18.8	2.5	8.9/10.1	4.8	270
Praia do Forte	PF16	40.0	−14.5/−16.2	3.4	8.4/9.0	2.7	486
Ubatuba	UB1	41.3	−14.9/−17.4	6.0	10.5/11.2	2.5	270
Ubatuba	UB2	45.0	−16.2/−16.6	1.0	9.8/10.0	1.1	162
Ubatuba	UB3	58.3	−15.8/−19.5	7.0	10.8/12.3	5.3	378
Ubatuba	UB4	53.3	−15.0/−17.3	5.2	10.1/11.7	5.0	324
Ubatuba	UB5	54.2	−19.8/−20.5	1.4	12.2/12.4	0.5	378
Ubatuba	UB6	61.4	−17.4/−18.3	5.7	10.4/13.4	21.0	324
Ubatuba	UB7	45.7	−18.6/−19.6	1.6	6.7/10.4	17.0	378
Ubatuba	UB8	39.7	−18.5/−20.1	3.7	5.3/6.6	10.3	216
Ubatuba	UB9	40.0	−18.1/−20.1	3.7	8.1/11.4	14.4	270
Ubatuba	UB10	44.7	−18.9/−19.8	44.3	6.9/11.8	48.1	378
Ubatuba	UB11	47.0	−19.6/−20.2	1.0	10.9/11.8	2.5	432
Ubatuba	UB12	37.0	−17.3/−19.3	3.6	5.6/6.0	2.0	324
Ubatuba	UB13	70.7	−17.2/−18.0	2.0	13.5/14.0	1.6	270
Ubatuba	UB14	34.0	−16.7/−19.7	4.6	8.4/9.8	4.7	486

of the jellyfish *V. velella* and several macroalgae to the diet of those green turtles with stable isotope ratios lying outside the mixing polygon. SIAR assumes that the variability associated with food sources and trophic enrichment is normally distributed (Parnell et al. 2010). To better restrict our model, we used elemental concentrations (%C and %N) in each prey (Claudino et al. 2013). Only the innermost layer from each turtle was included in SIAR, because older samples may correspond to foraging somewhere else and do not reveal local diet. Data are reported as means ± SD, unless stated otherwise.

3. RESULTS

Turtles ranged from 31.1–104.0 cm CCL in Praia do Forte, and from 34.9–70.2 cm CCL in Ubatuba (Table 1).

Variability in $\delta^{13}C$ values across carapace layers was similar in the juvenile green turtles from Praia do Forte and Ubatuba (Table 1; $F = 2.255$, $p = 0.144$). Conversely, variability in $\delta^{15}N$ values across carapace layers was much greater in the green turtles from Ubatuba than in those from Praia do Forte (Table 1; $F = 8{,}703$, $p = 0.006$).

The stable isotope ratios of most of the turtles from Praia do Forte (14 out of 16) were highly consistent across carapace layers (Table 1) and were also compatible with a local herbivorous diet (Fig. 2). Only 2 of the smallest turtles (measuring 31.1 and 38.1 cm CCL) had stable isotope ratios incompatible with a local herbivorous diet in at least some carapace layers (Fig. 2, panels P6 and P16). The layers outside the mixing polygon were more enriched than expected in ^{13}C, but not in ^{15}N. This suggests that unusual stable isotope ratios resulted from foraging somewhere else and not because of local mixed diets that included animal prey.

Conversely, only 2 turtles from Ubatuba (measuring 34.0 and 45.0 cm CCL) had stable isotope ratios in their carapace scutes compatible with a local herbivorous diet (Fig. 3, panels P2 and P14). The remaining 12 turtles (ranging from 37.0–70.7 cm CCL; Table 1) had stable isotope ratios lying outside the local mixing polygon and they varied largely across layers in $\delta^{13}C$, $\delta^{15}N$ or both (Fig. 3). Two of them (Fig. 3, panels P8 and P12) had $\delta^{15}N$ values lower than those expected for a local herbivorous diet; 7 had $\delta^{15}N$ values higher than those expected for a local herbivorous diet (Fig. 3, panels P1 to P6); and 3 had values both above and below those expected for a green turtle with a local herbivorous diet (Fig. 3, panels P9, P10 and P7). SIAR revealed that the stable isotope ratios above the mixing polygon were consistent with a mixed diet deriving approximately 20% of the nutrients from *Velella velella* and 80% from local macroalgae (Fig. 4). However, turtles from southern Brazil eating a plant-based diet were expected to have similar stable isotope ratios; thus, a recent arrival from more southern foraging grounds cannot be ruled out. On the other hand, $\delta^{15}N$ values lower than those in the mixing polygon revealed foraging somewhere else, and these were observed in 2 of the smallest turtles from Ubatuba (measuring 37.0 and 39.7 cm CCL; Fig. 3, panels UB8 and UB12).

4. DISCUSSION

It is no straightforward task to interpret whether an isotope ratio within a given portion of a turtle's scute is due to local trophic status or to prior foraging location, particularly if residency times are unknown and individual diet preferences exist. Turtles from southern Brazil were particularly challenging, as the stable isotope ratios observed in their more recent carapace scute layers were consistent with either a local omnivorous diet or a plant-based diet from Uruguay that would thus represent a recent arrival to the sampling area.

Despite those challenges, juvenile neritic green turtles from Praia do Forte and Ubatuba clearly differed in their diets and patterns of habitat use, as revealed by stable isotope ratios across carapace layers. Those from Praia do Forte had relatively consistent isotope ratios, which were usually consistent with an herbivorous diet based on local seaweeds. This evidence suggests that most of the turtles settled in the area and shifted to an herbivorous diet more than 1 yr prior to sampling. They also exhibited limited individual variability and few temporal changes in diet or habitat. Conversely, most juvenile green turtles from Ubatuba had omnivorous diets, with high levels of individual and temporal variability in stable isotope ratios, particularly in $\delta^{15}N$. This evidence suggests a more complex life history, with frequent changes in diet and habitat.

Previous research has already reported not only a rapid dietary shift from a carnivorous to an herbivorous diet after the settlement of juvenile green turtles in tropical neritic habitats (Reich et al. 2007, Guebert-Bartholo et al. 2011, Santos et al. 2011, Nagaoka et al. 2012, Bezerra et al. 2015, Gama et al. 2016, Jardim et al. 2016), but also a remarkable temporal consistency in diet and habitat use thereafter (Vander Zanden et al. 2013). In contrast, juvenile green turtles settling in

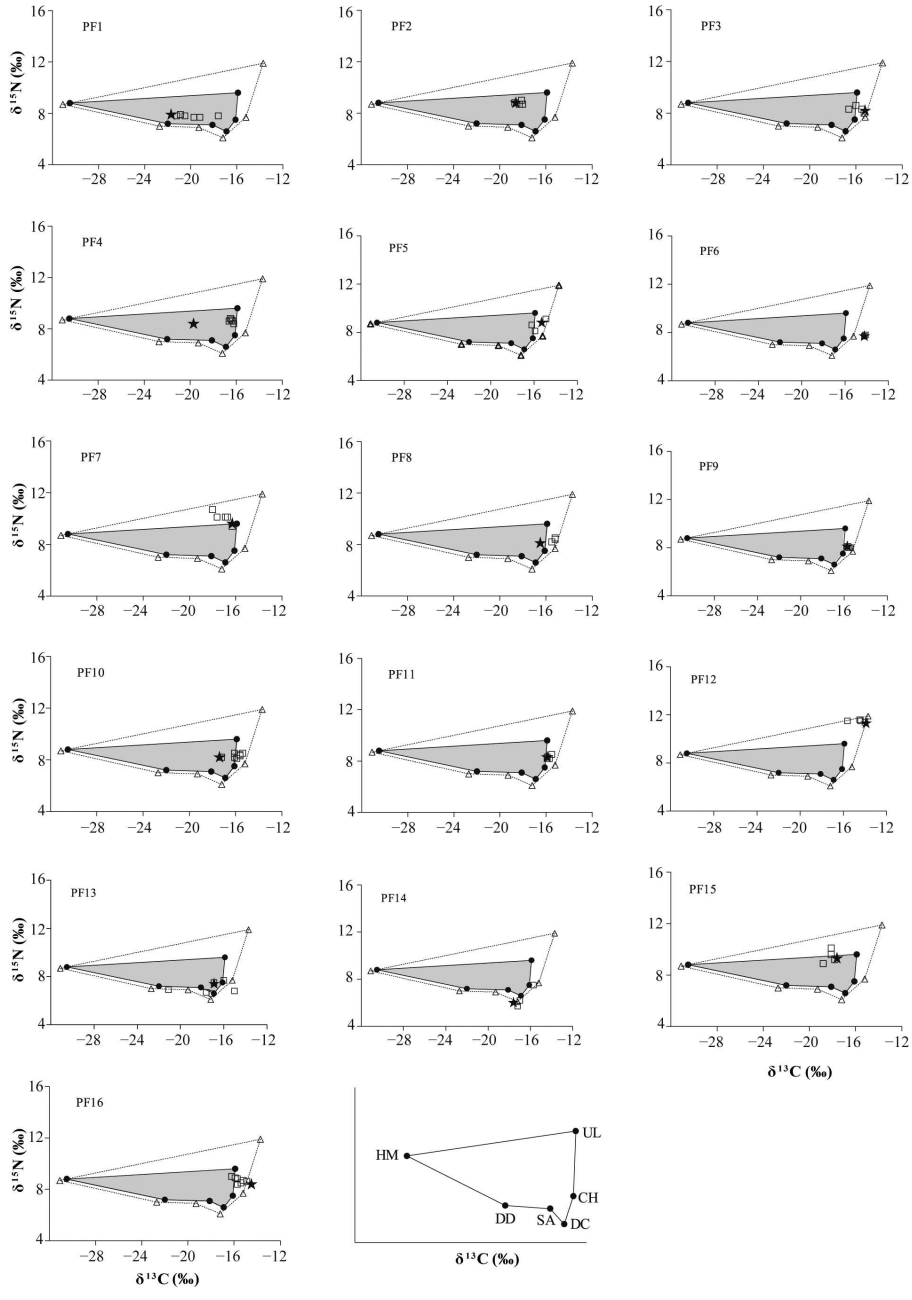

Fig. 2. $\delta^{15}N$–$\delta^{13}C$ biplot showing the position of 16 green turtles (PF1–PF16; see Table 1) in relation to the mixing polygon delimited by the macroalgae from Praia do Forte. The solid star denotes the innermost carapace layer and the open squares the older layers. The solid line connecting the solid circles shows the mixing polygon delimited by the average values of macroalgae, and the dashed line connecting the triangles shows the 95% confidence interval contour. The bottom right panel shows the position of each macroalga in the mixing polygon (CH: *Chondracanthus* sp.; DD: *Dictyopteris delicatula*; DY: *Dictyota dichotoma;* HM: *Hypnea musciformis;* SA: *Sargassum* sp.; UL: *Ulva* sp.)

warm temperate or subtropical habitats usually consume plant-based omnivorous diets and exhibit frequent habitat and diet shifts (Cardona et al. 2009, 2010, González Carman et al. 2012, 2014, Morais et al. 2014, Vélez-Rubio et al. 2016, Williard et al. 2017).

Campos et al. (2018) revealed the fast acquisition of a carbohydrate-fermenting gut microbiota by neritic green turtles after settlement both in Praia do Forte and Ubatuba, with no major differences in the composition of the gut microbiota at the 2 localities. Accordingly, the hypothesis should be ruled out that green turtles in subtropical and warm temperate regions have more carnivorous diets because of a delayed acquisition of a carbohydrate-fermenting gut microbiota (Cardona et al. 2010). Alternatively, higher levels of omnivory and frequent diet and habitat shifts in subtropical and warm temperate regions might result from a lower and highly seasonal food

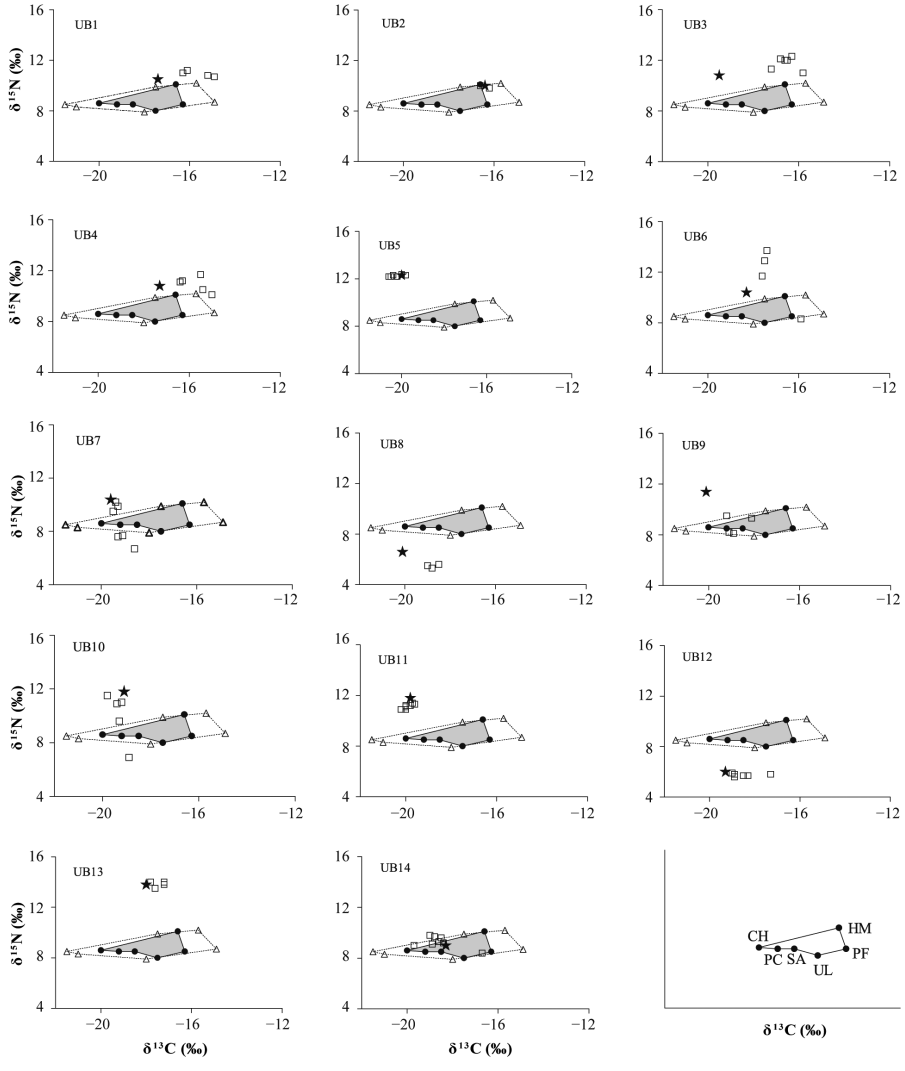

Fig. 3. $\delta^{15}N$–$\delta^{13}C$ biplot showing the position of 13 green turtles (UB1–UB13; see Table 1) in relation to the mixing polygon delimited by the macroalgae from Ubatuba. The bottom right panel shows the position of each macroalgae in the mixing polygon (CH: *Chondracanthus* sp.; HM: *Hypnea musciformis;* PC: *Pterocladiella capillacea;* PF: *Palisada flagelifera*; SA: *Sargassum* sp.; UL: *Ulva* sp.). All other details as in Fig. 2

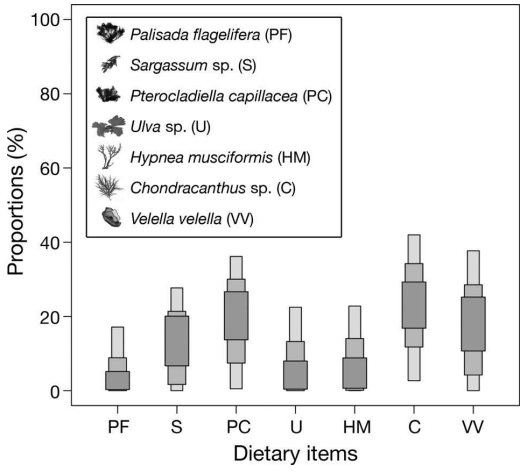

Fig. 4. Feasible contribution of prey to the diet of juvenile green turtles from Ubatuba (n = 12), according to the Stable Isotope Analysis in R (SIAR), including 95, 75 and 50% credibility intervals (light, medium and dark grey shading, respectively)

availability. If so, subtropical and warm temperate developmental habitats are indeed suboptimal, and juvenile green turtles settle there only because they drift there with the currents.

This is particularly dramatic in the western South Atlantic, where most oceanic juveniles from Ascension Island drift to north-eastern Brazil (Putman & Naro-Maciel 2013) while skipping past the mixed juvenile/adult foraging grounds stretching north to latitude 12° S, and they instead settle in southern developmental habitats. As adults occur only north to latitude 12° S, they must move north as they grow older, thus returning to areas they bypassed as juveniles (Fig. 1).

Laboratory experiments, satellite telemetry and genetic markers have revealed that juvenile green turtles ranging from 14 to 30 cm are able to sustain short periods of directional swimming to avoid areas and conditions unfavourable for survival (Prange 1976, Reich et al. 2007, Putman & Mansfield 2015). However, they probably lack the necessarily tight control of their buoyancy for settling in neritic habitats (Hochscheid et al. 2003). Indeed, even larger juveniles (38–48 cm CCL) may follow prevailing currents (González Carman et al. 2011).

Most post-hatchlings from Ascension reach north-western Brazil in less than 2 yr (Putman & Naro-Maciel 2013). Juvenile green turtles that age in the western South Atlantic are <35 cm CCL (Andrade et al. 2016, Lenz et al. 2017), although young green turtles from other populations can grow much faster (Turner Tomaszewicz et al. 2018). This indicates that only the fastest-growing members of each cohort from Ascension Island are probably large enough to settle in the neritic habitats of north-eastern Brazil upon reaching them for the first time. The majority of each cohort will likely continue drifting southwards along the Brazil Current until they grow large enough to control buoyancy and settle in neritic habitats.

The meandering of the Brazil Current far away from the continental shelf south to Cape Sao Tomé (Longhurst 1998, da Silveira et al. 2008) may delay settlement even more, because most oceanic juveniles reaching latitude 20° S are probably averted offshore and reside in the ocean for an extended period before once again approaching the continental shelf off southern Brazil and Uruguay (Putman & Naro-Maciel 2013). They will then be 3–4 yr old (Putman & Naro-Maciel 2013) and 40 cm CCL (Andrade et al. 2016, Lenz et al. 2017), which corresponds to the size at settlement reported for Uruguay (Vélez-Rubio et al. 2018). The high level of individual variability observed in the $\delta^{15}N$ values of juvenile green turtles from Ubatuba likely reveals the diversity of drifting trajectories during the pre-settlement and settlement phases of the life cycle.

In conclusion, this study demonstrates a diversity of ontogenetic trajectories for green turtles before and after settlement in the neritic habitats of the western South Atlantic, which likely results from broad variability in the rate of somatic growth of juvenile green turtles. This suggests that juvenile green turtles settle in developmental habitats south to latitude 12° S not because they are optimal, but merely because they serve as convenient end points in the oceanic dispersal phase once they grow large enough to become neritic.

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Microbiome

RESEARCH Open Access

Fast acquisition of a polysaccharide fermenting gut microbiome by juvenile green turtles *Chelonia mydas* after settlement in coastal habitats

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Abstract

Background: Tetrapods do not express hydrolases for cellulose and hemicellulose assimilation, and hence, the independent acquisition of herbivory required the establishment of new endosymbiotic relationships between tetrapods and microbes. Green turtles (*Chelonia mydas*) are one of the three groups of marine tetrapods with an herbivorous diet and which acquire it after several years consuming pelagic animals. We characterized the microbiota present in the feces and rectum of 24 young wild and captive green turtles from the coastal waters of Brazil, with curved carapace length ranging from 31.1 to 64.7 cm, to test the hypotheses that (1) the ontogenetic dietary shift after settlement is followed by a gradual change in the composition and diversity of the gut microbiome, (2) differences exist between the composition and diversity of the gut microbiome of green turtles from tropical and subtropical regions, and (3) the consumption of omnivorous diets modifies the gut microbiota of green turtles.

Results: A genomic library of 2,186,596 valid bacterial 16S rRNA reads was obtained and these sequences were grouped into 6321 different operational taxonomic units (at 97% sequence homology cutoff). The results indicated that most of the juvenile green turtles less than 45 cm of curved carapace length exhibited a fecal microbiota co-dominated by representatives of the phyla *Bacteroidetes* and *Firmicutes* and high levels of *Clostridiaceae*, *Prophyromonas*, *Ruminococcaceae*, and *Lachnospiraceae* within the latter phylum. Furthermore, this was the only microbiota profile found in wild green turtles > 45 cm CCL and in most of the captive green turtles of any size feeding on a macroalgae/fish mixed diet. Nevertheless, microbial diversity increased with turtle size and was higher in turtles from tropical than from subtropical regions.

Conclusions: These results indicate that juvenile green turtles from the coastal waters of Brazil had the same general microbiota, regardless of body size and origin, and suggest a fast acquisition of a polysaccharide fermenting gut microbiota by juvenile green turtles after settlement into coastal habitats.

Keywords: Tetrapods, Herbivorous, Microbial communities, *Chelonia mydas*, 16S rRNA, Fermentation

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Background

Herbivory has evolved independently in several groups of tetrapods belonging to diverse evolutionary lineages [1]. Unlike some invertebrates, tetrapods do not express hydrolases for cellulose and hemicellulose [2], and hence, the independent acquisition of herbivory required the establishment of new endosymbiotic relationships between tetrapods and microbes [1, 3–5]. As a consequence, the composition, abundance, and diversity of the gut microbiota of herbivorous tetrapods vary widely across groups, reflecting not only their evolutionary relationships but also their foraging habits and the location of the cavity of fermentation into the gut–hindgut vs. foregut fermenters [6–8].

Several groups of tetrapods have recolonised the marine environment after independent evolution in land, but only three of them are herbivores: sirenians (manatees and the dugong), the marine iguana (*Amblyrhynchus cristatus*), and the green turtle (*Chelonia mydas*). Sirenian diet is dominated by seagrasses [9–12] which are vascular plants rich in cellulose [13, 14]. Consequently, sirenians host microorganisms producing the enzymes needed for the fermentative digestion of cellulose [15, 16]. On the other hand, marine iguanas feed only on macroalgae [17]. The cell wall of macroalgae differs from that of seagrasses and other vascular plants in the abundance of sulfated polysaccharides and alginic acid and low levels of cellulose [18]. As a consequence, the microbiota of marine iguanas is characterized by the presence of some specific groups of methanogens and differs largely from that of terrestrial iguanas, despite a close evolutionary relationship [3]. Green turtles exhibit a much larger dietary flexibility than sirenians and marine iguanas, as they undergo a major ontogenetic dietary shift from animal-based to plant-based diets following settlement in coastal areas [19–25]. Nevertheless, they also exhibit a high level of regional variability in the degree of omnivory after settlement and the relative importance of seagrasses and seaweeds in their diets [20, 21, 23, 26–34].

The acquisition of a specialized microbiota is facilitated by lactation and intimate calve/mother relationships in mammals [35] and the consumption of conspecific excrements in marine iguanas [17]. On the contrary, the solitary lives of green turtles may delay the acquisition of a specialized gut microbiota, which in combination with the higher body temperature of larger turtles in winter may explain the improved digestibility and assimilation of plant material as green turtles grow [13, 20]. This is because green turtles are ectothermic, and the body temperature of inactive adult green turtles can be 2 °C above water temperature thanks to gigantothermy [36], whereas that of juveniles matches that of the environment [37]. It has also been suggested that mixed seagrass/macroalgae diets are uncommon in green turtles because the entirely different structure of polysaccharides in their cell walls would require different compositions of the gut microbiota [38]. In such case, frequent and short-term shifts in diet may reduce the efficiency of plant digestion [39].

Unfortunately, very little is known about the gut microbiota of green turtles, how it changes after settlement in coastal areas in association to the increase in the consumption of plant material, and the influence of turtle diet on microbiota composition. The only information available to our knowledge is about the microbiota present in the cloaca of pelagic and recently settled green turtles, which reveals a high prevalence of *Proteobacteria* and a low occurrence of bacteria associated to the fermentation of structural polysaccharides [40]. In this study, we characterize the microbiota present in the feces and rectum of young wild and captive green turtles from Brazil to test the hypotheses that (1) the ontogenetic dietary shift after settlement is followed by a gradual change in the composition and diversity of the gut microbiome, (2) differences exist in the composition and diversity of the gut microbiome of green turtles from tropical and subtropical regions, and (3) the consumption of omnivorous diets modifies the gut microbiota of green turtles.

Methods

Study area

Two different areas of Brazil were sampled in February to March 2016. Most samples ($n = 20$) were collected from subtropical Ubatuba (23° 26′ S, 45° 05′ W), in the northern coast of the state of Sao Paulo. Rocky reefs and sandy beaches dominate the coastline of Ubatuba [41]. A few additional samples ($n = 5$) were collected from tropical Praia do Forte (12° 38′ S 38° 05′ W), located 70 km from Salvador do Bahia. The coastline is characterized by the presence of shallow coral reefs with substantial air exposition during low tide [42].

Sampling

Fecal samples were collected from 8 turtles held in captive at the facilities of Projeto Tamar at Ubatuba and 11 wild turtles from Ubatuba. Some wild green turtles were captured alive in weirs ("*Cercos flutuantes*") used by local fishermen and consisting on fixed nets attached to the seafloor [43], and others were captured alive through free diving by members of Projeto Tamar (www.tamar.org.br), as part of the long-term study on the abundance and habitat use of green turtles along the Brazilian coast. After capture, curved carapace length (CCL) was measured with a flexible tape (CCL, notch to tip) and turtles were moved to the facilities of Projeto Tamar in Ubatuba. These turtles were confined in individual PVC tanks until the moment they defecated, between 24 and 36 h after capture, and then released back to the sea at the same place of capture.

Tanks had been previously disinfected with regular bleach. The core of each fecal pellet was accessed using sterilized forceps and sampled with a swab, to reduce as much as possible contamination from water. Additionally, rectal samples ($n = 5$) were collected with a swab during the necropsy of recently dead turtles at Praia do Forte.

Fecal and rectal samples were stored at 4 °C immediately after collection and then at − 20 °C until DNA extraction. No buffers were used. All procedures were non-invasive and conducted in accordance with guidelines from the Projeto TAMAR and ICMBio.

DNA extraction and next-generation sequencing

DNA was extracted from a subsample of 0.25 g from each fecal or rectal sample using the PowerSoil DNA kit (MO BIO Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. All DNA extracts were kept frozen at − 20 °C until further analysis. Massive barcoded 16S rRNA gene-based libraries in the *Eubacteria* domain were sequenced by using the MiSeq Illumina platform (Molecular Research DNA LP, Shallowater, USA). These gene libraries were constructed by targeting the V1–V3 hypervariable regions with the primer set 27F (5′-AGRGTTTGATCMTGGCTCAG-3′)/519R (5′-GTN TTACNGCGGCKGCTG-3′) as previously described in [44]. The obtained DNA reads were compiled in FASTq files for further bioinformatic processing. Trimming of the 16S rRNA barcoded sequences into libraries was carried out using QIIME software version 1.8.0 [45]. Quality filtering of the reads was performed at Q25, the default set in QIIME, prior to the grouping into operational taxonomic units (OTU) at a 97% sequence homology cutoff. The following steps were performed using QIIME: Denoising of sequence data using Denoiser [46], picking up of OTU reference sequences via the first method of the UCLUST algorithm [47] and, for sequence alignment and chimera detection, processing by PyNAST [48] and ChimeraSlayer [49]. OTUs were then taxonomically classified using BLASTn against GreenGenes and RDP (Bayesian Classifier) databases and compiled into each taxonomic level [50].

Biostatistical methods

A general lineal model (GLM) using locality (Ubatuba vs. Praia do Forte) as a fixed factor and turtle curved carapace length as a covariable was used to test the hypothesis that the microbial diversity of wild green turtles increases with turtle size and varies across localities. A general lineal model using origin (captive vs. wild) as a fixed factor and turtles curved carapace length as a covariable was used to test the hypothesis that the microbial diversity of green turtles increases with turtle size and differs between captive and wild green turtles from subtropical Ubatuba. GLMs were run in IBM SPSS Statistics 23. Multivariate principal coordinate analysis (PCoA) based on Bray-Curtis similarity distances was carried out on the OTUs incidence matrix using the CANOCO software package, version 5 (Microcomputer Power, Ithaca, NY, USA), to identify clusters of green turtles differing in the community structure of their microbiomes.

Results

The gut microbiome of 24 green turtles ranging in curved carapace length (CCL) from 31.1 to 64.7 cm was studied. A genomic library of 2,187,066 valid eubacterial 16S rRNA reads was obtained from their feces (Additional file 1). These sequences were grouped into 6321 different OTUs (at 97% sequence homology cutoff), ranging from 473 to 1952 in individual turtles (Table 1). The Good's coverage estimator on the percentage of the total species (as OTUs) represented in any given sample was above 98%, indicating that the observed species encompassed a very significant proportion of the entire sample populations. With this respect, the number of expected OTUs (Chao 1) ranged from 959 to 2818 and the Shannon index from 2.17 to 5.38 (Table 1). The number of recovered and expected OTUs in wild turtles form Praia del Forte was larger than those in wild turtles from Ubatuba and increased significantly with curved carapace length in both areas according to GLM (Table 2). However, the indices of microbial diversity did not differ between wild and captive turtles from Ubatuba (GLM; OTUs: $F_{2,18} = 1.750$, $p = 0.205$; Chao1: $F_{2,18} = 1.922$, $p = 0.179$; Shannon: $F_{2,18} = 2.445$, $p = 0.118$).

The dominant phyla in the majority of wild and captive turtles were *Bacteroidetes*, ranging 20–70% of relative abundance (RA), and *Firmicutes* with a 24–56% of RA (Fig. 1). In most of the studied turtles (Fig. 2), the predominant families within *Bacteroidetes* phylum were *Bacteroidaceae* and *Porphyromonadaceae*, while within *Firmicutes* phylum the predominant families were *Clostridiaceae*, *Lachnospiraceae*, and *Ruminococaceae*, with the exception of two wild individuals and one captive individual from Ubatuba. The bacterial community structure of these two anomalous wild turtles (UB7 and UB10) was characterized by a high RA of representatives from the phyla *Proteobacteria* (approximately 60% RA) and *Actinobacteria*, which in this latter phylum belonged to the *Mycobacterium* genus (1.2 and 4.7% RA in UB7 and UB10, respectively). The main OTUs of the former *Proteobacteria* phylum were related to *Burkholderia* spp. (*Betaproteobacteria*), *Sphingopyxis* spp. (*Alphaproteobacteria*), and *Pseudomonas* spp. (*Gammaproteobacteria*), which combined represented 49.5 and 38.3% RA for UB7 and UB10, respectively. Except for *Sphingopyxis*, these genera have been associated to the presence of *Staphylococcus* spp., in the phylum *Firmicutes* (5.0% and 3.5% of RA in UB7 and UB10, respectively). Regarding the bacterial community of the anomalous captive turtle

Table 1 Descriptors of bacterial diversity in fecal and rectal samples of juvenile green turtles *Chelonia mydas* from Brazil

Study area	Origin	Turtle	CCL (cm)	Total reads	OTUs	Coverage (%)	Shannon (ave ± SD)[a]	Chao1 (ave ± SD)[a]
Praia do Forte–BA	Wild	PF1	31.1	70,792	1589	99	4.69 ± 0.006	2015 ± 78
Praia do Forte–BA	Wild	PF2	35.0	111,405	1794	99	4.17 ± 0.008	1790 ± 88
Praia do Forte–BA	Wild	PF3	38.8	70,850	1997	98	5.14 ± 0.006	2523 ± 85
Praia do Forte–BA	Wild	PF4	40.0	90,045	1911	99	4.22 ± 0.008	2148 ± 87
Praia do Forte–BA	Wild	PF5	44.0	89,351	2211	98	5.05 ± 0.007	2466 ± 90
Ubatuba–SP	Wild	UB6	37.0	127,862	1217	99	2.16 ± 0.009	1071 ± 69
Ubatuba–SP	Wild	UB7	39.7	68,389	601	99	2.59 ± 0.006	956 ± 80
Ubatuba–SP	Wild	UB8	40.0	98,513	1954	99	4.70 ± 0.007	2053 ± 77
Ubatuba–SP	Wild	UB9	41.3	76,055	1947	99	4.82 ± 0.006	2389 ± 90
Ubatuba–SP	Wild	UB10	44.7	61,852	598	99	3.08 ± 0.005	953 ± 67
Ubatuba–SP	Wild	UB11	45.0	119,273	2150	99	4.37 ± 0.008	2036 ± 93
Ubatuba–SP	Wild	UB12	47.0	119,764	2206	99	4.47 ± 0.008	2050 ± 81
Ubatuba–SP	Wild	UB13	53.3	84,889	2006	99	4.60 ± 0.008	2264 ± 79
Ubatuba–SP	Wild	UB14	54.2	107,097	1670	99	3.24 ± 0.009	1657 ± 71
Ubatuba–SP	Wild	UB15	58.3	90,582	1951	99	4.53 ± 0.007	2187 ± 90
Ubatuba–SP	Wild	UB16	61.4	79,361	2179	98	5.15 ± 0.006	2540 ± 83
Ubatuba–SP	Captivity	UB17	32.5	103,168	2284	99	4.55 ± 0.008	2355 ± 88
Ubatuba–SP	Captivity	UB18	34.9	121,100	1481	99	2.88 ± 0.009	1374 ± 75
Ubatuba–SP	Captivity	UB19	38.6	56,987	1723	99	4.85 ± 0.005	2447 ± 77
Ubatuba–SP	Captivity	UB20	40.0	123,937	1442	99	2.79 ± 0.009	1302 ± 71
Ubatuba–SP	Captivity	UB21	41.3	101,478	2436	98	4.68 ± 0.008	2549 ± 93
Ubatuba–SP	Captivity	UB22	53.5	99,346	2079	99	4.17 ± 0.009	2118 ± 75
Ubatuba–SP	Captivity	UB23	58.6	70,520	2330	98	5.38 ± 0.006	2802 ± 77
Ubatuba–SP	Captivity	UB24	64.7	43,980	1036	99	4.70 ± 0.001	1875 ± 34
Range			31.1–64.7	70,792–127,862	1589–2436	98–99	2.16–5.38	953–2549

Fecal samples were collected at Ubatuba and rectal samples at Praia do Forte
CCL curved carapace length, *BA* State of Bahia, *SP* State of Sao Paulo, *ave* average
[a]Calculated upon sample rarefaction at 43000 reads

Table 2 Summary statistics of general lineal models describing the relationship between indices of microbial diversity in fecal and rectal samples of wild juvenile green turtles *Chelonia mydas*, sampling area (subtropical Ubatuba and tropical Praia do Forte) and curved carapace length (CCL)

Microbial diversity		*F*	df	*p*	r^2
OTUs	Model	4.155	2.15	*0.040*	0.296
	CCL	6.016	2.16	*0.023*	
	Area	6.205	2.16	*0.028*	
Chao 1	Model	4.517	2.16	*0.032*	0.319
	CCL	6.177	2.15	*0.027*	
	Area	7.671	2.15	*0.016*	
Shannon	Model	3.180	2.16	0.075	NA
	CCL	3.939	2.15	0.069	
	Area	2.708	2.15	0.033	

Microbial diversity is higher in tropical Praia do Forte and increases with turtles size. Italics denote statistical significance
NA not applicable

(UB18), it was characterized by a high abundance of the phylum *Fusobacteria* (27% RA). Such dominance was primarily caused by OTU7, affiliated with the microaerotolerant fermentative *Cetobacterium* sp. (96% of similarity to *Cetobacterium ceti*), also found in whale, dolphin, and porpoise gut flora (Bik et al. 2016).

When those three anomalous turtles (UB7, UB10, and UB18) were removed from the analysis, the abundance of *Proteobacteria* was consistently higher in captive (range 0.7–7.7% RA) than in wild (range 0.2–1.9% RA) turtles from Ubatuba (Mann-Whitney test; $U = 57.00$, $p = 0.046$). On the other hand, *Akkermansia* spp., belonging to the phylum *Verrumicrobia*, was found with a RA of 8–15% in captive turtles UB18, UB20, and UB21. It is noteworthy that in one of the wild individuals (UB14), *Akkermansia* was enriched up to a 30% of RA and, curiously, the microbiome of this individual was rather different from that of other wild turtles.

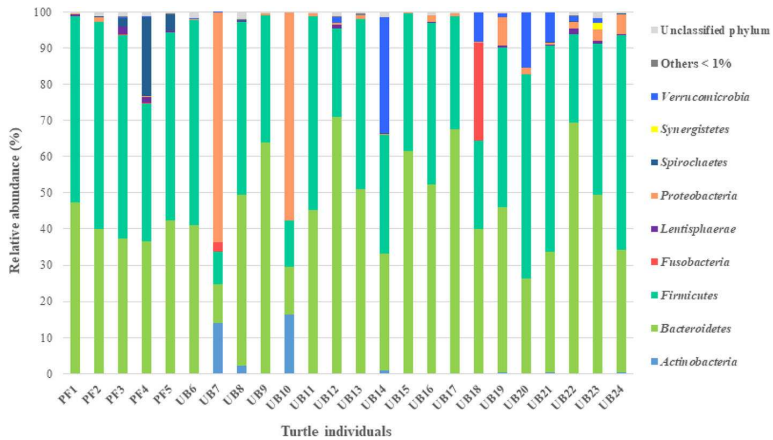

Fig. 1 Percentages of sequences from each individual turtle, fecal or rectal sample assigned at the phylogenetic level of phylum, according to the RDP Bayesian Classifier database with a bootstrap confidence above 80%. PF1 to PF5 = wild turtles from Praia do Forte; UB6 to UB16 = wild turtles from Ubatuba; UB17 to UB24 = captive turtles from Ubatuba. Taxa with a RA lower than 1% is grouped as "others"

The family *Clostridiaceae* comprised a ribotype (OTU1) that was predominant in almost all samples (from 1 to 8% RA). OTU1 belongs to the unclassified *Clostridiaceae* 1 subfamily. Interestingly, the RA of OTU1 in the wild turtles with the most dissimilar microbiome (UB7 and UB10) was < 0.1% RA (Figs. 1 and 2). Moreover, predominant OTUs of *Lachnospiraceae* and *Bacteriaceae* in those anomalous turtles were present at a comparatively low RA. On the other hand, representatives of the genus *Spirochaetes* were detected in all samples, but only in turtles from Praia do Forte this phylum appeared in significant amounts, especially in PF3, PF4, and PF5, where

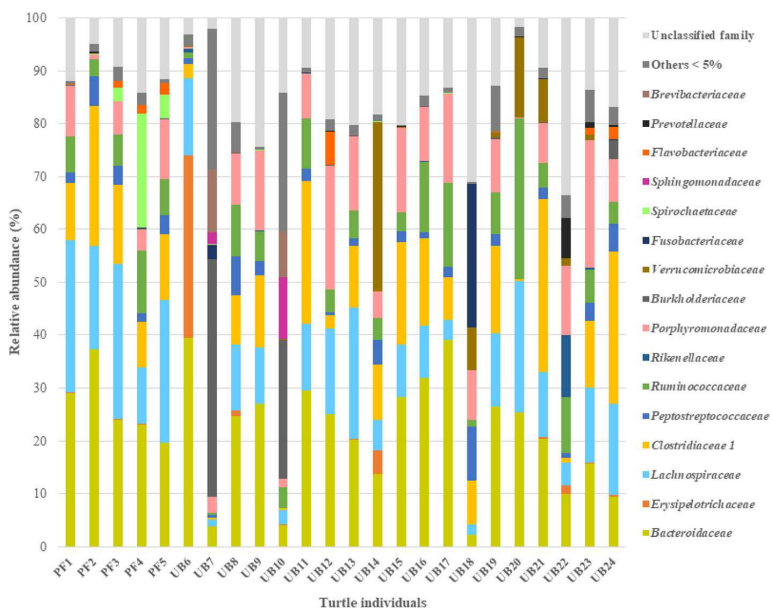

Fig. 2 Percentages of sequences from each individual turtle, fecal or rectal sample assigned at the phylogenetic level of family, according to the RDP Bayesian Classifier database with a bootstrap confidence above 80%. PF1 to PF5 = wild turtles from Praia do Forte; UB6 to UB16 = wild turtles from Ubatuba; UB17 to UB24 = captive turtles from Ubatuba. Taxa with a RA lower than 5% is grouped as "others"

OTU6 was predominant. This OTU was distantly related (88% in sequence homology) to *Treponema brennaborense* and might therefore correspond to an undescribed species. Furthermore, samples from Praia do Forte had a lower abundance of representatives in the *Actinobacteria* and *Verrumicrobia*, when compared to the Ubatuba individuals.

Multivariate analysis (PCoA of samples' Bray-Curtis distances based on OTUs incidence) (Fig. 3) showed three major clusters in relation to the microbial community structure of the gut microbiome from the studied turtles (Fig. 3). The smallest and more specific group confirmed the uniqueness of the bacterial community in the two anomalous wild turtles described above, UB7 and UB10. No significant segregation was observed between wild and captive turtles, but individuals from Ubatuba displayed a significant variability, and two major groups were apparent. The minor cluster encompassed the previously described individuals that were characterized by a relatively high abundance of *Akkermansia* spp., while a second larger one also contained the samples from Praia do Forte forming a very compact subcluster.

Discussion

Green turtles settle in the coastal habitats of the southwestern South Atlantic when they are 30–45 cm in CCL [34, 41, 51]. The results reported here indicated that most of the green turtles less than 45 cm CCL from Brazil exhibited a fecal microbiota co-dominated by phyla *Bacteroidetes* and *Firmicutes* and high levels of *Clostridiaceae*, *Porphyromonas*, *Ruminococcaceae*, and *Lachnospiraceae* within the latter phylum. Furthermore, this was the only microbiota profile found in wild green turtles >45 cm CCL and in most of the captive green turtles of any size feeding on a macroalgae/fish mixed diet. These results suggest a fast acquisition of a polysaccharide fermenting gut microbiota by juvenile green turtles after settlement into coastal habitats.

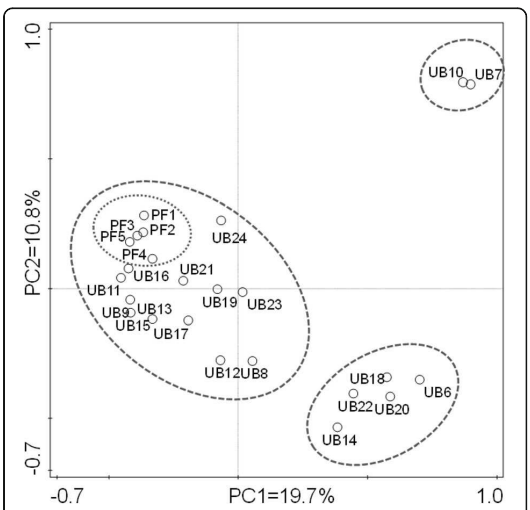

Fig. 3 PCoA biplot of the gut microbiome in Brazilian green turtles based on the Bray-Curtis distance matrix. Wild turtles came from Praia do Forte (PF1 to PF5) and Ubatuba (UB6 to UB16). Captive turtle came only from Ubatuba (UB17 to UB24). The percentage of explained variation encompassed by the two main axes has been indicated. The main sample score clusters (dashed contours) and the more specific subcluster from Praia do Forte (dotted contour) have been highlighted

A high abundance of *Proteobacteria* had been previously reported from the cloaca of pelagic (range 17.1–21.7 cm CCL) and recently settled (29.4–34.6 cm CCL) juvenile green turtles from Florida and from the gut of omnivorous marine fishes, but not from other groups of herbivorous vertebrates (Table 3). A high abundance of *Proteobacteria* has been observed also in two wild and one captive green turtles from Brazil less than 45 cm CCL (this study), but this is probably because they were immunodepressed and not because of recent settlement. We hypothesize that the prevalence of the *Proteobacteria* phylum in those three individuals was because of lesions from anthropogenic impacts [52]. The same is true for *Mycobacterium*, from the *Actinobacteria* phylum, a genus very uncommon in turtles but which includes several well-known pathogens for reptiles and amphibians [53, 54]. Furthermore, three captive and one wild turtle shared OTUs affiliated to the *Akkermansia* genus (*Verrumicrobiaceae* family). *Akkermansia* is a mucin-degrading bacterium commonly found in the human gut and recently isolated in reptiles [55, 56]. Several studies showed that the enrichment of *Akkermansia* induces gut inflammation and is associated with colonic diseases in mammals, but nothing is known about its pathogenicity in reptiles. It is also worth noting a small captive turtle (34.9 cm CCL) with a microbiota dominated by *Bacteroidetes* and *Firmicutes* but with a high relative abundance of *Fusobacteria*, a group occurring sporadically in carnivorous marine mammals [4].

High levels of *Firmicutes* are characteristic of the gut and fecal microbiota of herbivorous vertebrates (Table 3), as this phylum plays a critical role in the fermentation of complex polysaccharides [3, 57]. The families *Ruminococcaceae* and *Lachnospiracea* are particularly relevant, as both are obligate anaerobes with capacity to degrade structural polysaccharides into short-chain volatile fatty acids [3, 58–62] and occur in large numbers only in the gut and feces of herbivorous tetrapodes [3, 8, 62, 63]. Short-chain volatile fatty acids are indeed the main product of fermentation of plant material in the large intestine of green turtles [39, 64], and the analysis of the green turtle microbiota reported here revealed that *Ruminococcaceae* and *Lachnospiraceae* represented 3–30% of the OTUs recovered from the rectal and fecal samples of most

Table 3 Relative abundance of bacterial phyla to the gut microbiota of omnivorous and herbivorous vertebrates

Species	Diet	*Firmicutes*	*Bacteroidetes*	*Verrucomicrobia*	*Spirochaetes*	*Proteobacteria*	*Actinobacteria*	Other	Source
Teleosteans									
Acanthurus gahhm[1]	Omn/Alg	**29.5**	0.6	0.0	1.3	**49.4**	7.7	9.1	Miyake et al. (2015)
Naso elegans[1]	Herb/Alg	**97.4**	0.0	0.0	0.0	0.0	0.0	2.6	Miyake et al. (2015)
Naso unicornis[1]	Herb/Alg	**83.3**	9.0	**2.6**	0.0	2.6	1.3	1.2	Miyake et al. (2015)
Siganus stellatus[1]	Omn/Alg	**42.3**	11.5	0.0	2.6	**37.2**	0.0	6.4	Miyake et al. (2015)
Turtles									
Chelonia mydas[a,2]	Omn/Alg	6.5	27.1	0.6	0.0	**60.5**	0.1	5.2	Price et al. (2017)
Chelonia mydas[b, 2]	Herb/Seg	8.3	15.4	0.2	0.2	**66.6**	1.7	7.6	Price et al. (2017)
Chelonia mydas[c,e,3]	Herb/Alg	10.8	11.8	0.1	0.1	**60.7**	15.2	1.3	This study
Chelonia mydas [d,3]	Herb/Alg	**44.8**	**46.6**	**3.8**	1.3	1.1	0.3	2.1	This study
Geochelone nigra[3]	Herb/Ter	**81.1**	4.4	0.1	0.0	2.0	0.8	11.6	Hong et al. (2011)
Gopherus polyphemus[3]	Herb/Ter	**38.4**	**36.9**	**3.0**	4.4	< 3.0	< 3.0	7.4	Yuan et al. (2015)
Lizards									
Amblyrynchus cristatus[3]	Herb/Alg	**75.1**	8.2	1.0	0.0	0.6	0.6	14.5	Hong et al. (2011)
Conolophus spp. [3]	Herb/Ter	**63.9**	4.2	0.2	0.0	1.4	1.3	29.0	Hong et al. (2011)
Iguana iguana[3]	Herb/Ter	**74.0**	10.1	1.0	0.6	3.1	0.1	11.1	Hong et al. (2011)
Mammals									
Antidorcas marsupialis[3]	Herb/Ter	**75.6**	24.4	0.0	0.0	0.0	0.0	0.0	Ley et al. (2008)
Dugong dugong[3]	Herb/Seg	**57.5**	**42.5**	0.0	0.0	0.0	0.0	0.0	Eigeland et al. (2012)
Gorilla gorilla[3]	Herb/Ter	**67.4**	3.5	**10.5**	2.3	0.0	11.6	4.7	Ley et al. (2008)
Loxodonta africana[3]	Herb/Ter	**80.5**	2.5	**1.8**	0.2	10.1	4.7	0.2	Ley et al. (2008)
Ovis canadensis[3]	Herb/Ter	**64.0**	3.0	**2.7**	0.0	2.1	25.8	2.4	Ley et al. (2008)
Trichechus manatus[3]	Herb/Seg	**77.3**	19.5	0.0	0.1	0.3	2.0	0.8	Merson et al. (2014)

Bold type denote accumulated RA higher that 60%. Superscript numbers denote sample source as follows: [1] whole intestinal tract, [2] cloaca; [3] rectum or feces. Diet: ormnivores (Omn) or herbivores (Herb). Major group of plants in diet: algae (Alg), seagrasses (Seg) and terrestrial plants (Ter). Length of green turtles *Chelonia mydas*: [a] 17.1–21.7 cm CCL, [b] = 29.4–34.6 cm CCL, [c]39.7–44.7, [d] 31.1–64.7. [e] potentially immunodepressed individuals

juvenile green turtles, thus confirming their capacity to ferment structural polysaccharides. This suggests that juvenile green turtles with a *Firmicutes-Bacteroidetes* dominated fecal microbiota were plant-based omnivores or herbivores, which agrees with available dietary information [31, 33, 34, 65–68].

Interestingly, *Ruminococcaceae* prevail over *Lachnospiraceae* in terrestrial herbivorous reptiles [3] but the opposite appears to be true in marine iguanas [3] and in green turtles. Macroalgae are the staple food of both groups and differ from seagrasses and terrestrial plants in high levels of sulfated polysaccharides and alginic acid and low levels of cellulose [18]. This suggests that the prevalence of *Lachnospiraceae* over *Ruminococcaceea* in marine iguanas and green turtles is related to the similar composition of the polysaccharides in their diets. Nothing is known about the microbiota of green turtles feeding on seagrasses, but the profiles of the short-chain volatile fatty acids produced in the large intestine of green turtles feeding on seagrasses and those feeding on macroalgae differ [39, 64], thus suggesting potential differences in their microbiota worth exploring in further research.

Another major difference between the rectal and fecal microbiota of green turtles and those of other herbivorous vertebrates is the high abundance of *Bacteroidetes* in the former, a pattern reported previously only from dugongs (*Dugong dugong*) and gopher tortoises (*Gopherus polyphemus*) (Table 3). *Bacteroidetes* may contribute significantly to the initial attack on both simple and complex carbohydrates [69], and Yuan et al. (2015) speculated that the high prevalence of *Bacteroidetes* in gopher tortoises might be related to the seasonally low temperatures experienced in subtropical environments. However, *Bacteroidetes* had a similar prevalence in green turtles from tropical Praia do Forte and from subtropical Ubatuba (this study), thus suggesting that seasonal differences in temperature are unlikely to not induce major changes in the relative abundance of *Bacteoidetes* and *Firmicutes*, although samples were collected in summer in both areas. A high abundance of *Bacteroidetes* is neither characteristic of the gut microbiota of herbivorous chelonians, as they represent only 4% of the relative abundance of bacteria in the microbiota of Galapagos giant tortoises (*Geochelone nigra*) [3]. It is suggested that the high presence of this phylum in all the samples of green turtles from Brazil, except those of the three anomalous individuals, could be related to the presence of high levels of organic matter in coastal waters, which allow copiotrophs (such as *Bacteroidetes*) to thrive and dominate the microbial community structure [70]. Moreover, a recent study of gut microbiota of the loggerhead sea turtle *Caretta caretta* [71] found that *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* were the most predominant microbial population in turtle feces.

Spirochaetes is another group of non-cellulolytic bacteria associate with specific plant substrates during digestion [72], facilitating the breakdown of cellulose by co-occurring bacteria [73]. Within this phylum, the *Spirochaetes* members exhibit enormous diversity in a free-living or host-associated life, being pathogenic or non-pathogenic, and aerobic or anaerobic [74]. This phylum has also been reported to be a major component of the microbiota of gopher tortoises, omnivorous fishes and gorilla, but not in other herbivorous reptiles (Table 3). OTU 6, an unidentified *Spirochaetes*, was detected in all the samples, but only in the rectal samples of three individuals from Praia do Forte (PF3, PF4, and PF5) did it represented more than 2% of the relative abundance.

The fact that *Bacteroidetes* and *Firmicutes* were the dominant bacteria in the feces and the rectal samples of most juvenile green turtles less than 45 cm CCL, including four specimens ranging 31.1–35.0 cm CCL, indicates that they acquired a microbiota adapted to digest polysaccharides shortly after settlement. How this specialized bacterial flora is acquired by settlers remains unknown, but land and marine iguanas have been observed consuming conspecific excrements [17, 75], which certainly facilitate acquiring a plant degrading microbiota. Juvenile green turtles are not gregarious, but may form dense aggregations [31, 76], which might facilitate feces consumption and hence the quick acquisition of a bacterial flora adapted to digest polysaccharides. Alternatively, fermenters might be transferred through the diet, as they can be associated with algal surfaces [77].

Algae and seaweeds are typically rich in sulfated polysaccharides that are absent in terrestrial plants. Hence, microbiota from the phylosphere of seaweeds are characterized by high copy numbers of sulfatases in their genomes [78]. A recent study suggested that traditional sushi food, which is largely composed of seaweeds, significantly affected the gut microbiome of the Japanese population [79, 80]. It was then observed that carbohydrate-active enzymes (CAZymes) in the gut microbiome, which are absent in the human genome, were acquired by horizontal gene transfer (HGT) from the marine bacteria associated with seaweeds. Moreover, [81], reviewed several studies on the HGT phenomena between environmental and gut bacteria within the phyla of *Bacteroidetes* and *Firmicutes* in different organisms, including the grazer surgeonfish. Hence, it is well possible that the seaweed-based diet of turtles could similarly affect their gut microbiota by gene acquisition, considering that CAZymes and sulfatases are required for efficient seaweed degradation [82]. This topic merits further research taking advantage of the existing programs on captive breeding of green turtles by performing gut metagenomics analysis.

In any case, the fast acquisition after settlement in coastal areas of a microbiota adapted to ferment polysaccharides should enable green turtles to adopt an herbivorous diet soon after recruitment. This is the pattern reported from tropical areas [25, 83], but in warm temperate and subtropical regions, juvenile green turtles are best described as plant-based omnivore and only adults are primarily herbivores [19–21, 23, 33, 34, 37, 84, 85]. The results presented here indicate an increase in the taxonomic richness of the gut microbiome as turtles grow, but this is an unlikely explanation by the progressive ontogenetic dietary shift, because even small turtles had a high abundance of *Ruminococcaceae* and *Lachnospiraceae*. Consumption of animal material results into a slight and statistically significant increase in the relative abundance of *Proteobacteria*, as revealed by the differences between captive and wild healthy turtles, but the abundance of *Ruminococcaceae* and *Lachnospiraceae* remains high anyway. This suggests that omnivore is unlikely to reduce the capacity of green turtles to digest plant material.

Digestibility of plant material in green turtles increases with temperature [13] and the body temperature of juvenile green turtles inhabiting subtropical regions is close to that of water during winter months [86]. Conversely, the body temperatures of inactive adult green turtles can be 2 °C above water temperature thanks to gigantothermy [36], which explains why the digestibility of plant material by green turtles increases with body size even in tropical settings [13]. Interestingly, the apparent digestibility of plant material does not increase with body size in marine iguanas [86], because even very small individuals can rise significantly their body temperature through basking in black lava [17]. Green turtles bask regularly in the beaches of Hawaii and Galapagos [87, 88] and this behavior has been suggested to improve digestion, but beach basking has never been reported in other areas to our knowledge. If green turtles inhabiting subtropical and warm temperate regions do not bask in winter, the digestibility of plant material by small individuals can be compromised during winter, even if they support a specialized microbiota rich in *Ruminococcaceae* and *Lachnospiraceae*, which may explain the progressive dietary shift as they grow.

Conclusions

This study revealed that juvenile green turtles from the coastal waters of Brazil had the same general microbiota profile, regardless of size and origin (wild vs. captive; subtropical Ubatuba vs. tropical Praia do Forte). This indicates a fast acquisition of a microbiota with capacity to ferment structural polysaccharides soon after settlement in the coastal waters of Brazil and that the regular consumption of animal prey does not significantly reduce the presence of *Ruminococcaceae* and *Lachnospiraceae* and, hence, does not impair the capacity to ferment structural polysaccharides. However, subtropical specimens displayed a larger variability in the gut microbial community structure, which in the most extreme cases was clearly related to poor physical condition. In summary, there is no reason for a delayed ontogenetic dietary shift after settlement, unless low winter temperature reduces their capacity to digest plant material.

Additional file

Additional file 1: Phylogeny and incidence of gut eubacteria. (XLSX 1339 kb)

Abbreviations

BA: State of Bahia; CCL: Curved carapace length; NGS: Next-generation sequencing; OTU: Operational taxonomic unit; SP: State of Sao Paulo

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Availability of data and materials

DNA sequence data from the MiSeq NGS assessment was submitted to the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) under the accession number SRP114384. The bioestatistical data generated and analyzed during this study are included in this published article and its supplementary information files.

Authors' contributions

LC directed the overall research project. LC and FPB designed the experiments and drafted the manuscript. PC carried out the field work and was the first author. MG performed the DNA sequencing and bioinformatics processing. All authors participated in the analysis and interpretation of the data, and contributed significantly in writing the final manuscript. All authors read and approved the final manuscript.

Ethics approval

Field work in natural reserves and handling of wild animals was carried out under the authority of the Instituto Chico Mendes de Conservação da Biodiversidade–ICMBio (license reference ICMBio/SISBIO 52128-1), and of the Convention on International Trade in Endangered Species of Wild Fauna and Flora–CITES (license reference CITES 16BR020234/DF).

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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Los hábitats utilizados por las tortugas marinas durante su desarrollo varían notablemente en cuanto a características ambientales y disponibilidad de alimento. Comprender los factores que determinan la selección del hábitat y de la dieta resulta esencial para comprender la interacción que existe entre dichas especies y sus hábitats. Dicho conocimiento también proporciona datos para adoptar medidas de gestión y conservación destinadas a restaurar y mantener las poblaciones de tortugas marinas y así, su función ecológica en el ecosistema. Las zonas costeras de Brasil, Uruguay y Argentina albergan diversos hábitats de importancia para la alimentación de los juveniles de la tortuga verde, pero todavía existe un gran desconocimiento sobre la ecología trófica de esta especie durante la fase juvenil de transición desde hábitats pelágicos a bentónicos. En esta tesis se ha estudiado cómo se produce el asentamiento de las tortugas verdes en la costa de Brasil, incluyendo la selección del hábitat y la adquisición de un microbioma adecuado para la digestión de material vegetal. Los resultados demuestran que el asentamiento de las tortugas verdes a lo largo de la costa oriental de Sudamérica está fuertemente influido por la corriente del Brasil y que el cambio ontogenético es rápido en las zonas tropicales y más lento en las subtropicales, a pesar de la rápida adquisición de una microbiota capaz de degradar polisacáridos complejos en ambas zonas. Dicha microbiota resulta en una gran eficiencia en la digestión de algas, especialmente de las Rodophyta, pero no impide la digestión de presas animales. Por último, las tortugas juveniles seleccionan de forma preferente para su alimentación zonas de arrecife de baja rugosidad, someras y con poco coral vivo.